Diego Breviario
Jack A. Tuszynski *Editors*

Life in Science

Stories, Opinions and Advice for a New Generation of Scientists

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Preface

This book is intended to serve as an honest and clear manifesto in support of the profession of scientist. Being a scientist is one of the most stimulating, gratifying and important positions in modern society. Today, this is clear more than ever because our societies depend for their survival and prosperity on scientific knowledge and related technical developments. Almost any aspect of our present-day life: health, travel, communication, energy supply, etc. is based on scientific achievements and technological innovation. Scientists are actively acquiring new knowledge and transforming it into new applications for the benefit of humankind, the maintainance of high standards of living and the protection of our environment from degradation. New major challenges have recently emerged, such as the depletion of natural and genetic resources, the damage produced by pollution and climate change, the need for an adequate food supply across the planet and the development of new sources of energy from renewable resources.

All of these challenges call for a new generation of scientist to engage both their minds and hearts in order to take seriously the privilege and the responsibility of finding new creative solutions for the future. We believe that it will be helpful in this quest to follow in the footsteps of senior scientists. In fact, knowledge and experience must be passed down from generation to generation to ensure continued progress and to avoid repeating old mistakes. This, in fact, is the primary purpose of the book.

Twenty-two experienced and accomplished scientists who have worked successfully in a variety of fields have accepted the invitation to share their personal stories. They write about their lives in science and provide thoughts and reflections replete with sage advice for their younger colleagues. They have made many candid comments on their scientific interests, their vocation and enthusiasm for knowledge creation. As evidenced in the book, science is a vast and bountiful undertaking that can be explored and experienced from many different vantage points, from a theoretical level to the management of technical facilities. Accordingly, all authors used their personal styles, from the autobiographical to the philosophical, to describe their experiences and reflections. Although largely aimed at future and early-career scientists, the book also contains fascinating details about individual scientists and

the scientific environment that will be of interest to the general public, high school students and scholars alike.

We increasingly felt a need for this type of book within the vast scientific literature and thought it was about time to produce it. This is especially true now that, due to the COVID-19 pandemic, science has forcibly entered into all households through the media. However, science has not always been presented properly or understandably to the general public, leading to false interpretations and even conspiracy myths. In contrast, each essay in this book is a message of hope and commitment to a revitalized world confidently relying on continuing advances in science and their translation into beneficial technological innovations.

Milan, Italy — Diego Breviario
Edmonton, Canada/Turin, Italy — Jack A. Tuszynski

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Editors and Contributors

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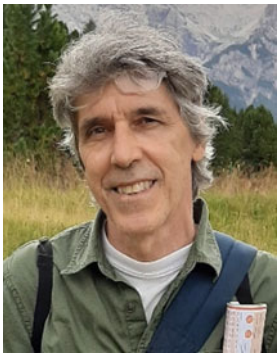

Diego Breviario worked for 40 years in the field of molecular genetics applied to yeast, humans and plants. His work took him to the National Institute of Health USA; North Carolina State University USA; and the Italian National Research Council. He has been scientific leaders of more than 30 projects, with more than 100 international publications to his name. He has been on the editorial board of the journals: *GENES*, *Transgenic Research* and *PLOS ONE*. He has received awards from Ciba-Geigy, Academia dei Lincei-Rotary, National Institutes of Health USA. He has also authored three novels on science, published in Italian. Diego has also prepared texts for educational videos in the English and Italian language. As an inventor, he appears on three released patent applications, one national and two European. He has served as an advisor for the EU and for national and international universities. Major expertise: genetics, genomics, cell biology. Major area of interest: agrifood.

Jack A. Tuszynski is Professor of biophysics at the Department of Physics, University of Alberta, and Professore Ordinario in biomedical engineering at the Politecnico di Torino in Turin, Italy. He obtained his Ph.D. from the University of Calgary. From 1983 to 1987, he was a faculty member at the Department of Physics of the Memorial University of Newfoundland in St. John's. Between 2005 and 2020, he held the prestigious Allard Chair in Oncology at the Cross Cancer Institute. He held the visiting professorship in China, Germany, France, Israel, Denmark, Belgium and Switzerland. He is on the editorial board of almost 30 international journals including the *Journal of Biological Physics*. He is Associate Editor of *The Frontiers Collection*, Springer-Verlag, Heidelberg.

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My Life Under the Star of Biology

Yaroslav Blume

Abstract Contributing to this book has been a small but very pleasant challenge. While it is very easy to write about an individual vocation, it is actually difficult to objectively evaluate your scientific achievements, because that involves balancing the subjective and objective aspects of your scientific activity which, of course, may follow different individual paths since, as stated by Diego: "different is the motivation, the ambition, the inclination, the intuition, the feeling, the education, the belief, the eligibility". But translation of this individual attitude, that is, the level of subjectivity, to the level of objectivity, which will be shared and perceived by the international scientific community, depends not only on the results that will be obtained using generally recognized methods of scientific research, own observations and logic, but also from your personal understanding on how to correctly use these tools. Having traveled the path in biology for more than 40 years, I witnessed the phase transition of biology from destructive to synthetic science. Of course, there is no clear dividing line between these two because the deeper we dive into the structure of the cell and its components, the more we understand the primary functional connections, we set the basis for the resynthesis of individual life processes, at the cell and even whole organism levels. This is the paradigm of what now we call the golden age of biology. Obviously, I was not only a witness of this transition, but also one of the participants in its implementation, passing the way from the basics of biochemistry to cell biology, genomics and molecular biotechnology. So, I will try to describe this path and how I see its continuation under the guiding star of biology.

1 Motivations: How I Developed an Interest in Science

Biology has been present in my life since childhood thanks to my father, Borys Blume, who was a school biology teacher. Naturally, back in those bright days of childhood, he introduced me into the world of living nature that surrounded us.

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Thanks, perhaps, to this, during all my school years, I was very attracted by classical biology: botany and zoology. Also, my father's life experience, who remembered well from his studentship the flourishing of genetics in the late 20s–early 30s, followed by its oppression by Lysenko during the notorious period of the 40–50s of the last century, provided me with a reasonable basis for an entire perception of modern biology.

That was how I came to Kyiv University being already a somewhat formed biologist. Having graduated from high school with distinction (I was awarded with a gold medal), I had an advantage at the entrance examination, which meant that I could be enrolled at the university if my comprehension of biology was confirmed as excellent. The entrance exam was hard but successful, and I became a student of the Faculty of Biology. My student period of life (1973–78) fell on a still turbulent period in the development of biochemistry, which increasingly fueled the development of molecular biology (Frey 2002). This trend coincided with efforts to formalize the educational process at my university. After the first year of study, we were suggested to specialize, separately from other biologists, in either biochemistry or biophysics. I chose biochemistry despite my personal preference was genetics. At that time, genetics in our country was barely reviving as an academic discipline after it had nearly collapsed during the difficult times of Lysenko's persecution campaign. As before, my decision to choose biochemistry was mostly determined by an advice and scientific expertise of the people closest to me. My elder brother, Oleg Blume, was at that time, as well as his wife Yevgenia Kopachevska, already an established lichenologist, maintaining strong scientific ties with colleagues in Europe and the USA. He emphasized that even the development of lichenology as a classical discipline would certainly require the use of both biochemical and molecular genetic tools. In fact, Oleg was one of the first to apply molecular genetic methods to solve specific issues of lichen taxonomy (Blum and Kashevarov 1986; DePriest 2004). It was he who advised me to focus on modern priority areas of biology development, and Yevgenia supported him, although she was very concerned about the development of classical areas of botany and zoology in Ukraine. So, I became a biochemistry student.

The peculiarity of our system of higher education was, and still is, that universities are structurally separate from leading research institutions. As in the Soviet Union times, we have universities with a research potential designed more to follow the educational process rather than to pursue advanced knowledge. On the other hand, research institutes, primarily those under the auspices of the National Academy of Sciences of Ukraine, despite all current challenges and chronic inability to carry out relevant reforms, are still an outpost of fundamental science and advanced research developments. Therefore I, a student of the Department of Biochemistry, had to face a personal challenge, had to set my personal path answering to the following question: where could I possibly acquire skills and practical research experience? Again, the choice was limited to two priority directions, which at that time sounded loudest in the Ukrainian academic community: molecular neurophysiology led by Academician Platon Kostyuk (Verkhratsky et al. 2010) or molecular biophysics and plant physiology led by Academician Dmytro Grodzynsky (Grodzinsky 1976). Since I liked plant biology more, I preferred experimental work at the Institute of Plant

Physiology, headed by Dmytro Grodzynsky, where I started studying the structure and functions of chromatin in plants. It was an invaluable experience for a young man who was looking for his ways in contemporary biological science. Influenced by the work of Don and Ada Olins, who at that time had just described the nucleosomal structure of chromatin (Olins and Olins 1974) and the discovery of the role of cAMP as a second messenger (Sutherland 1972), I began to investigate about the spatial structure of histones using electron paramagnetic resonance and nuclear magnetic resonance. These experimental endeavors led me into the topic of post-translational modifications of histones, the study of which gained a new lease of life on the edge of reconstruction of the nucleosomal structure of chromatin (Bannister and Kouzarides 2011). The department got intesively involved in this subject and I was offered, by my Head, Prof. Mykola Kucherenko, the possibility of joining the experimental laboratory where I could continue my studies on histone post-translational modifications induced by radiation injury.

The completion of the diploma thesis bridged me to postgraduate studies at Kyiv University, where as Assistant Professor at the Department of Biochemistry, I defended my Ph.D. thesis at the Institute of Biochemistry at the National Academy of Sciences of Ukraine (March 1982). I still believe that I was fortunate to have both a prominent academic supervisor and a rather hot research topic. Also, although a postgraduate student, I had sufficient freedom of choice for my own experiments and all resources needed for my research, which seemed to be a rare exception under the Soviet planned economy rule. Moreover, I already had a small group of junior colleagues (students and those who entered the postgraduate school after me), with whom I carried out additional research, beyond the scope of my dissertation objectives. I deeply appreciate the creative support I got from my academic supervisor. Due to such favourable opportunities, I defended my second thesis for the degree of Doctor of Biological Sciences (which is equal to habilitation) in February 1988 and became one of the youngest Doctors of Sciences not only in Ukraine, but in the whole USSR. Although I was already an Assistant Professor at the Department of Biochemistry, I did not feel enthusiastic about devoting all my further professional life to lecturing at the university, as it would have taken a major bulk of my working time (up to 1000 h per academic year). It so happened that I decided to move at the National Academy of Sciences of Ukraine to do research, even before defending my second thesis.

At that time, the star of Yuri Gleba glowed brighter and brighter in the sky of biological science, and in 1987 he invited me to join his team at the M.G. Kholodny Institute of Botany. His considerable efforts were then focused on obtaining somatic plant cell hybrids (Kumar and Cocking 1987; Gleba 1995), and I was suggested to investigate the cellular mechanisms of cytoskeleton function in such hybrids. Thus, I started exploring the structure and functions of microtubules and, in particular tubulin, their buiding block protein, thereby opening the door to the world of cell biology and plant biotechnology. In June 1990, we separated into the Institute of Cell Biology and Genetic Engineering, which I took over to run shortly after Y. Gleba's departure to the USA.

A little earlier, back in 1989, an agreement with Kyiv University was signed on setting up a Department of Cell Biology and Genetic Engineering, which would have been fully staffed with faculty and infrastructure by our Institute. Nowadays, graduates from this Department work not only in Ukraine, but also in a number of European countries, in the USA and Canada. This department issued a ticket to life to my wife, Alla Yemets, who, having received a MSc degree, successfully completed the Ph.D. program in cell biology at the department and came to work in our Institute. At present, she is a Professor, an Associate Member of the National Academy of Sciences of Ukraine and a member of the Scientific Committee of National Council of Ukraine for Science and Technology Development, the Ukraine's national think-tank. She was also a driving force for the establishment of the National Research Foundation of Ukraine. I cannot imagine all the years we have lived together without her support, and essential participation in the implementation of those research initiatives that allowed us to better understand specific details of the mechanisms of functioning of plant microtubules, including relevant tubulin amino acid mutations and post-translational modifications.

It is here worthwhile to note that I have always strived to remain active into research, not surrending to the heavy administration charges, although the life circumstances often pushed me to reach higher and higher levels in the management of science. Staying in science supporting and being part of a team capable of doing up-to-date fundamental research while confronting the difficult times of a new State formation, and the related socio-economic re-organization of society, has been quite a challenge and has obviously asked for some compromise of which I had already a taste, from 1992 to 2008, when Yuri Gleba, left the institute to work for American Cyanamid (later, American Home Products; Princeton, New Jersey) before founding first Phytomedics (USA) and then Icon Genetics and Nomad Bioscience, in Germany. During all that period, I was delegated to act as a Director of the Institute of Cell Biology and Genetic Engineering. Thus I had to take the responsibility for the correct functioning of the entire Institute while trying to develop and support my own laboratory. It thus turned out that the combination of research activities with a somewhat higher level of administrative freedom, always shared with the other research groups of the Institute, gave me exceptional opportunities to establish a team (and update it again after each wave of brain drain) motivated to solve cutting edge challenges in the vast field of cell biology, molecular genetics and plant biotechnology. So, it became quite consequential that after this productive period I was asked by the President of the National Academy of Sciences of Ukraine, Borys Paton (Osipian 2018) to reorganize a small institute by merging it with my Department at the Institute of Cell Biology and Genetic Engineering. This led to the establishment of the new Institute of Food Biotechnology and Genomics where I have been acting as the Director since then.

2 Work Done: My Personal Scientific Approach

Looking back at my pathway in science and analysing my attainments, I cannot see any particular reason to be dissatisfied with what I have achieved. Naturally, at the beginning of this path, during my student and postgraduate years, all my experimental outputs, even phenomenological or purely correlative, seemed to me great achievements, but discussing them with senior colleagues, deeply immersed in the perusal of scientific literature and actively participating in scientific events, I quickly adjusted my perception better defining my tasks. In fact, while I was growing a feeling of disaffection about the results of my PD thesis, which looked to me a bit shallow, I came across to the functional significance of certain post-translational modifications of histones, a more inspiring study to which I devoted a good share of my creative search.

In order to better clarify the functional role of some post-translational modifications of histones such as phosphorylation, acetylation, methylation and poly(ADP)-ribosylation, I worked out a protocol for the in vivo synchronization of DNA replication and protein synthesis during the cell cycle. At that time, most investigators used partial hepatectomy as the most attractive approach to address the same issue but it turned out to be inadequate, given the acute post-surgical effects of such an intervention at the level of the whole organism. Quite differently, I relied on the use of cycloheximide, an effective reversable inhibitor of protein synthesis, to synchronize the growth cycle in the cells derived from experimental animals. The idea of using this cell-based approach was successful, and the use of cycloheximide to block the cycle, turned out to be very effective not only for synchronizing hepatocytes at the phase of DNA replication, but also for analyzing the mechanisms of action of both radiations and radioprotectors on DNA and protein synthesis (Aslamova et al. 1983) as well as identifying the occurrence of specific histones post-translational modifications (Aslamova el al. 1984).

At that time, research on radiobiological effects and protection was a rather promising topic, well supported by the state, even as fundamental research. A combination of all these opportunities allowed me to deepen my studies on both the enzymatic systems that lead to the post-translational modifications of nuclear proteins and the relationships of these modifications with different phases of the cell cycle. Ultimately, I was capable of identifying certain regularities of the effect of histone post-translational modifications on the supramolecular (nucleosomal) structure of chromatin, developing an additional understanding of the biochemical mechanisms of regulation of the structural and functional properties of chromatin which are based on the intranuclear involvement of secondary messengers such as cAMP and cGMP.

Unfortunately, the Berlin Wall was then an obstacle to the wider publication of our research findings but my move to the National Academy of Sciences and the beginning of the research on the molecular organization of the plant cytoskeleton (microtubules and microfilaments), and their involvement in intracellular signaling coincided with the times of “perestroika” and the fall of that very wall. It so happened that my previous expertise in the field of histone post-translational modifications

could effectively apply to the study of post-translational modifications of plant tubulin (Smertenko et al. 1997). This turned out to be a great change because tubulins, compared to histones, allow a much wider range of possible practical exploitations, a perspective and a goal that, since then, I would always have treasured combining the development of biotechnological applications to the progress made in different research branches such as: cellular and genetic engineering, structural bioinformatics, genomics, molecular assisted breeding. That was probably due to the fact that I subconsciously followed the words of Louis Pasteur: "There is no such thing as a special category of science called applied science; there is science and its applications, which are related to one another as a fruit is related to the tree that has borne it".

When I was elected to the Academia Europaea (2021), my nominators and prominent scientists, members of that Academy, Prof. Ilan Chet (https://www.ae-info.org/ae/Member/Chet_Ilan) and Prof. Oleg Krishtal (https://www.ae-info.org/ae/Member/Krishtal_Oleg) formulated the following three reasons in favor of my candidacy:

- Top scientist in biochemistry and cell molecular biology of the plant cytoskeleton with original investigations on post-translational modifications of plant tubulin, including description of phosphorylation and nitration on tyrosines. He developed functional genomic approaches for understanding plant tubulin interaction with antimicrotubular drugs and for developing relative biotechnological tools to be used in practice.
- Multidisciplinary aspects of the nominant's research activity like development of plant structural bioinformatics, new approaches for plant genetic transformation, green synthesis of quantum dots as well as novel biotech aspects for generations of liquid biofuels.
- Personal contribution to the European scientific collaboration, representing the case of a Eastern European scientist capable of gathering top specialists in plant cytoskeleton and launching a new tradition of the meetings.

Also, my knowledge on ***plant cell biology,*** developed in the late 80s and early 90s gave me the opportunity to investigate the role of microtubules in the processes of plant somatic hybrids production and stabilization. My research team managed to obtain a number of plant cell lines carrying mutations in microtubular proteins that were successfully used by A. Yemets to produce symmetric and asymmetric somatic hybrids and create transgenic plants with resistance to antimicrotubular herbicides such as: dinitroanilines, phosphoroamidates, and phenylcarbamates (Yemets et al. 2008).

Simultanously and for the first time, we obtained cell-biological evidences of the role of tubulin post-translational modifications (phosphorylation, acetylation, polyglutamylation and tyrosination/detyrosination) in the regulation of plant microtubule function. Among them, we described the phosphorylation of plant tubulin on tyrosine residues (Blume et al. 2008), and reported about the functional significance of tubulin nitrotyrosylation (Yemets et al. 2011).

Currently, our attention is focused on cellular and molecular signaling mechanisms underlying the effects of abiotic factors on plant cells, and we are investigating about the role of cytoskeletal structures in these processes. We analyse the role and the place of cytoskeleton structures like microtubules and microfilaments, in the response of plant cell to ultraviolet (UV-B) irradiation, cold, high temperatures, heavy metals, herbicides with antimitotic activity as well as in the autophagy mechanisms. In particular, the ability of UV-B radiation to cause the phenomenon of programmed plant cell death was revealed and the participation of the cytoskeleton in mediating the action of UV-B was demonstrated. The mechanisms of participation of microtubules and post-translational modifications of their proteins (in particular tubulin acetylation) in the development of autophagy at the stage of autophagosome formation were also unraveled.

Eventually, these attainments in the biological study of the plant cytoskeleton led me to start research in the field of ***structural bioinformatics,*** in Ukraine. We were the first to develop original structural biological approaches for 3D-modelling of plant tubulin (Blume et al. 2003). Over the last decade, we have used and introduced new tools for the 3D-modelling of the main cytoskeletal and cytoskeleton-related proteins such as plant and bacterial FtsZ-proteins, tubulins, kinesins, protein kinases and phosphatases and for identification of the spatial effects of post-translational modifications of tubulin. Additional efforts have been oriented toward the creation of the databases and the search of low-molecular weight compounds with high affinity to the targeted cytoskeletal proteins; in silico high throughput screening of biologically active compounds and the molecular design of new compounds with herbicidal, fungicidal, antituberculosis, antiprotozoal, anthelmintic and antitumor biological activity.

An important part of my efforts has been and still is focused on ***plant molecular genetics, genomics and molecular biotechnological*** investigations as the development of the molecular markers for genes associated to specific agronomic traits, including QTL-loci; the development and implementation of the molecular genetic approaches for the identification of wheat and barley genes conferring resistance to the pathogens causing leaf, stem and yellow rust; the screening of the existing wheat and barley collections for the presence of resistance genes to these diseases; the study of the genetic diversity of wild relatives of cultivated cereals (e.g., *Aegilops* sp.) as a potential source of useful genes. The identification of genes encoding for proteins associated with the cytoskeleton, for their pyramiding in order to achieve resistance of new genotypes of cereals to highly pathogenic races of stem rust, continues to be particularly attractive to me.

A number of more technical oriented projects aimed at the improvement of the efficiency of *Agrobacteruim*-mediated and biolistic plant transformation and the development of new methods for gene transfer using nanoparticles, carbon nanotubes and other nanopolymers have been also successfully accomplished. In fact, transformation techniques for transgenic plant production with the desired traits such as herbicides or insect pest resistance have been developed in different plants such as: barley, sugar beet, potato, flax, finger millet, false flax (camelina), soybean, and, naturally, tobacco. Genetics experiments aimed to study the possible involvement of

cytoskeletal proteins in the acquisition of plant resistance to herbicides with antimitotic activity allowed us to identify and isolate the mutated forms of those tubulin genes responsible for the resistance trait and, eventually, to obtain plants resistant to these chemicals. New safe and environmentally friendly marker system for the selection of the transformed plant lines based on mutant tubulin gene has also been developed. Transgenic plants were used as "green factories" for the production of pharmacologically valuable products such as lactoferrin.

I particularly value our recent achievements in the development of new methods in ***nanobiotechnology***, namely the production ("green synthesis") of nanoparticles and quantum dots by biological systems (Borovaya et al. 2014). Completely original work accomplished on the development of these methods for obtaining quantum dots of various compositions, which can be used to visualize subcellular structures, including those of the cytoskeleton, is now of a particular interest for us (Yemets et al. 2022).

With my effective participation, new varieties of false flax, finger millet, sweet sorghum, and miscanthus for ***biofuel production*** were obtained using molecular breeding approaches. Original technologies for bioethanol production from sweet sorghum and finger millet have been developed as well as the technology of biodiesel production based on camelina oils (Blume et al. 2022a) and bioethanol for engines has been developed and the pilot equipment has been constructed.

Due to my scientific accomplishments, in 1995, I was elected an Associate member and, in 2006, a full member of the National Academy of Sciences of Ukraine. Also, I was awarded the Academy premia: after V.Y. Yuriev (genetics) (2002), after M. G. Kholodny (plant physiology) (2019), and honors "For Scientific Achievements" (2016). In 2011, the Parliament of Ukraine awarded me with the Certificate of Achievements, in 2012 I became the laureate of the State Prize of Ukraine in the field of science and technology, and in 2016 I received the Honorary State Title "Distinguished Person in Science and Technology in Ukraine".

My scientific outlook has been significantly impacted by my visits abroad as a Visiting Expert at the Canadian Food Inspection Agency (Ottawa, 1996), Professorship from DFG at the University of Freiburg (invited by Prof. P. Nick, 1997), visiting scientist at John Innes Centre during 1998–2003 (Norwich, with Prof. C. Lloyd), an invited professor at Shiller Univerity of Jena/Institute of Molecular Biotechnology (2001), a visiting professor at Antwerpen University (2005–2006, with Prof. J.-P. Verbelen) and the Cochran Fellowship in Agricultural Biotechnology (USDA/USA) in 2010. Due to that, when in July 2008 I was appointed Director of the Institute of Food Biotechnology and Genomics at the National Academy of Sciences of Ukraine, with which the Department of Genomics and Molecular Biotechnology headed by me was soon merged, I had already shaped a vision of how and where to direct the efforts in the development of a new academic institution. Professor Marc Van Montagu wrote to me the following: "*Dear Yaroslav, congratulations, that will be a very important opportunity and responsibility. Ukraine badly needs a restoration of its agriculture. Plant biotechnology can be a great help. Hope that you will receive the necessary financial support and that you can associate with the best of breeding capacity that is left, for developing novel crops*". Despite all the upheavals and difficulties that challenged the consolidation of the Ukraine's statehood (from the Global

Financial Crisis of 2008–10 to the current Russian invasion), over the short period of its existence, the Institute headed by me has become an advanced academic institution, with cutting edge developments not only in the field of cell biology, molecular genetics and plant biotechnology, but also in structural bioinformatics, population genetics of plants, food biotechnology, microbiological synthesis and biotechnology of liquid fuels (bioethanol and biodiesel).

3 Science Today and Tomorrow

"Omnis cellula ex cellula"—aphorism coined by the naturalist, doctor and physiologist François Vincent Raspail (1794–1878), from which Rudolf Virchow (1821–1902) popularized the second principle of cell theory: "Every cell has originated from another cell, by division of this one."

I decided to recall these catchphrases here because their profound meaning is directly related to what I have been doing for most of my life in science. Earlier, when I was a student at Kyiv University and nearly every day passed by the portrait of S.G. Navashin (1857–1930), and the room where he worked, I had no idea that my life pathways would have become so closely intertwined with his scientific work, directly connected with the two quotes.

Serhiy Navashin, being a professor at Kyiv University, discovered double fertilization in plants in 1898 (Korzh 2008) but the fact that, while describing double fertilization, he also paid considerable attention to the study of mitotic division in the generative cells of the pollen tube of the lily (*Lilium martagon* L.), has gone barely noticeable.

His early works in this direction, contemporary with that of the Polish-German scientist Eduard Strasburger, who actually introduced the terms "prophase", "metaphase", "anaphase" and "meiosis" (1884), and other coeval researchers, demonstrated that mitosis was based on a same mechanism of division, involving filamentous structures, in both the generative nucleus and the meristematic cells. At that time, researchers had long been unable to visualize and identify microtubules as the main structural element of the mitotic spindle but, as S. Navashin sharply noted, "Looking and seeing—these are two different things."

Much later, in the second half of the twentieth century, during a new wave of instrumental and methodological development of cytology, which, in fact, gave birth to a new biological discipline called "cell biology", it became clear that microtubules are a necessary component of the cytoskeleton of any eukaryotic cell and form not only the mitotic spindle, but also the interphase network (Brinkley 1997). Thanks to such structural and dynamic plasticity, microtubules not only ensure mitotic division, but also maintain the shape of cells, provide intracellular transport and mobility of flagella and cilia, participate in the positioning of organelles and sustain several other cellular events. Each single microtubule, capable of assembling with many others, is made up by the side to side adjoining of 13 protofilaments. Although this

rule of the lucky 13, the magic number, is quite strict, especially in plants, where such the structure of microtubules was established for the first time (Ledbetter and Porter 1964), some deviations have been described. However, it was just few years later (Borisy and Taylor 1967; Weisenberg et al. 1968) that the heterodimeric protein tubulin, the actual building block of protofilaments and hence of microtubules, was discovered (Breviario et al. 2013). So knowledge of the real composition of the microtubules went from the superstructure level of the mitotic spindle to the elementary, constitutive level of tubulin, in a direction, big to small (Borisy et al. 2016), that is commonly experienced in Science. Here I have intentionally refered to the history of the microtubule research to make it clear that when I joined experimental biology, the microtubules and, in fact, the mitotic spindle, were perceived as cellular structures not very well described and not fully understood. And when I shifted my research from the study of histones and the structure of chromatin to the study of tubulin and the structure of microtubules, the time coincided with the rapid development of modern cell biology. Such development was based, as I always say to students, on three pillars: in vitro cell culture, recombinant DNA technology, and in vivo visualization of cellular structures.

At that time, my vision of scientific problems and my immersion into the world of the cytoskeleton deepened thanks to acquaintance and cooperation, which sometimes turned into friendly relations, with scientists such as William Vance Baird and Don Durzan (USA), Clive Lloyd and John Doonan (UK), Konrad Boehm and Peter Nick (Germany), Vladimir Vicklicky and Pavel Draber (Czech Republic), and definitely, Diego Breviario (Italy). Some time later, I felt that the development of cell biology of the cytoskeleton was based not only on the three pillars just mentioned, but two more had to be added referring to: genomic and transcriptomic databases and structural bioinformatics.

Understanding these circumstances led me to organize several full-fledged symposia devoted to the plant cytoskeleton: “The Plant Cytoskeleton: Molecular Keys for Biotechnology” (Yalta, Ukraine, 1998), “The Plant Cytoskeleton: Functional Diversity and Biotechnological Implications” (Kyiv, Ukraine, 2002, jointly with C. Lloyd and A. Yemets), “The Plant Cytoskeleton: Genomic and Bioinformatic Tools for Biotechnology and Agriculture” (Yalta, Ukraine, 2006, in partnership with W.V. Baird and A. Yemets).

Thanks to these events, I was lucky enough to meet such scientists as Ilze Foissner (Austria), Jean-Pierre Verbelen and Kris Vissenberg (Belgium), Diana Simmonds and Larry Fowke (Canada), Pavla Binarova, Miroslav Ovecka, Jan Petrašek, Katerina Schwarzerova and Viktor Zarsky (Czech Republic), Catherine Bergounioux, David Bouchez, Anne-Catherine Schmit, Marylin Vantard and Cécile Raynaud (France), Frantisek Baluska and Dieter Volkmann (Germany), Einat Sadot (Israel), Clara Conicella and Alessandra Moscatelli (Italy), Tijs Ketelaar and Andre van Lammeren (Netherlands), Susana Moreno Diaz de la Espina (Spain), Patrick Hussey and Jeremy Hyams (UK), Federica Brandizzi, Karl Hasenstein, Bo Liu and Susan Wick (USA). Among the participants of these events were scientists, such as Consuelo de la Torre (Spain), who was one of the first elected ordinary members of the Academia Europaea in 1988, and Shoji Okamura of Japan, who fought courageously to solve the problems

of tubulin phosphoproteomics, and the Canadian Geoffrey Wasteneys from Australia, who is currently a Canada Research Chair in Plant Cell Biology and Professor at the University of British Columbia in Vancouver. Of course, all these contacts significantly contributed to the establishment, and further development of our research in Ukraine. That is to say that good international collaboration is a fundamental key for scientific success, regardless the place where you actually carry out your research.

In my life, there have been and still are active various science-related commitments and responsibilities, which contributed to the establishment of new bilateral contacts and to the broadening of my personal horizons. In fact, I have been: a member of the Advisory Panel on Life Science and Technology at NATO Scientific Affairs Division (2001–04); a member of the Steering Committee of Public Research and Regulation Biotechnology Initiative (since 2006), headed for a long time by Marc Van Montagu; the President and a member of the Board of Directors of the Black Sea Biotechnological Association (since 2010); the National Representative in the Programme Committee on Food Security, Sustainable Agriculture and Forestry, Marine, Maritime and Inland Water Research, and the Bioeconomy for EU Horizon 2020 Program (2017–20); the founder of the Ukrainian cluster of European Plant Science Organisation (EPSO). Along this way, I met many extraordinary scientists and prominent personalities who could hardly be named personally. But I cannot help mentioning such good friends and recognized scientists as Atanas Atanasov from Bulgaria, George Fedak from Canada and Edgar Cahoon from the USA. And up to now I am grateful to each one of them for creative collaboration in those areas of research that go beyond the framework of the cytoskeleton and shaped my current vision of genomics, molecular biotechnology, marker-assisted breeding and production of biofuels.

As for my vision of the further development of biology as a science in general, I like to quote Mark Twain when he said, “Predicting is a dangerous art—especially when it involves the future”. This is even more true now since In Ukraine we are currently living and working under conditions of a full-scale war, especially so for my family that have chosen science as the mainstream of its professional activities (https://www.chemistryworld.com/news/ukrainian-researchers-persevering-amid-war/4015691.article). Nevertheless, despite all the hardships and troubles of the martial law, we still feel we are living in the golden age of the biological sciences and we try to give our contribute to its progress.

Speaking about this, I think we can reasonably say that up to the first half of the twentieth century, biologists tried to disassemble the organism and the cell down to the smallest units, but, as the cultural and technical progress was taking place, they started moving on the reconstruction of cells and organisms, as well as those metabolic pathways that ensure normal life processes. Here, today, it is appropriate to say that we are only at the very beginning of a long and exciting reconstructive path.

In the early 2000s, I managed to organize a high-level on-line conference dedicated to hot issues in plant biotechnology discussing the use and the achievements of genetic engineering in view of their possible associated risks (Blume, 2002). Only twenty years have passed, and these issues have noticeably lost their sharpness,

because genome-edited products, which have resulted from the successful implementation of CRISPR technologies, have entered the consumer market. Ten years ago, within the framework of the journalism project Ukraine Next—a daily magazine in 24 h (https://issuu.com/ukrainext/docs/ukrainext), in the essay "Our place in the biological tomorrow", I briefly outlined my vision about the perspectives in biology and biotechnology. I was under the spell of the opening lecture given by Craig Venter at the congress of the European Federation of Biotechnologists held in Barcelona (2009). The man who, depending on the party, is either called an adventurer or a "superstar" was the one who, in June 2000, together with Francis Collins and in the presence of US President Bill Clinton, announced the deciphering of the complete human genome sequence. In Barcelona, he talked about the synthesis of the first living organism in the history of mankind, the so-called laboratory mycoplasma (*Mycoplasma laboratorium*). This organism contained in its genome the minimum possible set of genes, all synthesized artificially. It was at that time that I wondered: "What are we going to talk about in ten years' time?"

Now, that even more years have passed by, living material assembly of bacteriogenic protocells is a fact (Xu et al. 2022). Thousands of genomes of bacteria, fungi, animals and plants have been deciphered. By next-generation sequencing or high-throughput sequencing methods, we can now decode individual human genomes in a week and at reasonable cost (300–400 US dollars). What all this mean? At the very least, this announce the outbreak of a revolution in the way of diagnosing hereditary diseases which will soon bring us at the threshold of an indivual, "personalized" medicine. Hence, we can now cultivate legitimate hopes about the cure of some of the most relevant human diseases such as cancer, diabetes, Alzheimer's and Parkinson's, osteoporosis, cystic fibrosis and other illnesses.

Associated to this there are also new ways of developing pharmacology and gene therapy as well as further developments in synthetic biology. The time is not far off when we will be able not only to transfer blocks of genes to reproduce the synthesis of complex biomolecules or induce change in metabolic pathways, but also to work out reliable tools for developing entirely new organisms and cell factories fueled for a given purpose.

The beginning of the third millennium was marked by the birth of another new science discipline that is stem cell biology. While it is well known that each individual cell acts as a universal unit for the implementation of all the molecular biological processes, based on its inherent genetic information, it is the stem progenitor cells that are in charge of the correct cell reproduction and differentiation processes in a multicellular organism. Accordingly, the stem cells play a fundamental role in the development of modern biotechnology. In fact, stem cells can be isolated from any multicellular organism, propagated outside the body, and then differentiated into different tissues and even organs, which can then be used for transplants. Every month we receive new evidence of progress in this cutting edge technology. Literally, while writing these lines, I have read information about obtaining a mouse embryo with a beating heart from stem cells (Amadei et al. 2022).

I keep in my library the book "Life at the Speed of Light: From the Double Helix to the Dawn of Digital Life" with a gift signature of its author—the very same Craig

Venter I mentioned above. When I first heard his story about teleporting biological life to Mars, I was quite skeptical, to say the least. However, after carefully re-reading this book and comparing the author's thoughts with the seven-mile steps of genomics (O'Brien 2022), I am now well aware that the time is not far off, when the transfer of ordered genetic information for the purpose of reproducing a living organism at a distance will become as much a reality as the 3-D printing is today.

These are just a few words about the role of modern biology in the development of modern society and although it is not very easy to extrapolate them to predict what is stored for tomorrow, we understand that the cultural and technical progress based on Science cannot be stopped. It feeds an irreversible flow as it is that of our lives. So better we take time to think about it and see where and how we can possibly control its development. What can and should we do to make the fantasy of tomorrow a reality in our society? What will we use as a fulcrum to turn the world upside down? First of all, an unlost taste for serious science, which is not always appreciated by those on whom its further development ultimately depends such as: young people, who still hope to realize their professional knowledge and skills and human "islands" in academic institutions that have retained the ability to adequately perceive today's challenges and maintain decent positions thanks to their openness to the outside world and strong dedication to work. In our society, it is necessary to constantly fight for promoting both trust in science and respect for scientists. It is necessary to support those "islands" that tomorrow may grow to become a land and form new continents. Not only civil society, but also politicians should not only listening to them, but also realize that the future of their country directly depends on their ability to ensure the development of advanced science. And biology is a vivid example of how new realities that we sometimes don't see or don't want to see, are already knocking at our doors. For us, these realities are a hope for a better medicine, a healthy food, a healthy environment and an efficient economy.

4 Advice to the New Generation of Scientists

Certainly, my assertions about science, my own accomplishments in it and the vision of what place the biological science occupies in nowadays society and where it is going, are based on my personal life experience and achievements. However, I think it is appropriate to add here that technological progress and human welfare can only be successfully obtained by promoting the integration of Science with humanities, overcoming any kind of spurious segregation, concerning age or professional specificity. In other words, we, as a scientists and mentors, must fruitfully interact with the younger generation in the effort of making them better than us, as good parents aim to do for their children.

Although I already mentioned that, from the very beginning of my professional career, daily occupation as a teacher at the university did not particularly attract me, and I preferred to work for a scientific institution, I very quickly felt the need to build

bridges between the research institute where I was working and the university where I used to study.

That is why, I enthusiastically supported Yuri Gleba's proposal to set up a Department of Cell Biology and Genetic Engineering as a shared body between the university and the institute to directly raise the best graduates to work at our institute. However, when this happened, it cost me a lot of effort to organize the tuition process at the Department, to select students and engage them in the research activities carried out at our academic Institute. Afterward, when I headed the joint Department, my research laboratory, being an integral part of the academic institute, remained for a long time based at the Faculty of Biology at the University. Nonetheless, this work has turned out to be rather rewarding, since, at each wave of new entries, our Institute is often chosen by the best students. Only then, due to the freedom of choice in shaping the tuition syllabus, I began to enjoy my work of university lecturer teaching several courses, also with the help and contribution of Alla, my wife. More importantly, we were capable of involving the best professionals from the National Academy of Sciences, including those institute staff who at that time, were working abroad, as well as foreign colleagues. I recollect with pleasure, how in 1997 some of the lectures of my course on the cytoskeleton were given by Prof. Peter Nick, who at that time worked at the University of Freiburg. Having successfully defended her Ph.D. Thesis, Alla Yemets joined the teaching by giving lectures for our students. Her contribution to the development of the Department became especially tangible after her prolonged internship at the Syngenta Genomic Centre in Jealott's Hill, UK (International Research Centre now) in 2004.

After Ukraine had signed the Bologna Declaration in 2005, a gradual transformation of educational programs for university students was launched, and we continued training a separate group of master studentsi at the Department of General and Molecular Genetics where cell biology has become a separate educational program for MSc degree. In 2019, I was invited to take the position of professor at the Department of Biochemistry. Currently, Alla continues teaching genetics, while providing her share to our common course on genomics which I took over when the works on the decoding of the Arabidopsis (The Arabidopsis Genome Initiative 2000) and the human genome (Venter et al. 2001) were published. It was at that time that I received an invitation from the National University of Kyiv-Mohyla Academy to prepare, for the first time in Ukraine, a course on genomics. A year later, Alla and I started teaching such a course also at Kyiv National University. Actually, this was the beginning of my personal journey into the world of genomics which, apart teaching, contributed to the further expansion of my scientific interests and the initiation of new directions of my research. Among those students there was Rostyslav, our son, who started there his actual career of young scientist and continues it at the National Academy now as Ph.D. student (Blume et al. 2022b).

Nonetheless, our work with young people has never been limited to the training of university students. A feature of our academic system has been, and still remains, the fact that research institutes have always had Ph.D. programs and have been supporting Ph.D. and Dr.Sci (Hab.) defense degrees. For the past five years, our institute has already been training young scientists under new Ph.D. programs, which have become

the next educational level (after the MSc degree) on the way to a scientific career. This meant that we had to be licensed and accredited as an educational body, like any other classical university. In total, under my supervision and during this time period, 30 young scientists received a Ph.D. degree, and 6 scientists more, those who had me as the scientific advisor for the preparation of their Doctor of Sciences degree theses. The National Academy of Sciences of Ukraine acknowledged these achievements by awarding me with the honours "For Training New Academic Generation" (2017). It is quite natural that behind each of such cases of personal mentoring there has been a separate story of human relationships.

For such bilateral interactions, tutor-apprentice, to be effective and lead to the desiderable result, one needs to master a rare and precious quality throughout all his/her professional and personal life: listen to the opinions and the arguments of other scientists and people with extreme respect and full interest. It is not good enough to be curious and original, it is important to feel in somebody else's shoes, at least try to be quite tolerant in all aspects and quite warm toward people. Of course, this applies not only to the model of interaction between the mentor and his/her students, but also to the principles of building healthy and productive relationships between the manager and his/her subordinates.

At the same time, it is important to instil in young scientists the ability to honestly assess their attitude to science and find their own, specific interests in it. If for this purpose they need to change the team, and this might look the best way for effective professional growth, then they must do so. In accordance, they may apply to a different scientific topic or even move to another country being prepared and determined to follow it forward. These changes are expecially recommended, if one feels inadequate in his/her current niche of investigation, or feels a lack of fair judgments about his competence, value and a personal attitude. Also, if it turns out that your ambitious goals are unattainable, do not dispair since, once you have acquired a certain amount of scientific knowledge, there are many other parallel pathways to gratify yourself in the world of science that could fit you better, such as: technology transfer and business, and public educational activities. Anyhow, my most strong recommendation is to do as much as you can in science and for science, so as not to reproach yourself later for not doing something you could have done. This applies not only to my teaching and relations with young scientists. This is the motto of my own life.

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What Really Matters is not just Knowing "What", "Where" and "When" but also Knowing "How"

Gloria Bordogna

Abstract Human intelligence has been an object of investigation since the beginning of the research on information science to provide artificial agents with human-like decision making skills. This research field has led to the development of algorithms that try to simulate human reasoning. Several theories have been defined to model decisions in the presence of uncertain, imprecise and vague information, based on both subjective and qualitative criteria, expressed linguistically. Today, we are at an epochal turning point in which there are no longer attempts to reproduce human reasoning by machines, but algorithms are designed as networks of interconnected simple computational units learning to take decisions from examples. This data-driven paradigm simulates children learning from observations, so that their behavior evolves by accumulation of experience. Nevertheless, are we sure that purely learning from data is an effective sufficient method, not affected by bias, and that it can lead to fair systems that we can trust? Are we satisfied with completing a task without knowing "how" it was performed? Are we sure that children don't have, a priori, more complex and structured mechanisms regulating as well as directing their learning ability? Do we really want to throw away all the research that has been done so far, or can we retain it, so that knowledge of models and data-driven learning can play a synergistic role?

1 Motivations: How I Developed an Interest in Science

I grew up in a small town in the "Vandée Bergamasca", between the hills that extend North–South from the Orobic Alps to the plain, and East–West between two Alpine lakes, Iseo and Endine. The place was known to the Romans, who founded the thermal baths, still used today, exploiting the source of sulphur water for its therapeutic properties, and dug the pink marble from the hills, the stone of Zandobbio, which, over the centuries up to today, has been used to embellish the monuments of Bergamo Alta.

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The landscape was green, planted with wheat, corn, vineyards and pastures, ideal places where we kids roamed free. The horizon opens to the South–West on the plain where on clear days the acute view can glimpse the Monte Rosa and the skyline of Milan, at that time a destination for commuters, mainly bricklayers and metalworkers in the factories of the Milanese hinterland, nurses and housekeepers and some rare college students.

I believe that living surrounded by a varied landscape has influenced my need for different stimuli and the habit of looking at the horizon has somehow shaped my attitude of imagining beyond the limit of the perceived as well as my curiosity of knowing what's there.

I owe a lot to my parents who gave me the motivations for the choices I made and the strength to pursue them, and to my husband and girls who had and still have a lot of patience with me when I am absorbed in my thoughts.

Dad gave us the secular spirit and the love for the arts. He was a real "rare bird" in the strongly Catholic environment of Bergamo in the 1960s. He was a convinced atheist, despite or because of his training in the seminary where he had been sent as a child to pursue a career as a priest.

Mom was the daughter of a World War II prisoner in a labour camp in Germany, and as such she was sent to a boarding school for orphans where she developed a strong resilience and an independent spirit that she communicates to us every day, living peacefully alone in an isolated house at the age of 86.

In such an environment, in the 60s and 70s the echo of the events came through the "didactic" public television broadcasts, which together with the family and the school helped to stimulate my interest in literature, art and science.

I remember that my sister and I were eagerly waiting for the episodes of the TV dramas "*Eneide*", "*Odyssey*", "*Anna Karenina*", "*The Brothers Karamazov*", which generated emotions that were then enriched with the reading of related books. I used to look with curiosity at the mythical "*Intervallo*", which was an interlude broadcast between one program and another, unfortunately now replaced by advertising: the slide show of picturesque Italian landscapes with the "paradisiacal" toccata for harp solo by Paradisi as the soundtrack. This aroused my interest to visit those places and travel.

Comparing the experience of both commercial TV, which has led to the so-called "*Videocracy*",[1] and the Internet, with social networks and on-demand thematic channels that trap viewers making them addicted to television series and reality shows,[2] the single-channel public educational television, in a certain way, has contributed to an authentically egalitarian educational standards in Italy.

Having said that, I don't mean to cheer for the single channel state TV: when controlled by regimes it is a powerful tool for mass propaganda, persuasion and

[1] "*Videocracy—Basta apparire*", 2009, documentary movie directed by Erik Gandini (https://www.youtube.com/watch?v=EpEP2q0bj-s). (accessed on 10 January 2021).

[2] "*The social Dilemma*", 2020, documentary-drama directed by Jeff Orlowski that reveals how social media is reprogramming civilization. (https://www.thesocialdilemma.com/the-film/). (accessed on 10 January 2021).

circumvention, as we are still living in these sad times. On the other hand, social networks proved to be useful for directly witnessing events, and this is why they are censored by regimes when they offer another side of the story.

I have always had a strong attraction for the mystery of the starry sky. On the summer evening of the moon landing I was looking at the sky lying in a wheat field, playing hide and seek with my friends. I was moved by fear mixed with curiosity for UFOs, "*The question is: are we alone?*", which was fueled by the enthusiastic narrations of the space conquests reported by news, by Asimov's novels and by science fiction movies, such as "*2001: A Space Odyssey*" by Stanley Kubrick.

In particular, I remember the documentary "*Avventura*" which was worth watching even just to hear the opening theme song of the Beatles sung by Joe Cocker "*She came in through the bathroom window*" and the final one by Procol Harum "*A Salty dog*". It described the fascinating world of astronomical observations and exploratory space probes, such as Pioneer 10 and 11 and later Voyager, carriers of multimedia messages of the Earth civilization, diagrams, images and music, considered universal and therefore understandable to "intelligent" extraterrestrial beings. Certainly, an intelligence higher than the average of the earthlings was necessary to decipher the implicit message.

This is the humus in which my curiosity for science, passion for Russian literature and the pursuit of gratification in landscape and art grew.

Why did I finally choose Science, that is to say the degree course in physics?

Both for practical reasons, such as the greater job opportunities that a scientific degree offered compared to a degree in the humanities, and for social reasons: it was the time of the second feminist revolution in Italy. In 1976 Tina Anselmi, the first woman to be appointed minister in Italy, presented a law which prohibited gender discrimination to access work, career advancement and salary, and in 1979 Nilde Iotti was the first woman to hold the position of President of the House of Representatives. I was proud of choosing a science degree to strongly affirm gender equality.

Last but not least, by nature, I enjoyed solving geometry problems more than translating from Latin, and since I needed both objective and validated answers, not having the support of the truth revealed by a religion, it seemed to me that science provided the method to answer in an explicit, shared, clear and formal way, the question "*who are we?*", that is "*what is life*", "*what is intelligence*", and "*where are we going?*", that is "*what is the universe*" and " *how will it evolve*".

Nevertheless, my expectations as an enthusiastic student were soon dashed by the courses of the first years, which were mostly not very stimulating, or at times incomprehensible, where the technicalities were not accompanied by the explanations of the context or of the physical meaning. Out of desperation, I chose to follow a course on "philosophy of science", held by Giulio Giorello, dealing with Paul Feyerabend's anarchic vision of science and the role of intuition and creativity in the scientific process (Feyerabend 1983). These ideas guided me in my subsequent choices, together with some professors I have been lucky enough to meet.

Among these, I like to remember Prof. Piero Caldirola, who used to intertwine the lessons of theoretical physics with anecdotes of personal experiences in the context of international scientific conferences where he took part together with some giants of

physics. These narratives brought historical perspective on the origins of the theories, just as the experience of participating in a scientific community took on a certain daily perspective, which ignited in me the idea that one day I might experience.

I remember with sympathy Prof. Giovanni Degli Antoni, who took an active part in integrating technologies into the procedures of the Pool "*Mani Pulite*", in order to enhance productivity and transparency of acts in the Court process (Degli Antoni and Di Pietro 1994). He was a histrionic personality, from whom I learned the fundamentals of the Turing machine and artificial intelligence, which has been a constant thread of my subsequent research activities.

Finally, the mark left by Prof. Piero Mussio is unforgettable. He had a somehow unsettling personality, due to excessively high expectations he always placed in his undergraduates, tormenting them sometimes until late at night for the preparation of a presentation, but passionate and visionary, who initiated me into image pattern recognition and multivalued logic, and, above all, to the research activity that does not know Saturdays, Sundays and holidays. How many a Christmas Eve did I spend in the following years with my friend and colleague Gabriella preparing articles to be sent to the ACM SAC conference that had just that peculiar day as a deadline?

2 Work Done: My Personal Scientific Approach

The role of creativity and intuition in the scientific process are aspects that have always intrigued me. It's crucial for me to be able to put some creativity into what I do. Furthermore, I find the academic approach to the scientific method, according to which rationality is the only ingredient of the scientific method a bit tight.

I agree that "*science in any case evaluates and eventually absorbs*" (Appolloni 2014) but I also think that intuition and creativity, and therefore not necessarily observations, can be the engines of an "a priori" scientific hypothesis that is then better exposed rationally, explored and screened. This idea is well expressed by the famous quote of the legendary physicist Richard Feynman "*What I cannot create, I do not understand*".

I have always perceived experimental physics requiring the repetition of the same measurement process and the statistical synthesis of the results as suffocating.

On the other hand, theoretical physics was, and still is, in a situation of epistemological crisis, due to the impossibility of validating complex theories that make predictions on superhuman time scales (Dougherty 2016).

These considerations, coupled with a skeptical attitude, based on the belief that all scientific knowledge is provisional and subject to revision when confronted with new evidence, conditioned my subsequent choice to graduate in physics with a cybernetic focus, which left room for both creativity and validation of ideas.

Designing an algorithm and writing a code is a bit like writing a text, painting a picture or composing a piece of music. One has an idea, a hypothesis, there is a language to express it, and one then writes a piece of software, whose evaluation can include aesthetic criteria, such as cleanliness, synthesis, generality and reusability

of the code. However, also validation criteria are required, which are based on the correctness, effectiveness and accuracy of the results obtained by executing the code, as well as the reproducibility of results by third parties, therefore on a scientific validation. This process, in which the idea is validated on the basis of criteria that are as objective as possible, and can be faithfully reproduced and shared in an open way, is fundamental.

In the early 70–80s, Information Science was still in its prime in Italy, and the landscape was very promising for the country's future in this sector.

The spearhead of Italian computer science research was at the National University Center for Electronic Computing (CNUCE) a CNR institute since 1974. Initially, it housed one of the three most powerful IBM electronic processors in Europe and provided service to the Italian scientific community connected to the nodes of the first geographical network, having within it a double face of "service", for the management of both the systems and the network, and of "research", with "humanistic" activities of excellence on computational linguistics for Natural Language Processing (NLP), Information Retrieval (IR), and Musicology (De Marco et al. 2000).

Even the Italian industry, with Olivetti firm, where I carried out one of my first work experience together with two colleagues and friends, was at the forefront of the design of personal computers: the M24, was one of the first most innovative and competitive PCs on a global level.

In this context, where I witnessed the birth of the Internet and the Web, my experience, mainly carried out at the CNR, has been diversified. It ranged from the recognition of stars and galaxies in astronomical digital plates, to the representation of the content of textual documents in Information Retrieval systems, also known as Search Engines, to the automatic classification of remote sensing images for earth observation to identify anomalous situations such as flooded areas and wildfires. Finally, it also included the classification of time series of DNA barcode diagrams for the recognition of "species", a collaboration that gave me the opportunity to collaborate with Diego Breviario and his group.

Switching from one application to another was primarily dictated by various needs: affiliation with a new institute, research calls financed at that time, etc. Nevertheless, I have learned to make a virtue of necessity by choosing applications that presented new modeling challenges.

In fact, these apparently heterogeneous experiences actually conceal a very solid link, which is the approach I have always adopted to solve the various applications, based on "soft computing". It includes methods to represent and manage knowledge expressed in a linguistic way, to optimize automatic reasoning by simulating the selection and mutation typical of living organisms, and machine learning methods. These methods, that gradually became the real focus of my research, together with those commonly known as neural or artificial neural networks (ANN) are under the broad umbrella of artificial intelligence (AI), intended as the ability of artificial agents to model human reasoning and domain knowledge, and to learn from observations, i.e., from data, by evolving and generalizing their behavior so as to be able to face new situations by exploiting accumulated experience. **This implies the ability to model the uncertainty of information**, i.e., the doubt about its truthfulness. For

example, the ability to identify "fake news", and the imprecision and vagueness of information, i.e., the ability to interpreting and exploiting qualitative knowledge on the observed phenomena, the ability to recognize the exceptions to rules and the contradictions, and, ultimately, to adapt to the context. To this end, non-classical logics, such as multivalued logics, fuzzy logic, rough sets and possibility theory, were the formal framework that I exploited for modeling.

My small contribution was related to flexible decision making methods (Bordogna et al. 1997), based on multiple linguistically evaluated criteria, and their application in different contexts such as group decision making, documents relevance computation in Information Retrieval, personalization of search results in the recommender systems, and the fusion and classification of images and time series.

During the '90s, when Google was emerging, these aspects were very innovative and not yet well accepted by the scientific community. As a proof of this, when for the first time, in 1998, Larry Page and Sergey Brin proposed the PageRank algorithm for calculating the relevance of Web pages used by Google to the scientific community, the latter refused its publication at an important conference (Battelle 2005).

On the one hand, it has been very rewarding to do research in an area at the time of its infancy, and to see how the research topics, that were perceived as niche at the time, have become a leading sector of information sciences, with practical implications in decades to come.

On the other hand, **it was really sad to observe how companies used technologies mainly for marketing purposes**, such as for personalized advertising, e-commerce, and even to influence the vote of social network users by pushing personalized news based on their psychological profile (Matz et al. 2017).

There are still many aspects of people's life that could benefit from the research on AI: just think of the often disappointing experiences with the various chatterbots and virtual assistants we come across when trying to solve various situations. The creation of Question-Answering systems, understanding written or vocal user requests, specifically able to answer "how" and "why" questions by exploiting various knowledge bases and open data, possibly in different languages, and yielding a response in the user's natural language, is still challenging (Andreasen et al. 2021).

Turing predicted that by 2000 computers would speak English easily enough to cheat an average speaker about 30% of the time after about 5 min of dialogue. However, he was largely optimistic. At present, only Eugene Goostman, a software programmed with a credible personality to carry conversations like a 13-year-old Ukrainian boy, convinced 33% of judges that he was a human in the 2014 Turing test. However, his own creators argue that the judges were indulgent "*being perfectly reasonable to assume that a guy doesn't know a lot of things*."

Although the "Turing Test" still attracts the imagination of scientists and the public (just think of the sentimental conversations between "*Sam*" and the artificial intelligence in the film "*Her*") much has been debated on its validity to qualify what is intelligent. Specifically, knowing how to use language wisely is a different skill from knowing how to understand the meaning of a text or a question.

3 Science Today and Tomorrow

In the last decades, the data analysis methods have progressively changed from explicit models of symbolic representation of information to data-driven machine learning paradigms.

These latter approaches enable systems to learn how to perform a predefined task (for example recognition, classification, prediction, regression etc.), leveraging the information provided to them by empirical data, training data consisting mainly of raw data (such as color images and unstructured texts) that in the case of supervised learning have been previously labelled with tags (such as "faces", "vehicles" present in an image). Ultimately, they can learn to generalize "patterns", i.e., regularities associated with the labels present in the training data, in order to recognize the same "patterns" in unknown data, thus providing their classification or recognition.

These are artificial neuronal networks, statistical systems initially inspired by a simplification of biological neuronal networks (McCulloch and Pitts 1943). They consist of neurons, generally organized in layers and connected in a network. Each neuron is a simple computational unit that produces an output signal if the sum of the input signals exceeds a given threshold, or activation condition. The stable weights of the connections are not programmed but are learned by applying a back propagation algorithm that leverages the training data.

The availability of increasingly powerful computing infrastructures allowed implementing very complex artificial neural networks, the so-called "Deep Learning", consisting of dozens of layers with hundreds of millions of connections, and therefore an equal or greater numbers of parameters.

Their successful application has been made possible thanks to the availability of big data accessible on the Web, such as user-generated contents. They are created by unwitting users of social networks who express their opinions on the most disparate topics. This information, together with the user's profile, gender, age, network of followers, likes, geo-referenced and commented photographs, etc., constitutes a real boon for designing recommendation systems, personalized advertising, and for studying socio-political habits of particular social and ethnic groups residing in geographic areas of interest.

Even query log of search engines is analyzed by applying collective intelligence methods, to recognize events in progress or to formulate predictions, for example the spread of a flu syndrome before it is clear in a given region.[3]

Artificial agents have been created with superhuman abilities, such as computer vision (Cireşan et al. 2012), which is able to recognize objects moving in a space framed by a camera in real time more efficiently and effectively than a human being.

The astrophysicist Max Tegmark is visionary regarding the future scenarios of artificial superintelligence, and hypothesized life 3.0 (Tegmark 2017), in which the evolution of humans will lead them to become cyborgs. He warned of potential dangers of superintelligences by co-founding the no-profit organization "*Future of*

[3] Google Flu Trends.

Life Institute" which aims to foster research to minimize the future risks of their development.

In this data-driven framework, the original role of the scientist and software designer is greatly diminished: from being the algorithm creator the scientist is reduced to a mere tool for selecting data in order to train the network and to check the accuracy and "fairness" of the results produced. These tasks involve the selection of un-biased training data, so as not to jeopardize the ethics of the results. For example, to avoid designing security video surveillance systems discriminating the recognition of faces depending on gender or ethnicity.

For these activities, creativity and human intuition are mostly unnecessary.

Not only do scientists not define algorithms, and therefore do not participate in their creativity and intuition in the scientific process, but, in the current state of knowledge on "Deep learning", they are not even able to interpret the rules learned by the network to perform a certain task.

Also, the theories of twentieth century physics challenge human understanding, such as quantum mechanics and the theory of relativity. For instance, think about the Heisenberg's uncertainty principle, the phenomenon of entanglement, the particle/wave duality, and the relativity of time. However, through metaphors, a human interpretation is still possible, albeit apparently contradictory and counterintuitive from the point of view of common experience. For instance, think about the Schrödinger's cat paradox to explain the uncertainty principle, or the twin paradox to explain the special theory of relativity.

On the contrary, neural approaches are actually "black boxes". Ultimately, they offer "unthinkable" and humanly inconceivable explanations, or to put it like Arendt: "*demand not only the renunciation of an anthropocentric or geocentric world view, but also a radical elimination of all anthropomorphic elements and principles, as they arise either from the world given to the five senses or from the categories inherent in the human mind*" (Arendt 1977).

Although neural approaches can efficiently and effectively perform some technical tasks, they are currently not useful to produce science, that is, to increase our understanding of phenomena. They can recognize text in an image, perform robot tasks like disassembling/assembling components, prepare and serve cocktails, restore films,[4] simulate texts in the style of Shakespeare,[5] compose musical pieces in the style of Bach,[6] or paint in the style of Van Gogh,[7] activities that either promote 4.0 industry or exemplify potential of neural networks, but they do not explain why we like Shakespeare, Bach and Van Gogh.

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[4] Deep Restore: https://www.hs-art.com/index.php/research-main/deeprestore-menu. (accessed on 10 January 2021).

[5] Deep-speare: https://spectrum.ieee.org/this-ai-poet-mastered-rhythm-rhyme-and-natural-language-to-write-like-shakespeare. (accessed on 10 January 2021).

[6] DeepBach: https://sites.google.com/site/deepbachexamples/. (accessed on 10 January 2021).

[7] Vincent AI Art application: https://vincent.sabbir.dev/vincent/new-art/. (accessed on 10 January 2021).

Furthermore, the applicability of neural models in areas of social life that involve the assumption of legal responsibilities, such as autonomous driving and facial recognition in video surveillance systems, requires the explicability of the criteria, which means answering "*how*" they perform the tasks (EU, 2020).

With this criticism, however, I do not mean "*to throw out the baby with the bathwater*".

I believe that the future of research on Artificial Intelligence cannot renounce the use of neural methods, but needs to better understand them.

We are in a situation similar to that of the early nineteenth century, when the steam engine had already been invented, but the laws of thermodynamics were not yet known. At that time, the design of machines was based on empirical attempts, without exploiting physics to optimize performance (Fig. 1) (Lapini 2018).

Hence, **research on the fundamentals of AI methods is in its prime, with a wide range of goals to pursue**.

In the first place, the explicability of the neural models is needed in order to make explicit why the model made a particular prediction given a certain input, so as to communicate to non-expert decision makers the reason for the model's predictions.

Furthermore, the development of an information theory is needed for the optimization of Deep Learning models in order to be able to predict the accuracy and uncertainty that can be achieved by a given architecture. Last but not least, we lack the definition of mechanisms that preserve the intrinsic ethics of the learned models.

To this end, after this hangover of purely data-driven models, a research area that I consider promising is **to integrate neural models with soft computing methods,** in particular with semantic methods, which encode knowledge of the scientists and/or

Fig. 1 Digital painting made by Vincent AI Art application (see Footnote 7)

of the domain experts through rules, models or ontologies, **giving back the right role in the scientific process to scientists.**

On the one hand, a semantic representation of the rules learned from neural networks can support their explicability. On the other hand, the integration of a semantic representation of the principles of fairness in a neural network can lead to the definition of intrinsically more ethical approaches controllable by humans.

Only with more transparent systems, whose evolution can be adjustable and subject to constraints that reflect responsible and ethical principles decided by humans, can we delegate to machines some of the decision-making activities that are still purely human today, in the awareness that technology itself is neither good nor bad, but everything depends on its use.

4 Advice to the New Generation of Scientists

I'm not very good at giving advice, but I will try.

For years, I have hung up a sheet of paper in my office with various phrases that I consider significant to represent my personal scientific approach.

One of them is the Chinese motto "*Be bold in hypotheses and careful in verification*", attributed to the philosopher and writer Hu Shi. This is the attitude to scientific work that I find most stimulating and at the same time more fun for personal satisfaction. Obviously, it involves a humble approach to avoid "*reinventing the wheel*"; which translates into a careful and in-depth study and analysis of the scientific literature. If you find similar proposals, it makes sense to compare them with your hypothesis and identify the novelties of your proposal and the advantages, and to evaluate whether it is worth exploring it. Finally, it is necessary to allow the scientific community to evaluate the results obtained in the most impartial way possible, making available all the means to be able to replicate or refute the experiment.

Another motto is taken from a presentation that the American behavioral psychologist Peter Ossorio made at the "III International Workshop on Data Analysis in Astronomy", held in Erice in 1988, in which I participated by making my first speech in an international context. This motto reads "*It is not desirable for machines to learn: think of how much trouble a child causes as he grows up*".

This colorful metaphor expresses an indispensable principle for training machine learning algorithms: a careful analysis of the sensitivity of the models is necessary to establish the limits of applicability and the range of accuracy that can be achieved as a function of the variability of the input.

Finally, a humorous motto by the astrophysicist George F. Smoot, Nobel prize in physics in 2006, which I have listened to at the same workshop, says: "*If a theoretical physicist publishes a new theory, he will be the only one to believe it valid, if an experimental physicist publishes new results, he will be the only one to doubt them*".

I was able to personally experience how often this motto expresses a profound truth relating to the attitude that generally guides the scientific community in judging the papers submitted for peer reviews to conferences and journals. In the Information

Retrieval sector, proposals for new algorithms, although well formalized and theoretically sound, are not accepted if the evaluations are not carried out on benchmark collections, since the required validation criteria are purely empirical. However, this is not always possible. Think of new research tasks, never previously addressed by the scientific community. These proposals are often rejected because the authors carry out evaluations on collections of data generated ad hoc.

On the other hand, some experimental works report evaluations on benchmark collections but their reproducibility is not possible since the algorithms are not released openly, and therefore acceptance is based on an act of faith relating to the truth of the assessments.

Nevertheless, the replicability of the proposals should be guaranteed in any case, by publishing both the data and the code of the algorithms.

Finally, I would like to give some advice to stem the impact due to the research guidelines dictated by politicians and techno-bureaucracy.

Competitiveness is considered as a positive stimulus for scientific research. I have experienced it, and I must say that it leads to closure and not to the circulation of ideas, which is needed for cross-fertilization of the research. Often, researchers abstain from collaborations, fearing that their ideas will be stolen from them. In reality, collaboration enriches both parties. **Those who are afraid to collaborate are ultimately relegated to a dead end**: once the exploitation of the jealously guarded idea is exhausted, their ideas often atrophy, becoming self-referential and sterile.

Another questionable criterion is to reward excellence, that is "*when it rains, it pours*". Nevertheless, this does not help to sprout spontaneously grown seedlings that could become nice trees. As young researchers undergo their evaluation based on excellence, I recommend collaborating with national and international authoritative research groups. In this way, they will be able to carry out their activity in a fertile and protected environment, which, in the first years, will guarantee them the necessary means to concentrate solely on research activities, which will make them grow faster.

Besides that, **pay attention to the feedback received from evaluations based on bibliometric citations, which are a double-edged sword**. On the one hand, they allow to recognize the authoritativeness of the published works, and therefore, they provide useful indication on the research which is worth deepening. On the other hand, they can influence the "narcissus" researcher avidly seeking fame, encouraging him to a scientific production based on numbers rather than quality. The motto is therefore "*better few papers but good ones!*"

Finally, in order to navigate at the mercy of the constant changes imposed by politicians, I recommend creating strong relationships with international research groups, spending some time visiting institutes abroad, having international rather than national visibility. This may not help to grab the funds but it will certainly be more profitable and satisfying from a scientific and personal point of view.

I have never regretted not having a well-paid and safe job in an industry. The academic research environment, although harnessed and tyrannized by bureaucracy, is very dynamic and offers the opportunity to meet and get to know people from all over the world with whom to exchange ideas and experiences.

Travelling has always been a passion of mine, and research has offered me many opportunities to meet people from other geographical areas, nations and continents, speaking different languages, with different cultures and religions, yet united by a common language and intent, which are the universal scientific language and scientific information exchange.

Especially in present times, there is a fear of the return to opposing blocks in which the researchers of one block risk not being able to communicate with those of the other. I realize how lucky I have been to carry out my research activity in the last 40 years, in which we deluded ourselves that the barriers between people had been demolished forever with the fall of the Berlin Wall.

Precisely for this reason, I feel compelled to defend the internationality of science, which has no geographical, political or cultural boundaries. Therefore, peace is an essential condition necessary to deliver to the next generation of researchers an environment in which they can operate free from constraints and prejudices towards peers that depend solely on where they live and work.

To conclude, the possibility of being able to witness, serve, collaborate and contribute to knowledge gives great satisfactions. Although knowledge is uncertain and provisional, it is all we can reasonably count on to understand how reality works, and ultimately, to seek an answer to fundamental questions of humanity, "*How did we get here? How are we made? How will we evolve?*" At the same time, we must be well aware of the limitations on what we can understand.

I can't say it better than Bertrand Russell: "*Science tells us what we can know, but what we can know is little, and if we forget how much we cannot know we become insensitive to many things of very great importance*" (Russell 1945).

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Gloria Bordogna graduated in Physics at the University of Milan and after a scholarship at the Politecnico of Milano to carry out research on surface lighting for Computer Aided Design, she moved in Olivetti Tecnost, where matured the desire to do research. Therefore, in 1986, she joined the National Research Council (CNR) where she still works, currently as director of research at the Institute for the electromagnetic sensing of the environment. Her research activity concerns uncertainty and imprecision modeling by soft computing in information retrieval systems, databases and decision support systems. Her contributions and interests are related to methods for flexible query answering, mining, classification and summarization of textual documents and multidimensional heterogeneous georeferenced data from multiple sources.

Science is an Extraordinary Opportunity for Personal Growth

Diego Breviario

Abstract HUMILITY. What' s a DNA molecule? How is it capable of dictating a definite response? How many consequential and regulatory steps are actually needed to achieve the result? How, when and where are they controlled? When we inject in a human body an mRNA molecule how do we control its journey, the site and the level of its expression? When we inject an antigen into a human body are we able to predict the strength, the duration, the type of the immunological response? Are we able to control the changes occurring at the level of the different B- and T-lymphocytes populations? How can we explain the side effects of almost any drug? Do we know the pathways by which they work? These are just a few examples restricted to my own areas of interest that cannot find a conclusive answer and indeed may never find it if we consider that any effect, occurring in a different point of time and space, is likely exerted by multiple molecular and cellular events triggered either inside or outside of a given organism. And this has just to do with humans, a numerical fraction of the whole universe. Not to mention the quantum dimension. Now, do we have to stop doing research, because of these arguments? Stop looking for new answers to our ever-growing questions? NO and NEVER, because this is just the magnificence of our work: accept the challenge trying to uncover new fragments of an unlimited truth. This is also the reason why I have always treasured the most famous teaching of master Socrates when he said that he knew of not knowing. I believe that staying humble is the most straightforward way to grasp a further piece of knowledge.

1 Motivations: How I Developed an Interest in Science

CURIOSITY. My approach to science came relatively late and originated from a humanistic interest. At the time of my youth, the sixties, there was a great deal of interest in sociology and psychology. Urged by an intense political period, spread almost all over the western world, youngsters were idealistically searching new models of life while breaking obsolete social rules and enjoying a fantastic music,

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still unparalleled even nowadays. Grown up in a modest family and forced to attend a high school focused on chemistry, I ended up developing an instinctive interest in the organic base of the living matter wondering if anything, even human attitudes could eventually be traced back to it. I was someone like an anachronistic follower of Descartes' mechanistic view of human life or a pioneer of modern transhumanism theories, depending on the temporal side you want to take. I didn't care so much about intangible, vague matter of discussion or, better, I wanted to know how much of it could find a rational, organic, science-based explanation. Genetics was the field in which I choose to carry on this personal exploration with the idea that if everything was encoded into the DNA then it was just a matter of time to find the answer. This is a fideistic trait not too far away from some current thinking of the genome BIG DATA collectors. At that time, the influence of the environment on the way a genome could be differentially expressed was substantially neglected. Actually, Lamarck and any renovated version of his theory was ignored if not scorned. Cold war ideology, politically influencing science, was also a factor because the Russia of Stalin had promoted the idea of Lysenko by which the environment is stronger than genetic inheritance while the USA of the Statue of Liberty were discriminating amongst immigrants on the basis of their performance on IQ testing thus concluding that some populations were inferior to others, with the Anglo-Saxons as the champions of intelligence. Where was the truth? Speaking about intelligence can we even today explain what it is? How many forms of intelligence exist? Now that we have explored so many human genomes, do we have any genetic clue as to which nucleotides encode for it ? If we could insert the nucleus of Einstein into a human egg cell, mimicking the Dolly approach, would we generate a progeny of genius? If genetics cannot find the basis of intelligence, or any one of its multiple forms, how can bioinformatics work out their algorithms to develop robots that act like humans? Transported to the actual time, these were the kind of questions that were stirring my mind. **So, more of Mendel and less of Freud**. It was with this inclination that, while searching for a tutor of my thesis work, I asked my genetics teacher Prof. Giovanni Magni to help me in joining the lab of Prof. Luca Cavalli Sforza, a world-wide recognized expert/master in quantitative genetics, who was working in Pavia. I was unlucky since right at that time (1980), Cavalli Sforza moved to Stanford University in the USA. **Luck is a factor** as we will see. Ironically, and this gives the reader an idea of the weirdness of bureaucracy when science is ill-administrated, I have just recently closed (2022) an evaluation of a genetic test for lactose intolerance put in place in 2002 under the advice and consultation of Prof. Cavalli Sforza. So quantitative and population genetics were gone, and it would have been forever. I was left with, at that time for me, less intriguing, lab-based qualitative genetics, that by the way was almost in its infancy. Restriction enzymes were crude extracts from microorganisms prepared in the lab, separating columns were made by pouring gels into chemistry pipettes and I was happily transporting radioactive vials on a tray placed on my old bike to reach the closest institute equipped with a beta-counter. A kind of romantic, and dangerous, way of living science. What was supposed to be the occurrence of the moment, an unexpected change of plans, turned out to become a sort of philosophy of my way of

doing science, looking for different subjects of investigation in different organisms with different approaches at different periods of time.

In fact, ***the philosophy of change*** has sustained my freedom of learning, thinking and acting. Only in this way, by this attitude, I could preserve my enthusiasm, my wonder, the very base of knowledge. You are happily assisted in this by the ever-changing wonderful complexity of life, of the world. ***Panta rei***.

As a matter of fact, my thesis work on the time of synthesis, during DNA replication, of different families of human repetitive sequences was appreciable and not only assisted my knowledge on molecular biology techniques but, more importantly, drove my interest toward the less popular fraction of DNA, yet the most abundant, that doesn't encode for protein (ncDNA). Covering more than 97/98% of the Eukaryotic genomes was imprudently termed as selfish DNA or junk DNA even by very important scientists. At that time, I could not know that I would have spent several years in trying to understand the role of introns, elements belonging to that fraction of ncDNA. A lesson was about to be learned that is that the less obvious, the less evident, the less established is also the most attractive, surprising and exciting of the arguments. ***The dark side of the moon***. A course and a book contributed to further shape my personal inclination for science. In 1980 the University of Pavia held an EMBO course on DNA replication, recombination and repair with the participation, as teachers, of a group of the most well-known and respected scientists of the field led by the Nobel Prize winner, Arthur Konberg. I was strongly impressed by their conduct, by their openness, their helpfulness. They cared to spend time with us, answering our questions, transmitting in the simplest yet rigorous way their knowledge and teaching the art of reasoning. **Kings were dedicating time to peasants**. Incredible. Thus, science was a land of democracy where everybody is allowed to raise a question or to offer a line of thinking, a land where the strength and correctness of reasoning was the only thing that mattered. **Brain, not muscle**. In the meantime, I was reading the book entitled "Advice to a young scientist" written by another Nobel Prize winner, Peter Medawar. Another clue that prestigious, successful scientists cared about the rookies, the new entries, their younger colleagues to favor the flow of knowledge***, to raise children on the giant shoulders.*** The book of Medawar is full of advice and descriptions of the scientific environment with important and erudite references on ethics and philosophy. It starts with three important questions. How does one know if he/she is fit for science? What will be one's subject of research? How does one select a good place for his/her scientific training? Over there, in those pages, I found again my philosophy of change. In fact, the young scientist is warmly recommended to move out from his/her original lab, leaving his/her tutors and approaching new lab and subjects of research which I did, once graduated, leaving my Italian group to land in the USA where I was first involved in an immunology-oriented project and then in the discovery of the cellular counterparts of the viral oncogenes, that I searched for in yeast! This was the uttermost and most daring of the changes. But be aware as you'll pay a price for that: productivity hence career. **Freedom is not for free**.

2 Work Done: My Personal Scientific Approach

CHANGE. The major achievement of a 40 year-long career has been the development and the setting up of a method. Working out a method capable of providing reliable and consistent data has been the most intimately rewarding act of my scientific experience. **It is yours**! It is a product of your genius no matter how small it is. Experimental science depends on methods and if one thinks about it, the history of science is paved by methods starting from the *piano inclinato* of Galileo (1604) to genome editing (2012). Of course the one I have developed which is capable of providing, in an easy and reliable way, the genomic fingerprinting of any higher Eukaryote, doesn't aspire to that level of greatness but has provided me with many rewards. The first of them has been my freedom to do research. Let's describe the method in a few words and then discuss the associated aspects (see the right part of the Fig. 2).

It is the story of how elegant biology has been displaced by powerful technology. In fact, it has been through thousands of years that evolution has worked out a fine apparatus, the mitotic spindle, to ensure that, in Eukaryotes, the genetic heritage could coherently pass from mother to daughter cells. The spindle is principally made up by microtubules that bind, pair and move the chromosomes. Microtubules are made by filaments of alpha—and beta-tubulin, monomers added in a head to tail fashion. Hence, tubulins are key components for the maintenance and function of the spindle. As such, their primary amino acid composition is highly conserved. At a DNA level, this reflects in equally highly conserved nucleotide sequences that are only interrupted by two introns (variable, non-coding parts of the genes) at conserved positions in vertebrates and plants. So, if one placed a couple of primers at the two boundaries of the exons that flank the two introns, amplification done by PCR would produce fragments of different length, sequence and numbers in any genome, since that of tubulin is a gene family. **You generate a species-specific DNA code**, which is very simple and handy. I named it: TBP for Tubulin-Based-Polymorphism. This was worked out, and published in a patent, five years before the birth of the COBL (Consortium of Barcoding of Life) where DNA barcoding is instead more conveniently obtained by plain DNA sequencing of targeted mitochondrial or plastidial genes. I was creamed. The whole scientific community gathered under the flag of the COBL sponsored DNA barcode and our invention went neglected. Yet, my lab survived and our work was recognized at experimental, applicative and dissemination levels. These are the principal reasons. Experimentally, even the classical DNA barcode has its own limits, especially, but not only, in plants where the species boundaries are not well definable and there is the need to recognize varieties, landraces, wild species, hybrids. A method that doesn't require an a priori knowledge of the target DNA sequence may turn out to be very convenient. Another advantage is the application to mixtures like feed or food products where the TBP method can easily recognize the different ingredients down to a very respectable and useful quantitative limit. Here is where TBP turned out to be appreciated by farmers and industries allowing them to check their raw material against contaminations and frauds as well

as helping in assisting the release to the market of authenticated products. The money we received from these contracts was used to support our research on more basic scientific issues. Last but not least, the conceptual simplicity of the method favored the dissemination of key genetics concepts to students, farmers, and the general public, which was done by making videos, organizing events or delivering on-site lectures. Here to follow is just one of the stanzas of a small poem entitled TBP-DNA barcoding:

> *But the Microtubules stock/has* ***Tubulin*** *as the building block/This protein piles up in stalks/thank to conserved sequence docks/These* ***conserve aminoacid domains/****in the sequence of DNA are also well retain/so that is almost a game/to selectively amplify them and thus gain/an exclusive profile that renamed/as a* ***DNA barcode*** *acclaims/the diversity of life deserved fame.*

The story becomes even more interesting and instructive if one wonders how I ended up working on tubulin after my first experiences with human DNA repetitive sequences, immunology, under the tutelage of Prof Nicoletta Sacchi who became one of the most famous women in science, and virology. As already mentioned, looking for a biological role of the cellular counterparts of viral oncogenes I decided to address the question by working on the yeast *Saccharomyces cerevisiae*, a simple unicellular model organism that allowed sophisticated genetic approaches. This is a splendid organism with a refined genetics and a superior and precise DNA recombination system, natural precursor of genome editing. I attended a course at Cold Spring Harbor, at that time directed by the Nobel Prize winner James Watson, of the DNA double helix fame, under the training of Gerry Fink and Fred Sherman, two fathers of yeast genetics, and going back to my lab in NIH I was assisted by two other great scientists: Alan Hinnebusch working on campus and Kelly Tatchell who was at Penn State University. Under such an aura of greatness, I was able to give my small contribution to the role of the RAS cellular oncogene, primarily working on its pattern of expression in cell division and in response to external signals, facilitated by the availability of suppressors of its function. I was adopted by the yeast community, a very open circle of scientists continuously exchanging information and strains. I went back to Italy with this background and a lot of hope to further continue my work now that I had accomplished Peter Medawar's advice. But **life is never easy and one must be prepared for unexpected upheavals**. I was to join an Italian Institute that under the ghost direction of the President of the National Research Council (CNR) was at that time sponsoring its participation in the emerging HUGO (Human Genome) project thanks to the involvement of the Noble Prize winner, Renato Dulbecco. At first, my yeast expertise was appreciated since I set up, thanks to a collaboration established with Prof. Maynard Olson and Prof. David Schlesinger (University of Saint Louis), the megacloning of fragments of human chromosomes in yeast and the PFGE techniques for separating large size DNA molecules. Unfortunately, it soon turned out that participation of the Institute in the HUGO project was much more a matter of money and politics and much less of science. I spoke out about it loudly and had to quit. I joined then a Plant Biology Institute trying to rescue the authenticity of my yeast period, ignoring the

self-interest of humans. I could not go on working on yeast though and so I decided to translate my yeast interests into the two different issues of plant signal transduction and plant cell growth and division. Calcium protein-dependent kinases (CDPKs) and tubulins were selected as the two respective champions in the hope that one day I would have been able to uncover some functional link between the two. There was an even subtler reason. While CDPK was a somewhat confined issue, tubulin would have been, and indeed it was, and it is, a field of investigation that would have allowed me to move in many different directions satisfying my thirst for change and protecting me from any change in the leadership of the scientific direction of my institute, at the time under dispute by three major scientists who were working on gene expression, protein synthesis and accumulation and stress response, respectively. If you work on tubulin you can address all these issues, uncovering inherent unique aspects, and many more such as: DNA methylation and parental imprinting, pseudogenes, promoters, naturally occurring anti-sense RNAs, co-translational control and post-translational modifications, polyploidy, anti-mitotic drugs, embryo plane of division, motor protein interactions, viral propagation, pathogen attacks, plant morphogenesis, weed control, pollen development, endosperm ontogeny, cellulose biosynthesis. Actually, my group has been recognized, by the cytoskeletal community, for the work done in the characterization of plant tubulin gene families, their regulatory elements and pattern of expression (Breviario et al. 2013).

However, **it is the funding availability, or lack thereof, that will orient your research**. In fact, CDPK were the first to be abandoned despite the fact that for a while my group was a point of reference for many labs to ask for specific antibodies and cDNA clones. Studies on introns and their effect on gene expressions lasted longer but when it came the time of funding obtainable just with applied science projects I had to turn to ILP (Intron Length Polymorphism) that is TBP and thus we are now closing the circle of this short story which, I hope, should tell the reader about the vast possibilities of study and change that Science can offer and the resilience ability a scientist must have.

3 Science Today and Tomorrow

COMPLEXITY. Figure 1. What comes next depends on the idea you have of science, knowledge and progress. If you stand with Thomas Kuhn, who is considered the father of extant philosophy of Science, the progress in knowledge is neither granted nor linear. Each axiom must be experimentally verified to proceed with the acceptance of the current theory until the emergence of one or more anomalies, that cannot be explained, requires new thinking and propositions. This eventually leads to the definition of a new paradigm. In other words, knowledge in Science, that is different from progress in technology (see below), proceeds by discrete steps, and it is hard, if not impossible, to predict the direction of it while it is illusory to think of reaching the ultimate, revealing TRUTH. At the very least, this inference can be brought back to the birth of quantum physics, to the Heisenberg principle of uncertainty,

Fig. 1 My office, where chaos hides an order

and to Godel's incompleteness theorems about the intrinsic limitation of any logical system. Well known and often cited examples of paradigms are those that marked the change between the Ptolemaic and Copernican astronomy systems, the Newtonian revolution in physics, Einstein's theory of relativity, quantum mechanics and now is time for genetics to enter into this perspective (Fig. 2).

In fact, a century and a half of progress in genetics, starting from Mendel's laws of inheritance, has brought knowledge to a transition phase between an old over-simplified paradigm, where the central dogma has been the cornerstone, to a new paradigm yet to be defined. Premonitions such as the C-value (total DNA content doesn't correlate with complexity) and the G-value (estimated gene number does not correlate with complexity) paradoxes had been known for a long while but a decisive, yet problematic, contribution has been given by the massive sequencing of genomes that has revealed an extraordinarily large amount of dark matter, meaning the presence of non-protein coding DNA and RNA, the former accounting for more than 95% of the higher Eukaryote genomes and the later surpassing by far the sizes of the genome of reference. Neither can be easily traced back to the descending flow of information of the central dogma, from DNA to protein, since they are often attributed, but rarely demonstrated, to multiple regulatory functions and interactions. A dark matter that is still waiting to be deciphered and, when done, it could possibly lead to changes in the way we have been referring to genetics so far. For instance, what do we actually know about the molecular mechanisms defining speciation in plants

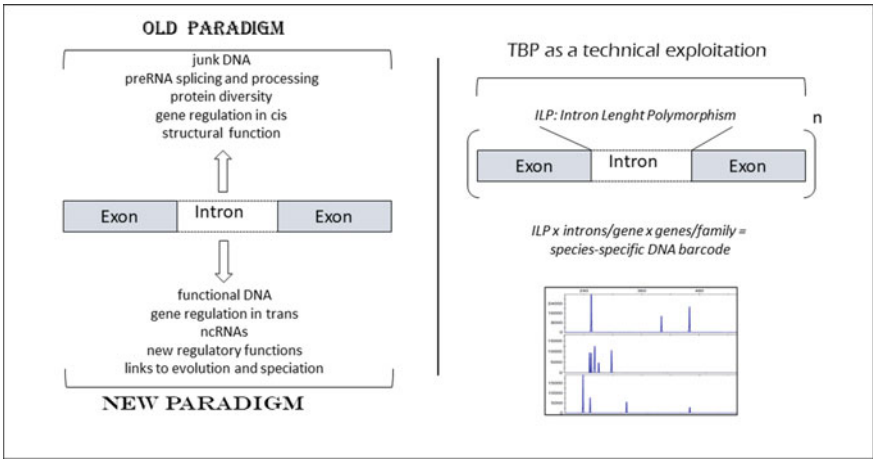

Fig. 2 The old and the new paradigm in genetics

where liberal outcrossing is defying sex barriers? Referring to my limited experience with the method described above, how comes that length and sequence variations in tubulin introns strictly correlate with speciation? It may be an effect or a cause but still remains an open and challenging question and thousands more can be similarly posed when investigating the complexity of genomes and transcriptomes. In order not to disregard proteins, always referring to the central dogma as the stronghold of the old paradigm, we should not forget the puzzling issues raised by prions. So, there is still a lot of work to do even if one restricts oneself to classical biology and genetics but of course we have to consider the contribution that may come from quantum physics to the possible unravelling of a new biological paradigm. After all, molecular genetics deals with the molecules world, not atoms, neither electrons, nor other subatomic elements, which together represent a higher and deeper level of resolution of biological matter. How are they going to impact on the current status of genetics knowledge? DNA mutations have been already explained through a quantum model. What will be coming next? And in which context? Reading the capital and visionary book of Erwin Schrodinger entitled What's life, which by the way I would make mandatory in any course of science, it is understood that the macro-genomic order that supports, preserves and inherently propagates biological life is dominant on the chaotic single- and sub-atomic events that would eventually lead an organism to its maximum of entropy that is death. By eating, drinking, breathing, assimilating, and very likely thinking, biological organisms replenish themselves of that amount of orderliness required to remain alive. Quantum biology is definitely an issue for next generation science.

