insects and other animals. Indeed, organisms that seem to be members of the *B. cereus* group have been isolated from insect digestive tracts and have been proposed to be gut-attached stages of the bacterium[10]. This predisposition of the *B. cereus* group may have been built upon and fine-tuned by the ready exchange of plasmids encoding accessory factors that allow the bacteria to exploit other animals or insects more directly as hosts and sources of nutrients.

All of this serves to underline the point that, whatever crude attempts are made by human beings, the true biowarfare experts are the bacteria themselves — they are constantly ready and exquisitely able to adapt to, and exploit, any environmental or pathogenic niche that presents itself. We can only hope that the availability of these sequences will assist those who are working to protect us against these agents of disease. ■

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Chemical physics

How to keep dry in water

Philip Ball

What does water look like close to biological surfaces? The question has provoked heated debate for decades. Experiments suggest that this 'vicinal' water may be markedly different from the bulk liquid.

Water is notorious for behaving strangely, but it is possible that we don't yet know the half of it. Amid the crush of biological macromolecules and membranes in a cell, the water of the cytoplasm rarely achieves thicknesses of more than a few molecular layers. Might this confinement between macromolecular surfaces induce behaviour very different from that of the bulk liquid? A flurry of recent papers[1–3], in *Langmuir* and *Physical Review Letters*, lends support to the idea — but there is, as yet, no consensus in sight[4].

Liquid water is held together by a random, fluctuating, three-dimensional network of hydrogen bonds. This unique liquid-state structure is responsible for many of water's anomalies, and it has long been thought to play a role in the hydrophobic interaction. This attraction between hydrophobic surfaces in water is one of the key stabilizing forces of protein folding and of multi-subunit protein assemblies.

Close to a hydrophobic surface the hydrogen-bonding pattern of water is disrupted, without the compensatory interactions that might operate at hydrophilic surfaces. How does water cope with this loss of stabilization? The classic model of hydrophobic interactions[5] proposes that, around hydrophobes, water molecules lock into a more rigidly defined cage that preserves hydrogen bonding in an 'ice-like' solvation shell. The attraction then results from the gain in entropy as some of this solid-like water is liberated by the overlapping of solvation shells when the two hydrophobes come together. It is an appealing picture, but turns out not to be supported by any evidence of increased ordering of water around small hydrophobic species[6].

Modern theories of inhomogeneous fluids show that in fact one needn't invoke water's unusual structure to anticipate that strange things will happen at interfaces. Capillary condensation in narrow pores shows how confinement shifts the phase diagram of a fluid. Conversely, when the interactions between a liquid and a surface are relatively unfavourable (as with water and hydrophobes), there may be a lower fluid density at the interface, leading in the extreme case to the formation of a gas-like layer (a process known as 'drying').

Lum *et al.*[7] predicted that this is just what should happen between hydrophobic objects of various shapes (plates and cylinders, for example, approximating protein surfaces): if the surfaces are large enough and close enough together, capillary evaporation creates a bubble between them. This theoretical model included none of the geometrical nuances of a hydrogen-bonded network; it was generic to any confined fluid.

Yet water will not give up its special status so readily. There is, for example, the mystery of the long-range hydrophobic attraction. Distinct from the shorter-range force that binds proteins, this interaction between hydrophobic surfaces seems to extend over distances of up to 100 nm — equivalent to many hundreds of molecular diameters. It has compelled some researchers to seek explanations, seemingly fantastic at face value, in terms of some kind of extended, collective ordering of the intervening water molecules.

The long-range hydrophobic interaction was potentially unified with the issue of drying at a single interface by the suggestion of Attard and co-workers[8] that the force might arise from the growth and bridging of submicroscopic bubbles between the surfaces. The bridging meniscus would pull the surfaces together. Tyrrell and Attard reported[9] that, using an atomic force microscope (AFM), they had observed pancake-shaped 'nanobubbles' on hydrophobic surfaces.

Might the formation of these bubbles be promoted by partial drying at the interface — that is, by depletion of water density in this region? At least two of the new studies support this notion. Noting that in the earlier work the AFM itself might have nucleated the bubbles, Steitz *et al.*[1] have used a less invasive technique — neutron reflectivity — to look at the interface. Neutrons are strongly scattered by deuterium (D), and the researchers look at 'heavy' water, D_2O, in contact with deuterated polystyrene, a hydrophobic polymer. The scattering densities of these two substances are nearly equal, so any inhomogeneities at the interface should show up in an otherwise 'uniform' sample.

Steitz *et al.* see a surface layer 2–5 nm thick with a density about 6–12% lower than that of bulk D_2O, which they interpret as evidence of partial drying. (It is hard, however, to rule out the possibility of an apparent density deficit arising from surface migration of protonated impurities, which scatter neutrons more weakly.) They also use an AFM to reveal a surface covered with flat bubbles, each 50–120 nm wide and up to 18 nm high. They propose that the thin depletion zone acts as a precursor to the nanobubbles (which might still be nucleated by the AFM tip itself).