Let's now go back to the concept that massive genome sequences have shaken the central dogma but still they have not paved the way to a new paradigm. In my opinion this is due, to a certain extent, to what I would call the survival instinct of scientists who find it more convenient and immediately rewarding to stick to the

coding part of genomes rather than bravely heading toward the dark matter. Scientists must publish to boost their reputation and to progress in their career and it is much more convenient and a lot easier collecting data and information on genes and their patterns of expression than muddling with the unknown. This would be part of a more sophisticated reaction that, as argued by Lakatos, brings scientists to shield the nucleus of the existing theory by a so called "protective belt". In turn, this is instrumental to **the easy construction of models to be applied under the most different physiological and environmental conditions**. Models are quite distant from data corroboration and methods realization. They can easily vary depending on the variables that are incorporated. Models built up on data collection are even less consistent, semantically incorrect, since are often presented regardless of their proper justification into the currently accepted axioms of the theory of reference. Because of their intrinsic inconsistency, models cannot even be falsified, in the most classical Popper view. **Models represent examples of inductive, sometime adductive knowledge which can yield different conclusions depending from different possible starting premises.** On the contrary, deductive reasoning is based on widely accepted facts or premises. Only when models are not opportunistically proposed, they can offer some idea on where to move for new investigations. In a way Plato had already warned us about this pernicious attitude when he made a clear distinction between **knowledge and numbers**.

Let's take a typical whole genome sequencing project that provides numbers, by search and definition. If the investigated organism is an Eukaryote it will be very easy to find out the number of the alpha and beta tubulin genes but the question is: how this numerical information is going to provide knowledge on even just one of the following issues? **Multi-tubulin hypothesis.** Since its first proposition in 1976 (Fulton and Simpson 1976) the question of why a highly conserved structural protein such as tubulin is actually encoded by a discrete and variable number of genes has not found an answer yet. Is it a regulatory or a functional issue or a mix of the two? Sporadic and not fully convincing evidence has been obtained so far and nucleotide sequencing is not going to help. **Intron length polymorphism**. The first question here is why tubulin genes conserve introns since, at present, no form of tubulin has ever been observed that could result from alternative splicing. Is thus a purely regulatory matter and, if so, why does intron length vary within the members of the same plant species and among the members of different plant species? Since speciation is the final result of the accumulation of DNA new arrangements and mutations, inevitably reflected in the length and sequence of the tubulin introns, it may be that the introns also influence the efficiency of chromosome pairing by some yet unknown ribonucleoprotein complex. After all, colchicine, a well-known anti-microtubular drug, it is used to overcome the sterility of hybrid species, by producing chromosome doubling. **Pseudogenes**. When looking for tubulin-like sequences within a genome you can also find a certain number of tubulin pseudogenes, forms of the genes that cannot encode a functional product because of the presence of several mutations that affect the correct frameshift. What actually are pseudogenes? The useless remnants of previously functional genes or an intermediate form that will eventually evolve into a new, more adapted tubulin isoform? That would be to say that the most evolved

form of a gene is when it loses its coding capacity. **Natural occurring anti-sense mRNAs**. For tubulin, they have been found at least in maize. The question then is: what's the role of these molecules and are they present in other organism besides maize?

All these issues ask for answers which cannot be provided by the simple numbers of a genome sequencing project, neither by their further bioinformatics elaboration but they rather call for a new, wild way of thinking that must be supported by a new scientist attitude for enduring the hardship of a long time spent in experiments that will not lead to easy publication. But, **what does the world really want: papers or knowledge?** As my friend Peter (Nick) says: a lot of data doesn't mean a lot of knowledge.

Once the current inebriation for models built up on any numerical assembly of known functional parts of the genomes will find an end and the scientists will start to look convincingly into the dark matter, withstanding a certain degree of unproductiveness, a fundamental step toward a new genetic paradigm will be taken. This goal could be more easily achieved if, simultaneously to the explosion of the BIG DATA era, some adjustments in the more general terms of doing science will also be introduced. I am here referring to a more careful and moderate rate of papers production (*Einstein: An academic career, in which a person is forced to produce scientific writings in great amounts creates a danger of intellectual superficiality*), on a stronger request for data repeatability (possibly with dedicated Journals), on a better control on raw data acquisition and elaboration, on the definition of new statistical limits of significance, since false positives may be orders of magnitude higher than real data and undesirable behaviors like that of p-hacking must be alienated. On the contrary, the time is due to publish negative results if the construction of the investigation plan is solid and to make reviewing a more responsible and gratifying job. So accepting the Kuhn model of progression of scientific knowledge, that cannot be linear as the whole is unlimited, there are **two quite distinct ways in which future science can be performed which we can call the accumulating and the breaking free way**, each one with its own role and references. A good quality accumulating science is based on experimental evidence, corroborating concurrent data and theories formulated in a new positivism milieu, qualified by high impact factors and citation index of the publications. The breaking free way, evidently moving toward the new paradigm, is based on wild discoveries, original ideas in a yet undefined new theory, supported by a post-modernism thought and qualified by a high disruption index. Technological progress is not necessarily bound to any paradigm. The old paradigm may contribute as well since it is a matter of practical tools and applications. This calls for the last consideration of this section which I leave to Peter Medawar in the following quote: *science must face the problems that trouble the humans struggling to find technical remedies and solutions but the direction to be taken, the priorities to be assigned, the distribution and coordination of the activities into the society go to politics that has to take the responsibility. Science provide new and diverse solutions but doesn't stand for a specific one.*

4 Advice to the New Generation of Scientists

WONDER

Aristotle stated that: "*Learning things and wondering about things, as a rule, is pleasant. For wondering implies the desire to learn and to know"* (Rhetoric 1371). I do strongly believe that science is for those people who are capable of wondering, who have a positive and enthusiastic way of looking at the miracle of life, who are constantly and intimately asking questions and try to find reasonable answers, who are not afraid of cultivating daring ideas and take the challenge to verify them. The one who is sustained by such a sacred fire will be content, no matter how many difficulties she/he will encounter. On the contrary, if one thinks to take science as an ordinary job, she/he will soon feel to be out of place and, by carrying on, she/he will eventually damage science and society. She/he will become an unhappy clerk or an ambitious bureaucrat but not a scientist. So the first basic question one has to answer is: **how I feel about Science?** If the answer is positive then the next question automatically follows: **am I fit for doing science?** Indeed, because the job is not that easy and, as I said, one must feel a strong commitment. Let's start by saying, partially contradicting what I have just written because fanaticism can be a mistake as well, that, once the commitment to science is there, one's important contribution can also be deployed on the path of accumulating additional evidence and data from experimental approaches designed to corroborate the extant theory. After all, there are **four roles** that a dedicated scientists can perform. **The explorer**, who produces new data, unravels new evidence, makes new discoveries. **The inventor**, who develops new methods, new materials, new algorithms sustaining innovation and technology. **The philosopher**, who takes the challenge of more fundamental and radical questions. **The teacher**, who has the responsibility of a correct education and of the dissemination of scientific theories, information and data. All the four types jointly contribute to improve human knowledge and their role in society is and must be fully recognized. This is more appropriately laid down in the European Charter for Researchers where rights and duties are also well defined.

Why the experimental job is so difficult? Hereafter, my multiple answers and humble advice.

The rate of success of even a properly planned new experiment is exceedingly low, This causes frustration. As I have always said to my students and young scientists, the percentage of success in a truly new experiment is less than 10. If you are not motivated you cannot seriously face such a high level of failure, that goes reiterated for any experiment that is not routine and confirmatory, and even there you may find problems.

You feel the pressure of being a productive scientist. Publish or perish, remember? If you do not manage this, and it costs energy, you may end up producing a series of irrelevant papers, or plagiarize the work of others or, even worse, publish biased and wrong data. You need your own money to work and competition is very high. Either you manage, because of your recognized expertise, to enter a consortium of people

who are interested in having you or you must be firmly convinced of your idea, your project, and yet it may not always suffice.

Writing a paper or a proposal is not that trivial and it may cost a lot of time, effort and energy and yet you may be facing failure, and sometimes you feel you have not been judged fairly.

Developing an entirely new method, not an upgrade of an old one, is a difficult task but it is even harder to make it fully reproducible in any lab and context. It requires time and patience and you are not likely to be funded for this.

Science is a very competitive area and yet you must be fair with yourself and colleagues. You should admire and not envy the ones who are better than you. You must acknowledge your limits and always try to improve your standing. **It is always better to be the last of the firsts than the first of the lasts**. At the same time you should not envy the ones who are successful even when you think that they did not deserve it. Once again, remember Aristotle and his saying: *dignity does not consist in possessing honors, but in the consciousness that we deserve them.* **Be content with your dignity**.

Follow your idea. Do not anticipate in your mind the results you will obtain from your experimental plans. Just do them and analyze the data. Do not think a priori of any impediment that will determine the failure of your approach. It is often a useless and ill-based speculation. **Do and then think. Do not think so that you never do**. Be aware that the stronger part of any of your experimental design is provided by the right controls. Controls are more important than results.

Do not follow the stream of the most fashionable science of the moment. Be an expert on something. At the next round science will come to knock at your door.

In presence of a recognizable and documented reputation for both, think and decide which you like better: a renowned large high technology lab with big numbers or a small science team with a more radical thinking?

Do not restrain yourself from conceiving several ground-breaking ideas, just apply for them. It is like in finance: you invest in many products but one will be enough to pay you back. You'll be content and have the lead of that field. You also will be content if and when one of your ideas, untimely and for this not financed, will eventually become a major field of investigation for others. That means you have a good brain, a good perception of science and on that you can count. That has happened even to me many years before the start of metagenomics. I was thinking and proposed to trace the geographic origin of cow milk, at cattle sheds, by making subtraction libraries (at that time there was no massive sequencing) counting on the presence of different bacterial strains, different feed and different bovine race DNAs.

Do not prolong indefinitely your training period, no more than 3–4 years says the European Charter, because that will make you dependent on your senior. You must leave her/his lab and change the subject of research to find your own way. On the other hand do not pretend to be a genius if you are not, and yet if you are you would not read these lines because you would instinctively find your way, as Einstein at the patent office of Zurich.

Do not be afraid of changing the subject of your investigations and be multidisciplinary in your approach establishing good and fair collaborations.

Do not value differently basic and applied science. You will damage both. Just make your choice. Ultimately science is performed to improve human life through newly acquired knowledge and tools. In fact, the old society based on private property has been replaced by the new one based on intellectual effervescence, the so-called Knowledge Based Bio Economy (KBBE). Distinction between basic and applied science, although the latter clearly depends on the first, may become fuzzy and petty and can be easily manipulated. **Just go for good, new and tangible results**.

Your salary, at least at the beginning and even longer in some countries, is going to be low when compared with less prestigious jobs but be content because you will have the freedom of thought and action, which you won't find in many professions.

Be a philosopher not just a scientist. Cultivate your mind with good lectures and classic novels. There are a lot of things that philosophers and writers can teach you. Take Karl Popper for instance and his falsification principle. Karl Popper believed that scientific knowledge is provisional—the best we can do at that moment, and this is in agreement with our post-modernism time.

We have now reached the end of this essay and I guess that the final question to ask is Schrodinger's: "*I", what is this "I"? If you analyze it closely you'll end up with the impression that "I" is just the facts, little more than a collection of single data made up by individual experiences and memories. Namely the canvas upon which they are collected.* This brings me back to my beginning and my appeal to humility.

ACTION

If you become a scientist and you really want to make a difference reassigning science to the field of freedom of thought where it belongs (remember the words of the Galileo in the Brecht play: … *What are you working for? I maintain that the only purpose of science is to ease the hardship of human existence. If scientists, **intimidated by self-seeking people in power**, are content to **amass knowledge for the sake of knowledge**, then science can become crippled, and your new machines will represent nothing but new means of oppression. With time you may discover all that is to be discovered, and your progress will only be a progression away from mankind. The gulf between you and them can one day become so great that your cry of jubilation over some new achievement may be answered by a universal cry of horror. I, as a scientist, had a unique opportunity. In my days astronomy reached the market-places. In these quite exceptional circumstances, the steadfastness of one man could have shaken the world. **If only I had resisted, if only the natural scientists had been able to evolve something like the Hippocratic oath of the doctors, the vow to devote their knowledge wholly to the benefit of mankind!***) then you could adhere to the following manifesto, or something alike. Hence, you could sometime place yourself in front of a mirror and read the OATH of the post-Galilean scientist.

I SWEAR TO FULFILL, TO THE BEST OF MY ABILITY AND JUDGMENT, THIS COVENANT

- I humbly recognize that life, in its more comprehensive definition, is marvelously more complex and perfect than I could ever grasp and that universal truth is just

unreachable and yet I will do my best to improve the knowledge of humans and the quality of the terrestrial life.

- I will remember that I remain a member of society, with special obligations to all my fellow human beings. Above all I vow to devote my knowledge wholly to the benefit of mankind and resist the intimidation of the self-seeking power people and their evil distortion of knowledge and applications.
- I will always stand for freedom of thought and free circulation of scientific information and data, fighting against manipulation and anti-Science. In accordance, I will always stand for peace and will be firmly and always against any war. I will not subjugate to any ideology and religion and I will not offer my knowledge to warmongers.
- I will make every effort to ensure that my research will be relevant to society and will not duplicate research previously carried out elsewhere. I will avoid plagiarism of any kind and abide by the principle of intellectual property and joint data ownership in the case of research carried out in collaboration with other colleagues, as also stated in the European Charter for researchers.
- I will remember that there is art to science and that collaboration, information and respect may outweigh the uncontrolled urge for publishing papers or any other kind of personal recognition.
- I will not be ashamed to say "I know not," nor will I fail to call in my colleagues when the skills of another are needed, a contribution I will gladly and duly recognized. On the other hand I will not pretend to be recognized for simple supports such as providing an information, a reagent, a cell line, a strain, a sequence information and stuff of this matter.
- I will respect the hard-won scientific gains of those scientists in whose steps I walk, and will gladly share such knowledge, and the new advancements I will be able to produce, with those who are to follow.
- If I do not violate this oath, may I enjoy life and science, respected while I live and remembered with affection thereafter. May I always act so as to preserve the finest traditions of my calling and may I long experience the joy of uncovering even the most tiny piece of new knowledge.

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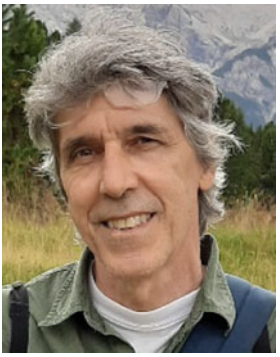

Diego Breviario Graduated in Biology at the University of Milan, Italy in 1980, he moved to USUHS, NIH and NCSU (USA) where he gained experience in molecular biology applied to the fields of human immunology and oncology. Back to Italy he continued his work on signal-mediated growth control characterizing the plant CDPK and tubulin gene families. Working on the control of tubulin synthesis, the main constituent of microtubules, he ended up in studying the role of introns in gene expression. In turn, because of some specific features of tubulin gene organization, he developed a new method for the easy genotyping of plants and vertebrates. He has been working for more than 30 years, at the Institute of Agriculture Biology and Biotechnology of the Italian National Research Council (CNR).

A Greased Pizza Paper Changed My Life

Piergiuseppe De Berardinis

Abstract I wanted to be a medical doctor, but several circumstances led to change my plans. I had the chance to go abroad and work on a Ph.D. project in an immunological topic. With Immunology it was love at first sight. When I was a medical student at the university of Rome I heard about the discovery of a new technique to produce monoclonal antibodies. I perceived the importance and the translational potential of immunological studies, and at the same time I was fascinated by the complexity of the immune system where small causes, such as antigen recognition, induce large effects (i.e. the cellular and humoral immune response in order to restore the homeostasis). I came back to Italy after my Ph.D. and obtained a permanent position at the CNR (National Research Council) in Naples, where I established a new lab and devoted myself, body and soul, to this undertaking, throwing my heart over obstacles. Coasting life in a science lab I learned to sail through my profession. Sailing new seas often in bad weather conditions I became aware of my limits, without ever giving up on my will to reach previously unknown ports. I learned with passion trying to treasure the mistakes. By telling my story I also want to share my mistakes, hoping they will be useful to those who wish to embark on this journey.

1 Motivations: How I Developed an Interest in Science

My childhood dream was to heal cherry trees, as in the metaphor of De Andre's song from the "Spoon River" anthology (De André 1971).

I didn't dream to be a scientist. With this hope, I found myself as a medical doctor in Rome in the early 80's. Just to dispel myths about the past and say something politically incorrect, I believe that free access to the medical faculty in those years did not reward the deserving, nor did it improve health assistance. But I will not elaborate on this any further as it would take me away from the main scope of this book.

Dedication: To my love, my daughter, and those who put up with me all these years.

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The indelible memory that changed my aspirations dates back to my first professional experiences. After so many sacrifices that my family had made to make me study, I could not afford to remain as a temporary worker in the university, and I gladly accepted to replace a medical doctor who worked in the popular "Trullo" district of Rome as a general practitioner. I immediately realized the futility of my role. What I was asked to do was to fill in prescriptions for specialist visits and drugs sometime useless and which had been recommended by other doctors.

I remember the day when a child sent by his mother showed up with a greased pizza paper with the name of the drugs to be prescribed written on it. It was a really enlightening event that proved beyond any possible doubt that it was not what I wanted to do with my life. I went to the university professor with whom I had done my thesis work (and who highly valued me) and I told him this episode almost as if it was a joke. He proposed that I participate to the competition for a scholarship from the "Cenci Bolognetti" Foundation to spend a few years in a research institution abroad. I gladly accepted, and thanks to some scientific projects to which I had contributed during the preparation of my thesis, I was able to obtain the scholarship. Destination: Department of Immunology of Middlesex Hospital in London. I moved there almost immediately, and while waiting for my first salary, I was hosted for a couple of months by an Australian girl whom a friend of mine had met that summer on a trip to Greece. She opened her house to me even though she did not know me at all. In those days, life also offered this.

I remember the emotion and the desire to do things, which I tried to convey with my eyes since I barely knew two words of English. I remember meeting with Professor Deborah Doniach, a giant of immunology. Well, unlike today, it was not uncommon at that time to meet these figures who have made the history of a scientific discipline.

After a few months I agreed to become a Ph.D. student in Marc Feldmann's laboratory at the Charing Cross Sunley Research Center. I remember the feeling, also due to my young age, of being in control of my own destiny. I remember those four years as a Ph.D. student, the nights spent in the laboratory and the joy of spending them there. I remember my final Ph.D. exam that lasted a whole day in the presence of Ivan Roitt who was one of my scientific myths, the author of the book on which all aspiring immunologists had studied. I remember his words and his appreciation for my work.

Having obtained the Ph.D. Diploma, my career was at that point definitely oriented toward the world of Research, and I was immediately invited by a senior CNR scientist whom I had met in London, to apply for a position at the Naples Institute of CNR, where some of his colleagues had requested a profile that met my skills.

At this point I must admit that my socio-cultural condition, which I would define with the words of the past as "petty bourgeois", probably made the permanent job condition triumph over other possible options. Unfortunately, it turned out to be a big mistake that I still regret today. However, at the time I was captured by the Neapolitan courtesy and by the beauty and charm of Diego Maradona's city (the Institute was located close to the stadium). Furthermore, and I would now say unfortunately, in the CNR Institute where I was working in Naples there was no consolidated expertise on the issues for which my position as a researcher was requested. This tickled my ego,

placed me in a position of greater independence and led me to ignore the difficulties and to believe that I could have made it alone. I therefore devoted myself, body and soul, to setting up my new laboratory, throwing my heart over the obstacles. If I have to take stock now of what I have managed to achieve both scientifically and in terms of my professional career, I should honestly acknowledge that I got a meager harvest than the effort. However, I know that I have paved the way to relevant but still neglected issues in the context of the CNR (the largest Italian research institution), and that I have trained young and enthusiastic students and post-docs, who are now my colleagues. I am sure that, starting from my mistakes, they will be able to carry on these issues in the best way, and that in some way they will also reward my efforts.

2 Work Done: My Personal Scientific Approach

In light of my experience and of my personal history, my working approach can perhaps be considered as that of a self-taught person and therefore quite outside the box.

As laboratory chief I have always adhered to an interview I read in the 90's, in which well-known scientists working in prestigious international institutions were asked what the ideal numerical composition of a wet lab in science should be.

Surprisingly, according to the responses of these prepared minds, the ideal laboratory had to be composed of five people including, in addition to the Principal Investigator, a staff scientist, a post-doc, a technician and a student. In short, quite the opposite of the "grandeur" displayed by many colleagues and many labs currently considered as leaders in their fields, who are often referred to as representatives of national excellence.

However, this so-called "rhetoric of excellence" should be called into question. In fact, the reference to excellence (which in itself appears as a noble intent) often serves to hide, in the scientific sphere, mere operations of power which do not do the good of science.

We should be aware that, without a basis of well-trained and adequately funded researchers, we will never be able to give birth and establish true scientific excellence. Regarding the formula of small research groups, it must be said that this dimension would be more advantageous in a local context of laboratories of similar size operating on related issues and connected in a network.

According to what I just said, the most important features I always valued when selecting young people to be trained in my lab were humility, the desire to learn and the disposition to dream. Rather than dwelling on the grades obtained in the university exams (which certainly have their importance), I have always preferred to evaluate the motivations and personal history of each candidate, trying to glimpse how those who want to do science will be able to overcome the difficulties that will be fatefully met in their training path. In my laboratory we share tasks. Trying to describe a typical week of work in the laboratory, I would say that Monday morning is dedicated to a lab meeting on the status of the experimental work, where we try to

dissect the things that did not work and decide how to deal with the new experiments, while Friday afternoon we often schedule a journal club on an article related to our research topics. As for the experimental activities carried out during the week, we try as far as possible to share the tasks of the projects (generally two) that are performed in the laboratory.

In light of this organizational and working context, and thinking back to our fulfillments and failures, the main price that we had to pay was due, at least in part, to the isolation of our research topics from the scientific issues addressed by the other laboratories in our institute and in the neighboring ones. This forced us to try to remodel the experimental lines to adapt not only to economic contingencies but also to the surrounding scientific environment. In fact, although science follows international objectives that go well beyond the institutional walls, there is no doubt that the environment you breathe in the physical workplace and the possibility to interact with the laboratory next door can make a great difference. Therefore, our skills as cellular immunologists, rather than in the analysis of the immune response in populations or in experimental models that required expensive investments also in terms of reagents, have been oriented toward less expensive approaches such as the formulation of new antigen delivery systems based on natural nanoparticles made up of bacteriophage virions.

This choice, let's say more of niche, allowed us to break through the wall of "Nature Biotechnology" (De Berardinis et al. 2000) with a manuscript exclusively authored by members of our lab (which I believe has happened very rarely in our local context), but which in contrast did not allow us to establish ourselves as a lab of reference either nationally or within the CNR.

It is now obvious, with the technological advances that occurred in recent years, that experimental planning has gradually changed, making it increasingly necessary to activate collaborations to absorb new technologies and make our projects more appealing. Even this approach is certainly more difficult when the expertise in the surrounding labs is focused on different issues. However, some seeds have germinated in recent years and in particular in my Institute the number of immunologists and reference laboratories has grown in the last decade. Moreover, a network called CIN (CNR Immunology Network) has been organized to connect the immunology labs displaced in different Italian CNR institutes, although the need to aggregate immunology labs in physical proximity still remains an unsolved issue. Overall, I hope for the future that the experience gained over the years will be treasured, so that those who will soon replace me in the leadership of the laboratory will be able to continue the new studies with even greater vigor. The esteem and the affection that binds me to the colleagues who will carry on the lab work makes me happy and confident for their activities and I wish them the best of luck.

3 Science Today and Tomorrow

It is not easy to define what science has been up to until now and what it will be like in the next few years. I think that each of us has his/her own opinion of the scientific world in which we grew up and worked. As a student at the University of Rome, I heard for the first time about the production of monoclonal antibodies. A compelling story that earned the Nobel Prize to the two protagonists, a young German post-doc (George Kohler) who worked in the Oxford laboratory of the great scientist Cesar Milstein. The discovery of a new technique for cell fusion, which allowed the unlimited production of antibodies by single clones of B lymphocytes, therefore defined monoclonal antibodies. A discovery whose developments are visible to all of us for the use of monoclonals in research, but also, and above all, in the clinical field of diagnostics and therapy.

I was struck by this discovery and applied to work on an immunological issue for my medicine graduation thesis project. Immunology was a kind of love at first sight to which I remained fascinated. Even today, when I read articles on immunological topics, I still feel like undertaking an imaginary journey into the complexity and beauty of nature and the mystery of complexity. As is known, the complexity of a system derives from the ability of any small cause to yield a large effect. Concerning immunology, an example is the antigen recognition which triggers the cellular and humoral immune response, with the consequent series of events that lead to counteract the pathogen and restore the homeostasis.

As recently explained by the theoretical physicist Alessandro Pluchino in his book "A walk on the edge of chaos" (Pluchino 2015), in mathematical terms, complexity has a signature given by the so-called "power law" and can be represented graphically by a curve on the border between order and chaos.

The complexity of the immune system depends on the intricate communications network capable of exerting multiple effects based on a relatively limited number of cell populations. The immune system has a lower complexity with respect to the nervous system, but it has greater possibility for intervention in the therapeutic field (Fig. 1).

Immunology is responsible for the most important discovery in the field of medicine in terms of costs/benefits: vaccination. As we know and have learned in these two years of pandemic, there are many and important scientific challenges that await us in the coming years and in which immunology, which is a frontier science, will be a protagonist. We imagine the ability to predict events and immediately identify new viruses that are about to spill over so that we can act promptly with adequate immunotherapeutic aids, or more simply to direct more sustainable social behaviors for the environment. Or imagine the role of the immune system, which must safeguard homeostasis and which is therefore based on individual experiences, in defining the new frontiers of the so-called precision medicine. This awareness makes me confident about the science to come and the role that immunologists can play.

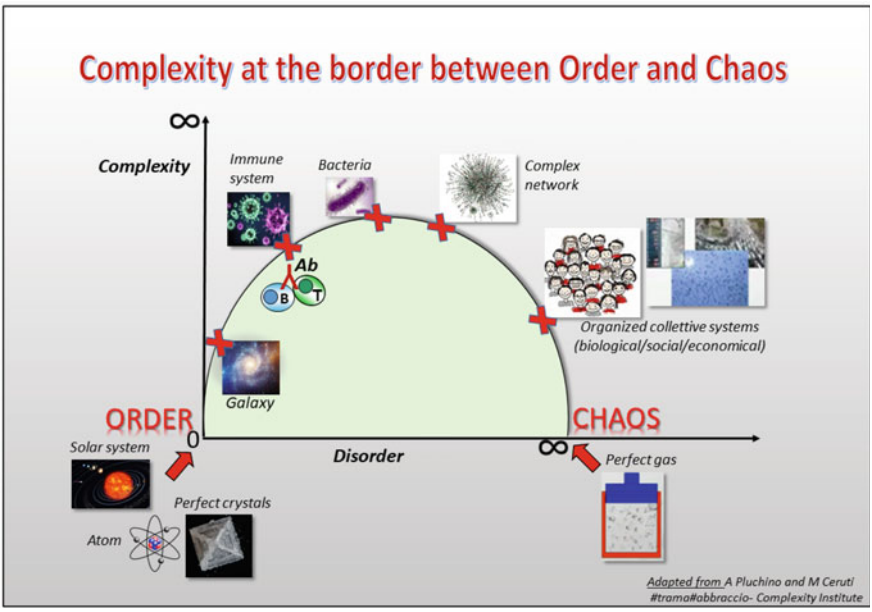

Fig. 1 Complexity

On the contrary, when we think of science in terms of policies to support science, then I am less confident, in light of the situation that has taken shape over the years in the world of science, especially in Italy.

The main issues that our country is facing relate to the following aspects:

Funding. Indeed, the total expenses for research and development carried out by our country are well below the European average.

Based on this evident gap, the Italian scientific community recently launched a call to support a proposal that was initially put forward by the physicist Ugo Amaldi,[1] who solicited the Italian government to double the national investment on research and development by 2026, so that it can reach 1.1% of the GDP.

With regard to the largest national research institution, the CNR, to which I belong, we know that about 90% of its ordinary funding (FOE) is used to cover staff salaries, and therefore the ordinary scientific operability of the institution is supported to a great extent by external funds obtained by CNR scientists to carry out their research projects.

Careers. The poor attention of CNR to research funding is accompanied by a substantial block of career progression.

What did all of this bring to? Unfortunately, rather than fighting to overturn these senseless policies, CNR scientists have found themselves increasingly frustrated and isolated, loosing their motivation to take part to the strategic choices of the Institution.

[1] Il Piano Amaldi per la Ricerca. https://www.scienzainrete.it/articolo/firma-anche-tu-piano-amaldi-raddoppiare-budget-della-ricerca/luca-carra/2020-09-30

In simple words, they have been unable to attend to the rights and duties established by the European Charter for Researchers.[2] Moreover, several trade unions supported the decisions of the management, thus diminishing the role played by scientists even further. All this has contributed to decreasing interest in science by the media and public opinion, which in turn leads to underestimate the role that research can play for the progress and the well-being of society.

Politics. Unfortunately, the choices made by the leaders of national scientific institutions in concert with politics are largely due to their inability to understand and grasp social transformations and to support existing and promising realities. For example, we have never managed to network many small industrious and innovative laboratories, which represent the backbone of our production capacity in the scientific field. On the contrary, much of the political effort were devoted to centralizing research activities according to a top-down scientific management scheme, referring to obsolete and no longer productive concepts of a "Fordist" type policy characterized by vertical control of the work process. However, research and innovation are based on creativity and can generate an economy based on new knowledge. The great economic leap made by vaccine manufacturers is here to testify this: immigrants scientists who founded small companies, giving a great contribution in the manufacturing of RNA vaccines.

Unfortunately, at the moment even the most recent choices made in our country seem to follow these old ways of thinking. The Italian government has recently adopted the strategic instrument of the European Commission named Next Generation EU, which recognizes a greater role for scientific research and includes a new funding instrument for science through the PNRR (Recovery and Resilience National Plan), even though it still does not meet the target set by the Amaldi's plan. How will these figures be committed? The premises are not reassuring. In fact, participation in the PNRR is organized vertically: at the CNR the president makes the decision on the research projects to be prioritized for funding and the head of the department asks the Institute directors to select 2–3 scientists in each Institute that will participate in that project. In recent years this wasteful scheme has been used by some Italian Regions for managing funding from the European community. All this is done in derogation from the principle earned by the liberal tradition, and generally shared, that free competition increases the quality of the product. Once again the base, that is the most fertile ground where creativity and true innovation stand, is ignored. Let's hope again that some critical appeals raised by eminent scientists (as the nobel prize winner Giorgio Parisi, https://www.italianpost.news/pnrr-warning-from-accademia-lincei-and-nobel-parisi-no-to-cathedrals-in-the-desert/) will help improve science policies.

In light of all this, what will the future be like?

A solid hope comes from the fact that our country, which is in any case a small entity compared to the European and international context, will have to adapt to social

[2] European Charter and Code for Researchers. Recommendation of good practice for researchers and employers and/or funders of researchers issued by the European Commission Directorate-General for Research. Istituto Euro-Mediterraneo di Scienza e Tecnologia. 2020-07-23. Archived from the original on 2021-01-05.

and economic changes to keep up with the pace imposed by the new social realities and preserve its competitiveness at an international level. In fact, the position rents will not guarantee sufficient dynamism, and consequently the new generations will in fact be able to cope with the changes and overcome the old logic of power. Other logics will inevitably impose themselves. However, quoting the "Antonio Gramsci's optimism of will" I am confident that "Science" will be ahead of its time and favor the emergence of a better society.

4 Advice to the New Generation of Scientists

To young colleagues I would recommend first of all to carefully evaluate where to carry out their work and to try to project themselves over time by not orienting their choices on immediate convenience. In light of my experience, I believe that in addition to the painting, it is very important to look at the frame and the exhibition gallery. In other words, make sure that the lab of choice belongs to an institute which prioritizes the topic of your interest. Once you become part of the lab, it is essential that you develop easy relationships with the other lab co-workers and that you establish good contacts with your colleagues in the neighboring laboratories to discuss the experimental activities from different perspectives.

Then, it will be very important to systematically reflect on one's work, never taking anything for granted. Find the time to consciously self-evaluate your contribution to the implementation of the experimental activity and do not be afraid to challenge yourself by moving in other directions, even by changing lab, city or country if you believe it might be useful for your personal improvement. Delaying choices sometimes can impair the possibility to improve one's position and aspirations.

More generally, once you have chosen the lab, remember to always have in your toolbox four available and fully functional instruments: (1) passion, (2) attention to detail, (3) humility and (4) hope.

(1) Passion will serve to go where it is difficult to get to, and to put up with difficulties. It is often defined as a fire and as such it needs dedication and sacrifice to burn. In fact, passion comes from the Greek word "pathos" which means suffering experience. Therefore, fueling the passion will involve a certain amount of suffering to support what we are passionate about. However, it will be the boost to move forward, improve and feel alive. As you already know, doing research will not be just any kind of job, it will not reward you economically, it will not get you many social recognitions, but if it is fueled by passion it will make you feel the emotion of being able to contribute to the improvement of knowledge. What is more vital for humanity than the advancement of knowledge? In addition, your passion in doing this work will give you the chance to share your achievements, in almost a real time, with a global world community that you may have the opportunity to meet together with the possibility to establish strong friendship with some of its members.

(2) Care means respect and a sense of responsibility. Think of your work as a cross-country run and not as a hundred meters run. The care and, above all, the attention to details requires not to be in a hurry, to know how to plan, to question oneself continuously and to know how to change perspective. Only by dedicating yourself to the attention to details you will be successful.
(3) Closely related is the third work tool: humility. Humility is the first quality I look for in students who ask to attend the lab. Humility is awareness of one's limitations, it is asking for help and accepting that sometimes you cannot succeed, without feeling guilty. It gives the strength to get yourself up after a fall. Being humble allows you to always be open to constructive discussion and self-criticism. Humility is therefore the ability to learn from others and to reconsider our beliefs every day based on comparison and new data.

    When my daughter was a child I asked her to learn poems by heart. I told her that learning a poem was not useless and boring. I knew that this humble exercise would allow her not only to enrich her vocabulary but also to understand different cultures, historical and social conditions and to store useful information that would come handy in due time. As Italo Calvino said in one of his famous interviews, learning poems by heart is also useful for old people. I would add that in the same way and in relation to your research topics, you should not disdain to review the basic topics with the same curious and attentive eyes of a student who always has a great desire to learn.
(4) Hope, the fourth tool, does not mean entrusting oneself to fate, but it means believing in what one does, trusting that the efforts will be rewarded and working towards this goal. Hope gives the strength and the will to add our small contribution of knowledge to the many others obtained before us and to those to come. Hope is to plant a seed and try to make it survive so that one day it can blossom.

Finally, it is especially important to remember that a good dose of humanity in doing things is no less important than the ability to perform experiments. In a world which is increasingly oriented towards individualism, to be a scientist puts you more at risk of losing your soul by isolating yourself in an approach based on cynicism and on attitudes of intellectual superiority, with the belief that you have the means to do it anyway. By losing empathy and the ability to relate (not just virtually) with your social and work community, you will lose everything. You will lose the strength to be indignant and to fight against injustices, you will lose the lack of vision for the future, you will lose the commitment to improve the present, and you will inevitably also devalue the effort of those who committed their life to Science before yours.

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Piergiuseppe De Berardinis MD specialized in clinical Immunology. In 1989 he obtained the Ph.D. in Cellular Immunology from the University of London (UK). Since 1990 he holds a permanent position at the CNR of Naples, where he still directs the lab of Immunology. Dr De Berardinis has committed his scientific career to studying the molecular and cellular mechanisms of the immune response. Recently, he focused his interest in the attempt to translate the results of his researches into immunotherapeutic strategies. He was entitled as PI of international research grants (EU, NIH, CNPq); has performed teaching activity in Italy and abroad (Università Vanvitelli; Universidade Federal do Rio de Janeiro, Brazil); has worked as associate editor of scientific journals (SJR Q1).

Gene-Rations

Roberto Defez

Abstract Today in the world there are about 10 million scientists and research workers and the number continues to increase; meaning that 90% of all scientists who ever existed are alive today. Timothy Ferris (*The Science of Liberty 2010*) links the explosion of scientific research to the development of democracy that is therefore, also linked to the right to vote. Scientific research can be viewed as one of the most sensitive thermometers of the degree of democracy in any country. Many governments have chosen to invest in higher education and scientific research; and scientists might be considered equivalent to the bricks of ancient monuments once erected by pharaohs and emperors. A scientific hypothesis is contrary to a belief, a prejudice, an economic, ideological or personal interest, and feeds on questioning, evolving and updating itself regardless of the sex, religion, or geographical origin of those who produce new verifiable published data. This is how evidence based scientific method can change public's opinion. In an era of decline of twentieth-century ideologies, scientific method is a tool that has immense idealistic potential. If on the one hand, scientific research in Italy suffers from many problems notably by being underfunded combined with a general lack of public trust; then, the "luck" given to scientists to cultivate freedom of thought and choose their research direction comes with enormous responsibility and the need to explain better the array of opportunities to grow and feed future generations, or gene-rations (These concepts have been described in Scoperta, 2018, Codice Editions, by Roberto Defez).

1 Motivations: How I Developed an Interest in Science

FROM THE BEGINNING. Who am I? This was the key question when I was 13 years old and about to enter lyceum. I was raised in a strange and wonderful family that influenced me in many ways. My practical and work-oriented father had a brilliant mind and changed work many times: mostly in construction and finance. By contrast,

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my mother Laura was more culturally oriented with many social activities and a life-long interest in reading. She was born in Milan and asked her father to offer her a trip to Rome for her 18th birthday, something quite unconventional in Italy in 1951. In Rome she met my father who was working there despite being born in Naples; and they married three years later. That explains why my older brother and myself were born in Rome. This means that we felt at home in any place in Italy, even though the barriers between different areas of the country were and are still deep and real. In addition, both my parents' families originated from elsewhere. My mother's family moved to Milan from the Adriatic coast, while my father's family was quite peculiar. My grandfather was born in Izmir, Turkey from a Jewish family who fled from Zaragoza some 5 centuries ago to avoid the Spanish Inquisition. My surname suggests that my ancestors were originally from Fez in Morocco and later immigrated to Spain. Despite living in Turkey since the late XV century, the language spoken in the family and the only language spoken by female members was Castilian (ancient Spanish). Five centuries without learning Turkish! Being raised at home by aunts, Castilian was the first language my father learned when his father escaped the fire in Izmir in 1919 to sell carpets in Naples, where he met my grand-mother, a Jewish lady born in Alba, close to Turin, before moving south to work in Naples. They settled in Naples and raised 4 children during fascism. When racial law came into force in 1938, the 4 children were expelled from school and had to study moving from house to house to visit their Jewish teacher that had been banned from both schools and universities. Even when World War II began they continued studying, and I like to think I inherited this fury against those who tried to repress my studying. In July 1943 my father's family was notified by the Order of the Prefecture to be sent to a concentration camp. They left their house early in the morning after a whole night of Allied bombing to walk down Vomero Hill to the Naples Prefecture to learn that the Prefecture had been destroyed by a bomb; and thus they could return home. I was probably born only because of a single bomb dropped on the Naples Prefecture on July 23, 1943. Two months later my father Leo and his older brother (Alberto) took part in the liberation of Naples from German Nazis: "The four days of Naples". Naples was the first big European city self-liberated from the fascist dictatorship. Soon after they voluntarily joined the resistance to liberate the whole of Italy from fascists and Nazis in April 1945. My uncle Alberto described all this in a few books and in an interview with the Steven Spielberg Foundation (https://vhaonline.usc.edu/ Search and then digit: Defez). I can assert that my deep Italian roots, as well as my repulsion to any invading army (we have today a dramatic example in Europe), my aversion to those who prevent fundamental freedoms and my belief that we are all part of the same human species are clearly derived from my family's history (Fig. 1).

There are two main lyceums in Italy: Scientific and Classic. The Classic is based on the study of Latin and Greek, which I avoided at the time, although now I'm reading classics such as the Ovidio Metamorphosis. I was in love with numbers and living organisms so I choose the Scientific Lyceum at 13 years old. It was October 1969 and the 68 age arrived in Italy a year later during fall 1969.

On December 12th internal terrorism started in Italy with a bomb that exploded in a bank in Milan to stop workers' strikes and students' demonstrations. Up to the end of

Fig. 1 Alberto Defez in his house giving the interview (https://collections.ushmm.org/search/catalog/vha45146) *Sources* CNR, via P. Castellino 111, 80131, Napoli (Italy)

the 20th century in Naples, politically motivated Italian terrorism and mafia/camorra assassinations killed an average of 100 people each year for decades. Politics was a central issue in my family, because of the war, because of the political choice (left wing) of my father's family and because school and society were faced with political debates and fights. However, I have always refused to be a political representative. I felt unable to teach anyone on how they should live and think, which road is the right one to take, or how to shape the country's future. Despite being attracted by political debate none of my friends and classmates became politicians. My feeling was that I was not ready to make strategic choices for the society, but I was ready to support someone else's decision by providing them with data, analysis and documentation. I am still doing this today and I have ended up being a consultant for many politicians of opposing parties, helping them to have a more solid scientific basis for their decisions.

At the very beginning I was fond of geology, as I love rocks and crystals, but in the summer of 1974 when I had to decide the Faculty I would attend, I chose Biology. This was a key moment in my life. I had the clear perception that as a biologist it was possible to interact with the life and the development of organisms, instead of only making observations as it would have been in Geology. At that time, I could have chosen many other professional directions that looked more promising for me such as: architecture, engineering, financial activities, etc., but I decided that I wanted to be happy while working, and not to work simply for money, being happy only during weekends and holidays. I decided to follow my passion and started to work with a standard (public) salary, despite being raised by professionals and entrepreneurs. Retrospectively thinking, I was very lucky to have the opportunity to decide my own path.

I was even luckier, because Naples became the place where modern molecular biology was first developed in Italy; and at that time one of the leading places in all of Europe. In 1962, Adriano Buzzati Traverso, the best Italian manager, endowed with a remarkable scientific strategic view, was asked to open in Naples a new research institute that took the name of International Laboratory of Genetics and Biophysics (LIGB). He was joined by brilliant researchers including a few Italians who had been working abroad, frequently as post-doctoral fellows in some Nobel laureate's molecular genetics laboratory. The group leaders were 30–40 years old and their informal style of living, the idea of being inhabitants of the world, and not of a neighborhood, the rejection of hierarchies, or authority based on a position of power were fascinating for a young biologist like myself. The LIGB institute appointed a truly international Scientific Council with half of the members from other countries. I still miss having such a diverse Scientific Council. Scientists such as Jon Beckwith or Sydney Brenner were leading the Council and group leaders had to present their research projects to that prestigious Scientific Council. In Pavia, in 1948 Adriano Buzzati Traverso was awarded the second chair of Genetics in Italy. As soon as he entered the University of Pavia he described the situation of funding, salaries, career prospects and research competitiveness and publications in Italy as "an undernourished fossil". This description is still appropriate today (Fig. 2).

Although I met some of the LIGB scientists before joining the Faculty of Biological Sciences, I was able to fully appreciate their relevance and cultural prestige only when attending their lectures at the University. Franco Guerrini, a full professor and a leader at LIGB, was one of them. He was both clever and merciless, capable of fascinating his audience, and yet would blow up at the deepest contradictions from each responder. He was a sort of midwife capable of nurturing an unlimited passion for scientific research, and, at the same time, of discouraging those who were uncertain about a scientific career: a true and strict mentor. I hope that I have been able to follow his teaching, which will be fully accomplished if I will be able to transfer my knowledge and attitudes to the next generation. On February 15, 1978 I joined a laboratory at LIBG where I was going to work on my Ph.D. thesis and 29 days later Italian terrorists kidnapped the leader of Italy's largest political party. He was killed few months later in May and the basis of democracy then wavered.

2 Work Done: My Personal Scientific Approach

NO AGREEMENT TODAY, NO AGREEMENT TOMORROW. After less than three months in the laboratory I understood that scientific research was my calling. I was working on microbial genetics and after just two months I decided to run a crazy experiment looking for the chromosomal position of a new *E. coli* gene that I had identified by mutagenesis. Since I had selected the precious mutation in a bacterial strain unable to mate with other strains, everybody advised me to try to re-isolate the mutant in a strain capable of conjugation, but I chose another approach. My hypothesis was that the mutation was close to at least one of the 50 known selectable

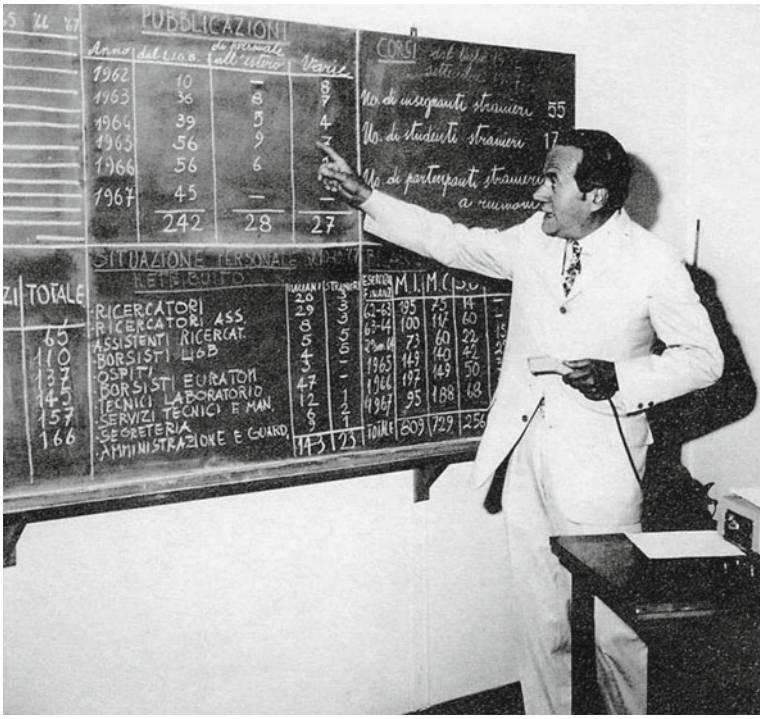

Fig. 2 Adriano Buzzati Traverso at IGB in 1967 (Credit photo to https://www.fondazioneadrianobuzzatitraverso.it/)

genes spread along the chromosome and, assisted by a good amount of luck, I was able to identify the new mutation by co-transduction. I performed that risky co-transduction experiment during the Easter holidays in 1978. This illustrates how I've always placed my research work before any other interest. Coffee, and ice cream in summer, often were my lunch. One year after that I found a fellowship looking for a candidate who was willing to join a research laboratory in France focused on plant-bacteria interactions. I had not yet obtained my Ph.D., could not speak French, and had no experience in this specific research area, but I was ready to leave attracted by this new research experience that, by the way, provided my very first salary. I spent 4 months in Versailles living and working alone for the first time, without knowing, at that time, that plant bacteria interactions would become my main research focus for the next 35 years.

Living in Versailles, fully dedicated to my work, was the best way to understand if scientific research was indeed what I was willing to do for the rest of my life, and it soon became clear to me that I wasn't interested in any other kind of work. I went back to LIGB in the spring of 1980 and then graduated on December 20th at the University of Naples. On that very day we were less than ten graduating students due to a city that was still suffering the consequences of a terrible earthquake that had

Fig. 3 My wife Maria Ciaramella (1958–2018) at the marriage, celebrated in Paris in 2014, of my close friends and scientists at Pasteur Institute: Gordon Langsley and Caroline Demangel

occurred 27 days earlier, causing hundreds of victims and endangering the stability of many buildings including my faculty's. One of the ten students was a very clever lady called Maria Ciaramella whom I married 12 years later (Fig. 3).

Once graduated, I received many offers to work in different laboratories in Italy, but with no or low salary. I was certainly eager to work but I also wanted to be economically independent, and so I decided to leave Italy. My university mentor, Pablo Amati, a lambda specialist who joined LIBG after a post-doc position spent in Matthew Meselson's (a Nobel laureate) laboratory, suggested that I apply for a post-doc position at the Pasteur Institute in Paris, where his phage lambda connections were still very strong. I received a fellowship to join Philippe Brachet's laboratory starting the summer of 1981. The research project was quite distant from my expertise, but I was ready to learn new subjects. That implied not only a lot of reading, but also a strong attitude and dedication to the experimental work. I stayed at the Pasteur Institute three and a half years working very hard, publishing few papers, but each of good quality. This makes me proud even now. In 1981 my first paper reported the results of the Easter 1978 crazy experiment. It was published in Genetics a highly respected although somewhat traditional journal, where Amati and Meselson also published their research. It was my way to acknowledge Amati's mentorship.

> While there, I was also involved in a new project dealing with the effect of the Nerve Growth Factor, produced by neuronal cells, on co-cultivated muscle cells, during their development. This work eventually resulted in the publication of a paper, of which I am very proud, describing how NGF releasing cells are able to establish a cholinergic synapse to maturate. https://doi.org/10.1016/0736-5748(86)90041-9.

To summarize this period of my career as a take-home message for young scientists, I spent my formative years in two leading institutions LIGB in Naples and the Pasteur Institute in Paris. Both nourished me with new ideas supporting my professional growth with active research groups and intense scientific discussion with group leaders who were not afraid of exploiting and developing novel techniques and challenging pre-existing theories. The rapid turnover of young students and international post-docs was also stimulating and instructive. Once more, the work prevailed on personal life and frequently I had to rush the last experiment in order to take the very last metro back home in Paris. That meant leaving the laboratory around midnight and being happy to find still open those small food shops in Montmartre that were selling hot French fries. Despite a working day that never lasted less than 12 hours, the friendships built in those years were and are still deep and intense. I still spend

my holidays with friends I made 40-years ago in Paris or at LIGB. These are friends and colleagues to whom I reached out to in desperate hope for useful information on anti-cancer immunotherapy for my wife's terminal tumor. An excellent scientific environment is an inexhaustible source of meeting new people and making friends. It can expose you to different ways of life, expertise and opinions.

Normal life and friends in science are the two faces of the same medal. When I was in Paris (1981–84), many events happened. One was the war between Great Britain and Argentina for the claim of the Falkland/Malvinas Islands. My apartment was a little bit larger than a normal "studio" in Paris and I love cooking. Many of my closest friends and colleagues were English and Argentines. Their different views resulted in heated discussions that often took place there, while eating pasta with Neapolitan ragù or a black spaghetti with cuttlefish ink. Cooking and preparing coffee have always been my way of meeting people, discussing and making new friends, while at the same time I came to understand that not all people interested me.

Stimulating scientific environment and open discussion and relationships experienced in those ten years of my training has been a gift for the rest of my life. A meaningful example of the stimulating environment that was absorbable at the Pasteur Institute at those times is that the only other light kept on very late at night was illuminating the laboratory in front of mine, where colleagues were hard working in the effort of isolating the HIV virus.

Also, the experimental group closest to my lab, was run by a microbiologist who had taken over Jacques Monod's old lab. Even François Jacob was still present and active at seminars and conferences. Together with Jean-Pierre Changeux, they routinely gave lectures on the philosophy of science at the College de France. At that time, spurred by the molecular biology revolution, the two addressed several issues like those of evolution, looking for some different interpretation of Lamarkian theory that could somehow be related to the complexity of neurobiology and immunology. The question was: why is the number of antibodies or synapses, far higher than the total number of genes that might code for that character in any living organism? Thus, science was discussed and embraced for its contribution to a deeper knowledge toward a new awareness and cultural progress, not just for its technical applications or for simple paper publications. They professed a science that is capable of supporting progress while reducing the space for religion and unsubstantiated fears, and a science that supports the scientific method of Galileo as an instrument to establish the basis of a discussion or of a political decision. This was a lesson that was to become the foundation for the public and social challenges I have faced as a scientist standing on the side of the non-negotiable rules of scientific method based on documented evidence, published and commented on by the rest of the scientific community; based on the reproducibility of experiments; on the transparency of data removed by any conflicts of interest: a scientific method that can be left unheard or marginalized, but on which no mediation, bartering, or negotiation can happen.

3 Science Today and Tomorrow

ANOTHER BRICK IN THE WALL. After almost 4 years in France, I wanted to return to Italy where I was offered the opportunity of a tenured staff position as a CNR researcher. Evaluating the potential of the place and the country, I chose to join a group that was doing research in the field of soil bacterial nitrogen-fixing symbiosis with leguminous plants: the same subject I was working on in Versailles when I was a student. I had the feeling that continuing with studies that I had performed at the Pasteur Institute on cell biology, NGF and neurobiology it would have turned out in a big failure because of the uncertainty of the funding, the disorganization of the facilities and the chronic delays caused by an overwhelming, penalizing bureaucracy. I have never regretted this specific choice: however, it might have been better to have done a second post-doc in the United States. At CNR, I got a tenured staff position within a group led by a senior scientist who was burdened with daily administrative responsibilities. This facilitated me becoming the leader of a quite large experimental group, working on the front line with the aim of contributing new scientific advancements despite daily endemic and structural difficulties.

In 1996, after few years of employment, a grant in Biotechnology disclosed new strategies, collaborations and opportunities. The work was to test the effects on biological nitrogen fixation of genes involved in regulation of different phytohormone biosynthesis by expressing each of them in the bacterial strain I had used to infect legumes. The strain was capable of fixing atmospheric nitrogen and convert it into ammonium or glutamic acid, and the assay was conducted with a gas chromatograph that measured how much ethylene (a double bond molecule) was produced by the bacteria colonizing the roots of the legume once they were provided with acetylene (a triple bond molecule, like di-nitrogen). Our hypothesis was that some hormone could enhance the gas triple bond breaking process by initiating the conversion to a reduced state. We found that promoting the expression of the main auxin (IAA, indole-3 acetic acid) had a remarkable effect on upregulating nitrogen fixation. A peak twice as high as any other construct harboring genes leading to the production of other phytohormones was repeatedly recorded. Moreover, plants looked much healthier. The plants were taller and the root nodules where the bacteria had settled on the roots were two or three times larger. Finally, the plant had more pods and seeds. That project is still ongoing and will have a long life, surpassing even my retirement. At that time professional photographers were used to produce the images to be published and I sent the plants by courier to my photographer without telling him which were the plants transfected with the two genes for the biosynthesis of auxins and those infected by the wild strain of the nitrogen fixing bacterium. The effect was so striking that even the photographer was able to tell them apart. Auxins were known to be involved in many aspects of plant growth and development such as root development and cell elongation, but nobody had considered the effects on nitrogen fixation. Recently, we have even shown a direct effect of IAA in binding DNA, altering its super-helical structure and preventing the relaxation of the negative supercoiling.

Improving the efficiency of nitrogen fixation with the view of transferring this possibility to plants other than legumes seemed to us a great breakthrough. Thus, before publishing the article, we decided to patent the method (from my side, by having my research institution as patent holder). It was 1998, and the feeling in the society toward biotechnological applications was frequently distrust. The Mad Cow outbreak (although opposite to the use of biotechnologies) diffused fears about the possible scientific applications to food or feed production. Unfortunately, it was the wrong time. In fact, it was in 1999 that the Seattle G7 international conference took place and biotechnology came out as a new form of pollution on the planet, responsible of all sort of disasters like environmental destruction, risk to human health, threat to traditions, cuisine, ecosystem and many other possible political or social damage. In that frame, scientists had become irresponsible people who by playing God, wanted to change Nature (with a capital N), also by producing Frankenstein's food. From that moment, a misguided and deceitful environmental lobby started to show researchers working on plant improvement with gas suits and masks, as if the plants were producing radioactive compounds. In line, the fruits would be represented pierced by a syringe to communicate that the public had been duped, and the scientists involved insulted as being hired by multinational companies that were poisoning the planet (Fig. 4).

With the other two colleagues who had conducted the experiment to increase nitrogen fixation, we decided to react when, in July 2000, a Green Minister of Agriculture (a striking contradiction given that cultivating is the opposite of leaving ecosystems intact) decided that it was forbidden to do research if biotechnologies were used. Even worse, it became necessary to assign to any resulting product the infamous acronym GMOs, conceived to demonize the technology. At first, our reaction was to write, in November 2000, a short appeal to protest against the choice

Fig. 4 Fake images of GMOs that never existed made to scare people before they can reason

of arresting biotechnological-oriented scientific research. That was the start for an enthusiastic endorsement that came from the most different kind of scientist, doctors, physicists, pharmacologists, etc.; and was somewhat unexpected. It was clear that the scientific community was exhausted by continuous financial deprivation and lack of consideration. A small group of journalists and professors who were experts in communication between science and society helped us to publish the appeal on the inside pages of the Sunday cultural insert of an economics newspaper. It was like getting off a cart pulled by an elderly horse and driving a formula 1 racing car. The issue of the marginalization of the scientific research was an ancient workhorse of the country's political left-wing parties, but this time the scientists (largely left-wing) protested against the obscurantist choices of a left-wing government, and the right-wing took advantage of the opportunity. On February 13, 2001 the library of the Chamber of Deputies offered to bring together the Italian scientists who had protested against the choices of the Government. I ended up being the first author of the appeal, also signed by two Italian Nobel laureates. I also coordinated the mailing list of the 1500 Italian scientists who subscribed to the appeal.

The media impact was devastating, not only for politics, but also for ourselves. All the national newspapers had the story as the first news of the day and the main news broadcasts opened the evening editions reporting about the "uprising" of the scientists. Later I would have learned that the press is always hungry for absolute novelties and that our fate had been decided that that day there was no other appeals or more tragic news to broadcast.

Three months later they would vote in the country's political elections and it was already known that the center-right would triumph: our demonstration went fully into the electoral campaign. This is why on that very day (February 13th 2001) delegations of scientists after the session at the Chamber of Italian Parliament, were invited to meetings with the candidate for prime minister of the center-left, of the center-right (who would really later win the elections and become the new Prime Minister), and also with the prime minister in office. Skeptical of these improvised and ephemeral meetings, only useful at giving news of the meeting to the press, I decided not to participate in those institutional meetings. So, instead, after the end of the meeting at the Chamber, we went for lunch with scientific journalists and with the professor of history of medicine with whom we had shared the main choices of appeal. The venue for lunch could not be more symbolic: Piazza Campo di Fiori in Rome, under the statue celebrating the martyrdom of Giordano Bruno, an indomitable heretic (Fig. 5).

The incredible media success was ironically followed by a systematic restriction to carry out research and applications in the field of GMO technologies, supported by a series of laws, shared indiscriminately by all political parties, which turn down the scientific research in the field and caused the lockout of several faculties of biotechnology. This occurred despite the evidence of data saying that many GMO plants are more productive, use less agrochemicals, prevent emissions of greenhouse gasses and are safer that unmodified plants for human consumption and the environment. What was at stake was infinitely more important than the simple application, study or experimentation of GMOs in the field. The question was that of the credibility, transparency and honesty of individual scientists and of the scientific community

Fig. 5 The placemat of the restaurant in Campo dei Fiori in Rome

as a whole. It was a question of fighting against all institutions, including those that provide research funds. This meant that by going against politics we were also penalized for obtaining research funds.

It was a matter of fighting against all the Italian ministries and against all the international misleading environmental organizations that have continued for years to spread lies and fears worthy of the worst medieval obscurantism. The choice I made was to play this game on David's side against a ferocious and invincible Goliath, by intervening in every location and place to speak against falsehoods, contradictions, myopia and the interests of companies and individuals to blame the GMO technology. In those occasions, I repeated several times speaking about my activities, that I never modified plants and I never selected a GM plant (only bacteria), but I realized that the matter was always dragged far beyond my personal activity and interest.

Nevertheless, I stood by my decision of devoting an increasingly important part of my work to science communication, despite all the personal and professional damage that this choice entailed. Up to now I have had about 40 speeches/participations a year, over the last twenty years, on issues relating to innovations in agriculture with contributions ranging from the writing of articles for newspapers to participation in national and regional television broadcasts, from conferences for schools to meetings for science exhibitions and festivals, including eight Italian parliament audits in 15 years, all on strictly scientific issues concerning GMOs, genome editing, or organic and biodynamic agriculture. Also, I have participated in Public Hearings including media emergencies (as well as agronomic) such as the death of 11 million olive trees in Puglia due to the arrival of the quarantined pathogen *Xylella fastidiosa*, or the media war against glyphosate herbicides that hid enormous commercial interests of various players of both agricultural or agri-food chemistry. The war with which Russia is bleeding Ukraine has brought agricultural issues back to the center of the media discussion for imports to the West of wheat, corn, soybeans, sunflower

oil and above all for the banning of Russian and Belarusian fertilizers. Themes that are perfectly in line with the experiments we conduct daily in the laboratory, where we now isolate endophytic bacteria from African wild cereals that provide nitrogen and protect various types of cereals from drought. This is the way the future should be envisioned and planned. **Projects that combine production of food in the context of climate change with the reduction of synthetic fertilizers and the adaptation of plants to changing environmental conditions.** I cannot give any advice to younger scientists as my road has been a highly risky one and some colleagues have been the subjects of personal attacks, physical intimidation to their private residences. Ecoterrorists have also planted bombs inside research institutes. When I hold seminars in some cities I have to notify the local police in order to be protected. Everyone has to deal with these risks and it is not certain that everyone has to act in the same way and expose themselves in person; but at the same time every young scientist has to deal with logic, experimental facts and his personal moral principles. And I have no teachings to give to anyone, but I can listen to the suggestions from others who have faced similar risks and challenges.

4 Advice to the New Generation of Scientists

BROTHERS IN ARMS. To summarize the whole matter I have just discussed, it seems that we have lost the war to support the study and use of GMOs, but at least our honor is safe. In fact, the European Commission offered to each country the ability to prohibit cultivation of GMOs at a national level with the Directive 412/2015 (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32015L0412) for reasons that are different from risks for health or the environment. EU thus admits that, despite there is no risk for the environment or health that can justify the denial to cultivate GMOs, social and/or psychological concerns overcome scientific evidences. As of today, 19 EU countries prohibit GMO cultivation for different reasons that go from social objections to landscape unfitness: whatever this means. At the same time, Europe imports and consumes over 30 million tons of GM soybeans per year as food and feed. This soy feeds the entire European livestock and therefore almost all cows, pigs, chickens and even farmed fish, as well as meat, ham, cured meats, milk, yogurt and cheeses, come from animals fed with GMO feed that cannot be grown in Europe. Included the best known, appreciated and exported Italian food products (DOP and IGP). Also, almost all the cotton we wear or handle in contact with our bloodstream, is GM: clear proof that no cotton patch has ever caused allergies or poisoning. Significant is the fact that these improved plants reduce the use of insecticides, promoting environmental benefits, and do not cause damage to health. To further refute the rampant anti-GMO misinformation I opened a blog in 2007 (now closed and transferred to Meta) where data, documents, interviews, documents and scientific articles were made available. Although many journalists have perfectly understood that the anti-GMO message is not based on facts, evidence and scientific articles they cannot fight this battle in place of the scientific community. I named my

blog "salmon", because it swims upstream against the current. The choice of name came years before GM salmon appeared on the market and was used only to highlight the fish's determination to pursue its goal, that is mating and spawning despite all the dangers it would face. The editorial I wrote in 2007 for the www.salmone.org proved years later to be a good omen for the appearance of the new technology of Genome Editing (Fig. 6).

> The "salmon" is a fragile and defenseless animal, but determined as few others. In the most important moment of its life, when it swims up rivers it's exposed to the aggression of hungry and merciless predators who without risking anything try to kill it, while it stubbornly swims upstream to reach its goal. The few who reach the goal will also die, but they do it to give birth to a new generation. The national scientific community, some cultural associations and a network of agricultural entrepreneurs who live off the fruit of their land, gathered under the acronym SAGRI (SAlute, AGRicoltura, RIcerca or Health, Agriculture and Research), must go up against the river of current thinking on GMOs. This in spite of all kinds of predators: almost the entire political class up to the top of Confindustria (the organization of Italian enterprise), large agricultural organizations, large-scale distribution and the environmentalist galaxy singing its catastrophic litanies to the inattentive media. There is objectively no hope of getting through unscathed, but at the same time it's not possible to hand over to our children a country that is so ignorant, so unscientific that it will once again make the fruit of myopic and provincial choices fall on their shoulders.

In my vision, as a scientist who has essentially lost his battle for the acceptance of the old transgenes technology, the CRISPR/Cas9-based method represents the way the new generation of scientist can embrace the challenges and move forward. The GMO burdensome past cannot cut the wings of the new generation's approach, effectively based on genome editing. Even so, my younger colleagues should always be aware that the lethargy caused by such a long lasting prejudicial bias on GMO will still continue for years, no matter if they are safe and respectful of the environment. A misleading "green" approach and the prejudice that scientists are "playing God"

Fig. 6 A bear biting a salmon

makes it even harder to go upstream. A young scientist that wishes to use scientific evidence in the public arena should be aware that she/he might be subject to public mockery (as recently myself http://extranet.greens-efa.eu/public/media/file/1/7922 to which I replied on the n.7 issue 2022 of https://scienceandethics.fondazioneveronesi.it/) and still be solid and confident in the scientific approach.

While waiting for the climate to change, those who edit mutations indistinguishable from spontaneous ones cannot be forced to fight the battle that my generation has valiantly lost. The legacy that I can leave is that it's useless to fight for a technology, for an additional publication, or for a better academic position if there is not an ideal goal to be reached. Pyramids are nothing more than a mass of bricks or stones without the project that holds together the foundations of mathematics, astronomy and engineering technology. I prefer to be a brick of a fascinating and evocative monument. But I have no moral suggestions to give to anyone and everyone has to look within himself/herself for the goal he/she wishes to achieve. Challenges are not always meant to be won. In my case the battle was fought to support the credibility of the entire scientific community. Working on food causes alarm in people instinctively and emotionally. GMOs have spurred these fears deep in people's minds, even though they are even healthier and safer than the plants from which they derive. Perhaps one of the keys to recognize the origins of these ancestral fears can be found in Ovid's Metamorphoses and in that mixing of the bodies of men with animals and of women with plants or rivers: as if some metamorphoses are legitimate while others are not.

Calestous Juma in his illuminating book: Innovations and Its Enemies. Why People Resist New Technologies (https://oxford.universitypressscholarship.com/view/10.1093/acprof:oso/9780190467036.001.0001/acprof-9780190467036), notes that Hephaestus has two unique characteristics: he is the only god of Greek mythology to be physically disabled, and the only god scientist capable of making unparalleled technological innovations. Hephaestus is conceived by Hera as a revenge for the betrayals of Zeus, but born ugly is thrown by his mother down from Olympus, thus becoming lame. Thanks to his workshops in the bowels of Mount Olympus (or Etna or any volcano) Achilles' armor and shield, the belt of Aphrodite, the bow and arrows of Eros, the chariot of Helios (Apollo), Pandora's box, in addition to the helmet and sandals by Ermes, are all made. Technological innovations bring envy and distrust. Just think of the feminine counterpart of Vulcan that is the most feared and isolated goddess of all the gods of mythology: the Goddess of Agriculture Demeter. I published the following text on Il Corriere della sera, La Lettura, on July 7th, 2019, page 6:

> The dim of twilight illuminates the Eleusinian plain in Attica. It is September 20, 480 BC. and a cloud of angry dust rises from the fields scorched by the arid summer. But that day the men are distracted and do not pay homage to the goddess of wheat who has granted them a bountiful harvest. The cloud is the result of the wrath of the offended goddess and will hit the Persian ships crossing those waters. Having offended the goddess of wheat will cost the invading army defeat in the naval battle of Salamis and with it the Persian aims to invade Greece will fade. If the world is what we know today, if the cultures of Egyptians, Jews, Greeks and Romans have come down to us, it is above all because our culture is the culture of wheat. Wheat with its symbols, its myths, its legends, technology, genetics and biotechnology where the Egyptians excelled, capable of giving life to fermented foods. The

Six Thousand Years of Bread by EH Jacob (https://www.amazon.com/Six-Thousand-Years-Bread-History/dp/1629145149) narrates these myths and events. These six thousand years are what forged our ancestors and are the foundation of Western civilization. Before we were hunters/gatherers who lived in sparse nomadic groups unable to release the refined potentials of the mind, emotions, fears and human relationships that flourish from common life in groups, communities, villages and then cities.

Only a settled people can set up an oven to bake bread: women are probably the source of the abrupt change of direction of human evolution. They observe that by planting seeds plants are born that if you take care of them will give a new crop. And the myth of wheat merges with the myth of the earth as a pregnant mother. The seed deposited in the womb of the earth will give birth to a new life. March 25 is the feast of the Annunciation to Mary of her conception, but it is also the day when the soil that will host the wheat seeds is plowed. August 15 is the assumption of Mary and also the moment of full ripening of wheat.

Dante compares Lucifer to a mill with three mouths, where he places Judas, Brutus and Cassius. (think of a mill as something like a GMO, as it is unnatural since it harbors the natural forces of wind and water to mill).

Agronomists such as Nazareno Strampelli (the father of hybrid granaries including Cappelli wheat) showed it first and followed by Norman Borlaug (Nobel Peace Prize winner for having quadrupled the yield of wheat in developing countries): without innovation and genetic selection we will not be able to counteract the ongoing climate changes, plant diseases, or defeat the prophecies of Thomas Robert Malthus to feed ten times more people (and better) than in his time (Fig. 7). Without innovation we will only have to rely on the dust cloud of some goddess, hoping she is kind.

The ongoing climate change, the war in the heart of Europe, the desertification of vast areas of the planet and resulting migrations, the extraordinary increase in the world's population and the desire to feed more and better, the conflicts over the use of water, are all factors that today are contributing to the dramatic food crisis. Norman Borlaug stated "If you desire peace, cultivate justice, but at the same time cultivate

Fig. 7 Norman Borlaug (left) shows to the USA vice-President Wallace (center) the problems of Mexican wheat in 1944. On the right, the Mexican Minister for Agriculture, Gomez. Photo credits: the Norman Borlaug Heritage Foundation

the fields to produce more bread; otherwise, there will be no peace" https://www.nobelprize.org/prizes/peace/1970/borlaug/lecture/). Without a vision of the future we will have a little hope of preventing new wars, of feeding enough people and of developing a safer future for the next generations. Without genetics, food will be rationed for the new generations: the "gene-rations".

Roberto Defez Researcher in Molecular Microbiology at the Italian National Research Council. Defez investigates the bacteria-plant interactions to understand the mechanisms leading from microbes phytohormone (auxin) biosynthesis to the increase of nitrogen fixation, plant salt and drought tolerance, to a reduced requirements for phosphate and an increase in plant dry weight and seed production. Work started with Rhizobium-legume symbiosis and now focuses mostly on cereal (rice, wheat) growth supported by endophytes isolated from harsh conditions. A collection of wild IAA (indole-3 acetic acid) producing endophytic strains triggering an increase in nitrogen fixation has been established. Defez is active in the public debate on GMOs, genome editing and organic agriculture. He is member of the Georgofoli Academy.

A Scientist Dreams, Ambitions and Realities

Khidir W. Hilu

Abstract The path towards my professional field started in a circuitous way. By the time I decided against dental school, botany was the only option left for me to pursue on the decisive college admission day. To top it off, I landed in my current specialty of plant systematics because I failed the class in my final exam as a college junior. I took that failure as a challenge and made a successful career out of it. Since then and by choice, my research has been focusing on plant biodiversity, tackling it at different taxonomic levels from species to kingdom. Understanding the dynamics of biodiversity and its synergistic interactions with the surroundings is a fundamental effort to reveal historic life events and bring attention to potential future ones including extinction threats. The field interacts with progress in science and technology, and thus, advances come in peaks and valleys, reflecting magnitudes of innovations and stagnations. Initial assessment of biodiversity patterns was egotistically based on plants' usefulness to humans, culminating in artificial classifications. Next peaks epitomize creative use of many thoughtfully selected traits, and introductions of chemical, developmental and microscopic ones. We transitioned towards comprehensive classifications. However, the work was untestable, lacking components we treasure in science: the empirical approach, and the scientific method. Yet, we thought we knew the "true" system(s) of plant classification until the era of biotechnology and bioinformatics arrived late in the twentieth century, turning our field upside down. The outcome was shocking but by no means disappointing. This latest revolutionary peak emerged with unparalleled excitement, presenting golden but rather challenging opportunities. Pioneering this in my lab became one of my career highlights. I did not, and you should not, be left behind by shying away from being in the forefront of progress. In fact, aim to lead it.

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1 Motivations: How I Developed an Interest in Science

I grew up in and around Baghdad, Iraq, with an illiterate mother and a father who only finished fifth grade. Science was nowhere in the picture. However, they both strongly promoted education and invested the little money they earned in our education. Their wishes and investments were highly rewarded. My parents' honest effort and motivation ignited in me the desire and will to achieve. The hard work and focus started early in my life. Fortunately, I loved school, learning excited me and exams were mere welcome challenges. So, the road started quite smoothly with the home support and the innate energy and desire. Well, until graduating high school and the crucial decisions on colleges and career choices were eminent.

Ironically, botany, plant systematics and biodiversity were never on my mind, and were rather foreign to me. I did not receive advice from teachers, mentors, or my parents. Wavering about careers, I tried, without success, admission to the police academy because my father was a policeman. I applied to become a commercial pilot because I enjoyed traveling, but neither did this work out for the naïve me. So, attending college was my next option, one that luckily was supported by my family despite their low income. I applied to dental school and was accepted on the spot, but then changed my mind because I did not like the looks of blood. Next, I considered liberal education, but changed my mind considering the narrow job market. After this wavering, the college of science sounded attractive. By this time there was only one department left with a vacancy, that was Botany. Luck and a little bit of thinking for the 16-year-old boy established the foundation of my career in botany. These experiences never left me and the lack of direction I experienced built in me the strong desire to mentor students and young Ph.Ds, which I will elaborate on later.

So, what landed me in plant systematics and evolution? As a freshman, I excelled in math and the math professor of my class, who was the department head, singled me out and asked if I would want to transfer to his department at the end of my freshman year, adding "you do not belong to botany"! My reply was a yes with excitement for math. Unfortunately, or rather fortunately, he left the country before the end of the year, and I remained in botany. In my junior year, I was required to take the undergraduate plant taxonomy course. It was interesting but did not turn on enough excitement to pursue it as a career. That is until the final exam when I was unable to identify the ornamental plant, crape myrtle (*Lagerstroemia indica*), an error that ironically accounted for more than half of the course grade. So, I failed my first plant taxonomy course, which was a heart breaker because I had never failed a course before. But we had the two summer months to study and retake the exam. I did not pity myself but instead took this first career failure as a challenge. My decision was to collect, key out and learn the names of as many plants that I could access in Baghdad. Passing the test was then easy. Interestingly, I relished the summer experience that brought me in intimate contact with the plant world and exposed me to the fascinating complexity of plants and their interaction with the environment. It was the turning point in my career, and my life as well. It taught me a valuable lesson that I carried

along with me to this day. In fact, I have five crape myrtles planted in our garden as a daily reminder!

Soon after that, an opportunity knocked on my door. Our Plant Taxonomy teaching assistant was about to go overseas for his doctorate. Noticing my interest and experience in plants, he recommended me as a replacement (Teaching Assistants are full time jobs offered to students that excel in the department). Being the top graduate in my class qualified me for the position. I worked on my master's degree in plant taxonomy while teaching the course until I was offered a scholarship to pursue my doctorate. I received admission in my top choice schools, Cambridge University, England, and the University of Illinois in the United States. Surprising to people then and now, I chose the latter because I was told that you will have to take a large number of courses in the American system, a situation that appealed to me due to my love of learning and knowledge. I worked under the advisership of J.M.J deWet and Jack Harlan in the Crop Evolution Lab, both are highly renowned scientists. Finding a university job in the United States for an immigrant like me was an evident challenge. After a temporary teaching position and two postdoctoral positions, I was offered a tenure-track position at Virginia Tech in 1982 where I taught and started my research career until retirement in 2019.

2 Work Done: My Personal Scientific Approach

In 4 years as a tenure-track faculty, you have one chance to show the department your national and international scholarly status and the unquestionable success as a researcher and a teacher. One of the colleagues in the department advised me early that the department relies on three factors for promotion: research, research, and research. I listened to his advice but decided that educating young scientists via serious teaching and advising should not be overlooked. My approach to research was to (1) incorporate the most recent advances in the field, (2) take on major projects and (3) do not always follow the ongoing currents but challenge the establishments and be innovative and a leader. It was an ambitious strategy for a young scientist but a good aim even if I accomplished part of my goals. I basically searched for potentially rewarding major challenges. It was the era when bioinformatics was in its infancy and computing was done via *punch cards*, a very primitive but effective modern means then. I capitalized on this approach and became a member of the new field of Numerical Taxonomy. This field allowed me to tackle my first large-scope study: the classification of the entire grass family Poaceae, the 5th largest family of flowering plants and ecologically and economically most important one. I started this major undertaking a year after my graduation and included a graduate student. As a temporary teacher and then a full-time postdoc, I had to find the time during these two years to do the work, which I did happily. The hefty paper was completed and published the year I started my permanent job at Virginia Tech. That pioneering work and subsequent studies raised me to prominence in the field of grass systematics at an early stage of my career.

Life is full of patterns if we consciously look for them. Throughout my career as graduate student and professional scientist I searched for patterns and was thrilled with what I encountered. One delightful observation started when I was a graduate student at the University of Illinois. I always loved libraries and literature search, and the University has some of the best in the nation, so I spent considerable time in them. This was immensely rewarding in broadening my knowledge beyond plant taxonomy. It led me to one particularly fascinating finding that turned into a major achievement. Often plant taxonomists and geneticists cross, for various purposes, plants of distinctly different features and recover progeny with characters that have simple Mendelian distribution of 3:1 or 9:3:3:1. What shocked me is that researchers overlooked the enormous evolutionary meaning of such patterns. Prominent changes of major features in flower, fruit and habit which were then construed as the result of numerous cumulative mutations appeared to be controlled by one or a few major mutations. I was tremendously excited by this discovery since it goes against Darwin's gradual evolution and in concert with Stephen Gould's theory of punctuated equilibrium. When that major contribution to science (Hilu 1983) appeared, some colleagues were excited while others were skeptical. One of the skeptical scientists was the late G. Ledyard Stebbins, one of the founders of the Synthetic Theory of Evolution, who was awarded the American National Medal of Science. He was my academic "grandfather" as my two major professors were his graduate students. I invited him once to present talks at our university. He was extremely upset that I challenged their theorem of gradual evolution, but he settled with the advice that I conduct further research on the subject. Professor Stebbins became one of my mentors. Later work shows substantial support to my findings, and I was told that my publication has become a classic paper in genetics.

For the next leap in my research, I decided to take a calculated risk. New research start emerging in systematic biology and evolution where bioinformatics (computer technology and software programing) is combined with molecular biology (gene and genome mutations), another novel and powerful field. The two in concert revolutionized our field. In fact, they turned our latest understanding of plant phylogeny (genealogy) upside down. They handed us a shockingly new picture of the plant tree of life, guiding us towards a radical restructuring of plant classification. What we thought we knew and understood suddenly appeared inaccurate. However, during the excitement we initially let our guard down by relying on one gene. Realizing the pitfall, the field exploded, and a variety of genes and genomes of varied evolutionary modes were employed and critically evaluated. Trees of life were reconstructed and timescale were estimated. But questions remain regarding intrinsic biases in the molecules used. It is crucial to be mindful that these findings are mere testable hypotheses. Future technological innovations may alter our understanding of biodiversity. I hope that we will not repeat the mistake made when we assumed that we knew it all! Science evolves as biodiversity does.

This is an exciting era shedding new lights and laying out new perspectives. I, among many of us, was taken away by its power of resolution and the immensity of discoveries it offers. So, it fits the criteria I put forth for my career, innovative approaches. However, implementing this innovative approach in my research

program was a risky enterprise since molecular biology is outside my expertise for one thing, and I had a limited time to demonstrate the required level of publications for my tenure. Venturing into a new field will delay the required publications. But the temptation and desire were overwhelming. For advice and assurance, I went to our department head to discuss the issue. As a molecular biologist, he understood the power of the field and its potential prospects, so he gave me the green light to start my training and build a lab with some financial support from the department. That initiative was one of the pillars of my career. I ended up publishing the first paper on the molecular systematics of the family Poaceae.

At the start, most colleagues were using the chloroplast photosynthetic gene *rbcL* to discern phylogenetic relationships among plants. That is because of abundance of sequences in gene banks due to its importance in photosynthesis, ease of sequencing, and reliable alignments (matching) of the sequences even among distantly related species as it mutates at a relatively low rate. Other slowly mutating genes were added for the reconstruction of flowering plants phylogeny. Shockingly, those genes individually did not result in identical phylogenetic tree. So, and rightly, combining sequences from different genes became the favored approach. However, the dogma of using slowly evolving (mutating) genes dominated the field. The argument was low mutation rate produces more phylogenetic signal and low amount of misleading noise. In contrast, rapidly evolving genes were considered to provide more noise than reliable signal and should be avoided in studying deeper level plant evolution. I questioned this theorem and decided to evaluate it. That was the time when I made a move that elevated my lab and career yet to another level and positively altered the field. The department at that point has already granted me tenure and promotion, so my job was secure. I decided to join a highly respected molecular biology lab at the Australian CSIRO institute in Canberra to gain experience in molecular biology and DNA sequencing. So, with a job security and knowledge, I made my move. After extensive search, I found a rapidly evolving chloroplast gene called *matK* that had been used a couple of times in molecular phylogenetics of closely related species. With a modest data set of sequences of this single gene, I constructed a phylogeny and compared it with what was available. To my delight, the structure of the tree based on *matK* alone was as good as those based on data from combining larger numbers of sequences derived from several slowly evolving genes. This finding clearly showed that there is an overwhelming power in the rapidly evolving *matK* gene, i.e., it possesses plethora of phylogenetic signal and was not impended by noise as others assumed. What was left is to convince the botanical community of these astounding results. I published several convincing papers from my lab that critically analyzed the *matK* in grasses and beyond.

I strongly believe in collaborations among labs. I always thought that in collaborations, one plus one could add up to 10! So, I invited a group of bright graduate students from the University of Bonn, Germany, to join my lab on the *matK*/rapidly evolving genes concept. They, as well as their advisor, were excited. We gathered immense amounts of data and analyzed them in several creative bioinformatic ways, and the outcome provided an overwhelming support for the approach. Then I invited several plant systematics from various countries to contribute their data on *matK* and

co-author a major paper on flowering plant systematics based solely on *matK*. The paper was a significant contribution to the field and has been very highly cited (Hilu et al. 2003). In fact, since the publication of that paper in 1983, the GenBank showed a significant surge in number of *matK* sequences deposited and the gene became a molecular choice in plant systematics rivaling the *rbcL* gene.

The other issue that faced me as a young scientist in this pursuit is how much challenge can I handle by expanding beyond the group of plants I am comfortable with, the grass family. Moving into flowering plants meant working on the largest and most dominant group of plants on earth. I started my doctoral degree working on the origin and evolution of an African/Indian crop plant called finger millet. The ensuing expansion months after my graduation to the systematics of grasses, the fifth largest flowering plant family, was a major undertaking for me at that time of my career. To demonstrate the efficacy of *matK* in plant systematics at deeper historic levels required the expansion to the immense group of flowering plants and even to seed plants by including groups like conifers and cycads. One must be comfortable, confident, and competent to be successful in such a move. There were already several labs to deal with in the United States, Europe and beyond that were well established in the field. So, as a young scientist one must be realistic about what I handle and how much one can give to such an enormous step. After critical thinking and evaluations of my academic and personal situation, I decided to proceed. Considerable amounts and time and effort were spent in the preparation process. My approach was to collaborate rather than to compete if possible. It was a fruitful approach at the start and throughout my career. After publishing the paper on flowering plants as a whole, I tackled various subgroups in detail. The collaborative approach also resulted in the very rewarding joint project on the assembly of the Tree of Life (AToL) project. Our team represents the leading labs and scientists from the United States and overseas and focused on flowering plants with about $3 million grant from the U.S. National Science Foundation. It was a gratifying project in terms of discoveries, major publications, and graduate and undergraduate student education. This collaborative project and others exposed our lab members over the years to legends in the field and established lasting cordial relationships. Although collaborations are not always free of some shortcomings, I still strongly recommend to young scientists such interactions.

Despite the hard work, the pressure of expanding the research program and the balance required between research-teaching and mentoring, the road was full of excitement and rewards. After the success of the flowering plants projects, I decided to venture into land plants. I proposed a collaborative study with two colleagues from the Mexico National University and the University of Bonn to contrast phylogenetic trees based on rapidly evolving *matK* genes and other slowly evolving genes. We placed the trees in a time frame using fossil records to show emergence dates of various plant groups. The study again showed that *matK* sequence data alone was as effective as sequence data from the other genes combined. The latest scientific move was to combine data and effort with colleagues working on fungi to learn about the co-evolution and diversifications of these two vital parts of biodiversity since

they moved to land over 700 years ago. The outcome was quite significant and was published in the journal Nature Communications (Lutzoni et al. 2018).

Equipped with scientific tools and knowing that there is complementary expertise from colleagues around you, one can be creative and think outside-the-box. My latest joyful scientific voyage was a study of the evolution of allergenicity in the peanut crop using the crop and numerous wild species in its genus. Using peanuts allergen genes sequencing generated in my lab by graduate and undergraduate students, I collaborated with a biochemist colleague and his graduate student to assess the evolution of allergenicity in the peanut genus. The colleagues generated three-dimensional models of the allergen proteins and we mapped their evolution on the phylogenetic tree of the genus. We supplemented these results with immunoblotting using sera from peanut allergy patients to estimate allergenicity. The study pointed out the molecular changes that intensified allergenicity in peanuts and circumscribed the regions that could be modified to reduce or even eliminate allergenicity (Hilu et al. 2019). It also provided information on wild species that can be used in human therapy.

I retired in 2020 but am still active in scientific writing. Scientific activities and endeavors in many of us never cease but continue and evolve.

3 Science Today and Tomorrow

My field of research does not create breakthroughs in technology but has been actively and continuously taking advantage of them whenever they emerge. This feature has kept the field on the move, advancing it to new heights in refinement and resolution of the patterns of plant classification and evolutionary relationships. Early scientists in our field were critical thinkers. They did not shy away from highlighting pitfalls, eliminating them, and building upon what then was regarded adequate. Artificial systems such as classifying plants based on their edible usefulness to humans was replaced by yet another artificial system based on medicinal applications. Then, they correctly thought that features from the plants reproductive parts are more reliable. Unfortunately, Linnaeus who spearheaded these efforts chose one character for initial classification, generating yet another artificial system. Increasing the number of features used in classification from both reproductive and vegetative parts is an example of building upon what existed and moving us to a more natural classification system. Simple mathematical evaluation of the data increased objectivity in classification, a welcome progress. Charles Darwin's introduction of the theory of evolution helped to move the assessment of patterns of biodiversity from a snapshot picture (phenetic) to an evolutionary system (phylogenetic). Finally, the usage of molecular characters from the genomes and the analyses of these large data sets with varied kinds of software using computers and supercomputers is brought us to where we are now.

One of the major differences between the current status of the field and previous periods is that we can assess patterns of biodiversity with the scientific methods. Our approaches and outcomes can be critically evaluated statistically. The sophistication

of these approaches continues to reach new heights. Different means are available to reconstruct the tree of life and above all to test and evaluate their accuracy. We have made outstanding strides to elevate the field to new heights. With this level of sophistication in the field, it is often advisable and profitable to collaborate with other scientists to bring together the skills of experts in molecular work, computer science, morphology etc. to achieve the most reliable and sophisticated results. It also increases chances to obtain the needed funding to finance the research. Another notable achievement is that our field is actively interacting with other fields like ecology, agronomy, forestry, medicine, paleontology, etc., providing ways and means by which our efforts can be utilized.

What I have presented is just the overall picture of where our field stands currently. The future is difficult to predict but new layers of sophistication will definitely emerge, driven by technological advances and creativity. We should keep in mind that future directions are determined largely by preferences in funding policies. I believe the status of the world with pandemics impacting human health, geopolitics severing localized or global economics, acute climatic changes that impact agriculture and food supplies, and uneven population growth will continue to provide new grounds for research in science and technology. It is gratifying to see how our field has been useful in dealing with the ongoing COVID-19 pandemic. Phylogenetics was implemented in tracing the patterns of emergence of mutational variants, the geographic area of their origin, routes of distribution, and the rates at which mutations emerge. These tools are also of notable value for refining computer models that focus on the expansions of existing pandemics and the predication of future ones. Bringing such information together helps global health organizations to plan for containment, designing new drugs based on mutation patterns to combat the virus and preparations for future events.

Extreme climatic change is also another major issue facing humanity. Global warming, droughts and desertification, floodings, and pollution, among others, are playing a large role in rates of extinction and loss of biodiversity. Phylogenetics could be very useful in discerning the distribution patterns of organismal extinction. For instance, phylogenetic and systematic studies can point out which group of organisms are more vulnerable for extinction and the geographic regions that are suffering the most from species losses. Such information will help us prioritize groups in need of preservation. Evolutionary history of lineages will reveal the differential rates of extinctions in lineages and which one(s) have suffered reduction in their species number and highlights the ones that are on the way to elimination. A good number of plants and animals have potentials for providing valuable drug products or have potential for domestication as food crops, rendering the focus on them and their phylogenetically related species a priority. Some of these plants are closely related to our crops and thus may have valuable genetic components that can improve the crops.

Relevant to my field, I see expansion in collaboration between computer scientists and engineers, software developers, and systematists/phylogeneticists. The latter group of scientists have been moving towards handling large number of species and using information from whole genomes. These are mega-scale datasets and

will require an immense amounts of computing power, speed and storage. Software developers are instrumental in the success and expansion of this arena since creative computer program will be required to speed up the processing of these data with the appropriate type of analyses required. A large number of iterations will need to be implemented in order to converge on statistically reliable pictures of patterns of biodiversity. For the field to succeed and advance, funding for educational institutions is a must since they tend to be non-commercial enterprises. So, novelty and sophistication in grant proposals with compelling reasons highlighting the uniqueness and urgency for doing the study are crucial for funding success. Availability of state-of-the-art computer hardware is essential for processing such studies. Computational time can be accessed from local or national supercomputers, but funds to establish such computers is again a major issue. I was involved in a grant proposal focusing on the establishment of a new supercomputer at my university, Virginia Tech, which we nicknamed the HokieSpeed Supercomputer. To support the need for such a supercomputer, we needed to gather letters from leading scientists in my field indicating the urgent need for such a supercomputer and the strong desire of the scientists to use it in their research. We succeeded in obtaining funding and the HokieSpeed supercomputer served our purpose and those of other colleagues. However, speedy advancement in computer science and engineering required the establishment of more advanced versions. So, progress in the ways to study biodiversity and the advancement in computer hardware and software go hand in hand, pushing each other to new levels. Such association exist in many other academic and industrial fields, and it represents the future directions.

I would like at the end of this section to remind us to look back at the status of the field of systematic biology and evolution hundreds of years ago, where we were then and where we are now, and the potential new directions it may be taking. The strides made are immense and speak loudly of human ingenuity, scientists' endeavors, and the innate desires to explore and succeed.

4 Advice to the New Generation of Scientists

Experience is a treasure that, if we learn from it, can enhance our chances of success regardless of our professions. Experiences could arise from personal achievements or mistakes committed via choices made unintentionally or consciously. They could also come from learning about other's fortunate and/or misfortunate actions. They are resources that we can freely dip in when made available. I would like to share the endeavors of my long academic road from the immature decisions I had to make while applying to undergraduate school to where I am now, an accomplished scientist with over 120 publications and 49 h-factor. I believe that young scientists can benefit from my experiences. I will divide this section into subsections:

Mentoring: It was a blend of luck and some sensible thoughts that anchored me at the start of a road and led me to where I am now. However, I wish I had a mentor at the very start that would have helped me make educated decisions and saved me

from potential unfavorable or disastrous choices. Seeking a mentor in your career at all points is quite desirable. Do not look at it as a weakness but as a strength of character. I was fortunate to have one while working on my master's degree and three since the start of my doctoral years. I owe them a lot. Both, the lack of mentors early in my career and the presence of astute ones afterwards motivated me to do a great deal of mentoring as a faculty and researcher. I do not regret the time spent on it. I made concerted effort to encourage students from their freshman college year on (as well as some high school students) to join my lab. We have shortages in the number of women in science as well as students from diverse backgrounds so my lab was quite supportive of minorities and women in science. I believe joining a research lab provides young people with (1) research experience, (2) enhance their focus and career directions, (3) a place where they can associate with serious graduate students, lab technicians and postdoctoral fellows, and (4) a professor that can give them an invaluable, solid letter of recommendation that speaks of their talent from concrete personal interactions not just observation from attending a class (Fig. 1).

I offered undergraduates a choice of the lab projects that suited their interests, paired them up with a graduate student/postdoc involved in that project and acted as the ultimate mentor. We had the tradition of conducting a weekly journal club. Lab members, regardless of their academic status took turns selecting a scientific article and lead the discussion each week. At the end of the meeting each member presents a statement regarding the successes and failures they encountered in their research during that week. This was carried out in an informal and friendly atmosphere which encouraged comments and suggestions from other members. Graduate students took this as a teaching opportunity and the undergraduates used it as a

Fig. 1 Diversity in my lab

learning and confidence-building chance. Although I spend a fair amount of time writing papers and grant proposals to support the research, I took the time daily to informally interact with the students discussing their progress. This nurturing was quite rewarding for the graduate and undergraduate students. Their success and the letters they send me after graduation speak highly of the time they spent in my lab. They considered it as their best academic experience and that they could have not been where they are had it not for that experience. This is my reward knowing that I made a difference in young people's lives.

Mentoring on an international scope: Some of us are fortunate to be working at institutions where state-of-the-art-technology is available in terms of lab equipment and supplies, computer hardware and software and technical assistance, as well as funding to support our research. But there are places in the world that are not so fortunate. I made a concerted effort to invite, mentor and support international scientists from those institutions. I helped Egerton University, Kenya, establish one of the first basic molecular biology labs to study genetic diversity of their native crop finger millet and trained their scientists in my lab and at their institution. Similar efforts were made for helping one of the laboratories in Morocco. I mentored and trained colleagues from Senegal, and doctoral students from Egypt, Jordan, Iraq, Indonesia, and South Africa. I sought funds from national and international organizations as well as from our university to support such efforts. In these efforts, we establish a foundation that hopefully will expand and start impacting others.

Mentoring while teaching: Although class teaching is often regarded as an effort to present information and knowledge to students, I tried to use it as a mean of mentoring too. The classroom is a place where you can interact with a large number of young people. To make use of this golden opportunity, I arrive as early and leave as late as I am allowed for the room. I walk around and take turns visiting with the students, asking them about their majors, their career interest, the preparations they have made for it. I offered advice about how they increase their chances of success. Proposing labs that can accept them to do research was welcomed; many students did not know that such thing existed. I highlight the future trends in the field and underscore the importance of some of the trends. As a young faculty, you will find this also an opportunity to recruit some excellent students to help you in your research program while they advance their own education and career. I welcome students who want further information to meet me in my office when I have the time or have them meet with graduate students. We do not always have the time to go the extra mile but if we do, it can make a difference.

Scientific Meetings: Attending scientific meetings are foundation builders of knowledge and careers. My doctorate major advisor did not like attending them, so I traveled with other faculty. I shadowed them to be introduced to the leaders in the field and ventured on my own to meet people and attend talks. My questions went beyond their research to their professional experiences. I listened carefully and learned. It did not bother or discourage me if some were in a rush to move on to another activity or if they did not remember me the next time I talked to them. Such effort could pay dividends when the people you met are on committees that decide your grant proposal or maybe even a job you applied to after graduation. I attended

a lot of presentations and paid attention not only to the scientific component but also to the method of presentation and the handling of questions. Attending was a notable excitement for me and the whole experience was enriching and rewarding. As a faculty, I strongly encouraged my graduate and undergraduate students to attend scientific meetings and helped them to cover the expenses whenever I could. At first, they needed my assistance in introducing them to my colleagues but then I noticed confidence building in them manifested by their independence. They started connecting names on publications they read with people they met and interacted with, deepening their interest. I encouraged them to contribute presentations or posters in meetings and worked very closely with them on the material to be presented, the proper organization and the effective way to present a talk. They were required to present it at least once in front of the lab team prior to the meeting. It is easier to start their presentations in a department seminar, followed by smaller regional meetings then moving to national and international meeting. This way you minimize the shock and pressure of starting at the top of the pyramid.

Scientific publications: Requiring graduate students to write at least one chapter of their dissertation as a scientific paper and submit it for publication prior to graduation is quite important. In fact, I required them to write each chapter first in the form of a paper ready for publication and then format it to fit the university dissertation requirements. This approach has multiple benefits. The students take advantage of the faculty experience in publication writing, which is an intricate undertaking. I recall the disappointment when my major advisor returned the first draft of one of my dissertation chapters when I formatted it into a publication version. It had more red pen corrections than the black ink. I thought I had written an excellent manuscript, so the revision was painful, but the experience was invaluable. So, I learned that talking to the students about the positive aspects of the revision first before handing them the revisions help built confidence and increased the quality of the second draft. Another benefit for writing up and submitting your dissertation chapters during your graduate program is that we normally become quite involved with the high demands of the new job regardless if it is academic or commercial enterprise. In academia, these involvements include establishing a new lab, preparations for teaching new courses, writing grant proposal to support your research, and the time-consuming departmental and university committees. One more advantage to publishing your work at the graduate stage, is the edge you secure in your job applications and grant applications. It will have a notable impact on the committee members when they notice your success at this early stage of your career.

Be very critical of what you publish in terms of accuracy and quality. It is not the number of publications alone that count but the impact of them on the scientific community that matters. I strongly emphasize accuracy in what you publish. Check and check and double check your experiments, scrutinize your data, and repeat the experiments. Should you obtain surprisingly good results, double check the work before you jump to publish it. Erroneous published results could damage the reputation of your lab and strongly impact your credibility. Have a colleague or two read your manuscripts before submitting them for publication. You can return the favor by reading their manuscripts to make the benefits mutual.

Creativity and foresight: In our field for instance, you see someone publishing a scientific paper on the systematics of a plant group and it impresses you. So, you follow the same theme in terms of the overall approach. This work will be lacking creativity. You will likely publish this research, but its impact will not be as effective as if you added creativity and ingenuity. Creativity will elevate the quality of your research and the status of your lab. Be very thoughtful about the questions you are addressing and methodological approaches that can address these questions more effectively. For example, see if geographic approach, genetic focus, or biochemical properties, etc. or combinations of them would fit your project. Be versed in a variety of techniques and tools and do not shy away from learning the old and the new and applying them separately and in combination in your research. Try to visit labs that are known for their expertise in some technologies to learn the techniques and explore possible collaboration if you see it fit or needed.

Part of our job as scientists is to observe, think, explore, and experiment. It is a wonderful profession. Patterns in life exist around us, and those of us who are acute observers and critical thinkers will spot them and start evaluating their biological meaning. Darwin is one example and his observation led to revolutionizing the field of biology. The list of creative thinkers is very long for such biologists, and you can be added to that list. Be an observant scientist and a critical thinker and do not take things at face value. One of my faults is that when I found one group of grasses to be very different in certain class of proteins, I just reported the finding. A colleague followed that report by creating a new subfamily of grasses for that grass group after reading my findings and observing other unique patterns for the group. However, in another case I used critical thinking when I observed in the literature numerous cases where genetic inheritance of traits was noted without realizing the significant patterns they display. Detecting the pattern led me to propose the single gene mutations as major factors in plant evolution and that gradual evolution is not the only means by which plant evolve as the majority of scientists believed. It explains, for instance, why we can find major differences among species in a single genus that are expected to separate or diagnose different genera or families. My proposal of using the *matK* gene and the rapidly evolving genomic regions to effectively resolve evolutionary and systematic patterns in plants also went against the grain of current thought. It was a major fight to assert this concept. But the outcome was all worth it and quite rewarding to me and to my field of science.

If you have a concept you believe in and possess at hand *substantial evidence* to stand on, come out and propose it with all the support you have gathered. That is how we move science forward and avoid stagnation. And, that is how you raise your standing to be an effective leader in your field.

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Khidir W. Hilu Born 1946 in Baghdad, Iraq. Received BS and MS degrees of Botany in 1966 and 1971, respectively, from the University of Baghdad. Awarded doctoral degree in 1976 from the University of Illinois, U.S.A. Joined Virginia Tech in 1982, became a full professor and remained until retirement in 2017. Supervised and mentored 24 graduate students, ~70 bachelor degree students, and 9 postdoctoral fellows, both US and international. Received numerous teaching and research awards. Research emphasized land plant systematics/evolution with focus on gene choices in phylogenetics, crop genetic resources and biodiversity, and gene evolution using a variety of tools including molecular and bioinformatic methods. Organized national and international symposia and involved in international research collaborations, including Fulbright, FAO and USAID. Professor Emeritus.

Uncommon Science and Common Sense: Shall the Twain Ever Meet?

Ajay Kohli

Abstract A book, a couple of friends and an unlikely relative guided me towards a career in science. Looking beyond the morpho-physiology and into the molecular factors was an early inclination. A stint in protein purification reinforced that with the science and the people around me. The initial haughtiness about being at the cutting edge of science through molecular biology was replaced with an appreciation that molecular biology could alleviate hunger, poverty, and inequality. The need to link the heady upstream basic science to the hearty downstream application became sacrosanct. An understanding was crystallized that the dissemination of the results was more important than the journals in which they were disseminated; that missing the boat on reporting novel findings does not take anything away from the value of the results. It also became clear that most excellent science is a sequential progression towards documentary evidence to illustrate common sense, and yet, excellent science also explains that which may not be easily comprehensible. In doing so over the centuries, science has caught up a speed which is difficult to keep pace with. Unless collaborative, transdisciplinary team efforts are not embedded in a pipeline approach, generating innovative products and processes is difficult. Given the funding scenarios dictated by market forces and short- to mid-term imperatives it is becoming increasingly difficult to advance the frontiers of knowledge. Young scientists must envision and clearly state the downstream practical application and timelines in their proposals. Equally, they must be effective communicators of their science to critical stakeholders in the intellectual property and market domains. Crucially, scientists must regularly expose themselves to new paradigms and not fear the unknown, for what is science if not exploring the unknown? This holds true for doing science at present when most learning becomes dated within a decade.

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1 Motivations: How I Developed an Interest in Science

The name John W. Kimball is etched in my memory. In 1980, an edition of his book 'Biology' stood on the shelves of a bookshop called E.D. Galgotia & Sons in the Connaught Place area of New Delhi in India, where I come from. The bookshop, one of the oldest shops in the area, closed its shutters in 2015 after 83 years of influencing four generations of booklovers.

Three other names are etched in my memory, two friends and a relative. They inspired my choice of a career in science. The first is GC., Dr. Sandeep Jaggi, now Director, Patent Strategy, Technology Platforms, Research, Operations and Analytics., Intel Corporation. He is my friend since we were six years old. We met in 1969 at the Oak Grove School in Mussoorie, a boarding school at 6000 ft in the foothills of the Himalayas.

The second is Sumitra Handa, my father's paternal aunt. We called her Bibi Ji, the Punjabi address for and elderly lady. A diminutive 'child-widow', she arose like a Phoenix from the ashes of her much older, sick husband. She never married again. In the 1930s, when there were few educated women in India, she studied to be a schoolteacher. She participated in the Indian Independence movement, was a fierce supporter of girl-child education, and espoused exploring traditional Indian knowledge through a lens of modern sciences.

The third name is that of Dr. Sanjeev Munshi, a protein crystallographer who went on to be the Executive Director of Business Development and licensing at Merck. We met in 1988 when he was finishing his Ph.D. at the Molecular Biophysics Unit (MBU) of the Indian Institute of Science (IISc) in Bangalore, India. He conducted postdoctoral research in Jack Johnson's lab at Purdue. Before he went there, unbeknownst to him, he cleared my mental cobwebs with his inspirational attitude to science and life and set me on the path to a career in molecular biology.

Within a day in June 1980, Kimball's book, Sandeep's friendship, and Bibi Ji's knowledge conspired to light a candle that illuminated a path. I saw Kimball's book at the Galgotia's and started flicking through it. I was studying biology as a Junior at High School then (grade 11, with a year to go to finish school). I soon realized that I was looking at, what for me then seemed like, a compendium of biology. I felt attached to it immediately, one, for the possibilities it held in answering all my questions in biology and two, for the epitome it was in the rigor involved in producing an authoritative document of such intellectual and academic insights. It wasn't the first 'doorstopper' I held in my hands, yet, marveling at the effort of putting together all the information only in this case, may have suggested an innate affinity to biology. I could not imagine letting go of the book despite the price, which in today's money would be around US$ 85. The average middle-class white-collar salary in India, depending on the location, is still between US$ 200–400 per month, not to mention confronting the situation in 1980 when the Indian economy was largely a closed economy and growing at a snail's speed. This price for the book was despite the paradigm popularized in India then by the TATA-McGraw Hill collaboration, of printing the original content on cheap paper with cheap labor to bring down the cost

of the books. Affording the original hard-cover publication was beyond imagination. Incidentally, my friend Sandeep was with me at the Galgotia bookshop to spend part of his scholarship funds earmarked for buying books. The INR 30/- he lent me then got me my first big fat book full of what I wanted to read, and I didn't have to return it within 15 days back to the library—it was mine. In a sense that day Biology became mine.

Flaunting my possession back at home, I saw Sumitra Bibi Ji, who was visiting us in Delhi, being interested in the cover image of the book—a micrograph of the sperm ready to fertilize an ovum. It remains to be analyzed if there was also a Freudian undertone in my interest for the book—to be able to display a 'sexual' image on my table in the form of a credible scientific book authored by a Harvard academician. We are of course talking of a society where till a couple of decades back young teenagers couldn't smoke or drink alcohol in front of their elders; let alone discussing sex-education. How those prudish societal pressures play out in life is an independent subject of research and deliberation. The 'out-of-respect' practice of not smoking in front of the elders still persists in pockets and families where the hegemony of internet is not pervasive.

Bibi Ji asked me what the image was about. At that point, to my ignorant self, she was no more than a sweet, elderly lady in the family who should not see me smoking anything or drinking alcohol, let alone engage in a discussion on reproductive biology. I am now ashamed to remember that to avoid the topic I brushed her off by saying that she would not understand. Lo and behold, for the next one hour, right there and then, in front of my mother, Bibi Ji gave me a full lecture on reproductive biology and especially on how the traditional Indian concepts of engaging in reproductive behavior take the act into spiritual realms, lifting them from banal intercourse. Watching my sweet little Bibi Ji morph into the teacher Ms. Sumitra Handa, brought out aspects of her personality that were completely unknown to me till that time; her being a teacher at the school in Amritsar, her overcoming the travails of a child-widow, her deep interest and knowledge in Indian culinary and ayurvedic medical practices, her sufferings in losing two of the three brothers at very young age, her sacrifices in keeping an extended family together through differences in matters of the family business of industrial furnaces, etc.

It was not so much the one hour of reproductive biology lecture but the next hour of Bibi Ji's history and future that stayed with me. It was an interest to understand people and organisms deeply. It later matured into looking at life at the molecular level beyond the morpho-physiology. I was fascinated by the permutational interplay of the simpler principles leading to the complexity of life. That amorphous seed idea kept me drifting to more basic sciences and while I studied organismal biology, my interest always lay in the molecular sciences from where to try and build an understanding of how organisms come to be and how they do what they do. What also became clear was that there would never be a compendium of biology. The nano- to macro-scale evolutionary processes, which manifest at the molecular levels and lead to differentiated organisms, will regularly add to the compendium beyond the apparent constancy of the regulatory principles.

On the same day that I potentially discovered my compendium of biology I also discovered, through an act of kindness and camaraderie of a dear friend, and through teachings from a humble and ordinarily extraordinary person, that there wouldn't ever be a compendium. What a paradox. While living with such paradoxes has always been part of human life, more apparent in the ancient and traditional cultures, this incident brought it home for me, and the paradox elevated itself to a Zen of acceptance. Within that one day I realized that the path is always in the middle where one is understanding more and understanding that one understands less. The journey had begun.

Coming to the influence of the third person in my choice of a career in science, Dr. Sanjeev Munshi at MBU, IISc. I joined the lab for an 8-month project on protein purification as part of the Master's degree program of the Indian Institute of Technology, Kharagpur (IIT-Kgp). The 1988 graduate session at IISc had started. I had been in the lab for nearly a month. Prof. MRN Murthy, our supervisor suggested the revival of the weekly seminar after the summer break, to present and discuss any latest papers of interest. Without any direct or indirect pressure to do so, Sanjeev offered to start off the seminars with a paper that he had read a day before. He made the best discussion of a paper I had witnessed till then. It was as if he knew all the top protein crystallographers of the world and had been interacting with them personally, he knew the intricacies of the tools and techniques they had used, he knew what was excellent about the paper and where there were gaps, and he also knew where that work could go in the future. How could anyone be so clued in at a time when internet was limited to a one-off email now and then? Only someone who had spent years reading and educating himself on the topic could present such an analysis of the paper. I still carry with me the inspiration from that day to achieve a stage of sustained declamation, discussion, and analysis, based on a deep and broad understanding of a subject.

Once before, a similar spark came from meeting my Botany lecturer Dr. Razza Abbas Khan of Lucknow University, in the vegetable market. He could categorize every single fruit and vegetable taxonomically, knew their center of origin, domestication history, nutritional benefits, and the combinations in which they should be eaten to ensure balanced nutrition.

The interesting point is that although Sanjeev first showcased it for me, many graduate students, and postdocs at IISc, could make excellent presentations. However, Sanjeev was one of the few who embodied a culture of intellect, diligence, rigor, perfection, and achievement with an additional flair for life, music, sports, food and endearing frivolity of sorts. A coterie of such people introduced me to the writings of the likes of Dirac, Sagan, Feynman, Dawkins, Gell-Mann, which steadily but surely altered my reading portfolio. Schrodinger's 'What is Life' and Hugh Prather's 'Notes to Myself' became my favorites.

By the time I had reached Bangalore, I had dabbled, at different stages of life, with middle school teaching (2 months), cost-accountancy (2 months), advertisement scout and article contributor for a local Hindi magazine (8 months), grocery store assistant (6 months), telemarketing (1 month) and toying with the idea of setting up energy-efficient and sustainable tissue culture lab in the mid-reaches of the Himalayas

using local natural resources for water and energy while leveraging low temperatures to contain culture contamination. Generating and selling bonsai plants was also an idea on the horizon. Indian Classical Music was always of interest through familial legacy, so getting into reviewing music performances was a rather strong urge. Since I am not blessed with the best voice and never took my interest and natural ability in playing some percussion instruments (Tabla, Bongo, Conga, Djembe etc.) to the next level, it was not an option anymore. However, none of those were as enticing after the IISc experience as pursuing an intellectual and academic career while retaining a connection with the outside world for a clear view on the impact of the work on the society. The journey had taken flight.

2 Work Done: My Personal Scientific Approach

One of my favorite Urdu language couplets is by a poet called Ameer Chand 'Bahar'. Like many Urdu poets he was a master at illustrating or capturing paradoxes in two lines, for example:

Hazār ilm kī zau se dimāġh raushan ho

Jo dil meñ ātish-e-pinhāñ nahīñ to kuchh bhī nahīñ

Much may the mind be luminous with the luster of knowledge

The heart missing an innate spark wouldn't acknowledge

The cited couplet refers to the eternal conflict between the mind and the heart, surmising, by way of one interpretation, that substantial mindfulness cannot compare to an obscure spark for a passion in the heart; that extensive knowledge in the mind may be void without gaiety in the heart. In a convoluted way it captures my take on the basic science (mind) and applied science (heart) whereby the best outcome of mechanistic understanding of a process is the application of that understanding towards a sustainable existence of the earth's biosphere, including humans and the earth itself.

It took a few years to accept that the basic upstream science should ideally have an impact on the socio-economic and/or environmental issues. During those years, a snobbish, haughty biotechnologist was tempered by plant breeders and agronomists into a humbler and more realistic agricultural molecular physiologist of sorts.

The stint at IISc was followed by a fellowship at the Genetic Engineering Unit (GEU) of the Jawaharlal Nehru University (JNU) in Delhi with Prof. HK Das. This was a time of the initial phase of exploring cereal rhizospheres for nitrogen fixing bacteria and their mega-plasmids for the *nif*-gene cluster. The vision was to transfer those mega-plasmids to the endophytes of cereals like rice and wheat. GEU-JNU became the site for lessons learned in the electron microscopy of the mega-plasmids from Nirupam Roy Choudhury. Molecular biology and gas chromatography techniques were also learned during this time.

Next, I moved to the International Centre of Genetic Engineering and Biotechnology (ICGEB), New Delhi. Rice and its insect diseases were the focus here. My own work focused on gene cloning and sequencing. The latter employed the large BioRad contraption for the ^{32}P Sanger dideoxy sequencing on a 40 cm gel (if I remember correctly). The gel was freshly prepared and poured between glass plates that had to be ultra clean and siliconized. The ACGT bands were read out on the gel manually for the sequence. Due to its large cylindrical shape and size with a revolving door of sorts, Richard Jefferson of the *gus*-gene fame once called it an orgasmatron during one of his regular visits. Genes like soybean trypsin inhibitor and chitinase were being used with a plan for rice transformation; the first commercial biolistic guns had just hit the market, predicting the end of an era for the protoplast-based rice transformation methods.

At ICGEB, fortunately again, I found a coterie of people who projected the vibe of a career in science being a daily dose of fun, inspiration, and learning. This might seem opposite to the impression generally given about science being too demanding and stressful. Not if one had people like Suresh Nair, Naveen Khanna, Raj Bhatnagar, and Sudeshna Mazumdar Leighton around. Naveen's greeting of 'How's She Doing', with full liberty to define who 'she' was, remains an enigmatic conversation opener and never fails to conjure a smile. Suresh remains someone I want to grow up to be. He epitomizes everything that a dependable man would be—paradoxically, the most accessible and inaccessible person; accessible to all for their problems, inaccessible to anyone for his problems, which he seemed never to have. Dr. John Bennett, the boss, was a beacon of inspiration who combined publishing single author Nature papers and a row of nine JBC (Journal of Biochemistry) papers, with declaiming Shakespeare in the corridors, mostly Hamlet. In the reflected glory of such personalities, their characters, and their achievements, how could I not find my favorite subject of molecular biology haloed, while it was still spreading its wings in the rest of India. Together we would save the world.

The next stint from 1993 to 1995, was at the International Rice Research Institute (IRRI) in the Philippines.

The larger-than-life Gurdev Singh Khush, World Food Prize Laureate, was leading the Department of Plant Breeding, Genetics and Biotechnology at IRRI. His rice varieties were being grown in multiple million hectares of South and Southeast Asia and had transformed the economies of countries like Korea, Taiwan and Vietnam. India was a huge beneficiary of these varieties, especially in the Punjab and Haryana regions (Damodaran 2022). Within the first few months at IRRI it dawned on me that molecular biology was a means to an end in addressing hunger, poverty, equality, equity, and sustainability. I felt I was on the right path with the realization that the intellectually stimulating part of scientific discovery and mechanistic understanding couldn't be divorced from its mandate of being in the service of humanity and the universe.

In the beginning of 1993 at IRRI I unpacked the components of the BioRad Biolistic gun and assembled it to standardize rice transformation. The first transgenic plants expressing Richard Jefferson's *gus* gene in his pCAMBIA vectors were obtained a year later in collaboration with the efforts of Ph.D. students HS Kim and

Behzad Ghareyazi. This was among the first rice transformation results in the public sector. What followed was discussed in closed circles in the corridors of conferences but not openly for want of an understanding of the causative principles. The *gus* gene was not expressing in the next generation although the gene was present. There was the nascent idea of transgene silencing doing the rounds then. John Bennett suggested that I germinate and grow the transgenic seeds on culture media containing 5-azacytidine. If GUS expressed in the germinated tissues, that would indicate reactivation of DNA cytosine methylation-mediated transgene silencing. In 1994 there were some reports on transgene reactivation by 5-azacytidine in the animal and fungal systems (Holliday 1993) but none in the plants. The germination experiment failed, and no GUS was expressed. That evening I went out with some friends to dissolve the frustration of the failure in beer and music. After two beers, the year-long experience of handling rice seeds for tissue culture came hauntingly back with an argument. For a germinating seed the primary source of the building blocks would be the seed reserves and not the externally supplemented culture media. DNA synthesis and replication would not incorporate the 5-azacytidine in such a scenario. It may then be an idea, to isolate and germinate the embryo on the supplemented media, à la embryo rescue (Jena and Khush 1984; Bridgen 1994). The minor inebriation dissipated, and I went straight back to the lab at 10 pm to start the experiment. Five days later, to my utter joy, my first critical and incisive idea had worked. The embryos and the germinating tissues were all blue with GUS expression. Professors Diana Bowles of York University and John Gray of Cambridge who visited IRRI and saw these results suggested that we should publish the work in *The* Plant Journal, which was only 2 years old then. Some last few minor bits couldn't be wrapped up because I was to leave soon for my graduate studies in the UK. The work was published in Rice Genetics in 1996 (Kohli et al. 1996). A few months later, in 1997, a similar result was published by Kumpatla et al., from Texas A&M in Plant Physiology (Kumpatla et al. 1997). From then on, rice was my research system for the next 8 years, which involved Ph.D. and postdoc at the John Innes Centre (JIC), Norwich, UK with Prof. Dr. Paul Christou, a 'never say never' man.

At JIC, in the Baulcombe lab, I saw the unfolding of the principles underpinning our demonstration of the *gus* transgene reactivation. It was an awesome experience to see the steady progress, almost on a monthly basis, in teasing out the mechanisms underpinning transgene expression and silencing. I was overawed to be sharing the coffee table at the JIC café with scientists whose work was laying the foundations of the modern plant sciences. Meanwhile, I concentrated on answering the question—what happens to the transgenes when they enter the cells and where and how do they integrate in the genome. The complex patterns of transgene integration were illustrated and related to methylation mediated silencing over the next 4 years as part of the Ph.D. Those principles were then used during the postdoc period to generate the only *indica* rice library of transposon insertion mutants (Kohli et al. 2001) in a multi-partner collaboration led by Prof. Andy Pereira, then at the Plant Science Centre of the Wageningen Agriculture University and now at the Arkansas University. Increasing the efficiency of rice transformation with the biolistic approach

using efficient selection markers (Twyman et al. 2002) was also a critical undertaking during this time.

JIC was a treasure trove of brilliant minds from across the globe although it must be said that the fun group among them was the Iberic fraternity. Some wonderful friends from that time are still in touch and still enrich and inspire me. The journey from JIC back to IRRI in September 2008 was through the University of Washington to look at the role of non-coding DNA of the human genome, laying the principles of ATAF sequencing. This was followed by 4 years at the University of Newcastle upon Tyne, to come back to molecular agriculture with rice and *Jatropha* as the research systems for food and biofuel respectively.

The work with *Jatropha* for biodiesel from the seed oil led to the discovery that most *Jatropha* accessions from around the globe were 98% genetically alike. Yet, there was a wide spectrum of phenotypic differences. We hypothesized epigenetics to underpin the visual differences. It was during this work that I came in touch with Diego Breviario whose interest in understanding the molecular basis of life and appreciating the consciousness basis of living is our common ground. Our work was reviewed at Nature Biotech and New Phytologist without any major concerns, but more proof was demanded for the epigenetic basis of differentiation. Once again, due to retracing my path back from the UK to the Philippines this time, we could not follow up on those requirements and could not publish this work in a 'reputable' journal. We thus put it out online (Popluechai 2009). However, papers in standard journals later confirmed these results (Yi et al. 2010) with additional proof.

This was the second time we missed striking it big with novel results, the first time being our work on transgene reactivation with 5-azacytidine. With the *Jatropha* work, the effort was to understand the gene expression patterns in different accessions for the phorbol ester synthesis genes in the seeds. The aim was to obtain seed oil free of phorpbol esters. The laborers harvesting the seeds manually are exposed to the latex containing phorbol esters which can cause skin cancer (Fujiki et al. 2017). To that end we succeeded (Popluechai 2009) but our effort to link it to upstream molecular biology of epigenetics was wanting. Since then, till now, my approach to science and our results is the thrust on the rigor of our science, on the utility of our results and on the fact that the results should be public in whatever way possible. Saving the laborers harvesting the *Jatropha* seed from skin cancer would be any day bigger and better than the 'big' paper that did not happen.

3 Science Today and Tomorrow

Science today is progressing at a more rapid pace than ever before, and it will only get speedier. Discoveries in human evolution, cognition, quantum computing, artificial intelligence (AI), climate change and matter (elementary particles at one end and planets, stars, and the universe at the other end) are improving our understanding of what exists and how it works. Many discoveries are not a result of targeted attempts to enhance human existence and experience. Yet rapid advancements of some, such as

the CRISPR system over the past decade, are evidence of the possibilities of quickly elevating a discovery to the benefit of humankind in medicine (Katti et al. 2022; Ledford 2020) and agriculture (Zaidi et al. 2019). The generation of the COVID-19 vaccine in a record time is another evidence for the capacity to expeditiously attend to human maladies. These examples, and others in AI, data science, communications, and graphic design, serve to improve human experience and entertainment through a direct connection with product delivery. Such delivery is, in turn, tied to monetary benefits. Under the circumstances where financial gains largely dictate the demand, discovery, deployment and dissemination of research products, the funding for research on esoteric areas such as evolution, cognition, quantum physics, rare earth chemistry, astronomy etc., is a continuous challenge. Even in areas such as plant, animal, and environmental sciences it is difficult to find funding unless a direct link is established to the sustainable development goals (SDGs).

We cannot forget that the relative swiftness in finding solutions to many natural and anthropogenic problems today flows from seminal discoveries made over the last few centuries without the explicit link to the 'market'. Science for esoteric discoveries and understandings should continue to prosper. Unfortunately, many scientists who could be the critical mass for furthering the horizons of knowledge, have lost their way in chasing publications, presentations, and fellowships. A rising number of publications do not add to knowledge and a rising number of products and patents are never utilized. This is a crime against society.

Lack of funding for the science that is not tied to the market, but funding for redundant science conspires against a sustained rate of better understanding our space, time, matter, and organic existence. Recognizing the reasons for such myopic decisions could perhaps help conjure up some solutions. The problems of political short-term expediency; lack of a systems level vision, pursuance of favorite legacy agendas, and the use of inadequate metrics that elevate mediocrity to senior scientific, and powerful political and financial positions can only beget mediocrity. Even the basic training and recruitment processes for prospective scientists needs an overhaul. Science is a mainstream interactive profession. It is rather well paid in many cases, especially if it is linked to delivering on market demands through small, medium, or large enterprises. **The 'entrepreneurial' science maintains quality controls due to its direct link to market-driven delivery processes and financial bottom lines. However, most public sector science, where there is more scope and need for esoteric science, suffers from silos, fiefdoms, fraternities, lobbying, multi-dipping and artificial inflation of quality. Thus, the quality of science has been progressively deteriorating in many public sector entities.** Simultaneously, there is a mushrooming of highly profit-driven educational and research entities with non-optimal facilities and standards. These entities churn out additional non-optimally trained graduates every year. Their technical excellence in tools of the trade is being rebranded as creative scientific endeavor. Consequently, the proportion of 'good' scientists that can creatively innovate is exponentially reducing. The limited funding in science is not being apportioned to the most deserving and creative ideas. Unfortunately, even when the scientists and their science are excellent, two aspects can drown their voice and productivity: a lack of ability to effectively communicate their science to a larger

audience, and the lack of understanding and/or interest in intellectual property and market forces. This ends up in some very good science never seeing the light of the day. There is a clear need of overhauling the training of scientists, so they appreciate these two aspects as critical to a good career through excellent science.

Putting numbers to quality defies the basic difference between quality and quantity. The metrics of papers, books, presentations, fellowships and grants have been hijacked to establish those scientists with impressive numbers as 'stars'. There is no doubt that excellent scientists will have several durable papers, and prestigious fellowships but there is no guarantee that all scientists with large numbers of papers, books, presentations, fellowships, grants, and senior positions will be excellent scientists. The quality of science must be delinked from such metrics. Yet, because the shorter path to maximized quality could be in maximized quantity; approaches such as the altmetrics route to measuring the amount of general attention that a paper has gained, irrespective of the journal impact factor, is a step in the right direction. "*Altmetrics are metrics and qualitative data that are complementary to traditional, citation-based metrics. They can include (but are not limited to) peer reviews on Faculty of 1000, citations on Wikipedia and in public policy documents, discussions on research blogs, mainstream media coverage, bookmarks on reference managers like Mendeley, and mentions on social networks such as Twitter*"[1]. Although social media can also be influenced, yet after a certain threshold, and after a certain time, it is the genuine trail blazing or the utility value of a result that attracts attention. How can we mainstream something like altmetrics, without the digital media caveats? How can we avoid the anthropocentric lens, which puts 70% of the top 100 altmetrics score garnering articles to be human health[2] related. Nevertheless, the highest altmetric score of 11,980 is for an article titled 'How Diversity Works'; an essay of sorts on the advantages of putting people from different backgrounds and intellectual capacities together (Philips 2014) something that is illustrated in the last paragraph of this semi-autobiographical essay.

A concept like the Internet of things (IoT) should be considered, which can establish the utility of a result by considering multiple factors. Perhaps one version could include, and another could exclude the lens of human health to highlight impactful reports. It could span academia (citations in journals, research thesis, altimetric score), downstream utility (downloads, patents, reference to the papers or authors in patent applications, company profiles etc.) and popular culture with broad mandates (news and views). The durability and continued interest over time would be an important metric. Accounting for scores based on the total number of authors of a paper, where in the authors' list the name appears, how many different sub-branches of a subject have been tackled by the author during his/her career, how many presentations in conferences evolved into publications; return on investment through product, process, policy or added novel knowledge; such information can establish a pattern that can be assessed for utility in hiring and research fund disbursement.

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[1] What are Altmetrics. https://www.altmetric.com/about-altmetrics/what-are-altmetrics/.

[2] Top 100 Altmetric. https://www.altmetric.com/top100/home/about/#:~:text=The%20highest%20ever%20Altmetric%20Attention,in%20Scientific%20American%20in%202014.

3.1 Uncommon Science and Common Sense

Science has a specific definition, but common sense has a few connotations e.g., an understanding of something common to most people; a cumulative and integrative sense arising out of inputs from all the human senses; a sensitivity for other humans shared amongst all; an understanding shared by 'common' people and not necessarily by the 'elite'. The goals of science and common sense have been said to be different and yet "*scientific thought stops with obviousness understood in fundamentally the same way as in commonsense, at where things do what they do because of what they are*" (Ogborn 2006). Unfortunately, most science has reached a stage of common repetitive science. Uncommon trailblazing science denoted by excellent science is less and far between. For the matter of our discussion here the meaning of common sense that I refer to is for a sense (feeling) shared by most people about something. In this regard, is common sense of value for conducting excellent science?

Excellent science explains that which may not be easily comprehensible. However, excellent science is also a sequential progression of documentary evidence for common sense-based understanding of the various phenomena. In either case a wider audience, other than an expert group, should relate to the scientific endeavor. An example of good science could be a report on the identification and characterization of a transcription factor, which regulates the set of genes that increase the panicle number in rice. This would potentially lead to a rice variety with increased yield. However, till it results in a novel, high yielding rice variety, it has no penetration. In that context, it is common sense that an increasing population needs more food. Thus, breeding a high yielding rice variety caters to that common sense but it includes the non-mechanistic knowledge of when and how much nitrogen and water to be applied, while also starting from the knowledge of when to sow the plants so that there is a minimal likelihood of rain for 25 days after pollination. Depending on the knowledge, if the variety is a short, medium, or long-term genotype, the field preparation and sowing must happen within a particular week, three to six months before pollination. A lot of such observational, common sense-based knowledge on organic manure and date of planting is now categorically excellent science as captured by nutrient and water use efficiency, or by remote sensing for environmental and climate variables and their predictions. **Hence, common sense could be a guide to the scientific path pursued to test if 'a feeling' is correct or wrong.**

In one specific case in our lab, a QTL was identified by the molecular breeders for rice yield under drought at IRRI. The next steps were the fine mapping of the genomic region, identifying the underpinning candidate gene and validating it through transgenic plants. Both yield and drought tolerance are complex traits. It would be almost miraculous if a single gene were responsible for yield under drought. Common sense would dictate that going from point A to B through C and from Y to Z through C, at the same time, could not be done by a single vehicle. We hypothesized, based on the genes within the QTL region, that a large part of the QTL would be necessary to maintain its effect on yield under drought. Such a premise led us to look for reduction in yield in a couple of recombinants, rather than looking for maintenance of yield

in multiple events. We thus fast-tracked the proof for the need of multiple genes for the functionality of the QTL (Dixit et al. 2015; Raorane et al. 2015a, b), against the prevalent trends of fishing out a single causative gene.

3.2 Science in the Future

If we are to survive as a human race, even with the capacity "*to boldly go where no man has gone before*" (Day 2005) this "*mote of dust suspended in a sunbeam*" (Sagan 1994), the earth, must be cared for because for now, we have nowhere to go. Our obsessions to be 'successful', whatever that means, either as islands or at the expense of other islands will be our downfall. The earth and everything it supports, is an integrated biome and our consciousness must see that singularity. Schrodinger said "*consciousness is a singular, of which the plural is unknown … in the same way (as) Gaurisankar and Mt Everest turned out to be the same peak seen from different valleys* (Schrodinger 1944). Paul Davies in his recent article 'Does new physics lurk inside living matter'[3] implicates the singularity demonstrated by single-celled slime molds that come together to behave as a single organism or the ants and bees that take a decision after exchanging complex information to collectively overcome any barriers to feeding or reproduction. Life is thus seen as an additive outcome of matter and the consciousness of information gathering, processing, relaying and converting into action. The advent of the internet seemed to be a great unifying factor by using the innate attributes of information, leading to people being far more connected across space and time. The question to be solved by this and the next generations of scientists is whether the singular consciousness is real, and whether the 'perceived' chaos generated by the apparent lack of unification is part of the unification itself. The exceptional science and the common sense that 'feels' and proposes this unification, shall the twain ever meet—or is there something more to it.

We have come rather far from our hunter-gatherer days and our advancements in all sectors have most probably followed the Moore's law of lesser time taken to advance further. However, while some societies are advancing rapidly others are dragging their heels—the question to ask is how valid this discrepancy is and will it ever cease to exist, perhaps more critically, should it cease to exist. From most of our common understanding of societal norms we are given to understand that such a discrepancy should either not exist or be minimal if embedded in a cultural context of hierarchy (Steckermeier and Delhey 2019). Towards that end, for the foreseeable future, we must act as one, like the organismal raft made by the ants on water. Holding that as the guiding light, science and scientists must move towards:

(i) gathering all the data in all the domains as pieces of puzzles and
(ii) generate new data that solves the puzzle instead of generating redundant data.

[3] Does new physics lurk inside living matter? Paul Davies Citation: Physics Today 73, 8, 34 (2020). https://doi.org/10.1063/PT.3.4546.

Whether the new data to be generated is redundant or not will depend on how much of the previous data has been gathered and curated. Yet, looking at the science being funded by major donors it is not difficult to see that a lot of it is prone to repetition, mostly in the name of location specificity to the benefit of 'less advanced'. Hopefully, soon, with the exponential growth in Big Data analytics, resources will be allocated and spent more judiciously to avoid redundancy and that machine learning and artificial intelligence will reduce the time taken to get to the results by avoiding repeated human follies across the globe. Does this mean that only the data-based science has a future? No. The micro- or nano-evolutionary aspect of the physical and biological systems will ensure that 'data' always has something to catch up with. Which events will lead to a major physical or biological change may not be easy to predict.

In the short-term the survival of the present and the next couple of generations depends on recreating favorable conditions for biological systems under the climate change scenarios. The now famous pillars of sustainability—society, economy and environments must be the drivers for judicious allocation of funding for integrative, transdisciplinary research with clear sights on deliverables, whether they be new crop varieties, farming systems, medical interventions, physico-chemical advancements, environmental ameliorations, financial instruments, or societal equity.

The integrated transdisciplinary approach of **D**emand assessment, gene **D**iscovery, product **D**evelopment, product **D**eployment & dissemination and finally policy and impact **D**istinction makes up the virtuous 5D research circle that characterizes the IRRI research pipeline (Verma et al. 2021) through a systems biology approach (Pabuayon et al. 2020). Not all entities can include all the 5Ds, which makes a strong case for dependable partnerships. None of this is possible without the passionate staff at all levels internally, and without the support of collaborating partners externally. Hence transdisciplinary capacity and partnership development are core to success (Hellin et al. 2020).

When looked at through such a lens the resources spent on upstream science will not have value without feeding into a product pipeline in applied science research institutes such as IRRI. Teasing out a mechanistic understanding of natural and life processes furthers the frontiers of science and knowledge and is the noblest of undertakings in cumulatively understanding the universe and our purpose of being on this planet and in this universe. However, the pressure of deliverables through science to society, economy and environment are not unknown to any scientist now. Under such circumstances it would be foolhardy not to integrate one component of delivery into the other for the final product. For example, the project to convert rice from a C3 to a C4 photosynthesis plant is an ambitious moonshot initiated at IRRI. Over more than a decade the project has provided incisive insights into the rice leaf biochemistry and morpho-anatomy (Fouracre et al. 2014; Wang et al. 2016; Johnson 2022). It is now being revisited to integrate translational products and socio-economic considerations (Kohli et al. 2020) for the return on investment (ROI). Unfortunately, there is a sizable section of scientific endeavor yet not integrated as a delivery component at locations where the mandate is product delivery. Also unfortunately, such islands of endeavor

are convinced that they are complete in themselves or with their preferred partnerships with some islets far ashore.

4 Advice to the Next Generation of Scientists

For the next generation of plant scientists who wish to contribute to better the human existence I can only provide pointers in rice science. After all, rice is the staple food of 56% of the human population, it is grown by 25% of the world's farmers on 10% of the global cultivated land, employing 35% of the world's irrigation water and 15% of the world's fertilizer use. It accounts for 13% (US$ 206 billion) of the world's annual crop value. At a product driven institute like IRRI, conducting basic upstream discovery science was challenging: one, because a formal pipeline connecting discovery to utility was amorphous and two, because it was low priority for funding compared to breeding, agronomy and climate research—for all the right reasons of course. However, if an envisioned pipeline could be crystallized, then the value of upstream science feeding into the product delivery would become apparent. Overtime this has come to be and IRRI is strongly positioned to deliver molecular science-based products over and above the normal marker based molecular biology employed for genomic selection in its present incarnation. For example, looking for a rice genotype with low glycemic index (GI) was mostly an exercise of finding a needle in the haystack of the rice germplasm. However, now specific genes that contribute to low GI have been identified (Anacleto et al. 2019) with the potential to convert any genotype into a low GI variety. Similarly, a rice amidohydrolase gene was characterized as the guanine deaminase (Gotarkar et al. 2021) (*OsGDA1*) gene. The biochemistry of *OsGDA1* in converting guanine to xanthine was well known, especially in tea and coffee plants, but the gene encoding the enzyme was not known in plants. It was characterized as a negative regulator of root hair proliferation useful in drought tolerance, most likely through epigenetic controls influenced by metabolite flux that alters the S-adenosyl homocysteine (SAH) content, which in turn affects DNA methylation (James et al. 2002). Further, the wild species *Oryza longistaminata* genomic segments were introgressed into the cultivated *Oryza sativa* for an extended stigma to facilitate outcrossing for hybrid rice production (Prahalada et al. 2021). The near isogenic lines (NILs) were assiduously cleaned up to cancel linkage drag. The underpinning gene was cloned and validated through transgenic studies of silencing and overexpression. The case of rice tolerance to 14-day submergence through a single gene (Xu et al. 2006) is now classic scientific literature while the deployment of the submergence tolerant rice varieties in the farmers' fields is exemplary in public sector product dissemination and impact analysis (Emerick and Ronald 2019; Raghu et al. 2022). Upstream molecular studies have also allowed us to provide examples of how breeding for increased yield in the 60s and 70s has led to varieties that cannot survive adverse environments (Vikram et al. 2015) unlike their original landraces

and wild species. Therefore, the state of affairs now calls for new breeding strategies (McCouch 2004).[4]

The critical areas that remain unresolved in sustainable rice cultivation globally are the following:

1. *Nitrogen use efficiency (NUE).* Harnessing the associative or rhizospheric nitrogen fixation for cereals remains a daunting challenge despite major strides in understanding the underpinning genes and gene networks of the microbes and plants involved. With more than 40 years of research in the area and excellent papers elucidating the individual aspects of nitrogen fixation in microbes such as *Azospirillum*, *Azotobacter* etc. perhaps an integrative understanding is hampered by a few missing pieces of the puzzle. Success in this area of research will be the biggest game changer in the sustainable, climate smart, regenerative agriculture in the future. Obtaining an exhaustive understanding of the genetic, protein and metabolite networks and how they feed into each other and influence each other is a major goal of the omics biology in the coming years. Once again this can be facilitated by Big Data feeding into AI-mediated machine and deep learning, leading to hypotheses to be tested experimentally. It must be considered that NUE is a complex of nitrogen uptake, mobilization and utilization. The latter two aspects do not necessarily depend heavily on the first, hence provision of nitrogen through associative or rhizospheric methods is only part of the solution. We have recently observed that rice yield increases in lines that accumulate xanthine, allantoin and urea in roots. This yield increase does not occur through increase in panicle or spikelet number or through increase in 100 grain weight. It occurs through more spikelets getting duly filled, which implicates nitrogen remobilization from the roots. This hypothesis was tested in the field by growing lines in 50 and 25% less nitrogen and still obtaining the original yield.
2. *Resistance to certain biotic stress.* The DNA sequencing, gene cloning, rice transformation and transgenic plants have come of age since 1992, but the problem of rice resistance to borers and hoppers persists and leads to major crop losses annually still, not least because of the anti-GM sentiments in many countries. In a turn of events, the evolution in the sequencing technology has come to the rescue. Sequence of the 3000 rice genomes (2014) and an additional nearly 1000 more in public databases provide inroads into functional allele mining, especially from landraces and wild species, which can circumvent the redundant need for transgenic validation. However, certain other projects such as the Golden Rice reiterate the importance of transgenic plants because of leveraging genes that are not available in the rice pan-genome. Many critical traits, including yield, have now been addressed through combining the power of allele mining and gene networks.

[4] OneRice Breeding Strategy. https://www.cgiar.org/news-events/news/irri-overcomes-barriers-and-bottlenecks-towards-a-rice-breeding-revolution/#:~:text=However%2C%20according%20to%20IRRI's%20Senior,with%20common%20terminology%2C%20resource%20management%2C.

Publication of one such result on yield through panicle branching, without transgenic validation of the candidate gene, but through the analysis of the differential alleles (Pasion et al. 2021) has set the stage for such an approach becoming a mainstream method. Hopefully soon some useful genes against borers and hoppers within the rice wild species or landraces will also be similarly utilized to address these longstanding challenges. Sheath blight, false smut and some viral diseases are also similarly difficult to address. One viral disease in rice was indeed addressed through wide hybridization of cultivated rice *Oryza sativa* with wild species of *Oryza nivara* (Nuque 1982). Now of course we have been able to show the utility of molecular tools to transfer a very short segment of *Oryza longistaminata* into *Oryza sativa* for the trait of high outcrossing in a largely self-fertilized crop, for generating hybrid rice (Prahalada et al. 2021). Similar approaches can be used once specific genes for the purposes are identified in the wild rice species and landraces.

3. *Direct seeded rice.* The problems of water shortage and methane (CH_4) and nitrous oxide (N_2O) greenhouse gas (GHG) emissions from paddy fields posit a very difficult scenario for rice cultivation in the future. With most of the hungry, malnourished, and poor still heavily dependent on rice, methods must be found to cultivate rice sustainably to use less water and restrict GHG emissions. One such solution is direct seeded rice (DSR). However, DSR is prone to weed infestations and other maladies. Hence, traits that facilitate DSR must be mainstreamed in rice breeding. Weed competitiveness through anaerobic germination, seed vigor, root strength, canopy development, early flowering, nematode resistance, herbicide tolerance etc., are all traits that are useful for DSR and make up substantial gene and genotype discovery projects. Success of DSR feeds into water, labor, land, fertilizer and capital input reduction without compromising yield and grain quality. Due to all such reductions, it is financially and environmentally beneficial to the farmers and humans respectively. DSR amenable varieties can be used in transplanted conditions but vice versa is not true. Hence DSR breeding is the future of rice improvement.
4. *Grain quality for biofortification.* Rice is used as a grain more than any other cereal/staple. Hence maintaining the eating and cooking quality of rice grains is more important than in any other grain crop. Moreso because the preference of eating and cooking quality changes in countries, regions, topographies, and cultures. Having mega-varieties of rice that are grown in more than a million hectares is already an achievement and a testimony to how breeders and molecular geneticists have achieved grain quality acceptable to a large section of the population in some regions. However, since most grain quality traits are starch dependent, we have, along the breeding timeline, lost some useful traits of high protein, resistant starch, minerals, vitamins, and secondary metabolites from almost all mega-varieties and elite parental lines. Recent unpublished results from IRRI suggest that a gene that increases the zinc and iron content in the rice grain, and makes it nutritionally superior, must be dysfunctional for the plant to yield more grain. Unwitting selection has therefore been practiced for decreased mineral content in the grains of high yielding rice varieties. There is an imminent

need to build back these traits in lines with elite yield characteristics. IRRI has started concentrating heavily in this direction but there is a lot of work to be done to ensure that the rice consuming population of the world also gets sufficient mineral and protein nutrition from rice while consuming rice that is low in glycemic index (GI). The last advice any rice consuming diabetic wishes to hear from the doctors is the need to reduce rice intake. There are genes and genotypes waiting to be discovered that can substantially reduce the GI without compromising yield and eating/cooking qualities of rice. Bright young biochemists, geneticists, omics experts and breeders are needed to make rice healthy as it can be. IRRI's initial forays in this area hold a lot of promise but many more efforts are needed.

5. *Grain development.* This is a rather underappreciated area of plant development when the aim in many projects is to have more and better yield. Grain development is a unique process sustaining life activities almost till harvest under the most restrictive micro-environments of desiccation, pH, ionic strengths, cell death etc. What sustains these life activities under conditions that are not conducive to normal cell survival and even after cell death. The normal replication, transcription and translation processes stop long before life activities continue to feed into a developing grain. The role of encompassing husk tissue has been elaborated but not exhaustively. Yet the developing endosperm itself retains life activities for long. We have evidence that small unannotated seed proteins that are highly stable have critical roles. These proteins are not easily degraded, are desiccation tolerant, can spatio-temporally organize into multiple oligomeric forms to conduct different enzymatic or structural functions. Exploring the panoply of such proteins and their multiple functions remains largely unchartered territory and can influence the grain quality in ways that are industrially useful thus leading to financial implications as well.

Statement of the above five points does not preclude the importance of several climate change related adaptation or mitigation mechanisms being explored for biotic and abiotic stress tolerance and relating aerial and sub-terranean processes, including root biology. However, there seems to be a critical mass active in those research areas while the above five points illustrate areas where critical mass is not engaged, yet the topics are critical for rice cultivation and consumption in the future.

In undertaking such research, the young scientists are faced with the dilemmas of choosing the right countries, labs, supervisors, projects, and approaches. Not everything falls in place at one point of time. The philosophical and practical approach to take is firstly to accept this shortcoming and then explore where and how most of those aspects can be available as desired. A good place to start is to look for labs in universities, institutes and companies that are well integrated from demand to impact. As a Ph.D. student or Postdoc one doesn't do more than the necessary field and/or lab work. However, being around people who are engaged in the various aspects of the value chain pipeline, one can see what one is taking from and what one is feeding into as part of the pipeline. The word pipeline mostly has negative connotations and invokes lack of intellectual freedom. Let's face it, even if one had

full intellectual freedom to understand, for example, collision physics on a billiards table, one would wish to find a player who can use that information and improve in that game. To obtain the wherewithal to achieve that understanding is the upstream part of a pipeline and finding a player to use that information is the downstream part of the pipeline. It's time we saw a pipeline as the most useful part of a research project to create impact.

The second part, after a decision on the entity, is deciding the supervisor. This will depend a lot on what one wishes to address in one's research but equally could be easy if one is open-minded to follow the research path of a desirable supervisor. What is a desirable supervisor? There may be many traits to look for as far as scientific aspects are concerned but a good supervisor could be someone who:

1. Empathetically differentiates between a team member as a person and as a student/employee.
2. Is openminded and collaborates not only with top shots abroad but within the entity as well.
3. Has set routines and processes but is not punctilious or fastidious.
4. Is an equal mentor for all and treats everyone without bias.
5. Is conscientious to recognize and credit the successes and discusses failures constructively.
6. May be running routine experiments but the results feed into answering bigger questions.
7. Has the capacity to extrapolate and hypothesize on what the results mean at the systems level.
8. Is regularly productive without an obsession to publish/patent every year.
9. Is equally respected by the peers, seniors and juniors as a person and as a professional.
10. Is embedded in an egalitarian community of professionals to foster confidence.

Such a supervisor would open doors of opportunities that can compensate for the lack of any facilities. Dedicating time to the team members would not be an issue for someone like this. To find such a supervisor one would need to have the right set of questions and scenarios, hence a couple of internships/stints with prospective supervisors would set the stage. It doesn't matter whether such a supervisor has a large group or a small group. There is no harm in learning from immediate seniors because within a good large group the character of mentorship would percolate down. Similarly, it is not important if one goes for public or private research entities. Both have their pros and cons. The aim of a positive balance sheet in a private company is a great guide to what needs to be done in how much time and under what constraints. The public sector freedom requires self-discipline, sometimes imposed by the supervisor, donor, or larger mission of the entity, in engaging in the most critical aspects before spreading oneself thin. There are very few labs that now engage in basic science without a vision on where the results will be of use downstream. The clearer that vision the better the research project to take.

An important point, especially in view of the statements above on publishing, is that the results should go out in one way or another. If it happens in a 'top' journal:

great, if not, it must still be out there for others to assess its soundness and utility. It is critical that one is convinced of the validity of the results and has a vision on what the caveats are and how to address them.

A few words on the attributes of potential scientists, rather potential professionals in any field and even potential entrepreneurs. To quote Vygotsky's concept of the "Zone of Proximal Development" (ZPD) which posits that *"human potential is theoretically limitless; but the practical limits of human potential depend upon social interactions and residential environment. ZPD is the distance between the actual developmental level as determined by independent problem solving and the level of potential development as determined through problem solving under adult guidance or in collaboration with more capable peers". In theory, then, so long as a person has access to a more capable peer, any problem can be solved.* It goes to suggest that it is important, especially for scientists, whose job it is to solve problems, that they expose themselves to the fears from accosting new people, places and events because, as a paraphrasing of Jordan Peterson's speech, "*we learn on the edge of the unknown; that edge is right where we confront that which is intimidating and frightening to us at the rate that we find optimal, where we are gripped with an intrinsic sense of meaning, and so the sense of intrinsic meaning itself is a measure of the edge of fear—and that's where all the new information is*". Let's train the next level and next generation of scientists not to be afraid of fear.

And a final suggestion, do not delay the start in the expectation of an understanding of where to reach. Even those who advice you do not know where you will reach. It's your journey, no one, not even you know the destination. You will make a destination of every station on the path, there is no *final* destination. That is how the path becomes happiness, because there is no path *to* happiness. An expected clarity and guidance on where to reach can bring momentary peace when you get there, but your real journey will begin from there. As said in one interpretation of two couplets of the Bhagvad Geet, "We are kept from our goal not by obstacles but by a clear path to a lesser goal". Looking for recognition is the lesser goal. The bigger goal is service. The beauty is, that service leads to recognition. Extrapolating this philosophy to conducting the experiments would make one an excellent and a happy scientist who is not divorced from common sense.

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Finding My Purpose in Life: Science as a Path for Self-discovery

Eric Lam

Abstract Life is all about making choices. While the degrees of freedom that one may have to choose from can vary greatly between individuals, a common dilemma for people is to choose a career path as one transitions from youth to adulthood. How we approach this is a very personal journey, and often, one does not really know the destination of the path that we chose when we start out. I mean, how "could" anyone know what the best career opportunity may be 15 or 20 years down the road? Or how our own perspectives and values may change as we mature and age? As technologies evolve ever more rapidly while our planet's environment and geopolitical outlook continue to deteriorate, how are we to attain financial security and maintain self-fulfillment at the same time? In other words, to get to a happy space with contentment when we become an adult. I am not wise enough to give specific answers to these questions, but I believe over the years, I have made some observations and encountered experiences that may speak to these common anxieties about making an important choice at certain junctures of our life. By sharing these anecdotes and opinions alongside to those of the other accomplished scientists in this volume, I hope my personal journey down the path of scientific discoveries would add more "food for thought" and assist our young readers to make their brave move with conviction and optimism. A caution: NOT choosing is in itself a choice and procrastination will likely makes the work needed to realize your goal much harder later on.

1 Motivations: How I Have Developed an Interest in Science

FINDING MY PATH IN A NEW LAND. If I look back at my chosen career path of science, and more specifically Plant Biology, I think my interest in understanding the natural world probably took its firm hold in the late 1960's around the time when

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I was 10 years old. Those were the heady days of the Moon Landing by the Apollo missions and the launch of the original Star Trek series on U.S. television. My family could not afford our own television set in Hong Kong at the time, and I remember hanging around after dinner outside shops and stores near my apartment building that may have a black-and-white television set turned on. There I watched with fascination the amazing events such as touching the surface of the moon for the first time by humans from Earth and imagined exploration of the galaxies with fantastic technologies in the not-too-distant future. Little did I realize then, my innate affinity for exploration and wonder about our place in the cosmos were being awaken. My curiosity was kindled and fortunately for me, this flame remains still to this day after more than half a century.

In 1970, my parents took the family from Hong Kong and immigrated to New York City. I was twelve and the youngest child along with a brother and a sister. I did not understand at that time what courage my parents must had to transplant the whole family to a new land, and the amount of sacrifice that they would have to make in the decades afterward. All that for the sake of better opportunities for their children. For us, and likely for many other families that moved to the United States without higher education, resources, or marketable skills, immigration was a traumatic experience financially and psychologically. We basically had to start from the bottom of the social ladder and work our way up, learning all the intricacies of the new society that we transplanted into, starting with learning to speak English—my parents never did learn English in part because of their more advanced age and daily struggles to make ends meet. During this tough time of my passage from adolescence to adulthood is when I first began to question my purpose and what is life's meaning. One can wake up quickly to the fact that the "American Dream" can be fraught with pain and suffering, when one began to encounter prejudice, bigotry and layers of bureaucracy. While my parents did not leave me with material riches, what they taught me is their work ethics, pride, kindness and optimism about the future. Their firm belief that education and knowledge is the most important key for our success and well-being shaped the course of my career choice. I was a good student in elementary and high school, despite my poor English in the first couple of years, while I have always enjoyed and did well in art. I especially liked my science classes and my high school biology teacher, Mr. Lucas, who was the first person that introduced me to photosynthesis and the world of plant biochemistry. I was fascinated by this fundamental process that provides the energy and oxygen which all animals depend on to stay alive. Growing up, I heard personal tales of survival from my mother about famine caused by the Japanese invasion and occupation of China during World War II and later on during the Cultural Revolution time in China. I thus have an early appreciation of agriculture as the bedrock for human society and the most fundamental of our essential needs. My interest to understand plants better was further sparked by the intricacies and beauty of the first steps of the process to capture energy from the sun and convert it into chemical energy as food stuff while making the oxygen that we need to stay alive.

After graduating from high school, I attended the State University of New York at Stony Brook for 3 years as an undergraduate with a double degree in Chemistry and

Biochemistry. While there, I conducted research for two years, first working with *Euglena* under Dr. Lyman and later focused on mitochondria bioenergetics under Dr. Tu. There, in lab classes and in my first taste of real laboratories, I found that I enjoy laboratory research immensely and have the aptitude for devising experiments independently. I was fascinated by how discoveries were made in different fields with very diverse sets of tools and model systems and thus read scientific journals voraciously. I especially enjoyed browsing the *News and Views* section of *Nature* for example in order to learn the cutting-edge development in many fields of science. Reading is also fundamental for me to learn how to write a scientific report as well, as I understood early on. However, the experience of trying to publish one's findings could be daunting. I still remember typing excitedly my first short paper for submission to a journal and drafting the graphs and charts for the data by hand in the wee hours of several nights. Then in a few months, I experienced the bitter taste of my first rejection with critiques from reviewers. After a week of gloom, overcoming my dejection and feelings of injustice, I went back to work and addressed the issues raised, followed with resubmission, etc. Thankfully, after another six months, the joy of receiving its acceptance by the journal and finally the ecstasy of seeing my name as an author in print! This experience along with subsequent successes in publishing additional papers during my undergraduate years solidified my decision to follow a career path in science. The competing choices at the time was becoming a painter or professional tennis player, both activities I enjoyed since my high school time. However, the odds for my ability to sufficiently excel in these two professions to enjoy some degree of financial security are much lower than that of getting a degree in the sciences. Another key deciding factor for me is that I found the process of knowledge discovery a truly exhilarating experience. For a moment in time, one could be the first person to hold on to a new scrap of knowledge about nature and which could add incrementally to our understanding of the world that we are in. To me, this is a special feeling that touched a personal chord in my conscience and relates to my later reflection about life's motivational force. I resolved then to pursue a career as a scientist and run my own laboratory to discover new knowledge in the area of Plant Biology.

2 Work Done: My Personal Scientific Approach

PATH TO MY "OWN" SELF-ENLIGHTENMENT (A 40-YEAR LONG JOURNEY). During the third year of my undergraduate work at Stony Brook, I applied to graduate schools with advice from Dr. Lyman, who strongly suggested that I study for my Ph.D. diploma at the University of California at Berkeley. This choice was made because of my interest to carry out photosynthesis research to make contributions that would improve agriculture and help alleviate famine in the world. During the 40-year period between 1950 and 1990, UC Berkeley was one of the hotspots of basic photosynthesis research in the world, starting with Melvin Calvin's Nobel Price-winning research that utilized carbon isotope to trace the biosynthesis

of carbohydrates and other organic compounds from fixation of atmospheric carbon dioxide. Enhanced by my successful research effort as an undergraduate, supported by having 3 publications already in press or in print at time of application, I was accepted to the Biophysics Ph.D. program at UC Berkeley with a full scholarship. In 1980, I left my family for the first time and traveled to Berkeley to begin my research career on the West Coast. I fell in love with the famous California sunshine and the laid-back atmosphere of the Berkeley campus. Since I did not know any of the professors in the program nor did I know what research topic interests me the most, I decided to attend introductory seminar classes related to photosynthesis in the first year to get to know more about the program and professors before committing myself to a laboratory. In the meantime, however, I secured a spot in the laboratory of Dr. Lester Packer in the Physiology department to study the mechanism of bacteriorhodopsin, also known as purple membrane. Dr. Packer was a well-known researcher in the field of oxidative stress, free radicals and human health. However, he also had a broad interest in bioenergetics that included the mechanism of light-dependent charge separation model systems, such as bacteriorhodopsin. This remarkable protein from a salt-loving bacteria called *Halobacterium halobium* is the simplest light-dependent proton pump known and uses retinal as the pigment for light absorption, in a fashion very similar to the opsins in our retina. I have known of Dr. Packer's laboratory in this field from my readings during my undergraduate days and wanted to learn about this interesting protein while getting settled into the new environment. Having my own support and armed with my short but convincing vitae, I was accepted into the Packer laboratory as a rotation student in my first semester and started working with a project to study the structure–function relationship of this simple transmembrane proton pump. With my prior experience working with rat liver mitochondria and membrane protein purification in the Tu laboratory, I quickly learned to purify the purple membrane patches from the bacterium and carried out chemical modification studies with various chemical reagents in an effort to determine which type of amino acid residues are important for the physical properties and function of this protein. During the 1970's, the age of molecular biology has just begun, and site-specific mutagenesis and protein structure determination were hardly routine. Thus, while the approach of chemical modification would be considered clumsy and inconclusive by today's standards, I was able to work hard and generated several small papers describing the effects observed upon treatment of purple membrane with reagents that modify different types of amino acids, with some showing interesting shifts in the chromophore's spectral properties. While my hard work and results from this project have been productive in terms of publications, I was dissatisfied with the ambiguity of my results, and I did not see a clear vision of how I could progress any further to reach more definitive conclusions without additional technologies to provide the tools needed.

By the end of my first year at Berkeley, I have attended several classes in plant biology and one special topics class on photosynthesis light reaction pathways given by Richard Malkin (hereafter referred to as Dick). During the early '70s, working together with Alan J. Bearden in Berkeley, Dick has applied the technique of electron spin resonance (ESR) to discover and study electron acceptors in the path of

energy transduction from absorbed photons in the reaction centers to their ultimate chemical carriers in the chloroplasts. I was fascinated by the new revelations that this technology has enabled and was also encouraged by new reports from several laboratories that have successfully isolated membrane protein complexes containing different components for the electron transport system from Photosystem II (PSII) to Photosystem I (PSI). It seemed to me that this complex pathway was then poised for new discoveries which can reveal the molecular mechanisms for energy capture and utilization by the chloroplast. I approached Dick in the Spring of 1981 to request a rotation in his laboratory starting at the end of my first year and he accepted me after an interview. I worked in Dick's lab that summer and began in earnest my Ph.D. thesis project to unravel the biochemical and structural complexity of proteins required for conversion of light to chemical energy while producing oxygen as a byproduct. It also began my long friendship with Dick and his family that continues to this day. While I appreciated all the great mentors that I have been fortunate enough to encounter throughout my career, the time that I have spent in Dick's lab and the warmth and sincere care that Dick and his family have bestowed on me have changed my life perspective. They showed me what mentors ought to do to help nurture the younger generation. In addition to the camaraderie that we had, such as sharing coffee in the morning before we start work in the lab, Dick and I often have informal chats about all-things photosynthesis. Most importantly, Dick encouraged me to strike out and explore my own ideas and often let me try things that he was not too convinced of or interested in. This is one of the most enjoyable time periods that I have had during the formative years in my career, with little responsibilities except to explore and learn while having little external pressure. In addition, as I mature in my knowledge in the field and begin to make significant contributions, Dick generously supported my attendance to national and international meetings, as well as to introduce me to many top researchers in the field at the time. These important activities helped to lay the foundation for the next step of my career.

My graduate work in the Malkin laboratory focused on the optimization of methods to purify active complexes involved in the light reaction of photosynthesis. These included PSII with or without the oxygen evolving complex, the cytochrome b_6/f complex, and PSI. Once these membrane-associated complexes were solubilized and purified from each other, they were then systematically characterized biochemically and functionally to define the minimal functioning units. For my thesis work, one of the achievements that Dick and I accomplished was the total reconstitution of photosynthetic electron flow from water oxidation by PSII to the reduction of NADP to NADPH by ferredoxin via the soluble ferredoxin-NADP reductase enzyme, together with all the resolved complexes and purified cofactors (Lam and Malkin 1982). This system allowed us to test competing hypotheses at the time on the specifics of donor/acceptor relationship between various components of the complex electron transfer pathway. Nearing the end of my thesis work in 1983, I attended one of the Photosynthesis Congresses and heard a lecture by Mary-Dell Chilton on the successful demonstration of stable plant transformation by a soil bacteria called Agrobacterium, using its newly discovered Ti-plasmid as a vector to direct DNA transfer and integration into the plant genome. This dramatic feat excited

me tremendously since it opened the door for much more precise experimentation than ever before to study genes and proteins in a living plant. I was convinced then that the molecular approach to precisely alter the sequence of a gene or its encoded protein would be the way to unravel the secrets of plant biology and advance agriculture. This conviction led me to seek out Dick's advice as to where I ought to go for my postdoctoral training. At the time in the early 1980's, it was the dawn of plant molecular biology when cloning of a plant gene was a high impact achievement worthy of a publication in *Nature* or *Science*. The race was on to isolate promoter elements of such genes that are active in plants and define the architecture of the controlling elements and relevant transcription factors, much like the bacterial promoter system worked out by pioneers like Jacob and Monod in the early 1960's. The sentiment in the field at the time was that like in the case of bacteria, we expected getting our hands on the promoter element and its corresponding transcription factors will quickly resolve their regulatory circuits. We were rather naïve in retrospect, as we learned in the subsequent decades.

Several prominent plant molecular biology pioneers in the U.S. that focused on nuclear encoded genes at the time were Robert Goldberg (UCLA), Nam-Hai Chua (Rockefeller University, referred to as Nam), and Elaine Tobin (also at UCLA), to name some of the top researchers. Dick turns out to know both Nam and Elaine quite well through their common interest in photosynthesis and plastid biogenesis. I first met Elaine because of my growing interest in duckweed, an aquatic macrophyte that was considered a model plant at the time, back in 1983. Our interest in duckweed was aroused due to the discovery of a duckweed mutant that was impaired in photosynthesis, and we suspected that it might have defects in electron transfer between the two photosystems based on its phenotype. Part of the effort we spent in the Malkin lab during the early 1980s was to generate antibodies to major components from each of the 3 electron transfer complexes. From a discussion between Dick and I, we thought it would be interesting to examine this mutant duckweed using both the ESR technique and immunoblot assay to query the components within the cytochrome b_6/f complex. After a phone call with Elaine, who was working with this plant at the time to identify and clone genes from duckweed, I soon went down to Southern California for a visit and obtained both the wild-type and mutant duckweed strains from her lab. Our work positively identified loss of the Riske iron-sulfur protein in the cytochrome b_6/f complex as a likely lesion in this mutant. In addition, we found that most of the other peptides within this membrane protein complex became unstable and thus are present only at very reduced amount, likely indicating the importance of this protein in mediating inter-subunit contacts. After I published this work before leaving Dick's laboratory, I stopped working on duckweed until thirty years later, as my career's focus came back full circle which I will mention later in this story.

At the end, I applied to work with Nam at the Rockefeller University, in part because I was impressed by his seminar at Berkeley during his visit in the early 1980s and because I wanted to move back to the East Coast to be closer to my family. I first know of Nam's name from his excellent work on organellar protein synthesis and transport in the days when coupled processing of signal peptide sequences of nuclear encoded proteins destined for organelles was being elucidated. However, his

seminar on his laboratory's more recent projects to combine tissue culture and in vitro molecular biology for deciphering the secrets of gene regulation using transgenic plants impressed me. I arrived back to New York City in the summer of 1985 to start the next phase of research training at the venerable Rockefeller University where much seminal work in biology had been carried out. While it is a small university in terms of student numbers and campus size, it boasts probably one of the highest numbers of Nobel Laureates per capita for a single institution that I have known. It featured many large laboratories that took up better part of a whole floor in some of its buildings. The Laboratory of Plant Molecular Biology is on one of the top floors of the Tower Building, located in midtown Manhattan, with views of the East River flowing by outside some of its windows. In this venue, I learned how big science is done in a large laboratory with more than 15 postdoctoral researchers that are smart, eager and industrious, all working on various research topics in plant molecular biology. We have access to state-of-the-arts equipment and reagents, including our own in-house oligonucleotide synthesizer when it first became available. Nam's laboratory that I worked in, and learned molecular biology techniques and approaches, opened my eyes to what it takes to compete at the highest level of science. Rubbing shoulders with top-notch postdoctoral researchers such as Robert Fluhr, Ferenc Nagy, Steve Kay, Pamela Green, and Cris Kuhlemeier, to name just a few of my contemporaries at Nam's lab, helped me to think deeply about research problems and properly frame the questions. The four and a half years that I spent at Nam's lab complemented in many ways my graduate training in the comparatively small lab of 6–7 people at Berkeley. I believe I have benefited from both settings to teach me about the pros and cons of both types of laboratories.

My projects at the Rockefeller University revolved around the many facets of cis-elements and trans-acting factors that combine to regulate gene expression. I spent several years devising ways to make transcriptionally competent nuclear extracts from various plants and tried to establish a robust in vitro transcription assay. While we have some successes, it was never quite good enough for large scale purification of the individual components required for promoter-specific transcription. What activities we had with crude extracts often were rapidly lost as we started purification procedures. However, using the nuclear extracts that we have learned to make, I was able to optimize the assays for monitoring and characterizing sequence-specific DNA binding proteins that interact with active promoter sequences. After 3 years of research, we have generated functional results for different cis-acting elements, showing that a single copy or multimers of a simple 21 base pair sequence can produce strikingly distinct patterns of promoter activity in transgenic plants. For example, addition of one particular 21-basepair sequence, called *as-1* for Activating Sequence-1, can turn a normally leaf-specific promoter to become highly active in the root of plants. In the last couple years of my work in Nam's lab, a big leap forward for me was the cloning of one of the first sequence-specific binding plant transcription factors, TGA1a and TGA1b, from tobacco. This was a collaboration with Fumiaki Katagiri, then a graduate student in Nam's lab, who adopted quickly the new method, devised in Philip Sharp's lab in 1988, of cDNA phage libraries screening with binding site multimers to isolate the genes for the binding factors

of interest. The result was my first publication in *Nature* as a co-author (Katagiri et al. 1989). These key advances also enabled me to rapidly generate many additional results with other cis-elements that I have already characterized and begin to formulate the next series of questions and projects that I could tackle in my own lab. I started my job search in academia at the Spring of 1989 and accepted an offer to start my own research group at a new center (the AgBiotech Center) that was being built in the nearby Rutgers University of New Jersey, just across the Hudson River from New York.

I started my own laboratory at Rutgers University in the summer of 1989. My first two years at New Jersey was a hectic time both in terms of working to set up my laboratory from scratch, writing grant proposals, recruiting people, and to start new experiments for different projects. By then, I also had three young children and we bought a modest house near the university with financial help from my brother. It was a busy, but also exciting time in my career. It was the heyday of biotechnology, and the U.S. economy was good, which translated into ample Federal and State funding for research and education in the plant molecular biology area. I was fortunate to be awarded with a coveted NIH grant in 1990, as well as additional grants from NSF and the USDA over the next couple of years. Together with my support from the AgBiotech Center, my research was well funded, and I was able to recruit students and postdoctoral researchers to my team without much trouble in those days since my publications were at the front of the field at the time. In the remaining paragraphs of this section, I would like to summarize what I think are my key independent contributions to plant biology and focus my description on how and why I ventured into these areas as I navigate my own path of research after leaving the wings of my graduate and postdoctoral mentors.

1) *Targeted disruption of a non-selectable gene in plants.* Back in the early 1990s when I started my lab, functional genomics tools were essentially limited to increase or decrease of a gene's transcript by overexpression of its sense or anti-sense coding region. We quickly found that this approach is confounded by a plethora of limitations such as position effects of the transgene, inadequate suppression, potential off-target effects, and unintended gene silencing, to name some of the most obvious issues. After working very hard with these approaches for four to five years, we found that few if any clear functional insight for our cloned transcription factors could be gleamed. I was convinced then we need to establish targeted gene knockout in the field to enable more definitive approach for determining gene functions. Working with my postdoc Zhonghe Miao for several years, we developed a gene targeting vector and reported its efficacy with Arabidopsis callus tissues for targeted disruption of the TGA3 locus in 1995. This vector was used in 1997 for *in planta* Arabidopsis transformation in Marty Yanofsky's lab to successfully generate a targeted disruption of the AGL5 locus after screening more than 750 transgenic lines. This was the first report of targeted disruption in plants of a non-selectable gene via homologous recombination (Kempin et al. 1997).

2) *Programmed cell death in plants.* In 1994, I was promoted to Associate Professor with tenure and was feeling more adventurous to explore risky ideas. One that came to me at the time is whether we could endow plants with a novel photoreceptor that can enable it to utilize photons in the green spectrum since chlorophyll absorbs light only in the blue and red spectral regions of visible light. Bacteriorhodopsin, a protein that I have worked on as a young graduate student, appears to be a perfect fit for this task since it absorbs light specifically around the yellowish-green region and reflects blue and red light. I convinced a new postdoc in my lab at that time, Ron Mittler, to take on this idea and determine if transgenic tobacco plants expressing bacteriorhodopsin can be made to absorb green light upon addition of the missing retinal chromophore. We found and reported in 1995 that our transgenic plants expressing this foreign gene resulted in ectopic cell death lesions and later demonstrated that these plants are more disease resistant to plant pathogens such as tobacco mosaic virus. This chance discovery demonstrated that expression of this single ion channel in plants can activate innate immunity that included spontaneous cell death activation. Since then, my lab has been working to understand the complex pathways that link programmed cell death to plant defense activation and stress responses (Lam 2004). Over the past twenty years, we have focused mostly on examining the structure and function for two types of cell death regulators that are highly conserved across multiple phyla: the metacaspase protease family and the Bax Inhibitors-1 cell death suppressor. This story is an example of serendipity that led us into the cell death and disease resistance field.
3) *Chromatin and genome organization.* By 1995, I have also begun to appreciate the difficult challenge for reverse genetics approach in organisms such as higher plants where gene families exist for many of the transcription factors, and crosstalk between pathways known and unknown makes mild quantitative effects very ambiguous. While we have shown that gene targeting for any locus is in principle feasible in Arabidopsis, the amount of work required is daunting as a routine method. By then, effort from multiple laboratories in Europe and the U.S. to create insertion mutant libraries using either transposons or T-DNA as mutagens were also beginning to be contemplated for Arabidopsis. In parallel, whole genome sequence for this model plant was also underway. The combination of these development made it likely that libraries of mapped insertion lines could become available for the community soon. I thus decided to turn the effort of my young lab to explore new grounds that I believe will be more fertile for novel discoveries. A key difference between eukaryotes and prokaryotes is the increase in DNA content per cell as well as their packaging into chromatin within the nucleus of eukaryotes via histones and other accessory factors and scaffold proteins. While the architecture of the nucleus and its enclosed genome has remained mysterious for decades, earlier observations in *Drosophila* and mammalian cells have shown that activation of certain genes can be correlated with changes in their subnuclear locations. This and other related reports on the effects of physical location of a gene with respect to the other parts of the genome on its activity suggested to me that the ability to track a gene's 3-D

location in a living plant could be a key tool to begin to address this aspect of gene regulation. This was a feat that would have been difficult to imagine in earlier days before 1995. However, it was just a year before that Marty Chalfie reported the remarkable discovery of a portable fluorescent protein, the green fluorescent protein (GFP) from jellyfish, with no requirement for addition of a chromophore (Chalfie et al. 1994), and the biology community was all excited about its possibilities. I began to try to come up with ways of utilizing GFP for tracking chromatin DNA in situ for live plants. The obvious challenge is to increase the number of GFP molecules to obtain sufficient fluorescence signals that can be localized to a specific sequence embedded in a known location of the genome. Eventually, my experience with multimerizing DNA binding elements to increase their strength of enhancer activity suggested that insertion of multimers for a heterologous cis-element can be used as a beacon within the genome that we could then "light up" with the corresponding DNA binding protein fusion with GFP, so long as it would not bind significantly elsewhere in the genome. But what sequence to use and how many copies would be necessary? As it happened, the exact same strategy was being contemplated by the labs of Andrew Belmont and Andrew Murray working with mammalian cell cultures and the budding yeast *Saccharomyces cerevisiae*. They demonstrated the efficacy of the method by using the well-characterized bacteria DNA binding protein *Lac* repressor and multimers of its target sequence *Lac* operator. This was further developed by 1997 in John Sedat's laboratory to monitor and quantify constrained movement of interphase chromosome for the first time. By then, GFP application in plants was finally getting a foothold from the realization that the original coding sequence of the jellyfish gene contains a cryptic splice site which prevented its ability to be expressed in the intact form when using plant cells. Discovering this critical issue, Jim Hasloff and colleagues in the U.K. finally solved the GFP expression problem in plants with mutated versions that removed this splice site. I remember visiting Jim's lab soon afterwards and obtained his latest mGFP vector as well as discussed with him my interest. With encouragement from Jim, I began to assemble the required pieces for this system from Aaron Straight and set out to test them in my lab. In 1999, I wrote an application to the new Plant Genome Research program of the NSF and obtained my first multimillion dollar grant to implement a chromatin charting project where we would map and quantify the transcription potential and diffusion coefficient for many insertions which we will create with a multimerized *Lac* operator tag. With this funding that enabled us to purchase one of the most advanced 3-D imaging system available at the time and recruiting a talented postdoctoral fellow, Naohiro Kato, we reported in 2001 the first transgenic plants in which we could visualize and track the movement of individual insertions. For the next seven years, I assembled a team in collaboration with Robert Martienssen and David Spector's groups at Cold Spring Harbor Laboratory to carry out the Chromatin Charting project. It culminated in a publication that described a collection of 277 transposant lines with our custom designed construct for insertion tracking and transcription potential monitoring using a luciferase reporter gene (Rosin et al. 2008). Examining a set

of transposant insertions clustered on one end of Chromosome 2, we carried out detailed studies that generated information about potential modes of epigenetic regulation of gene activities. Unfortunately, our project was not renewed because our transgenic approach and use of repeat elements were criticized as prone to artefacts. In addition, the advent of chromatin structure investigation using a new technique of proximity crosslinking combined with Next Generation DNA sequencing technology to query chromatin organization was cited as a more superior approach. While I do appreciate the advantages that the new technology (which is called HiC) has to offer, the functional data and mobility information that we could generate from our insertion lines remain a unique resource to study chromatin behavior in live plants. As an example, the quantitative description of DNA mobility along the length of a chromosome arm that we reported for different cell types in live plants is still an uncommon set of results to this date.

4) *Duckweed as a novel model crop plant for research and applications.* When our chromatin charting project ended around 2008, it coincided with the time that I was promoted to Distinguished Professor and was appointed as the Director of the AgBiotech Center. I was also awarded a sizable training grant from the NSF to support a large cohort of graduate students for over 6 years in the renewable energy area of research at Rutgers University. Through managing this large, cross-campus interdisciplinary training grant, I gained a deep appreciation of the looming crisis of Climate Change and our unsustainable dependence on fossil fuels. These are clearly existential challenges for our generation and all the scientific evidence that have been gathered since the 1970's indicate that our species would be running out of time very soon to find the right set of solutions for avoiding a global catastrophe that will confront humanity later in this century. In addition to formidable challenges in the geopolitical and policy fronts, finding solutions for feedstock production that is environmentally friendly while economically attractive are especially difficult. While billions have been invested in algal and cellulosic biofuels, they remain non-competitive because of the high costs for their production. In early 2009, I was reunited with duckweed as a research subject due to a combination of circumstances. A central figure in this turn of events is my colleague Todd Michael, who joined Rutgers as an Assistant Professor in 2007. Todd's main research interest is plant genomics and we quickly became good friends sharing common interest to develop advanced NGS at Rutgers, which culminated in getting support from the Waksman Institute at Rutgers to purchase the first SOLiD platform on campus at the time. Among the myriad plant species that Todd started genome sequencing projects on, he added duckweed (aka water lentil) to his list from a suggestion by Randy Kerstetter, who was also a professor at Rutgers at the time. However, in 2009, Todd decided to move to work at Monsanto in St. Louis but called me one day to ask if I would be interested to manage a large collection of duckweed germplasm. It turns out that the company Biolex, which had been working to commercialize duckweed-based human therapeutics, was going out-of-business and they were essentially cleaning out their stock collection of duckweed strains that were brought over to the U.S. from the Landolt collection at Switzerland decades ago by Anne Stomp.

Todd had been trying to get these strains from Biolex before but without success, and just before he was to leave Rutgers to move to Monsanto, this opportunity arose but has a short timetable for us to decide. We both agreed that this is too valuable a resource to refuse, in which case Biolex will likely discard them and these clones could be lost forever. At that time, I happen to have resources available from the Biotech Center as well as students from the new NSF training grant. So, I agreed to take care of this duckweed collection and that is how I restarted my research on these tiny plants in 2009. Since the ~650 clones of duckweed arrived to my lab from that time, it became a work of love for me to take care of them over these past 13 years. What sustained me over these challenging times of initiating a new area of research from the ground up is the firm belief that these plants could hold the key to a powerful new platform for feedstock production that will be cleaner, more productive and climate resilient than traditional crops. Their small size, simple aquatic habitat, and growth by vegetative budding make them amenable to scalable production without need of arable land (Fig. 1). On the other hand, unlike microalgae, their floating nature and size enable their easy harvesting by simple filtration and hydrology.

As I learn more about these tiny plants in the past dozen years, I became convinced that they offer an untapped natural resource that can play a key role to transform the way agriculture can be carried out. However, to make this plant model competitive for external funding has been more challenging than I expected in 2009. Chief among the needed foundation to enable the duckweed community to thrive in terms of attracting support for research or development are unified standards for nomenclature, broad access to well-curated germplasms, high quality genomic resources, and integration of societal engagement through commercialization efforts. The latter is especially a key to sustaining the support and expanding the impact from research with these plants far into the future. Working with a core group of duckweed research colleagues, including Todd Michael and Klaus J. Appenroth, I believe we have achieved many of these aims over the past dozen years, which are described in an invited review

Fig. 1 Duckweed in the wild and as a crop. *Left panel*: a pond in a garden is covered with the duckweed Wolffia (Hamilton, New Jersey) with the author shown on the left next to his brother Roger. *Middle panel*: freshly harvested duckweed (Landoltia spp.) grown on hydroponic system. *Right panel*: dried duckweed from hydroponic cropping system

published in the *Plant Cell* (Acosta et al. 2021). Currently, my own research on duckweed has two prongs: one is to elucidate the induction mechanism for turions, a form of dormant duckweed that sinks to the bottom of lakes and ponds and allow the plant to "hibernate" over the cold winter months. Another topic is the characterization of the duckweed associated microbiome, with the objective to uncover the basis for assembly of a stable microbial community that can benefit plant heath. Over the past seven years, I have also tried to learn how to farm duckweed effectively in order to understand the challenges for its commercialization. While I first started with ponds at a local nursery in New Jersey, I switched about five years ago to focus on establishing a modular, vertically integrated system with the objective to build a scalable platform that can be replicated quickly as well as fit for automation. The vision is to pave the way for eventual creation of a hands-free agricultural platform that can be driven by all the advanced technologies of the 21st Century such as robotics, artificial intelligence and wireless controls. Working with a graduate student Shawn Sorrels, we have founded a company Planet Duckweed (www.Planetduckweed.com) two years ago and are now moving toward the fund-raising phase to make duckweed products a reality in the marketplace. There is a lot of new tools and knowledge in the business arena that I must learn in the coming years, and I look forward to those challenges. Overcoming them should help make the duckweed field a vibrant one for investment by government agencies as well as commercial ventures.

3 Science Today and Tomorrow

HUMAN VERSUS MACHINE. In my own experience over the past 45 years working in science, especially in the biological disciplines, I have witnessed truly remarkable progress in knowledge acquisition—not only in the number of game-changing discoveries, but also the rapidity that these new advances have been realized. While I mentioned a few in describing my own journey in the last section, another simple tool that took over Biology practically overnight is the method of DNA amplification called polymerase chain reaction which was first reported in 1985. Another more recent technology is the famous CRISPR/Cas gene editing method that almost became household names in biology and medicine since its first publication in 2012. Of course, a more dramatic advance that truly permeated our society is the advent of digital technologies driven by computer hardware, software and satellite communication capabilities. Artificial intelligence will enable the use of big data that are being accumulated worldwide at a truly remarkable speed that would not have been thought possible merely a decade or so ago. A case in point is the recent advance of the AlphaFold platform published in 2021 that made significant improvement in our ability to predict protein folding structure based solely on the amino acid sequence (Jumper et al. 2021). These examples signify to me that scientists will need to have broader knowledge to utilize and assess what various data and machines can provide, as well as their limitations. In other words, a generalist with broad visions and skillsets may be more able to work effectively with interdisciplinary teams having two or more

complementary approaches or capabilities. The state of information availability and their proper "filtering" is another interesting shift of the paradigm between human memory needs and personal judgement. With a simple smartphone, an average person on this planet can now readily access more information on most topics than one can digest completely—think how many pages of a Google search output does an average person bothers to read. So, a new challenge for us in this information age is finding the right data and knowing the way to assess their validity as well as to integrate them. This challenge is true for us in this epoch for politics as much as with science. For scientist, the ability to design the right experiments for the proper questions, and to then assess the data with the right methods will be the "art" behind the laboratory bench that gives our work its personality. I do not think that this will be replaced by a computer algorithm in the near future, just yet.

TECHNOLOGIES: OPPORTUNITIES AND DANGERS. At the societal level, the awareness of how powerful technologies can offer both prosperity and catastrophes at a global scale has never been more acute in human history than in the past century. Advances in particle physics directly led to the creation of nuclear weapons in addition to nuclear power plants. The arms race during the Cold War era directly threatens the survival of the human species along with other life forms on a planetary scale. To this day, stockpiles of nuclear warheads continue to exist in an increasing number of nations and the threat of a nuclear holocaust remains. In contrast to the clear and present danger of the "bombs", the existential threat posed by the unintended consequence of rampant use of fossil fuel is much more insidious and challenging for human nature to deal with. While the rate of ocean temperature warming and rise of atmospheric carbon dioxide content has been steady and alarming during the post-industrial era, there is little political will to make serious adjustment to our fossil fuel-driven economy worldwide. Introduction of agriculture about 10,000 years ago was perhaps the first technology that changed much of the landscapes and altered the representation of plant and animal species on arable land of our planet over the millennia. In contrast, global warming catalyzed by greenhouse gas emission from massive fossil fuel utilization is impacting all parts of this planet's surface chemistry in merely a couple of hundred years. Humanity is currently undergoing an experiment on a planetary scale since this rate of rapid warming and rise in carbon dioxide in the atmosphere have not been seen before from ice core studies that go back 800,000 years. The outcome is uncertain, because like all good experiments it has no precedent. If unchecked, most models predict dramatic rises in sea levels, coupled with large extremes in climate patterns and rapid loss in biodiversity globally. These predicted effects is already evident over the past decades with dramatic disappearance of most glaciers worldwide as a vivid example. While policies and human nature appear too slow to change in response to this threat, I believe good technologies based on new science are urgently needed to mitigate the effects of Climate Change as well as to provide new tools that can disrupt our historic dependence on arable land and a predictable climate. Thus, new technologies and their associated science for the next millennium would need to be cognizant of their potential impact on the earth system as much as their immediate societal benefits. Some of the current efforts to minimize pressure on global deforestation to create pasture for meat production by

advancing plant-based or artificial meat products is a forward-looking approach that is gaining acceptance in the marketplace. With more optimization of the relevant food science and product development in the future, I could imagine perhaps in a decade or two, when we could revert pastures back into carbon sequestering forests all over the world. Similarly, improvement in vertical farming technologies could help minimize excess fertilizer runoffs while producing fresh produce close to the consumers, which could decrease transportation cost while lowering their carbon footprint for production. These are but a few examples of topics for investigations that could produce important solutions needed for climate resilience in the coming decades.

ECOSYSTEM OF SCIENCE AND BUSINESS. Another aspect of the evolution in scientific enterprise is a shrinking division between basic and applied research. One reason for this tendency is that while basic research driven primarily by curiosity of the natural world serves as a foundation for novel discoveries, those topics that are most relevant to societal needs typically garner significantly more funding from either government agencies or commercial sources. Human health related topics and key crops for agriculture are two examples of subject areas that are usually among the more well-supported. In the last two decades, there is also a growing trend of academics turn entrepreneurs who start their own commercial startups to develop their academic research results into products, often with early support by their institutions. This has created a growing ecosystem between the business and academic worlds where the potential for conflicts of interest can be substantial. That being said, I believe a good scientist can be an excellent Founder to translate good research results to benefit the neediest segment of the population, and not necessarily for the most financial gain. While there are examples of academic researchers who became successful entrepreneurs, it is very rare for a successful businessperson to shift successfully into academic research. This is in part due to the rather lengthy process required to gain the experience and track record needed to compete for a tenure-track position in an academic setting. My own introduction to the commercial aspects of basic research is through involvement in filing patent protection for discoveries that I have made in the Chua lab as a postdoc. Later, I have also learned to file patent protection for discoveries made in my own laboratory at Rutgers University. From this experience, and now with my aim to ensure that we could help develop duckweeds into a new sustainable crop, I have grown to appreciate that the business world works quite differently from academic research and a critical skill for a successful entrepreneur is to be able to build a core team with complementary skills in order to deliver a successful product. A big challenge is consumer education through advertisement, which one can leverage the available social media in today's world to reach the global market effectively. In the long term, my vision is to create a successful automated agriculture platform that can integrate basic research directions, such as duckweed microbiome research, together with optimizing a hands-free plant production system that can produce high quality food anywhere by everyone. Getting this technology to disadvantaged populations in the world to help democratize availability of nutritious food would be realizing the dream that I had 45 years ago to help mitigate famine by the fruits of my research.

4 Advice to the Next Generation of Scientists

HOW EVOLUTION HELPS ME TO RATIONALIZE MY OWN COMPASS ON THE PATH. I like to finish this chapter with a note of optimism. Stay curious, I'd say to the reader, and maintain your wonder about our world and our lives. In this story, I have shown two examples of serendipity that have consumed a large percentage of my career to work on: the topics of cell death in plants and duckweed as a new plant model. What they served is to illustrate that often we do not know the actual destination of the journey that we may take, but one has to trust one's own heart or intuition. Looking back, I chose these paths because I thought they were not crowded already with competing labs and I believed that I could do something interesting that has not yet been done by others. Most importantly, I felt they could have a big impact if my work is successful. This last point is a crucial one for me, and it circles back to the end of Sect. 1 when I described why I chose the path of science as a career.

A famous quote from the physicist Steven Weinberg is that "the more the universe seems comprehensible, the more it also seems pointless" meaning that there is no proven design for our existence and our destiny has not been prescribed (Weinberg 1977). However, that is not to say that our existence is meaningless—we come, we go and eventually the sun goes dark, and humanity will disappear in time. As the great science communicator Carl Sagan aptly puts it "our species is young and curious and brave and shows much promise" (Sagan 1980), what we need is for some of us to do the right thing at the right time, and keep humanity as a whole on the evolutionary path to a greater future for our species. That, I believe, is what our "purpose" could be. Knowledge, provided by good science, is like candles in the dark of the cosmic universe, and one after another, they can add to light the way for our next step in a path without fear and prejudices. At this juncture of our evolution, it seems that humanity is likely at the brink of a Climate Catastrophe which we are seeing the dramatic beginnings of over the past decades, with worsening climatic patterns recorded every year and species are disappearing at an alarming rate. While politicians are continuing their debate as to what policies to enact, we as scientists, artists, engineers, or entrepreneurs could all find our own niche to create and to build solutions, both small and big, to help make our planet more hospitable and peaceful. That perhaps would be the best mental driver to help you stay focused and persevere through difficult challenges on your career path: believing in what you want to do is important for now and that it will lead to a better future for all.

Once you have made your choice to pursue science as your calling, how could one start to learn all the necessary knowledge and skills required for an independent position? I hope Sect. 2 of my chapter has illustrated my own personal journey through this formative phase of a science career. Chief among the important ingredients for cultivating your skillsets is an interactive and vibrant laboratory as well as a research-active institution with a strong science culture. Through finding the most interesting publications in your field, I believe you would be able to make a list of the laboratories in the world that you admire the most in terms of the quality and depth of the work that they have published. Try to visit them if possible and if you can attend scientific

meetings, listen to their talks to get an impression of their intellectual and personal qualities to see if they are someone you like to emulate. In other words, are they likely to be a role model for you? While you are contemplating these people and places that you are likely to aim for, prepare yourself as best that you could by working hard at where you are at the present time. Assuming that you are at the undergraduate stage, volunteer to do research at a lab that is working on a subject of your interest and just take a deep dive into the work to see if you can learn it well enough to produce some significant data by the time you are nearing graduation. This would help you to compete for entry into a good laboratory. Once you are in a laboratory, bear in mind that there will be competition, either real or perceived, in any setting. You need to demonstrate your abilities and accomplish the goals of your project, but you also need to be considerate of others in your laboratory environment so that you can help create camaraderie in the lab for the benefit of all. Often, the network of colleagues and fellow students/postdocs that you work together with would be in the same field with you for many years to come. What I learn over the years is that productive collaborations with other laboratories can help me accomplish much more of my goals without having to support many people in my own laboratory. In other instances, it enabled me to compete effectively for grant proposals by bringing in technologies that I have no experience in. Your reputation as a good and fair colleague is just as important as your scientific abilities in your peers' eyes, so learning good people skills is an important asset for your career. In spite of all good intentions, however, there are always unfortunate situations when friction can occur. My advice is to treat others with kindness in these situations and never let the challenging times change your values and goals. Remember that we are all in this evolutionary path together and our lives' purpose may be more similar than most of us realize.

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A Biologist by Chance and Necessity

Patrizia Lavia

Abstract Jacques Monod's *Chance and necessity* was my life-changing book. It turned me from being a prospective student in humanities, as I had always thought I would be, into a student in biology and, later, a Life Sciences researcher. In my research journey, I have been fortunate to live through an extraordinary time, in which thrilling discoveries have been accomplished. In the twentieth century biology has been revolutionised by our understanding of the genome. Jacob and Monod exemplify the strength of idea-driven, intuitive, almost handicraft activity yielding conceptual breakthrough. They announced an era, later called the molecular revolution era, that symbolizes to me the human quest towards progress. The curiosity, strive, efforts, and persistence that lead to discovery found a fertile humus in the molecular revolution: novel ideas were seeded, blossomed and generated new hypotheses, new efforts, and yet new discoveries in a collective effort to understand as complex a problem as the organization of our genome and molecular evolution. It has been an extraordinary privilege to see that progress develop. We have acquired the ability to work with DNA: the discoveries that followed confronted us with unprecedented questions and possibilities. Modern biology is now changing with the development of powerful technologies. It is progressing through high-resolution, automated techniques, generating "big data" requiring artificial intelligence for interpretation, and drawing global profiles of cells and organisms. We must regard these "big" approaches as knowledge-generating tools that can open up now venues we could never have explored otherwise, yet must remain aware that data generation must not overshadow human curiosity and intuition. We are still only beginning to understand fascinating processes in the life sciences. There is still much to be expected for those who have the passion, drive and patience to live through the lights and shadows of research.

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1 Motivations: How I Developed an Interest in Science

My motivation to study biology was a revelation to myself, which I had not suspected until the age of 18, when a book marked a turning point: "*Le hasard et la nécessité"* (*Chance and necessity*), by Jacques Monod. That happened by chance—and the book's name contained what was going to have a fatal impact on me.

I had always enjoyed learning at school. In the early '70s, my last three years in High School were amazing: the world was changing around us. People all over the world demanded freedom, civil rights and peace; we would sing to "*Give peace a chance*" and John Lennon's *Imagine.* The future seemed to hold a promise of change. We were exposed to a tourbillion of novelty in music, movies, literature, and philosophy. In high school we were fortunate to have excellent teachers who helped us reasoning through these ideas. I was looking forward to taking humanities at University. Everybody in my family had taken studies in humanities or law, which also seemed a natural culmination of my education at that point.

Then came the Monod book, in my last year in High School. Like many of my generation in the '70s, I was fervently committed to the ideal of equality and justice. I haven't changed my mind as to the ethical value of justice and equality, but I realize that my views then had the inflexibility, strength, and naiveté of a teen-ager with a dogmatic approach to reality. I am sure my assays in philosophy conveyed a complete lack of nuanced, critical reasoning, and yet, at the same time, a genuine desire to gain knowledge. Our Philosophy teacher tried to stimulate our critical thinking. In addition to ordinary lectures, he used to assign books for us to study and report on to the class. My assignment was "*Chance and Necessity"*, by Jacques Monod, Subtitle: "*an Essay on the natural Philosophy of modern Biology*" (Monod 1970). I think my Professor intended to stimulate me to think about how knowledge of reality can be gained and how it feeds back on our thoughts, beyond preconceived ideas. That was a revelation. I read about the "mystery" of the genetic code and the "mystery" of the functioning of the brain, as Monod described them. I came in contact with topics I had only marginally encountered up to that point. I was struck by the concepts flowing from the book, which shattered my programme to take humanities at University. Now I thought nothing could possibly be more interesting and worth dedicating one's efforts than biology.

Years later, I met Agnes Ullmann, who had been a close collaborator of Jacques Monod in Paris and the author of "*The origins of Molecular biology—A tribute to Jacques Monod"* (Ullmann 2014). Mme Ullmann was asking everybody at that party what had moved them towards biology. When I told her my story, she encouraged me: that's a wonderful story, you have to let your Professor know!

My former Philosophy Professor had moved to France and I had lost contact with him. Years later the occasion arose. With my schoolmates we organised a reunion to celebrate the 40th anniversary of our Baccalauréat and did a systematic search to invite our former teachers. After a bit of web searching, I found his e-mail and invited him to join our reunion, then added a few personal lines, telling him I was sure he couldn't possibly remember having assigned to me "*Chance and necessity*"

forty years earlier, but that book had changed my life, after which I had taken biology and spent the rest of my life in research. My Professor's reply was moving: "I know few who can gratify a teacher so much. It has made me happy in more than one way: for the somewhat narcissistic feeling of learning that I played a role, albeit a very modest one, in determining your professional orientation. Above all, I am delighted to hear that you now carry out as noble and essential an activity as the one you have chosen, so please accept my whole-hearted congratulations".

Thanks to my Philosophy teacher, *Le hasard et la nécéssité* has been my life-changing book.

2 Work Done: My Personal Scientific Approach

I can't trace my personal path without sketching the context. My first acquaintance with research in biology took place at an extraordinarily stimulating time—possibly, one of the most exciting times in biology.

I enrolled in Biology at Sapienza University in Rome in 1973. In that same year, Herbert Boyer and Stanley Cohen accomplished the very first genetic engineering experiment: they were the first to transfer a plasmid from one bacterial type into a different one, proving for the first time that genetic information could be transferred across genomes. Only a few months earlier, Paul Berg had created the first recombinant DNA molecule, demonstrating that a gene could be isolated, manipulated, cloned and amplified in the manipulated version, thus propagating novel genetic information. It is fascinating to listen to the account of those discoveries by Berg himself (https://www.youtube.com/watch?v=ZVK5MHieDAM) and read the account of how he got to make these discoveries in his Nobel lecture (Berg 1980), a prize shared with Walter Gilbert and Frederick Sanger, the pioneers who devised the method for DNA sequencing, starting the journey to deciphering the genome.

Listening to my University professors debating the implications of those molecular discoveries was a blessing for a first-year student. These groundbreaking discoveries raised hopes for those who saw science as progress, but triggered fears in those who saw scientists as Frankenstein creators. The implications of these discoveries stimulated thoughts I may define "à la Monod", reaching beyond the boundaries of Universities and research centers. At that time it was impossible to anticipate the long-term consequences of transferring DNA from an organism to another one. In 1975, Berg and other world-leading molecular biologists who had developed the recombinant DNA technology held the historical conference of Asilomar, California, where they established self-imposed regulations for manipulating genes and genomes (Berg et al. 1975). The Asilomar conference marks a milestone in self-reflection on science by scientists, where they established a model for self-regulation. The Asilomar manifesto read: *"Although there has as yet been no practical application of the new techniques, there is every reason to believe that they will have significant practical utility in the future"*. As knowledge advanced in the following years, the regulations evolved, but the insight of the Asilomar scientists in foreseeing the impact that

genetic engineering techniques were going to have remains unsurpassed. Looking back, I realise how privileged I have been to start my studies at the time of a scientific and cultural revolution. Biology was revealing the unexpected, seeding novel ideas, evolving fast, and promising life-changing progress. The feeling of embarking in a wonderful adventure was there. With progress advancing, I was confident that some answers to the fundamental questions asked by Monod could now be expected.

Besides the understanding of how to manipulate genes, a growing understanding of how genomes are organised was beginning to accumulate. It was becoming clear that, in higher eukaryotes, genomes are composed of diversified regions, some containing the genes that determine our characters, while others contained repetitive DNA that did not encode protein products. It also became clear that the genomic DNA was not "naked", but was associated with proteins that gave it a higher-order organization. That triggered an era of fundamental experiments to understand whether that organization had a functional significance, possibly related to the capacity of expression of different genome regions. Many laboratories began to study the genome organization during development and across species. It emerged that genome regions containing protein-coding genes, defined "euchromatin", shared "organisational" features that rendered them preferentially accessible to the machineries for replicating the DNA, transcribing the information in messenger RNA, and repairing any occurring damage, compared to the "heterochromatin", mostly made up of non-coding repetitive DNA. Scientists were puzzled about all that genomic material apparently devoid of coding functions: was it residual "junk" from evolutionary remnants, or did it have some purpose? We now know that this part of the genome has important regulatory roles in genome function and in evolution. That was the context I had the privilege to live through as a student: an era of groundbreaking discoveries fuelling continuous curiosity and enthusiasm.

In 1976 I started my thesis in Drosophila genetics and, a few years later, my first post-doc in human cytogenetics. The "fil rouge" in my early research experience was the effort to understand the functional organization of the genome. In the Drosophila project, I characterized differential features in the non-coding fraction (heterochromatin) of the genomes of closely related species, but could not formally underpin their evolutionary function. I felt a mixture of enthusiasm and frustration, as I felt the field had exceeded my capacity: I had remained on the surface of observational correlations, but had not identified a mechanism for how things worked. Nevertheless, my internship marked an extremely important time that was going to leave a mark on me: my mentors had shown me how to ask an interesting question and how to build up a scientific reasoning.

By the time I finished University, it was clear that we have a lot more genomic DNA than is actually expressed at any given time in any given cell. A concept was beginning to take shape: different genomic regions must be endowed with some reversible capacity to enable or disable their expression. Many laboratories had come to realize that the DNA in mammalian genomes could be reversibly modified at one of the four DNA bases, cytosine, via the addition of a methyl group. Intuitively, that modification could have provided the sought after, reversible switch regulating the genome expression. Early discoveries held promises in terms of understanding

genome functions, and also hinted at possible therapeutic opportunities for certain genetic diseases: indeed, turning a specific gene on or off by acting on its epigenetic control might now be envisaged as a tool to correct a pathological phenotype. The following decades have uncovered a broader array of finely tuned mechanisms to achieve that regulation, in addition to DNA methylation, shaping the field of epigenetics.

After graduation I joined the Human Cytogenetics laboratory, still at Sapienza University, whose research focus was on ribosomal genes. These genes exist in multiple copies, some of which are active, while others are silent, providing an informative system in which alternative functional states can be studied for genes with identical DNA sequence. We could demonstrate that ribosomal genes transmit their methylation status through cell division and that the newly generated cells inherited therefore the "blueprints" for expression of specific genes via their DNA methylation marks. With those results, we had entered the arena of epigenetic control. When, on a heavily rainy day, we were walking to the main post office in Rome to mail our manuscript to a US-based journal, my supervisor Marina Ferraro slipped on the wet pavement, fell over, and the manuscript single sheets fluctuated all over the street. Computers were still to come and we had nothing like a "virtual memory" of our work. Without the faintest sign of giving up, Marina just got up at once, walked to the middle of the street and—as if that was the most natural thing on earth—arrested the chaotic traffic in Rome waving at the cars, with the risk of getting continuously ran over, until she picked up every single sheet! Eventually we managed to submit the paper, which had a good recognition, compensating all the efforts and risks!

My research training in Drosophila heterochromatin and in methylation-directed gene regulation represented key experiences in what was going to be my future research. Albeit intertwined with errors and frustration, they were never disjoined from enthusiasm and a desire to learn more. In 1984, I was awarded a position at the Italian National Research Council (CNR) and, at almost the same time, an EMBO fellowship I had previously applied for. The CNR—the largest public research organization in Italy—allowed me to accept the EMBO fellowship while postponing my research start-up. I will always be grateful to the CNR for that. As an EMBO fellow, I joined the MRC Mammalian Genome Unit in Edinburgh, one of the top laboratories in the DNA methylation field, then directed by Ed Southern. He and Adrian Bird had devised methods to recognize methylated and unmethylated regions in the genome, rendering epigenetics studies amenable to unprecedented molecular detail. That led to the discovery that the human genome, albeit being largely methylated overall, contained discrete regions, or "islands", free of methylation, associated with expressed genes. At that time, the idea of achieving the human genome sequence was still far away: thus, the identification of unmethylated DNA islands as gene landmarks provided a molecular tool to identify new genes, including disease-causing genes, from the then undeciphered genome. In addition to that important outcome, which we would now define "translational", enormous progress was being made in understanding epigenetic control.

My experience at the MRC in Edinburgh also showed me a very different lab model from those I had known. Those were the years of Mrs Thatcher and public

expense was being cut down in the UK, including in public research. Nevertheless, despite of financial cuts, the resources were intelligently utilised to sustain a rational organization, with excellent technical services, a collaborative attitude of the leading scientists and a constant effort to foster independence in the post-docs and facilitate their independent growth.

Such a system would have been unthinkable of in Italy. The inadequacy of funding remains a major issue for Italian research, with obnoxious consequences at least at three levels. First, it directly damages research in that it prevents many research groups from accessing experimental and infrastructural conditions of excellent quality. Second, it limits intellectual diversification, pushing practically all biologists to develop "fundable" projects with some applicable outcome (e.g., cancer, infections, genetic diseases, etc.), with severe limitations to fundamental research, when every discovery that has proved useful to human health comes from fundamental research. Third, it pushes scientists to oscillate between an individualistic strive to prevail over others, and an opposite effort to access the limited financial resources via "alliances" that are often more political than genuine scientific collaborations. That limits the cross-fertilization that comes from true scientific exchange and represents a self-defeating limitation of the Italian system—which includes otherwise many excellent, courageous scientists. Science is a collective effort and needs humus to grow: it needs confrontation, frank discussions, and collaboration. All major scientific discoveries have become ripe in a community. The Asilomar conference is the paradigm of self-critical science: no single scientist could have reached the intellectual, scientific and ethic insight that they reached as members of that community.

When I returned to the CNR in Rome funding was not as limited as it presently is. I have had the opportunity to establish my group. In Edinburgh I had cloned some new genes for being unmethylated, but their function was unknown. On characterising them, one turned out to encode a regulator of the GTPase RAN, which regulates macromolecular transport in and out of the nucleus. A series of unexpected findings led me to find a link between the mammalian RAN GTPase, the cell cycle and mitosis, which I have continued to pursue, with various ramifications, in my career. It has been difficult at times. Sometimes we have underplayed our results or have not pushed at the right gear. But, overall, I have enjoyed it a lot. I have had the pleasure and the honour to witness the field take shape and to play a small part in studying RAN and nuclear transport receptors in cell division, which has been both exciting and rewarding.

3 Science Today and Tomorrow

From reductionism to systems

Biology is literally "the discourse about life". What we have learnt in the last three centuries has been wonderfully gratifying in elucidating the mechanisms of life. In the

last decades, we have come to appreciate that the molecular complexity of systems—an organism, a cell, or an organelle—relies on multiple circuits, networks and interactions, each made up of many molecules that operate simultaneously, dynamically, and influencing one another in complex programs. The reductionist approach consisted in altering one single gene or protein at a time to isolate the specific function of that gene or protein. That has yielded the founding stones, which we can now combine and build upon to understand the big picture. In the last few years, tools have been developed to study living systems at a global level, generating"big data" in high-throughput (globally profiling many genes, many transcripts, many proteins, many modifications) and high-content (to what extent, in which spatial localisation, in relation to which temporal events, associated with which other variations) modes. Artificial intelligence (AI) is growingly called upon to process and rationalise these data.

The process of understanding in past and future science

A recent webinar has elaborated on the concept that "*the lab of the future will not be bounded by walls*." The bottom line is that the scale of the experimental work has moved to a level that incorporates automated pipelines, robotics, connectedness, digital results, AI-driven analyses, transforming not only experimental practices but even the laboratory setting: fewer benches, more integrated platforms, larger space for computer hubs and workstations, virtual space to manage and store large datasets. For scientists of my generation, who identified "their" bench with their second home, it is a big transformation. Our experiments were mostly handcrafted; no robotics for sample handling, no automated pipelines, no artificial understanding of the data. Experiments took energy, patience, perseverance, restarting over and over again, adjusting conditions, even gestures. These crafting efforts generated a mastering of the experimental process, yet entailed a slow progress and a small scale of information that are no longer adequate. We have probably been the first generation of scientists to train in the recombinant DNA era, and the last to take notes by hand in a notebook. For us, writing down lab notes and drawing schemes by hand was an integral part of the process of understanding. Do technical innovations trigger the same learning processes in the researcher's brain? Every time I think about this, for example getting numerical measures from multiple series of stacks of a digital picture, I think back of Walther Flemming (1843–1905), considered the founder of cytogenetics (Paweletz 2001) and a striking example of understanding by drawing. Flemming understood chromosome segregation while drawing the process, 65 years before the DNA was known, and before its structure motivated the famous anticipation of the mechanism of replication: "*It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material*" (Watson and Crick 1953), which entailed segregation of the replicated molecules at some point. When Flemming illustrated mitosis (Fig. 1), he was not even aware of Mendel's laws for the segregation of characters.

Using large Salamander cells, and aniline-derived dyes, Flemming was capable to accurately describe the sequence of events leading to chromosome partitioning during cell division in a manner that has remained unsurpassed in intuition: he realised

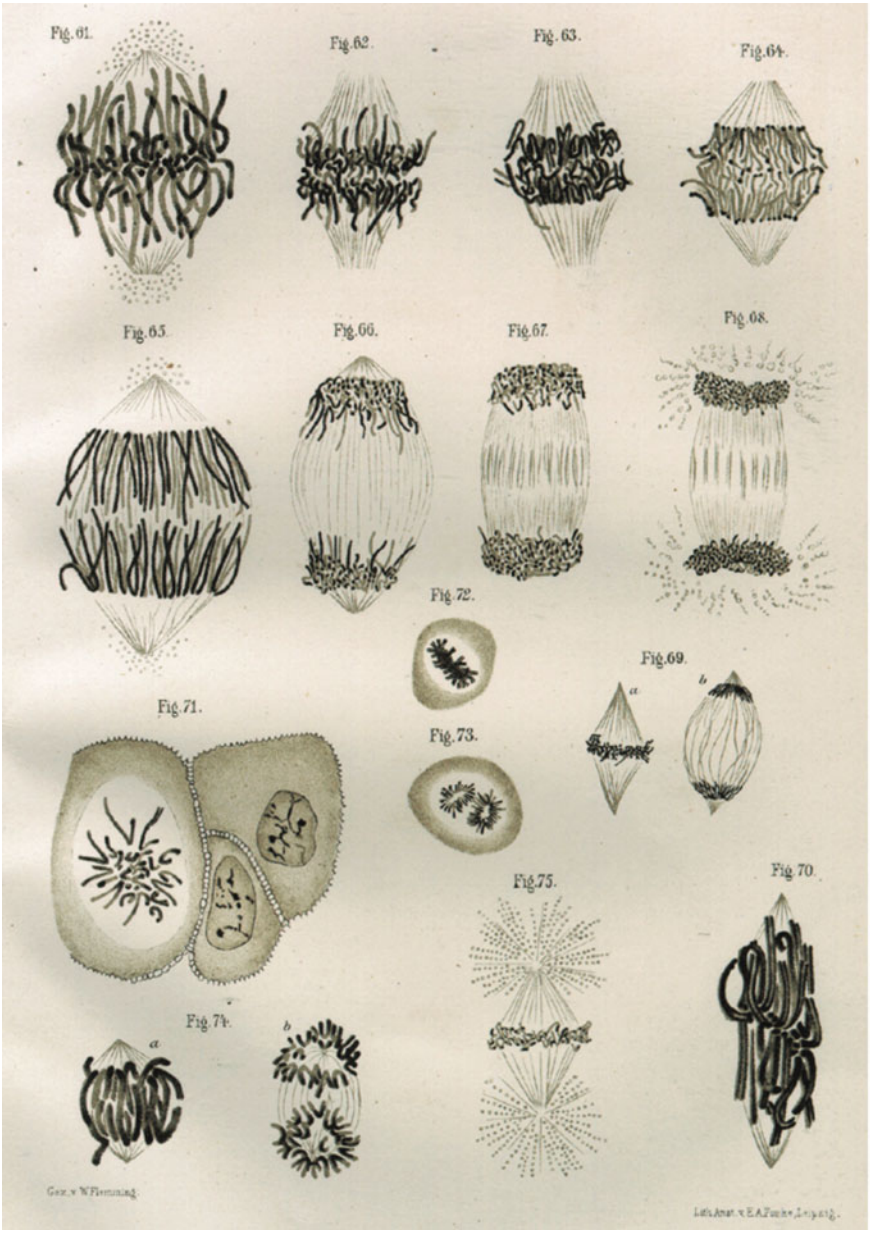

Fig. 1 Flemming's original drawings of cell division, representing progressive stages of the process of chromosome partitioning in two daughter cells, before being aware of Mendel's laws of heredity

that the nucleus contained stainable material (the chromatin) that could organize in threads (the chromosomes), that split longitudinally at some point; each half then moved to opposite sides of the cell. He first understood that all cell nuclei came from a predecessor nucleus (he coined the phrase *omnis nucleus e nucleo*, after Virchow's *omnis cellula e cellula*)(Flemming 1882).

The implications of Flemming's work for heredity were only fully understood after the recognition of Mendel's principles of heredity. Flemming was gifted with extraordinary intuition and drawing has likely nurtured his intuition. An interesting article in the E-life series on "Philosophy of Biology" speculates, "*drawing may be a way to better understand a biological process and to explore and develop scientific ideas"* (Anderson et al. 2019). The passage from handicraft to automation marks revolutionary change in experimentation and might change not only the object of our knowledge, but also the very structure of our learning processes.

3.1 What's Ahead in Science and Technology in the Life Sciences?

With the increase in the informational power in the life sciences, it is important to remain alerted on possible issues. The automation of experimental processes, the global profiling of cells, tissues, and organisms, are generating more data than the human mind can control, requiring artificial intelligence-driven analyses to be made sense of. This level of study clearly increases enormously the information capacity of biology. Might there be a risk that the interpretation of the data is eventually entirely transferred to an AI?

A related question comes from the notion that making advance on the large scale represented by complex phenomena in which multiple components interact dynamically requires large groups, but just putting together people with different expertise will not suffice: we need a common intellectual language and mindset capable of mutual understanding across as different frame of minds as those of "wet" and "dry" laboratories. With some foresight, the EU has made efforts to support multidisciplinary training projects for "new generation scientists", which have now been running for almost 20 years. After this long, we may ask: have these programmes been effective? Can the "new scientists" move easily from biological reasoning and experimental design of biological goals, to mastering in silico experiments and AI-driven data analyses?

The development of technology has become central to research, and may even grow to overshadow the purpose of a scientific question rather than providing a set of tools to develop it. Original ideas with true science-advancing power, disruptive rather than descriptive, have historically formed from the combination of two sources: the "humus" offered by the collective knowledge reached by the scientific community, and the focussed development in small groups, offering a compact, agile environment in which the fertilization, discussion and testing of ideas can be achieved

promptly. Let's take, for example, the "RNA interference" defence system of plants and its adaptation to silence unwanted genes, or the CRISPR-Cas9 system to edit gene sequences: both of these discoveries come from innovative ideas conceived in small teams, with tremendous applications for human health and biotechnology recognized with Nobel awards. At the scale of "systems" understanding needed to dissect complex processes, the unexpected, odd intuition that might generate a good idea may no longer find opportunities to arise.

As we become more aware of the biological complexity compared to the era of reductionistic approaches, but also of disruptive science by small groups, new funding issues arise. Large groups working on a large scale need correspondingly large grants. The issue is not just the need to find big investors, but also quality investors. Are funders going to be willing to invest large financial efforts on an expensive project, if it entails a fraction of a risky idea? Can public institutions afford them? What is the chance for an emerging scientist to access an opportunity to develop a good idea? These are some of the challenges that I think modern biology is going to face, that my generation has not been confronted with.

It is difficult to delineate plausible scenarios as the effort to grasp complexity (the "systems" level) builds up, and yet we need to keep track of our "human" intelligence. A beautiful, condensed article by Paul Nurse offers inspiring, stimulating thoughts, suggesting that paradigm shifts should be consciously considered to make sure that research is led by theory and knowledge (Nurse 2021): an article that all students and scientists should read and meditate about when thinking where research in the life sciences is heading to.

4 Advice to the Next Generation of Scientists

Biology is expanding in novel directions, gaining a reach it has never had before in health, reproduction, evolution, the environment, food, and in the use of biotechnologies for the benefit of society. Biology is one of the sciences called to address central aspects of our life on this planet (and maybe on others, as NASA and ESA programmes suggest). That sets the grounds for a wonderful, exciting time for new starters. The more we understand the better we can apply that understanding to challenge inequalities, both among individuals, among species and among areas of the globe. That can make a strong drive in wanting to do research in the field. Young scientists may be confronted with a broad range of new professions and will have chances to think about which path best fits their nature.

While preparing this chapter, I have travelled back through my own journey through research, recalling the knowledge I have been exposed to, re-weighting turning points, still resenting the inevitable mistakes, but reviving the feelings of expectation and excitement. The stories I have recalled (Drosophila heterochromatin, the inheritance of methylation of ribosomal genes, the fortuitous cloning of a RAN regulator by proximity with an unmethylated DNA island), have paved my research path and have given me the opportunity to project towards important biological

processes, with a long-standing focus on the RAN GTPase network in the cell cycle and mitosis. But that sort of approach would not be at the adequate scale today, as we become aware of biological complexity, as I have described in paragraph 3. A corollary is that researchers in biology will need to approach complexity with multifaceted skills, including collaborative and communication skills, superseding the romantic idea of the inspired scientist pursuing their little piece of knowledge. Because the practice of research in the life sciences is undergoing such transformation, it is difficult to give any advice to young scientists. But a couple of things remain true. First, I am convinced that the moments of joy and reward haven't changed in nature. Researchers still feel good when their experiment looks good, no matter how tiny a tile of a big piece it may be. If the feelings of stress and struggle prevail, then it may be worth reconsidering if you truly want to be in research. Research can be tough, full of repetitions, subjected to go astray any minute, stressful, so if you don't feel the reward of the beauty of the experiment, then there is little compensation, and in the long run it may become difficult to sustain it. Second, intuition has still a big part to play. My advice could sum up to that: cherish intuition. A good idea often grows from a well-formalised intuition. An intuition might come from anywhere, while listening to a seminar distant from your subject, or in a conversation apparently unrelated to your actual project. It may come from something you read, or come across unexpectedly, then sticks in your brain and eventually triggers connections you would not have rationally envisaged. To make a good connection that will generate a worthy idea, we need to find moments where we keep to ourselves—we need that moment of silent synthesis.

Both of these points may sound trivial, but if I distil my own experience, then I see that those two moments—conceiving an idea from intuition, and getting good-looking results—make the drive that renders all efforts worth doing.

I haven't touched yet on personal ambition, yet it has a role in research. It is very personal for each one of us. Ambition, like competition, has a dual value: it can either push us to try and do as best we can to gain more knowledge and understanding; or, it can push us to do best in order to prove ourselves and be recognized. These are two distinct scenarios. The best reflection I have found about this was offered by Robert Pirsig in his *Zen and the Art of Motorcycle Maintenance (Pirsig,* 1974*)*, a rather popular book in the 1970–80s. Without any idea of giving it any moral nuance—which would be out of place here—one type of ambition is ego-less, it is the ambition driven towards understanding something outside of me; the other type is ego-centered, it is the ambition to prove myself. There a mixture of both in every researcher, and each type of ambition may prevail under different circumstances for every one of us. I will quote Pirsig: to illustrate how the ego affects the quality of research, he uses a metaphor, describing the motivation for a pilgrimage to a holy mountain in the Himalayas.

> "He never reached the mountain.
>
> After the third day he gave up exhausted, and the pilgrimage went on without him. He said he had the physical strength but that physical strength wasn't enough.

> He had the intellectual motivation but that wasn't enough either.
>
> He didn't think he had been arrogant but thought that he was undertaking the pilgrimage to broaden his experience, to gain understanding of himself.
>
> He was trying to use the mountain for his own purposes and the pilgrimage too.
>
> He regarded himself as the fixed entity, not the pilgrimage or the mountain, and thus wasn't ready for it.
>
> He speculated that the other pilgrims, the ones who reached the mountain, probably sensed the holiness of the mountain so intensely that each footstep was an act of devotion, an act of submission to this holiness. The holiness of the mountain infused into their own spirits enabled them to endure far more than anything he, with his greater physical strength, could take [….]".

Now, Pirsig's view may appear naïve and clashes with the dominant demand that achievements be made, and within a defined time schedule. That description must not be taken literally, but it is worth accepting the suggestion to check, time and again, whether we are regarding ourselves as the fixed entity. We should avoid being blinded by worries about our next achievement, our performance, and our success. Those who think research is a quest to assess one's own value will be bitterly disappointed, because there will be many more hardships than success. As Pirsig put it, you have to climb the mountain selflessly, not to assert yourself.

Concluding with Jacques Monod

I have opened this chapter with Jacques Monod and, in conclusion, here he comes again, with one of the most profound remarks a scientist may formulate, accompanying the Obituary published in the *Nouvel Observateur* magazine (Serres, 1976) (Fig. 2): *"In science, self-satisfaction is death. Personal self-satisfaction is the death of a scientist. Collective self-satisfaction is the death of research. It is restlessness, anxiety, dissatisfaction, agony of mind that nourish science"*.

I couldn't close with a more intense remark, which I would like to dedicate to all those that are starting in research in the life sciences. Any additional word would spoil it.


Monod

SES REPONSES AU
- NOUVEL OBSERVATEUR -

• J'ai écrit, dans ma leçon inaugurale au Collège de France, au grand scandale des marxistes, que le langage a fait l'homme au moins autant que l'homme a fait le langage. Cela signifie que le langage a contribué à l'évolution physique de l'homme et pas seulement à son évolution culturelle. • [...]

• En science, l'autosatisfaction, c'est la mort. L'autosatisfaction personnelle, c'est la mort du savant. L'autosatisfaction collective, c'est la mort de la recherche. C'est l'inquiétude, l'anxiété, l'insatisfaction, le tourment qui nourrissent la science. •

• J'ai toujours pensé que le Nobel était plus malfaisant qu'utile dans la communauté scientifique elle-même mais il reste extrêmement important dans la communauté en général. •

• "Tout ce qui existe dans l'univers est le fruit du hasard et de la nécessité." Quand j'ai découvert cette citation de Démocrite, j'en avais presque les larmes aux yeux : penser que cela avait été écrit il y a 2 500 ans ! •

Jacques Monod au quartier Latin en mai 1968

Fig. 2 *Le Nouvel Observateur* issue of 07/06/1976, reporting one of the best Monod's quotes in a dedicated homage soon after he had passed away

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An Unexpected Journey: From Experimentalist to the Human Developmental Biology Resource

Susan Lindsay

Abstract The Human Developmental Biology Resource has enabled human development research and the understanding of congenital disease for over 20 years. I was involved in its inception in 1999 and ultimately became the Resource's co-Director for nearly 15 years. How did my scientific journey lead me to this position? I started my career as a research scientist in 1980, following the traditional pattern of Ph.D. then post-doctoral positions, initially studying the human X chromosome and searching for X-linked disease genes. By the mid-1990s, characterising and understanding gene expression patterns during human development was an important part of my work, partly because my searches for disease genes weren't fruitful and partly because of my interest in embryology. At the time, studying human embryonic tissues was an unusual thing to do: it was expected that animal models would provide the important answers. My colleagues and I, however, thought that investigating human development directly could provide key insights into human congenital disease. The difficulty was that human embryonic tissues required for this research are intrinsically challenging to obtain: raising ethical, practical and experimental issues. Thus, as my career progressed, I became more involved in establishing the human tissue bank which in 1999 became the Human Developmental Biology Resource (www.hdbr.org): a significant international resource that has been expanding and innovating for more than twenty years. Personal circumstances and funding possibilities contributed to the decisions leading me to this fundamental change in role: from making scientific discoveries to enabling researchers to carry out ground-breaking work which otherwise would have been difficult or impossible for them. Now at the end of my career, I see very clearly how valuable and how vital a part of scientific endeavour are service organisations which facilitate research by providing much needed resources and I'm proud of my contributions to HDBR.

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1 Motivations: How I Developed an Interest in Science

Biology was my favourite subject at school and this enthusiasm survived even the teacher who, during the lessons on reproduction, scurried from side to side at the front of the classroom crying "no questions, no questions". The teacher was male, and it was an all-girls school! My next biology teacher (Miss Stevenson) was excellent, answering lots of questions and sparking my interest in developmental biology and genetics, subjects which I took forward to university in Glasgow in 1976. Scottish undergraduate degrees have 4 years of study, which gives scope to try different subjects in the first and second years building towards an honours course in the third and fourth years. Although I'd studied genetics in my second year, the same year as introns were first reported, I chose zoology because developmental biology was a strong element in a wide-ranging zoology honours course.

I wanted to undertake a Ph.D., but I always had a whole range of scientific interests rather than one burning question. I also had a romanticised (i.e. highly unrealistic!) idea of what science is and what being a scientist meant: it was people who were "lone geniuses" working away in isolation and having a "eureka" moment or moments. Despite being uncertain about whether I could become a scientist, I decided to look for Ph.D. places and was accepted by Marilyn Monk at the UK Medical Research Council (MRC) Mammalian Development Unit at University College London (UCL). As often happens, my Ph.D. subject area changed: from investigating X inactivation in embryonic mouse germ cells to studying differences between human active and inactive X chromosomes, trying to find molecular mechanisms involved in X inactivation. This was fortunate as the technologies I needed to learn were at the cutting edge of molecular genetics, a fast-moving new field, and X inactivation is intrinsically interesting: why does one of the two X chromosomes in female mammals get switched off?; how is this one chromosome chosen and is it the same X in every cell?; how does it actually happen?

As I moved from Ph.D. to post-doctoral positions although I could construct a connecting theme, there was no one specific question or area that I was driven to pursue. Partly because of this and partly because of funding, my motivation was more in the day-to-day and the question or questions I was addressing in each project. I mostly enjoyed working in the laboratory although it was discouraging when experiments failed and it was unclear why, often leading to many rounds of troubleshooting! However, I also found excitement and even wonder during my experiments, such as the first time I precipitated human DNA and thought about the myriad of possibilities, opportunities and questions left to answer in this amazing gloopy substance in the test tube. I'm sure many scientists have had this moment.

2 Work Done: My Personal Scientific Approach

(1) The shift from experimentalist to resource director

I was fortunate to work with excellent scientists at many points in my career. They were also very different from each other which gave me several models of what a scientist is and highlighted many of the elements of what makes up "Science".

First steps towards being a research scientist

My Ph.D. supervisor, Marilyn Monk, was a highly individualistic scientist who was both meticulous and creative. She had a very particular way of approaching her research including a leap of imagination to place herself "in the cell or nucleus" aiming to see different perspectives. She was a mouse embryologist investigating the formation of the primary germ layers; the role of X inactivation in these processes and using the choice of inactive X as a marker for differentiation. My project was to look for differences between human active and inactive X chromosomes at the DNA level and, as part of this, to bring molecular genetic techniques from the National Institute for Medical Research (NIMR), Mill Hill to Marilyn's lab at the MRC Mammalian Development Unit, UCL. At that time (the early 1980's) I was learning very new technologies: using restriction enzymes (some of which I had to prepare from scratch!), Southern Blotting and DNA hybridisations.

One of the things I learnt from Marilyn was the importance of controls and that thinking about them helps you to understand whether the experiment you're doing is the experiment you intend to do! She had a variety of horror stories but one in particular stays with me to this day. On a visit she made to a laboratory where a Ph.D. student was explaining his project to her, she asked what happened when the petri dishes of different bacterial strains were placed under the UV lamp in a different order. The student realised that his data did not show UV susceptibility of different bacterial strains but rather the different strengths of UV output across the machine.

I studied differences in DNA methylation between active and inactive human X chromosomes. The picture was confusing and didn't fit with the straightforward hypothesis that increased global methylation was involved in gene or chromosomal inactivation. Over time it emerged that DNA methylation was one of the important mechanisms involved in X inactivation but that it was specific changes in specific regions that were critical. The DNA methylation field and the variability of results and shifting response to them showed me that often a new mechanism is hailed as *the* answer to a particular question, then exceptions are found and the mechanism is deemed not to be the answer at all. As evidence accumulates, a more nuanced understanding is reached of the complexity of the problem that the question is addressing.

First post-doc: still fascinated with DNA methylation

My first post-doctoral position was with Adrian Bird at the MRC Mammalian Genome Unit in Edinburgh. It was an exciting time to join Adrian's group as they

had just identified "CpG islands", a new and, as they discovered later, very common kind of promoter (Illingsworth and Bird 2009).

Paradoxically, although CpG dinucleotides are less common than expected from nucleotide composition, there are specific regions in vertebrate DNA where they are clustered and abundant, the CpG islands, and cytosine DNA methylation is key to both phenomena. In the mid 1980s, before the human genome sequence was published, finding genes in human DNA was difficult and laborious. The discovery that CpG islands were promoter sequences potentially gave a whole new way of identifying genes as some restriction enzymes could be used to find the clusters unmethylated CpG dinucleotides.

My project was to see whether identifying CpG islands in cloned DNA (where all DNA methylation is removed) was an effective way of finding gene sequences on the human X chromosome. Satisfyingly, it was in three out of the four clones identified (Lindsay and Bird 1987).

Adrian Bird embodied my preconception of a scientist, highly intelligent and focussed on his research but not, I soon realised, working in isolation. His team, including excellent technicians and research officers, as well as students and post-docs, made important contributions to the development of his work.

Second post-doc: from MRC to university

I worked in MRC units in both my Ph.D. and first post-doctoral position. At that time (mid 1980s), the MRC funded the research in units so individual group leaders generally did not seek external funding. This was beginning to change and for my second post-doc, Shomi Bhattacharya introduced me to grant writing which from then on was an integral part of my life. Shomi was based in Edinburgh at the MRC Human Genetics Unit and also at Newcastle University. When he moved to be fully in Newcastle, I went with him. While in Edinburgh, Shomi and his colleague Alan Wright were one of the first groups to use molecular tools to identify a chromosomal region where a disease gene was located (Bhattacharya et al. 1984). The disease was an eye disorder, X-linked retinitis pigmentosa, and positioning the locus along the chromosome, although a very major step, was just the start of the work. I joined the group as they were searching for new markers to refine the position of the gene and beginning the search for the gene itself. This was a very different type of project for me, particularly because family studies (following the inheritance of a disorder in relation to different alleles of a marker or markers) involved the human element of meeting family members who made the crucial contribution of genetic material (usually from blood) underpinning the entire project. It was also my first experience of research where inputs were necessary from people with very different expertise, including genealogists, clinical geneticists, genetic counsellors and bench scientists as well as close collaboration between staff in the NHS (UK National Health Service) and in the university. Shomi set up one of the early NHS molecular diagnostic laboratories which ran alongside his research group, and this proved a very fruitful model both for research and for translating research results into clinical tools.

A change of direction

In the early 1990s Shomi took up a professorship in London and, for family reasons, this time I didn't go with him. Tom Strachan then became Professor of Human Molecular Genetics in Newcastle and I continued with family studies and projects aimed at identifying disease genes involved in X-linked disorders. Although Tom's group and others in Newcastle were very successful at identifying disease genes for a range of disorders, my projects did not reach this goal. This was very disappointing but one of the approaches for screening possible candidates led to what was to become my main focus for the remainder of my career. Many genetic disorders have their origins during embryonic or foetal development and it was expected that the genes responsible should be active at relevant times and in relevant tissues during development. Tom and the head of Clinical Genetics, John Burn, set out the case for human embryo research (Burn and Strachan 1995) and they, along with Stephen Robson, an excellent, research-active obstetrician, and I collaborated to gain funding from Wellcome for a pilot project to collect human embryonic tissues for gene expression studies. The project started in 1996 and from the outset, we wanted to study mRNA expression. This meant that the tissues had to be collected quickly after the termination of pregnancy and processed with great care to preserve as much high-quality mRNA as possible. The success of our methods was clear from the reproducible and specific data gathered for several studies: for example of *HLXB9*, a major locus for Currarino Triad (Ross et al. 1998) and *SHOX*, which underlies some aspects of Turner Syndrome (Clement-Jones et al. 2000).

Genesis of the Human Developmental Biology Resource (HDBR)

Also in the early 1990s, a group at the Institute of Child Health (ICH), London, had a pilot project funded by the MRC to collect and carry out gene expression studies on human embryonic and foetal tissues. They, like us, recognised the importance of not wasting any of the tissues collected and so both groups provided tissues to other researchers. The complexities of creating our collections within a robust ethical framework and the increasing demand we foresaw for the tissues, led to successful applications for joint funding from the MRC and Wellcome, resulting in HDBR being established in 1999 (www.hdbr.org; Lindsay and Copp 2005). HDBR has been continuously funded by the MRC and Wellcome for more than twenty-three years. In London it has been led throughout by Andrew Copp and in Newcastle, firstly by Tom Strachan and then myself, when I took over in 2004 until I retired in 2018.

In the early years, the prevailing orthodoxy was that studying animal models (e.g. *Drosophila*, chick, mouse) would tell us everything that was important about human. Our work, for example on *SHOX* a gene that doesn't exist in mouse and others (e.g. Fougerousse et al. 2000) showed that this wasn't always the case. Furthermore, there was a very interesting change as the field moved from identifying genes underlying genetic disorders, to studying their function and to trying to find therapies. It became crucial to know precisely what happens in human (e.g. timing and site of expression and specific gene involved). As time went on, the importance of studying human development directly became much more widely accepted: for projects aimed at

understanding and developing therapies for specific diseases and, more generally, for understanding human development, particularly brain development, at a molecular genetic level.

(2) HDBR: major elements

Tissue collection, ethics and guidelines

Throughout my involvement with HDBR, first as co-investigator in Newcastle and then as HDBR Newcastle Director from 2004, the ethics of collecting and using the tissues were of paramount importance. Cultural sensitivities, legal requirements and practical considerations all make collecting human embryonic and foetal tissues difficult in many places and impossible in some. In the UK, the 1989 Polkinghorne Report set out guidelines for the use of human embryonic and foetal tissues in research based on the presumption that, if possible, using these tissues for research was beneficial. The main principles it identified were:

- the decision to terminate the pregnancy must be before and separate to requesting consent to donate the tissues for research;
- there should be no discussion of the specific research the tissue would be used for i.e. consent would be generic;
- the tissue should be anonymous and held by an intermediary body, separating the research team from the medical team caring for the donor.

From the beginning, HDBR has followed the Polkinghorne Guidelines, acting as an intermediary body between researchers and the medical staff caring for the donors. Consent was obtained by research midwives or nurses and in the early days, in line with Polkinghorne, gave very little information about the types of research that might be carried out. Following several scandals relating to human tissue and a general change in the UK public attitude to consent, the Human Tissue Act was passed in 2004, followed by the setting up the UK Human Tissue Authority (HTA; https://www.hta.gov.uk/) in 2006. The HTA regulates, licenses, and inspects a wide variety of activities concerning human tissues and produces codes of practice (e.g. code A—consent, code E—research) for guidance. The other crucial regulatory bodies are the Research Ethics Committees (RECs; https://www.hra.nhs.uk/about-us/committees-and-services/res-and-recs/research-ethics-service/) which scrutinise proposals for human research. From its inception HDBR has also had a Steering Group which has independent scientists as Chair and co-Chair and includes a lay person and, in later years, a legal expert with an interest in tissue banks.

Consent was a major issue and, as I outlined above, ideas of what was appropriate changed radically over the nearly 20 years I was involved with HDBR: from a position where providing essentially no information about the research was appropriate to a position where to be meaningful, donors had to be given some information about the research in order to make a decision. Generic consent was allowed but you had to indicate the types of research that might be carried out and specifically address any areas that might be sensitive or where donating tissue might have future consequences. One example of the latter, which was explicitly addressed in more recent

HTA and REC guidelines, was sequencing of embryonic and foetal DNA and RNA. Even though the tissues we collected were anonymous (HDBR had no identifying details about the women who donated them), as computing power increased and the programmes for searching and comparing sequences became more sophisticated, there was the theoretical possibility that the sequences from the foetal tissues and the woman could be linked: for example if the woman had donated a sample of her own DNA for sequencing to a private database (e.g. one of the ancestry-searching companies), along with her personal details and the security of that database was compromised. We altered the patient information leaflet and consent form to take account of this concern and others over time, but it was the skilled research midwife and nurse team who took the consents and answered questions who really helped women to understand clearly what everything meant. We were fortunate in HDBR Newcastle to have a senior research midwife, Allison Farnworth, involved over many years and her input to planning and governance meetings helped me, and my team understand and appreciate the reasons why women wanted to donate their tissues; the most common one given was so that some benefit might come from a difficult situation.

The importance of defining terms

At the beginning, HDBR Newcastle collected only embryonic tissues, from approximately three until eight weeks of development (Bullen et al. 1998). The embryonic period in human is divided into 23 Carnegie stages (CS) based on features that are present (e.g. during limb, eye, ear development), embryo size and age (O'Rahilly and Muller 1987). This is a critical period when all major organ systems form. Developmental age starts with the fertilised oocyte and is described as either post-conception or post-implantation. Something that can cause confusion is that obstetricians use the term gestational age which begins approximately two weeks earlier, at the time of the last menstrual period. There is further confusion if researchers use gestational age but mean developmental age! Another confusion arose with the new field of human embryonic stem cell (hESC) research. The term embryo was then often restricted to only the time when hESC could be derived i.e. 0–14 days of development and the period after that was called foetal development. We used developmental age with the embryonic period being 0–8 weeks. In the foetal stages that follow, there is significant growth and further differentiation of all systems. As for many aspects of science (and life!) it is always important to define your terms.

Keeping track as HDBR expanded

In the early days, HDBR Newcastle embedded most of the tissues it collected and provided tissues as sections on glass slides. This meant that, depending on its size, a single tissue could generate hundreds or even thousands of glass slides. It was clear that tracking all these slides would be very important and not an easy thing to do. We started with an access database for Newcastle samples and finally had a custom database built because, by that time, the details of tissues from both London and Newcastle were held jointly in the database, which was updated in real-time with information on the tissues (how, where and when they were collected, developmental

stage), how they were processed (e.g. wax embedded, frozen, DNA and/or RNA prepared), which project individual slides, tissues or other material were assigned to and when slides were returned. As you can imagine, this is a very large database as many tissues have been collected and processed into thousands of items and HDBR has contributed to more than 750 projects (Gerrelli et al. 2015).

A resource that provides services is very different from a research group. Shortly after I became HDBR Newcastle Director, I sought help and advice from Ann Curtis who headed the Molecular Diagnostic Service in Newcastle. She kindly showed me the rather daunting folders of standard operating procedures, risk assessments and policies for every aspect of the service. I hadn't thought about formalising our day-to-day activities in such detail but began to do so, with the help of my colleagues. It was an eye-opening process! Of course, we had experimental protocols but not, for example, procedures for recording version number or standard dates for review. We also didn't have any knowledge of how the services the university provided us with (e.g. computing, electricity supply) were risk assessed, backed-up or supported. Fortunately, by the time that information was required by the HTA, we were part of Newcastle Biobanks (https://www.ncl.ac.uk/biobanks/), which covered all the tissue collections in the Faculty of Medical Sciences and had, amongst other staff, a quality assurance manager who had expertise in many aspects covered by the HTA licence. So all we had to do was provide the information we had on HDBR's workings!

Collaboration is all: interactions between the HDBR sites and with researchers

At first the great majority of the material sent from HDBR Newcastle was to groups outside Newcastle. HDBR London, on the other hand, was initially set-up for research groups based in London to collect tissues directly from the HDBR laboratory and take them back to their own laboratories for processing. So at the start, probably because of the size of the "interested research groups" pool in Newcastle and London, the two HDBR sites had very different set-ups. Expertise grew on both sites and there were exchanges of ideas, protocols, and policies. By the time we had the joint custom-made database, both sites had extended their activities considerably and were operating jointly in much more standardised ways (Gerrelli et al. 2015).

Our interactions with many research groups went well beyond simply providing material (tissues/slides/cells/DNA/RNA/protein). Our knowledge of human development helped with planning which stages were appropriate to include for the specific questions the researcher was investigating. Some researchers visited the laboratories to show us techniques which we then used to tailor the material we sent for their experiments. We set up an in-situ service which carried out gene expression studies which was advantageous for research groups whose expertise was in other fields but who needed the gene expression data to add to their evidence, often for a publication. For us it meant we had the opportunity to capture images of all the data. As I will discuss in the next section, I felt very strongly that it was important to make publicly available all the data that we could.

An important long-term collaboration for me personally and for HDBR Newcastle was with Gavin Clowry, a researcher in Newcastle whose interest in cerebral palsy led him to study gene expression in the developing human brain, particularly the

cortex. HDBR Newcastle originally didn't collect foetal stages but began to do so in response to requests from researchers. We then had to gain the anatomical expertise for these stages as we had earlier for embryonic stages and Gavin worked closely with us to share his knowledge of the developing foetal brain. I collaborated with him on several of his studies of cortex development (Clowry et al. 2018) over a time when new technologies and wider access to human developmental tissues (with a major contribution from HDBR) enabled significant gains in understanding to be made by many groups both of key developmental processes and of the roles of specific genes in a wide range of disorders of the cortex (Molnar et al. 2019).

Nothing stays the same: new technologies and innovations

In the last decade or so advances in sequencing technologies have reduced the cost and increased the speed of sequencing both DNA and RNA, making large-scale projects feasible. I was keen that HDBR kept updating its services. One example was a collaboration to produce systematic RNA sequence data from different brain regions from approximately 4 to 17 post conception weeks (PCW). The datasets were deposited in ArrayExpress [now ArrayExpress in Biostudies) and the details and links are available from the HDBR website (https://www.hdbr.org/expression (Lindsay et al. 2016)].

The huge changes in the capacity for generating and analysing very large quantities of data and the increasing number of significant results showing human prenatal development as a critical time when many genetic diseases arise, have led funding bodies to support major research programmes on human development. In the UK, the MRC and Wellcome have been very forward-thinking and provide funding to large-scale programmes such as the Human Development Cell Atlas (https://www.humancellatlas.org/dca/) and The Human Developmental Biology Initiative (https://wellcome.org/press-release/wellcome-funded-initiative-unlock-secrets-human-development). HDBR is an integral part of both programmes, providing national and international researchers with material (e.g. Behjati et al. 2018).

How to capture, analyse and make image data public

DNA and RNA sequence data can be made public in relatively straightforward ways and there are a number of accepted repositories e.g. European Bioinformatics Institute (https://www.ebi.ac.uk/). For image data, such as those generated in many projects HDBR contributed to, it is more difficult as the precise location of the section or cell within the tissue is often important and not easy to specify consistently. This is even more difficult for developmental stages where there are large changes in shape, size and cell composition of organs over time.

I found the solution for HDBR in a collaboration with Richard Baldock and Duncan Davidson who co-headed the Edinburgh Mouse Atlas team (https://www.emouseatlas.org/emap/home.html [archive only now]) and had developed a gene expression database (EMAGE) and a suite of software for analysing and comparing gene expression patterns, including for mapping them to 3-dimensional (3D) models of each stage of development (Christiansen et al. 2006). James Sharpe, a member of their team, developed a novel method for generating 3D models of mouse embryos

(optical projection tomography, OPT; Sharpe et al. 2002). We were able to generate 3D OPT models from all stages from Carnegie Stage (CS)12 to CS23 (approximately 4-8PCW) which had a much higher resolution than any of the models then available (https://hdbratlas.org/3Dmodels.html).

My 3D spatial awareness is not strong and mapping data to 3D models helped greatly with visualising and understanding results generated in different experiments, particularly when we identified and "painted" anatomical structures in the models, initially for the developing brain in collaboration with Luis Puelles and his team in Murcia and later for other organ systems (https://hdbratlas.org/organ-systems.html).

The importance of an excellent team

Over the years I was supported by an excellent team in Newcastle. In particular Steve Lisgo, HDBR Newcastle's Resource Manager, was key to the success of HDBR from an early stage. Steve was involved in, and led many of, the changes, expansions and innovations. Amongst numerous other talents, his people skills help foster excellent working relations with HDBR London and the researchers around the world who use HDBR, as well as encourage and support the scientists and students who have been part of the Newcastle team over the years.

3 Science Today and Tomorrow

There are many different tissue collections and over the last fifteen years there has been a drive towards optimising and harmonising them as well as making it easier for researchers to find the samples they need for their research. In Europe in 2014, this had the logical outcome of establishing the EU Biobanking and BioMolecular Resources Research Infrastructure—European Research Infrastructure Consortium (https://www.bbmri-eric.eu/). BBMRI-ERIC's Directory enables researchers to find a biobank with tissue samples they're interested in, for instance from patients with a particular disease. It also provides tools and expertise to help biobanks e.g. with ethical, legal and social issues. I think it's likely that this drive will intensify, particularly alongside the trend in many countries to regulate human tissue banks (e.g. by the Human Tissue Authority in the UK).

For HDBR and other biobanks collecting human embryonic and foetal tissue, the major worry is the availability of tissues in the long-term. They are vulnerable to changes in legislation surrounding termination of pregnancy (such as has happened in the USA recently) as well as changes in clinical practice. Fortunately, new technologies, such as spatial transcriptomics (Williams et al. 2022) which HDBR now provides as a service, are enabling large quantities of data to be gathered from small samples, helping to make the best use possible where available tissues are limited. The development of human stem cells and methods to differentiate them into specific tissues have provided powerful tools for understanding disease causation and testing possible therapies. The gold standard is to validate stem cell differentiation against

what happens during human development (e.g. Collin et al 2019) and I can see tissues being requested for such studies for some time to come.

Our two databases are very important to HDBR. The tissue collection and project database I described above, although large, is a standard relational database containing text and numerical data. The gene expression database, on the other hand, is image-based and presents a much more difficult problem. It is a huge and expensive task to develop and maintain image databases where it is possible to search and cross-compare the data within them. We were fortunate to be funded by US NIH from 2002–2009 for the initial development of the human gene expression database and generation of 3D models and following that, MRC and Wellcome supported the continued mapping of gene expression data as part of HDBR. When Richard Baldock retired, the EMAGE database stopped being updated as did our database. All the images are now available on the Image Data Resource (http://idr.openmicroscopy.org/) which is an excellent repository but it is not set up for cross-analysis of data in 3D models. The Allen Brain Atlas has developed a Brain Explorer that allows these comparisons for their adult mouse brain gene expression data (https://mouse.brain-map.org/static/brainexplorer), however it is not yet available for their developing human data. I hope that technologies will evolve that make storing image data; identifying anatomical domains in 3D models and analysing multiple gene expression patterns within them much easier and more affordable. Funding for image-based gene expression databases is difficult to obtain but if the technologies genuinely allowed searching and comparisons in 3D space, with related anatomical domains, then I think that the substantial aid they provide to understanding complex spatial relationships would be a very strong argument for the significant funding that is needed.

4 Advice to the New Generation of Scientists

'Do what you're interested in' was my starting point but the carefully crafted CVs of Ph.D. student and post-doctoral applicants that I've seen suggest that much more thought is needed nowadays about where (in subject and place) you're aiming as well as knowledge of what might be needed (skills, additional qualifications etc.) to get you there. Fortunately, I also think there's much more help and information available now, from university careers offices to the websites of institutions and research groups you might be interested in. The latter often have "meet the team" short biographies of people at all different stages of their career which can help you to think about what might be needed.

It's likely that your scientific career will change over time so you will need to be prepared to train and retrain and continue seeking help and advice (a hopefully enjoyable experience!). I realised when I became a supervisor that it was very much easier to supervise someone who came to ask for help and said when they didn't understand or something wasn't working. In retrospect I think I must have been difficult at times to supervise as I often felt that I should tackle things by myself and, I'm sure, there was much less discussion than I certainly would have benefited

from. So, if you can, recognise what you find difficult, seek help, and use all the resources available to you to overcome these difficulties. Equally, testing your ideas and enthusing or being enthused by your peers and colleagues adds greatly to enjoying your work.

Don't be disappointed when things don't work out as you expect: this is often a pointer to the need to rethink your experiment and make some changes. At times it may also suggest that there is a need to change some aspect of your job or career aspirations. There are many ways in which failing at something is helpful and can have positive outcomes. I realise this is easy advice to give and I certainly felt failures keenly and often took them personally: it took some time to realise that this wasn't a useful way to think of them. It's hard, for example, not to be disappointed when grants are rejected. Some grant referees and grant bodies, however, are extremely good at helping you to understand how to make improvements. Grant-writing has also become much more professional and there are now many more resources to help you improve your techniques. Universities (like other organisations) are helping their staff to improve and strengthen their grants, recognising that this should improve the hit rate for gaining grants which is in their interests too. Being part of grant reviewing processes is very helpful in strengthening your craft and, similarly, reviewing papers improves your skills as a paper writer.

There are many ways of being a scientist and many kinds of contribution that scientists can make. It's helpful to be aware that your career is for the long term and will change over time, which can be a good thing. At different phases in your career the skills needed for your work will change, often in unexpected ways, so keep learning new skills. Working and collaborating with people from different disciplines also keeps work interesting and challenging. A lot of my work was with computer scientists which gave me new perspectives on many problems. Learning new technical terms wasn't so difficult but recognising when we were using the same term and meaning very different things was tricky.

Nowadays there are a multiplicity of careers for scientists: one person's ideas often need many people to help refine, test, and implement. As I found out, this includes scientists generating and running the gamut of resources that modern multidisciplinary science demands.

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Susan Lindsay obtained a first class honours zoology degree from Glasgow University (1980) and a Ph.D. from University College, London (1984). After a short time in a Los Angeles laboratory, she was a post-doctoral scientist at the MRC Mammalian Genome Unit and the MRC Human Genetics Unit in Edinburgh, before moving to Newcastle University (1990) where she was lecturer, senior lecturer and finally Professor of Human Developmental Genetics from 2006 until retirement in 2018. Research interests were in the molecular genetics and 3D modelling of the developing human brain and in leading a unique UK resource of human developmental tissues.

A Scientific Path to Global Citizenship

Fiorella Lo Schiavo

Abstract Here I tell the story of what has been my unexpected and exciting scientific journey that impacted my working life. In my case, entering the world of science has been due to a particular historic time, that has introduced my generation into an international context, both politically and scientifically that contributed to changes in traditional and local lifestyle choices of the youth. During my undergraduate and graduate courses in Naples, I participated in student movements stemming from international wars and civil rights battles. This experience was a turning point that allowed me to leave a traditional path and set a new course of life. At that time, in Naples, the International Institute of Genetics and Biophysics was attracting an international group of scientists, offering the Italian scientific community an opportunity to access modern biology. In this institute, I learned genetics, but not only that. In fact, I learned how to live, work and thrive in an international environment. This period represented a turning point in my life. Because of these experiences, I began my career by moving to other places to expand my initial biological knowledge and my cultural background. In Pisa, I had the opportunity to meet very good mentors that allowed me to make the final decision to be a scientist. It has been an incredible journey that I enjoyed very much and I hope that my stories, opinion and advice could explain why so and, at the same time, can convince many young people to consider this unique career.

1 Motivations: How I Developed an Interest in Science

TIME FOR A CHANGE! Being born in a family of lawyers, I was thinking of becoming a lawyer myself, following the family tradition. But at the end of the sixties, when I concluded my secondary school, times were changing very fast. I was exposed to and participated in student movements that changed perspective and renewed the lifestyle of my generation and of the whole society. A wave of novelty and modernization hit Italian universities in the wake of the student movements in

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America and Europe where these movements were born, particularly at Berkeley and in Paris. This experience projected my generation into a more international context where big political themes such as the Vietnam War and the battle for the conquest of civil rights were shacking the world, becoming part of every day's life.

In this new worldview, I changed my mind deciding to enrol in a STEM faculty. Physics was considered the gold option at that time but, even if I was a good student, I was afraid to enter in such a hard field. So the decision was to study Biology. My father was very happy with that, and my choice was considered at home the beginning of a new family tradition.

During my university studies I was very lucky, because at the beginning of the sixties (1962), Adriano Buzzati Traverso founded the International Institute of Genetics and Biophysics (IIGB) in Naples within the National Research Council (CNR) with the aim of organizing a centre for excellence in basic research on biology. The idea and the hope behind this initiative were to contribute to the enrichment of the Italian scientific community with modern biology. The laboratory was organized by putting together young brilliant Italian scientists returning to their country after gaining experience from abroad and adding visiting foreign scientists. Some of these scientists established new and modern courses at the university, particularly in biophysics and molecular genetics: completely new disciplines at that time. I had the opportunity to follow these courses and write my thesis in IIGB labs. In that international environment, I got in touch with "Big Science", so to say. At the beginning, everything was new and exciting. Living every day in contact with very famous scientists was strange but rather very attractive. Their life always in movement around the world to attend meetings and giving seminars in prestigious universities of different countries opened my mind to other ways of living. After the initial excitement, a couple of years of hard work started, that I would consider the time of my greatest cultural enrichment. I learnt how to carry on my experiments through a scientific method that became since then my way to address not only scientific questions but also to approach daily life problems. What a great teaching it was!

So, the approach to political issues and the knowledge of what big science meant can be considered the beginning of my adult life that let me come out of a tight provincial life to entering in an early globalised world. On the basis of these experiences, I decided to try to become a scientist. This choice represented the desire to belong to a community without borders in which people coming from different countries met together to address scientific questions with clear minds and exchange their results to let science and innovation move forward. From that time on, my curiosity was challenged and funnelled in solving scientific questions.

Being embedded into this Neapolitan environment and having as teachers the highly respected Paolo Amati, Marcello Siniscalco and Franco Guerrini, my background turned to be very strong in genetics. And genetics has become my way to address biological problems during my career. At the beginning, I started working on the regulation of a bacterial operon and on this I wrote my thesis in Napoli. Then, I moved to Pisa for a post-doctoral position, where I began to focus my interest on mechanisms involved in differentiation processes. To gain a better grasp of this field, I joined for one year Prof. Garcia-Bellido's lab in Madrid to address development

problems in *drosophila meganogaster* carrying on experiments strictly with a genetic approach. When I went back to Pisa, and in agreement with my head of lab Prof. Mario Terzi, I started my experience in the field of somatic cell genetics of plants that became the major interest of my scientific life.

Mario was very experienced in animal somatic cell genetics. However, his mentor and old friend Guido Pontecorvo was trying to convince some of the member of his group to move into the plant field. And I accepted this suggestion.

My expectations for the life of a scientist have not been disappointing. I had the opportunity to live in intellectually lively environments in different countries where I worked and established friendships with talented colleagues. These networks around the world provide scientists with a sense of belonging to a community beyond borders, devoted to a greater cause.

This represents a big privilege that is unique to this type of work. So if I have to summarize what I found in science in general terms I could say a *modus vivendi* that is a rational way to face life, in good and bad times…. and that is not a small thing!

If I had to choose an artist that could represent my experience of life, I would like to think of Matisse, a painter that began his career proceeding in the path of the traditional painting of his time. That was until he had as a teacher Gustave Moreau at l'Ecole des Beaux-Arts of Paris, who taught his students to stay out of the tradition in order to follow their own visions. Matisse was very innovative as a member of the group of the *Fauves,* but he kept his own soft style where colours played a very important role.

2 Work Done: My Personal Scientific Approach

BUILDING A CAREER, ONE STEP AT A TIME. My scientific life consists of a long journey that allowed me to land in many ports, starting from Naples, moving on to Pisa, Madrid, Nottingham, Berkeley, Potenza, and lastly Padua, where I've been for almost 30 years: an excellent safe harbour for long stays. It has not only been a physical but also an intellectual and emotional journey that has contributed to my cultural growth leading me to choose the themes I dealt with. I would like to start by saying that I did not focus on a unique, main biological question along my carrier.

In Pisa, at the "Institute of Mutagenesis and Differentiation, CNR", after my first experience on bacterial molecular genetics in Naples, I focussed my scientific interest on somatic cell genetics of plants, at that time a poorly exploited research field, that can be considered central to my scientific research interests. In this quite new field, I started by setting up procedures, already applied to somatic animal cells, to isolate carrot cell mutants, obtained spontaneously or after mutagenesis, looking initially for mutants resistant to cytotoxic action of drugs. As these cell mutants could regenerate the whole plant, this was considered to be a fairly rapid method of obtaining new useful variants. The other important use of these mutants was as selectable genetic markers for the selection of somatic hybrids after a fusion event, considered then a difficult task.

After that, I decided to take advantage of the unique features of plant cell cultures to be totipotent to address several developmental problems in order to uncover biological mechanisms controlling these processes. It was known that plant cell cultures originating from some species own the ability to regenerate the whole plant. This was done mainly through two different developmental pathways, either successive organ regeneration or somatic embryogenesis, a process resembling zygotic embryogenesis. In particular, I investigated the process of somatic embryogenesis in *Daucus carota* being many carrot cell lines able to produce millions of somatic embryos in liquid cultures, essentially free of undifferentiated tissues. In addition, in this experimental system it was possible to control embryogenesis experimentally to achieve synchronized development and uniform embryonic stages isolated *en masse.* These unique features of carrot culture made it the model system for this kind of studies at the beginning of the eighties of last century. On this basis, we decided to isolate temperature-sensitive (ts) carrot lines capable of regenerating plantlets at the permissive temperature but blocked in various ways at the non-permissive temperature. The aim was to attempt a temporal dissection of the development process. In fact, this type of conditional mutants was previously proved to be essential tools in the genetic dissection of development of organisms as diverse as *Drosophila, Caenorhabditis,* and *Volvox*. The characterization of some of these ts variants showed a different response pattern of sensitivity to the non-permissive temperature, allowing us to identify in this way distinct steps during the embryo process. When these results were published, we entered in contact with Renee Sung at Berkeley that was carrying out similar experiments in her lab. I moved to her laboratory to work together on this field, considered central in plant biology at that time. It has been an unforgettable experience! California was really another world compared to Italy. And at that time, my only experience abroad was Madrid in joining Prof. Antonio Garcia-Bellido's lab, where Spanish customs and habits resembled very much those of my country and, in particular, of Naples, my hometown.

In Renee's lab I joined the on-going research to continue our work on somatic embryogenesis. In particular, the comparison by two-dimensional polyacrylamide gel electrophoresis (PAGE) of cellular proteins from somatic embryos and unorganized proliferating cells allowed the identification of a number of embryo-specific abundant proteins. The remarkably small number of them was probably due to the fact that the genes responsible for embryo formation could be already expressed in proembryogenic masses during unorganized growth and, therefore, escaped detection in our analysis. After (what would be later remembered as) an epic workshop held in Italy in San Miniato in 1985 that gathered all people working on somatic embryogenesis from around the world, we started to collaborate with the group of Ab van Kammen and Sacco de Vries, from University of Wageningen. Together we were able to identify glycosylated extracellular proteins as essential for the differentiation of carrot suspension cells into somatic embryos. Consistent with these results, we showed that one of our previously ts mutants, in particular ts11, characterized by a block at the globular stage, was not able to perform proper glycosylation at the non-permissive temperature. This result indicated that the activity of certain extracellular proteins was essential for the transition of globular to heart stage of somatic embryos, making

this transition conditional on a correct modification of oligosaccharide side-chains of secreted proteins. Later on, from these proteins, a glycoprotein was purified, which allowed the completion of somatic embryo development of ts11 at non-permissive temperature, whose function was identified as a glycosylated endochitinase (Terzi M. and F. Lo Schiavo Somatic embryogenesis. In Plant Tissue Culture: Applications and Limitations S.S. Bhojwani ed. Elsevier, Amsterdam, 1990 p. 54–66).

After these studies on somatic embryo development, I consider one of my major contributions to the field, moving on the same path, I started to investigate the action mechanism of auxin, its perception and its effects on signal transduction in embryogenic cell cultures. Later on, I focussed my interest on the isolation and physiological characterization of potassium ionic channels and the definition of their functions during various carrot developmental stages. All the studies on somatic embryo development anticipated and opened the way to the successive work on embryo development in Arabidopsis plant.

Another subject I investigated with great interest by using cell cultures as model systems was the unveiling of the basic mechanisms of organ senescence and, in particular, the understanding of programmed cell death events as the final stage of cell senescence. Only more recently, in the last years of my career, I began to study the mechanisms of salt tolerance in a crop plant as rice and, in particular, the role of signalling molecules as calcium and ROS induced by abiotic stresses, in particular salt stress. These studies were carried out not only in plants, but also in rice cellcultures that represent my true passion and major expertise (Formentin E.,… Lo Schiavo F. (2018). Transcriptome and cell physiological analyses in different rice cultivars provide novel insights into adaptive and salinity stress responses, Frontiers in Plant Science, vol 9, p. 204).

This work on environmental stresses aiming to obtain resilient crops will be a challenging research field in the near future and for the decades to come, when high temperature, drought and salinity caused by climate change will significantly impact plant growth and development. Without new advances in research and biotechnology, the inevitable result will be a dramatic decline in crop yields. The effects of climate change need to be considered along with the increase in world population, projected to reach 10 billion by 2050, implying the necessity of a substantial increase in food production! So, more food is required in a sustainable way, in times of altered climate conditions.

I would like to conclude this section by saying that I consider myself a lucky woman because I had the opportunity to do a job that I have been liking so much since the very beginning.

3 Science Today and Tomorrow

HOW SCIENCE CHANGED OVER TIME. I belong to a generation of scientists that has taken part essentially to two different ways of doing science. At the beginning of my scientific activity, I was embedded in labs where the work was “artisanal”,

meaning that the groups were small, the experimental procedures quite simple and experiments carried out on a limited scale. Behind each experiment there was a lot of thoughts and the results required a lot of discussion. In simple words, it was more a theoretical than experimental work. During the years, many changes have taken place, in particular due to the introduction of innovative techniques in our field of cellular and molecular biology. The opportunity of whole genomes sequencing of various organisms, of identifying all the proteins present in a tissue or a cell, of knowing some categories of metabolites present in some organs of a plant, etc…, has implied a switch from the study of a single gene, protein or metabolite to an omics-based approach. These changes opened a new prospective either in the organization of science or in the type of biological questions to address.

In plant biology, the sequencing of numerous crop genomes together with the innovative technology of genome editing has opened the way for important applications in agriculture. In fact, we expect to see quality traits involved in plant resilience necessary to maintain and increase crop yields improved: a pressing requirement in times of climate change. We can foresee a different way to take up farming that will be tailored to the species, sustainable, smart and ready to feed the ever-increasing population in future years. What a big challenge for the young generation of green scientists!

All these new techniques require different lab organizations to allow scientists to switch from an artisanal to a large-scale work organization. To do so, investments not only from a single grant group, but also from institutional resources were needed to build common platforms that allowed all members of a given department or a research centre to have access to them. As I mentioned above, this has given (and continues to give) the opportunity to ask new biological questions but, beyond common platforms, it has implied development of new multidisciplinary approaches. In fact, the immense amount of data coming out from omics- based experiments require bioinformatics knowledge and the integration of several different competences in order to answer the new biology questions.

The integration of different knowledge that is allowing a fast-moving progress in biology can be applied also to other fields, in particular, to some areas of engineering as robotic, home automation, artificial intelligence etc… that are providing incredible achievements nowadays able to quickly transform our daily life and behaviours. A new way to move science forward!

All these accomplishments are derived from development and advancement in basic research as results of many years of hard work based on tight international collaboration inside the scientific communities.

We experienced all this during the recent Covid-19 pandemic. A tight collaboration among labs, along with generous public economic support, have contributed to develop a vaccine at breakneck speed and to collect information on the diseases so that new drugs to fight this new virus could be developed in short order.

Another important aspect I would like to underline here is on how the role of science recently has been changing in our society.

As a scientist I have taken part for most years of my career to an international inclusive scientific community born after the Second World War. In this community,

members from various countries were able to gather in scientific meetings in good as well as in difficult times when geopolitical divisive events happened, such as the Gulf War, the attack on the Twin Towers, and so on.

As a matter of fact, the scientific community, owning a unique language, represented and still wants to represent a meeting place above the parts, able to build bridges never walls, seeking to advance a vision of a human society that evolves towards models of solidarity and sustainable development based on international collaboration and multilateralism, with what is defined "scientific diplomacy".

In recent years all the innovative technologies, coming out as application from basic research, have a geopolitical interest, particularly for the most powerful countries in the world, as never before in recent human history. The economic competition among countries is increasing very fast and in the present times technological achievements play an essential role for geopolitical supremacy. All this has implied in the last years a reduction, when not an abrupt interruption, in scientific collaborations and a change in the usual atmosphere present in labs all over the world for the fear of scientific espionage.

The model behind the community of scientists draws a freedom space without borders that implies freedom of thought, expression, and gender equality that should be a model for our societies.

In reality, like never before, now is the time for the scientific community to be of fundamental importance to play the role of keeping open all the channels of communication to allow a dialogue for solving the big crisis we are living in these days. This *super partes* community has the duty to help solve contrasts between nations, when my generation is experiencing for the first time a war in the middle of Europe.

The scientific community is called upon and has the responsibility to provide all its capability to help countries to overcome difficulties and re-establish bridges, breaking down the walls where they have been built. And I retain the hope that this will be done!

4 Advice to the Next Generation of Scientists

GIVE IT A GO! I would strongly recommend to a young person to enter into science today.

In this session I will try to explain why. Entering into science and becoming a scientist represents a big privilege because of the uniqueness of this type of job. Understanding deeply how to apply the **Scientific Method** is not only a *conditio sine qua non* to do this job, but it becomes a personal way of dealing with life. Under this point of view, entering into science is a totalizing choice, in fact, it is a *modus vivendi.* It is a job requiring great dedication, being not an office job with a precise schedule, but an all-encompassing experience in which the mind is always connected with daily problems, either scientific or academic. On the positive side, this job can provide great satisfactions. Nothing is better than unveiling a new mechanism or a

process that you are investigating for a long time. Discovering something new in the field of interest is emotional and priceless. It introduces each scientist in the long arc of scientific discoveries, providing a deep sense of participation to important events of humanity. Even if discoveries are not revolutionary, each one feels that can give a small contribution to the collection of human discoveries. In fact, all of us should not forget that scientists build on the work of many who have come before. It is a bit like a relay race. Each scientist does a little part and the next generation of scientists have to pick up the baton and do their part of the race.

At least two features are required to become a good scientist: curiosity and humility. Curiosity for natural surroundings is the first and perhaps the most important feature. It is an important behavioural trait that has to enlighten the whole life of a scientist, helping to keep an active mind that allows to always think of new things. In addition, to be humble is a categorical imperative, considering how little we know and how much there is yet to discover. Science teaches scientists to be humble and this will be another behavioural trait that should be adopted in all aspects of life. A prerogative and general scientific teaching is to make decisions on the base of consistent scientific data. This can be considered obvious in the world of science, but it is of fundamental importance when critical daily life problems have to be faced. A clear and recent example of this is how to behave in pandemic times, in which a rational way to proceed is of great help. Another important teaching coming from practising science is to recognise when some thoughts are wrong. After testing with experiments and hypothesis, if the results are against the initial idea, the hypothesis is ruled out and another one is coming out and new experiments will be performed to test it. By doing so, a scientist will always have an open mind to new hypotheses to test and, as a consequence, no prejudices can harbour in its mind. An open mind is required for doing science but also to face and enjoy daily life.

To be introduced and continue in this job, good mentors are of paramount importance. In fact, they play a role not only in the cultural growth of their students, but also letting the best qualities of a student emerge, by providing tailor-made advice to them. I would like to suggest to all the young people that will try to embrace this job to be ready to change as many times as necessary their initial place until the right mentor and the right area of interest will be found. It is unlikely that you will find what you are looking for at the first try! Don't be afraid to change all the times you feel like if you are not working with the right people, or you are not embedded in the field of interest you are looking for.

I owe my entire career to the guidance of a number of people that I have had the luck of meeting during my scientific life. I would like to mention here the ones that have played a fundamental role as Vittoria Nuti Ronchi and my main mentor Mario Terzi, I met both in Pisa, that really helped me in making my decision of entering science. Another important person has been Renee Sung of Berkeley University, where I spent an unforgettable post doc and where I became aware of what it really meant to participate to an international community of scientists.

Now that I am practically at the end of my scientific journey, I can say that I consider myself a lucky woman because I have had the opportunity to work a job that I liked so much since the very beginning, and I still love very much. Many

colleagues and friends met in all these years contributed to this fantastic journey. I consider all of them excellent travel companions that contributed in different ways to my career.

I hope that these thoughts and comments can help the youth to pursue a career in what I consider one of the best jobs ever.

Fiorella Lo Schiavo Present position: Senior Professor of Plant Physiology, Dept. of Biology University of Padua. Initially the research focus was on plant somatic cell genetics studying embryo development in carrot cell mutants. Later, research interests turned to the study of potassium ionic channels and their functions during plant developmental stages. Then, the basic mechanisms of organ senescence, in particular programmed cell death using cell cultures as model systems were investigated. Recently, the role of signalling molecules as calcium and ROS induced by abiotic stresses in model plants and in a crop as rice was investigated.

Chemistry or Biology: That Is the Question

Enzo Martegani

Abstract My first interest was for chemistry. I was fascinated by the possibility to make simple experiments with reagents commonly used in any house or readily available in a drugstore or in groceries such as baking soda, vinegar, bleach, ammonia, slaked lime, iodine, quicksilver. I also tried some electrochemical experiments with low voltage batteries and salt solutions obtaining the electrodeposition of copper, zinc or water electrolysis. Then I "discovered" organic chemistry with its almost infinite possibilities to generate new compounds with a marked interest for naturally occurring molecules like alkaloids, plant pigments and dyes, and natural flavors like terpenes, organic esters etc. Subsequently I realized that the most complex and sophisticated chemistry was invented by nature in the generation of living organisms. Living organisms in fact use organic chemistry to make an incredible variety of compounds and to construct the macromolecules (DNA, RNA, protein) and lipids that working together generate the complex phenomenon that we called "life". At that point my choice was made and I decided to study biology with a focus for biochemistry and molecular biology and this opens the way to a long career in research laboratories and universities.

1 Motivations: How I Developed an Interest in Science

The beginning: I don't remember when and why I got interested in Science, but surely it was a very early predisposition related to a curiosity and a thirst for knowledge that occurred very early, probably during the period of primary school (6–10 years old….). I was fascinated by nature, by flowers and plants and I remember our old teacher (a nice woman of about 55–60 years old) took us out of the classroom in spring time and brought us to green fields showing the blossoming of flowers and the buds on tree branches ready to open for a new beautiful season. My interest in biology increased when my parents gave me as gift a small microscope (really

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it was a toy, not a serious one…) but at that time (I was about 10 years old) was a continuous discovery of new wonderful things, like the different type of pollen spores, the delicate structure of flowers, small insects etc. These early observations were also documented by hand-made drawings to record my observations. However at the end of the primary school my interest in science widens to the other scientific matter like physics, chemistry and astronomy with the help of books provided by older relatives. During this period (between 10 and 14 years old) I became deeply interested (may be even fixated) in chemistry and not only I tried to do small experiments in my kitchen, but I studied also basic stoichiometry laws and calculations. My obsession for chemistry took me to choose as a secondary school a technical one (Chemistry, really I graduated in Nuclear Chemistry in 1969) (Fig. 1). During this period I continued to do experiments in the kitchen sometimes with hazardous results, like the synthesis of iodo-acetone (a strong tear gas), or experiments with explosives that once gave fire to the kitchen table! But the real turning point occurred when at the age of 17 I read a book of Biochemistry (The Biochemical Approach to Life, by Frederic Jevons, Edizioni Scientifiche e Tecniche Mondadori, 1965). I was fascinated by the complexity of life and realized that chemistry was relevant for living things so that it would be better to study Biology and Biochemistry instead of Chemistry alone. And this actually happened: I got a Master degree in Biology at the University of Milano, with an experimental thesis in Biochemistry followed by a specialization in Biological Research.

Biology became my principal interest and was relevant for all my working life, but I had also interest for other more technical things, like the understanding and the realization of electronic devices (audio amplifiers, radio receivers and transmitters, oscillators, etc…) and I remember that during one summer I read and studied books on the theoretical basis for working with Radio and Television equipment and performed several radio transmission experiments with home-made radio-frequency generators. Later I was also interested in computers and in coding, initially in the lab where we had the fortune to have a Digital PDP-1 computer (just at the beginning of ‘80s) and then also in my home with the Commodore VIC-20 and several personal computers. In the free time I was (and I am still now) also interested in astronomy (I have an 8-inches telescope) and in playing bass guitar and/or keyboards in a local band.

2 Work Done: My Personal Scientific Approach

Don’t care about money. Although my family was typical working-class people, I have never been interested in accumulating wealth as such but instead always tried to do a job that would stimulate my thirst for knowledge and give me intellectual satisfaction. This was the basis of all my work focusing on experimental research activities without disdaining theoretical-speculative activities associated with a university teaching function. As a result I have been teaching biochemistry and molecular biology for more than 40 years through the whole career development: fellow, assistant professor, associate professor, full professor. Taking an average of

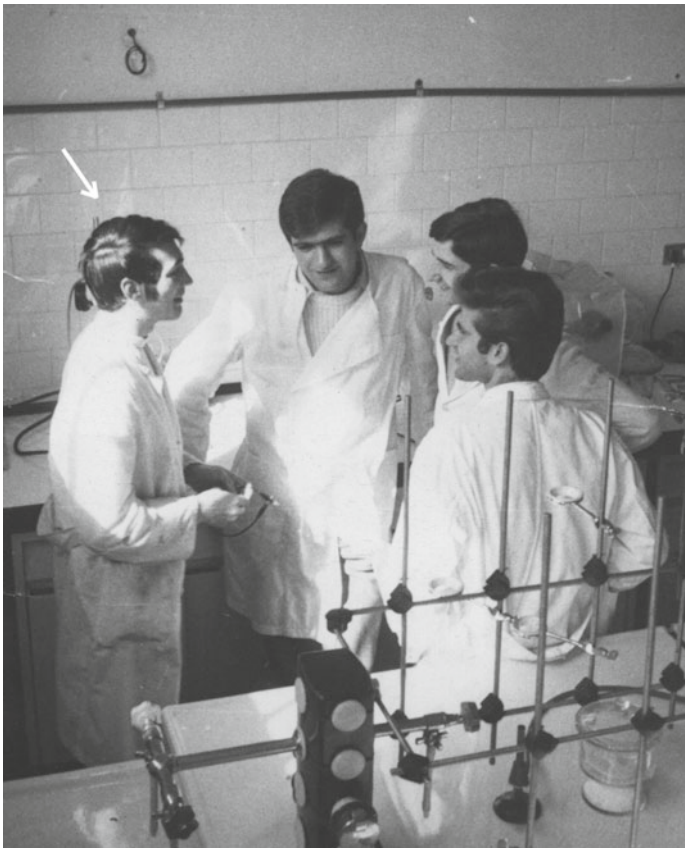

Fig. 1 ITIS-Stanislao Cannizzaro, Rho (MI), Italy, 1969. A photo taken in a laboratory of Chemistry during a discussion with classmates. The author is marked by an arrow

200 students/year, I have taught to more than 8000 students over my career. Of course teaching occupied only a fraction of my working time and most of the time was occupied by research activities done in the laboratory and also at home. The work of a scientist (or of a researcher) is not really a job but a way of life. Your work does not end when you leave the laboratory but continues outside since it is often not possible to stop to think about your experiments and your positive or negative results. It is a sort of continuous occupation and some of the more interesting and positive products from this thought process were originated by ideas generated in this way. It could be after dinner or before going to sleep, or even during summer holidays!

In the course of my experimental work I followed many fields of research that however could be grouped in almost three different topics: (1) Molecular mechanisms that regulate growth and cell cycle in eukaryotes; (2) yeast biotechnology; (3) systems biology and modeling.

The first topic is one that gives me major interesting results with also an unusual jump from budding yeast to mammalian brain. This story started with the biochemical characterization of a temperature-sensitive (ts) cell cycle mutant of budding yeast called *cdc25*. This mutant showed and interesting phenotype: when transferred to restrictive temperature (37 °C) it stops growth in a way resembling the lack of nutrients, suggesting that the principal defect was in a mechanism that links nutrients sensing to growth regulation (Martegani et al. 1984).

With the effort of my young colleagues David Baroni and Gianni Frascotti and under the supervision of Prof. Lilia Alberghina we cloned the *CDC25* gene of *S. cerevisiae* in 1985 (Martegani et al. 1986). This gene was then found to code for a 180 kDa protein that activates the two Ras proteins (Ras1 and Ras2) present in budding yeast. In fact this protein was the first GEF (Guanine Nucleotide Exchange Factor) identified and its activity was found to be essential for the activation of adenylate cyclase and of Protein Kinase A that in turn regulates growth of yeast cells. In the following years we studied the regulation of this pathway, (now called cdc25/Ras/cAMP) and several other GEFs were discovered in lower eukaryotes (*S. pombe*, *K. lactis*, etc…) all characterized by the presence of a well-defined and conserved RasGEF-domain (or CDC25 domain) of about 250 amino acids that is responsible of the interaction with Ras proteins and catalyzes the exchange of GDP with GTP, thus generating the active form of Ras (Ras-GTP bound). At that time however, no Ras activators have been identified in mammalian cells, although it was well known that mammals have different form of Ras proteins (h-Ras, K-Ras and N-Ras) that are involved in the control of cell growth, differentiation and tumorigenesis. A relevant fraction of human tumors showed activating mutations in a Ras protein. Starting from this observation we were convinced that GEFs should be present in mammals and we decided to use yeast as a tool for identification and cloning of mammalian GEFs. Using limited resources and the effort of many students, we made a cDNA library in yeast expression vectors using RNA extracted from mouse brain. We chose the brain since the nervous system is known to be rich in h-Ras. The library was used to transform a *cdc25* ts mutant strain of budding yeast, and we recovered clones able to grow at the restrictive temperature (37 °C).

These clones were characterized and we found that all of them contained a mouse cDNA responsible for complementation of the *cdc25* mutant defect. This cDNA was sequenced and found to code for a protein containing a well conserved CDC25 domain, that we called Cdc25Mm (Mm for *Mus musculus* the scientific name for mouse…) (Martegani et al. 1992), now renamed RasGRF1. This was the first mammalian Ras activator to be found and identified. My interest for regulation of small G-proteins continued in subsequent years with several works done either in budding yeast (Fig. 2) and in mammalian cells and with some interesting efforts to develop small inhibitors for Ras protein activation in collaboration with colleagues organic chemists (Peri et al. 2005).

The interest in yeast biotechnology was a relevant follow up of the necessity to use molecular biology and recombinant DNA technology in yeast as an obvious improvement for studying mechanisms of signal transduction and growth regulation in *S. cerevisiae*. Indeed this aspect was also supported by the necessity to develop

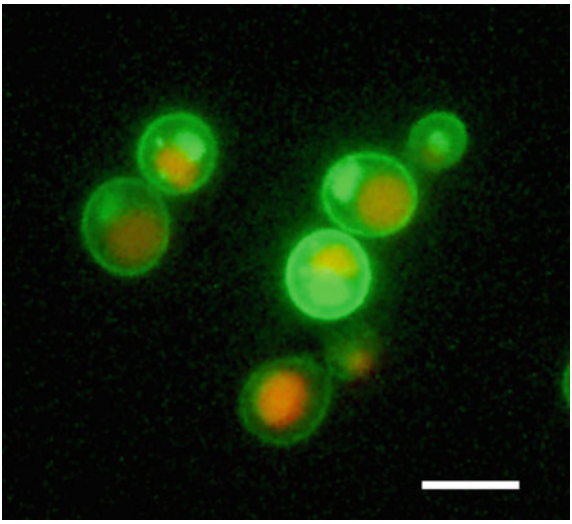

Fig. 2 Localization of active Ras (Ras-GTP) in budding yeast cells. Yeast cell were transformed with a plasmid expressing a fusion protein between eGFP (enhanced Green Fluorescent Protein) and three Ras binding domains (RBD-3) (Broggi et al. 2013). The fluorescence microscope image shows the localization of Ras-GTP in the cell periphery and in the nucleus (green fluorescence), while the vacuoles are evidenced by a red fluorescence. Bar = 5 μm

Biotechnology in Italy and we had several specific grants by the CNR (National Council of Research) on this topic. To learn the basic techniques for recombinant DNA I spent a few months in the laboratory of Prof. Vittorio Sgaramella at the University of Pavia (a pioneer of recombinant DNA research in Italy) and then a short period in the autumn of 1981 at the University of Sussex in Brighton (UK) in the laboratory of Dr. Paul Nurse to learn the basic methods for yeast transformation (https://www.nobelprize.org/prizes/medicine/2001/nurse/biographical/). The more interesting results of this research line were the expression in yeast of several heterologous proteins, like maize zein seed protein, maize B32 endosperm albumin, beta-galactosidase, human tissue-plasminogen activator (tPA), the development of computer controlled fed-batch fermentations and the generation of engineered strains able to grow and produce ethanol on cheese whey or to produce lactic acid by fermentation.

The development of mathematical models for cell growth and cell cycle was due to a singular coincidence of interests and events that occurred after my graduating in Biological Sciences, in July 1973. A few months after my Thesis defense I started for a obligatory military service, and I used part of the free time available to study System Theory and Dynamics using an universitary handout with a friend of Napoli. At the end of military service I had a fellowship of CNR for working in the lab of my thesis supervisor, Prof. Lilia Alberghina who was also very interested in System Theory and mathematical modelling of cell growth and I was immediately involved in developing this topic. We used a computer simulation approach (with a mainframe computer facility at the University of Milano) to model the dynamics of growth and cell division in eukaryotes and in bacteria, indeed this was a side-work since my primary activity was to stay in the laboratory to study growth, RNA synthesis and protein turnover in the filamentous mold *Neurosposa crassa.* In the following years Lilia Alberghina started a collaboration with Prof. Luigi Mariani, director of

the Laboratory of System Dynamics and Bioengineering at the CNR (LADSEB) of Padua that give us a strong mathematical support for the development of cell cycle models and modeling of yeast populations in the period 1980–1989 (Alberghina et al. 1986). After that my interest in modeling and computer simulations weakened but it was still present for several years to further re-emerge when a new collaboration with colleagues of the Department of Computer Science of my University started in the period 2005–2015 with the development of a comprehensive model for the Ras/cAMP/PKA signal transduction pathway in yeast.

3 Science Today and Tomorrow

Has science moved from an artisanal to an industrial dimension? That's not true at least for biology. The industrial dimension is especially relevant for technology which is an application of known knowledge but not for the true discovery which is "curiosity driven" and subject to serendipity, therefore in itself not predictable or industrializable, but reserved for the thought and genius of the individual researcher and obviously to chance. The discovery of new phenomena in biology, their understanding and use has also recently occurred thanks to the intuition of individual researchers and without the need for stratospheric economic resources. Just think as an example to the discovery of the CRISPR-Cas bacterial systems that have revolutionized our ability to modify eukaryotic genomes (including higher plants and animals) in a simple and inexpensive way, within the reach of any small laboratory (Lander 2016). Therefore, I believe that in biology the craftsmanship dimension is necessary to truly arrive at new developments that increase our real understanding of the "life" phenomenon. Does this mean that biological research is cheap? Not always there are some aspects that require substantial investment of materials and time and that allow the generation of large amounts of data (like large genome sequencing, metagenomics, transcriptomics, and all omics "sciences" in general). This type of research can be industrialized but it is a routine research that generates data and resources (a lot of data) but not a true knowledge of the mechanisms and causes! It is certainly useful for biomedical and/or biotechnological applications but in my opinion, it leaves little room for revolutionary ideas that change the way we understand living systems. Obviously in other scientific fields the new discoveries, and therefore the expansion of knowledge, are often linked to expensive technological developments that can only be addressed at a national or transnational level, see for example nuclear and subnuclear physics or discoveries related to space and to the exploration of solar system and extrasolar planets.

In the course of the last 50 years a real revolution occurred in Biology and I had the choice and also the opportunity to live and work in this straightforward period. The breakthrough happens with the "discovery" of the recombinant DNA technology that open the way not only to a better understand of the basic mechanism that generate "life" but also to a possibility to directly modify the code and to enter in the "button room" of a living organism as stated in meeting by Renato Baserga,

an Italian scientist that worked for a long time in Philadelphia (Baserga 2006). Now we have the complete sequence of the genome of many relevant organisms, and among them the human genome. These data are freely available in the Genomic Data Banks (GenBank, Ensemble, etc.) but these sequences tell us that something of relevance is lacking in order to achieve a real understanding of the information presents in these genomes. For example consider two relevant mammals, human and mouse. The genome of both was completely sequenced, both genomes encode for a similar number of proteins (about 20,000 protein coding genes), the human and mouse proteins are very similar (more than 95% of similarity in most cases), both genomes contain a high proportion of repetitive sequences (LINE, SINE, ERV, DNA transposons), the size of the two genomes is comparable (around 3 Giga-bases), but where is the difference? Why similar genomes generate so different organisms? What are the key differences in terms of genome encoded information between a mouse and a human being that are causally linked to the final outcome of two distinct species? We don't know but the deep understanding of the genome information and how it is decoded is still a frontier of our knowledge!

4 Advice to the Next Generation of Scientists

Accelerate a path of independence and seek for a position in an Institution (University or Research Center) well equipped with shared instruments and up to date facilities. This will allows a young Ph.D. student and/or post-doc to carry out good research without having high budgets. This can also be available in Italy, but the real frontier is the world and therefore it may be useful or even necessary to move abroad where the selection of proposals and personnel is more based on merit and where perhaps there are more opportunities to make a good start to a career in prestigious laboratories, perhaps under the mentorship of established scientists (not necessarily a Nobel Prize winner). This would allow you to gain good experience, learn new techniques and obviously have good publications in journals with a high impact factor that will also be useful in view of a possible return at home.

However, do not be a slave to the Impact Factor when striving to publish a good work. It is important to publish in international journals, with peer review (https://www.nobelprize.org/impact-factors/), and try not to follow fashion, but your inspirations and ideas that initially might be considered extravagant or not "fashionable" can at the end be a passport for new discoveries. Think of now famous scientists who have been refused the publication of their work in prestigious journals: just to give an example to Hans Krebs who saw his fundamental work on the discovery of the citric acid cycle (later called the Krebs Cycle) rejected by *Nature* and then published in *Enzymologia* (Krebs and Johnson 1937). Or to scientists who have had the recognition of their discoveries only many years later like Barbara McClintock (McClintock 1951), (https://www.nobelprize.org/womenwhochangedscience/stories/barbara-mcclintock). The publication in an international journal guarantees you the authorship of an original idea that, if valid, sooner or later will be recognized. For additional information on this point see also the paper of Stephan et al. (2017).

Another important point for a researcher is the ability to see what others do not see, often new discoveries or new phenomena are available but few are able to grasp the meaning, just think of Alexander Fleming, many researchers had plates polluted by molds, but they were usually thrown away without further investigation, (Fleming 1929) (https://www.nobelprize.org/prizes/medicine/1945/fleming/facts/) or of Watson and Crick, the double helix was there, there were the diffraction images and biochemical data, but only they could see it (Watson and Crick 1953). It is important to know how to seize the opportunities that are offered to us while maintaining a great curiosity and an open mind on wide horizons. Research activity often forces us to overspecialize in a specific sector and topic, but it is necessary to expand our knowledge even on fields apparently very distant from the specific topic we are working on. This will allow us great flexibility and the possibility to imagine and understand interesting events and phenomena that might otherwise escape our appreciation or be underestimated.

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Enzo Martegani Born just at the half of past century (1950): graduated in Biology at the University of Milan, Italy in 1973 and then acquired a Specialization in Biological Research (University of Milan, 1975). Working activity: Fellowship from National Council of Research (1974–75), then Assistant professor, Associate professor and Full Professor of Molecular Biology (1994–2021, University of Milano Bicocca), retired in 2022. As researcher initially studied macromolecular syntheses and growth regulation in *Neurospora crassa*, then regulation of cell cycle and signal transduction in budding yeast and in mammalian cells using biochemistry and molecular biology. He taught Molecular Biology for almost 40 years to graduate students.

Living is Searching

Peter Nick

Abstract The contribution uses the scientific career of the author to demonstrate one main point: questions are more important than answers. Questions help us to transform information into knowledge and questions are the raw material for scientific explanations. When we are young, we ask questions, later we get accustomed to giving answers. Therefore, the more important part in the life of a scientist are the beginnings. The scientific life of this author began with the childhood question, how plants think, even though they do not have a brain. In the retrospect narrative inspired by this book project, the author finds out that this childhood question has been shaping his entire scientific development, although it adopted different shapes. This inner development is narrated in the context of a professional career that started from a small village in the German mountains over studies in Freiburg and St. Andrews, being confronted with a different mindset during a postdoc in Japan and a road full of obstacles till becoming a full professor in Karlsruhe. Since this chapter is mainly thought for researchers in the early phases of their career, the part of becoming is given in more detail than the part of being. What was important to keep going? What type of mentors one should look for? How to balance professional dedication and private life? The chapter ends with some conclusions as legacy for the next generation to encourage them on their path.

1 PROLOGUE IN VENERATION OF XENOPHANES: Why Questions Are More Important Than Answers

I spent a free, wild, and happy childhood on an ancient castle in a tiny little village in the Allgäu, a mountainous and remote region of South Germany. A long and intricate history had left many remnants that were allowed to wither in dignity and pittoresque decay, hiding numerous secrets and wonders that inspired my vivid imagination. One of our favourite games was "explorers", which meant that we took some candles,

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wooden swords (in case that we would be attacked by rats), and a little crucifix (in case that the attack would come from other worlds) to find our path through the numerous passages that had been driven into the castle mound. With a lot of adrenalin and inspiration, we eventually managed to establish a map of this subterraneous labyrinth and the day arrived, when we had found out everything. I still remember the deep disappointment when I got aware that our expeditions had eliminated all mysteries of the castle.

Many years later, I was a young student of biology in Freiburg then, I was taught by the impressive and stern Hans Mohr, a reputed plant physiologist, that science only advances by falsification of the hypotheses, we had first established with a lot of effort to explain a phenomenon. I did not know about Karl Popper then but needed some time to digest this harsh message and asked one of my other teachers, Rainer Hertel (one of the founder fathers of auxin research), whether there were no alternatives, more positive ways to find scientific truth. He smiled at me through his thick horn glasses that were already outdated those days and asked me back, what I thought, how *Escherichia coli* found the "truth", which in that case would be the same thing as a food source. I remembered, how he had explained to us, that this poor little thing, too small to perceive a chemical gradient at once, because the concentration differences between front and rear of the cell would be overwhelmed by Brownian fluctuations, uses a different approach: swimming straight, as long as the concentration of the attractant increases and searching a new direction by tumbling as soon as a drop in concentration tells that it had swum offtrack. He waited, until the thought could form in my mind and then said—"see, this is, what Popper wanted to say". In the next session, he introduced me to the word by Xenophanes which runs, in the translation by Popper himself (1998):

> The gods did not reveal, from the beginning,
>
> All things to us; but in the course of time,
>
> Through seeking we may learn, and know things better.
>
> But as for certain truth, no man has known it,
>
> Nor will he know it; neither of the gods
>
> Nor yet of all the things of which I speak.
>
> And if by chance he were to utter
>
> The perfect truth, he would himself not know it;
>
> For all is but a woven web of guesses.

I learnt from Rainer Hertel that it is the tumbling, not the straight swimming, that helps *E. coli* to find its path. Or translated to science: it is the question, rather than the answer that helps us to find our way through the "woven web of guesses". I understood—it was exactly, what I had experienced during the explorations of my childhood. The bliss is in searching, not in arriving. This lesson has accompanied

my scientific life since then and still does. This is also the most important lesson, which I try to pass on to my students.

2 Motivations: How I Developed an Interest in Science

HOW I FOUND MY FIRST QUESTION. To find out the own calling can be a challenge, living in a time and a society full of opportunities and distractions. However, we should never forget, that freedom of choice is a privilege bestowed only on a small minority of humankind. Most people on our planet do not enjoy this freedom and must readily accept, what allows them to survive. Even in our privileged societies, individual choice had been the exception, rather than the rule, over centuries.

I feel myself double privileged. First, I was always allowed to have a choice. My parents, whose childhood had been shaped by the war, always told us children that we could not expect much of a material legacy, but that we are free in deciding our professional career and that they would always support us on our path, no matter, what we decided to do. My second privilege was that I did no need to search long for that what I wanted to do. Instead, my decision to become a researcher and to explore the mysteries of living beings, came in a single blow, very clearcut, without any doubts or sways.

I still remember this day very clearly. I was a boy of four years, and the decision was linked with a spruce tree that grew in front of our house. The tree was old, and time had left its traces upon him. This tree was very important to me, and I went there every day. For some reason, I had arrived at the conclusion that a big knothole in its trunk would represent a kind of an ear, and so I talked into the trunkhole about my thoughts, worries, and imaginations. And it would seem to me that the tree listened patiently and sometimes murmured in response. As different as this tree was from me, our relation was somehow personal, I had the impression that there is "someone", not just "something". That particular day, I woke up in the morning from the noise of motor saws, and when I looked out of our kitchen window, I saw, how woodchuckers had already cut down the tree to a short stump. I was too shocked and confused to accept reality. These men that stood around the stump, joking and smoking, had just murdered my tree and they obviously did not even understand, what they had just done. Didn't they see that they had killed "somebody", a living being endowed with the joy of being alive?

This shock made me think and inspired my first scientific question. Apparently, it was not evident for other people that my tree had been "somebody", because it was just too different from us people. Nevertheless, the tree was able to think, I was quite confident about this point. When my father came home from the office for lunch, I asked him "Daddy, how does a tree think?" My father smiled, and told me that trees do not think, because they do not have a brain. What a disappointing answer! I immediately contradicted "I know that they have no brain, but I want to know, how they think, even though they do not have a brain!". As a response, my father just

smiled once more and patted my head. Thus, it was clear, the adults did not know anything. I had to find out for myself!

To become a scientist in a remote village in the Allgäu was not an easy endeavour. There was no internet that would allow me to find answers to my questions, and although I learnt to read very early, the bookshelves of my parents, while containing a lot of interesting stories, did not give any answers to my question, it even seemed that nobody had ever even asked it. So, I started to think by myself.

2.1 What Science is About: To Make Mistakes and then Correct Them, Without Much Ado

The Catholic nuns that were running our *kindergarden* did not appreciate own thinking, nor did they appreciate the many questions, I asked them. I was told to shut my mouth and be quiet. So, I decided, not to go to the *kindergarden* any longer. Every morning, I took my lunchbox, said goodbye to my mom, and pretended that I would go off to the *kindergarden.* Instead, I disappeared into the woods, strolled through the thickings and climbed on my favourite tree, from where I could observe the wildlife. When the bell on the castle tower announced noon, I came home as if returning from the *kindergarden.* It took a year, until my mom found out, by accident. Fortunately, this was a few weeks before school started, a village school, four classes in a room. In the cities, pedagogy was experiencing a thorough turmoil in those days, the old concepts became overthrown in favour of new ideas, such as anti-autoritarian education. But this was still far away and completely unheard in our village. However, my teacher, Fräulein Krug, was a naturally born pedagogic talent, she really loved her children, each one of them and she tried to support everybody on the own path, at the individual pace. She was patiently answering to my questions and when she did not know the answer, she simply told me that she did not know, but then gave me a book and asked me to find it out by myself.

After so many years, I still remember an incident that deeply impressed me. We were talking in the class about the way, how animals survive the winter; some would sleep, some would survive as an egg, others would search in the snow for food. I had observed that the Brimstone Butterfly comes out as soon as the snow starts melting and I asked her, how this butterfly could develop so rapidly from an egg to an adult, if in the snow life activity would be so slow. I proposed that this butterfly might freeze and survive the winter, already being an adult, when the snow melts. My teacher said that this would be certainly wrong, no animal could freeze and survive. When I came home, I looked it up in an old book on insects, which I had found in my parents' bookshelf. And indeed, the Brimstone Butterfly was told to be the only insect that can survive down to −20 °C. Very proudly, I brought the book to my teacher and showed it to her. She read it and then plainly said "Well, Peter, now you have known it better than me, and I was wrong". I was thunderstricken by her response, mainly by the attitude of this response. Although she was an adult and I was only a little boy,

she admitted without any ado that her opinion was mistaken. Only many years later, I understood that she wanted me to learn a lesson, which was much more important than the hibernation practices of the Brimstone Butterfly. I am very grateful for this lesson because this is, what science is about: To make mistakes and then correct them, without much ado.

Soon after, one of my most important wishes came true—for my birthday, I got a little department-store type microscope, which opened me the miracles living in the water drop. I was now looking at anything that came across and learnt a lot. I was especially fascinated by flagellates such as *Euglena* because those were considered the basepoint of life. I was observing and drawing them and was impressed by the complexity of their behaviour. To find special literature on these creatures was really hard but with the help of a dedicated bookstore in the neighbouring town, I succeeded to get three antiquaric monographies from the turn of the century. I devoured those books, even reading foreword and index several times. What I found very striking was the fact that the borderline between plants and animals was quite permissive in these creatures. Thus, plants obviously were not principally different from animals since they derived from the same origins. I wondered whether the nucleus might be something like a cellular brain which would be shared between both life forms, and I squeezed out all information I could from my three monographies but found the information there quite vague. I also felt progressively limited by the lacking resolution of my department-store microscope and went to the local optic store to find out more about real microscopes. The prize, around 3000 DM, was clearly beyond my budget. Fortunately, I just had become fourteen, which meant that I was allowed to search for a vacation job. I worked very hard—first, as a woodchucker in the forest, later, as workhand on construction sites. After two years, I had accumulated the money and proudly went to the store to order an Olympus CHX light microscope. Two weeks later, a car from Freiburg came by and a very astonished salesman handed over a big parcel to me, a schoolboy.

The new equipment advanced my studies a lot. Eventually, I was able to see the details, such as flagella, the nucleus, and even the contractile vacuoles. From a pond behind our village, I got a sample, where I found my first model organism, a zooflagellate called *Peranema trichophorum*, which was well observable, because it did not swim too rapidly and in addition was capable of ameboid movements. I built a simple cultivation chamber, where I could keep this creature for up to several weeks, providing pond water through a cotton thread and from time to time feeding with *Euglena*, which I kept on a decoct from our local cheese (my mother refused to enter my room for weeks). I noticed that *Peranema trichophorum* was somehow capable of recognising its environment. When it encountered an inanimate particle, such as a piece of debris or a sand grain, it retracted and then searched a way around the obstacle. When it met its prey, a cell of *Euglena gracilis*, it protruded a stick-like organ at its front, slat its victim open and devoured it by phagocytosis. When it met a fellow, a deadly battle ensued, which usually ended by the larger cell devouring the smaller. Sometimes, when the cells were similar in size, the battle remained undecided, which attracted additional cells that joined into the fight, which was then

hard to follow. When they separated again a few minutes later, one or two of the cells had disappeared. Apparently, this flagellate displayed cannibalistic manners.

This phenomenon had really caught me—how could it be that such a primitive cell was able to show such complex behaviour? Obviously, the cell was able to sense touch and this sensation must be processed somewhere, integrating additional information, such as being inanimate, being prey, or being a fellow *Peranema* cell. I came back to my old idea of the nucleus as a cell brain—would this be the place, where all these stimuli are processed? How could I find out? I developed the crazy idea that it should be possible to stimulate the cell at different locations with a fine glass needle and measure, how long it would take until it responds by a contraction. To make a fine needle was the easy part of it—I had got Pasteur pipets from our pharmacy and had learnt to draw thin capillaries over a candle flame. But how to handle the touch in a manner precise enough for my experiment. I did not know about micromanipulators at that time and even if I had known, those devices would have been unreachable for me. So, I invented a system of levers using my Märklin metal kit, where I could handle my glass needle with sufficient precision to conduct the experiment. To keep things simple, I decided to do only three settings—touching the rear (intermediate distance to the nucleus), touching the middle (very close to it), or touching the tip (very far from the nucleus) of the cell. After some exercise, I became quite good at it and persuaded my younger brother to sit by me and take the time with a stopwatch. I remember, how excited I was, when I started to plot the times over the three positions and saw a clear increase of reaction time over distance to the nucleus.

In the meantime, two years had elapsed, and I decided to tell my biology teacher about my work. When I showed him the data (including drawings, I had made with ink), he encouraged me to publish this. I had never thought about publication, but then wrote to the editor of *Mikrokosmos*, a traditional light-microscopy journal, describing them my story. They told me, I should write it up and gave me some hints, how to do it. I replied that I would not have access to microphotography, and they agreed that I could document my observations by ink drawings. So, I was writing my first scientific manuscript on my mother's mechanical typewriter and sent it in to *Mikrokosmos*. It took a few months, until I heard back. In addition to the editor, a reviewer had read my paper and suggested that I should come up with an explanation for this cannibalistic behaviour. I was reading in my three monographies and found out that *Peranema trichophorum* was thought to be a distant relative of *Euglena*, which had lost its chloroplasts. I came up with a kybernetic model, where I proposed that there are surface structures that are still conserved, such that under my conditions, where feeding the prey had increased the density of this carnivorous flagellate, also the likelihood of an encounter with a fellow rather than a cell of prey would increase. Due to cannibalism, the number of cells should decrease, and this should also make this behaviour disappear, until a new wave of population growth would bring cannibalism back—an implication of my idea that I was able to confirm experimentally. I even made suggestions, how one might find out about the nature of the inducing factor. The culture filtrate of a dense culture might elicit this behaviour in a culture with only few cells, which would allow to distinguish the mechanism from an alternative,

where the behaviour requires physical interaction to become manifest. My revision was then accepted, and two years later, just right for my 20th birthday, it appeared in print (Nick 1982)—I was already at university then.

3 Work Done: My Personal Scientific Approach

HOW MY FIRST QUESTION SHAPED MY SCIENTIFIC PATH. To move to Freiburg University was a real excitement for me—the town with its a bit more than 10^5 inhabitants was something like Metropolis for the village boy I was in those days. The biology faculty followed a very high standard with respect to teaching quality and dedication. Actually, all prominent figures in research turned out to be great teachers and I learnt a lot, both in terms of science, but also with respect to personality. Dispute was cultivated and it was public, and from the very beginning we were taught to foster doubt and critical thinking, and to question even the most venerable theories. I was grabbing all what I could get, and this extended beyond biology. Greedy for knowledge, I was visiting also courses in ethnology, philosophy, or history. Over eight years in total, I studied Russian literature, attending the legendary course of Svetlana Geier, who re-translated with us the entire work of Dostojevskij. According to her opinion, the Germans had not had the chance to really understand this author, because, so far, he had not been properly translated. I was impressed by her precise language, unfolding the different connotation of a single word, her never-ending struggle for the correct expression, and her big perspective spanning different countries and cultures. Science is strongly dependent on language, and thus, I profited a lot from her course. Our biology curriculum was very broad, so I delved into anything from ecology till biophysics, and probably my teachers needed quite some patience, when I was getting on their nerves after the lecture.

A theme that returned to me repeatedly in different contexts was the question of integrity. What is an entity? Is it more than just the sum of its parts and the interactions between them? Is an ecosystem something which exists only in our minds, or is it there in the real word, does it act in a holistic manner, like an organism? Is a lichen, emerging from the symbiotic interaction between algae and fungi, a new kind of life form, or is it just the combination of the two symbiotic partners? These were the kind of questions, I discussed passionately with my prof in biophysics, Eberhard Schäfer. He was strictly denying that the whole is more than the sum of its parts and so we fought a lot—I listened to his courses, although I did not intend to choose biophysics as subject, but rather was considering going for geobotany (which in Freiburg was shaped by the school of Plant Sociology, dealing with plant associations as shaping element of ecosystems). But for some reason, I was attracted by Eberhard Schäfer's lectures, there was something fascinating about the intellectual challenges to translate biological phenomena into logical elements that could be mathematically addressed and modelled. One day, in the middle of our fight, he stopped suddenly and asked me, whether I would like to do a research project in his lab. I was stunned—it was just out of my scope. But he continued to tell me that they had observed that a maize

seedling, although not able to respond to red light by a phototropic curvature, seemed to *remember* the red light and later would change its response to blue light. And then came the decisive sentence: "Nobody understands it, nothing is known, and you are completely free, how you want to address this." He had got me.

3.1 You Are Completely Free, How You Want to Address This

Already the following Monday I started work in his lab—I learnt to meticulously standardise my system, etiolated maize seedlings. The caryopses had first to be watered for a given time, sown on a particular type of tissue, the air bubbles had to be rolled out with an empty bottle, the caryopses had to be sown equidistantly, embryo up, and the boxes had to stand in a particular region of the light field, which I had to adjust to homogeneity with a variation below 5% (which took me hours), and, eventually, two rounds of selection had to be added to get coleoptiles of exactly the same length. The system had been conceived by Dr. Moritoshi Iino, a Japanese guest scholar, who had driven precision to a stage that even 2° of curvature made a significant difference. The red light to induce the memory effect was extremely weak; when the shutter was open, a beeper signalled to me that the light was on. I was not able to see anything, but my maize seedlings clearly did see something. In the beginning, I worked with a night vision device, which a former Ph.D. student had smuggled in from the military, but later my body knew by heart, where things were in the dark room, which became my home during those days. I was working very hard, combining the different lights in different directions, timings, and fluences. When I stumbled out of the dark room in the evening, I had collected hundreds of data points, and slowly a complex, but clear curve began emerging from the data cloud. Above the little desk, where I was inserting my data points into a graph with a pencil, there was a framed quotation by the famous Karl Hartmann, a pioneer of phytochrome research, who had set up the illumination system ten years earlier from bits and pieces he had acquired from an abandoned burlesque theatre: "If you are not able to generate quantitative data here, you are on the wrong place here."

I started to make hypotheses about the curve that I could infer from my data. Eberhard Schäfer proposed that each cell would act autonomously, measuring the local quantity of red light by the phytochrome system and that the memory could be reduced to just a gradient of red light across the coleoptile. I calculated the consequences and got a curve that looked quite different from my observations. I proposed an alternative idea—that the coleoptile would recognise the direction of the two light qualities in a holistic manner and then *decide*, whether to increase or to decrease the bending. If this was right, I should get the same result if I imposed a certain *relative* gradient of red light, no matter, whether this gradient was established with strong or with weak light. I started to do this experiment, but time was running out, because a few weeks later I was supposed to start a year abroad, at the University of St. Andrews, Scotland. I was now increasing the pace of my experimentation and had to get up very early in the morning to have my maize seedlings done. One day, during

one of the many farewell parties that were celebrated on my behalf, I remembered in the morning at four that I had forgotten to sow my seeds. So, I decided to bring the whole tipsy company to the institute, and we sneaked into our laboratory. They were impressed by the red-light chamber and my discipline in arranging the maize seeds equidistantly. The deepest impression, however, made our discussion room with its central table that was plastered with empty beer bottles, and the blackboards sketches with weird formulae and cartoons, and, last, but not least, our beer-bottle collection that meanwhile comprised all letters of the alphabet. While we were sitting there, drinking, and joking, the night guard passed by, but since such sights were familiar to him, he just shuffled further, without taking any suspicion. My hard work payed off—my data clearly discarded the hypothesis of cell-autonomy and supported my holistic decision model. I still remember the bliss I felt when I plotted the points.

3.2 Scottish Interlude—How Different Parts of a Plant Talk to Each Other

But it was already time to pack my things for one year at St. Andrews University in Scotland. I thought about ways, how to continue my experiments and even took a parcel of the respective maize cultivar with me. However, the parcel did not make it—the custom officer in Victoria Station decided that this would endanger British agriculture and ransacked the caryopses. St. Andrews was a tiny, but romantic town on top of a cliff, and I rapidly merged into the international community there and tried to get what I could, although the Bachelor system with its block courses was not as free as I was used to from Freiburg. I soon started to work on my Bachelor project. Since I was a foreign guest and, thus, a bit outside of the system, I did not have a true mentor taking care of me. This was, what I preferred anyway, I wanted to work freely. Listening to the seminars in the institute, I soon found a topic that attracted my attention, heterophylly in the semi-aquatic plant, *Pogamogeton natans*. This plant produced different leaves, depending on submergence. A Ph.D. student at the Institute of Botany had discovered that in clear Scottish ponds this switch happened already before the leaves reached to the air. Coming from a photobiological group, I started to test the idea that it was the light quality that decided over the transition. Indeed, after a long struggle against algae that tended to overgrow my plants, I was successful to change the leaves by increasing the ratio of far-red light over that of red light, indicative of a phytochrome effect. This led me directly to the next question: the young leaf primordia were generated at the tip of the plant that was already reaching out into the air, but still the leaves that developed from these primordia were of the submersed type. Where was the light actually perceived? On site, in the tip, or in the older leaves? I started to cover up different parts of the plant using aluminium foil and soon found out that it was the older leaves that sensed the light quality, sending a signal to the meristem, where this signal would steer the differentiation of new primordia. I even tried to find out, how fast this signal migrated, but the variability in

my experimental system did not allow to tell this with reasonable resolution. I was writing my first thesis, which was a challenge, because English was not my mother tongue, but my mentors appeared to be quite impressed.

Of course, the year in Scotland was not only filled with science, but I also travelled a lot, joined several of the university clubs. On a sunny spring day, I was taking my lunch in the Russian Club, when the radio announced that Chernenko, the senile last dictator in a row of senile Soviet dictators had died. A new General Secretary was elected the same day, it was March 11, 1985. I listened to the speech of this new leader, whose name was Michail Gorbatschow, and I immediately understood that this was a historic moment. Hard to believe from the perspective of today, where the story that had started then, has inevitably ended. I also delved deeply into the British society, which in some aspect appeared really exotic to me. I also witnessed the conflict around the Miner Strike, which lasted for over a year and really disrupted the society even in this tiny university town. The gap had developed to a degree where students from both sides (coming from quite disparate social backgrounds) frequented separate pubs. As foreigner, I was alien anyway and had the privilege to converse with both sides, which led me to the deep conviction that the ability for compromise is crucial for solving any problem in society.

3.3 How to Sense Direction—Everybody by Himself or all Together?

In summer 1985, I returned to Freiburg and rapidly finished the remaining courses and examinations to start my diploma thesis, again in the Schäfer lab. My story had in the meantime been pursued by a Ph.D. student and was already published. I was not satisfied about this, the holistic behaviour had been ignored in that paper, and I was disappointed. But my prof came up with a new idea: He told me that there was a very old paper by Johannes Buder, who had asked a similar question as me, and recommended me to read that and develop a new idea, which I should pursue then in my diploma thesis. I had to go to the cellar of the well-equipped Freiburg university library to find this paper, which had been published right after World War I. The title "Neue phototropische Fundamentalversuche" (New fundamental experiments on phototropism) was unusual and immediately attracted my attention. The author summarised work he had done during war time under very difficult circumstances, partially he had to work with candles, because the electricity was shut down (Buder 1920). He had designed a very elegant approach using a custom-built light-fibre which allowed him to stimulate a coleoptile of oat (since Darwin's days the favourite model for phototropism research) from inside out, such that the direction of light was opposed to the gradient over the entire coleoptile. If each cell would act autonomously, which was the general concept of that times, the tropistic bending would be defined by light direction. If the coleoptile compared the light perceived in different flanks, i.e., if it acted as an entity, it should bend the other way round

following the gradient and opposing the direction of the light beam. The coleoptiles followed the gradient, not the direction, which meant, they acted as holistic systems. I was turned on. Many years later, I even repeated those experiments and tested the behaviour of microtubules in this context (Nick and Furuya 1996). I was discussing with Eberhard Schäfer, and he convincingly explained to me that such a holistic sensing would not work for gravitropism, because, here, each individual cell must sense the sedimentation of the amyloplasts independently. This discussion brought me to my idea—I guess, he had already conceived this earlier, but he generously allowed me discovering it by myself—what would happen, if a photo- and a gravitropic stimulus would be administered simultaneously? Would the two curvatures just add up by cell-autonomy or would there be deviations indicative of a "holistic decision". He told me that the seedlings, during their bending would experience a counteracting gravitropic stimulus making them straighten up again after some time, which would introduce additional complexity into my already complex experimental design. He adviced me to put the maize seedlings after stimulation on a so-called clinostat, a device rotating the seedlings slowly around their axis, such that any asymmetric gravity during bending would be excluded.

Since I had already been trained in the methodology, my work advanced quickly. I used two set-ups—one, where the two stimuli were acting in parallel and one, where they were opposing each other. For each set-up, I recorded a so called fluence-response curve progressively stimulating the dose (for light such a dose is called a fluence). The opposing set-up was not very interesting to me—the curvature induced by the gravitropic just subtracted neatly from that evoked by the phototropic stimulus alone. This merely additive behaviour supported a cell-autonomy model. The parallel stimulation was much more interesting. Here, for weak stimulation, the angles added up, but if the phototropic stimulus reached its optimum, suddenly something unexpected happened, since the interaction began to turn antagonistic. Apparently, the coleoptile had a limited capacity to process directional signals, and when this capacity was crossed, it responded qualitatively different to the light. This pattern was clearly holistic, as if the coleoptile made *decisions* that were qualitative in nature and not just the sum of their components. To document my readouts, the coleoptiles were glued on a specific tape and then xerocopied, such that the angles could be measured using a custom-built digitiser coupled to a calculator. After my diploma thesis, I had two piles, each of one meter height of xerocopied maize seedlings, which seems pretty pleistocenic from current-day perspective. I even succeeded to mathematically model my data and clearly could discard the original additive interaction model. The manuscript was already written on an electrical typewriter coupled to an antique personal computer. When I gave the first version to my postdoc colleague from Britain for language edit, it came back completely covered with notes in red ink, such that I hardly could find my text. In the iterated version, the red area had already decayed to half and after version four, I could submit and was successful (Nick and Schäfer 1988a).

3.4 Progress by Accident: A Tale About Grey Geese and Maize

Of course, I was quite proud about my first "real" scientific paper resulting from my diploma thesis, but as often in science, the real decision came not from this paper, which even the reviewers found quite complex and hard to digest, but from an accident that happened during this time. As to sustain my living, I had to work in several jobs at the university. I was teaching courses, and, in addition, worked as a lab manager in an analytical company processing blood and urine samples from patients. This company was around one hour by bike from Freiburg and so, I had to bike a lot those days under a very frosty November moon. One evening, after a long day of research work, teaching, and work in the company, I cycled home and suddenly remembered that I had forgotten my experiment launched in the morning. Since it was clear that I could discard those data anyway; and tired and hungry as I was, I decided, to go home and let the seedlings be seedlings and the clinostat turn around. The next morning, I went to the lab to dismantle the experiment and found to my surprise that my seedlings had bent on and on all night long, assuming the shape of pigtails. I was struck by their curious look, but then, I started to think: If a light pulse of 30 s was sufficient to cause an everlasting bending, this meant that the seedling, by this first stimulus, had assumed a stable asymmetry across its axis.

I felt reminded of the little grey geese of Noble Laureate Konrad Lorentz, who had discovered that these little creatures, while hatching, would accept any moving object that came across their sight, as mother for the rest of their life. The impressive proof for Lorentz' "imprinting theory" were the geese that followed him everywhere, no matter, whether he was taking a swim in the lake behind the institute, or whether he was entering his lab. Were my maize seedlings "imprinted" in a similar way by the first light pulse they encountered, when they "hatched" from their caryopses? This idea already led to the next experiment. If there was "imprinting", it should remain stable against the temptations of subsequent stimuli coming from a different direction. I started to do the experiment immediately, and was disappointed: the second, counter-directed, stimulus overran the effect of the first "imprinting" stimulus completely. Again, I got pigtails that curved the whole night, but now in direction of the counter stimulus, as if they had completely forgotten about the first light pulse. I tried to digest my disappointment and wondered, whether it might take some time, until the "imprinting" became stable, like the human mind that also needs around 20 min to memorise something in a stable manner. Thus, I increased the time interval between the first and the second stimulus and probed by prolonged rotation on the clinostat, in which direction the bending would develop. Up to 90 min, the second stimulus won completely over the first, but if I allowed the first stimulus more time to exert its effect, something strange happened: the coleoptile briefly started to follow the second stimulus, but then suddenly stopped and bent in the direction imposed by the first stimulus. After a few hours not any trace of the second stimulus was detectable. Thus, the coleoptile had developed a spatial *memory*, and this spatial *memory* was stable even through times, when it was not manifest as a curvature. The coleoptile

took a decision, which was all-or-none. In fact, when the challenging counter-pulse was administered just at the time, when the spatial memory was fixed, the population became very noisy—some already bent very strongly in the direction of the first pulse, some still bent very strongly in the direction of the second pulse, and a few could not decide and remained straight.

When I was writing up my second paper on this "spatial memory" (Nick and Schäfer 1988b), I suddenly understood that, unconsciously, I had returned to my first question from childhood. "How do plants think, even though they do not have a brain". Although this paper remained purely phenomenological, without a single molecule being addressed, it has remained important to me since, because it was a kind of enlightening experience, where my future path emerged from the mist. With this point, my "scientific childhood" had inevitably ended, and I felt that I had found my vocation as scientist.

4 Science Today and Tomorrow

FINDING ANSWERS AND NEW QUESTIONS. My discovery induced me to ask further: When a short light pulse of a few seconds was able to imprint a spatial memory that was stable over several days, there must be some structural correlate on the cellular level. In the human brain, memory is encoded by the mutual connections of the neurons. The cellular correlate of these connections are ramifications that are kept in shape by long and stable bundles of microtubules. While reading about these fascinating organelles, I learnt with astonishment that they had not been discovered neither in animal cells, nor in the context of the division spindle, as I had naïvely assumed. Actually, a biophysicist, Paul Green, had predicted them in the year of my birth due to considerations on expansion growth in plant cells (Green 1962), and one year later, two cell biologists, Ledbetter and Porter (1963), had looked for these predicted structures and discovered these "micro-tubules" underneath the plasma membrane of plant cells by transmission electron microscopy. Using the same technique, the team of Paul Green was able to show that reorientation of these microtubules by ethylene can induce a tilt of cell expansion from elongation to lateral thickening (Lang et al. 1982), because microtubules serve as guiding tracks for the movement of cellulose synthase complexes that will lay down the cellulose fibres in parallel to the microtubules and, thus, define the direction in which the cell can expand. I wondered whether these reoriented microtubules might be the cellular correlate of my spatial memory. A methodological innovation that became accessible just at that time also for plant tissues, immunofluorescence (Lloyd 1987), helped me to address this.

The idea to address "spatial memory" in terms of microtubules was not only fascinating for me, but also appealed to Eberhard Schäfer. He proposed me to apply for a Ph.D. fellowship from the German Scholarship Foundation. They had selected me already, when I was still at school, and they had also funded the tuition fees during my year in Scotland. I applied and I got the fellowship, so that I could start

with my Ph.D. straightaway without the need to keep myself alive by side jobs. My path was now spread out clearly in front of my eyes. I learnt how to label microtubules by immunofluorescence, after carefully peeling off the epidermis, since this tissue was known to control the elongation of the entire coleoptile by limiting its extensibility. In fact, microtubules underwent a re-orientation during phototropism. In the dark, they were oriented perpendicular with the cell axis. In the illuminated flank of the coleoptile, they realigned parallel to the cell axis, while they maintained their transverse orientation in the shaded flank. A similar reorientation could be evoked by decapitation of the coleoptiles, such that the cells became depleted from the growth hormone auxin. Conversely, by addition of exogenous auxin, microtubules could be turned back to their original transverse orientation. I followed the time courses of these processes and arrived at a model, able to explain phototropic bending: a lateral blue light pulse caused a shift of auxin from the lighted to the shaded flank, the cells in the lighted flank would become depleted from auxin, this would induce reorientation of microtubules into longitudinal arrays, such that cell elongation would not be any longer sustained. In contrast, in the shaded side, growth would proceed unrestrained, and the resulting gradient of growth would lead to the bending. To strengthen my point further, I could also show that this mechanism worked for gravitropism as well. The resulting paper (Nick et al. 1990) was widely read and still is my paper with the largest number of quotations.

However, my original motivation was not to explain phototropic bending, and I thought anyway that my explanation was too simple to be entirely true. The cell has numerous tools to rapidly control growth, for instance by activating ion channels culminating in changed flexibility of the cell wall, or by breaking down starch, such that the turgor pressure is increased. Why should the cell go for a much slower mechanism, such as realigning microtubules in a new direction? Such architectural changes would rather be meaningful to obtain stable changes. My original fascination for microtubules derived from directional memory and so I asked what happened to the gradient of microtubule orientation after the bent coleoptile had straightened again. If microtubules were in charge of the instantaneous growth response, this gradient should have disappeared. I tested my implication and was happy to find it wrong. The microtubule gradient persisted, no matter that the bending had already disappeared long ago (Fig. 1). In other words: microtubules behaved in exactly the same way as spatial memory was predicted to behave. Using complex combinations of stimuli, clinostat treatments, and waiting times, I could corrobate this parallelism and show, how microtubule reorientation developed in response to a directing blue light pulse and acquired stability at the same time, when the spatial memory became fixed. This memory effect was unique for blue light and could not be mimicked by a mere gradient of auxin, even though this gradient induced similar curvatures (Nick and Schäfer 1994). However, I was able to cancel the memory by cytochalasin D, a compound eliminating actin filaments, the second important player of the plant cytoskeleton.

My phenomenological approach had reached a stage, where the complexity of my experiments made it progressively difficult for others to follow my reasoning. I remember the comment of one anonymous reviewer of a paper stating that he

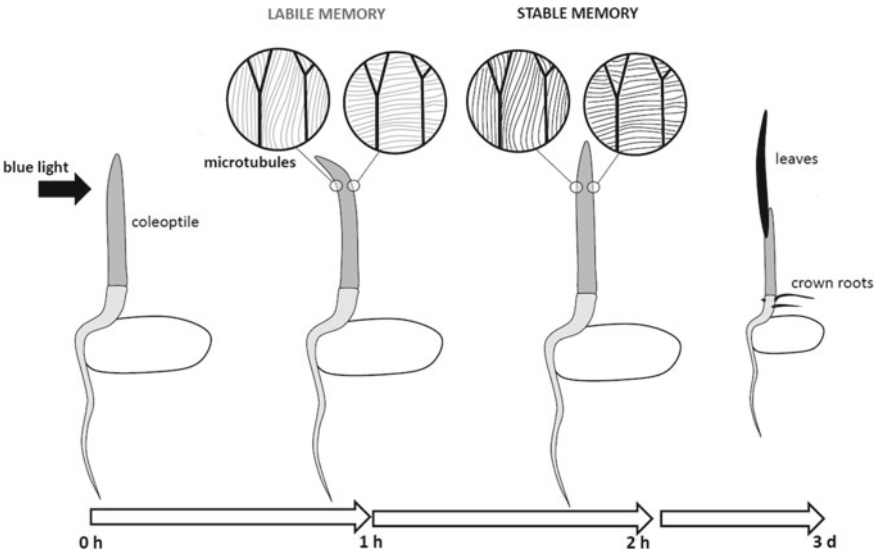

Fig. 1 Spatial memory is embodied as a stable gradient of microtubule orientation. When a maize coleoptile is stimulated by lateral blue light, it will bend and microtubules at the lighted flank will assume a longitudinal orientation, while they remain transverse in the shaded flank. This gradient is first labile, because it can be inverted by a light pulse from the opposite direction. However, 2 h after the inducing light pulse, the microtubule orientation is fixed, even though the bending has disappeared. A few days later, this memory guides the emergence of crown roots

found this great science, but very hard to follow and that he had difficulties even to understand my questions, not to speak about my answers… Anyway, the time of my Ph.D. had come to an end, which was also true for my scholarship. It became clear to me that I would need to search for molecules to understand, how directional memory would guide microtubules.

4.1 Fighting in the West, Journey to the East

Around this time, it was the last year of my Ph.D., a Japanese professor, Masaki Furuya, paid a visit to our institute and listened attentively to my account on my experiments and ideas. He was asking very stimulating questions and seemed to like my work. Moreover, he appeared to be a nice and generous person. When we were finished, he suddenly asked me, whether I would like to come to his lab in Japan. He told me that the Japanese government had launched a new policy to attract foreign scientists to Japan in order to stimulate scientific creativity. As part of this policy, they also offered prestigious scholarships. Since it was clear to me that a scientist needs to see the world to get confronted with other ideas, I was already pondering a postdoc. However, to go to the US, which in those days was the usual way for a

young scientist from Germany, was not really appealing to me, because the US was certainly not different enough for my taste. To go to Japan and delve into a culture and way of thinking that was far from mine, appeared much more attractive. I did not think long and answered that I would come.

I designed a project to elucidate the biochemical changes underlying the blue-light induced spatial memory and sketched down some ideas, how candidates could be identified and validated. To my pleasant surprise, the project went through smoothly, and I would start in Tokyo at the start of the next financial year, which in Japan begins April 1.

The journey to the East belongs to the most impressive memories of my life. I did not go for the easy way taking an airplane, although the Japanese government offered to pay for everything. Instead, I decided to use the Transsiberian Express to Wladiwostok, from where I hoped to catch a boat to Yokohama. It was autumn 1989, the Iron Curtain that had separated Europe for all my life, had collapsed without a single shot. I was in the middle of events—in October 1989, I had travelled as translator with a delegation from my hometown, Freiburg, to Lviv in the Ukraine to negotiate and sign a city friendship. This friendship is still active and more important than ever in those days of war, because ordinary citizens organise transport of food, medical goods, and important commodities, such as emergency generators, from Freiburg to Lviv and on the way back take women and children to Freiburg. Who could imagine this in those days? Just the day after our arrival in Lviv, the Berlin Wall fell, and everybody was tantalised. In the weeks to follow, one country after the other threw off the chains of dictatorship. In November, I was in Budapest to get the ticket for the Transsiberian Express on the grey market. For a Westerner, it was still not possible to do this officially, but Hungary, which in those days was at the forefront of fighting for democracy, just ignored the old rules, and so I could get both visum and ticket for a few DM. In January, I defended my Ph.D., *Versuch über Tropismus, Querpolarität und Mikrotubuli* (Attempt on Tropism, Transverse Polarity, and Microtubules), which raised some turmoil, because I had started off with Goethe's famous poem on the *Ginkgo* tree from the West-Eastern Diwan (Goethe 1819). This poem deals with polarity and allowed me to develop my concept. I was accused of mixing poetry and science and one professor even threatened me to make the thesis been turned down, if I did not withdraw and rewrite it. I refused because I found Goethe's description of polarity exactly to the point. As a consequence of my refusal, I had to face a very harsh review process, where additional external reviewers were asked. One of them even died during reading my thesis, as I learnt years later. It took several months, until the faculty arrived at the conclusion that this thesis was *summa cum laude* (excellent), I was already a postdoc then in Japan and waited urgently for my Ph.D. certificate. Nevertheless, looking back, I still would act the same way, although it had brought me into trouble.

So, I left Germany, on an icy winter day early in 1990, travelling through the dramatically changing countries of Eastern Europe and the incredible widths of a starving, but free, Russia. On the way, it was somewhere in Western Siberia, I learnt that there would not be any ship from Wladiwostok before May, because the sea was frozen. Fortunately, I could bribe a railway agent to change my ticket for Beijing,

where I arrived in the dawn of a cold day in March, after ten days without food (the train restaurant had closed soon, because the cook sold the food to the local population during the rare stops in Siberia). The train entered Beijing, moving very slowly through the baracks along the rails, and thousands of people lined our path, conducting their morning Tai Chi, a sight as surrealistic as from a dream. China had been less lucky, the Tiananmen upheaval in June 1989 had been quenched in blood, and on all my ways through Beijing I was followed by secret agents that did not even try to pretend that they were civilians. Still, I was impressed by the hospitality and curiosity of the people, and really enjoyed floating in the streams of thousands of bicycles that were moving as smoothly as the Tai Chi practitioners I had seen the day of my arrival. I heard rumours about a ship from Shanghai to Kobe and managed to organise a train ticket—three days on a so-called hard seater (which meant a wooden bench). The ship really existed, and it was not even difficult to get a ticket. Thus, on the last day of March, just in time, I reached Tokyo successfully ready to start my new life as a postdoc.

4.2 Learn About Yourself, by Understanding the Other

The lab of Prof. Dr. Masaki Furuya was among the cutting-edge groups in the field of plant photobiology, mainly phytochrome. Furuya-*sensei* (literally "the one, who lived before me"), as I called him, following the Japanese habit, was a very energetic person. At that stage, he had already retired twice, first after finishing his professorship at Tokyo University, then, after he had helped to build up the National Institute for Basic Biology in Okazaki. Now, he was a central figure in the Frontier Research Programme at the RIKEN Institute in Wako-shi, just at the city border of Tokyo. The Japanese government had decided that creativity should be boosted by attracting foreign scientists and adopted a policy of *kokusaika* (internationalisation). I was one of the first people profiting from this new policy, but since Japanese do things very thoroughly, when I left two years later, there were already more than 200 foreign scientists in the institute. As I was not working primarily on photoreceptors, I did not belong to the core of the group. Moreover, I brought my own money, which gave me a certain independence. I started off to do biochemistry, looking for microtubule-associated proteins that were responsive to light. Furuya-*sensei* was a demanding, but also a very supportive boss. When I needed anything, he would move all his levers such that I could meet the respective person. So, I learnt to use a very efficient affinity method to purify plant tubulin, which had been developed by an extremely modest, but skillful biochemist, Mizuno-san. This method is still of use in my lab to our days. Furuya-*sensei* had spent ten years as postdoc in the US and knew in person almost everybody who was important in plant biology those days. In addition, he invited many scientists to visit and discuss. So, I learnt a lot during that time, polished my discursive skills, and also profited from the analytical education I had obtained in Freiburg.

Of course, I also wanted to see the country and learn something about its culture. The fellowship funded very generously language courses in Japanese, and so I took four hours a week and got up every morning at five to learn *kanji*, the Japanese characters. Although I never reached to a stage, where I could read and enjoy Japanese belletristics, I came along quite well and also travelled quite a bit. This was not so easy, because the fellowship did not foresee any vacation, but I had an implicit agreement with Furuya-*sensei*. When I wanted to ask for a leave, I just put a manuscript for publication on his desk and asked for a "business trip". Everybody knew that this "business" did not exist, especially, when, after one year, I was travelling through China, Mongolia, and Siberia, but as long as the formalities were maintained, it was fine. During those two years, I absorbed this completely different culture and way of thinking and was astonished that, by doing so, I did not only learn a lot about Japan, but also about Europe. Never before in my life I had to explain my way of thinking. Now, I had to and found out that my way of thinking was not just "human in general" as I had assumed before in my naïvety, but that it was also specifically "European".

After my first year, three events shaped my path:

4.3 A Fancy Microscope Allows to Ask a Fancy Question

Furuya-*sensei* entertained a long-lasting cooperation with Hitachi, and they had built for him the large Okazaki spectrograph, a unique device for photobiological experiments. It was basically a gigantic light source generating the entire spectrum emanating in a huge circle. Basically, each nanometre was expanded to around 10 cm and the light was strong enough to elicit biological responses. Now, Furuya-*sensei* had convinced them to build a microscope, which would allow for microbeam irradiation, combined with an infrared optics, such that one could search the target cell without the need for visible light (which would activate biological responses). This fancy toy was just ready and now waiting for applications. I once had the chance to talk to the CEO of Hitachi at occasion of a dinner, organised by Furuya-*sensei* in gratitude, and I asked this leading manager, what the benefit was for them in building such devices as the spectrograph or the infrared microbeam irradiation microscope. The answer of the Hitachi manager was surprising for me—they would not get any benefit for the moment, but the technological challenges they had to solve in order to meet Furuya-*sensei*'s demands might help them in 50 years from now. I often remember this answer, when I read about another example of short-sighted economical leaders in the West.

I did not have to think long to come up with an application: during my work on the spatial memory in Freiburg, I had understood that organisms have to make decisions that are qualitative. Whether my coleoptile followed the first, imprinting stimulus, or whether it decided to follow the opposing, new stimulus, is a decision as fundamental as a pregnancy. It is all or none, there is no room for any graduality. This decision not only guides short-term bending, but will also define, on which side the root system is laid down several days later (Fig. 1), as I had already observed at

that time, although published it only after my return to Europe (Nick 1997). Such qualitative decisions require some kind of signal amplification, which means that, at the transition point, the population will be very noisy, because some individuals decide for the left, while the others decide for the right. This was exactly, what I had found and what inspired my interest into threshold phenomena in biology.

I did not need to search for long. Hans Mohr, the founder of Freiburg biology, was working on the effect of light on plant development, and his favourite model case was the induction of anthocyanin in cotyledons of White Mustard (*Sinapis alba*). A pulse of red light activated the photoreceptor phytochrome, leading to the accumulation of the red pigment anthocyanin (Fig. 2a). The Mohr lab had standardised this system to such a precision that it was even possible to relate the amount of extracted anthocyanin to the percentage of active phytochrome (Fig. 2c). The degree of precision was impressive. One of my student jobs consisted in preparing the seeds for these experiments. After they had been selected for size and uniformity, the final test, they had to pass, was to let them roll on an inclined board—only those that rolled straight without deviation, were allowed to enter the experiment. The Mohr lab had shown that the readout showed a threshold, with anthocyanin forming only if activated phytochrome crossed a certain threshold. I wondered whether this model might be a good candidate for Furuya-*sensei*'s fancy microscope and proposed to address this phenomenon on the single cell level, since all the experiments before had used irradiation of the cotyledon as a whole, such that potential interactions between different cells would remain unnoted.

Furuya-*sensei* liked the idea and not only gave green light to me, but also invited (and funded) a research visit of Eberhard Schäfer, who was interested as well.

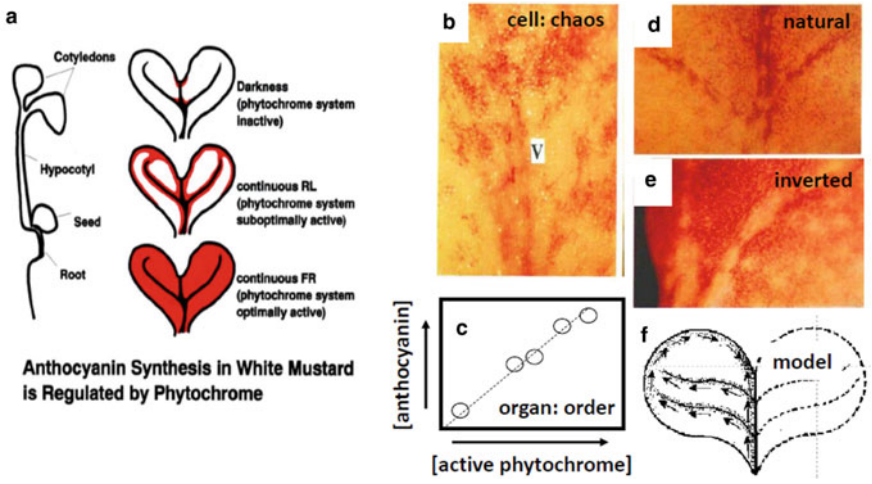

Fig. 2 The anthocyanin pattern in the model plant White Mustard (*Sinapis alba*) emerges from chaotic cells talking to each other by a transportable signal and, thus, creating together an orderly pattern of the entire organ. Summary of the microirradiation experiments (Nick et al. 1993)

What followed, were several weeks in green safelight (which is not perceived by phytochrome) at the microscope. In total, I conducted more than 2500 microbeam irradiations and analysed the resulting anthocyanin pattern. The results were astonishing. I observed that the individual cells followed an all-or-none pattern—a given cell either decided to produce the full set of anthocyanin and turned red, or it decided not to respond at all and stay white. Even neighbouring cells behaved differently, producing a very stochastic readout (Fig. 2b). This was a shocking contrast to the high precision that had been found in the Mohr lab by extracting the entire cotyledon. When I irradiated individual cells, I observed in most cases that only the irradiated spot activated anthocyanin, indicating that the cells responded to the local intensity of light, they perceived. However, if I directed the beam on the leaf margin or the leaf vein, where cells were more elongated, the result suddenly turned global. Interestingly, this global effect was in some cases stimulating, in other cases it was repressive. I was even able to invert the natural pattern, where veins and leaf margin were coloured, while the intercostal cells remained white. By stimulation of specific spots, I could generate leaves that were a mirror image—now veins and margins were white, while the intercostal cells turned red (Fig. 2d, e), breaking a paradigm announced by Hans Mohr that patterns are inherited, but just their expression would depend on the environment.

After all, I could explain the complex patterns by a model, where a factor required for anthocyanin was transported along the leaf veins from the leaf base to the leaf margin (Fig. 2f). Those cells, where phytochrome had been activated, attracted this factor, such that other cells were depleted from it (leading to the observed repression). The individual cell responded all-or-none, but the cotyledon as an entity responded in a gradual fashion by integrating the individual cell responses via systemic signals. The fact that even neighbouring cells differed strongly in their threshold led me to propose that this would allow to reconcile high sensitivity (requiring efficient amplification leading to an all-or-none output) with the need to still adequately respond when strong signals differ in intensity. Thus, cellular noise was not something, the plant tolerated, it was something that was actively maintained to serve the needs of plants that have to adapt to signals that often differ by several orders of magnitude. Furuya-*sensei* was quite pleased that this story (Nick et al. 1993) ended up in a prestigious journal, and it became clear that he was looking for ways how to support my path into science.

4.4 If You Are in a Conflict—Listen to Your Guts

I was able to meet in person many people, which I had previously known only from literature. As I learnt later, Furuya-*sensei* had also discussed with them about possibilities for my future career. One day, the famous and powerful Nam-Hai Chua came for a visit. He was heading two labs, one at the Rockefeller University in New York, the other in Singapore. I was impressed by his sharp mind and straight questions, but I also felt a bit intimidated by the air of extreme ambition he emanated.

He seemed to be interested in my anthocyanin story and asked very stimulating questions. Later in the evening, I was just sitting on a sofa, chatting over a beer with my lab mates, he suddenly joined and asked me straightaway, whether I would like to guide his lab in Singapore. From surprise, I almost fell from the sofa. He explained to me that he had assembled there many laborious researchers from China that were quite skilful, but that the lab lacked conceptual guidance. The position would be for five years and if I wished, I could stay forever. Although this offer was almost immorally attractive for a young postdoc, who did not know, on what to live after the fellowship ended a year later, I felt somehow uneasy and asked for a night to sleep over. He conceded this but told me that I should agree the next morning, he would not be the person to ask twice.

I did not sleep very well that night, but close to dawn, I dozed off. Early in the morning, I was suddenly awake and felt very clearly that I should decline the offer, although I could not give any rationale for my decision. After the breakfast, I told Nam-Hai Chua that I would not take the position. He was honestly surprised but kept composure. To his honour I have to say that he never showed any resentment on my behalf afterwards. I felt a bit embarrassed in front of Furuya-*sensei*, who had probably arranged this proposal in the backstage. He was astonished as well, but when I explained him that my gut-feeling had clearly told me that it would not work, he understood immediately and said something unexpected. This offer had been certainly a great chance, he said, but to his opinion my vocation was to act in the science of my own culture, and not elsewhere. After that I did not need to wait for long to get a second chance. Already a few weeks later, a French lady came for a visit—Anne-Marie Lambert from Strasbourg, I knew her name from pioneering work on plant tubulin and wonderful movies she had generated from microinjecting fluorescently labelled tubulin into plant cells. It turned out that she had been a friend of the Furuya-family since many years and Furuya-*sensei* used all his charms to make her stay unforgettable, including even a visit in the old Shogun capital Nikkô with a night in the garden house of the former emperor. At the occasion of a banquet in a traditional restaurant, where the Tokugawa Shogun had feasted three centuries earlier, Furuya-*sensei* came up with the information of a brand-new funding programme by the Human Frontier Science Organisation Fellowship that fostered transcontinental interactions, and, before the meal ended, the plan was born that I should apply for a postdoc stay in Strasbourg and work on microtubule-associated proteins. I was as surprised about this turn of event (as I had been over Nam-Hai Chua's offer before), but this time, my gut-feeling was positive, and I agreed immediately.

4.5 If You Get a Chance, Grip It—Think Afterwards!

Half of my time in Japan had already passed when, one day, Furuya-*sensei* approached me with a new request: he told me that the Ministry of Agriculture, to which he was an advicer, entertained a so-called *Gamma-Fierudo* (the Japanese pronounciation for Gamma-Field) around 100 km North of Tokyo. There, they would

generate mutant collections of many crop plants, including rice. Everybody now would go for mutants in *Arabidopsis* and these mutants had been useful to understand the function of photoreceptors. Would this not be a great option for doing similar things in rice, for instance, searching for a phytochrome mutant? As scientists, we were funded by the taxpayer, he continued, rice as most important staple crop in the world would certainly be more relevant than *Arabidopsis.* Actually, I was already rather booked with my other projects, but I noticed that Furuya-*sensei* insisted as he never did before, because he came up with the same proposal five times during subsequent days, and I understood that this was the Japanese way of giving an order. I proposed that I would be willing to do that, if, in addition for searching phytochrome mutants, I would be able to search for cytoskeletal mutants. Of course, I did not utter this so bluntly, but in the Japanese style, stating that it would be a great idea to search for phytochrome mutants and at the same time search for mutants affected in the growth response to phytochrome, such as microtubule mutants to get a comprehensive view on the signalling and responses to phytochrome. I noted that, for a heartbeat, he looked at me with a somehow examining look, but then he immediately seemed delighted and told me, he would call them immediately. I also noticed a kind of pride, as if his student had mastered some of his implicit lessons.

Already the next day, I got a call from a Japanese researcher, who excused continuously that he was not able to speak English, and suggested me, whether I might kindly consider paying a visit to their humble institution (which was funny, because actually it was a favour I obtained from their side). The visit in the *Gamma-Fierudo* was a bit eery—my train stopped in a tiny country-side station, and the very shy, but kind researcher, Yamane-*san*, picked me up with the car. We were driving through a lonely forest, while I spotted tumours at several trees and saw blackbirds with marbled white plumage. The *Gamma-Fierudo* was a gigantic circle surrounded by a huge dam. A really big cobalt bomb in the centre irradiated anything around for twenty hours a day, and a siren announced, when it was moving underground for four hours, such that busy gardeners could run out from the shelters to do the cultivation work. I learnt from *Yamane*-san, how they had generated the mutant population of a rice variety called Nihonmasari, and he proudly showed me his trick, how he was cultivating the seedlings on a floating mesh to simulate the conditions of a rice paddy (this simple, but ingenious method, which was not published, turned out to be useful in our lab for the decades to come—hereby, I pay tribute to its inventor).

I returned from the trip and, indeed, a few days later, several parcels arrived with more than 6600 mutant lines, and I started my work. First, I calibrated the system using the *Nihonmasari* wild type and learnt a lot about the physiology of rice. My Freiburg legacy has taught me that a thorough knowledge of your system is the key to success. Afterwards, I was conducting several screens, using Yamane-*san*'s floating-mesh method, searching for mutants with elevated resistance to different antimicrotubular drugs, mutants with altered gravitropic behaviour, and, of course, also mutants where the response to red light, inducing phytochrome, was altered. I was working very hard, spent weeks in the darkroom, and I squeezed out all what was possible from this collection till the last caryopse. Some of these mutants are still in use in my lab even nowadays, so this work was well invested. However, it was difficult

to propagate the selected plants in that institute because there were no greenhouses, not to speak about rice paddies. The only facility was a large phytochamber, where my Japanese colleagues raised some tobacco plants under continuous light. I spent hours every day to get my mutants growing, which was not an easy job. I was glad to see them thrive after all, but unfortunately, they did not intend to flower. My time in Japan was coming to an end, it was already January, and my plants were almost taller than me, but did not show any sign of flower development. In my desperation, I phoned to Yamane-*san* and learnt that this variety of rice, coming from Honshû, the main island of Japan, was a short-day plant. So, since it was not possible to set the chamber on a short-day regime, I had to go every afternoon at four and bring my plants to bed, waking them up the next morning at eight. Indeed, end of February, I noticed the urgently desired flag leaves announcing flowering, and end of March, I could harvest the seeds. Only one of my mutants did not set seeds, although it had flowered. It was a mutant, found in the screen for a lacking phytochrome response, which produced very long leaves that were creeping on the ground, which inspired me to baptise it as *hebiba* (Japanese for 'snake-leaf'). Apparently, this mutant was male sterile. Fortunately, I had kept not only the homozygous mutants (the mutation is recessive), but also two of the heterozygotes, and one day before my departure, I harvested five seeds from them. At this time, I still did not know, where I would be after my arrival in Europe, because the outcome of my application for the Human Frontier Scicnce Programme had not yet arrived. Following my principles, I planned to return to Europe without the use of airplanes. Anyway, my next stay (if it got funded), would not start before June, such that I was left with a gap of three months. The other mutants had been sent already to the address of my former institute in Freiburg, and I decided to take the precious five seeds of *hebiba* on my body, in a little plastic bag, which I kept inside my shoe.

4.6 Journey to the West, It Is Good to Go Out, It Is Good to Come Home

And so, the last day of March 1992, I left Japan, on the ferryboat to South Korea, not knowing, how and even whether I would continue my scientific career after my arrival in Europe. When I was on the gangway, I was called out and a clerk handed over a fax to me. It was the message from the Human Frontier Science Programme congratulating me that I had got the fellowship. The fax had arrived early morning in the institute. How Furuya-*sensei* managed to find out about my whereabouts and forward the fax, I still do not know, but of course this news filled me with great relief. The journey was full of adventures and impressions—after two weeks in South Korea, a country that just had returned to democracy and was full of contrasts and change, I took a boat to Qingdao in China. The two countries still did not entertain diplomatic relations at that time, and therefore this boat officially did not even exist, but it was already full of South Korean businessmen. In China, I managed to get a train ticket

to Mongolia on the black market. I had to go a Roast Duck Restaurant, where I was guided into a kitchen in the back, already wondering, whether this endeavour would have a happy end. It did, and so I ended up in a train filled with Mongolian smugglers that had filled all the cabins with suitcases and bags. Since a Mongolian visum was very expensive and my Mongolian friends had adviced me not to do it the official way, because it would take months, I arrived in the town of Er-Lian in the Gobi Desert and persuaded the obviously helpless custom officer that he should know that as a German citizen I would no longer require a visum, because due to the re-unification, Mongolia and Germany were now brother nations. He seemed sceptical, but due to a sandstorm he obviously had no way to verify my fairytale and after four hours he returned my passport with a stamp. The following weeks passed in a similar manner, the Soviet Union had ceased to exist, now there were many new borders with new rules that nobody knew, and I smuggled my seeds in my shoe undetected till the border between the Ukraine and Hungary behind Ushgorod. The Ukrainian custom officer seemed determined to upgrade his meagre salary, because he searched all my luggage and even my clothes, but to no avail. At the end, frustrated, he commanded me to take off my shoes and found the bag with the seeds. I explained him that this is for science, but he did not seem impressed. So, after all, I asked him, how much, and after some bargaining, he accepted fifty US $. When I look at the value these five seeds had brought to my science later, this bribe was well invested.

Soon later, it was a warm and humid summer day, I entered the *Institut de Biologie Moleculaire des Plantes* in Strasbourg, and joined the lab of Anne-Marie Lambert. With exception of a Ph.D. student, who defended those days, I was the only male—in the institute, the lab ran under the name "The Lambert Girls"—and I was also the only non-French. Despite this double minority statues, I merged in quickly and learnt a lot about biochemistry. The molecular work was complemented every Friday by group meetings, where everybody was supposed to bring a product of own culinaric activity, which allowed me to considerably expand my cooking skills (when I left the lab two years later, I got as farewell as gift a great book "La Nature dans l'assiette" (Nature on the Plate), a collection of vegetarian dishes arranged through the seasons of the year, which is still a source of inspiration for me. In addition to biochemistry, both in scientific and social contexts, I learnt quite a bit about tissue culture, and I applied these new elements to the questions, I had brought from Japan.

4.7 Sometimes, Nature Does not Want to Reveal Its Secrets

Since microtubules can reorient in response to light, there must be associated proteins that interact differently with microtubules, depending on signals from the environment, and this interaction is the core of the fascinating ability of plants to adjust to the conditions by changing their shape. My host lab had developed antibodies against microtubule-associated proteins from neural tissue that recognised proteins from plants, and they also had developed approaches to purify such proteins through their interaction with microtubules. I got the chance to apply these tools and approaches

to coleoptiles from maize and rice and discovered two proteins that were cross-reacting with the antibody and bound to microtubules but appeared and disappeared depending on the status of the plant photoreceptor phytochrome (Nick et al. 1995). I worked then more than a year to purify the two proteins, in order to obtain peptide fragments that would, in the future, allow to clone out the respective genes. For the protein that prevailed in non-growing cells, this approach was successful, and I identified Heat-Shock Protein 90 as microtubule-associated protein (Petrašek et al. 1998; Freudenreich and Nick 1998). The second protein, much more interesting for me, since it was activated when microtubules reoriented into transverse orientation, was purified as well, but when it was sent to our cooperation partners that did the sequencing, apparently some accident happened, and most of the sample got lost, such that I remained only with very few fragments that did not lead to any known protein (the coverage of databases was not comparable to that what we have nowadays). There was no time left to relaunch this purification, and although I have later tried several times to catch it by other approaches, this protein has remained a phantom that lured me to interesting places since, but till now preferred to stay elusive. Maybe, I will catch one day, but maybe, I should accept that, sometimes, Nature does not want to reveal its secrets.

4.8 The Path to Independence Leads Through a Valley of Uncertainty

I had now, over the years, walked on a path coming from the realm of phenomena (spatial memory), over cellular mechanisms (microtubules) to the world of molecules (microtubule-associated proteins). Should I continue along these lines, analyse further, and try to reach towards the level of genes, or should I rather go for a synthetic approach and try to integrate the lower system levels into an understanding of the entire organism? The rise of molecular genetics as dominating approach was already felt those days. The use of *Arabidopsis thaliana* as model organism, the genome project for this organism, and T-DNA mutant collections that allowed for reverse genetics was attractive and became soon the main flow in the plant sciences. Should I join this flow, which also would make it much easier to find a position in a university? Although I was quite impressed about the work of the Gerhard Jürges group on embryogenesis mutants in *Arabidopsis thaliana*, my gut feeling told me that this was not my path.

At this time, I not only crossed a crucial point of my scientific path, also my private life changed fundamentally. Our daughter was born, which made me aware, how happy and blessed my life was. Of course, it was a challenge as well, the following three years, I learnt that a man can keep going even with little sleep. It was also clear that the more adventurous period of my life would need now to become steadier, since I was bearing the responsibility for a young family. In Germany, it is very hard to get a permanent position in academia. Actually, a professor position is

almost the only way. To be able to apply, one needs a degree beyond the Ph.D. This so-called habilitation is basically a second book, along with experience in teaching. I applied for a habilitation fellowship to return to Freiburg, where my family lived already (I commuted every day, which meant to get up at five in the morning, which at that time was anyway the preferred activity time of our daughter). I also started teaching, first at the trinational École Supérieure de Biotechnologie Strasbourg, later at Freiburg University. I liked teaching and still do, my repertory was broad, from evolution, over plant anatomy, till modern methods, physiology, cell biology and biotechnology, and I profited from the memory of my excellent teachers I could enjoy as a student. My application for the fellowship was successful and I even got a position for a Ph.D. student. In the Lambert lab, a Ph.D. student from Bonn, Andreas Freudenreich, was working next to me as a visiting fellow. I had found out that he was not overly happy about this Ph.D. and that funding after his return was unsecure. Furthermore, I found him nice and original in his thinking, and I had also learnt that he liked adventures. So, I did not think twice and asked him, whether he would like to come with me to build up a lab in Freiburg. He did not think twice either. Thus, in summer 1994, we started in Freiburg. Soon, students appeared from different places of the world and wanted to work with me, although my budget was quite moderate, and I could not offer salaries. My principle was to guide them by inspiration, not by power. It was a great feeling to suggest an idea and see, how somebody else was developing something from that idea.

Just before I finished my time in Strasbourg, I was invited to a Cell Biology conference in Prague to talk about my microirradation story on pattern formation. After my talk, a smiling man with a moustache approached me and asked, whether he could show me something. It was Zdeněk Opatrný, at the Institute of Plant Physiology at the Charles University in Prague. He revealed to me data from a tobacco cell line which he had initiated long back, during the days of the Prague Spring and that had grown as unknown treasure in the shadow of the Iron Curtain. This line underwent a fascinating cycle of cell divisions forming a small file of cells that behaved like a small organism and obviously were "talking to each other". I was tantalised and we started to cooperate, a relationship that is still alive, almost three decades later. He sent students to my lab that helped us to establish the culture, which is still thriving in our lab, although I must admit that I later switched to the more widespread tobacco BY-2 system, because it is easier accessible to molecular biology. We profited a lot from the decades of experience on cell-culture systems of our Prague partners, and tobacco cells have helped us to get insight into the functions of several proteins that connect to microtubules and steer their functions. We also could use them as "minimal organisms" that helped us to understand, how cells, through a self-referring oscillation composed of actin filaments and auxin transport can organise themselves into an organism (reviewed in Nick 2010).

A second line of research addressed the cytoskeleton as a sensory structure. Already in Japan, I had worked on the role of microtubules in gravity sensing, inspired by discussions with Rainer Hertel. I found that, in addition to their role in guiding the growth response, microtubules were needed to perceive the direction of gravity. This sensing was linked with a very dynamic subpopulation of microtubules (Nick et al.

1991). Zdeněk had sent me a young student, Kateřina Schwarzerová (in the meantime she is heading the lab in Prague), to work on the behaviour of microtubules in response to cold stress in winter wheat. While it turned out to be difficult to visualise microtubules in this system, her short stay made me think whether the known cold sensitivity of microtubules might be used by the plant as a kind of thermometer.

But before I could go on, however, I had to work on my habilitation, which forced me to think on the unifying theme of my different projects. I understood that all these projects were dealing with the relationship between cells and the organism they constituted. Whether it was the anthocyanin pattern, where chaotic individual cells responded all-or-none and communicated to generate a smooth and precise readout of the entire organ; whether it was the microtubules that sensed the direction of gravity and translated this into the bending response of the entire organ; or whether a cell activated phytochrome leading to a re-orientation of microtubules which then stopped the growth of the coleoptile, the question behind was always on the relation between the whole and the part. How does it work, if there is no brain, no big boss that rules everybody? Again, I recalled the question from my childhood ("How do they think, even though they do not have a brain"). Writing my habilitation thesis was rewarding for me, although it was sometimes difficult to write it under conditions of a young family—parts of the thesis I had to type with our lively daughter on my lap. Over the years, I had found out that the quest for the "whole" that organises the parts had inspired many great minds from the time of Greek philosophy. My favourites were Ovid, Heinrich v. Kleist and Goethe, and I enjoyed connecting my experiments with their thoughts and concepts, and also paid quite some attention to the rhythm of my language. After six months of writing, I handed in my thesis *Einzelzelle und Pflanzengestalt* (Individual Cell and Plant Shape). I was not overly surprised that the echo in the faculty was controversial. Some found it great, others were furious (the same persons that had already attacked my Ph.D. thesis years before). Again, I was accused of being unscientific because I had mixed science and culture. I was asked to retract and rewrite it in a less provocative way, just giving my papers and some general summary. But I refused because I did not see the point to keep science separate from culture. The scientific method relies on separating observation and interpretation and on a professional way to ask questions, search for answers, fail with these answers and find new, better, questions. But as long as these principles of scientific work are kept, there is no reason, why a scientific text should not also connect to culture. Science is a central part of human culture. So, I decided to fight it through. The following weeks were not overly relaxed, but after all, the majority of the faculty arrived at the conclusion that this thesis was unusual, but of quality. I got my *venia legendi* (the right to teach) and just the next day, my first Ph.D. student defended his thesis under my newly acquired responsibility. After everything was over, the head of the habilitation commission came to me and told me that he had never gone through such a turmoil before, and that he was not even understanding, where all this passion came from. He concluded "You see, science is like a salami, where everybody cuts off only a slice. And you come and take the whole sausage! This is scientific aristocratism!" I answered that his judgement was perfectly right, but that I would stand up for this.

What followed, were two years of extreme uncertainty. The first time in my life I did not live on a fellowship but needed to find short-term contracts, which were difficult to get in the German academic system that basically foresaw only professor positions. Together with other young scientists in a similar situation, I even thought about setting up a start-up (detection of transgenic food), but when we had finished our business plan, it turned out that such a company had just started already, even in our town, Freiburg, and was very successful, since Greenpeace had stopped a boat from the US full of transgenic soybeans, such that the topic attracted a lot of public attention. Twice I was in the situation that I came home Friday evening and did not know, whether it would go on next Monday morning. It always did. The science went fine, my network of colleagues grew, and students wanted to join my team as well, because they had known me as dedicated teacher. I had set up a new type of seminar, where hot topics from widely read papers in high-ranking journals were presented by one student, but where a second student had to play the *advocatus diaboli* and presented a paper that falsified the celebrity (usually these were from solid, but not as high-ranking journals). Then we discussed, what had happened here, and what type of scientific mistake was behind the flaw. Many of my later students came from this seminar, all of them had strong individualities and were somehow "out of the box".

During that time, I also got to know Diego Breviario, who proposed to apply for EU-funding. Twice we failed with two proposals that were scientifically attractive, but obviously did not fit into the policy of the EU system. I became so annoyed by this political component that I wrote up a third proposal, mainly motivated by satirical intentions. It was on microtubules, our common theme, and it drew upon a finding from one of my rice mutants, where a truncated tubulin conferred resistance to microtubule herbicides. We suggested that this could be used as selective marker for transgenic plants—in contrast to the antibiotic resistances used hitherto, it would not be a foreign gene, but a gene that was already present in the plant, just mutated in one base pair (if it were in our days, I would have added that one might engineer this with CRISPR-Cas). With the tongue in my cheek, I thought of a catchy title and called then the whole thing "EcoTub". To my complete surprise, this proposal got funded and so Diego myself and Paul Christou at John Innes started to cooperate. Honestly, this project was not really successful if I look at it from today's perspective. Nature just did not like to have tubulin be messed up (the transgenic cells obviously suffered from the engineered tubulin). However, the project allowed me to extend my group, and I even got a second lab in a new building, where I had additional infrastructure, such as a climatised dark chamber to get my rice to flower. As I had done already in Japan, I needed to transfer the plants from the greenhouse to the chamber—since it was around one km away, I used the bike trailer of our daughter and always caused a lot of ado at the traffic light, when I approached the waiting bikers from behind with my jungle of man-high rice plants in the carriage…

4.9 Never Give Up, at the End Things Will Join

End of the millennium, my career situation stabilised after all—first, I was able to catch an assistant professor position (only temporary, though) and I succeeded to acquire a Junior Research Group by the Volkswagen Foundation. This programme was new and encouraged interdisciplinarity. The new spirit was also manifest in the unusual selection process, where, after a first round of expert reviews, the shortlisted candidates had to convince a jury about the merit of their project. My idea was to look into the dynamics of the cytoskeleton because I had repeatedly encountered the limitation that my microtubule images were just snapshots, but that I could only indirectly infer the processes behind them. I had a quarter of an hour, and in my committee, there was not a single person with a background in biology. I decided to use a metaphor from the theatre world and asked my auditory to imagine someone watching a Shakespearean tragedy in the theatre but falling asleep after the first act and waking up only at the end, when the stage was filled with corpses. This were the way, how a cell biologist feels, when looking on microtubules after stimulating the cell with signals. After my talk, one member of the jury approached me, introduced himself as theatre scientist (I had not even known before that this science existed), and told me "See, I did not understand a word from what you were saying, but I got the spirit that it is significant". I got the grant, which gave me almost 2 million € over five years!

My team grew further and with a pioneering spirit, we explored new fields—for instance, by microinjection of fluorescent tubulin which we purified from calf brain into gravity-stimulated maize seedlings, we could show that the reorientation of microtubules was going through a chaotic stage, where the individual microtubules were either transverse or longitudinal, but where the frequency of those that became longitudinal, increased (Himmelspach et al. 1999). So, even on the level of microtubules, there was an all-or-none decision, as I had seen repeatedly earlier (for instance, in my microbeam irradiation experiments on the anthocyanin pattern). Through the hard work of a Ph.D. student from Tatarstan, Albina Abdrakhamanova, we could show for winter wheat that microtubules, indeed, were thermometers that had to yield in order to induce frost hardiness (Abdrakhamanova et al. 2003). Interestingly, the "hard guys", wheat varieties from Siberia, where those, where microtubules were most sensitive. In the meantime, we have dissected many molecular steps of the underlying mechanism (a recent review is given in Wang et al. 2020) and are currently investigating whether the microtubule thermometer is rather a kind of a clock that measures the timing of a stimulus.

Using the "minimal organism", the tobacco cell line from the Opatrný lab in Prague, a gifted Ph.D. student (actually coming from Diego's group), Prisca Campanoni, discovered that the cell files were all even-numbered, while odd-numbered cell files were rare. Obviously, the cells talked to each other, before they divided. When I discussed this strange phenomenon with a theoretic physicist, Bernd Blasius, at one of the yearly interdisciplinary meetings that were organised by the Volkswagen Foundation for their fellows, he told me that he had been looking for

something like this for years, because he was interested in modelling ecosystems. He asked me for the data, and a few weeks later he came back and told me that he was able to model Prisca's data by assuming that the cells behaved as oscillators that were synchronising their divisions by weak coupling, but that it worked only, when he assumed that the coupling was one-sided—a cell would talk to its right-hand, but not to its left-hand neighbour. While he was rather sceptical that something like that should exist, I asked him to model what would happen, if the weak coupling was interrupted. He did and sent me the data—I asked Prisca to do the experiment, blocking the directional transport of auxin by an inhibitor called 1-Naphthylphthalamic acid (NPA). She did the experiment and found, what our colleague from physics had predicted (Campanoni et al. 2003). In the following years, we could show that actin filaments became bundled, when the cell was depleted from auxin, and that this was inhibiting auxin flow, such that auxin was not exported from the cell, leading to a debundling of actin, which then restored auxin export. This actin-auxin oscillator (Nick 2010) was the base for organising individual cells into a minimal organism. My childhood question for the"somebody" in a plant that is lacking a brain, found an unexpected answer. A plant organism is not a body, an object, but rather a rhythm (for a philosophically stained overview of this research see Nick 2013a, b).

A fourth breakthrough from this time with the Volkswagen Foundation was the discovery that my *hebiba* mutant which I had smuggled in my shoe over the long way with the Transsiberian railway, was not blind at all. It did see red light, and it was endowed with a functional phytochrome photoreceptor. However, the mutant mixed up light and dark. We reckoned that a growth hormone, probably auxin, might be affected in this mutant, and with the group of Elmar Weiler in Bochum that was able to measure the minute amounts of plant hormones in tissues, we investigated the response of different plant hormones to red light. As often, it was an accident that solved the case—our colleagues just set up a new method to measure jasmonic acid, originally discovered as component of jasmin scent, and since our rice samples were still sitting on their bench waiting for being discarded, they decided to check their method on them. Later in the day, I got an excited and exciting call—surprisingly, rice was packed with jasmonic acid, but the *hebiba* mutant was completely void of this hormone (Riemann et al. 2003). Years later, supported by the matchmaker skills of Furuya-*sensei*, my Ph.D. student Michael Riemann succeeded, during his postdoc in Japan, to find the mutation in a gene called Allene-Oxid Cyclase (AOC) that had been hit away by the gamma-rays in the *Gamma-Fierudo* and therefore was not able to provide the precursor of jasmonate (Riemann et al. 2013).

I could have gone on like this for years, but the suspense from unemployment by the grant from the Volkswagen Foundation was limited—five years. I had to find one of the rare professor positions. In Germany, this is not possible in the institution, where you did your Ph.D. One needs to go elsewhere. In 2002, I was participating in more than 10 rounds of "singing" as these events, where shortlisted candidates have to present themselves, are called colloquially. The effort was considerable, but at the end I remained with three options, two of them as an institute director. The decision to go for Karlsruhe, was exclusively based on private grounds—in the meantime, our son had been born and my family did not want to move around in Germany.

Karlsruhe was the only place, where commuting on a daily base would be feasible. So, I picked Karlsruhe. The arrival was sobering—from ten professor chairs, seven were vacant; in rankings on teaching, Karlsruhe biology was on the third position—from the end; the faculty was hierarchic; the institute was romantic, but from the nineteenth century. It was a construction site. I remembered a sentence, which our Latin teacher, a conservative, but just man had taught me, when I was a teenager "*Mallem hic primus esse quam Romae secundus*" (I prefer to be the first here, rather than the second in Rome), said to be uttered by Julius Caesar, while being in a village in Gallia. I was not sure, whether I would be able to compete with Julius Caesar, but I was pretty sure that the place where I had stranded was not Rome. However, I was determined, not to give in, but built up my lab from scratch—fortunately, I had a very good relationship with the people from the workshop of my institute in Freiburg, and they helped me a lot. For instance, they built a custom-made gene gun for me, which costed me only 300 € (and a bottle of red wine) rather than the commercial version, which was more than 20 T€. They revived an antique ultracentrifuge, and also helped me to find second-hand equipment for my new lab. However, it was clear that I needed to force my new university to invest something. Fortunately, soon after arrival, I had been asked to become the Dean of Study Affairs, because, due to the vacancies, all my colleagues already installed before me, were on other duties. Moreover, I had inherited the task to introduce the new Bachelor/Master system. A construction site is also a chance—when everything is crumbled, it is easier to build something new. So, I started to work and reformed the teaching system, against a lot of resistance, but the thought of Julius Caesar helped me to look at this struggle from a larger perspective…

4.10 Whether a Place Will Be a Good Place Depends on What You Make Out of It…

In order to improve my strategic position in addition to having the lead in study reform, I applied for a prestigious institute director position in Salzburg, Austria and was successful. This move helped me to exert pressure on my own university. They had to offer me a leading position and real money to buy good equipment, advanced microscopes in the first place. Most important, they had to offer me a permanent position. After so many years, I could stop worrying about the future of my family and my own existence, which was a great relief.

I will not describe the following fifteen years up to now in the same detail as I did for my early career. It was the time of harvesting the fruits of my former work. I was able to build up a large group, which at peak times counted more than 40 people from many countries, languages, and cultures. I even had to split it into three subgroups, dealing with Cellular Biotechnology, with Plant Stress, and with Applied Biodiversity (I had also become the director of the Botanical Garden).

I will now briefly address three questions that I discovered through these years and that I want to work on in the seven or so years that are left till my retirement:

My old question, how a plant cell can develop directionality and translate this into spatial organisation of the entire organism, had inspired an approach, pursued in my Cellular Biotechnology group, where cells were stripped from any direction by digesting off their cell wall and then let them develop a new direction. Making use of GFP-tagged cytoskeleton marker lines, we could then follow, how the nucleus searched the centre of the cell, while actin filaments and later microtubules organised a new directionality (Zaban et al. 2013). To find out, how individual cells communicate, we needed a new technology. Here, I profited from the interdisciplinary interaction with engineers in my university. We developed a microfluidic chip system that allowed to impose directionality to individual cells by preformed artificial cells (physical stimulus), along with a superimposed chemical cue, a gradient of auxin to see, how the cells will decide about their direction (Zaban et al. 2014). Could we use such chips to mimick the chemical interactions between cells in a tissue? It took a few years, but eventually, we succeeded (Finkbeiner et al. 2022) and could demonstrate that cells in suspension stop dividing, when they feel lonely, but can be induced to enter division, if treated with a conditioned medium from a dense culture. We have already found a molecular candidate for this "social hormone", but we are still not entirely sure, whether we have caught the right fish.

As often, this purely scientific interest led to applications—we could show that secondary metabolites are often produced in a kind of "chemical LEGO", where different cells have to cooperate, and we were able to show the concept by producing the anti-Alzheimer compound nornicotine in cell culture (Rajabi et al. 2017), or by combination of different cell types, the long-desired anti-tumour compound vincristine (Finkbeiner et al. 2022). Could we use this modular principle also for producing valuable compounds from rare and endangered plants? At the moment, we are working on *Cephalotaxus hainanensis*, an almost extinct tree from the Chinese island of Hainan, producing very potent anti-tumour compounds. It is so rare that the trees have to be guarded, because people come at night to steal the bark, which is sold for eight times the price of gold. Based on the transcriptome (Qiao et al. 2014), we work with the team of my former Ph.D. student, Fei Qiao, on reconstructing this pathway by chemical and biotechnological means. This is really tedious work, but we want to demonstrate a new principle and so we just go on drilling. Moreover, our results led me to a new viewpoint—we saw that some of these enzymes localise to mysterious protrusions of the plastids, so-called stromules. Originally discovered by a German cell biologist, Schimper, in the nineteenth century, they were later forgotten and rediscovered in the 1990ies by means of GFP-technology. The original idea of a plastid network that would be physically coupled, could be falsified by elegant experiments with light-switchable forms of GFP directed to the plastids (Schattat et al. 2012). We found out that these stromules can be induced by the stress hormone jasmonate, but also by oxidative stress. They seem to touch other organelles, mainly mitochondria that are oxidatively challenged, and this brotherly tap on the shoulder of their stressed fellows seems to help them to overcome the stress. Thus, the organelles, we know from the textbooks, are apparently much more

interconnected than we think. What we see in the microscope, are just snapshots of dynamic processes, and stromules seem to act on the biochemical and molecular events in a cell. Form rules over Matter? This is one of the questions, I would like to address in the years to come—I am sure, I will not answer it, but the path is more important than the goal.

The *hebiba* mutant that I had smuggled in my shoes was male sterile, but it brought a lot of fruit. We discovered that it coped well with salt stress (Hazman et al. 2015), an emerging problem in many countries like Egypt, Vietnam, or Bangladesh, were the rising sea level makes the soil progressively saline. When we followed this up, we discovered that the stress hormone jasmonic acid, similar to human adrenalin, has two faces—if activated rapidly and degraded rapidly, it is a very important signal that activates adaptation to different stress factors, such as salt or drought. However, if jasmonic acid is produced and stays, this will initiate cell death, and the resources of the dying tissue are mobilised into the young parts of the plant, from where new organs can be reformed, once the stress episode is over. We observed similar phenomena also in grapevine cells and I wondered, how the same signal can mean "life" or "death", just depending on the timing of its birth and decay. In several months of hard work, I developed a model that could explain this phenomenon (Ismail et al. 2014). But how to prove this idea?

In fact, we developed a way, how we could engineer stress signalling with an optimal time course. The details are complex—we used a promoter from a salt-inducible transcription factor, driving a jasmonate-signalling protein where we had cut out a small piece needed for the decay of this protein. The whole cassette was introduced into tobacco cells or real rice plants. Upon salt stress, jasmonic acid was produced due to the salt stress and would have initiated cell death. But since our salt-inducible promoter became active, the jasmonate signalling protein was made and silenced jasmonate signalling, and, because we had cut off the piece needed for its decay, this "off-switcher" remained on all the time. Indeed, the tobacco cells and the rice plants were now able to survive on salty water (Peethambaran et al. 2018).

In the meantime, we found out that also the response to cold stress was depending on temporal patterns. Here, it is microtubules that measure the time and evaluate the stringency of stress and its duration. The outcome is then signalled to the nucleus where different genes are activated that either activate cold hardening, or cold-dependent cell death, such that the resources can be rescued for the younger parts of the plants that will launch a new round of development, once it is getting warm again. To our surprise, this signal is a novel and very exotic microtubule motor, which we baptised Dual Localisation Kinesin. It is running wrong way on the microtubules and when microtubules diassemble in response to cold, this kinesin moves into the nucleus, where it acts as a gene switch and activates Cold Box Factor 4, a master switch for cold hardening (Xu et al. 2018, 2022). Wonders over wonders, which wild imagination could conceive such a crazy mechanism? The older I get, the more often I get astonished.

Salinity, drought, but also untimely cold snaps are events that become accentuated due to global climate change. In parallel, new diseases emerge and spread and challenge agriculture. Over many years and numerous publications, we have dissected,

step by step, the signalling controlling immunity in grapevine. I will not describe the details here, they are very complex, and my brain had to crack really hard nuts to understand this—here, my logical education, which I had obtained as young student in the Schäfer lab in Freiburg, helped me a lot.

I rather want to formulate the question that emerged from this long and complex path. I want to understand, how plants can distinguish the different types of stress. Do they have different signals for each stress? The answer is a clear: no—it is a handful of signalling events, which are used over and over again, jasmonic acid being one of them. However, by combining different signals in a specific temporal order, each stress type leads to a specific signature, which conveys different meanings. In other words: plants use chemical "words", and they combine them in a kind of "grammar". To test this idea experimentally, I asked my former Ph.D. student to challenge rice with different types of osmotic stress where different components were either given separately or in combination (Hazman et al. 2016). The result was intriguing—the plant did not simply add up the response to the components, but each stress combination was processed as a new type of sensory quality—similar to us, when we perceive the combination of blue and yellow as green, even though there is no light of the green part of the spectrum.

In the meantime, we search on the cellular correlates of this holistic property and found out that microtubules are an essential element of "reading" the grammar. I got the honour to be invited to write a review on this idea on behalf of the 50th anniversary of the discovery of microtubules and I took the occasion to develop the idea of a stress grammar (Nick 2013a, b). At some point in the middle of writing, I understood that I am just again asking the question of my childhood "I want to know, how they think, even though they do not have a brain!" The answer is that they use chemical signals just as we use words and that the order of these words in time gives meaning, just as we can generate language by creating rows of words. If I am lucky, I will be able to decipher the "grammar of plant stress", which will allow not only to understand the secret language of plants, but also to make this knowledge useful, in order to render plants more resilient against climate change. Of course, this plan is too ambitious to come true, but it does not matter. According to Camus, one needs to envisage Sysiphos as a happy man…

When I negotiated with the university to ward off the call to take the chair at Salzburg in 2004, one of my conditions was that the university would preserve the Botanical Garden, which was at stake and repeatedly at the brim of being closed down. They agreed, but the deal was that I should demonstrate that the Botanical Garden is relevant to research (under my predecessor, the Botanical Garden was just left alone and was basically a place where plants were grown without any connection with the research at the institute). I thought about a strategy and developed the idea that a Botanical Garden is a place, where plant biodiversity is established and maintained. In a technical university, where engineers dominated, I would need to show that this is useful. Thus, I coined the idea to protect and to valorise biodiversity, focussing on crop plants. Still in my Freiburg time, I had started to cooperate with the State Institute of Viticulture, because they had asked me for support in understanding the cell biology of *Plasmopara viticola*, the pathogen causing Downy Mildew of Grapevine, one of the

most severe diseases in viticulture worldwide. This pathogen is native to the US but was introduced by accident to Europe in the nineteenth century causing tremendous damage and still requiring intense chemical plant protection (around 70% of the total consumption of fungicides in Europe). It was already known that wild grapevines in the US were resistant to the pathogen because they had evolved together.

But what about wild grapes in China? I started to establish a collection of wild grapevines from all over the world, connected to many stories and wild anecdotes, which I will not tell now, because I want to finish the chapter. We found out that some of the wild grapevines from China were resistant as well, but by a different mechanism—they were able to confuse the flagellate zoospores of the pathogen, such that they could not find the entry point into the grapevine leaf, the stomata, and continued to wander around, progressively frustrated, till at some point they tried to develop a mycelium on the surface of the leaf that would die off soon after. We reckoned that the zoospores could sniff out were to go through a chemical signal emitted by the stomata, a kind of mouth odour of the plant. I could convince Prof. Boland at the Max-Planck Institute for Chemical Ecology in Jena to help us finding out, what the signal was. They agreed, and so I drove to Jena with several grapevine plants, a German variety called Müller-Thurgau, and one of the Chines grapevines, called *Vitis jaquemontii*. I still remember, how people stared at me, when I entered the high-speed train with my grapevines that were almost as tall as me. The lab of Prof. Boland had a sophisticated Gas Chromatography/Mass Spectroscopy platform and analysed what the plants were emitting. A few days later we had first candidates for the mouth odour that differed between the German and the Chinese grapevines. We tested those for their ability to attract the zoospores and found out that a small aldehyde, nonanal, was responsible. The Chinese grapes emitted this compound from everywhere, such that it became impossible for the zoospores to find out, where the stomata were. In the process, we also discovered, how the searching worked—as long as the concentration of the attractant was increasing, they were swimming with their flagellae straight, if the concentration was decreasing meaning that they have missed their target, they stretched out the shorter flagellum perpendicular such that the cell was circling around, changing direction, and then trying again to swim straight. In this way, zigzagging, they ended up at the stoma, where they attached and entered the leaf. Actually, these spores are finding their path in the same way as scientists do (remember the start of this chapter).

Soon, the news about our exotic collection spread and I was asked by the Ministry of Agriculture to help them in preserving the almost extinct European Wild Grapevine (*Vitis sylvestris*). A last population grew at the Ketsch peninsula, in an alluvial forest between Karlsruhe and Mannheim. The task was to collect twigs from those grapes, make them regenerate and multiply them for resettlement in the wild in places, where the Rhine had been re-naturalised. To collect these grapes was an adventure because they were growing as lianae in the top of the Ketsch jungle. My technical assistant, Ernst Heene, who was both, a hunter and a native from the Palatinate, a part of German, where the relationship with vine is genetically encoded, caught fire and promised to get all of the wild grapes for our garden. In some cases, he even had to shoot down the twigs from the treetops. The project was successful,

but we were not content in just helping this species to survive, we also started to investigate the immune responses of these plants, as said above, had been working intensively on the signals that regulate grapevine immunity. To our surprise, many of these wild grapevines were able to ward off *Plasmopara viticola*, although they never had any contact with this pathogen from North America. We found out that they were endowed with a strong basal immunity and rapidly accumulated resveratrol and viniferins, defence compounds that kill fungi very efficiently (Duan et al. 2015). For one of our champions, called Hördt 29, we could even find out the reason for this ability—this wild grapevine had evolved a special version of a promoter for a gene switch that turns on the enzymes producing resveratrol (Duan et al. 2016). We demonstrated this, by inserting this promoter sequence in front of luciferase from firefly and shooting this construct into grapevine cells by a gene gun. When these cells were stimulated by signals, the promoter became active and luciferase was made, which we could measure as a light signal with a luminometer. The promoter version from Hördt 29 was much stronger, explaining the better basal immunity.

We understood that our collection of *Vitis sylvestris* was a treasure, full of genes for resilience, which could be used for breeding, because this ancestral species can be easily crossed with domesticated grapevine. Since we knew, which gene variants are relevant, the breeding process can be accelerated, because the offspring of a cross can be checked already at the seedling stage for the desired version of the resilience gene (so-called marker-assisted selection or smart breeding). We have launched this already with Hördt 29, which is resilient not only to various diseases, but also to other stress factors. The news of our collection spread, and I got a request from the Chinese Academy of Science, whether they could sequence the genomes of our wild grapes. First, they wanted the plants, but I refused to give them away. Later, we agreed on a deal—they would get high-quality DNA and we would get access to the genome sequences. In the meantime, we have assembled the entire gene pool for *Vitis sylvestris* that has survived in Germany along with a good part of genotypes from other European countries, and we have established a genome database, such that we can look up for each gene of interest, which variants exist in our collection, go to the garden, pick the leaf, and clone out the respective gene to investigate its function. We have identified resilience factors against many diseases including Grapevine Trunk Diseases that emerge now in consequence of climate change, but also resilience factors against drought, salinity, or heat, problems that will become progressively relevant for agriculture. We want to use this treasure to help breeding new grapevines that can cope with the consequences of climate change.

The grapevine project was something like a paradigm to demonstrate that plant biodiversity is not only relevant for Nature, but also for us, humans. We have added other projects along the same line. For instance, we established a gene bank for Crop Wild Relative which is now part of the Plant Genetic Ressources of the Germany; or we assembled a collection of reference plants for genetic authentication to hunt faked food products, exotic plants that, due to globalisation, enter the European market and are hyped as "super-foods"; or we could show that different Mint species use their scent to kill their competitors by attacking their cytoskeleton (Sarheed et al. 2020). All these projects link scientific curiosity with application (we call this strategy

hypothesis-driven application). “Pure” scientists often tend to look down on applied science—I strongly disapprove this attitude, and I concur with one of the teachings, I had heard from Furuya-*sensei* again and again: “We are all paid by the taxpayer. You should always be able to explain to the taxpayer, what you are doing, and how this, what you are doing, justifies being paid by tax-money.”

The work in the Botanic Garden was thriving, but one day, we received a threat that was existential. A wealthy software company approached the university with the offer to build several huge buildings for informatics, some they would use themselves, others they would rent out, and some would be given to the university. It should be central and close to the Campus. In the eyes of these technocrats, the Botanical Garden appeared as an unbuilt area which was worth a lot of money and was an ideal site. I had to fight a long and sometimes lonely battle against my own university, and if I had not demonstrated before that the biodiversity established in the Botanical Garden is of value also for the taxpayer, I would have lost the battle. Even so, it took many years, and I felt like David against Goliath. I did not use a sling, but I had to employ the entire repertory of tricks which I had been taught by Furuya-*sensei*, also activating my entire political network to resist. Eventually, they promised a new garden at a site nearby with new greenhouses and even a new institute building. The budget, which is considerable, has just been approved by the parliament end 2021 and I hope to see the new garden before I retire. This investment would then also secure the existence of the Botanical Garden after my retirement, it is the only material legacy, I want to leave behind (otherwise, I consider only the legacy from our actions and inspirations as real, but here I make an exception).

4.10.1 Don’t Be Afraid from Taking the Lead—It Is All About Communication in the First Place

I want to conclude this chapter with some remarks about leadership, because scientists often forget that their path will lead them one day into a position, where they have to lead others, and to my opinion, they are rarely prepared for this. The conventional model for leadership is that of a hierarchy, often accompanied by psychological pressure. As I know from my own career, young scientists are extremely vulnerable and dependent on the benevolence of their bosses. To push them to work even harder, seems an easy job, since their perspective is unsecure over many years. However, I strongly despise this approach—it is immoral, it is exploiting, and it is not sustainable. Science is rooted in strong personalities. Personal motivation is the most important driving force (and the only driving force that matters to my opinion).

Thus, I did not even try to lead such a big group by hierarchy. Instead, I tried to learn from the way, how plant cells organise themselves into an organism. They do this by communicating with each other all the time and by synchronising their individual rhythms. Following this principle, I mainly worked on rhythmic communication, which means that I have frequent meetings with all three subgroups, but also with the gardeners, the technical staff, and of course, with the entire institute. While I leave a lot of individuality to everyone, also with respect to working time, I insist

that everyone participates in these meetings, and when somebody does not show up, I do ask for the reason. My friend, Diego Breviario, had introduced to me, some years ago, the work of John Mattick, who wrote an inspiring article about the role of non-coding sequences in evolution (Mattick and Gagen 2001). He pointed out that it is not the number of genes that determines the complexity of an organism, but the number of their interactions. If the number of nodes in a system grows by a factor of n, the number of interactions needs to grow by the n^2. I took this very seriously in my group and worked mainly on the structure of interactions and on clear communication channels. The second duty I have is to inspire my people, to help them in finding the story in their data and to support their scientific and personal development by discussion and advice. One lesson, I have learnt from Furuya-*sensei* is, that as a leader, I always need to respond, if somebody asks me, and if I have no time now to deal with the matter, I need to give at least this reply. He taught me that the more one advances into a leading position, the more obliged one should be to communicate and respond to everyone—the only power that is real is the virtue of communication. Of course, I am not naïve and know that this virtue is often ignored, but a moral value remains in effect even if it is ignored by many.

I enjoy helping young people from all over the world to develop on their path. I also enjoy teaching young people and watching them, how they find questions and answers, and I greatly enjoy the freedom to shape my work following my interests, to develop ideas, and make them tested experimentally. Of course, there are also less pleasant aspects of my work, such as hunting for the money to run my lab or sitting in endless committees. Of course, I also have to act on the political level, which is not the thing, what really interests me. However, I also have learnt to use power, if it is necessary, but the cases, where I have to rely to this means, are rare, and if this happens, it is directed against the bureaucracy or the leaders of my university, not against those that depend on me. In the times, when I suffer from this aspect of scientific life, I recall Furuya-*sensei*'s lessons, who not only taught me many tricks, but also a perspective on science as a common endeavour of humanity that we get as legacy from our teachers and that we have to pass on in good shape as legacy to our students. This perspective helps me to live through the less pleasant aspects of being the head of a chair at university. Overall, I cannot imagine any other work that would give me that degree of fulfilment.

5 Advice to the Next Generation of Scientists

BELIEVE IN YOUR QUESTIONS! The previous section was quite detailed, and this had a reason. All these examples, highlighting my scientific path, tried also to illustrate some general points or advice that I want to pass on to the next generation. In the final section, which will be much shorter, I try to formulate those points explicitly. In this explicit form, these statements may appear a bit abstract, but this is intentional. To render an advice fruitful, you need to translate it for the own path. Each path is different and individual (this is already the first statement).

5.1 Try to Stay Rooted

If you are looking for a predictable, stable, and regular life, it is probably not a good idea to become a scientist. As you can see from this chapter, moving around in the world as well as coping with unsecure perspectives are typical elements of a scientific career. It is important that you balance this in your personal live. Only then will you have the energy to stand the volatility of scientific life. Your cultural background, important habits that come with your upbringing (for instance, food or music), a close relation with loyal friends, or following a non-scientific passion will help you to withstand the unsecurity and challenges arising from living as a scientist. It is inevitable that you also will move through times of conflicting demands—for instance, when you have small children, it is not possible to work late hours or to visit a conference, even if your boss would like you to. One needs to find compromises. This does not mean at all that you are a bad or non-dedicated scientist. It just means that, as a human, you are more than just a scientist. When you have to make important decisions, always hear what your beloved ones think about it. If they think that this move may be good for your career, but bad for them, you have to take this seriously, and if the move would disrupt your family or relation, decline it.

5.2 Search Mentors that Do Not Instrumentalise You

Especially during the early part of your career, you rely on mentors, which brings you into a state of dependency. Their benevolence and support are not only needed to help you through the formal steps of your scientific curriculum. If you do not have your own funding, for instance, through a scholarship, you may need a working contract from them. After you have defended your Ph.D., you will need them for reference letters that open doors to other opportunities. Last, but not least, biology is an experimental science, and to be competitive, you need a certain infrastructure and funding for your experiments to proceed. Your mentors, on the other hand, are also subject to competition for funding and sometimes also for their standing in science, especially, when they are driven by personal ambitions to climb up the hierarchy of scientific and political power. To find the right mentor is pivotal, therefore. Some mentors consider their students as tools to support their own path, rather than as young scientists that strive to develop their own scientific profile. Of course, it is difficult to predict, how it will turn out to work in a given group, but you should keep your eyes and ears open, before you accept an offer to join a given lab. What type of paper does the lab publish—do you see that each Ph.D. student comes up with his or her story, or are these papers with numerous authors, possibly published in high-ranking journals, but with many former lab students sandwiched in a long list of authors, and just the one, who was happy enough to join the lab, when the story was ready to be completed in the life time of a Ph.D. student, had the profit in form of a first-authorship position? How is the mentor talking about his people? Do you have the feeling that there is an

atmosphere of respect and cooperativity in the lab, or are the students all working separated, possibly even against each other? Are there regular group meetings, are the discussions there open and supportive, or is there an air of fear and mistrust? Most importantly: do you feel that the mentor is a happy person with a generous and supportive character? What are the motivations behind the research of the group—are they mainly thinking about money and reputation, or are they genuinely interested in understanding Nature's mysteries, are they interested in using science to make our world a better place? If you feel that some of these questions would be answered by "no", you should look for a different mentor, because there is nothing worse for young scientists than a mentor that tries to use them as instruments to boost the own ego.

5.3 Follow Your Gut Feeling

The path of a scientist is a path through a jungle. Whether your path will lead you somewhere, you can never tell. Actually, nobody can tell. You need to pass uncharted territory, often under unclear or even unsecure circumstances. The temptation to get hold of anything that appears stable is strong, therefore. To refuse an opportunity that is offered to you may appear like acting insane. However, don't let yourself be overwhelmed, but ask for a short time to think and then listen to your guts. Do you feel comfortable in presence of the person who offers you the job or place in the lab? Or do you perceive some kind of uneasiness? If so, follow your gut feeling and not your neocortex that will provide you with numerous rational reasons, why you should accept the offer (although the same neocortex is as readily able to provide you with numerous rational reasons, why you should not). Decisions are often depicted as branching points that will decide your future path (I have to admit that I also did this repeatedly during this chapter). This may not be totally true. It is not the decision, but what you make out of it, what really matters. Why should the paths through the jungle only split up? They might as well merge at a later point—I have experienced this several times. In order to develop out of a decision something which is fruitful, the circumstances along the path are more relevant than the question, whether you had turned to the left or to the right in the beginning. Such a decision is usually a multifactorial situation, which very quickly goes beyond that what we can grasp rationally. Your gut feeling acts in a holistic manner, it may perceive the unspoken signals of a person, promising you a pink future, it may notice that the facial expression of the person does not entirely match with the content of the words. The discrepancy may be subtle, and your consciousness may not pick it up, your gut feeling will. It is rarely scientific content that decides about success or failure, but the atmosphere in your lab, the support by the others, the spirit of inspiration that provides you the energy to go on and overcome the many difficulties and frustrations that usually line the path of a scientist. If the atmosphere is bad, it will suck the spirit

out of you, and you remain like a zombie, filled with fatigue and depression, which will make you fail inevitably. Your gut feeling can protect you from that. Take it seriously.

5.4 Ambition is Fine, But It Should Not Be Personal

As I tried to convey to you in this chapter, science is motivated by questions, sometimes by bold questions. To follow this up, requires a certain degree of megalomania, and this is perfectly fine. Because it is clear that you will need to pass not only through periods of hard labour, but also of frustration, and your vision needs to carry you through all that, often over many years. However, this ambition should be directed on your idea, not on your person. Whether it is you or somebody else, who discovered something is actually irrelevant. Did you know that the half-time of biological knowledge is around five years? In five years from now, half of the knowledge has become irrelevant, in ten years, only a quarter is still valid. So, even the most prestigious breakthrough will become inevitably annihilated by the course of time. What your name is in science, matters only for a few years, what matters more, is the impulse you gave to science, it will survive you, inspire those coming after you and bear fruits that are quite different from those you anticipated.

5.5 Do It Your Way

Doing science successfully, requires a long training and a lot of skills. If you do not apply a method appropriately, you will simply fail to get results. One needs to respect the rules of handicraft to become a good artist. However, there is a level beyond methodology, and, here, it is important that you follow your question, and not the question of somebody else. If you find something new, it is rarely easily understood or adopted by the majority. Usually, you will encounter scepticism and sometimes even resistance. Don't yield too easily—if somebody gives you a good ground that your idea is wrong, then you have to yield, but only then.

5.6 Have Fun

Knowledge does not fall from sky, it has to be searched, often through blood and sweat. One needs a certain degree of stoicism to go through all that, but one can only do so, if one is able to feel the bliss of finding out a secret, to make connections between parts that seemed unrelated before and now begin to make sense. So, do not forget to have fun in doing science, cherish the moments, where something which you

predicted became manifest in the experiment you conducted to test your prediction. This is not only perfectly alright, but it also keeps us going.

5.7 Trust and Be Trustworthy

Scientists are individuals, but they are never alone. Your question crystallised from the work of others that have asked other questions, and your answer will inspire those that come after you. This vertical line is accompanied by a horizontal plane—at the same time as you follow your questions, others follow theirs and those that support each other will advance. Cooperation will also help you to integrate different viewpoints on your matter. Never forget that observation is never objective, nor is experimentation. Both have a purpose, coming from the reasoning you invested before setting up the experiment. During this reasoning, you had to reduce the complexity of reality to some aspects, which you can and want to address in your work. Perhaps you have chopped off an important aspect that you will not see then. Your colleague, who looks at the phenomenon from a different angle, my see, what you don't. Thus, cooperation is the core of science, and it must never be sacrificed to competition. Cooperation is based on mutual trust. Search people, whom you trust, and be trustworthy for others. Only then will you be able to become a good scientist.

5.8 Be a Citizen of the World

Science is and has always been crossing borders of language, culture, and geography. It is amazing to see, for instance, how during the early Middle Ages, when Christians and Muslims were caught in everlasting warfare, scientists from both sides exchanged ideas, concepts, and methods. While science always requires personalities, it is and should be independent of collective constraints. Whether the scientist, who proposed a theory, is from Patagonia, or from Germany, is completely irrelevant, as well as if this person is male or female or something else, relevant is only the content of the theory proposed by this person. You can also turn this argument: If science is independent of all separations that are artificially constructed by humans, it is also overarching all humans, beyond these separating categories. Scientists are, thus, members of a community that follows a common set of virtues:

- *Justitia.* To be successful, they need to seek for fairness—since nobody of us knows the truth, we always have to weigh the arguments in favour and against a theory, even if it has been developed by ourselves. If we neglect fairness, we will, sooner or later, run astray in the jungle of the unknown.
- *Veritas.* As scientists, we know that we will never be able to see the world as it really is. At best, we will move in our work towards something like scientific truth. The only thing, we can do, is to be authentic, work, think, and speak carefully,

avoid mistakes to the best of our knowledge and correct them, when our knowledge has grown far enough to see that we went astray.

- *Temperantia.* To go on our path, which is often not an easy path, we need enthusiasm and inspiration. When, after a long struggle, we succeeded to extract a piece of consistent reality from the fog that surrounds us, we feel a bliss, because we have created an image of the world that fits together and allows us to see a part of the beauty of Nature. This bliss comes with a risk, though. We can get drunk from it and easily overstretch the image we have created. Like Narziss, who fell in love with his own mirror image, we stop then to continue our path. Especially in such moments, it is important to pour some modesty into our temporary victory considering that our image has been created by reducing the complexity of reality to some aspects we can grip and humbly acknowledging that even the most powerful theory is only an approximation to a far more complex reality.
- *Fortitudo.* To search a path in the fog means, one has to walk, where nobody else has walked before, leaving footsteps on virgin ground. Whether this path will lead somewhere, or whether it is a dead end, we cannot know before we have walked it. This needs courage. Without courage, there is no science. Even when our path has been successful and we arrived on a hilltop that allowed us to oversee part of the landscape, it will need even more courage to tell this to others that have not been there and first are reluctant to follow.

So far, a translation of Aristoteles Cardinal Virtues into the world of science. Whoever follows these virtues as part of scientific professionality, will also need to live those virtues as a person, because personal authenticity is a precondition for the professional use of these virtues. In other words, the professional virtues of scientific work establish a framework that can be used, when people from different backgrounds encounter. Scientists are citizens of the world. Citizenship has some consequences—a citizen is a free member of a city, but on the other hand, a citizen bears responsibility for the city. Science can only thrive in an atmosphere of freedom, freedom is not a gift from the Gods, it has to be achieved and cultivated by everybody of us, every day.

5.9 Cultivate the Art of not Knowing.

Science is not a religion, and it does not enshrine a "truth" whatsoever. It is more something like a scouting art—while we are searching for our path in a forest, nobody of us has the privilege to fly like an eagle over the landscape and see, where these paths are leading to. We can only pursue our own path as long as it appears successful to us, when we get stuck, we have to change direction. Whether the new direction is better than the former, we will only find out by walking. As long as it is clear to us that we are not in the position of the eagle, but in the position of those walking in the forest, all is fine. But this is not enough. We should not be angry about the infinity of this forest, which will never be grasped by our minds. In the opposite, we should

be happy about this infinity, because it will keep our fascination alive, our fire burn, and our steps move on. It is the process of walking that matters, not the path that we have achieved.

So, just go ahead!

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More is Better Than One: The Strength of Interdisciplinarity

Alfonsina Ramundo Orlando

Abstract Bioelectromagnetism, a scientific niche sector that studies the interaction of ElectroMagnetic Fields (EMF) with biological systems, is an interdisciplinary field of research. The following are just a few examples of current unanswered questions that still foster my interest. Could the exposure to Extremely Low Frequency (ELF) electric and magnetic fields generated by power lines, and Radio Frequency (RF) electromagnetic fields emitted by radio antennas and wireless networks cause possible adverse health effects? Could RF field exposures at realistic power densities cause systemic body warming in humans? Is warming the main cause of any observed RF fields effects? How can we explain RF field subtle effects or non-thermal effects at absorbed power level well below the existing safe exposure limits? Do we know RF field long-term effects? Do we know the biophysical interaction mechanisms between RF fields and biological systems that are caused other than a change in temperature? The rather complex structure of the human body and dosimetry—evaluation of the dose of RF fields when the human tissue is exposed- makes difficult the studies and the reported effects to be described by classical dose–response relationships. Furthermore, the intensity and frequency and/or modulation—the physical parameters of the electromagnetic field itself-dependence is not consistent with classical physicochemical responses of living systems to physical or chemical agents. Does this mean that we have to stop searching for answers? No, I don't think so. These are the intriguing issues that encourage the creativity of scientist across disciplines, who look for answers that will enhance humanity's knowledge as well as how we relate as humans to electromagnetism.

1 Motivations: How I Developed an Interest in Science

I enrolled in the Faculty of Pharmacy for family motives (my favourite aunt was a pharmacist), not because it was my preferred subject matter. However, later on I was to appreciate this choice, given the number of hours spent in the laboratory.

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I have always been fascinated by hands on research that made me feel active and elated, then, I became captivated by biochemistry; the study of matter made up of atoms that combined together form biomolecules; the study of complex chemical reactions and metabolism. What beauty existed within the understanding of all this knowledge! Every day, on leaving the university grounds, I found myself in front of the "Istituto Superiore di Sanita" (ISS), the National Institute of Health of Italy. I was curious and knew that inside that building, important medical research studies took place and that some Nobel Prize winners had carried out their researches there: biochemists such as, Ernst Boris Chain, who was the co-discoverer of penicillin, or Daniel Bovet, who discovered antihistamines. It is important to know the reputation of the place that will influence the choices of where to invest one's future, therefore, I did everything to get into ISS. In the Laboratory of Toxicological Biochemistry directed by Prof. Vittorio Silano, a pure biochemist, I found what looked like an "artisan's workshop"; a Master of great professional experience, who knew how to command even through his humanity; he was surrounded by talented biologists and chemists full of **enthusiasm**, who were assisted by highly qualified technicians, one of whom had worked directly with Daniel Bovet. There was passion; time spent in the laboratory seemed to fly and with total commitment I performed the tasks given to me. I was educated in the precision of measurements, **honesty** in recognizing a negative result, as well as critically interpreting every sort of scientific evidence, and always questioning my (and their) own ideas. I acquired experience on a great number of techniques that would later permit me to deal with the complex problems of biochemical research. In 1982, I was chosen among various research fellows to follow the studies started by a researcher (who was leaving for an internship), on the purification of a new α-amylase—an enzyme that hydrolyzes starch and glycogen-different from other α-amylase, which is well characterized and produced by *Bacillus subtilis*. The positive results were the subject of my first-first authored publication. This period of time in the laboratory at ISS proved fundamental for my scientific maturation, because it allowed me to acquire more awareness and familiarity with experimental work.

At the laboratory, there was a strong interest in ecotoxicology—a multidisciplinary field that integrates the concepts of toxicology and ecology—thus we began to study the enzymatic cytochrome dependent monooxygenases P-450. This system represents the main path of metabolism and drug detoxification in the human body. It was known that this metabolic system could also play a role in the transformation of some chemical substances (present in the environment) into carcinogenic substances. However, it was not known whether this metabolic system was present in some organisms situated on various levels of the biological scale, that were used in the laboratory as bioindicators since they were particularly sensitive to the changes made to the ecosystem that they inhabit. Thus, quails, trout and water fleas (*Daphnia magna*) appeared on our laboratory benches from which we had to extract the liver in order to characterize the said drug metabolism system. It was a very time consuming and meticulous job; imagine how much time and patience it takes to excise a sample of the liver from a water flea, a crustacean, measuring only a few centimetres. But, it turned out to be worthwhile as well, not only regarding the results of the experiments—we

had discovered that even the water fleas have this metabolic system- but also because we received international and national funding that allowed us to buy laboratory equipment, never imagined possible before, such as a very expensive CARY double wavelength and beam spectrophotometer with which one attains superb differential spectra of the cytochrome P-450. Invitations began to arrive requesting us to present our results to the most important congresses; and, in my case, be chosen to participate in prestigious courses of study, such as the one promoted by UNESCO at the Stockholm Arrhenius Laboratory. There I was able to converse with scientists who represented all my bibliographic references like Joseph W. De Pierre, who was the first to characterize the P-450 metabolic system in a rat liver. However, it was my participation in a Summer School on the multidisciplinary evaluation of environmental risks on human health—held at the University of Siena in 1988—that was to consolidate my interest in interdisciplinary science. I felt at ease with other participants coming from various disciplines and other parts of the world (some of whom I am still in contact with today), as well as with professors, who ranged from entomologist to "disastrologo", from biochemist to philosopher.

In 1990, prestigious scientists such as Nobel Laureate Rita Levi Montalcini, Enzo Bonmassar, and others decided to open a scientific "research area" at CNR in Rome, focused on interdisciplinary studies. I joined as a tenured researcher one of their Institutes to follow my interests in the development of biomimetic systems that imitate the function and the structure of a more complex biological system. Their applications can be diverse from the biological (proto cell, bioreactors) to the therapeutic sector (drug and vaccine delivery).

Later, my encounter with a group of electronic engineers collaborating with Prof. Guglielmo D'Inzeo, initiated my studies of Bioelectromagnetism. An area of study that was then developing, the purpose of which was to understand how living organisms interact with EMF, that is to say, that portion of the spectrum whose electromagnetic waves, these days, come exclusively from artificial and technological sources produced by man for electrification and radio communications. Gugliemo asked me to give lectures in his course, at "La Sapienza" University in Rome, on plasma membrane and its artificial models (i.e., liposome). He was always present at my lectures and, at the end of each one, he would continue to ask for more clarifications and insights, experiences that sometimes lasted as long as the lecture itself. I found the approach required by these studies intriguing (interdisciplinary, by its own nature); although it did call for a considerable effort to make engineers, biophysicists, pharmacists and biologists to break away their specialized language and find a common ground of communication. The results were such that dozens of students from electronic engineering applied for Master or Doctorate's theses in my laboratory. At that time, there was no Faculty of Bioengineering! My expectations were high; it was well-known that the cell membrane was the primary target of the interaction between EMF and living organisms, however, by applying a simplification to this very complex cellular system, we would have been able to give a substantial contribution, at a molecular level, to the understanding of the biophysical mechanisms at the base of this interaction.

2 Work Done: My Personal Scientific Approach

I would never initiate a discussion with "We, the biochemists", since I find it a bit annoying when those from other specializations do. I never felt like being a member of a corporation or association no matter how authoritative or respected. I consider the researcher's skills at the same level as craftsman or creative artist with a practical understanding of the problems at hand, as well as the knowledge of the most appropriate ways to deal with them. I have always worked with dedication, constancy, and intelligence on my practices trying to improve and, therefore, innovate them. I have never been driven by the urgency to increase the number of my publications or to think that publication metrics are the primary means of being evaluated; and, I never stopped questioning my self, my colleagues, and the world around me regarding what I was doing. There are an infinite number of questions going on in the heads of people involved in science.

In the case of Bioelectromagnetism the questions that emerge can affect different fields of research: (*i*) biological ones: can we use the EMF, to which probably living organisms are not adapted, as probes to study the functions of living organisms? (*ii*) medical ones: overlapping external EMF with consequent modification of a physiological process can alter electromagnetically active processes of cells? and (*iii*) health: can the growing and continued exposure to EMF, virtually ubiquitous in the environment, have long-term consequences on human's health? Under no circumstances, today, are there any definite answers.

With respect to health—my specific interest in the ELF electric and magnetic field—notwithstanding the initial scepticism of the scientific community with respect to the American epidemiological studies that, in 1979, had indicated a three-fold increase in the incidence of leukaemia in children residing near power lines; more than 2500 articles were published on the biological effects of EMF in the next thirty years. Clearly, though, the ELF-EMF generated for electrification have *very low* field frequencies and, therefore, carry much less energy than those involved in different chemical or physical phenomena that normally occur in biosystems and, based on the limits set by law, they are also at low intensity. Consequently, they do not directly interfere with the cellular electrical activities (for example nerve endings, muscles or heart). Despite the above, numerous studies in vitro (on cells) and in vivo (on animals) had indicated biological effects on cellular proliferation, on biomolecules (DNA, RNA, proteins), increase of cancer, and some functions of the cell membrane, such as the transport of calcium ions and ligand-receptor bonds. Due to its electrical properties and its function as a barrier to the outside, the cell membrane was indicated as the primary target of interaction with EMF and, most of all, the calcium ion (Ca^{2+}), whose physiological role as second messenger is to allow an external signal to propagate within the cell thus modulating several biochemical processes. Based on this ion, several mechanistic models have been identified, highlighting a crucial point for bioelectromagnetism regarding the interaction between ELF-EMF and membrane components, e.g. cyclotron ion resonance, a phenomenon related to the movements of ions in a magnetic field. However, these mechanisms were not able to explain all of

the biological effects observed up until then, suggesting the possibility that there were multiple mechanisms or that there others primary targets should be considered. The latter was exactly what we explored together. With electronic engineers, with whom I collaborated, we focused our attention on the very components of the membrane (i.e., phospholipids) rather than free ions (i.e., calcium). To be able to do this, we needed a simpler model system than whole cell. The simplification of the complex world has always fascinated mankind, however, in the process of simplification, a scientific approach must always be adopted.

At that time, I was studying bioreactors in which an enzyme capable of catalyzing the study reaction was enclosed within a liposome. The liposome made up of phospholipids, mimics the structure and the function of the cell membrane, but compared to the latter, it has a decreased level of complexity in its molecular composition, thus allowing studies of the interaction of EMF with cell membrane at molecular level. The first series of experiments were disappointing because the effects of the ELF-EMF were at the limit of statistical variation and not reproducible, most likely, due to the fact that the detection of the effect on the membrane permeability was indirect: the sample was exposed to ELF-EMF and later the enzymatic activity was measured.

Was it possible to reveal the possible effect in real time during the exposure of the sample itself to reduce artefacts or a possible reversibility of the action? I pondered about this question day and night, even dreamt about it. Yes! The effect could be revealed in real time! It was the time of celebration when the new ELF-EMF exposure device, nicknamed "chick", due to its yellow colour, arrived at the lab. Inside this device it was possible to co-locate both the sample and the optical probe used to measure the enzymatic activity during the ELF-EMF exposure. Thus, the detection of the effect on the permeability function was direct in this case. We were the first to have a rigorous 'real time' experimental set-up, which was later adopted by the entire scientific community of bioelectromagnetics for in vitro studies. The experimental results were excellent and, above all, due to a brilliant piece of thinking by two of our young engineers, a clear correlation was found between experimental data and theoretical analysis and, consequently, a mechanism of interaction was hypothesized and proposed. They demonstrated with mathematical calculations that the energy (even if low) transferred by the ELF-EMF to a dipole, a component of the polar head of the phospholipids, was sufficient to give a small tap to the dipole and consequently make it rotate—like a balloon attached to a rod—thus modifying the very position of the phospholipids in the liposome. That was just enough to create a small free space, which, in turn, could alter the permeability function. The proposed biophysical mechanism, based on Larmor precession theory, made it possible to predict more specific biological effects as a function of well-established parameters of exposure to ELF-EMF. If our theory was right, then it had to also work on alternative membrane models ('theory testing is comparative'): that is what I verified developing several new artificial membrane models over the years. In one case, I was able to obtain, due to a *weak* serendipity (we had left the sample in the fridge for one night without processing it immediately), the first reconstitution of a functional gap junction in pairs of closely apposed lipid bilayers, as experienced in cells. Intercellular

communications mediated by gap junction channels plays an important role in many cellular processes. Recently, we have built a bioelectrical model of a neural axon to study the effects of microwaves on the propagation of the electrical signal, with possible future medical applications.

Even though we obtained important results, regularly published in respected scientific journals, the topic was not considered to be sufficiently trendy for scientific journals with a very high impact factor. The difficulty of defining a dose for EMF exposure, due to the characteristics of frequency, intensity, modulation and exposure time, does not allow for the evaluation of the biological effects with a classic dose–effect relationship, and this creates perplexity about the possible sensitivity of biological organisms to EMF.

Besides that, thanks to the previously mentioned new experimental models, more targeted, rigorous and screening in vitro studies are being conducted today regarding the characteristics of EMF, laying the basis for future discoveries in the field of Bioelectromagnetism.

To conclude this section, I would like to emphasize the importance I have placed on the combination of research (producing knowledge) and teaching (transmitting knowledge), which, in my opinion, must remain indissoluble. In this regard, with considerable organizational and financial efforts, I founded an International Summer School at the CNR Research Area of Tor Vergata-Rome for the promotion and development of studies and research in the pharmaceutical, biomaterials, and tissue engineering and molecular simulation. The characteristic of this school is its interdisciplinarity. Postdoc students from all over the world and from different academic fields (physics, engineering, chemistry, biology, medicine, mathematics) follow highly specialized lecturers on cell model systems, given by internationally recognized professors, along with practical exercises on high level techniques and technological instruments (e.g. advanced sensors, precise analytical methods, enhanced imaging capabilities as well as sophisticated computational tools) conducted by physicists, engineers and mathematicians in the laboratories of the CNR Area.

3 Science Today and Tomorrow

The phrase that best summarizes my view of science was said back in 1986 by Rita Levi Montalcini, Nobel Laureate in Medicine: “Science is the only thing that distinguishes Homo Sapiens from the rest of living creatures. It must be **cultivated**, certainly not blocked.” Science must be cultivated because science proceeds in steps, and with the help of those who come first to those who come later, and mutual collaboration among scientists, it adds new discoveries to earlier discoveries in a continuum. It needs time “Tempore patet occulta veritas”—“The passing of time unveils the hidden truth” [was the motto on emblem of some of Sir Francis Bacon’s texts]. Therefore, typical of those who do scientific research is a sort of continuous and confident dedication driven by a desire *to unveil* something, proceeding by the sound method of trial and error in order to offer objective and reproducible answers,

but not *absolute unchangeable truths.* Science therefore needs to be not only today but **always**. Yet society today has managed to upset this cornerstone, and on a par with everything else around us, it has become a here-today-gone-tomorrow fad.

Let us examine one case. As I mentioned in the previous section, numerous studies performed in the 1990's had indicated a possible correlation between exposure to EMF and the occurrence of harmful effects on human health. The term EMF, incomprehensible to most people, made its way from electrical engineering textbooks to newspaper articles. Even though the term remained largely misunderstood, it became familiar/common. The perception of the risk from exposure to EMF at an industrial and radio frequency (RF) spread and grew in the public mind to such an extent that decision makers were prompted to promote targeted research programs-selecting the type of questions in advance and narrowing their depth as much as possible, hoping to obtain *certain* acceptable answers and enabling the issuing of recommendations and regulations. Since science cannot be asked for answers it is incapable of giving, the results of these studies were not the absolute truth the bureaucrats hoped to receive. The results were devoid of cause-and-effect correlations with refutable epidemiological data and with contradictory in vivo and in vitro studies, meaning that no risk could be determined with sufficient certainty. The only well-established thing was that the power (measured in Watt/m^2) of RF fields had to be kept very low to avoid thermal effects. Therefore, exposure levels were set in the main EU countries, mainly based on the EU Council's 2001 precautionary principle, which had to be complied with-in order to protect the health of the population. Subsequently, the public's attention to these issues declined—it has been shown that humans are not rational at all when it comes to risk assessment—and as a consequence that of the decision-makers who are so very sensitive to public opinion. Over the last few decades very limited economic resources have been devoted, particularly in Italy, to *progressive* research programs—worthwhile ones according to Imre Lakatos's classification—because they delve deeper into topics. In those cases, curiosity-driven research, i.e., useless science, is welcome, much to the chagrin of EU agencies who favour projects with timescale prediction of the milestones (in year 1 discover this, in year 2 discover this) and fund on a publication basis (total number of publications in journals preferably with a high impact factor that strongly depends on the number of their readers, who in turn privilege hot topics and abstracts that attract attention). Meanwhile, industry and technological progress—though indirectly still a product of science—has moved on with all its social consequences. And here we are today with the advent of 5G—the fifth generation technology for mobile broadband networks that surpasses the 4G LTE commonly used today—that promises connectivity beyond all predictions and unprecedented integration with the virtual world -from driverless vehicles to the Internet industry, from smart cities to machine-to-machine communication (Internet of Things = IoT). To be able to live with such heavy data traffic we have to accept: (1) a change in currently used frequency channels to higher RF fields, and (2) an explosion of traffic between base transceiver stations (BTS). We are not prepared for this, and do not know if there may be risks for the health of the population. In Italy, to allow the 'applicability' of 5G and in the future of 6G now under development, exposure limits will have to be raised at least tenfold, in terms of electric field strength

from 6 to 61 V/m, at the expense of the precautionary principle. It is desirable for future research to delve into the issue of the impact on the humans of the frequencies to be used in 5G and 6G. For example, we do not know what are the non-thermal effects even regardless of their power, nor do we know their long-term effects. Above all, however, we need to identify a relevant biophysical mechanism for the action of RF fields that is fundamental for interpreting non-linear results that do not fit into the usual cause-and-effect patterns. Thus, efforts should no longer be directed solely toward innovation and potential economic gains; they must also be directed toward avoiding undesirable consequences. Are citizens sufficiently informed about this? Scientific outreach (i.e., explaining the subject to the uninitiated) is needed more than ever and will need to be done with fairness, timeliness, and in comprehensible terms.

The great revolution nowadays is to develop and heighten people's numerical/scientific literacy because science must become part of our lives to build a better world.

4 Advice to the Next Generation of Scientists

I believe that participating in scientific research to learn and acquire new and greater knowledge of nature is a fascinating 'craft'. You may object, with cause, as I have been a scientist my whole life, but I am truly convinced of this. I deeply love my field of work and I truly believe that it can be extremely useful and helpful to humanity, though science may not always have an answer to every issue we have in today's society. For example science does not tell us who we are, or what values may benefit us all, it does not tell us how to live in peace or in a more equitable and fair world. It would be fair to acknowledge the value and relevance it simply has. If you have indeed chosen to pursue a profession in the sciences, but you are still unsure or undecided on about which field to enter, then challenge yourself by exploring other subjects, by asking questions, read materials and study. You could possibly explore these other fields in science other than those of your thesis or doctorate. Familiarity with scientific literature, while participating in lectures and conferences, is very useful in providing you with a deeper understanding of the opportunities, and finally with the laboratory you wish to join to expand your learning and your overall exploration of your selected field. In my opinion, a good environment, for a young researcher to grow and learn is a sort of 'renaissance artisan workshop', a smaller laboratory where to truly learn the craft, with a team of creative artists. In a creative laboratory the young researcher would be part of a smaller team, where the tendency to compete and envy, are curbed by teamwork and common goals. Teamwork will provide the young researcher with the tools, camaraderie, support that are necessary to explore her/his own individual creativity, (one must not forget that freedom in research and creativity are complementary and without the first the second cannot thrive).

Be mindful and use care when choosing a mentor and teacher, one with great professional experience (so that you will have the opportunity to learn from real-life

experience, often not found in books that you have read during your academic career). Your mentor should be recognized among peers, and must be able to support your growth by sharing their experiences with kindness and empathy. The relationship should evolve and become familiar where as a young researcher you have the opportunity to learn daily, working, perhaps imitating at first and then creating your own way to investigate. A mentor should transmit technical knowledge to young researchers as well as allow them to experience the social and cultural environment of the scientist profession as well as expose them to the inter- and trans-disciplinary nature of science. In the twenty-first century it is essential that scientific work becomes trans-disciplinary, where work transcends the confines and boundaries of traditional scientific fields, where creativity allows for new research and knowledge. It is relevant to fully comprehend the interconnections that are formed by the multidimensional nature of our world. Once you have decided on the field you wish to pursue, put in your best effort to realize your goals, don't give up and never surrender to mediocrity, always aim to be the best you can be. The road will be uphill (saying this from personal experience), and does not always get easier as you progress in your career.

In the past we used to go to the library to research a topic, today when we are interested in a new subject or research we type a few words on a search engine and we access information. In the best case you might find 'just' a few hundred articles that you should study. You may choose to spend a few years reading all that has already been written or you may select just a few, (but what is the criteria you use to select them?). The risk is to rediscover what has already been found and written about or in a subject leading to a dead-ended. I do realize that today, at least in Italy, a young researcher, with inadequate pay, in order to advance their career must "publish or perish", the latter option being more likely. Though the 'push' to publish as much as possible might impact the quality of your publications, find the courage if you can, to rebel against that pressure to publish in excess. Writing a scientific research article, should require an in-depth understanding and knowledge of the preceding publications, and should be original, essential, simple, clear, rigorous, honest and complete. By explaining your discoveries, synthetically, even if they represent years of work, and carefully using words with the goal to harmonize the overall context to make it approachable by others, will allow you to gain humility and perspective. Remember research is an instrument of knowledge, not a tool to gain power or fuel competition.

In the laboratory you should work with rigor, method and patience (anything observed once needs to be repeated or replicated for it to have relevance), with total dedication and closing your eyes (i.e., ignoring) when facing difficulty (in that way you can face challenges that others, more critical and acute, would not confront). These factors are essential to your success and personal satisfaction. In scientific research you are absorbed by progressive revelations, even if they may be a small contribution to the general advancement of knowledge, it gives the researcher great satisfaction in the moment of discovery, however small, only you could have placed that tile in the mosaic. That tile in the future will allow you, or someone else; to take that starting point and continue from they're towards new discoveries. The essence of scientific research is sharing discoveries and collaboration. Take your time when

elaborating experimental results: learn to understand the significance of revealing anomalies (serendipity); have doubts (they are the root of knowledge) welcome them without fear but as a possibility; tolerate a few errors as they may be necessary, at times beautiful (the tower of Pisa), or at times fertile (Fermi and the slow neutrons); be open to challenge your own ideas and those of others, science gives us objective answers and not absolute truths (Einstein *vs.* Galileo).

Try to share your knowledge—teaching and propagating science- education and science are important for society, and vice versa. If we can divulge scientific knowledge, sharing the derived advantages for humanity, so that public opinion may view science in a much more favourable light and influence the decisions of politicians in regard to the importance of funding scientific research. This will also lead a larger number of young people, hopefully an increasing percentage of women, to pursue a degree in science.

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Wondering Why: Limitless Curiosity

Sara Patterson

Abstract What is science? For me it is curiosity and the study of almost anything. I believe that in contrast to an engineer or a physician, as a scientist I ask questions that I have no idea as to what the answer is; and then I try to solve the mystery. It can be inspired by the need to know more about something to inspire or facilitate new solutions to challenging problems (such as understanding plant growth in order to improve yields of food crops under dynamic climate conditions) or specific knowledge regarding how things were created or work.

1 Motivations: How I Developed an Interest in Science

I grew up in Cleveland Ohio in a suburban neighborhood with excellent public schools. On weekends, the family would attend church and then travel to one of the Cleveland Metropolitan Parks to hike and have brunch. I recall learning how to identify fungi, plants, birds and many creatures. In the summer we would spend five to six weeks traveling across the US—hiking, camping, and exploring the National Parks. I recall fishing with my dad in the early mornings, grilling fresh caught wild salmon and even a huckleberry pie that we cooked in a reflector oven. We would periodically stay in a motel so all could shower and my father would disappear for a couple days of business meetings. It was pretty idyllic and firmly established my love of the outdoors.

Once home, I would wander in the nearby woods and collect plants to transplant to a small space in my parents' yard where I was permitted to have my own garden. Often, I would experiment in making dyes from different plant parts including petals, leaves, stems and roots. I remember collecting campanula, bloodroots, violets, dandelions, and buttercups. Some worked well and others not so great: an auspicious beginning to my scientific inquiry. My interests expanded and I remember becoming obsessed with mushroom and fungi as well as hunting for salamanders in the woods.

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When unable to go to the woods, I would climb to the top of the tall sugar maples in our yard and read for hours in the upper boughs.

Once every couple months, the family would travel to Indianapolis to visit my mother's parents. My grandfather was my hero and still is. He was a quiet soft-spoken journalist who was passionate about curtailing nuclear proliferation. I'd never known about his story as a journalist and only after he died did I learn that he had witnessed the atomic bomb tests and how they had impacted his life. I had known that he'd grown up in the foothills of the Appalachian Mountains and worked many jobs to help support the family. One job included working on a commuter train that resulted in helping and befriending a philanthropist who ultimately funded his college education. As a grandfather, he told stories of giants, trolls, and fairies and how they lived in the woods hidden from view. My grandfather and I often took walks in southern Indiana collecting geodes and crayfish, and on these excursions he enhanced my knowledge and respect for nature. There were fruit trees and a small vegetable garden at the house. I had two cousins who lived with my grandparents and their dad. We would always publish a mini-newspaper with columns by each grandchild and news of the visit. My grandmother had been a home economics teacher and I recall the kitchen always filled with incredible smells.

In high school, I volunteered teaching math in inner city Cleveland schools, demonstrated in protests against the Viet Nam War, participated as a Nader Rader and even boycotted the local grocery store. My sister shared many of my views and although our parents were often embarrassed, they accepted our differences with grace. I recall buying a backpack with some Christmas money that I had received and the angst and dismay my parents expressed. They couldn't understand how a young woman would want to go camping or trek across the east coast on a bicycle. I was always pushing boundaries, and did surprisingly well in all subjects in school: and thus I felt that the world was open to me. Little did I really understand about discrimination whether it is family, class, religion, race or sex.

As an undergraduate, it was a time of dissent in the United States and the Viet Nam War. I avoided the sciences and leaned heavily to the humanities focusing on intercultural studies, religion, and majored in English. My curiosity and love of learning was undiminished and I obtained a teaching certificate for K-12. I also took some upper level graduate courses in English, but unfortunately was dissuaded by the focus on critique of critiques of recognized literature. Although science and math were always easy subjects for me, I narrowly believed that the only careers for those who studied the sciences would be engineering, military, or the medical field. My goal was to become a teacher and share my love of learning and it seemed as though the humanities would be the path.

That path was redirected after listening to a talk by Frances Moore Lappé, who wrote *Diet for a Small Planet*. Thus, my Senior Year in college, I took as many science and math courses as possible and applied to graduate schools in the plant sciences. I was going to "help solve the world's food crisis and protect the environment". It was an exciting time socially and politically. Many of us were starting to worry about the environment, becoming vegetarian and conservationists. My family and friends had

known me as the outdoors tom-girl with her own small garden and curious about everything, so they were not surprised.

So, I moved across country from the East Coast to the Pacific Northwest and began graduate school in the plant sciences at Oregon State University. The work was relatively dull but did not subdue my love of Botany or growing food crops. Shortly after finishing my Masters degree, I spent two years in Seattle teaching Introduction to Horticulture, Botany, and Plant Tissue Culture at a Community College. A newborn and a partner looking for a job limited my opportunities; and after two years in Seattle, I found myself in Philadelphia. There I also garnered a part-time faculty position at a small four-year college an hour north of Philadelphia. The commute was long and with a young newborn at home, I looked for work closer to Philly and found a research position in a plant science lab at the University of Pennsylvania. I combined my field skills, my plant tissue culture and basic plant biochemistry and was quickly introduced to the rapidly evolving world of plant molecular biology and protein tools. It was an exciting project transferring the *Bacillus thuringiensis* (BT) insect resistance gene into tomato and analyzing the stability across generations and functionality. I was in the right place at the time, as I was one of the few scientists with a strong plant biology background as most plant molecular biologists at that time came from animal, yeast or bacterial studies. The project introduced me to the world of patent battles, depositions, and what it felt like to be drilled by five Monsanto lawyers.

Again we moved, and I followed my husband to North Carolina State University where I started my Ph.D. work on *Zea mays* (corn). Unfortunately things did not work out there, and a year later I found myself in Madison, WI at a Biotech Company as a single parent. But life's ups and downs can be a blessing and the disappointing Biotech job evolved to a new partner, more kids, my Ph.D., and a faculty position at the University of Wisconsin. Research was exciting as the field was rapidly changing and we cloned and characterized the first receptor kinase, TMK1 (Chang et al. 1992). I diverged from the lab's main research and began my inquiries on abscission and cell separation using Arabidopsis as a model plant. It was by chance that one of the mutant plants that I was characterizing had a delayed abscission phenotype and provided the avenue to a unique niche of research that was the foundation of much of my academic career.

Somehow, I've balanced family life, friendships, gardening, baking bread, research, teaching, and mentoring over the last several decades. In general, it's been a joy with new discoveries at many steps. I've been fortunate to be able to travel and maintain contacts with colleagues around the world and enjoy their foods and culture. All of life has been an adventure for me. And, sharing those moments has been so much of the journey.

2 Work Done: My Personal Scientific Approach

How to describe a personal approach? I like to think of each experiment as an empty canvas with an infinite number of possibilities. Often the question is unexpected, as it may have evolved from new observations. While a young graduate student at Oregon State, I found myself making novel observations and asking unexpected questions. While researching disease resistance in green beans, I identified progeny from random field crosses that generated beautiful bright red edible bean pods. Unfortunately these were hybrids and the brilliant red pods were lost after several generations. Even years later, I tried to repeat the cross but I never determined the parentage or the genes responsible for the flavor and coloring. Sometimes, I believe my diverse background provided a naivety and openness to see things differently and ask questions outside the mainstream. Being a scientist seemed so much easier than studying English literature or Philosophy as I always felt I could challenge my assumptions and determine if they were incorrect.

For me, scientific inquiry takes passion, commitment, technical expertise, lots of energy, attention to detail, and a bit of serendipity. One must add "wonder" and curiosity. The Scottish philosopher Adam Smith wrote of "wonder" as the "quality of experience with a distinctive bodily feeling—'that staring, and sometimes that rolling of the eyes, that suspension of the breath, and that swelling of the heart'" (Prinz 2013). And for me it is so true, as there seems to be nothing more exciting than the unexpected discovery. Descartes and Socrates also wrote of "wonder" as a motivation for philosophical inquiry. So, for me as a scientist "wonder" has always motivated me: leading to questioning, and the willingness to challenge the dogma and consider new perspectives.

Another critical factor inspiring me as a scientist is love of learning and being able to convey that love, curiosity and wonder to others. The desire to understand and learn new concepts rather than memorize facts is so important. Not only does this develop a deeper understanding, but leads to new questions and further exploration of ideas. Similarly, one should never hesitate to admit lack of understanding. I was an English major who embraced science upon graduation from college and thus, I had many gaps in my knowledge. While this could initially be viewed as a handicap, I believe it was an asset allowing me to feel less encumbered about my lack of understanding or knowledge.

My experiences in the field of abscission, the loss of organs from a plant, were amazing. It was an open book, as previous scientific research had been quite focused with researchers using limited approaches. I was able to use the model plant Arabidopsis and develop a genetic approach and use newly established molecular tools. We could order mutants that had identified gene knockouts or create an individualized population of plants by mutagenizing with multiple approaches. These tools included chemical mutagens, mutant populations created by random DNA insertions using Agrobacterium and irradiation. My lab applied an array of approaches characterizing plants based on morphology, physiology and genetics. I always emphasized the importance of "knowing your organism" to all my students. Even in daily walks,

I find that I repeatedly see new things that have always been there. Sometimes it is the direction from which I have approached, while other times it might be changes in sunlight or shadows, or just pausing in a new location. Most recently, I discovered that I could see Mt. Rainer, the highest volcanic peak in the contiguous United States, on my daily walk if I paused and looked southeast. I had been taking this same walk for almost two years and never seen the mountain. It was awesome, and a fact I now share with dozens of neighbors. In the lab, similar experiences can be identified as I find that often when we take a fresh look at results with an open mind, we may see new things. And, this can lead to new questions and discoveries.

Viewing scientific inquiry as a process rather than a single task is a valuable lesson. Learning versus memorizing, the ability to accept uncertainty, to consider new approaches, the willingness to be challenged as well as patience were critical to my success as a scientist. Careful experimental design (limiting variables), persistence, resilience, learning from each mistake or unexpected result, designing new experiments, and ongoing critical reflection are all part of the process. Again, patience and careful reflection.

As a scientist, not only do I love asking questions, learning new things, and discovering the unexpected; but, I also am motivated by inspiring others and sharing my scientific approach. Opening new doors to students, postdocs and colleagues is a critical part of my approach. Sometimes presenting one's scientific findings at a meeting easily does this. This not only leads to learning moments, but also can help establish collaborations, and develop respect and long lasting friendships. These friendships may be departmental or within the University, but often are across borders around the world. The ability to inspire young scientists and exchange ideas with others that have diverse backgrounds has been an incredible benefit throughout my career.

Last, I believe that the scientific process should ultimately lead to unifying concepts resulting from inquiry and reasoning. Data is collected using a variety of techniques and hypotheses are formed. These hypotheses are tested by new experiments and additional data collected as needed. Often the simplest explanation may be the best, yet it is always important to have an open mind. This belief is shared by many scientists and has guided many; and examples include Descartes, Galileo, Newton, Darwin, Einstein and dozens of others. For more on scientific philosophy read "Why Simplicity Works" by McFadden 2021. In many ways my scientific inquiry has always followed the principles above, but it has also been spiritual—looking for meaning.

3 Science Today and Tomorrow

Science and its future can sometimes be incredibly discouraging. There are challenges for funding; failed experiments; disrespectful and unethical colleagues; 'dead-wood', lazy and seemingly incompetent lab members; and public disdain for science. Yet despite these challenges, the future of science continues to be promising as new

high-impact and transformative research advances are made. In the US, this process is threatened by the public's eroding trust in science, lack of transparency, and lack of oversight. Despite the gloomy picture, I believe that scientific inquiry will always continue to make advances.

In the US, the competitive process for federal funding of scientific research is generally considered quite good. Foundations such as the National Institute of Health (NIH) and National Science Foundation (NSF) have provided funds for scientific research for decades. NSF was established in May 1950 and its mission was to promote basic scientific research. And, although NIH was established in 1887, funding of basic scientific research has routinely declined over the last century. However, in 2002 a new directive to fund basic science was implemented; thus providing additional funds for new transformative science. While I applaud the U.S. funding agencies, I might suggest more frequently replacing some of the permanent program officers in the funding agencies, as sometimes they develop a narrow vision after years at the job and biases towards specific investigators or universities. Too many researchers receive funding because they previously received funds and their publications are greater in number. This can all be a consequence of the fact that since they have previously received funding, then they have more people to conduct the science, and thus more publications. There are often limited reviewers willing to critique the science and some have prejudices against a competitor, an institution, or implicit gender or racial bias. As scientists, we can step-up by volunteering our services to review and advocate for revised guidelines to guarantee impartiality.

Securing funding for basic scientific research has always been an uphill battle and federal budgets often swing with administrations; and yet, I continue to be hopeful. Communication with legislators and the general public has contributed to general interest and increased funding. In addition, the elimination of wasteful government spending or pork-barrel projects would free up additional funds. Senator William Proxmire of Wisconsin was well known for his "Golden Fleece Award" which he bestowed upon what he considered the government's most wasteful spending. Similarly, the automatic yearly allocation of funds to a plethora of areas should be revisited more frequently and more carefully evaluated. Also, encouraging for the future of science is that philanthropic support for basic sciences has been increasing. Significant sources include the Gates Foundation, the Paul G. Allen Family Foundation, the Rogovy Foundation and individuals such as Jeff Bezos and MacKenzie Scott. The University of Wisconsin College of Agriculture and Life Sciences lists over 600 sources for funding including federal and private foundations. So, despite challenges to obtain funding, resources are actually increasing and more and more projects funded. Many are truly transformative, and the National Science Foundation website has a list of the "Nifty 50" or discoveries that have become familiar to most of society. Some of these include the discovery of antifreeze glycoproteins, understanding the effects of acid rain, the value of circadian rhythms, plant growth and heavy metal and salt resistance, and edible plants as vaccines. As society embraces basic scientific breakthroughs such as these, the appreciation for science will continue to expand.

However, there is also a critical need to review and update ethical guidelines as well as provide more transparency to the public on the research that is funded. The integrity

of scientific research is dependent upon the design, presentation and interpretation of experiments; and scientists need to be trusted and honest. Environmental ethics are also a concern and as scientists we must recognize our responsibility to the community and society. Unfortunately, not all scientists are ethical and institutions need to do a better job training our young scientists. This can include required classes, workshops and discussion groups. Recognition of other's rights, undue pressures for publication, funding, and international recognition can be addressed. Additional issues to be discussed can include fabrication of data, taking credit for another researcher's data, observation of misconduct and whistle blowing. Universities need to establish strong guidelines and ethical review boards for inquiries regarding misconduct or mistreatment of researchers. Although a slow process, this is happening across the US.

If science will continue to thrive, it will be important to question the reliance on quantitative metrics as these are subject to manipulation and bias. Metrics including number publications, citations, patents, and students trained can have significant effects on hiring, successful grant writing, and promotion. Having been in a department where many of the faculty publications were incremental, it was confounding and frustrating to compete when one's goal was a quality publication rather than just quantity. The good news is that here are initiatives to curtail the promotion of quantitative metrics including the San Francisco Declaration on Research Assessment (DORA).

Historically Americans have voiced objections to certain types of research. As a scientist, I have worked at several places where protests turned violent resulting in building destruction and loss of life. At the University of Wisconsin protests have emerged repeatedly against specific research. The bombing of Sterling Hall in 1970 targeting the Army Math Research Center, killing one and injuring others, forced many to re-evaluate attitudes towards military research and trust in the administration. Subsequent protests on campus over brain trauma research with simians and studies on deadly flu viruses using 'gain of function' technology have further deteriorated community trust. Public oversight in the U.S. has been consistently eroded since major policy changes in 2017. In the US, both Francis Collins, the Director of the National Institute of Health, and Anthony Fauci, Director of the National Institute of Allergy and Infectious Diseases, feel that gains from this research far outweigh the risks. This opinion is not shared by all scientists and there are many who believe that poor experimental design or accidents could inadvertently create lethal pathogens. The distrust in science by much of the American public clearly contributed to recent vaccine hesitancy and rejection of predicted consequences of climate change. We must curtail this trend of increased secrecy and less rigorous ethical review and insist on more transparency.

My faith in the future of science is both my enthusiasm as a scientist and the incredible breakthroughs made in the last decade. Discoveries about the brain and sleep are revolutionary and previously unimaginable. Similarly, insights into the human gut and its role in health and susceptibility to disease are equally impressive. For, the astronomer, one might cite the recent photos of black holes. As scientists continue to collaborate and exchange ideas across borders, these discoveries will

flourish. It takes incredible persistence as well as creativity, skill and luck to develop new ideas and redirect. But, the scientific advances made in the last several decades are truly impressive. I believe that despite all the challenges; science will always continue to advance, as the quest for meaning is so fundamental to humans.

4 Advice to the Next Generation of Scientists

My advice to a young scientist is work in a lab (or field work) for an extended time as an undergraduate or a technician (maybe more than one, but best to get an in-depth experience rather than jumping lab to lab). When looking for employment, consider volunteering, as once a mentor sees your commitment and zeal, they will often find a hidden source of funding. Writing grants and presenting your research no matter how inconsequential it may seem will also serve you well. I was in my 40s when I finally went back to get my Ph.D.; and I had worked in 5 different labs, so I felt that I knew what research was about and different approaches. I knew that I wanted an advisor that would allow me to make mistakes while learning, yet one who would engage in heated discussions and theorize. My research experiences were fairly broad and I'd also taught large University classes, classes at a small college, and classes at a Community College. I'd mentored over fifty individual students and I loved it all. I wanted a Ph.D. and stayed in academia so I could keep doing what I loved: definitely not for the salary.

I always want to say that one needs to "taste it" if you go on for a Ph.D. because it is hard and challenging work, and low pay. And, when I went back for my Ph.D. at the University of Wisconsin, I had the bug. Groups of us would huddle as one of our colleagues developed a Western, a Northern or waited to see if our plants had new phenotypes. We were all excited! Long hours at work might have annoyed my family, but not me as I pushed ahead or waited eagerly for the next result. It was not uncommon for me to go back to work at nine or ten in the evening and stay well past midnight after a family meal and a couple hours of down time. Designing and performing experiments, and experiencing "awe" were motivating factors. I also had the goal of becoming a professor, managing research projects, and inspiring new young scientists.

My energy was boundless as the work was fun. But, I also tried to never forget about my priorities in life. These will differ amongst people, but balancing commitment to family, friends and taking care of oneself is incredibly important and difficult. My approach was to give each "TASK" my full effort, thus being more efficient and creating more time for additional undertakings. Choosing a career is a balance of life's objectives; and choosing quality of life combined with security are often in the forefront. It is also important to realize that there is seldom a single path; and the best path will be that which permits you to blossom and flourish.

I selected the program of study based on the research possibilities, the degree requirements, and the promise of funding. I did not compare program differences in terms of funding, as for me most important was being inspired by the research; and

while there were differences it seemed that over time these were fairly minimal. So, I recommend deep discussions with the potential advisor, discussions with students in the degree program, and a visit to lab meetings if possible. The publication record of the faculty is useful and conversations with current and past graduate students can always help understand what you'll be encountering and what to expect. Websites today allow one to easily see where previous lab members are and gage if that is a direction you would want to pursue.

Similarly the selection of what University to attend is incredibly important. As the Director of the Science and Medicine Graduate Research Scholars program at the University of Wisconsin from 2008 to 2019, we strove to provide a home and a community for minority underrepresented graduate students in the sciences. Thus, finding a lab and community that will allow you to feel welcome and safe is so important. In addition, finding an advisor or site of employment that will provide opportunities for professional development, networking, and additional resources on campus or in the scientific community should be considered. As individuals, we can have many mentors; so don't worry about finding all the desired qualities in one advisor. In selecting a research advisor or graduate committee, consider the strength that each faculty brings to the committee and your comfort discussing challenges with each member. All of these criteria may not be immediately obvious when considering an institution or later employment, but always remember what characteristics you value in colleagues and mentors.

Having been an English major, I had many deficiencies in undergraduate classes, so I can't emphasize how important it is to embrace those gaps in learning and expand one's understanding. Not only will it facilitate understanding of concepts, it often leads to new interests and new questions. While being self-driven and independent are strengths, never rule out asking for help. Recognize your weaknesses as well as strengths and ask questions. And at the same time, be thoughtful with questions and consider whom you are asking, the timing, and the clarity of the request and the method of communication. Always deliver questions as a request and not a demand.

In summary, as a young scientist keep an open mind, attend meetings, present, network, and always strive for the truth. Time management is critical and complete focus or 100% effort on each task will serve you well. Just as not worrying about having enough time with friends while working, I also recommend not worrying about where the next job will be as it's critical to focus on the question at hand. Resilience, persistence, and learning from mistakes are all valuable tools. There is so much anxiety today as one worries about the future. There is no denying that there is uncertainty in the world, but recognizing the value of embracing the present is important. There are many problems that we cannot instantly solve and thus prolonged worry about these issues will just consume your time. As a retired professor, a parent and a grandparent, I tell my family "baby steps". Be satisfied with incremental progress and don't expect instant transformative research results. So, work hard, play hard at times, and always remember life priorities.

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Sara Patterson Professor Emerita of Horticulture. University of Wisconsin, Madison. Patterson's research focused on understanding the mechanisms that regulate cell separation and adhesion in floral organ abscission in Arabidopsis. Patterson also applied this work to economically important crops such as cold-hardy grapes, olives, and fonio (a West African orphan grain). Patterson was also the faculty director of the Science and Medicine Graduate Research Scholars program, a fellowship program for underrepresented graduate students.

From Molecular Biology to Science Diplomacy: A Long and Winding Road

Giuditta Perozzi

Abstract As young kids we view science as a fascinating world of breakthrough discoveries, and it was probably this childish idea that drove me into choosing biological sciences as a field of study. Like all aspects of our existence, however, life in science can take unpredictable paths and my personal journey through it was hardly linear, mostly as a consequence of close encounters with unavoidable obstacles, and partly reflecting my constant desire for new challenges. Yeast molecular genetics was the field where I moved the first steps during my Ph.D. experience in the United States, and molecular nutrition/nutritional systems biology is the broad scientific area of my professional lifetime since returning to Italy. Walking backwards the route of Darwinian evolution, I spent the last decade of my life in science studying food microbiology, until a final sharp turn led me to conclude my career within the transdisciplinary field of science diplomacy at the Italian Ministry of Foreign Affairs. On the verge of retirement it is hard to say whether this non-linear path had more pros or cons, but it leaves me with the rewarding feeling of having learned a lot, and I must thank for this all the people I met and teamed with along the way. Being them mentors, collaborators, young fellows or students, sharing part of my path with them has always provided precious take-home lessons. Regrettably, I felt all along that the outcomes of my hard work were more valued and supported outside than within my own institution.

1 Motivations: How I Developed an Interest in Science

I did most of my studies in Rome, up to the Laurea, which in 1978 was the highest University degree one could achieve in Italy. I had attended a high school with main focus on humanities. Funny enough, but crucial for my choices later in life, my high school had an incredible asset for a humanities journey: a science lab! I had the fortune of being enrolled in one of the classes that could exploit this precious resource, dedicating an extra hour per week to experimental lab work. I can now

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say with reasonable certainty that this was key for my future choices. Thinking back to those five years of high school, I believe that they sow the seeds of what later became my strong interest in science. I still have exciting memories of the time we used to dedicate to the experiments, which gave us the opportunity to learn the basics of life sciences: looking into a microscope at live microorganisms within a drop of stagnating water, identifying tissues of dissected prawns and the developing structures of chick embryos (there was no need for ethics approval of the protocols at that time…), discovering our AB0 blood group on color strip tests using a small drop of our own blood… it was quite an advanced training program for those times! When the time came to make my choice for a university program, I was certainly more at ease with science than with Italian literature and ancient languages.

I joined biology at the university of Rome. Molecular biology was in its infancy at that time, and the power of genetic engineering was the rising star. Like many other students I fell into its fascinating perspectives and shaped my panel of courses toward the goal of becoming a molecular biologist. However, my first attempt to enter this exciting world was unsuccessful. Not many labs had yet started to apply molecular approaches, and in the few who did, applications for a thesis work greatly exceeded the maximum number of students they could host. So, I completed my thesis work in a human nutrition lab at the National Institute of Nutrition in Rome and then applied to Ph.D. programs in the United States, where I was accepted by the Department of Biology at the University of Rochester, upstate New York. My dream of playing with DNA finally came true when I joined the laboratory of Satya Prakash, focused on the molecular genetics of DNA repair systems in the yeast *Saccharomyces cerevisiae*. Yeasts were the first eukaryotic organisms that could be genetically manipulated with fast and relatively simple techniques, thanks to their unicellular nature and well characterized genetics, which led to the availability of a broad collection of mutants that could be easily transformed with exogenous DNA. These features made it possible to clone genes by complementation of mutant functions and to achieve targeted gene disruption by homologous recombination. Yeast cells were also found to host self-replicating high copy number plasmids, which were quickly adapted as versatile cloning vectors.

For the following six years I learned how to use the powerful tools of molecular genetics to clone and sequence yeast genes, characterize their functional expression and study their regulation. The field was moving fast, being part of it was extremely exciting, and by the time I had learned enough to be independent in designing and conducting the experiments, I was also convinced that I wanted this to be my future field of work. With the plethora of fully sequenced genomes that are available today in open access databases, and used as we are now to high throughput automated sequencing, I can hardly believe that the experimental work reported in my Ph.D. thesis was "only" the cloning and sequencing of a 2 Kb long yeast gene and the initial characterization of its regulation by UV light! However, as crazy as it may seem today, it took two full years of hard work to clone the gene, subclone its restriction fragments one by one in the M13 vector used as template for DNA synthesis with chain-terminating nucleotide analogs (the Sanger sequencing method, Sanger et al. 1977), separate the bands on polyacrylamide gels, and finally, but even more strikingly,

to *eye-read* the resulting band ladder from the exposed gels and to *hand-write* the unknown genetic code on endless sheets of my lab notebook…it definitely makes me feel old more than anything else I ever did in my life! (Fig. 1).

One year after my return to Italy, the encounter with Sancia Gaetani, leading a nutritional biochemistry lab at the National Institute of Nutrition in Rome, introduced a new turn to my scientific path. She proposed that I join her lab as a post-doc to contribute the molecular biology expertise to their ongoing biochemical studies on the regulation of protein synthesis in response to nutritional stimuli. She had a long-term vision based on recently published evidence of a transcriptional regulatory mechanism mediated by an exogenous dietary molecule, the vitamin A metabolite

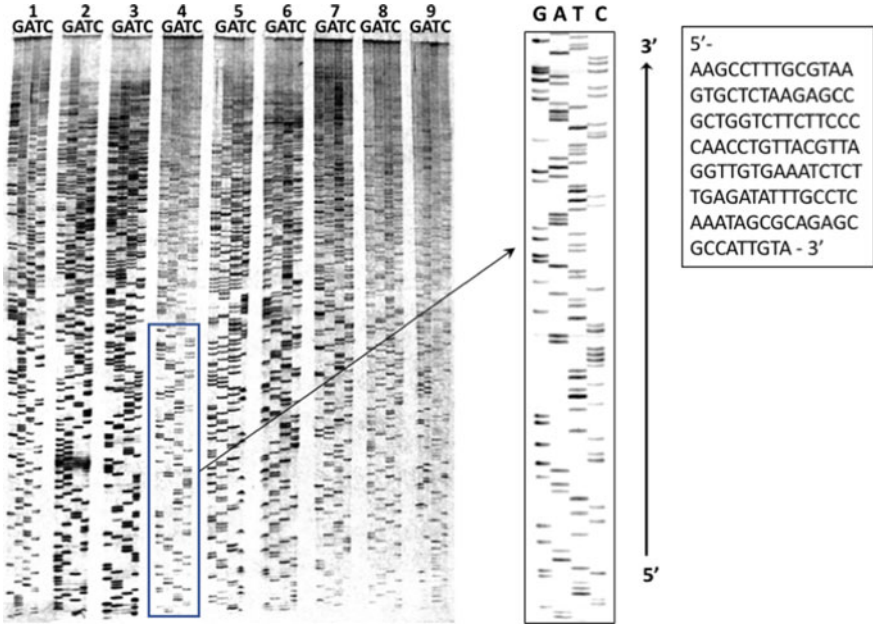

Fig. 1 Left side Exposed X-ray film of a polyacrylamide gel displaying the ladders of bands resulting from sequencing reactions of 9 different DNA templates (labelled 1–9) with chain terminating nucleotide analogs (the Sanger method, Sanger et al. 1977). The DNA sequence of each template is derived from the bands in the corresponding 4 lanes (labelled G, A, T, C) which were loaded with 4 separate reactions containing the same template DNA but only one of the 4 specific chain terminating analogs of the normal dNTPs (usually a dideoxy-nucleotide). Acting as a specific inhibitor of DNA polymerase, the ddNTP indicated on top of each lane interrupts DNA synthesis when randomly inserted in place of the corresponding deoxy-nucleotide. The presence of ^{32}P-labelled dATP in all reaction mixtures allows to visualize the bands after overnight exposure. The resulting sequence is determined by reading the ladder of bands in each group of GATC lanes from bottom (5′ end) to top (3′ end). Right side: enlargement of a portion of the band ladder for template DNA #4, taken from the lower half of the gel where the bands are well separated allowing easier reading. The corresponding DNA sequence is displayed within the box in the upper right corner starting from the first two AA at the bottom of the insert (The exposed film was kindly donated by Simona Baima, CREA-GB)

retinoic acid, in mammalian cells. The molecule could bind to, and activate, a transcription factor of the nuclear receptor family with homology to steroid/thyroid hormone receptors (Giguere et al. 1987). It was indeed a breakthrough discovery as until then, transcription factors regulating gene expression in higher eukaryotes were thought to respond exclusively to intracellular mediators or circulating hormones. This conceptual revolution forever changed the science of nutrition, which had been traditionally viewed as a branch of human physiology/biochemistry, paving the way for the new field of molecular nutrition aimed at elucidating the mechanisms of nutrient-dependent regulation of gene expression in mammals. In light of all this, Sancia's intention was to expand the ongoing projects in her lab by including the powerful approaches of this emerging research area, and to that aim she was recruiting young scientists with molecular expertise, many of whom had just returned to Italy after a research experience abroad. The scientific goal appeared to be quite challenging, the environment was stimulating, all the ingredients seemed to be well set to start a new adventure, and this is how I turned myself into a molecular nutrition scientist.

2 Work Done: My Personal Scientific Approach

Molecular nutrition, and later on nutritional systems biology, have been my major field of interest ever since. In line with the aims and scopes of this book, I will focus mainly on the initial challenges that led to the establishment of my own line of research, as I believe they might be more instructive for young scientists at the beginning of their scientific career.

When I was hired as a permanent staff scientist, a few years later, I had become familiar with working on more complex systems than unicellular yeasts. I had also realized that the complexity of higher eukaryotes was far more difficult to address with molecular tools, and that the overall effect of nutrients and other dietary molecules on gene expression was often dependent on chronic, long-term exposure, yielding milder outcomes in the short-term than those observed in microorganism. It seemed therefore reasonable to try and change the approach, seeking to identify regulated genes rather than screening nutritional stimuli for potential regulatory effects. Intestinal epithelial cells represent the first selective barrier encountered by dietary molecules for their internalization. They express all the functions required for selective nutrient absorption during their differentiation process, which parallels the constant renewal of the epithelial monolayer covering intestinal villi from the stem cells localized in the crypt compartment. The chances of isolating novel genes expressed in these cells and whose regulation might be linked to nutritionally relevant processes, appeared therefore higher than in other tissues. It was a shot in the dark, but we were fortunate enough to succeed. Using subtractive hybridization libraries we isolated several cDNA clones differentially expressed during intestinal cell differentiation and focused on one of them, whose sequence encoded a novel protein with a histidine-rich motif predicted to bind metal ions. Transition metals

are essential micronutrients in mammals as they participate to a multitude of metal-dependent cellular processes, but their role in cellular regulation depends on the chemical nature of each specific metal. While we were running binding assays to identify the metal specificity of the subcloned portion encoding the His-rich sequence, serendipitous cloning of the homologous mouse gene was reported by another group using chromosome walking. Guided by its chromosomal localization, corresponding to the mapped locus of an inherited mouse zinc deficiency syndrome (*lethal milk*), they could immediately identify zinc as the specific metal bound to the protein, and we spent one more year of further work before being able to publish our data. I believe this is a good example of the crucial importance of choosing experimental approaches.

After this upward beginning, the work in my lab focused for almost two decades on the study of zinc transporters belonging to the ZnT family and their involvement in zinc-dependent regulation of metabolic functions. It has been a rewarding experience that led us from single genes to genomics, from the isolation of other members of this protein family to in silico studies of the cellular zinc proteome and its possible role in health and disease, within a stimulating niche where even a small team like ours could survive and add its own contribution to the field. As it usually happens in science, our achievements were often the result of fruitful collaborations, both within and outside our country. Among them, a prominent place goes to my invaluable mentor and friend Arturo Leone, who introduced me to the international community studying micronutrients and trace elements. Regrettably, he left us and the world too early (Bonatti 2006). Later on, many colleagues and collaborators within the micronutrient genomics team of the European NuGO network (Nutrigenomics Organization) played a key role in the acquisition of the nutritional systems biology angle (https://www.nugo.org/).

Over the years, the scientific strategies of my host institution progressively shifted toward a more pronounced focus on food research, drifting away from fundamental science in favor of more applicable technological approaches, until the whole National Institute of Food & Nutrition (INRAN) was eventually merged into the Agricultural Research Council (CREA) as a result of political, rather than scientific drivers. When a storm comes at sea, one must adjust the sails to stay on course. Adjusting to the new scenario without giving up entirely the nutrition research expertise acquired in so many years of hard work, and unfortunately no longer appreciated in the new environment, was not an easy task, but with a bit of luck and thanks to my previous experience with microorganisms, we had the opportunity to start a parallel line of research on food microbiology. Growing scientific interest was in fact being devoted to foodborne bacteria, especially in light of their potential capacity to interact with the human gut microbiota through the food chain, thus contributing uncharacterized live strains of environmental origin that could carry beneficial (probiotic) properties or unfavourable traits (antibiotic resistance genes). The study of foodborne bacteria and of their interactions with resident gut microbes could be addressed with molecular tools and could also integrate our longtime experience with gut-derived tissue culture cells and animal models, so it slowly became the main focus of the lab. It was also my last contribution to experimental work. Partly due to the turmoil

of life, and partly to my restless nature, another sharp turn in my personal scientific journey was in fact about to occur.

Four years ago, in 2018, I caught the opportunity to join Science Diplomacy (SD) at the Italian Ministry of Foreign Affairs and International Cooperation as a science expert, representing my institution and its specific field of interest within international scientific cooperation programs. This time it was a real paradigm shift, quite different from the previous turns, a change of perspective that I would have never imagined for myself before then. It was, and still is an enlightening experience which added a new perspective to my overall vision of science as well. As defined in the Madrid declaration (https://www.s4d4c.eu/s4d4c-1st-global-meeting/the-madrid-declaration-on-science-diplomacy/) released by the EU funded project S4D4C, SD is a trans-disciplinary field interfacing science, technology and foreign policy, based on the intrinsic capacity of science to support team work above and across boundaries. In other words, the international nature of science is viewed by SD as a powerful mean to be fostered and implemented toward the establishment and maintenance of an open dialogue and peaceful relations between peoples in different countries, even under difficult political and diplomatic conditions. In Italy, this conceptual framework translates into funding a broad spectrum of activities that can promote international scientific cooperation (i.e. scientific workshops and events, access to large multilateral research infrastructures, support to inter-governmental research organizations), with the majority of the available funding being devoted to supporting joint research projects run by Italian scientists with their foreign colleagues in countries whose governments have signed scientific cooperation agreements with Italy. Integration of the scientific expertise is required all along the process, and the scientific network supporting diplomatic work is composed of a team of science experts in Rome acting in synergy with scientific attachés to the Italian Embassies around the world, who have a more in-depth knowledge of the scientific landscape in other countries. Scientific attachés are also pivotal to promote Italian science abroad and to connect with Italian scientists working in foreign countries. All scientists involved in this network, at home and abroad, work in tight connection with the diplomats, who set the basis for this trans-disciplinary field bridging and integrating such different professional backgrounds and cultures, and whose specific expertise in foreign policy is the key to a successful and effective action of SD. I have been working in this field for the past four years and found it indeed very enriching. What I most enjoyed of this dramatic change from my previous life as a wet lab scientist is multifaceted: the acquisition of a broader scientific angle, a longer-term perspective of the impact of science on society, and not the least, the awareness of the added value of international collaborations beyond their major purpose of joining forces and expertise to advance scientific knowledge.

3 Science Today and Tomorrow

My generation has witnessed amazing scientific achievements in all fields. An example from my specific field of work is the full sequencing of the human genome released by two independent consortia in 2001, which represented a groundbreaking scientific revolution of our lifetime, accelerating by light years the translation of DNA structure into function and paving the way to diagnostic and therapeutic applications of molecular biology. If I had to name the key driving forces that generated most of these achievements and that will likely drive the future ones, what comes to my mind is: technological advancements and multi-disciplinary approaches.

The contribution of technological advancements to scientific leaps is precious, and it is constantly providing us with powerful means to reach our objectives with unprecedented pace. However, with a view toward the future I fear that this very dependence of science (as well as of our everyday life) on technology might lead to imbalance the equilibrium between these two disciplines, slowly blurring their borders and eventually turning technology from a powerful mean to the final aim of scientific research. The growing demand for "applied" science, as opposed to fundamental science, by funders, policy makers and research institutions is already evident and represents the most remarkable sign of a world that does not value the advancement of knowledge unless it can deliver "innovative" and tangible outcomes in the short term. Science and technology have proceeded in parallel for centuries, feeding and complementing each other with the results of their advancements. The main objective of science has been the understanding of natural processes which exist and are in place irrespective of our attempt to reveal them, while the power of technology has been the capacity to build on the acquired knowledge and expand it with its own research angle to create the new, the "non-existing before". In one of his most famous quotes, Louis Pasteur said that "*There is no such thing as a special category of science called applied science; there is science and its applications, which are related to one another as the fruit is related to the tree that has borne it*". Along the same lines but with his own angle, the Italian philosopher Emanuele Severino (https://en.wikipedia.org/wiki/Emanuele_Severino) expressed an interesting definition of the difference between science and technology in his invited talk at the event for the 50th anniversary of the milestone Watson and Crick paper describing the double-helical structure of DNA (Watson and Crick 1953). In his view, science shows the potency of nature as much as technology displays the power of humanity. Whether we share or not his thoughts, if the demand for constantly new technologies eventually overthrows the need to support fundamental science as well, we will not be able to draw the best out of both disciplines in a not so far future.

Referring again to my recent experience with Science Diplomacy, the economic impact of innovative technologies and of their industrial applications is very dear to the political and diplomatic world, as they contribute to the progress of national economies, ultimately affecting the wellbeing of society as a whole. Acknowledging the need to support both scientific and technological achievements so that they continue to feed each other in the long-term, Italian SD developed distinct

funding schemes involving independent foreign counterparts. Bilateral scientific calls including the approaches of fundamental science are negotiated and jointly funded with the foreign Ministries of Science, representing academia and research institutions in other countries, while industrial calls aimed at supporting technology transfer are co-funded with foreign bodies whose action is targeted to achieve innovation, for example the Israel Innovation Authority (IIA) with Israel, and the Global Innovation Technology Alliance (GITA) with India. This latter scheme supports joint ventures between companies, academia and research institutions.

Trans-disciplinarity, on the other hand, has to do with complexity. The complexity of our research questions has grown tremendously, in parallel with our capacity to address them. However, working in a specific field of science requires a high degree of specialization, while the global challenges of our century (climate change, food security, sustainable agriculture, progressive erosion of natural resources—including traditional energy sources—, to only name a few), are too complex to be approached within a single, although highly specialized field of study. Although the pressing need to provide solutions to these and other challenges encompassing multiple disciplines will likely increase over the next few decades, the most successful achievements of trans-discliplinary approaches were reported to be limited to the involvement of relatively close subject areas (Stock and Burton 2011). In order to provide effective contributions to the common goals, more distant subjects need to be connected into truly integrated new trans-boundary disciplines, just like the application of advanced computational tools to the study of biological systems gave rise to the powerful trans-disciplinary field of bioinformatics. I believe that future science will greatly benefit from inclusion of new trans-boundary disciplines in the educational system, yielding a new generation of scientists who can truly integrate different fields of science into a common language and successfully address complex goals.

4 Advice to the Next Generation of Scientists

Endurance is the first adjective that comes to my mind when I think of what a scientist mostly needs. Working in science inevitably leads to successes and failures, and negative results are unfortunately more frequent than we expect. Don't get yourself overwhelmed with frustration, try to identify the causes and get ready for a new start. Negative results can be very informative if properly analyzed and they can also suggest what went wrong. So be resilient and flexible, always bear in mind your scientific goals and proceed with a different approach if deemed necessary.

Experiencing different labs, especially in the early years of your life in science, is a fundamental step in the learning process. In the past few years at the Ministry of Foreign Affairs I was struck by the ease with which all of its personnel (not only diplomats) constantly moves back and forth from foreign countries, even the most distant ones, challenging themselves with new professional and personal lives for several years at a time. The international nature of science calls for this model to be pursued as well, especially at a young age when it can shape a long-lasting

collaborative attitude, but it is always a rewarding experience to be considered at any stage of a scientist's life. Mobility of students and early career scientists is greatly valued within a specific funding scheme offered by Italian SD, aimed at promoting the establishment of international collaborations by providing support for staff exchange between collaborating labs in different countries. My personal experience abroad is limited to the years of graduate studies in the United States, but I have no doubts that it laid the foundation of the many international collaborations and intense networking that marked my life in science all along, leading me to learn a lot while sharing part of my path with great people in many different countries.

I am tempted to conclude my few advices with the one used by Steve Jobs to end his famous and touching speech at the 2005 Stanford Commencement (https://news.stanford.edu/2005/06/14/jobs-061505/), quickly turning into one of the most cited quotes of our times ("stay hungry, stay foolish"). It perfectly fits the romantic idea of a scientist by summarizing in two simple words the essence of what is really needed: curiosity, ambition, endless learning, open mindedness beyond and against conventional thinking. They are all very important drivers of success in science but, if I may dare to add, always to be pursued without losing touch with your own reality. I learned the hard way through my personal experience that our working environments play a crucial role in shaping our scientific paths, as different countries, but also different institutions within the same country can greatly differ also in their research priorities, which in turn affect their funding and recruitment strategies as well as the opportunities they can offer to their scientists. There are certainly better and worse places to do science, but if you manage to pursue your own scientific goals within the overarching strategies of your country/institution, it is one of the very few jobs that can be done anywhere with full satisfaction. The only place you should escape from, is where you feel you are not learning enough…

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The Funny Story of an Unpredictable Molecular Biologist

Paolo Plevani

Abstract I was quite uncertain in accepting to give my personal contribution to a book focused on "Life in Science". In fact, now that I am retired due to age limit in an Italian public university, it was like reconsidering all my life in that perspective. However, when I thought about that, I decided that my Life in Science was, after all, very happy and, I have to admit, also quite successful. Of course, as in all "Lives", I had to face good and bad times, but I cannot imagine for myself any different life outside a biological laboratory. Although I had several different interests in life (like having a wife, two children, playing tennis or travelling around the world, preferentially in underdeveloped countries) I spent about 10 h/day in the laboratory (except when I was engaged in doing the activities I just listed above !!). Moreover, some of the best friends I had and I still have, have been my scientific colleagues or students. For these reasons I thought that the best way to give my contribution on the topic of "Life in Science" was not to take it too seriously and to describe some facts and stories that may highlight how success in science is unpredictable and it is strongly dependent on the people each of us had the chance to meet during our scientific career.

1 Motivations: How I Developed an Interest in Science

I must admit that, before I started doing research in biology, I did not have a particular motivation for science. Indeed, my science teacher in high school was the least engaging of all the teachers in my school class. I was more attracted to study humanities, philosophy or mathematics and physics. Also for this reason, many years later (when I was already a University professor of Molecular Biology), I decided to establish, together with some colleagues, the CusMiBio (University Center of Milan-School for the dissemination of Biosciences) with the aim of offering high school students and their professors the possibility of using university laboratories properly equipped to carry out laboratory activities able to excite students to research and to

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facilitate the interactions among secondary school, university and research in biology and medicine. I consider the success of CusMiBio, which has now been active for over 17 years, as one of the most important achievements of my scientific career.

In the absence of a precise motivation towards one or the other of the university faculties I followed my father's suggestion to enroll in the Faculty of Medicine and Surgery. However, despite having passed the exams of the first two years I realized that even that Faculty could not fully involve me. So, after a break of a few months and my involvement in the student movements of 1968 I decided to leave the Faculty of Medicine and Surgery at the University of Milan and to enroll in Biological Sciences at the University of Pavia. This choice was essentially motivated by the fact that Biological Sciences was a shorter Degree Course and it gave credit for most of the exams I already passed in Medicine and Surgery. Moreover, for political reasons it was even better to "change the scenery"!

Arriving in Pavia, I went to visit the Institute of Genetics which, at that time, was located inside the Botanical Garden of the University because some friends had told me that it was considered an excellent institute and, among other things, it was very close to the small and scruffy apartment that I shared with three other students. No other choice was ever more fortunate for me!

At that time, the Institute of Genetics and the laboratory of the National Research Council directed by Arturo Falaschi were located inside the same building where several researchers between 30 and 50 years old with various experiences abroad, especially in the USA, were working very actively. They gave me the chance to talk to them and describe me their research experiences and future lines of research. For the first time I found myself in front of people who demonstrated a real passion for the work they were doing. The environment was informal and very different from what I had known at the University of Milan at that time. The researchers of Pavia greatly involved the young people who attended the laboratories to prepare the degree thesis, frequently invited foreign speakers to present scientific seminars and the atmosphere was very relaxed, even if the amount of work required of the students was very high and left little free time to study for the preparation of the exams.

In short, despite being students, they were considered an integral part of the research group and the students had the opportunity to be included among the authors of a scientific publication if their work was considered an integral part of the research activity of the whole group involved. It was necessary, therefore, to be able to team up with the sole final goal of achieving results that could be published in a prestigious scientific journal. This attitude has been an important experience for me and I have always tried to apply it also in my subsequent scientific career.

I believe that the passion for the work that each of us is performing is essential in all types of activity a person is choosing in life, but this is particularly true for any individual who has decided to do research. The curiosity to know is probably the spark that triggers the desire to devote oneself to research, but it must be supported by a real passion for one's work and a certain amount of creativity in order to have some hope of success. Success is then linked to a series of often unpredictable external factors. You have to grow and work in the right environment by interacting with colleagues who push you and also accept to critically interpret any kind of scientific

evidence and to always question everyone's results. Many of the colleagues I have had the pleasure of working with have also become my best friends! Solitary "research" in biology is almost always destined to fail as it is necessary to be able to build a close-knit and collaborative working group that faces a problem often from different perspectives. The daily work of a researcher is certainly hard and studded with challenges: from the experiment that gives conflicting results to one's expectations, to the need to integrate one's experiments with the time required for a continuous study of research activities in other sectors more or less close to one's own. However, no fatigue or disappointment is comparable with what one feels when realizing that one is observing and understanding a phenomenon never understood before. That is, when one is making *a discovery*.

2 Work Done: My Personal Scientific Approach

Certainly influenced by the discovery of the DNA structure and the decoding of the genetic code in the 50s and 60s of the last century, when I arrived at the University of Pavia I chose to work for my experimental thesis in the laboratory directed by Arturo Falaschi at the CNR who had recently returned from the USA after having worked with Arthur Kornberg, Nobel Prize winner for the discovery of the enzyme DNA polymerase in the bacterium *Escherichia coli.* As sometimes happens, Kornberg (a great scientist with whom I was lucky enough to interact over the years) won the Nobel, despite having at that time identified the wrong DNA polymerase! In fact, (as Kornberg himself discovered a few years later) the polymerase he first identified is not the main enzyme involved in DNA replication, but plays its most important role in DNA repair. Thus, the Nobel Prize to Arthur Kornberg was widely deserved and paved the way for the thousands of research projects that in the following 40–50 years highlighted the numerous molecular processes taking place on DNA (replication, repair, recombination and transcription) that are intimately interconnected with each other precisely to maintain the stability of the genome (DNA) in all living organisms.

Therefore, my passion for DNA and the control of its stability began when I was a student and it has continued to this day. However, it became very soon clear that an exclusive biochemical approach to understand the function of proteins involved in DNA metabolism could have great limits, as mentioned above regarding the discovery of the first DNA polymerase by Kornberg. Instead, an ideal scientific approach might require the integration of biochemical and genetic approaches based on the isolation of defective mutants in one or the other of the molecular processes taking place on DNA.

Since the field of studying DNA stability in prokaryotes and viruses was already quite crowded and involved powerful research groups, it was perhaps more appropriate to deal with the problem of DNA stability in eukaryotic organisms, although it was clear that this was an undertaking full of obstacles. The first problem was to decide which organism would be better to work on. The most obvious choice could have been to work with human cell lines; however, at those times, the manipulation

of human cell lines was quite limited. Shortly after graduation I came into contact with the laboratory of Prof. Giovanni Magni at the University of Milan who was a well-known geneticist working on the mechanisms of mutagenesis using the yeast *Saccharomyces cerevisiae* as an experimental model in eukaryotes. For a series of reasons that it would be too long to describe in detail in this context, yeast had then become over the years the most recognized model organism for the study of molecular mechanisms in eukaryotic cells.

A few years after graduating in biological sciences and obtaining a Master Degree in Biophysics (at that time there was still no PhD programs !!! in Italian universities), I read in the Journal of Biological Chemistry an article in which Lucy Chang's laboratory in the US had managed to purify two DNA polymerases from the yeast *Saccharomyces cerevisiae.* Lucy, in turn, came from the laboratory of Fred Bollum who first purified a DNA polymerase from an animal tissue (calf thymus) and was Kornberg's great competitor on the American scene. Through Giovanni Cassani, (known in Pavia, and who had worked with Lucy and Fred on another famous enzyme, known as "Terminal Transferase" or TdT, which has the ability to synthesize DNA molecules in test tubes), I managed to get accepted as a post-doctoral fellow in their lab first in Farmington, CT and then in Bethesda, MD in the USA. The research project consisted in purifying a DNA polymerase that was able to initiate DNA replication and that, therefore, was able to start the synthesis of DNA "de novo", that is, without the use of pre-formed DNA or RNA molecules, since all DNA polymerases known at that time were unable to initiate DNA synthesis "de novo".

Working day and night and using monoclonal antibodies produced against the only partially purified enzyme, we were able to identify an innovative immuno-purification procedure that allowed us to isolate in a few days from yeast cells extracts an enzyme capable of replicating DNA molecules using RNA molecules as "initiators" or primers, similar to what Kornberg and others had discovered in prokaryotes. Fred Bollum and Lucy Chang had sensed that the use of specific antibodies could be a powerful tool to quickly purify the proteins with which these antibodies interacted. Thus, in the laboratory a project was started to produce monoclonal antibodies capable of recognizing specific replicative proteins, and the first choice fell on a DNA polymerase able to start DNA replication in yeast. One of these monoclonal antibodies was able to purify to homogeneity in a single step what was then called DNA polymerase α-primase, a complex consisting of 4 subunits of 180, 74, 58 and 48 kDalton, which was subsequently also identified in all the other eukaryotic organisms analyzed.

This was perhaps the most important discovery of my early scientific career and paved the way for other interesting research studies. The polymerase activity is linked to the p180 polypeptide while the activity required for the synthesis of initiating primer RNAs lies mainly on the p48 polypeptide although the p58 polypeptide also performs important catalytic functions. Back in Italy after three years of work in the USA, the possibility of producing substantial quantities of the Pol α-primase complex has allowed us to isolate the individual subunits of the enzyme complex and to produce, in collaboration with researchers from the Zooprophylactic Institute in Brescia, monoclonal antibodies against the individual subunits. This work was

the result of the collaboration with Gianfranco Badaracco whom I had met in Pavia and who continues to be one of my greatest friends. These antibodies were essential to understanding the biochemical functions of the entire complex, but they also paved the way for another collaboration that opened up new fronts in our scientific research. In those years, in fact, the new frontier of gene cloning through recombinant DNA technologies had begun. Among these technologies, in fact, the way to be able to clone a specific gene from DNA libraries had also been identified, if you had antibodies against the protein encoded by that gene. Together with Giovanna Lucchini who had also just returned from the USA where she had worked with Gerry Fink, one of the pioneers of gene cloning and their study in yeast, we went on the hunt for the genes coding the 4 subunits of the Pol α-primase complex in DNA libraries of *Saccharomyces cerevisiae* created in the lambda bacteriophage. Over the course of several years of intense work, with the support of numerous students who later became established researchers, we isolated all four genes encoding the subunits of the complex and produced a variety of mutations in those genes that have been essential to elucidate the different functions of the Pol α-primase complex in the replication, repair and control of genome stability in yeast and many of these conclusions have been extended to other eukaryotic organisms.

It is worth pointing out another quite interesting aspect related to this genetic approach. If a mutation inactivates a gene and that gene encodes a function essential to the cell's viability, it is likely that the mutation could be lethal to the cell. However, not all genes in an organism encode functions essential for cell viability and, although the gene is essential, many mutations can cause a hypomorphic phenotype compatible with cellular life. However, if the protein encoded by a certain gene interacts with other proteins, it is possible that combined mutations in two genes coding for proteins interacting with each other cause a lethal phenotype when the two mutations are present together. This so-called "synthetic-lethal" phenotype has also been widely used in our group to identify genes that code for proteins interacting with each other in different processes related to the control of DNA stability in yeast cells.

This work and the whole experience of my scientific career have largely substantiated my original belief that a successful work in the field of biology can only be obtained by combining different approaches like, in my case, genetics and biochemistry. This multidisciplinary approach, further extended to other subjects, will be more and more relevant in the development of the science of the future.

3 Science Today and Tomorrow

Describing what science is today is essentially impossible because the question is too broad to be summarized with simple definitions but my major bet would be on quantum biology or something of that kind where the knowledge on cells and organisms will integrate with physics and philosophy, which over the centuries, played a major, if not unique role in the interpretation of the phenomena.

If we ask scientists what, in their opinion, is the most important scientific theory, we will probably have different answers from researchers working in biology or in physics. Many biologists might answer that Darwin's theory of evolution by natural selection was perhaps the most brilliant idea ever conceived. Most physicists, on the other hand, might answer that quantum mechanics is the foundation on which much of physics and chemistry is based and it allows us to understand the fundamental blocks on which the entire universe is built.

Quantum mechanics, for a molecular biologist like me, has always been (and continues to be !!!) a mysterious theory. According to quantum mechanics, the behavior of an elementary particle (neutrons, electrons, protons, photons, etc.) is predictable only in a probabilistic way: for example, we know that a single uranium atom will emit radiation, but we do not know when this is going to happen. Such a limitation, however, does not exist for macroscopic systems composed of millions of atoms put together. Moreover, in the quantum view, there is a duality between a particle and a wave: all things around us are composed of tiny and distinct particles (atoms, electrons, protons, etc.). Energy, like light or sound, on the other hand, is transmitted by waves and not by particles. Quantum mechanics began at the beginning of the last century when it was discovered that sub-atomic particles sometimes behave like waves and that light waves sometimes behave like particles!! But that's not all: one of the most bizarre features of quantum mechanics is the "correlation", whereby two particles that have been in contact with each other, but then move away on opposite sides of the universe can be, in principle, still in communication with each other.

Quantum correlation was the natural result of the mathematical equations of the pioneers in this field, but its implications were so extraordinary that even Einstein refused to accept them to the full. In 1925 Werner Heisenberg, who worked in Niels Bohr's group, established that the world of atoms is a "spectral" place that crystallizes into a definite existence only when it interacts with a measuring instrument. Heisenberg showed that one can design an experiment to establish the precise position of an electron at any given time, or another experiment to measure the velocity of the same electron. It is, however, impossible to design an experiment in which to measure with arbitrary precision both: where an electron is and also its speed. In 1927 this concept was expressed in the famous "Heisenberg uncertainty principle" which has been confirmed hundreds of times in laboratories around the world. In short, a great confusion for me that let me think that quantum mechanics is too complicated for a poor molecular biologist!

As I described in a previous section, the scientific problem that most fascinated me was the study of DNA replication and the mechanisms involved in maintaining the stability of genomes. Watson and Crick conclude their historic work published in Nature in 1953 in which they present the model of DNA structure with the following sentence "It has not escaped our attention that the specific coupling of the bases postulated here immediately suggests a possible mechanism of replication of the genetic material." That is, that DNA replication is semi-conservative: the two strands of DNA can separate and each of them can act as a template to create a complementary strand, thus generating two identical copies of the original DNA double helix.

In 1944 Schrodinger (another of the "fathers" of quantum mechanics) argued that the extraordinary level of precision observed in the mechanisms of genetic inheritance could not be explained by classical laws and proposed that genes were some kind of "crystal" that he called "aperiodic". That is, crystals with a structure similar to normal crystals, but modulated in some way: for example, with different intervals or periods between repetitions. He proposed that these modulated repeating structures contain genetic information and that, like crystals, their order can be encoded at the quantum level. Was Schrodinger's intuition (ten years before the discovery of the structure of DNA) correct?

DNA is indeed a repeated structure (the nucleotide bases) and it is also true that it is an aperiodic structure in the sense that each repeated unit can contain one of four different bases. But aperiodic crystals do not necessarily encode information at the quantum level. To address this issue we need to look at the structure of DNA in more detail. The coupling of the two strands of DNA is based on the formation of hydrogen bonds between complementary base pairs: two hydrogen bonds to join A with T and three hydrogen bonds to join G with C. These hydrogen bonds are formed by individual protons (hydrogen nuclei) and the position of protons is determined by quantum, not classical, laws; it follows that the genetic code that underlies life is inevitably a quantum code.

Life on our planet could not have evolved if copying the genetic code onto DNA had always been perfect and never made any mistakes. Life would have become totally extinct if it had not adapted to the new conditions that have been created on our planet over billions of years. It can be calculated today that the percentage of infidelity in the replication of the DNA of the human species is about 10–20 errors for each replication of our haploid DNA genome that has a size of about 3.2 billion nucleotides.

There is also another aspect to consider: in the second article in Nature also published in 1953 and entitled "Genetic implications of the structure of deoxyribonucleic acid", Watson and Crick suggested that a process called "tautomeria" could be one of the causes of mutations. Recall that the hydrogen bonds that hold together the base pairs on DNA correspond to a shared proton and protons are quantum entities with both wave and particle characteristics, so they behave like a wave moving between the two bases. If the two protons that hold the two genetic letters together (for example A and T) jumped to the other side of their respective hydrogen bonds they would be closer to the other base and this would provoke the creation of alternative forms of each base that are, in fact, called "tautomers". If a base is in its rarest tautomeric form during DNA replication, the new DNA strand will have a different base pair from the original. For example, tautomeric T can only pair with G instead of A, as it normally does: it follows that the new DNA strand carries mutations that will be inherited from the offspring.

We know that the genetic information on DNA replicates in a specific phase of the cell cycle (called the S phase), but we also know that the reading of the information contained in DNA does not take place specifically in the DNA replication phase, but whenever DNA is transcribed into RNA and then translated into proteins. A feature of this process of "reading" the genetic information contained in DNA is that some

genes are read more frequently than others. If reading DNA during transcription is a quantum measurement, then the most frequently read genes should be subject to greater perturbation and this would lead to higher percentages of mutations. Experiments conducted in yeast, murine and human cells have shown that this is indeed what really happens. On this subject there has been a long debate on the relationship between quantum mechanics and genetics and the problem is not yet solved. It is however certain that the knowledge of quantum mechanics is essential to address the central problems connected with the understanding of the physics of life and only future research work will be able to clarify the many questions still left without a satisfactory answer. Therefore, I do think that the time has arrived in which biology, and its related fields like genetics, physiology, and biochemistry must meet quantum physics to unravel new mechanisms and establish new paradigms.

For this reason, I believe that a continuous interaction between biologists and physicists is a good way to go in the near future.

4 Advice to the Next Generation of Scientists

As it is said in a famous song (Bocca di rosa) by my favorite poet-singer (Fabrizio De Andrè): "… Feeling like Jesus in the temple, it is known that people give good advice when they cannot give bad examples …" That's why I have some fear to write what I think on this topic, but I'll try just hoping not to be taken too seriously!!!!

As I have already written about the motivations that led me to try to do research in biology, when I was a student I was not at all convinced that being a researcher/biologist would be the best way to follow in my life. It all happened for a series of events and casual encounters. For this reason, I advise every young scientist/researcher to read the book by Jaques Monod "Chance and necessity" which is an important reading to give rationality to our beliefs and hopes.

The first thing to do is to believe in ourselves and think that nothing is precluded if we really believe that it is possible to do so. This is the initial part that is perhaps also the most important and unpredictable because "believing in oneself" depends on a series of personal factors that have happened to us since we were very young. In my lucky case, surely what has been most helpful to me throughout the entire life has been to grow up in a supportive family that was always available to spur you on, solicit and help you even when any specific situations or results were not among the most enthusiastic.

A second point that I consider very important is to always try to inform ourselves about the aspects that can influence our choices, so that these become the most logical possible. If you are following a degree course and you think that doing research can be an activity that could really involve you, a very important moment is the choice of the degree thesis. First you need to identify the discipline that you consider most relevant and similar to your scientific interests and, immediately after, identify who may be the teacher who will follow you during the thesis that must necessarily be an experimental thesis if you really think you can become a researcher in biology. The

suggestion I have always given to my students was to go to PubMed and download the list of publications of the possible candidates they have identified to get a better idea of the quality of the publications obtained in the laboratory they identified. Remember that "quality" is certainly more indicative than "number" of publications and one of the first things you have to learn is how to judge "quality", a rather complex aspect. Moreover, from the publications it can be deduced how much the laboratory you have identified is a collaborative environment open to interactions with other national and international laboratories and available to "contaminate" itself with similar scientific disciplines. Personally, I believe that it is better to do the research connected with the degree thesis in your country of origin in order to acquire defined scientific bases and personal security that can be positively used abroad at a later stage.

Once the degree is obtained, the time begins to make another important decision about one's future: to be accepted into a Ph.D.? Where? In the same university where you obtained your degree or in a different institution in your own country or abroad? On these points my opinion and personal experience is that if you have found yourself well with the teacher and the research group connected to her or him it may be a good choice to continue the research work in the wake of what was done during the thesis, obviously trying to acquire new skills both theoretical and experimental. After about five years of intense work in a specific area of research all the good young people I know (really many) are able to fly alone, maybe taking a few slips, but without falling.

After this phase another equally crucial one begins: at this stage, in fact, you should have already acquired the personal conviction if you really want to become a researcher or you prefer to do another job almost always more remunerative economically and, in any case, where it is better to live this new period of scientific growth. Both in one case and in the other, in my opinion, this is the moment in which to make the choice to spend a few years in a laboratory in another country: it is only in this way that you become really independent and it is a unique experience that, certainly in my case, was the most important. Having traveled around the world extensively, there is nothing like an experience in the USA for a European to gain a more complete view of the way(s) of doing research but be aware that even the best planning could encounter unexpected difficulties and be prepared and confident to change your programs and expectations accordingly. Here is my short but quite instructing story.

After my experience in the USA I felt confident that I could compete for a position with any other researcher and so I decided that I would return to Italy to try contributing in some way to the country I always had in my heart. This was a dangerous choice! Back in Italy my job (which was renewable annually) as an assistant at the University of Eastern Lombardy (E.U.L.O), now become the University of Brescia), had been taken by somebody else. However, in my head I had several research projects to carry on and I decided to try to go on working without any salary counting on my wife's salary as a teacher and the help of our parents and maybe the help of good friends working in other labs. In fact, in order to have a chance to work, I often returned to Pavia to ask for reagents and support in my old laboratory and I must say that they have always helped me. In Brescia I worked next to the

laboratory of Human Anatomy whose professor seeing me work like crazy for free offered me a job as an assistant of Human Anatomy. I told him that I knew practically nothing about Anatomy and he replied not to worry and to devote myself to my research interests that he would have taken care of Human Anatomy. Incredible!!! In the following months, an unforeseen and unpredictable event occurred. Since in the three years I spent in the USA I had had a position as Teaching and Research Associate in a prestigious University, the Italian Ministry of Education established that I was suitable to occupy a position of Researcher in an Italian University, a new role that replaced the previous one as Assistant Professor. I was thus contacted by Giovanni Magni (Professor of Genetics at the University of Milano) who offered me the position of Researcher at his Department, where I have always worked until the end of my University career. I would occasionally go back to Lucy and Fred in Bethesda USA, with whom we carried out a series of joint research programs. There my troubles ended and my career took off.

This is just to say that, even the most wise of the advices can be made irrelevant by the unpredictability of the events and that luck also plays a huge role in our lives. Nevertheless, it is by arming yourself with rock-solid determination and a good confidence in your scientific competence that you will eventually succeed. You can get it if you really want it, is the title of a famous reggae song.

Paolo Plevani graduated in Biological Sciences at the University of Pavia where he also obtained a Ph.D. in Biophysics. After 4 years as a post-doc in the USA he came back to Italy acting first as Researcher and then as Associate Professor of Genetics and Full Professor of Molecular Biology in the Department of Genetics, University of Milano. In 2018, he was nominated Emeritus Professor of Molecular Biology in the same university. His major research interests are: DNA and RNA metabolism and the mechanism controlling genome stability and cell cycle progression. In 1996 he was elected Member of the European Molecular Biology Organization (EMBO) for his scientific contributions.

Finding My Path, Trusting My Voice

Natasha Raikhel

Abstract I was a budding pianist immersed in music in Leningrad, in the Soviet Union (now Saint Petersburg, Russia), when I started over, giving up sheet music for the study of ciliates. In a second starting-over story, I became a refugee after emigrated to the United States, where I switched to studying carbohydrate-binding plant lectin proteins, dissecting plant vesicular trafficking, and isolating novel glycosyltransferases responsible for making cell wall polysaccharides. I track my journey as a plant biologist from student to principal investigator to founding director of the Center for Plant Cell Biology and then director of the Institute for Integrative Genome Biology at the University of California, Riverside and then being elected to the US National Academy of Sciences. I discuss implementing a new vision as the first and (so far) only female editor in chief of Plant Physiology, as well as how my laboratory helped develop chemical genomics tools to study the functions of essential plant proteins. Always wanting to give back what I received, I talk about my efforts to develop female scientist leadership in different countries and a constant theme throughout my life: a love of art and travel.

1 Motivations: How I Developed an Interest in Science

I was born in Germany. My father, Vladimir, a surgeon, and my mother, Alexandra, an X-ray technician, had met at a hospital in Germany, where both of them had been sent to work by the Soviet Union during World War II. In 1948, when I was a year and a half old, we came back to the Soviet Union. I was lucky to have very good parents. They loved me and my younger sister dearly, and we had a psychologically secure, happy, and loving home.

My childhood was not like that of many scientists, who fondly remember an interest in animals and plants and many hours spent exploring the outdoors. Studying piano since age six, I spent much of my childhood inside. I was a quiet child, and the long hours at the piano suited me. Constantly at the music school, I grew up exposed

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to symphonies and opera. Combined with the books that filled our apartment—our father read to us constantly—this created an enriching environment for me and my sister. I learned to be extremely disciplined, practicing for many hours a day. I attended a specialized music school that included a regular grade school in Leningrad (now Saint Petersburg). Even early on, I was certain that music and playing concert piano would be my life.

When I was in my last year of specialized music high school and planning to continue my music education, a teacher for my conducting class had a chat with me that turned my life upside down. It was the first time somebody questioned whether I could successfully compete as a concert pianist the rest of my life. I knew the career was fiercely competitive and required more than just incredible skills, but had grown up playing to receptive and appreciative audiences and thought I could make it. I started to question my abilities and aspirations and came to realize that although I was a very capable student, I would probably never be one of the select few at the top. After this realization, I was devasted and thought my life was over; however, I made a decision to start all over again. I dropped my last year of music school, went to evening high school, and worked as a music teacher to pay private tutors to learn chemistry, math, physics and biology. My life at this time was spent almost entirely working and studying, but the hard work paid off and I was able to pass exams and enroll in the Biology Department at Leningrad State University. There I continued studying hard to catch up with fellow students, many of whom came to the University from specialized physics, chemistry, math, and biology schools.

In retrospect, I am glad that I had a serious musical education, thanks to the dedication and support of my wonderful late parents. Studying for many years in a dedicated music school taught me to value and know high culture, be disciplined and organized. These traits have helped me in the past and continue to help and sustain me today. Later I have greatly appreciated the fact that my conductor teacher was very honest with me and I always tried to be very honest with my students and postdocs.

At the University, I was trained as a classical cell biologist and at that time it mostly meant working as an electron microscopist. I was working with unicellular organisms and was not a plant biologist. I married my fellow university classmate Alex Raikhel and we had our first son, Eugene in 1975. In 1978, I flew to Baku, Azerbaijan, to collect samples from the Caspian Sea, one of many such trips I had taken since becoming an assistant professor. After a few days, Alex and Eugene (almost three-year-old) joined me for a week of vacation time. On the return flight, on May 18, our plane crashed in a potato field between Moscow and Leningrad, killing some of the passengers on board. It was a large plane with many travelers, and the crash was a horrific experience. Our small family survived, but my perspective on life changed completely. Because my equipment and samples did not survive the crash, I had to get a statement for my institute from Aeroflot—the only available airline in the Soviet Union at the time—detailing how the equipment and collected samples had gone missing. This led to one of my most direct experiences with the Soviet system: The airline told me that I needed to go back to Baku, because as far as they were concerned, there had been no airplane crash! After some insistent prodding, I received an official paper statement that the crash was an “unexpected

landing". For me, it was the last straw: It became clear that we could not live in the Soviet Union anymore.

My husband and I disliked the Soviet system wholeheartedly and read samizdat, but we were always very careful with whom we shared our views; I would say we were silent dissidents. However, we knew that, being Jews, we could officially emigrate from the country. The saying at the time was that Jews were exchanged for grain from the United States. Later, I learned that this was due to an effort spearheaded in the United States by Senator Henry "Scoop" Jackson.

When I decided to leave USSR I already defended my Ph.D. and was an Assistant Professor at the Institute of the Academy of Science. I remember my laboratory supervisor at the Institute—a Member of the USSR National Academy of Science who had a chance to travel abroad—telling me I must have lost my mind to leave such a desirable position. He also said it was unlikely that I would get a job as a scientist in the U.S.; most likely, I would be sweeping the streets of New York and never have close friends. How wrong he was!!! In fact, we met later at an international cell biology meeting and he apologized for his prognosis.

I immigrated from USSR with my husband, Alex, also an Assistant Professor at the Institute of Zoology, Eugene and my mom, to Athens, Georgia in 1979 with a personal fortune of 25 dollars. Both of us had to start all over again as postdocs. My memory is that I felt completely lost and wondered how I could and would ever make the language, scientific and social transitions required to survive and succeed in this country. The late seventies were also not an easy time in the States: there was the Iranian crisis, the energy crisis, very high inflation and tremendous concerns about a recession and high unemployment. Our second son, Vincent, was born in Athens, Georgia in 1984 and after seven years on soft money we both were able to obtain our first permanent jobs in the U.S.

During the first year, at the University of Georgia I continue to study free-living ciliates. And even published a paper but honestly, I was bored. So, when I was offered it, I accepted a postdoctoral position in the cell biology laboratory in the Department of Botany. There was much excitement coming from the department at that time, and it was infectious. The department was active and fast paced, with many interesting lectures and a lot of buzz about isolating genes and doing gene transformation in plants—a stark contrast to the slow and swampy Department of Zoology, where I was before.

I later realized that the high-energy activity and enthusiasm were thanks to Professor Joe Key's vision for making the Department of Botany a leader among modern plant molecular biology departments. Key is also credited with helping to advance plant molecular biology in the United States. I experienced his knowledge first hand making first cDNA library and cloning wheat germ agglutinin in his laboratory. In the Botany Department I worked with graduate student Michael Mishkind, joining his project on lectins—proteins abundant in grains and legumes that bind carbohydrates and play an important role in plant immunity and responses to stress. We focused on wheat germ agglutinin. Michael taught me immunocytochemistry, and together we discovered that this protein is expressed in specific cell layers—the coleoptile and the embryonic root—of different grains, such as barley, wheat, and rye.

This lectin had different and interesting patterns of expression in these different agricultural plants. In wheat, it was expressed in the outer layer of the coleoptile; in rye, it was expressed in both the outer and inner layers; and in barley, it was completely absent from the coleoptile. We published the results of this work in Science which was one of the reasons, a few years later, that I was offered a position at the Plant Research Laboratory (PRL) at Michigan State University.

I did not know at the time that the PRL was the premier place in the country for plant biology. When I was invited for an interview, I proposed to extend our study of wheat germ agglutinin to understand the cell type–specific expression of these proteins and related lectins, both in monocots (such as rice and barley) and in dicots. The project was almost impossible to do then, and even today it is still very difficult in monocots, which have complicated polyploid genomes and long-life cycles and are difficult to transform. This type of study is easier in dicots, such as Arabidopsis and tobacco plants, but wheat germ agglutinin and other lectins we were studying are not present in these plants. The PRL was a very international institute at that time and I absolutely fell in love with it and was incredibly happy that I was offered a job there; my husband was offered a job at the Entomology Department. We moved from Georgia to Michigan in the summer on 1986. We packed our car, loaded with the boys, our cat, and the possessions we had accumulated by then, along with a little trailer full of monoclonal antibodies and frozen material in a nitrogen tank attached to the back (would have never allowed these days!)—not as dramatic as the move from the Soviet Union to Georgia, but still a major change for our family.

I became an Assistant Professor at the DOE Plant Research Lab when I was 39 years old. My colleagues and mentors at the PRL provided a local environment that was both stimulating and challenging, encouraging me to be the best possible scientist I could be. What I achieved was also due to the chance of time. I am a product of the age of molecular biology and now genomics with its rapidly expanding knowledge base and incredible information systems made possible by technological growth. This lucky moment in history has allowed all of us here today the privilege to be pioneers of new and fascinating frontiers.

In my lab, we studied cell biology of WGA and other chitin binding proteins and by the early 1990s, my laboratory was engrossed in trying to understand the mechanisms of trafficking of these proteins thorough the endomembrane system. Suddenly, I had found my research niche: intracellular organelle and membrane trafficking. We began to explore what the propeptides recognize and bind to and how vesicles move from one place to another, continuing to use a variety of standard biochemical, molecular biology, and genetic approaches.

I can give a simple analogy to explain protein trafficking. If we think about airspace and flight plans, we know that there are specific routes/pathways for flights and specific rules regarding air traffic. There are hubs where planes could be changed if malfunctions develop or detained if serious natural or man-made events occur. Protein trafficking in the cells is similar. After protein is synthesized, it has to be delivered to the proper part of the cell to perform its function. Sometimes protein arrives at the wrong place and the cell compensates for this mistake with built-in system redundancies. However, if the hub is extremely important, the mistake cannot be corrected

and the cell dies. This analogy, while simplistic, explains why we study the "rules and regulations" of protein trafficking in the cell. Many basic "rules" in eukaryotic cells are similar but as multicellular organisms, plants possess many specialized cell types, each surrounded by a cell wall and organized into tissues and organs. In addition, many proteins in plants are encoded by many similar genes (we call them multigene families). There are more multigene families in plants than in other organisms, probably because plants are sessile organisms and need more different ways/opportunities to deal with stress and environmental changes. Also, proteins function in various complexes with other proteins and this complexity brings specificity for a particular function. Therefore, we study the intricate network of "rules and regulations" for protein trafficking because it is key to the proper functioning of organelles in the live cell. I owe a great deal to my PRL colleagues, especially Professor Chris Somerville, who was an Arabidopsis expert and was particularly instrumental in advising me.

Another important project our laboratory began while at the PRL was the study of cell wall biosynthesis in collaboration with Prof. Kenneth Keegstra, a fellow plant biologist at the institute. In 1999, we were one of the two laboratories to isolate the first glycosyltransferase, an enzyme that decorates xyloglucan in the Golgi apparatus. Xyloglucan is a polysaccharide that is involved in plant cell wall biosynthesis. Subsequently, we found other enzymes that add other types of sugars to xyloglucan. Working on this project, I learned more about carbohydrates, chemistry, and gas chromatography than I had ever known before.

Working with energetic students and postdocs has always been the fun part of running a laboratory. What was hard in my transition to the PRL was the pressure of writing and waiting on grants over and over again. I was relatively lucky, I realize, in that I was always awarded national grants and in addition had support from the US Department of Energy thanks to the PRL's unique relationship with this department (the PRL was the only place in the country receiving millions of dollars of grant money for plant research). But with the Department of Energy's support came incredible pressure to perform and to publish in high-impact journals. In the beginning, it was quite overwhelming for me to balance writing grants, teaching, and running a laboratory.

Alex and I inevitably brought our work home with us. By the time we moved to Michigan, our sons knew all about their parents' proteins of interest. A few years into our time at Michigan State University, neither of them wanted to hear about genes, proteins, or their parents' model organisms. "No more wheat germ agglutinin or vitellogenin [Alex's mosquito's protein]," they would declare. I remember our younger son playing "family" with his animal toys: After the animal family had finished their dinner, he had the mom lion say to the dad lion, "It is my turn to go to the lab." The dad lion disagreed, thinking it was his turn. This was amusing but also an accurate depiction of our family life: Either Alex or I always tried to go back to the laboratory after dinner, while the other stayed home with the boys. On weekends, we spent Sunday together as a family (unless there was a looming grant deadline) but also divided Saturday into laboratory and family time. We talked about our research at home a lot, which might have deterred both boys from studying biology, but at least

they both saw how much we cared about and loved our work. Perhaps that is why Eugene chose an academic career and is now a cultural and medical anthropologist and professor at the University of Chicago.

There were many positive aspects to the structure that our careers gave our family life. We traveled a lot, both within the United States and abroad, and always took the kids with us. Both of our sons grew up aware of the world and its cultures and comfortable in new and unfamiliar situations. We constantly took them to concerts and museums, which is why, I would like to think, Vincent became interested in and studied music and became a professional composer. Vincent was very successful as a composer, but at some point, he realized that the competition in the music world is unbelievable. While I was visiting him in New York now quite a few years ago, he told me that he had decided to go back to school to become a medical doctor. He spent two years at Columbia in New York taking general science requirement courses and working as a tutor, entered to a medical school at Cornell University in New York and is now a physician at VA hospital in Seattle, WA. In a way, it seems to me that he has followed my path from music to biology via medicine.

2 Work Done: My Personal Scientific Approach

At the PRL, I had initially felt that I could only handle running my own laboratory—managing students and postdocs, writing grants, and teaching. But then, little by little, I realized that I could do more outside the scope of the laboratory, to give back. I had come to the United States with so little—no professional network to speak of, a language barrier, and no knowledge of the academic system—yet I had received mentorship, support, and career advice.

In 2000, as I was recovering from breast cancer treatment, I was asked if I would consider becoming the editor in chief of Plant Physiology, an international monthly journal that covers a wide range of plant science and is also one of the oldest plant science journals. By then, I had been on the editorial boards of several plant-focused and general science journals and was familiar with the work involved. Yet I had never been at the helm, and I saw the position as a new challenge and an opportunity to make a positive impact on the journal. The previous editor in chief, Maarten Chrispeels, who had held the position for eight years, did an incredible job, turning the journal into a future-facing, modern journal with high review standards. When I started, I felt the journal was poised to make a leap forward that would highlight the latest technology-driven insights in plant biology and widen its readership. I knew it would be a lot of work, but I also felt that it would be a fun challenge that would make a difference in the plant science community.

I set out to increase the visibility and influence of the journal and to not only move the content into the postgenomic era, but also energize the presentation of the content. I was able to invigorate the editorial board, bringing on associate editors who were leaders in their specialties to create an international and diverse team. To my amazement, every single one of the 71 scientists whom I asked to join agreed to

do so. We had scientists from 13 countries other than the United States, including Mexico, Italy, Argentina, and Israel. I am also proud to say that I was the first and (so far) only female editor in chief in the journal's 90-year history.

In the five years that I led the journal, Plant Physiology became the first plant-focused journal to publish genomic and systems biology research. We moved the journal online and managed to increase its impact. It was a very active and stimulating five years, and I could not have done it without incredible Plant Physiology staff and my personal assistant.

The editorship term was five years, and when I was asked to stay on longer, I realized how hard it is to stay creative and innovative on the same project. Perhaps this is different for others, but I have limits. The journal was a huge part of my life for five years, but in imagining an even brighter future for plant biology research, I stepped down in 2005. I was ready for the next step in my life!

So, after years at the PRL and the experience of being an editor in chief of Plant Physiology, I began to see that I could take on a leadership role. The PRL had offered me a director position, but I knew that I could never have a major leadership role there because that is where I had grown up as a scientist, so to speak. I had learned much from the PRL's director, Prof. Hans Kende, who had become a good friend because of our mutual love of science, music, and art: how to be a proactive leader, how not to let a situation escalates, and how an institute thrives when there are significant resources thanks to a well-supported infrastructure. Researchers can flourish only when they are well supported. Hans, who was instrumental in the success of the PRL, had shown me firsthand that this strategy works like a charm. He was an excellent scientist and a very smart human being, and I have tremendous respect for him. After observing him in action, I soon had my own opportunity to be a leader.

In 2001, was recruited by the University of California, Riverside (UCR). I moved my laboratory to the UCR where I became the founding director of the Center for Plant Cell Biology (CEPCEB). I loved the PRL and initially did not want to move. At the time, the UCR Department of Entomology was recruiting my husband. He had wanted to move away from Michigan's harsh weather for some time, but I had not been ready. But in 2001, the idea, for me, was becoming increasingly conceivable. Our younger son, was finishing high school, and our older son was already in graduate school at Princeton University. (By this time, our younger son had changed his name from Andrew to Vincent. He made the decision and changed his middle name to his first name at age 12 when I was on sabbatical in Australia, citing as his reason that there are too many Andrews.)

Both Alex and I were invited to visit UCR that February. I saw the ubiquitous citrus trees in bloom everywhere, and thought about how Shauna and Chris Somerville had already left the PRL for California and that, after ten years, it was perhaps time to find a new challenge. Both Alex and I received offers from UCR. I was hired by the chancellor, Raymond (Ray) L. Orbach; the dean of the College of Natural and Agricultural Sciences, Mike Clegg; and the department chair, Elizabeth (Betty) Lord, as a university distinguished professor and was given the Ernst and Helen Leibacher Endowed Chair and the chance to build a center for cell biology. But with formidable institutions like the University of California, Los Angeles, and the

University of California, San Diego, close by, I told Orbach that a center specifically for plant cell biology would be more realistic. I had a vision that the center, with an infrastructure of core facilities, would be open to all UCR researchers, regardless of whether they studied plant biology. Many scientists in the United States like to have their own equipment, but coming from Russia, I had learned that infrastructure that can be used by everyone helps everyone. I asked for new staff positions to run the core facilities—a bioinformatician, a microscopy facility coordinator, and eventually genomics instrumentation and proteomics experts, who would enable and empower the work of faculty and students—and the chancellor agreed.

At the time, in the late 1990s and early 2000s, the advent of genomics and proteomics technologies had increased the gene-to-researcher ratio exponentially. Researchers were now able to create snapshots of thousands of molecules inside the cell simultaneously. With this new systems biology approach in mind, the vision of the CEPCEB was to create a place where these technological tools would be accessible to all UCR researchers and where interdisciplinary work among biologists, engineers, computer scientists, chemists, and other researchers would increase our understanding of the complexities and dynamics of cells and tissues. I officially started in June 2001 as the founding director of the CEPCEB. Because Vincent was still in school in Michigan, for half a year we commuted so that he could finish school; we then finally moved to California in January 2002. I am proud to say that after just a few years three-quarters of the new center's faculty and staff were women, and that this is still the case today. By then, Orbach had been replaced as chancellor by France A. Cordova who later became the director of the National Science Foundation and she and I worked closely to bring the center together. It officially opened in October 2002. I was able to draw on my experience at the PRL and what I saw as improvements that could be made for the new center. Later, Cordova asked me to take over the directorship of the Institute for Integrative Genome Biology (IIGB), of which the CEPCEB is one of the centers.

When we celebrated the CEPCEB's tenth anniversary I transferred its directorship to my colleague and good friend Professor Julia Bailey-Serres and continued as director of the IIGB until my retirement in July 2016. Of course, the process of building the CEPCEB was not without challenges. Together with my colleagues, I worked like a horse to coordinate the center's facilities, hire the best creative new faculty possible and hire staff, create a new facility for chemical libraries, apply for several training grants, and organize workshops, which, in the end, changed the culture at UCR. At first, there were some who were not supportive of the way the center was coming together. But I just concentrated on my task to create a democratic, all-inclusive place for great plant research and research in general and tried to tune out any negativity. Eventually, I think many people saw that the CEPCEB was inclusive and that it made UCR a well-known biology research institution, but this took more than five years to achieve. I was elected to the National Academy of Sciences (NAS) in 2012. In May 2016, when Julia Bailey-Serres was elected to the National Academy of Sciences, we hosted a fantastic event. As far as I know, this makes the CEPCEB, IIGB and the UCR Department of Botany and Plant Sciences the only institute and/or department in the United States with four women who are members of the National

Academy of Sciences (Susan Wessler, Natasha Raikhel, Xuemei Chen and Julia Bailey-Serres). I also don't want to forget our CEPCEB colleague and friend the NAS member Sean Cutler.

When my laboratory moved to UCR, we continued vacuolar and protein trafficking projects. However, while still at the PRL, I had begun to think about the limitations of genetics for studying the essential processes of plants' endomembrane trafficking systems, including vacuole trafficking, endocytosis, and exocytosis. Endomembrane movement is complicated and dynamic, and we still know little about how plants regulate endocytic and secretory pathways. I saw the merits of using chemicals to perturb these essential processes in plants, as had already been demonstrated with mammalian cells. The small molecules would allow us to bypass the problems of redundancy and lethality, which are common features in plant genomes. We could increase or decrease the concentration of the chemicals, making the phenotype conditional in a way, with the added ability to wash out the chemical and reverse the phenotype. That is the power of chemicals.

I am most grateful to the Botany and Plant Science Department for giving me the Ernst and Helen Leibacher Chair. When I came to UCR in 2001 I wanted to set up a chemical biology platform for my own program and also the CEPCEB. I applied funds allocated by the administration for CEPCEB's inception to establishing microscopy, bioinformatics and later proteomics cores and to updating our Genomics capabilities. However, chemical biology/genomics was a new kid on the block, so-to speak, and I had to first see for myself whether or not the approach would be useful. This is where the Ernst and Helen Leibacher Chair funds became essential since I did not have outside funding for this project. Using this fund, I purchased the first chemical library of two thousand compounds and my lab performed the first chemical screen. Little by little, starting from scratch and making tons of mistakes, we began to learn how to design and conduct chemical screens. From the beginning, I understood that when screening several thousand compounds, it was important to design screens with very simple phenotype readouts. Eventually, we learned how to do this type of work and after we validated these chemical biology screens in our laboratory in collaboration, with the laboratories of Professors Jirı Friml and Eugenia Russinova at Ghent University in Belgium. Several CEPCEB's laboratories started to use the chemical libraries and perform screens, and incredible collaborations developed between biologists, chemists and bioinformaticians and computational people. We were successful in obtaining an IGERT grant based on this chemical genomics platform, insightful and groundbreaking papers were published, and CEPCEB /IIGB and UCR became known in the States and abroad for using and contributing to science with this technology. It does not mean that chemical genomics is CEPCEB's only contribution to the field of plant cell biology: there are many other fields for which CEPCEB is known.

I have been fortunate to be surrounded by strong and caring female scientists both at the PRL and at UCR. I have developed incredible relationships with these women, some of whom remain my closest friends, like my colleagues Professors Gloria Coruzzi (New York University), Joanne Chory (Salk Institute), Susan Wessler (now at UCR), Vicki Chandler (now at Minerva), and June Nasrallah (Cornell University)

and many more. Among my favorite memories is the 1994 Plant Molecular Biology Gordon Conference, where several of us, including the organizer, Vicki Chandler, found that we were, perhaps inadvertently, at the helm. As a comparison, only Gloria and Vicki had been speakers at the previous Plant Molecular Biology meeting. So now the momentum had changed, and colleagues dubbed us "the Power Women" that year. It was a pivotal time and a turning point: amid the laughter, we suddenly realized that it was women who were in charge of most of the sessions and giving the major talks. We recognized that we had a strong voice, and from that time on, we were really a team. We knew that we had had an impact when colleagues would later ask, "Who is going to be the next Power Women group?".

The support of fellow female plant biologists has been invaluable experience and growth as a scientist. Actually, this is now common in our field: We are supportive and thoughtful about our female colleagues and especially support and help develop young scientists in general. When I moved to UCR, I met Professor Betty Lord, who was the chair of the department and was instrumental in my hiring. Later, Betty became my close friend. After she and her husband moved from Riverside to Pasadena, I followed them. I have now gone full circle: from downtown Leningrad when I was young to downtown Old Pasadena now!

3 Science Today and Tomorrow

I have always been a cheerleader and supporter of female scientists and young people in general. But it was when I began to travel to Japan and then China that I found that I could make a true impact, particularly on the science careers of women, providing them with mentorship to reach their potential and being a voice of authority regarding their skill and intellect. As part of the Japan Society for the Promotion of Science's Fellowship for Research that I held in 1996 while on sabbatical in Japan, I encountered many brilliant female scientists who were resigned to work under the auspices of male researchers even though they were just as talented and capable of running their own laboratories. For two of these women, I am proud to say that I helped them to secure laboratories of their own. This prompted me to be bolder as I realized that I could encourage a cultural shift to bring women into the spotlight—not only as scientists in their own right, but also as leaders in the scientific community. After I completed the sabbatical, I began to travel frequently to China to give scientific talks on my laboratory's research. I saw that women made up the majority of the audience at my talks, and yet the scheduled meetings with scientists from the institutions, I visited took place only with men. When I did meet female researchers, it was mostly because they came up to me after talks to ask career questions in addition to scientific ones. Many of them were timid and conveyed the familiar "I am not good enough" sentiment about themselves.

These experiences inspired me to take action. I have worked directly with several universities in China as well as two institutes in Beijing and Shanghai, providing an example and mentoring the female scientists to be more self-confident and to

learn that their opinions and voices matter. I was head of an advisory board of a new joint institute between the John Innes Centre in the United Kingdom and two Chinese Academy of Sciences plant biology institutes located in Beijing and Shanghai, where we are trying to change the culture and incorporate better standards, especially for young researchers. There are many fantastic scientists in China, but the culture within the scientific community there has been slow to evolve and needs to embrace international scientific values: encouraging women and men in science to be bold researchers and to become prominent scientific leaders, and providing mentorship to young researchers to become more open to sharing their information and more independent scientists, rather than using their gifted hands to do only laboratory work. Although there have been many improvements, there is also still much to be done. However, I have encountered only encouragement and support in my efforts, and I have hopes that the culture is slowly changing for the better.

Another, perhaps lofty, project of mine is to foster a bigger and hopefully lifelong appreciation of visual art and music among younger scientists. Music did not turn out to be my profession, but it has been a running theme throughout my life. Music and art have enriched my life and sustained me through divorce, cancer, career stresses, and many other problems. I go to concerts and the opera in Los Angeles and the Metropolitan Opera in New York and Europe frequently and have a baby grand piano at home. At the PRL, I had a requirement for graduate students and postdocs leaving my laboratory. They had to have visited the Art Institute of Chicago at least once before leaving!

I have always felt a need to give back whenever I have found something that works well for me in my career, and I am now trying to do that with music and the arts. One project is to incorporate music into the agendas of science conferences. I have convinced the organizers of a science conference in China to add two 20-min classical Chinese and Western music performances in between scientific sessions. We call these exercises “creative disruptions.” There are many nearby universities with music departments, and we plan to have a chamber orchestra perform for the researchers in attendance. But my grand plan was to bring together scientists and artists from different disciplines—architects, musicians, animators, painters, cell biologists, mathematicians—and have them mingle their ideas. My gut feeling is that these two groups have a lot to learn from each other and will find ways to inspire each other. For example, one of Jonas Salk’s visions for his institute was to have the creation of art and science together. The science is going well, and it is time to focus on art as well!

My career has taken me all over the world, and I am thankful for that. I plan to continue to enjoy my wonderful children and grandchildren and would like to play an active part in their lives. I am traveling and often hiking a lot in the States and all over the world.

With a friend, I annually traveling to two national parks in the United States and one special place abroad. I also visit Italy, annually a country I love deeply and where I have several close friends. I find that scientists are particularly adventurous, seeking to go abroad to study and teach. My career has facilitated interaction with people from many different countries and cultures—scientists who have come through my

laboratory and the universities where I have worked and those I have met through conferences and sabbaticals—and some of them and their families became my friends for life. These friends all over the world have been a happy by-product of being a scientist. At the September 2016 European Network for Plant Endomembrane Research conference in Bordeaux, France, which I have attended every year since its inception, I was presented with a marvelous gift. The illustration, commissioned from a Belgium caricaturist (See Fig. 1), shows me seated at a baby grand piano on various textbooks in English and Russian, with the Golgi apparatus, endoplasmic reticulum, and other cellular components inside the piano; the chemicals my laboratory worked with coming out as music; and three women representing my support of women in science. The female sculpture placed as one of the piano's legs represents my general appreciation of art (I have quite a lot of art at home, and purchased this particular sculpture when visiting Luxembourg).

People wrote very nice things on the back; I was overwhelmed and completely surprised by this gift. At the retirement symposium organized by the IIGB, the scope of the international scientific family and community, I have helped to create was before me. I have mentored 47 postdoctoral fellows and 25 graduate students, some of whom were able to attend the retirement symposium. Seeing so many of them in the same room, along with many of my colleagues and friends from UCR and those who had flown in from different parts of the United States and around the world, was a humbling and gratifying experience.

Fig. 1 My caricature

4 Advice to the Next Generation of Scientists

I think that after reading my story above, you, future scientists, can clearly see my advice. Believe in yourself, find what you are interested in, do not afraid to ask many questions, work very hard and be open, kind and inclusive.

In biology research, when you ask a question, you always end up somewhere unexpected. Biology takes you on its own path and that it why it is so exciting and extremely interesting. My motto has always been: listen to nature. My laboratory never stayed on a true course of trafficking. We have contributed to protein machinery, cell wall biosynthesis, and plant physiology. I like to think that we tried to always be attentive to what nature was showing and teaching us. When my laboratory was at the PRL, we had already made observations that the function of trafficking is not only to deliver molecules from one place to another, but also to act in signal transduction crosstalk, development, and many other important events in the life of a plant. Every time we did a trafficking experiment, we saw interactions with hormones, with flowering, and with other components. We did as much as we could to make sense of the observations but then would always steer back to our main focus of plant cellular trafficking. We used all possible new technologies to answer our questions: we always learned something new! The postdocs would then take these interesting observations, start their own laboratories, and continue the path of unexpected biology findings.

I also would like to encourage young people to seriously consider research in plant sciences. Why plant science and plant cell biology are vital for everybody. People love and enjoy plants but not often remember how essential plants are for the well-being and health of humans and for a sustainable global environment on our planet. All animals including humans use plants in their diets. Most of the world farmland is already in use and currently feeding 7 billion people. However, by the year 2050 our planet will be populated by approximately 9 billion people and it will be necessary to use all available technologies and knowledge about plant growth and development in order to feed such a huge population. Animals, including humans, breathe because marine plants produce around 70–80% of the oxygen on our planet, allowing us to be here! We are clothed and make furniture largely from plants. Plants do not need us for all the above but we definitely need plants! We know a lot about the way plants develop (seeds germinate, seedlings grow and develop, plants flower) and respond and adapt to environmental conditions on a genetic level. We need to know much more, however, about what is happening on a cellular level to proteins in live cells during development or in response to different environmental conditions. If we know how a live cell works, we can think of clever ways to strengthen its "durability" and usefulness. This information is critical to addressing the challenges we face with the increasing worldwide demand for food, global warming, and clean energy production. We need young vibrant people to join our efforts to learn more and more deeply about plant science!

And finally, I would like to encourage young people to learn more about arts and music: this will make your life fuller and much richer.

I hear very often now about what an exceptionally difficult time it is for young people, and I agree that it is not easy. But it has never ever been easy! I hope that reading a part of my story young people see that one must believe in reaching for the stars. I never dreamt of being where I am today, but I always worked hard and loved doing science, working with people and love music and arts- all these events have made my life meaningful and exciting.

Acknowledgements I would like to thank my family, my former students, my postdocs and collaborators, and my colleagues and friends, who have made my life so rich, so full, and so meaningful. Several paragraphs of this piece were reproduced from my article published by Annual Review of Plant Biology with the journal permission.

For those who interested in more information about my life can refer to Natasha Raikhel article "Firmly Planted, Always Moving" published by Annual Review of Plant Biologists, in 2017, vol.68: 1–27

Natasha Raikhel studied the endosomal and vesicular trafficking to vacuoles in the model plant Arabidopsis thaliana. This important understanding of the basic biology of the plant cell endomembrane system can be translated to agriculture, to produce better crop fitness and increased production. She served as editor in chief of Plant Physiology, a major journal in plant sciences, and continue to be very active in several journals. She was the founding director of University of California, Riverside (UCR) Center for Plant Cell Biology and director of UCR's Institute for Integrative Genome Biology. Now retired, she has organized art exhibits and concerts at various European and Chinese scientific conferences and is working with universities and institutions in China, Europe and the USA to mentor and promote young scientists.

A Kaleidoscope of Colours and an Unexpected Journey

Giuliana Rubbia

Abstract Physicist, female, red-haired, with computer science basics learned on the field, from mid'90s I engaged myself in different contexts trying to play a concrete role in supporting knowledge creation. Information dissemination, science communication, research support services and enhancement of human capital, complementary to any investigation, have been my battlefields, with earth sciences being the long lasting application domain. People met by chance or on purpose in Italy and abroad, with different professions in research, as well as local and global challenges, controversial traveling companions, pushed me in a non-linear professional trajectory. I took part in the Information and Communication Technologies revolution, putting users at the centre of the story. Feeling the need for a more positive working environment, I started dealing with gender equality and codes of conduct, stumbling in research integrity. Finally, I landed in the river bed of Responsible Research and Innovation, where the circle closes, and both researchers and citizens share concerns and strive to find solutions. I invite young scientists to keep their uniqueness and take responsibilities as individuals, while hybridizing their disciplines at any time to widen their visions. We are all precious tiles of a big puzzle.

1 Motivations: How I Developed an Interest in Science

It happened by chance. At the very beginning, during the last years of high school, I thought of enrolling in archaeology or philosophy, because reading the story of archaeology by C. W. Ceram arose my enthusiasm as much as the first philosophy lessons about Socrates and Plato. I choose to study physics, instead, because of the fantastic marks I got, surprisingly, and because physics seemed to offer more opportunities to find a job (Fig. 1).

I got these very high marks because at a certain point of the year I started to *really* study the subject. If the teacher would have questioned me earlier I would certainly have taken a very low mark and the story would have been different. But that morning,

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Fig. 1 Happily, relationships between art and science are increasingly taking the scene in science communication discourses. The image is a tribute to the Italian artist and performer Armando Ilacqua (1930–2015) who was my mentor in new media art and whose inspirational words proved restoring in hard times. *Armando Ilacqua, Untitled 2004, private collection, Milano*

I was ready with homework, just back from full immersion in the physics program, and I was late for school due to heavy snowfall in Milan. I arrived in the middle of the second hour, that of gymnastics, which the teacher usefully used to question me about the motion of a sphere along an inclined plane, the interference in optics, and much more. It was 9 on 10, a mark which has been given very rarely, according to the teacher. This success repeated in the year's second quarter, albeit with less surprise.

Therefore, I decided on physics without hesitation, and one summer morning when my parents and I brought the cat to be visited at the veterinary University, I took the chance to hand over the registration forms to the secretariats nearby. Sometime later, a particle physics student told me that during his internship at CERN in Geneva, he had met a person with the same family name. I knew from my parents that he was a cousin from Gorizia. Afterward, in 1984, Carlo Rubbia won the Nobel Prize in physics, a fact that had no major consequences in my life, except to make me feel much more inadequate as a student when I was struggling to pass the exams. For some dozen years I was asked if there was any kinship, which caused me some discomfort, to be honest, since we spent very distant lives, and I would like to be noted for my own achievements, instead of the possible kinship with a …Very Important Scientist. I chose the complementary courses in the electronic-cybernetic field that I found very interesting, systems theory and information theory the most fascinating, and I graduated with a thesis on the interpretation of radar images on precipitation systems.[1]

[1] Mussio et al. (1992).

The late 80s were the years in which letters from companies arrived in your mailbox inviting for interviews, and I received some of them. But somehow, thanks to word-of-mouth among professors, I was sucked into a whirlwind of further post-graduate education. I began a period of scholarships, on a different topic: Computer Assisted Learning in a research program with IBM. Then I continued with an inter-disciplinary Ph.D. in Image Science in Biology and Medicine, the latter with the Image Processing and Interpretation Group of the University of Milano, where I graduated. This period proved very formative. I worked on automatic pattern recognition in digital images, visual user interfaces and human computer interaction, and adsorbed the fundamental notions of data management systems and participatory design.[2] With biologists and physicians in particular, my colleagues and I experimented in the real world what Ladislav Tondl called "progressive semantisation",[3] that is trying to have a dialogue and understand each other, starting from different semantic universes and backgrounds, speaking different languages and jargons. In our conversations in the laboratory, "structure" was the most ambiguous word, that could identify both a set of pixels in a digital image and an arrangement of atoms in an amino acid-chain molecule, for example. At the beginning I felt a bit disoriented, then I solved it, and now I realize that this situation is very common when collaborating with experts in different domains. On the other hand, Ph.D. working conditions have been stressful. Although we were passionate and motivated, we were under constant pressure and some of us felt uncomfortable. Once completed the four years doctorate, thanks to a friend, a researcher himself that introduced me, I got my first, fixed-term, contract. I proudly swiped the badge at the Institute for Multimedia Information Technologies—ITIM at the National Council of Italy—CNR: it was the mid-90s, and the project was that of the National Group for Protection against Earthquakes—GNDT, to disseminate seismicity data and earthquake catalogues of Italian area. In the following years, both ITIM-CNR and GNDT were disbanded and people moved into other research organizations.

I was invited to join the Seismic Risk Research Institute IRRS-CNR, which in turn became part of the National Institute of Geophysics and Volcanology (INGV)[4] at the beginning of the new millennium, where I got a permanent position in 2001 as a technologist, the first female technologist to be hired at INGV—Milano, where I still work. Please note that according to the current labour contract of Italian public research organization,[5] people involved in research activities are in fact recruited as "researchers" or "technologists". The former is devoted to knowledge advancement, the latter to technologies that support such advancement. As I am writing, a new contract is being negotiated.

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[2] Bianchi et al. (1996).

[3] Tondl (1981).

[4] Istituto Nazionale di Geofisica e Vulcanologia, www.ingv.it Accessed 30 Jun 2022.

[5] Contratto Nazionale Collettivo del Lavoro del comparto Istruzione e Ricerca 2016–2018 (Labour Agreement—Public schools and research organizations) https://bit.ly/3HmvLgT Accessed 30 Jun 2022.

2 Work Done: My Personal Scientific Approach

A LEAP FORWARD**.** As a matter of fact, when I started working, I found myself doing something totally new, for which I was not prepared in detail, and on which I lavished all my enthusiasm.

During the seismic crisis of September–October 1997 in Central Italy, my working group and I hurriedly set up a website dedicated to the earthquakes occurring in Umbria and Marche regions, to supply information and preliminary results of the activities and investigations carried out by GNDT's researchers and technicians.[6] The September 26, 1997 Umbrian-Marche Apennines earthquake represents the *first* Italian large seismic event on the occasion of which the Internet was intensively exploited to exchange and disseminate data, information and news[7] to a broader audience. In those years, in fact, the Internet was still in its infancy in Italy. There were no templates to refer to, unless reports provided by the Geological Services of the United States—USGS. "Il primo terremoto sulla grande rete", as a journalist titled his article in a newspaper, the day after, was the experience with which I had the baptism of fire. Later on, I did have the privilege of designing the first INGV institutional web portal and co-coordinating its development for several years, until the L'Aquila earthquake, in 2009.

In the middle of my career, I attended a master, of which I heard about accidentally. It was run by the MIP School of Politecnico di Milano,[8] dealing with Open Innovation and Knowledge Transfer. The course would be paid by my institution, according to a funded professional development plan, with a programme complementary to my expertise. What a big opportunity! Indeed, both this course and the project work about research support services[9] allowed me to get in touch with colleagues and teachers of different backgrounds and turned my energies to other fields of activity. Soon after that, I moved from Milan to Rome, at the central premises of INGV, where I joined the Central Administration and did have the opportunity to collaborate with several units during times, such as the science outreach lab, the disciplinary office, and the grant office.

From 2010, worth noting was the experience with INGV's Guarantee Committee for Equal Opportunities, Employee Wellbeing and Non-Discrimination at Work (CUG), in its first edition. According to Italian laws,[10] the creation of such a committee is mandatory in public structures; each CUG has several duties towards the hosting organization, such as making proposals, providing advices, and monitoring progress to develop equal opportunities, enhance well-being in the workplace and fight discrimination of any type.

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[6] Padula and Rubbia Rinaldi (1999).

[7] Rubbia and Camassi (2008).

[8] Today, Polimi Graduate School of Management https://www.som.polimi.it/en/ Accessed Jun 30 2022.

[9] Rubbia et al. (2014).

[10] In accordance with provisions of article 21 Law no. 183/2010 https://bit.ly/3Hhigz2 modified by government directive no. 2/2019 https://bit.ly/3Ofndef Accessed 30 Jun 2022.

Such experience regarding human capital brought many challenges: it was carried soon after the instituting law came out, thus there were no examples from which to draw inspiration; the mandate was broad but resources and know -how were limited at that time; a free university course in equal opportunities and networking with other committees resulted providential. Women scientists' associations, such as the Italian Women and Science, Donne e Scienza,[11] and the European Platform of Women Scientists (EPWS),[12] turned out to be the right places where to learn and contribute, in particular regarding gender issues.

It has been a very formative and exciting period, although proved frustrating for some sections of the route. The committee in fact coped with relevant topics such as prejudices, discrimination, and harassment, to mention a few. We established collaborations with counselors and other committees, wrote the first code of conduct against physical and psychological harassment while organizing seminars about equal opportunities. It has been hard work, which is still ongoing, the biggest problem being the dominant culture. The urgent request for Gender Equality Plans made by European Commission[13] as a requirement to fund research programmes in EU countries' public bodies, including higher education institutions and research organizations, should help a lot.

In a word, what has pushed me forward most of the time has been the desire to contribute and innovate, to do something that has not been done before. What arrogance of thought! (Good) ideas alone are not enough, an effective organizational structure and good support are needed!

During my *life in research*, I played several roles, tackled new positions from scratch, worked with people with different expertise and backgrounds, belonging to both research and administrative staff, and external consultants as well: biologists and physicians, seismologists, geologists, social scientists, IT managers, project managers, EC officers, communication officers, lawyers, and academic developers. These encounters prompted me to constantly change focus and enriched me with both human and professional experiences.

3 Science Today and Tomorrow

We have witnessed a great evolution in science, which has become tremendously social, with the increasing urgency to get out of the ivory tower and rethink science communication, engaging society more closely.

[11] Donne e Scienza, Italian Women and Science Association, www.donnescienza.it Accessed 30 Jun 2022.

[12] European Platform of Women Scientists, www.epws.org Accessed 30 Jun 2022.

[13] European Commission, Directorate-General for Research and Innovation (2021). Horizon Europe guidance on gender equality plans https://data.europa.eu/doi/10.2777/876509 Accessed 30 Jun 2022.

Earthquake science in particular is progressing both in technology and in its relationship with society. In the last 25 years, acquisition, processing, and communication systems have undergone a great evolution. Regarding the Italian area, the 2009 April 6 L'Aquila earthquake kicked off communication via social channels,[14] with enrichments and refinements added on the occasion of other large events, such as Pianura Padana 2012[15] and Central Italy 2016,[16] and constantly upgraded. After the December 26, 2004 Sumatra, Indonesia earthquake and the strong March 11, 2011 Japan earthquake, which caused a devastating tsunami and the incident of the nuclear plant in Tohoku, tsunamis began to be studied more systematically worldwide.[17] The Italian Tsunami Alert Center (CAT-INGV) has been established, and begun its monitoring activities on strong earthquakes and sea level variations in the Mediterranean Sea, assuming the role of National Tsunami Warning Center for Italy.[18] Tsunami risk perception still remains low among the population,[19] and further research is necessary to improve the whole management cycle: "risk communication needs to move on from traditional one-way communication models (e.g., scientists communicate the scientific results to the society) to models that envision constant dialogue and active participation of all the societal actors",[20] a recommendation that goes beyond this phenomenon and has a general validity.

Bucchi and Trench (2016) compiled a ten keywords glossary aimed at making more transparent the terms used in science communication and science in society.[21] They highlight the paradigm shift in science-society relations of the last decades: from "communication" to "dialogue", from "public awareness of science" to "citizen engagement". If it is true that audiences have information gaps, the approach should not be that of filling these gaps with a one-direction approach (the so-called deficit model) but to work on what people know and what their questions and concerns are. From the initial efforts in science popularization, we passed to a more structured, socalled "third mission" activities.

Regarding Equal Opportunities, much has been done, but still, there is a large room for improvement. Since 2022 organisations applying to any part of the funding programme Horizon Europe are required to have a Plan aiming at Gender Equality (GEP), covering the following areas: (a) Work-life balance and organisational culture; (b) Gender balance in leadership and decision-making; (c) Gender equality in recruitment and career progression; (d) Integration of the gender dimension into research and teaching content; (e) Measures against gender-based violence, including sexual harassment. The Guidance provides organizations with precious suggestions to cope with all these aspects. But, so what at the individual level?

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[14] Amato et al. (2012).

[15] Pignone et al. (2012a).

[16] Pignone et al. (2012b).

[17] Lorito et al. (2021).

[18] Amato et al. (2021).

[19] Cerase et al. (2019).

[20] Rafliana et al. (2022).

[21] Bucchi and Trench (2016).

Harassment in particular is a delicate subject, a troublesome issue both for those who experience it individually and for institutions that must deal with it. A female researcher's testimony published anonymously in Nature in 2016[22] depicted very well the problem in its contingency and consequences in career development. After the explosion of the #me-too movement, gender-based violence and sexual harassment fully entered public discourses in different contexts, being these topics addressed more systematically in academia, which is not free from these harmful and unwanted behaviours. In Italy, sanctioning procedures are stated in the labour contract, thus they should be known and applicable. Troubles persist both in denouncing on one side and in minimizing on the other. But micro-aggressions and downsizing the problem do damage.[23] The situation is similar over countries[24] and there is still the need "to establish a culture of zero tolerance toward sexual harassment and violence".[25] Changes will require time. In my opinion, beyond training and awareness initiatives, concrete support from peers and top management would make the difference for the community.

4 Advice to the Next Generation of Scientists

STRUGGLE FOR YOUR DREAMS AND BE AGENTS OF CHANGE. If we look at the European Charter for Researchers and the Code of Conduct for Recruitment,[26] and particularly at those principles addressed to researchers, we read that "researchers should" do this and that. When I became aware of the Charter, I found these suggestions very inspirational and therefore quote partially hereafter. The Charter, conceived in 2005, provides guidelines that are still appropriate after fifteen years, although a review is ongoing, to revamp it.[27]

Beyond the principle of research freedom, researchers should "adhere to the recognised ethical practices appropriate to their discipline …, make every effort to ensure that their research is relevant to society …, avoid plagiarism …, be familiar with the strategic goals governing their research environment and funding mechanisms …, be familiar with the national, sectoral or institutional regulations governing training and/or working conditions …, be aware that they are accountable towards their employers, funders or other related public or private bodies as well as, on

[22] Sexual harassment must not be kept under wraps. Nature 529, 257 (2016). https://doi.org/10.1038/529257a.

[23] Fajmonová et al. (2021).

[24] Avveduto et al. (2019).

[25] European Commission, Directorate-General for Research and Innovation, Horizon Europe guidance on gender equality plans (2021) https://data.europa.eu/doi/10.2777/876509.

[26] The European Charter & Code for researchers: https://euraxess.ec.europa.eu/jobs/charter-code-researchers Accessed 30 Jun 2022.

[27] Review of the European Charter and Code of Conduct for the recruitment of researchers https://cdn2.euraxess.org/sites/default/files/policy_library/ttf_goal_2_results_v1.0.pdf Accessed 30 Jun 2022.

more ethical grounds, towards society as a whole, …adopt safe working practices …, ensure that the results of their research are disseminated and exploited …, their research activities are made known to society at large in such a way that they can be understood by non-specialists …, establish a structured and regular relationship with their supervisor …, seek to continually improve themselves by regularly updating and expanding their skills and competencies …".

In other words: be aware of rules, and make sure you know the rules of your working environment, either to follow them or to champion changing them.

Passion and perseverance are obviously top keywords. During the thesis period, two supervisors accompanied me: one was a male physicist, and one was a female engineer, from Poland. If I look back, I think that she has been very inspirational to me, implicitly suggesting that there were no barriers for a woman in hard sciences, nor the need to disguise as a man. To advise young women scientists, in particular, I borrow the words of distinguished professors recently interviewed by the European Platform of Women Scientists: "be brave and follow your heart. We are all afraid at times—scared to make a big move or try something new. But don't let that stop you from achieving your dreams, please don't aim low because of fears of failing. Take advantage of opportunities, even if they make you feel nervous. Take a deep breath and go for it!",[28] "be fully yourselves, avoid adopting typically male models, and just totally follow your curiosity, and passion. You should be proud of being women scientists",[29] "discuss with women's scientist peers the experiences that you have in your career. Ask questions about what surprises you or strikes you as questionable. Learn about the impact of gender on perceptions of women and men, their qualities and behaviors. Celebrate your successes, learn from your mistakes, try to keep your curiosity".[30]

I would add some suggestions, capitalizing on my experience.

Volunteer for your institution, your commitment matters. In my opinion, it would be very rewarding to serve as a member or representative on a committee or board. You can become closer to those mechanisms governing your institution, learn and contribute. In my institute, coordinating activities or serving in dedicated working groups have been valuable experiences for me. Coordinating a project at national level made me aware of different situations, that were unconceivable if I would have worked only from a local perspective. When I started reporting to a governing body, the so-called Public Function Department, I familiarized myself even more with forms and regulations. The same happened collaborating in the working groups for the drafting of personnel regulations and code of ethics. Volunteer as well for reviewing and partaking in expert groups. This would make you more aware of the state of the art and widen your perspective.

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[28] EPWS interview to Prof. Karen Vousden, Francis Crick Institute in London, April 2022. https://epws.org/woman-scientist-karen-vousden/ Accessed 30 Jun 2022.

[29] EPWS interview to Prof. Silvana Badaloni, University of Padua, March 2019. https://epws.org/woman-scientist-silvana-badaloni/ Accessed 30 Jun 2022.

[30] EPWS interview to Prof. Mineke Bosh, University of Groningen, November 2019. https://epws.org/woman-scientist-mineke-bosch/ Accessed 30 Jun 2022.

Apply gender lens and commit yourselves to be agents of change, you will improve the quality and societal impact of your research. While analysis of participation of women in science is already underway, and positive actions are in place or foreseen, integration of variables such as sex and gender in questions, methods and impact of research, which implies new perspectives and approaches, is still to be structured. In many areas, gender knowledge still needs to be created. In particular, natural hazards "are gender-neutral. But impacts are not".[31] There are differences in hazard perceptions, health effects, exposure to additional risks in the occasion of events…. Studies at national levels, for example, show that, although gender has been recognised as an important factor, "current Disaster Risk Reduction policies and practices do little to challenge existing, unequal social and institutional structures; instead, they accommodate the gender status quo",[32] "the main factors of gendered disaster vulnerability are inadequate disaster education, a lack of protection measures, and cultural issues".[33] There is room for improvement in targeted information and communication, citizen science contributions, disaggregated data collections. In recent literature, several studies start from the concept of women's vulnerability during an event, to arrive at that of resilience, reconsidering the event as an opportunity for women to be not only victims, but agents of change, and consequently to improve conditions for both women and men.[34] Engendering science matters.

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[31] Erman et al. (2021).

[32] Yadav et al. (2021).

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Giuliana Rubbia Graduated in Physics at the University of Milan, Italy, in 1989, I have embarked on a non-linear career path with a strong inter-sectoral mobility. After a Ph.D. in biomedical images interpretation and a fellowship in Computer Aided Learning, I continued with Information and Communication Technology at Italian National Research Council (CNR) and later at National Institute of Geophysics and Volcanology (INGV). I co-developed first on line seismology databanks of Italian area and served as president of Committee for Well-being and Non Discrimination in its first edition. Joining Central Administration in Rome, I widened my experiences in supporting knowledge creation and research policies, including science and society activities, human resources excellence, ethics and gender equality. Senior Technologist, now at INGV Milan.

Biased Random Walks on the Scientific Landscape

Jack A. Tuszynski

Abstract In the middle of World War II while in exile at Trinity College, Dublin, the famous Nobel-Prize winning Austrian physicist Erwin Schroedinger wrote a book called "What is Life?", which affected several generations of biophysicists although he himself was not trained in biology but was a genius who grasped the main questions in all of science. He didn't answer the question posed in the title of the book but opened an area of future investigations by just pointing to some key open issues in biology seen through the lens of physics. Biology cannot be explained by physics from its first principles but it must not be in contradiction with the laws of physics. The dialogue between these two vast areas of science has been beneficial to both. It has allowed biology to use sophisticated mathematical and experimental tools of physics and, conversely, biology challenged physics to address the issues of complexity, hierarchical organization and coordination of heterogenous parts into a unified whole which differs from the simplicity of physical systems. I was trained as a physicist but at a mid-career point embraced the challenges posed by the complex biological systems and tried to advance the various aspects of biophysics using my training as a backbone on which new ideas about living systems can be developed. While the transition from physics to the life sciences took me out of my comfort zone, I never regretted taking this leap into the unknown territory. An even more uncomfortable and risky step took me to study consciousness, a field which grapples with the definition of its object of interest: the human mind. While I now consider myself a full-fledged member of the life sciences (and physical sciences) community, consciousness studies is still an area where I feel more of an amateur than a professional. As I now approach retirement, what has been my hobby over the past 30 years or so may become my main activity. In the short essay below I briefly describe my adventures with science and share my thoughts with the reader about what it is and what it can or should be.

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1 Motivations: How I Developed an Interest in Science

Science and academia have been my constant intellectual home and, at times, a refuge from the turbulence of life in general and politics in particular. I grew up in an atmosphere of great respect for science and scientists. It was a different era though. My early heroes and role models were quite naturally Sir Isaac Newton, Albert Einstein, Lev Landau and Richard Feynmann, since I'm a physicist by training, but also such mathematicians as Stefan Banach and Luigi Lagrange, Carl Friedrich Gauss and Leonhard Euler. Outside physics and mathematics I didn't have any real heroes. I held an overly idealized view of science seeing it as a pure pursuit of the truth about the nature of the universe in which we live. Also, science was for me an intellectual refuge, an oasis of tranquility away from the turmoil of politics and social upheaval. I grew up in socialist Poland at a time that to be successful in almost any field other than science, one had to "pledge allegiance" to the socialist party. This was a Faustian bargain and I wanted no part in it. This was also partly the reason why I left Poland for Canada shortly after graduating in physics. However, my first "love" was mathematics because it was so elegant and objectively beautiful as an intellectual edifice which is least affected by human biases and corruption. Maybe it even exists on its own as a set of Platonic ideals? A little later on I moved, somewhat reluctantly, to physics, at first theoretical physics due to its closeness to mathematics. This move was due to my early successes in physics compared to mathematics, which was my ambition but I didn't have sufficient mathematical talent. I wasn't cut out to be a mathematician, as I realized later on. Physics has a different appeal to me compared to the allure of mathematics. It teaches us how all things in the world work and not only that, but more importantly, it uncovers universal laws that govern physical matter and its processes. Its amazing ability to generalize empirical observations and derive laws of nature using the scientific method has given all of us the technological achievements that afford the unprecedented quality of life we enjoy today. Only much later in my life as a scientist did I develop deep appreciation for other fields of science, especially biology and chemistry, which I completely ignored as a student. Physics, being often referred to as the queen of science endows its practitioners with a somewhat undeserved arrogance and hubris. Physicists have a tendency to view the rest of science, especially biology, as "stamp collecting" as attributed to Sir Ernest Rutherford. This attitude has been enforced by the amazing feats of human insights into the nature of things achieved by the theoretical physicists who provided the most fundamental understanding of how the universe works. These were the true geniuses who developed quantum mechanics, namely Max Planck, Erwin Schroedinger, Albert Einstein, Werner Heisenberg, Paul Dirac, Wolfgang Pauli and others. When I studied physics as an undergraduate, I dreamed of becoming one day a scientist like these intellectual giants. Alas, this area of physics had been more or less completed when I entered into science. I then got fascinated by a topic which was "hot" at the time, namely phase transitions and critical phenomena. As an aspiring adept of science, I attended my first conference

and met some of the key practitioners of this field. I remember talking to the then-Soviet physicist Alexander Patashinski (who with Valery Pokrovsky had just missed getting a Nobel Prize for the discovery of the renormalization group theory, which was instead credited solely to Kenneth Wilson). My enthusiasm was dampened when he told me that "this dinner had already been eaten", meaning the train has left the station and it's time to move on.

2 Work Done: My Personal Scientific Approach

This didn't deter me from trying to find new and important aspects of critical phenomena and this helped me in subsequent decades understand systems outside physics that undergo bifurcations and phase transitions. They include biological, social and political phenomena. I contributed modestly to this field of physics by introducing descriptions of non-Gaussian fluctuations and nonlinear coherent phenomena. I even wrote a scholarly book on these topics, which took five years to complete, was published by Oxford University Press and sold around 300 copies, being a commercial flop. I am very proud of it and wouldn't change a word or an equation in this monograph.

This period also brought me back for a few years to my first love, namely mathematics, because I needed to learn how to solve nonlinear differential equations and analyze chaotic dynamics and fractal processes. I truly enjoyed this period of my career because my mind was expanding exponentially in various directions including biology and chemistry. I also engaged for the first time in interdisciplinary collaboration, with first-class mathematicians such as Pavel Winternitz, Michal Grundland and Peter Clarkson from whom I learned a huge amount of mathematical knowledge that propelled my research in physics for many years thereafter. Later on, crossing disciplinary boundaries has become one of my greatest joys and gave me great satisfaction related to being involved in an uninhibited discovery process. In fact, at about the same time, when I was developing my scientific credentials, I started noticing a less pleasant aspect of working in physics, namely fierce competition, which sometimes borders on pathology. At, what I thought was the peak of my career, when I was promoted to full professor at only 35 years of age, my idealistic perception of the society of scientists as people dedicated to the truth and nothing but the truth, was dealt a brutal kick in the groin. My enthusiastic and unbounded thrust into the newly created field of high temperature superconductivity was met with scorn, disdain and dismissal. As a result, I lost all my research funding and seriously contemplated quitting science as I perceived myself to be an intellectual pariah due to the criticism I received from anonymous reviewers. This, however, turned out to be in the end the best thing that ever happened to me as a researcher. To change the scenery, I took a sabbatical leave and spent over a year visiting a number of major European labs having chosen my base in Duesseldorf, Germany with close proximity to many research centers in Germany, France, Belgium, the Netherlands and the UK. My

ego and my wallet were slightly boosted by the award of a von Humboldt fellowship that allowed me both the access and funds to visit some of the best places on the European research map such as Heidelberg, Oxford, Paris, Brussels and Copenhagen. Most importantly, I found a new groove, which was the just emerging field of computational biophysics. This was to become the bane of my scientific existence for the next two decades and a catalyst for subsequent changes down the road. I returned to Canada energized and excited to start a new research area in my home department, the Department of Physics at the University of Alberta. Simultaneously, another interest has been steadily growing in my mind, in a completely unplanned and unintended manner.

In 1991 I was asked to co-organize a conference in Arizona, which was entitled: "What is Life". This was a momentous occasion for at least two reasons. First, it led me to an amazing community of interdisciplinary scientists focused on the understanding of life processes through the lens of physics and chemistry. I met some of the most brilliant scientist of that era such as Alwyn Scott, Arthur Winfree, Steen Rasmussen, Christopher Langton and others. The conference introduced me to such newly forming concepts as artificial life, nanotechnology, protein assembly and what was to become my fascination for the next two decades: microtubules. The second reason for this event to become transformative for me occurred at the conclusion of the conference with Stuart Hameroff's challenge to embark on a common quest to understand consciousness as a physical phenomenon. While I was a reluctant player in this nebulous field of consciousness studies (because of my straight-laced attitude as a mainstream physicist), the positive feedback I kept receiving for my work in this new field, compared to the disappointments encountered in condensed matter physics, kept me involved for much longer than I ever thought. The next twist in the direction of my research came also out of the blue. I was by now yearning for interactions with the broader community of life sciences researchers since my background in biology and biochemistry was very modest to nonexistent then. My home department at that time had no other professors interested in anything vaguely related to biology, so I felt like a stranger in my own "country". By chance, an endowed chair in oncology was established when I was giving courses on biophysics on a voluntary basis to students in the faculty of medicine. Since my research was by then strongly focused on microtubules, which are instrumental in cell division and hence provide a strong connection to cancer, I successfully applied for this position. The next 15 years were a dream come true for me as I was able to unleash my creativity and receive funding for my research at levels several times greater compared to the highest levels before this transition. My only objective and self-applied pressure were to make a difference in the fight against cancer. That became my new mission and a major challenge. Without going into details, I had to reinvent myself as a scientist in order to become a singularly pragmatic, goal-oriented and disciplined strategist, fundraiser, empirical thinker and a tireless promoter of the cause. The fact that I was released from teaching duties for 15 years helped me achieve the goal of finding a promising drug which is now in clinical trials against metastatic bladder cancer. This was made possible by funding from various agencies, including charitable donations and most importantly, the financial support of the Allard Foundation. This privately-funded organization

has been incredibly generous and without its support none of my work in the 15 year-period would have been possible. Personal involvement and generosity of Mr. Chuck Allard have been instrumental in bringing my project to completion. Having worked both in North America and Europe, I can confidently say that the North American culture of private support for public science is much more advanced than in Europe.

3 Science Today and Tomorrow

THE EVOLVING NATURE OF SCIENCE. In the first two decades of 21st century we have been witnessing a tectonic shift in the way modern society functions. Economic globalization has led to internationalized competition in all aspects of our lives. No sector of the economy is immune to the process of internationalization. Information technology, especially machine learning, is becoming a major force transforming everything we do. Our modern society increasingly depends on advanced technologies and our economy is becoming information-based and knowledge-driven. Newly emerging international leaders in the latter areas are increasingly coming from countries that invested heavily in restructuring and modernization of their research and development areas. Embracing this trend has often propelled these, one-time sleepy backwater places, to pole positions in the global race for the share of the world's GDP. Many examples can be cited here but suffice it here to mention such countries as Singapore, Hong Kong, South Korea, Switzerland, Israel or Luxembourg. A result of this situation is a somewhat regrettable outflow (brain drain) of many young, well-educated budding scientists or students who flock to places, which provide not only better income potential but, most importantly, greater opportunity to succeed on the global stage.

Translation of academic knowledge is seldom easy and not always possible. Often, it takes a very long time and sometimes forever. For example, quantum mechanics was discovered more than a century ago but it took several decades to apply it to such areas as electronics, laser technology or MRI imaging. Nonetheless, those who foresee the applicability of a new scientific result into practical uses, tend to reap enormous profits and gain superstar reputation. This reputation, more than anything else, acts like a magnet to attract more like-minded creative people. California's Silicon Valley is proof positive of this effect. Particular examples include the search engine Google, developed by the then two graduate students at Stanford Sergey Brin and Larry Page. Likewise, the multibillion-dollar company Facebook was nucleated by Mark Zuckerberg and several of his fellow undergraduate students at Harvard. The Weizmann Institute in Israel boasts generating several billion dollars in revenue from licensing a medicinal compound that became a blockbuster drug for multiple sclerosis and propelled the Israeli pharma company TEVA, which licensed it, to become the largest generic drug manufacturer in the world. In many instances a single successful effort underwrites the cost of many years of failed trial and error by many! How does one anticipate such amazing windfalls? The simplest answer is by being always aware

of the potential to turn a scientific discovery into a profitable technological invention and by working in an environment that constantly reminds one of such possibilities. EMBL in Heidelberg employs a small but nimble unit of technology transfer officers who, on a daily basis, talk to researchers there about their latest discoveries in order to sense if there is an opportunity to commercialize them. I had several other opportunities to see examples of deliberately promoting research commercialization that works. The tiny Grand Duchy of Luxembourg boasts the highest GDP in Europe with virtually no natural resources to draw on. It was a poor country some 200 years ago when it relied mainly on agriculture. A century later it transformed itself into a coal-and-steel industry leader. In the last few decades Luxembourg has become Europe's banking hub. It has now started a new transformation into the information technology age. The relatively small but extremely affluent country of Switzerland devotes a very high percentage of its GDP to research and development (R&D). South Korea tops this list and has become a technological powerhouse. After World War II, South Korea's GDP was comparable to that of Kenya. Today it is on par with Austria. Switzerland (just below Sweden) is a country with no natural resources but has the highest percentage of Nobel Prizes scaled to the country's population. Switzerland has created a technology transfer organization called CTI (or KTI in German) that matches small and medium size companies with academic research groups and supports generously their joint projects. It also incubated about 200 start-ups with a greater than 90% success rate for profitability. Germany has been an industrial giant for a few centuries. However, it has not rested on its laurels. Germany provides billions of euros for international research collaboration with German scientists through the Alexander von Humboldt Foundation and academic exchanges for students and scientists from around the world through the German Academic Exchange Service (DAAD). Germany's support for industry-academia cooperation is best exemplified by the Fraunhofer Foundation, which covers 50% of the cost of joint projects, mostly industry-generated. I visited an amazing research commercialization incubator placed in Munich and called "Biocenter LMU". It spawned almost two hundred clinical trials for the medicinal chemistry molecules created in the Munich area, which is unprecedented for academic research and received several billion euros of direct industrial funding as a result of semi-annual meetings between representatives of the German industry and researchers from the Munich area. In Canada, MITACS is a network of centres of excellence that provides hundreds of industrial internships annually for graduate students and PDFs in mathematics, computer science and physics. This has become a global initiative with hundreds of Canadian and foreign students working on mathematical and computational solutions to problems of practical importance to the private sector. This has become a runaway success that helps students become competitive in the marketplace and makes Canadian industrial sector internationally competitive. England's venerable Oxford University (oldest in the UK but younger than Bologna) invented a framework called the Oxford Problem Solving Workshop where private sector companies or public institutions fund intense, typically one-week long, brain storming sessions during which graduate students under the guidance of their professors crack tough problems proposed by industrial sponsors. All parties concerned benefit as a result.

Can any of these examples be successfully implemented elsewhere? I strongly believe so. How? These types of efforts are usually made gradually but persistently and with careful leadership. In the next part of this document, I'll discuss some ways and means of a radical transformation aimed at not only reversing the brain drain of talent from one country but bringing international expertise and youthful energy to rejuvenate its academic sector.

I visited many of the places where research translation has been a very important aspect of their function. I tried to learn from these examples and implement them whenever possible. My personal learning curve was never steeper than during the period of my life when I worked in oncology. This is a quintessentially interdisciplinary field which links basic and applied sciences with medicine, technology and also pharmaceutical industry. I also became much more appreciative of the work done by legions of cell biologists, oncologists, pharmacologists and geneticists. While they don't solve complicated nonlinear differential equations challenging the intellectual prowess of a mathematical physicist, the complexity of living systems such as cancer cells is mind boggling. Understanding this level of complexity requires a different skill set than the one needed to succeed in quantum physics. Naturally, I became quite humbled by the enormity of the problems faced in oncology and other fields in the life sciences area. At the time of writing this piece, I'm back in physics trying to find a new equilibrium following another "phase transition" in my life as a scientist. This is unexpectedly challenging as I'm no longer imbued with the same sense of mission that guided me through the period spent in oncology. Time will tell what happens next. I am open to exploring new horizons in science and beyond. The meanderings on the scientific landscape that I described above taught me to expect the unexpected. As somebody wisely stated: "when man makes plans, God is laughing". Almost everything of significance in my scientific career happened (seemingly) by accident, but apparently, according to another wise person, there are no accidents. This then would be my main piece of advice to young scientists: "be open to change, be prepared to do things you were never trained to do before and enjoy the voyage of discovery".

I will end this short essay with some observations about how science has evolved over the past few decades that I've experienced first-hand. At the start of my career, the emphasis was on old-fashioned scholarship: thinking a lot, teaching well and publishing your work in solid-reputation journals. Life was fairly simple. We had support staff, secretaries and motivated students. Today, the job description has changed a lot from the olden days. The university professor has become a small-business manager. It is now required to be constantly hustling for funds, administering these resources, which requires business and accounting acumen, publishing in "prestigious" journals and teaching in such a way that students give very favorable evaluations. One has to be a marketer and a PR expert because we need to "sell" our products, which is now more and more commonly referred to as IP (intellectual property). Those scientists who build the largest groups, receive the biggest funds from the most prestigious granting agencies and publish in the top-tiered journals. They are the modern day "heroes" of the scientific community. Somehow, I feel that science has entered into a Faustian bargain with those who run this enterprise, i.e.

the administrators and the politicians. Ideals and visions are gone. Intellectual giants such as those I named above are nowhere to be found. I'm worried that in today's science there is no room for another Einstein, Newton or Dirac. Science has become big business at the service of its paymaster. I don't want to end on a sour note, so let me say what I think are some positive aspects of the new way science is done. Only about 20% of the PhD graduates end up staying in the academia, the rest find jobs in the industry and the public sector or create their own companies. This is a very good sign as highly educated young people who understand how science is done enter into other sectors and elevate their quality standards. Secondly, being practical and goal-oriented is also desirable. What we need is a healthy balance of unfettered creativity and rigorous methodology. We need to allow some brilliant scientists to be unencumbered by the strait-jackets of granting agencies. As the famous philosopher/financial expert/flaneur Nassim Taleb aptly stated, most major transformative technological advances occur as black swan events, i.e. completely unforeseen. Hence, even the most enlightened calls by the most sophisticated granting agencies cannot predict those discoveries. We should therefore, accord some of the top thinkers the creative freedom they need and deserve.

It may be premature to identify precisely the areas of science, which should be at the centre of the next major efforts to discover and innovate because this is very much context-dependent, both from the point of view of timing and geography. Nonetheless, several fields are currently undergoing explosive growth in this new knowledge-based economy and they should be seriously considered. These could include artificial intelligence, robotics, big data analytics, nanotechnology, precision medicine, nano-pharmacology, diagnostic imaging, nano-neuroscience, evolvable biocomputing, quantum biology, biomimetics, sustainability, etc. Importantly, this should be built not only on the existing strengths of a particular place but also with a competitive assessment of the global landscape. Also, it should be stressed that the idea is to generate innovation and research translation based on basic science excellence and not necessarily applied science as the starting point, as has been traditionally the case. While top-down direction in applied research should not be abandoned, I believe emphasis should typically be placed on the opposite direction: bottom-up, i.e. from new fundamental ideas to their commercial applications. EPFL in Lausanne, Switzerland is a very young academic institution that started its existence as a thoroughly average technical university. It joined the ranks of elite universities such as Princeton and Heidelberg when a newly appointed President decided to focus its mission only on the areas where EPFL can compete with the best in the world. Today, EPFL is consistently ranked in the top 50 or even top 20 in the various world university rankings. Initiative and creativity of the researchers, irrespective of their age and position should be rewarded. Collaborations within the institute and with other organizations should be encouraged, of course, paying careful attention to IP issues. Below, I only list in bullet form some ideas for a range of activities that should be included within the framework of a successful creativity-driven center for research translation:

- Recruit small and medium-size companies to participate in joint research translation projects
- Provide entrepreneurship training for graduate students and PDFs
- Form consortia with local companies for cutting edge research programs
- Start industrial problem-solving workshops to attract the attention of companies
- Expand the framework of trainee internships in the industry taking MITACS as a successful example
- Promote the region as a hub for innovation and develop a reputation for unbridled creative freedom
- Recruit talented graduate students irrespective of their country of origin on a much larger scale; fund entrance scholarships for the best and brightest no matter where they come from.
- Promote the flow of cutting-edge technology projects applied to solve industrial problems, which require collaboration among departments and an industrial partner. This would stimulate both internal cooperation and an open-minded vision in technology development.
- Lobby the government to provide tax incentives to private companies and individuals for supporting academic research, funding endowed chairs, establishing scholarships, awards for excellence, etc.
- Provide support for investigator-initiated international research collaboration, especially in areas of commercialization with countries that have strong programs
- Form consortia with local and foreign research institutions.
- Establish prizes for the best spin-off, best innovative idea, best patent, etc.

Another important point to stress is multi-disciplinarity of the approaches to be promoted and a visionary aspect of at least a fraction of the projects to be funded with a mix of low- and high-risk projects to maintain profitability in the long term. In a nutshell, working in such a creative environment should become a dream come true for the most ambitious and motivated young minds and would emulate the "cool" and trendy environment of California's Google research center.

As Malcolm Gladwell aptly stated in his book "The Tipping Point", a tipping point can only occur if there is a small but highly dedicated group of people that are willing to make a difference. Therefore, it is imperative to recruit a small number of influential figures in local politics, industry, main-stream media (for public relations effects) and academia. However, based on my experience, many academics are rather conservative in their attitudes and only those who are not complacent or comfortable with the system should be invited to strategize on this issue.

4 Concluding Remarks and Some Advice

Magic happens when we move from day-dreaming and active imagination to action. Nike's motto should serve as our inspiration and hence we should "**just do it**". The beautiful city of Florence waited patiently from 1296 until in 1436 to see the

completion of the construction of the cupola of the Duomo. At the time, Florence did not have the necessary funds available for Filippo Brunelleschi's work but Cosimo de' Medici believed in the cause and was convinced that the needed money would be eventually found. Money was indeed found, in spades one might add. Today, Florence's Duomo is admired as one of the greatest architectural masterpieces of all time. What a shame it would have been to abandon such a marvel of architectural genius. I'm convinced that in 2023, there must be some leaders of the European industry and politics who see beyond the bottom line and wish to be remembered, like Cosimo and Lorenzo "Il Magnifico" de' Medici, for the legacy left for Europe's future generations of scientist and entrepreneurs.

Finally on a more personal note, I'd like to say that I feel enormous gratitude to all my collaborators, trainees, students and colleagues around the world for making my scientific life so rich in positive experiences and satisfying interactions. There are too many of them to mention all here in this essay but I wish to acknowledge the long-lasting collaboration with the late John Dixon of the University of Warwick. I learned a huge amount from all of them. I'm also very much aware of the fact that very few people have the good fortunate of making a living by doing the things we, as scientists, love and have a real passion for. We do what we love and get reasonably well remunerated for it. We learn new things every day and interact with young creative minds on a daily basis. We travel around the world and no matter where we are we speak the same language of science. This is priceless. Science is and should be without borders. Science also allowed me to transcend geographical borders. I was fortunate to have lived in Poland, Canada, Germany, Belgium and Italy. I had the privilege to spend prolonged periods of time in Denmark, France, Switzerland, Spain and the UK. I gave seminars on five continents and made friends in too many places to list here. Can one ask for anything more? We should always follow our passion, which is a cliché, but it rings true to me. We should also appreciate the good fortune of being able to follow our passion for science as a profession.

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Finding My Way Through the Nuclear Landscape

Enrico Vigezzi

Abstract Almost all of the matter we are made of resides in atomic nuclei. Nuclei are many-body systems governed by the laws of quantum mechanics, and present an amazing variety of patterns, depending on the number of protons and neutrons which compose them. New experimental facilities are greatly expanding our knowledge of the nuclear landscape, getting close to the limit of nuclear stability. Theory strives to improve our knowledge of the strong force and to deduce the nuclear properties from the interaction between their constituents. On the other hand, nuclei are wonderful examples of self-organized systems, showing much evidence of collective behaviour, that can be understood on the basis of elementary degrees of freedom. I outline my itinerary as a theorist investigating nuclear structure and nuclear reactions, stressing the importance of the collaboration with experimentalists and the importance of feeling part of a research community.

1 MOTIVATIONS: How I Developed an Interest in Science

I grew up in a family devoted to the humanities. Both my father and my maternal grandfather were history professors at the University of Milan; my mother translated books from French and German into Italian. Humanities books were the masters of the house, and only a few popular science books appeared around here and there. As for me, I liked math a lot in middle school, but then in high school I did not find teachers to help me nurture this interest. As for physics, it seemed to me a distant subject, also because of the soulless manuals that I was supposed to study.

At the same time, I was bursting with curiosity, especially for languages. I really enjoyed translating from Greek and I was attracted by different alphabets. I amused myself with language, creating wordplays. In this regard, I was aware that the mathematical language represented a door to a different world that I might never discover.

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I remember the summer of 1976 after graduating from high school, and my uncertainty about the choice of university major. I read some introductory books to scientific subjects. I remember that Feynman's *Lectures on Physics* struck me a lot, as they were much more fun and interesting than anything else I had studied until then in physics. Biology was also of great interest to me, particularly the central question of the origin of life. But somehow, physics appeared to me as more fundamental, more rigorous.

I went to enroll in university on one of the last days before the deadline. As I was leaving home, I was still uncertain and torn between geography and physics. The decision to pick up physics, in which the desire to emancipate myself from my parents certainly also played an important role, determined my entire working life.

I was not disappointed by my first years at the Department of Physics of Milan University. When I reached the end of my four-year degree, I asked Giulio Casati, a physicist well-known for his studies on statistical mechanics, to supervise me in my Master's thesis. The subject was stochastic electrodynamics, a somehow heretical theory, which sought to provide an interpretation of some aspects of quantum mechanics and quantum electrodynamics based on the existence of a random electromagnetic field. I learnt the fundamentals of quantum mechanics and how to write a computer program. I fondly remember the punch cards brought with caution to the computing centre. However, I did the job mainly on my own, and ultimately I didn't get a real introduction to research work. Above all, my work was somewhat academic in the worst sense of the word and led to no clear further development.

I went through a period of crisis, until fate decided my future for me. I sought advice from Francesco Resmini, a brilliant experimental physicist, who had recently returned to Milan from the US, where he had worked on the construction of a new superconducting cyclotron at Michigan State University. In Milan, Resmini was directing the construction of a new Italian cyclotron, which was later moved to the Laboratories of the South, in Catania, where it is still in operation today. Instead of heeding my uncertainties, he picked up the phone and called a group of accelerator theorists in Zurich, as far as I remember. The line was busy, so he called another number and spoke to Ricardo Broglia, an Argentinian theoretical nuclear physicist working in Copenhagen. He quickly arranged an appointment for me with Broglia who was to come to Padua some time later. This swift development, so contrasting with the confused thoughts I had had during the previous months, struck me very much.

The fact that I knew essentially nothing about nuclear physics did not matter; which was a good lesson, for someone like me who was often mulling over all the different aspects of a situation in a sometimes exhausting way. I was greatly saddened when Resmini passed away prematurely shortly afterwards, leaving an unbridgeable void for all of Italian nuclear physics.

I then won an international scholarship from the National Institute of Nuclear Physics (INFN) and in the spring of 1984 I found myself in Denmark, at the Niels Bohr Institute (NBI) in Copenhagen.

The Institute for Theoretical Physics (see Fig. 1), as it was originally called, had been created in 1921 by Niels Bohr, who was one of the fathers of quantum


Fig. 1 Left: The Niels Bohr Institute in Copenhagen. Right: A famous photo of a meeting in the Auditorium in 1930. Some of the fathers of Quantum Mechanics sit in the first row: from left to right, Oskar Klein, Niels Bohr, Werner Heisenberg, Wolfgang Pauli, George Gamow, Lev Landau and Hans Kramers. © Niels Bohr Archive, Copenhagen

mechanics, and represents a mythical place for twentieth century physics. Many of the most famous physicists had discussed sitting on the wooden benches of the Auditorium, as shown by the pictures hanging on the walls. It was an intimate and suggestive place. In the Institute one could appreciate first-hand one of the wonderful aspects of research: the true equality between researchers, the complete openness to scholars from all over the world. Among other things, the Institute was one of the few places that also welcomed physicists from the Eastern block, during the years that preceded the fall of the Berlin wall.

At first I was very shy, and I remember my surprise when one day a man who had just arrived in the office across the hallway came to my own office. “May I introduce myself? My name is John Wheeler”. He was one of the great physicists of the twentieth century, Feynman’s supervisor and the one who gave black holes their name. This lack of formalities, this naturalness and simplicity, rather different from what I had experienced in Milan, helped me.

Nobody seemed to care about my lack of knowledge of the fundamentals of nuclear physics. I was entrusted by Aage Winther, a well-known expert of nuclear reaction theory, with the task of writing a computer program to calculate the cross sections of heavy ion collisions using an innovative formalism. I met once with Per Rex Christensen, an experimental physicist who had written a first version of the code, I was given a couple of relevant references, and then went on to start working. This would be my main research work for the next three years.

At the time, the NBI represented one of the main centres for the study of the structure of atomic nuclei. Ten years earlier, Aage Bohr, the son of the great Niels, and the American Ben Mottelson had won the Nobel Prize for their studies conducted in Copenhagen, sharing it with James Rainwater. Around Bohr and Mottelson, a group of first-class theorists and experimentalists had developed and constituted a point of reference for guests and young researchers from all over the world. In particular, there was a fairly large group of Italians and Spaniards, who at that time collaborated intensely with Ricardo Broglia. Fairly quickly, I formed a strong friendship with

Francisco Barranco, who was to become, together with Broglia, the main collaborator of all my future scientific activity.

One of the best known and most fruitful aspects of the activity in Copenhagen consisted in the collaboration between theorists and experimentalists. The collaboration arose spontaneously from the history of the Institute and from the common interests of research. The experimental physicists worked in another location, at the Risø accelerator which was at an hour's drive from Copenhagen. Following a long tradition, joint seminars were held at the Niels Bohr every week.

These close interactions led to my involvement in 'high spin' nuclear physics. The possibility of exciting nuclei and imparting high angular momentum to them was revealing spectacular aspects of nuclear structure, such as the possibility that the nuclei would acquire extreme deformations; and the advent of new techniques for detecting gamma rays emitted during the de-excitation processes provided unique information on the structure of quantum many-body systems. Bohr and Mottelson had received the Nobel Prize precisely for their profound understanding of nuclear deformation and rotation.

The collaboration of those years, in particular with Bent Herskind, the leader in Risø, my experimental Italian colleagues Angela Bracco and Silvia Leoni, as well as the Danish theorist Thomas Døssing and the Japanese theorists Masayuki Matsuo and Yoshifumi Shimizu, represented for me a fantastic introduction to the world of fundamental research. The work developed side by side with the experimentalists and this led me to appreciate the fundamental role of the comparison of theoretical results with experimental data.

2 Work Done: My Personal Scientific Approach

A few years after my stay in Copenhagen, I was hired by the National Institute for Nuclear Physics (INFN). INFN researchers often share their research activities with faculty members from university physics departments, even if they are not required to teach. Ricardo Broglia had become a member of the Department of Physics of Milan University, and I joined the theoretical physics group that he was about to create. In the following years, Pier Francesco Bortignon, who had also worked in Copenhagen in close contact with Broglia, and who was a great expert of many-body theory, joined us.

It would not be appropriate to provide here a summary, even a schematic one, of the themes I studied together with my research group in Milan. I would rather like to sketch the basic lines of approach I have followed. But let me first recall some essential features of my playground, namely the nuclear landscape.

At the core of every atom is a nucleus, surrounded by a cloud of electrons. The nucleus is characterized by the number of positively charged protons and electrically neutral neutrons that compose it. It contains more than 99.9% of the atomic mass but occupies an extremely small fraction of the atomic volume, of the order of 10^{-15}. The nucleus obeys the laws of quantum mechanics. As such, it is characterized by

a ground state of lowest energy, and by a series of distinct excited states of higher energy in which it can be found when it is disturbed by an external force. In addition to their energy, nuclear states are characterized by other properties, such as angular momentum and parity, and by the intensity of transitions to other states.

The nucleus is governed by the complex interplay of three forces of different nature, which are exerted between the nucleons (i.e., protons and neutrons) that compose it: the (mostly) attractive strong interaction, the repulsive electromagnetic interaction among protons, and the weak force that converts neutrons into protons or vice versa. Due to the balance between these forces, nucleons can only be bound together and form a nucleus if the number of protons (Z) and the number of neutrons (N) are not too large ($Z \lessapprox 120$, $N \lessapprox 170$) and not too different between them.

The resulting nuclear landscape is sketched in Fig. 2, in which observed nuclei are displayed by squares, whose colour depends on their lifetime. Only a small fraction of nuclei (a few hundreds) are stable on the human timescale and lie along the 'valley of stability' (black squares in Fig. 2). It is estimated that about seven thousand nuclei can be bound and are stable with respect to the strong force; they are contained within the proton and neutron 'driplines' (red curves in Fig. 2), beyond which nuclei become unbound. About half of the bound nuclei have been observed. Most of such nuclei decay under the action of the weak force, with lifetimes that are long (see inset of Fig. 2) on the nuclear timescale (10^{-22} s) but can be short on the human timescale.

Many of these 'exotic' nuclei then do not exist naturally on earth, and are created and studied in the laboratory, thanks to the continuous progress in extending the frontiers of the known part of the nuclear landscape. New accelerator systems have been built to reach more and more 'exotic nuclei' far from the valley of stability. On the other hand, exotic nuclei are produced in stellar environments and the knowledge of their properties is very relevant for the study of the astrophysical processes that lead to the creation of the elements.

In a nutshell, the objective of the experimental physicist is to determine the states of a nucleus and their properties by designing experiments and using different measurement techniques, while that of the theoretical physicist is to conceive models that accurately reproduce these findings, and possibly predict those that will be measured in subsequent experiments. Nuclear properties are very sensitive to the number of protons or neutrons and can change considerably by changing Z or N by just a few units.

Nuclear physics has been evolving for over a century into a mature science. In the popular image, it is unavoidably connected with the nuclear bomb and with nuclear energy, but as for my scientific activity, it is an area of fundamental research which has developed many well-recognized models and schools. The Copenhagen school, in which I was trained, puts its focus on the identification of a limited number of degrees of freedom, which define the 'collective', self-organized behavior of the nucleus. One places oneself at a 'mesoscopic' level, avoiding to explicitly consider the interactions between all the protons and neutrons that make up the system. In the words of Bohr and Mottelson, in the preface of their famous monograph on Nuclear Structure (1969):

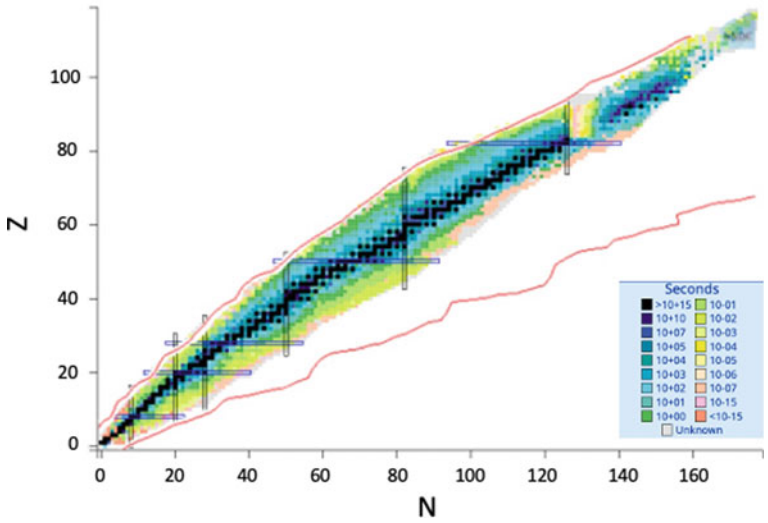

Fig. 2 Map of the nuclear landscape, the playground of nuclear physicists. A given nucleus is characterized by the number of protons (Z) and neutrons (N) it contains. Known nuclei are represented by squares of different colours. Stable nuclei are few: they lie along the 'valley of stability' and are marked by black squares. Specific ('magic') numbers of protons and neutrons are associated with increased stability; they are convenient "landmarks" in the map and are indicated by double lines. Most nuclei are unstable under the action of the weak force that converts neutrons into protons, or vice versa. The colour scale indicates the value of the lifetime associated with each nucleus. The red curves indicate the estimated positions of the proton and neutron driplines, beyond which nuclei become unbound

Some of our colleagues have argued that a proper presentation of the subject should start with the Schrödinger equation for the nuclear many-body problem and proceed by appropriate approximations to derive the observed nuclear properties.

We view the subject, however, in a rather different way. In the study of a many-body system such as the nucleus with its rich variety of structural facets, the central problem appears to be the identification of appropriate concepts and degrees of freedom that are suitable for describing the phenomena encountered.

Some of these degrees of freedom of the quantum system may correspond to quantities that lend themselves to a classic image: one can thus speak of the 'shape', of the 'surface' or of the 'rotation' of a nucleus, at least if the nucleus contains 'enough' nucleons. This implies that the nucleus has broken the homogeneity and isotropy of space, defining a direction, although the fundamental interactions obey these invariances.

In the approach sketched above, the nucleus is a finite quantum many-body system characterized by a mean field and by the existence of a surface. To a first approximation, the nucleons move 'independently' in such mean field, generated collectively by all of them. A considerable part of my research has been related to the study of the interweaving of the motion of individual neutrons and protons with the dynamics

of the nuclear surface, based on Nuclear Field Theory. This theory was developed by Daniel Bès, Bortignon, Broglia and other colleagues in Copenhagen, in close collaboration with Bohr and Mottelson, and is able to take into account the fact that this surface is in turn created by the coherent motion of all protons and neutrons. This dynamical interplay can be studied in the easiest way in spherical nuclei, while during my stay in Copenhagen I had mostly been involved with deformed systems.

In the case of weakly bound nuclei close to the drip lines, surface effects are very strong and modify the mean field properties in an essential way, in particular for 'halo' nuclei. The latter are light nuclei which lie at the edge of stability and whose density extends far out from the center of the nucleus.

It is important to mention that the structural properties of nuclei are often revealed in scattering experiments, in which two nuclei collide and only the final products of the reaction are measured. To interpret the experimental results, one must then make use of theoretical tools that consider not only the internal structure of a single nucleus, but also its interaction with the other nucleus. The transfer of one or more nucleons from one nucleus to the other during the collision provides essential information about the nuclear 'shell structure' and about the correlations existing among nucleons. The connection of structure and reactions is a complex and fundamental subject, especially in the case of the study of halo nuclei, which are very fragile and can be easily broken during the reaction. We could show that our analysis of the structure and stability of halo nuclei based on the interplay of nucleons with the surface is indeed consistent with the measured cross sections. This was possible thanks to the essential contribution of Gregory Potel, a specialist of transfer reactions who worked with us in Milan for several years and is presently at the Livermore National Laboratory in the US.

The Copenhagen approach that I have very schematically outlined above needs several elements from nuclear phenomenology for its quantitative implementation in a specific nucleus. There are other approaches that aim at reproducing the bulk properties of nuclei (energy, radius) and some of their spectral properties across the nuclear landscape using a limited number of parameters. These parameters are usually fitted using the properties of a specific set of nuclei, and then applied to a large part of the nuclear landscape. This approach leads to the definition of nuclear density functionals, in parallel to the analogous approach in the physics of condensed matter. I have collaborated with Gianluca Colò and Xavier Roca-Maza, experts of density functional theory, on some applications of this approach, which allows to have a global reproduction of the nuclear landscape.

The theories I have mentioned above can be contrasted with a 'reductionist' point of view, which seeks to derive the properties of a nucleus from a more 'fundamental' point of view, starting from the 'bare' interaction between all its components. One of the crucial problems is how to derive the strong force acting between nucleons taking into account that protons and neutrons are not elementary objects, but are composed of quarks and gluons that obey the laws of Quantum Chromodynamics (QCD). In the last twenty years, many steps forward have been made in this direction, related both to important theoretical developments in Effective Field Theory and to advances in computational power. It is now possible to derive bare microscopic interactions

between two and three nucleons from systematic approximations to QCD based on the properties of few-body systems, and to apply them to compute 'ab initio' at least the basic properties of nuclei composed of many neutrons and protons. This is a topic of great interest, which is currently attracting many of the young researchers working in nuclear physics. In recent years, I had the opportunity to follow more closely these developments collaborating with Carlo Barbieri, who has joined our group in Milan a few years ago and employs bare interactions to study nuclear structure with Green's function techniques.

Ab initio methods are successful in reproducing experimental data in light nuclei, while it is an open question whether they may be able to reproduce experimental nuclear spectra in heavy nuclei.

The nuclear landscape therefore appears as a laboratory, where, by increasing the number of nucleons of the system, it may be possible to investigate the connection and possibly the merging between an approach based on the reductionist hypothesis (the possibility to compute the property of a complex system based on the laws obeyed by its constituents) and an approach related to the concepts of emergent properties (properties that are not contained in the constituents) and symmetry breaking, intimately associated with the definition of elementary degrees of freedom.

In any case, even if the interactions were perfectly known, calculating the spectrum of a medium-mass nucleus would remain an extremely complex task to accomplish, requiring advanced techniques in many-body physics and a large computational effort, which increases substantially as the number of nucleons increases. It is not easy to relate the resulting discrepancies between theory and experiment to possible shortcomings of the present microscopical interactions. In many cases, one must limit oneself to examine only some of the experimental data, to formulate models which quantitatively 'reproduce' the data and to 'interpret' them, identifying regularities and correlations that reflect the fundamental characteristics of nuclei and their way of reacting to external forces, and possibly predicting the result of experiments not yet carried out, which serve as stringent tests for every model. In practice, if a model is falsified it does not have to be thrown away; rather, it is a question of understanding its limitations, trying if possible to improve it, or to extend it. Compared to the naïve image of the theoretical physicist I had as a student, over time I realized that the agreement between models and reality is necessarily only approximate. On the other hand, I was able to appreciate the rigour necessary to draw the consequences from a given model. This rigour is essential to allow the comparison between different theories; repeatability of calculations is the theoretical counterpart to repeatability of experiments.

Theoretical models generally lead to mathematical equations that can be solved by analytical formulas or much more often by numerical computation. I have always liked the moment of concrete comparison between the prediction of the theoretical model and the experimental reality, as well as the challenge to determine whether the discrepancies arise from an error in the calculation procedure or from the inadequacy of the model. I have always liked the numerical calculation too, the patient work of efficiently translating formulas in a computing program, even the sometimes unnerving effort of correcting the errors that inevitably creep into the procedure. Over

time, I learned the importance of proceeding step by step, without getting carried away by the desire to arrive earlier to the conclusion; as well as the importance of carefully documenting what has been done—how many times have I realized that I was no longer able to accurately reproduce a calculation made just a few weeks earlier!

A rewarding aspect of the activity of a theoretical physicist lies in the possibility to apply one's favourite models to other fields of physics. In our group, Broglia fostered studies of diverse systems which can be investigated with profit with the techniques of nuclear physics. For example, there are very important parallels between the behavior of matter in nuclei and in condensed matter, particularly concerning the phenomenon of superconductivity. It is suggestive to realize that the phase transition occurring in some metals, which brought to very low temperatures can conduct electricity without producing resistance, has a correspondence with the behaviour of some nuclei, despite the enormous difference in scale. I have also used the experience accumulated in the study of atomic nuclei to produce models useful for understanding some aspects of the structure of the so-called neutron stars—extremely compact systems (once or twice the mass of our sun, compressed within a radius of 10 km, almost 100,000 times smaller than the radius of the sun!) which are the remnant of massive stellar explosions and are mostly composed of neutrons.

I would also like to recall an important aspect to which I have devoted more time in recent years, namely that of the relationship of young people with science. In particular, I collaborate to the Asimov Prize, a project that concerns scientific dissemination in high schools, and which involves about 12,000 Italian students. These students are asked to read a recent educational science book, write a review about it and give it a mark. The book receiving the best marks wins the Asimov Prize. For many students, this is the first occasion to read a scientific work beyond textbooks. For teachers, this prize represents an opportunity to extend their courses in the direction of currently debated research topics.

More generally, science outreach has acquired much relevance, and I think that young scientists should devote some time to this rewarding activity.

The research activity I have outlined has been carried out mostly in collaboration with a rather small group of colleagues. On the other hand, it has been developed in the framework of the larger INFN research community, which determines many of the concrete conditions for carrying out the research, above all concerning the funding. I would like to conclude this section by briefly describing the organization of INFN, which I think is interesting from several points of view. The Italian research system—unlike individual researchers, who are generally highly regarded—is often criticized under various aspects—some examples are the lack of funding, the excessive bureaucracy, the minor role that researchers play in defining the research activities, or the difficulty of access to it for young people.

INFN is partially a happier place. It was born in 1951 and is based on the strong tradition of Italian physics, linked to the figure of Enrico Fermi. INFN deals with fundamental research in the field of elementary particles, astroparticles and atomic nuclei, as well as several applications, for example in accelerator physics or medical physics.

What characterizes INFN is its self-government system. Apart from a few representatives of the ministries and a general manager, the governing bodies are made up of physicists who are members of the INFN and are elected by colleagues; the President is also designated by the Board of Directors of the Organization.

Another characteristic feature of INFN lies in the existence of five scientific committees, linked to the main areas of activity and made up of scientists elected by colleagues in the various Units. The scientific committees manage the research funds assigned each year by the Board of Directors. This organizational structure therefore guarantees—within certain limits—the participation of the researchers in scientific directorial decisions. It also allows researchers to devote part of their activity to scientific management and organization for a few years, without necessarily taking this on as a full-time occupation. During my career, this structure has helped to make me feel like a member of a large research community, especially when I was elected into the Board of Directors as the national representative of the researchers.

3 Science Today and Tomorrow

The title of this Part can refer to the main directions of current research development. At least in the field of fundamental research in physics and astrophysics, the role of Big Science will remain essential for the foreseeable future, although its pace will be determined by the available economic resources. What may change is the balance between the various laboratories, the role of the different countries—in particular concerning the competition between CERN and China in the field of particle physics—but probably without substantially altering the way of organizing and conducting these large scientific enterprises, which require resources which are at the limit of those available in the richest countries or are based on international funding.

Big Science is very expensive experimental research, which is often expected to bear fruit after decades. And it is great when this becomes true, as was the case, for example, with the discovery of the Higgs boson, or, more recently, with the observation of gravitational waves generated by the collisions of neutron stars and black holes. Based on Einstein's theoretical prediction, this observation was achieved thanks to the collective effort of thousands of researchers from many countries who have conceived and built detectors of extreme sensitivity at the frontier of technology such as LIGO in the USA, VIRGO in Italy, KAGRA in Japan and GEO600 in Germany. The existence of gravitational waves was expected, but their measurement will enable us to explore a new dimension of the universe. Soon after the site of the neutron star collision that produced the gravitational waves event GW170817 in 2017 was identified, the radiation generated after the event was detected, thanks to the collaboration of observatories all around the world. These observations provided the first direct evidence for the nucleosynthesis of heavy elements, after 60 years of speculation.

Several other international enterprises aim at providing answers to fundamental questions which are of primary interest to large scientific communities. To mention just one among the most ambitious ones, the Webb Telescope has just recently delivered its first images after 20 years of preparation and will start to investigate the most distant galaxies in space and time.

In my research field, the Facility for Rare Isotope Beams (FRIB) has just come into operation in the US. This is a system for the production and measurement of exotic nuclei, which will greatly expand the number of known nuclei and will bring us closer to the limit of nuclear stability. In Europe, the international accelerator facility FAIR (Facility for Antiproton and Ion Research) is being built in Germany. It will provide beams of all the chemical elements that will allow research on exotic nuclei as well as on antimatter and astrophysics, producing matter in conditions similar to those found in very dense stellar environments.

These large installations have a driving effect on all research worldwide. They require the commitment of many scientists, both to define the best procedures for their realization and for their actual construction. They cause keen competition for their use, which requires special panels to choose the best experimental proposals among those submitted by research groups in many countries. For example, the committee that gathers FRIB users includes about 1600 scientists from more than fifty countries.

Despite the driving role of the large collaborations, Big Science is often criticized, among other things because it involves much politics and bureaucracy. Furthermore, its goals—however ambitious—may represent gratifying verifications of widely accepted theories (even in the case of the Higgs boson or of gravitational waves). In fact, the key to fundamental issues such as the nature of dark matter and dark energy, or the unification of general relativity with quantum mechanics, may arise from experiments on a much smaller size or from new theoretical elaborations that can change the overall direction of research in the field. At the same time, no one knows where the awareness of unknown unknowns—of what we are not even aware we don't know yet—will originate: radical change is never easy to see coming.

Historians and sociologists of science stress the importance of serendipity in the processes leading to scientific discoveries. Great advances may originate in unexpected ways; sometimes casual discoveries even represent solutions to problems which emerge only at a later stage. Actually, this is one of the motivations for the economic support of free, not oriented research.

In conclusion, a good balance between Big Science and free investigations represents an important element to preserve the vitality and charm of fundamental research in the future.

A different question related to the future of science concerns the relation between "data" and "models". I will limit myself to mentioning one of the most debated points, namely the role of artificial intelligence. There is no doubt that technologies such as machine learning associated with the use of Big Data can have a strong impact on research. Most of the physicists of my generation follow the traditional scheme: theoretical or empirical "models" are compared with observations to be falsified (or if you want to stay in a comfort zone, to confirm the validity of their starting

assumptions). Thanks to computational power and ever-growing experimental techniques, models can become more and more elaborate and data ever richer and more precise. But the question becomes qualitatively different when one asks whether it is possible to do without models—and without theorists: *The end of theory: the data deluge makes the scientific model obsolete*, was the provocative title of the article by Carl Anderson, editor in chief of the Wired magazine, published in 2008 and spurred by Craig Venter's DNA sequencing revolution.

Through deep learning, robots can find associations between data that escape human analysis, just as they can beat any person at chess, following strategies that escape our understanding, even in retrospect.

The underlying question is whether it is possible to extract physical laws directly from the data, according to a strictly inductive approach. A position that appears far from the historical evolution of modern science, if we think, for example, of the role of Galileo's 'thought experiments', in which the ability to abstract the effect of friction from the kinematic data made it possible to establish the fundamental laws of motion.

Part of the research on complex systems aims at simplifying data, finding collective coordinates, fundamental degrees of freedom that allow the scientist to 'interpret' a physical system. If a way is found to 'understand' how the neural network comes to recognize a given pattern that escaped human intuition, perhaps an important step will be taken in research. After all, a person still has to choose how to feed data into the system, at least until the time when a committee of computers will approve research projects submitted by other machines.

4 Advice to the Next Generation of Scientists

For many young people, passion for research is so strong that they have no doubts about the path to take after obtaining their Master's degree.

Sometimes, however, I have met young graduates interested in research but unsure whether to take the first step, which usually consists in competing for a doctoral scholarship. I think it is almost always worth it. The experience of a Master's program may not be enough to assess whether you are cut out for this job, and even for your mentor to be able to give appropriate advice. Once you have completed a Ph.D., you should have a clearer idea. In any case, this is an experience that can later help to find satisfactory employment even if you do decide to abandon scientific research, although in Italy, even today, there are work areas in which a Ph.D. is not considered a qualifying title.

For those who aspire to remain in research, spending several years abroad with postdoc positions is an essential condition to obtain a staff position, or at least to a tenure track. For most young researchers this should not constitute an impediment, on the contrary it represents a rather natural condition and a stimulating incentive, if the string of precarious positions is not protracted for too long. It is good to be aware, however, that the question becomes thorny if the years of postdoc become

many, particularly if you have a family or if you intend to create one. It is important that universities and research bodies facilitate researchers who have children as much as possible, even if they are in non-permanent positions.

A general piece of advice may be not to hesitate to apply for a permanent position when there is an opening in a research field in which you have skills, without fear of not being good enough for it. Sometimes a search committee selects scholars based on their potential, even if they do not have a long publication list.

In various cases people find a partner right during this period and in the same area of research—as it happened to me. Finding two research positions in the same field in neighboring places is often very difficult—and in some countries it is even (I think unfairly) discouraged. This leads to sometimes painful sacrifices. I think that a young scientist should be prepared to change his/her research field, without following the obsessive drive to stick to a single path to complete a task, but instead taking a moment to reassess his/her approach. There are also areas of research in which it is possible to continue to do research at a satisfactory level in the private sector.

Medawar, in his well-known book addressed at young researchers, *Advice to a young scientist*, remarks that *who wants to make important discoveries must study important problems*. It is of course an excellent suggestion, but not always easy to put into practice, especially because you may not have the right tools to assess the importance of a problem by yourself. However, a parallel and more pragmatic advice can be to verify if your supervisor has an adequate network of collaborators, for example by asking if it would be possible to carry out part of the doctoral research in collaboration with other scientists and in other locations. It is also useful to go to conferences and present your work once significant results have been achieved and to attend seminars from other fields, although without exaggerating. Indeed, it is not always easy to maintain the right balance between two equally important needs: on the one hand, that of mastering a field of research and producing significant works, of making oneself known, in order to be competitive in the search for the next position; on the other hand, that of being able to place one's research in a broader context, and not to lose sight of different research fields in which it is possible to successfully enter and which can be fascinating.

It should also be said that the period of the Ph.D. and the first postdocs represents a formidable opportunity to establish collaborations that can later translate into very close working relationships and friendships that arise from a consonance of scientific interests, in a period in which much of your and other people's time is being devoted to them. This is very important for everyone, but especially for theoretical physicists, whose work tends to be more solitary than for experimentalists, who usually work in larger groups. One of the most delicate points, at least in my experience, is that of feeling as an integral part of a group, of a research community. Your research can involve long periods of individual work to tackle difficult problems, which require total concentration and dedication. But it is important that—in addition to the desire to overcome the challenge—you keep the sense of working towards a higher common goal.

Acknowledgements In Copenhagen I met Bénédicte Million, who was to become my wife, and Francisco Barranco, with whom I developed most of my research activity. I thank both of them, as well as my daughters Natalia and Serena, for their suggestions.

Enrico Vigezzi is a physicist, Director of Research of the Italian Istituto Nazionale di Fisica Nucleare (INFN), settled in Milano. After graduation, he spent a few years at the Niels Bohr Institute of Copenhagen, where he started to work on the theory of nuclear structure and reactions. In particular, he studies the renormalization processes associated with the coupling of quasiparticles and collective vibrations, and their effects on the physics of weakly bound systems, as well as on transfer reactions. He has also been involved in various aspects of the organization of INFN.

The Curious Job of Trying to Look at Things as They Are

Alessandro Vitale

Abstract So many interesting things were happening when we baby boomers were students. I have not started studying biology with a strong motivation. But the idea of doing things not done before and, most of all, trying to understand the unknown, slowly started fascinating me. Above all, I was lucky enough to encounter mentors that trusted me. Living up to their expectations, and later to the expectations of the larger scientific community, has perhaps been the strongest driving force for my work as a scientist, since science is above all a social activity. I am well aware that I have not always succeeded.

1 Motivations: How I Developed an Interest in Science

In 1969 Italy had opened access to any undergraduate university major to all students graduated from high schools, independently on the school specialization. Thus, in the fall of 1971, after five years of science high school, I could choose whatever I liked. I was undecided between foreign languages and biology. I chose the latter, in spite of the fact that my marks were better in the former—the English teacher was great.

Like many teenagers, I had developed a liking for ecology, so that was my plan of studies. However, the teachers made me soon aware that ecology was not such a promising field in Italy. I changed my plans towards biochemistry, even if the ecology class turned out to be the most fascinating I followed. In the spring of 1977 I had just obtained my Laurea (Master) degree in Biological Sciences at the University of Milano and I was a teaching assistant at the Institute of Botany—where I had worked for my thesis on sugar metabolism in germinating seeds—when news arrived that a nearby new Institute of the National Research Council (CNR), offered a fellowship to study the biochemistry and genetics of maize storage proteins. At that time fellowships were the best you could have after a Laurea to continue in academic

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research: in Italy, Ph.D. programs only started in 1983. The news said the CNR institute wanted to transform maize seeds to improve their protein nutritional value. Wow! That was something! Sounded like science fiction to me. I got the fellowship, at the Istituto Biosintesi Vegetali of the CNR, which in 2001 became the Istituto di Biologia e Biotecnologia Agraria.

I immediately discovered that plant transformation was still a dream in 1978, but my project was nevertheless very interesting: trying to understand how many different storage proteins are in maize and whether they are different modifications of the products of a handful of genes or are encoded by a large gene family. The second was true and I discovered that a strange protein was also part of the family—notably, many years later I started again working on that protein: first love is eternal, it is said. My supervisor, Ennio Galante, gave me precious and generous freedom on how to develop my research, and the other PIs of the large maize group also helped and inspired me: the late Carlo Soave and Angelo Viotti. Importantly, we were in strict collaboration with Francesco Salamini, one of the great plant geneticists of the world, at that time Director at the Istituto per lo Studio della Cerealicoltura of the Italian Ministry of Agriculture. It was intellectually very involving, we had very lively discussions and many visits from expert colleagues from around the world.

CNR had a fantastic program of fellowships to do research abroad. The maize group had close contacts with the Max Planck Institute for Plant Breeding Research in Köln, and in 1981 Carlo Soave proposed me to go there, one of the great places for plant molecular biology—the first transgenic plants came from there in 1983. But something had happened to me the year before. Roberto Bollini, another staff researcher of our institute, had asked my help on experiments aimed at understanding the genetic heterogeneity of common bean seed storage proteins. The questions were similar to those we were asking for maize proteins, but an important difference is that the bean proteins *traffic* within the cell whereas those of maize do not. I will detail this in the next section, but here the important point is that Roberto had been involved in this topic while on sabbatical at the laboratory of Maarten Chrispeels at the University of California, San Diego, and was still collaborating with him. In 1981 Maarten was looking for another young researcher to join the lab. Roberto asked me whether I was interested. I applied for a CNR fellowship to do research abroad, I got it and in July 1982 I was in San Diego, studying the traffic and biochemical modifications of bean storage proteins. The month before I had become staff CNR scientist, thus I left on leave from my job at CNR.

My spouse Anna could not take long leaves from her job as a social worker at the main maternity hospital of Milano, and our son Luca was born in April 1981. Anna and Luca were "always on jet planes" between Milano and San Diego. Not easy at all, and quite sad at times. Nevertheless, my almost two years in Chrispeels lab have been fundamental in my path to become a scientist, and important also for my personal growth. The scientific environment was fantastic, and Maarten did anything possible and more to make me feel at home and love science, and supported my work. That was the big turning point of my scientific life. Since then, I have never left the field of protein traffic.

Back to Milano in 1984, I joined again Roberto's lab and slowly started to build my own research group. In the fall of 1998, Maurizio Cocucci, at that time head of the faculty of Agricultural Sciences at the University of Milano, asked me to become adjunct professor for a new class for the major in Plant Biotechnology. I had total freedom on how to organize it and I decided to teach the biotechnological implications of plant cell biology, with the main focus on the exploitation of what was discovered about protein synthesis, the different subcellular compartments and what proteins need to reach them. I termed the class *Cellular engineering*, and I was a professor from 1998 to 2006, when the growing responsibilities at CNR made me decide to stop teaching. Teaching has been a really rewarding experience, because several highly motivated students joined my lab for their thesis work, but mainly because the confrontation with students is such a joy, a continuously refreshing experience and a school of life. I regret giving it up.

An important change happened in 2000. Transgenic plant research for crop improvement was very active in Italy during the last years of the 80s and all the 90s. But the anti-GMO movement was growing everywhere in western societies. The perception of plant biologists as the most romantic scholars rapidly turned into that of servants of the multinational corporations that want to destroy nature. Propaganda was very well orchestrated, as it is still now. In 2000, a member of the Italian Green Party became Minister of Agriculture. That was the end for transgenic plants in Italy. Together with other colleagues, among which I want to thank Roberto Defez, we started science popularization activities to fight against irrationality and nonsense and to promote a rational approach to the use of genetic technologies in agriculture. During the years, we set up specific groups on these topics within the Italian Society of Plant Biology, the Italian Society of Agricultural Genetics and the Italian Federation of Life Science Societies, mainly thanks to the strong dedication of Michele Morgante, Felice Cervone and Gennaro Ciliberto, who at times have served as Society or Federation presidents. This very challenging and at the same time very stimulating work is still ongoing, taking a relevant part of my time.

Last turning point has been in 2019, when Gabriella Colucci CEO of Arterra Biotech, established in Napoli, asked me to join the Scientific Committee of the company. I had short scientific collaborations with companies before, but seeing science within a company has opened a completely, fascinating new world to me. Again a new challenge, and again I have been very lucky: at Arterra I feel being in a family and in an exciting enterprise at the same time.

2 Work Done: My Personal Scientific Approach

Protein traffic is a major topic of cell biology, awarded with several Nobel Prizes. This is especially true in eukaryotes—which mainly include animals, plant and fungi—because their cells, unlike those of bacteria, contain many internal membranes that delimit what are defined as *intracellular compartments*; such separation is essential to allow proper functioning of the many thousands of biochemical reactions occurring

in a given eukaryote cell, avoiding negative interferences among them. The problem of protein traffic arises because protein synthesis occurs almost exclusively outside these compartments, in the cellular soup, termed *cytosol*, where compartments are contained and from which they are separated by their enclosing membranes. The different proteins must therefore contain specific *signals* that target them to their correct destinations. Understanding which are these signals and the mechanisms that recognize them is the topic of *protein traffic* studies.

Molecular biology is rooted in the facts, discovered in an amazingly short time between 1940 and 1960s, that genes are made of DNA, which is transcribed to produce RNA, which in turn is translated into proteins, which perform most biochemical reactions that allow life. There is more than this in a cell, but understanding the structures of genes, RNA and proteins, how they work and how their activities are regulated remains the major aim of molecular biology. The enormous information on entire genomic DNA sequences and on the three-dimensional structures of proteins that has been accumulated in the last twenty years by structural biologists, as well as the ease with which DNA sequences can now be manipulated and transferred to organisms, not only have made us understand how life works and evolves with time, but on the whole they have also provided immense, invaluable knowledge and tools to help the work of biochemists, physiologists and cell biologists. I have never worked in structural biology and large scale sequencing; mine has always been a *small scale science* lab, nevertheless I recognize the scientists that use large scale approaches and very complex equipment as the heroes of contemporary biology. What my lab has done over the years has been to study protein traffic in plant cells using a number of proteins, changing their sequences through recombinant DNA, reinserting the modified DNA in plant cells and see whether our hypotheses on the function of specific segments or individual amino acids were right or wrong. We have also applied this knowledge to try finding strategies to improve recombinant protein production in plants, but anyway this has been the approach, and it is a good example of reductionism: the bet that complex systems can be understood by dividing them into smaller components that can be more easily studied individually. I have never really understood what holistic science is. Even the study of how the expression of the entire genome of an organism is regulated in different tissues seems to me a reductionist approach; all molecular biology is reductionist, almost by definition. And it works.

Defeats are more instructive than achievements, I will therefore dedicate some space to what has been perhaps my most important failure. It occurred early, not long before I moved to UCSD. A collaboration of our CNR group and that of Salamini had identified increased accumulation of an unknown protein. This could be seen as a thicker band in electrophoresis gels, in a natural maize mutant (termed floury2) that has mildly reduced accumulation of storage proteins, which in maize are termed zeins. We found that the protein was enriched only in the seed endosperm, and especially in the subcellular compartment where zeins accumulate, termed the endoplasmic reticulum. Importantly, genetic analysis by crossing different maize mutants showed that when other, independent mutations that highly repress the synthesis of zeins are present together with floury2, the amount of the unknown protein reverted to normal

level, in spite of the very low zein accumulation. There was therefore a complex correlation between the amounts of zein and this unknown protein. As we stated in our published study, one hypothesis was that our results "may suggest a functional relationship between this protein and the zein synthetic-secretory system". But which was the identity and function of this protein? In electrophoretic gels, proteins are separated based on their molecular mass, which in turn depends approximately on the number of amino acids that form a given protein (in most cases this varies in the 10^2 to 10^3 range). The apparent mass of our protein was curiously similar to that of a family of proteins whose synthesis is increased in plant and animal cells upon heat stress, termed heat shock 70 proteins (HSP70). Heat stress denatures proteins and this may lead to permanent aggregation and therefore loss of activity. HSP70 transiently associates with denatured proteins inhibiting aggregation. There is more request of HSP70 proteins during heat stress, and therefore a complex signaling mechanism induces their enhanced synthesis. The point is that the HSP70 proteins known at that time were known to be located in the cell soup, the cytosol, and not in the endoplasmic reticulum where zeins are located. I thought it unlikely that our protein was an HSP70. I then left the project, concentrating on my plans of what to do in San Diego. In 1986 Sean Munro and Hugh Pelham published a fundamental cell biology paper showing that there is an HSP70 in the endoplasmic reticulum of rat liver cells and that this protein is the same that a few years before had been identified in cells that produce antibodies and therefore termed immunoglobulin binding protein (BiP). In 1990, the group of Salamini and at the same time, but independently, the US maize research group of Brian Larkins published that the unknown maize protein was BiP. There are now thousands of publications on BiP, because it is a fundamental protein that assists the synthesis of hundreds of very important proteins, such as antibodies, cell receptors, blood proteins, protein hormones, viral proteins, all seed storage proteins; BiP is also a master regulation on how animal and plant cells respond when something goes wrong with the synthesis of one of these proteins; this signaling mechanism, implicated in practically all aspects of the life of eukaryotic cells and important in many human genetic diseases, is termed unfolded protein response. Larkins group later found that the reason why BiP increases in floury2 seeds is that the floury2 mutation affects one zein gene: it is a single amino acid mutation that does not allow correct folding of the encoded zein protein. This mutated protein remains misfolded, has negative effects on the other zein proteins, leading to reduced zein accumulation and induction of the unfolded protein response which enhances BiP synthesis. This occurs only in seeds, because zeins are not synthesized in other parts of the plant. The reason why other maize mutations suppress the increased synthesis of BiP in floury2 is quite simple: those mutations inhibit the transcription of numerous zein genes, including the one encoding the zein mutated in floury2.

My laboratory has later produced numerous studies on plant BiP and the plant unfolded protein response, studies that have been considered important contributions. I have co-authored two university textbooks—I am especially thankful to those who invited me in these adventures: Paola Mariani, Maarten Chrispeels and Natasha Raikhel—and I am among the authors of papers cited in others. I have even received prestigious awards from the Italian Society of Plant Biology and the

American Society of Plant Biology and I have served in editorial boards of major plant biology journals. I am very happy for all these recognitions, somehow with the impression of being overrated. But we could have been the first research group to discover BiP, if we had been more open minded. Prejudice is disaster in any human activity, and science does not stand as an exception.

3 Science Today and Tomorrow

I am a baby boomer, born in Milano in February 1953. At elementary, middle and high school we were so many. Classes and schools exploded with pupils and students: there was simply not enough space, and also a want for more teachers. Not all of them were great. It was probably first year of middle school (it means I must have been 11) when the mathematics and science teacher was telling us "some people say we are descended from apes, but you know it can't be: the opposite may be true but not this". I remember this as if it were now: I thought "She does not know what she is saying. She can't tell this to a class". This episode indicates I already had a grasp on how evolution can work, meaning I was already enough interested in scientific thinking to reject explanations from authority when they sounded really wrong. But I must admit that I do not know very well where such interest came from. My father was a medical doctor; I think I used to ask him questions when I was a child. He passed away in the summer of 1971.

Being science a social enterprise, it is subjected to the tensions between passion of spirit, moral responsibility, economic costs and expectations, political approval and public acceptance. This means that every society has the science it deserves; since it is very difficult to make predictions on the future of the former (remember the *end of history*, popular in the 1990s, and look at the present global situation), uncertainty on the latter is guaranteed.

Due to the limited space, I will only make some observations on the conflict with public acceptance and its effects on science future developments. Scientific thinking has always experienced uneasy relationships with traditional human thinking, to put it mildly. The physicist and popular educator Alan Cromer provides two complementary explanations for this (Cromer 1993):

> Science is the heretical belief that the truth about the real nature of things is to be found by studying the things themselves. Traditionally, scholarship has meant the study of books – the older, the better.
>
> Science is the search for a consensus of rational opinion among all competent researchers.

Cromer is actually reporting an excerpt from the following paragraph by John Ziman, the theoretical physicist who became also a very well known, authoritative figure in the sociology of science:

> Science is not merely published knowledge or information. Anyone may make an observation or conceive a hypothesis, and if he has the financial means, get it printed and distributed for other persons to read. Scientific knowledge is more than this. Its facts and theories must

> survive a period of critical study and testing by other competent and disinterested individuals, and must have been found so persuasive that they are almost universally accepted. The objective of science is not just to acquire information not to utter all non contradictory notions; its goal is a consensus of rational opinion over the widest possible field (Ziman 1968).

This is practically identical to the definition given by the Encyclopædia Britannica (1962):

> To summarize, science is a search for judgments to which universal assent may be obtained – universal, that is, on the part of those who understand the judgments and their bases.

Those who understand the bases, *all competent researchers, competent and disinterested individuals* are the keywords here. Science is not about truth, which scientists leave to religions. It is about observation, discussion and agreement. Agreement not with anyone, but with researchers that can understand and therefore have the competence to make experimental observations that could prove wrong a conclusion or a theory. This is why, in 2018 and with some scandal, the Italian virologist Roberto Burioni could state that *science cannot be democratic* (Burioni 2018). Burioni, an advocate of rational thinking and experimental evidence against nonsense, was tired of being involved in public discussions about vaccines with popular DJs and actors. And rightly so. However, and equally rightly so, in democratic societies the final decisions on whether to allocate money for specific scientific enterprises and how to accept the consequences of scientific discoveries are taken by the people through their elected representatives. This creates conflicts between science, wide sectors of society and at times institutions, not only regarding the technological applications of scientific knowledge—weapons, vaccines, stem cells, nuclear energy, transgenic crops are a few well known examples—but about acceptance of scientific knowledge itself: evolution and whether human races exist can be cited in this sense.

Public support for science has increased almost unbelievably in the last hundred years, mainly because the investments have paid off. Vaccines, plant breeding, engines, global positioning systems and spaceship travel have one common characteristic: they work because they are based on fundamental scientific knowledge that has passed the approval of expert scientists. Also nuclear physics-based atomic bombs work: given the importance of military spending, the success of the Manhattan project may have had a major role in the increased public support for science in the post-WWII era, whether we like this or not.

Science however seems to be now victim of the esteem and authority it has won in institutions. The anti-intellectual movements that have spread in western societies in this new century have linked what they call *official science* to authoritarianism, big government, multinational corporations, esteem for the rich and contempt for the poor. Interestingly, this affects almost equally the political right and left, only the specific scientific issues being distinct: liberals object GMOs, chemicals use, nuclear power and are obsessed by "eating natural"; conservatives don't think that climate change is occurring, object evolution and any science related to sex and gender. The anti-vaccine ideology has an individualistic, anti-authoritarian flavor surpassing political boundaries. The internet and social networks have complicated the issue

further. Lies, anti-science thinking and nonsense that very often mask economic interests now easily spread even if one does not have the financial means mentioned by Ziman in the pre-internet era.

What to do then? More engagement of scientists in communication with the general public is good but may have little effect if primary school teachers have not been trained to defend rational thinking from ideology-driven beliefs. The education to scientific thinking must start from school, before it is too late, for the power of traditional human thinking is too strong. Regarding the daily work of the scientist, what again John Ziman wrote in 1971 seems appropriate:

> I feel the need to preserve the collective skills, the expert knowledge, and the delicate social organization of the scientific community from the pressures of an ignorant public, a shameless press, rapacious money-makers and opportunist politicians. A certain aloofness, a slight distance from everyday affairs may be the only way of preserving these islands of sanity in a crazy world, not as refuges but as watch-towers and safeguards against far greater evils. That is the paradox: social responsibility in science must not be too concerned about today, for tomorrow also will come (Ziman 1971).

4 Advice to the Next Generation of Scientists

I find it impossible to give advice on how to choose a laboratory where to be trained. My main suggestion is: be lucky. Whether a laboratory is already well established or famous does not count that much. You may be poorly trained and considered in a famous research group where there are too many people, and you may learn and be highly productive in a small laboratory that has just been established by a brilliant, enthusiast, empathetic young researcher. My second suggestion is: if possible, talk with the young scientists and students of the lab where you think to apply. They know how is the atmosphere in the lab and how they are trained and considered. For what concerns choosing your project, don't be influenced too much by how fashionable is a topic at that moment: it is much more important how much fascinating it is for you. Working in the lab must be a joy.

Wherever you are, after a certain time go away. I usually remind people that science has been a globalized social activity way before the term globalization had been invented. Science has no borders and is at odds with patriotism. Bruce Springsteen's cry *We gotta get out while we're young* is an excellent advice for young scientists. Apart from short visits, I have only been away from CNR at UCSD, in 1982–83, and that was the best scientific time in my life. I should have done more sabbaticals out of Italy, that was the mistake I regret the most. Changing scientific environment is refreshing and opens your mind. Do it more than once when you are young, and also later on.

Be a voracious reader. Keep yourself constantly updated. You must become in a short time an expert in the field of your project, more than your project leader. It is not very difficult, it only takes time and enthusiasm. Read in detail more than one paper every week. And when you find something you think is interesting, bring it to the attention of your colleagues and your project leader. Read books on science and

by scientists. Two had a profound influence on me. *The eight day of creation* by the science journalist Horace Judson (Judson 1979) is an extraordinary account of the rise of molecular biology. Many of the makers of that revolution were still alive and were interviewed, making history so lively. They are shown in action, as characters of an epic play: it is scientific activity as it is. *Uncommon sense* by Alan Cromer (Cromer 1993), which I have mentioned above, is based on the author's observation that scientific thinking, not to be confused with technology, goes against the grain of the human mind, appeared only once in history in classic Greece, and can disappear forever at any time. It is a fascinating and educational view of scientific activity. But there are certainly many more great books on science that I don't know, of course.

In 1996 plant cell biologist David Robinson organized in Göttingen a meeting of a handful of European laboratories working on protein traffic in plant cells, where young scientists were asked to discuss their latest, unpublished results in the most informal way. Since then, this was repeated every year, growing in number of participating labs and at a certain point starting to change location each year around Europe. Even if the increase in participants, and perhaps also the advent of powerpoint, have inevitably affected informality, those yearly three days of discussion have been and are still invaluable. Attend seminars, give seminars, and go to scientific meetings, especially small, focussed, informal ones. Attend journal clubs and if there is not one (unlikely but possible) be the first to establish it. Listening to seminars is an excellent school for scientific thinking. Presenting your results is a very good way to put order in your mind on the meaning of what you have done and to understand if your research strategies need corrections. Join at least one scientific society. Scientific societies are the scientists' families, and help you to have a broader vision of your activity.

Talk with people. If you have doubts on how to conduct an experiment or to interpret unexpected strange results or anything that did not work as expected, first talk with your closest colleagues. The person next to you in the lab may very well have suggestions or has encountered the same problems before. She or he is more likely to give you a reliable answer on a technical point than your project leader. Exchange your doubts and ideas with your colleagues. Science cannot be done in secret. It is a social activity, because it is too easy to make mistakes. It is a good idea to also treat those of other labs as colleagues and potential collaborators, not competitors. There is competition in science, of course, and it will happen that others take unfair advantage from your data. But don't be paranoid: most of the times you will instead find stimulating advice, precious collaborations and sometimes new friends.

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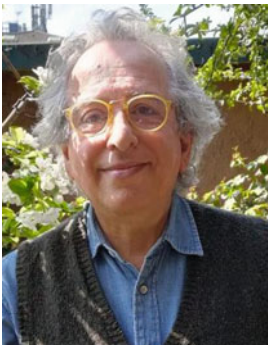

Alessandro Vitale Throughout my scientific career, I have been interested in molecular cell biology, especially protein synthesis in plant cells. We have applied these studies in developing strategies to improve the nutritional value and accumulation of seed proteins, which are the major sources of proteins for human nutrition, and to exploit plants for the production of recombinant proteins. By participating in official hearings with Italian legislators, public debates, the popular press, book chapters and other outreaching activities, I contribute to the actions of the Italian scientific community to stimulate rational, science-based, approaches to agriculture.