Jensen *et al.*[2] find a similar depletion zone in X-ray reflectivity measurements of the interface of a heavy-alkane monolayer floating on the surface of water. Again, the density depletion is around 10% of the bulk water density, corresponding to about one H_2O molecule for every 0.25–0.30 nm^2 of surface. But the depletion layer appears to be thin — less than 1.5 nm, perhaps reduced by 'capillary waves' that ruffle the surface of the free monolayer.

Another neutron-reflectivity study has been conducted by Schwendel *et al.*[3], who confess that their studies on contrast-matched mixtures of D_2O–H_2O against alkylated surfaces show a density deficit in the first 2 nm or so that is so large as to be unphysical. They suspect that nanobubbles or other air inclusions might be distorting the results. In a personal communication, Thomas and co-workers indicate that they have also found a water depletion layer at a hydrophobic surface using neutron scattering, extending over a thickness of about 1.8 nm. They are confident that their surface is free of nanobubbles, and fairly sure that protonated impurities are not responsible for the result.

These studies are starting to produce a consistent picture of hydration at hydrophobic interfaces: a thin layer of low-density, somewhat gas-like water that precedes complete capillary evaporation between two such surfaces, and helps to nucleate nanobubbles that create an apparently long-range attraction. But that may not be the end of the story. For example, Yaminsky and Ohnishi[4] argue that experimental imperfections (protrusions from the surfaces), rather than nanobubbles, create the long-range hydrophobic force, and claim that their surface-force measurements show nothing inconsistent with the standard theory of colloidal interactions — that is, nothing unique to water — down to separations of 3 nm or so (at which point the attraction between the surfaces is so great that they spring into contact).

Moreover, would we necessarily expect 'depleted' water to be really more gas-like, or instead more ice-like, with enhanced ordering, as originally supposed[5]? Evidence of ice-like water inside a (hydrophobic) carbon nanotube at room temperature has recently been seen in simulations[10]. And what are the consequences for the hydration environment of proteins, where, for example, a local change in water density might be expected to affect important quantities such as pH and salt concentration? In other words, what does the physics of confined water have to say about cell biology? ■

Philip Ball is a consultant editor for Nature.

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Molecular biology

Complicity of gene and pseudogene

Jeannie T. Lee

'Pseudogenes' are produced from functional genes during evolution, and are thought to be simply molecular fossils. The unexpected discovery of a biological function for one pseudogene challenges that popular belief.

Pseudogenes are defective copies of functional genes that have accumulated to an impressive number during mammalian evolution[1]. Dysfunctional in the sense that they cannot be used as a template for producing a protein, pseudogenes are in fact nearly as abundant as functional genes[2,3]. Why have mammals allowed their accumulation on so large a scale? One proposed answer is that, although pseudogenes are often cast as evolutionary relics and a nuisance to genomic analysis, the processes by which they arise are needed to create whole gene families[4], such as those involved in immunity and smell. But are pseudogenes themselves merely by-products of this process? Or do the apparent evolutionary pressures to retain them hint at some hidden biological function? For one particular pseudogene, the latter seems to be true: elsewhere in this issue (page 91), Hirotsune and colleagues[5] report the unprecedented finding that the *Makorin1-p1* pseudogene performs a specific biological task.

Hirotsune *et al.*[5] had been analysing mice in which copies of a fruitfly gene called *Sex-lethal* were randomly inserted in the mouse genome. In the course of their studies, they encountered one mouse line that died shortly after birth from multi-organ failure. As this occurred in only one mouse line out of many, the results could not be explained by aberrant *Sex-lethal* expression. Instead, the authors attributed their finding

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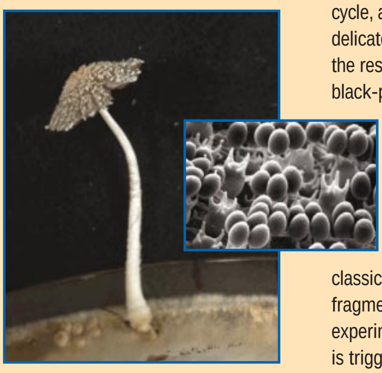
Genetics

Suicidal mushroom cells



URSEL KÜES; INSET, DAVID MCLAUGHLIN

Programmed cell death — apoptosis — is a universal phenomenon among multicellular organisms, and is especially important during development. Genetically orchestrated mechanisms of cell death have also been found in single-celled protists and yeast. Writing in *Fungal Genetics and Biology* (**39,** 82–93; 2003. doi: 10.1016/S1087-1845(03)00024-0), Benjamin Lu and colleagues describe a remarkably simple version of apoptosis in the ink-cap mushroom *Coprinus cinereus* (pictured).

Lu *et al.* studied apoptosis in mutant strains of the fungus that have defects in spore formation. Spores are formed by meiosis, which is the same type of cell division that halves the number of chromosomes in the egg and sperm cells of animals. In the mushroom, meiosis occurs in a synchronous fashion, sweeping across the gill surfaces underneath the cap, reconfiguring and sorting the chromosomes within 10 million spore-producing cells called basidia (inset). Mutants of *C. cinereus* called 'white-caps' are infertile: their basidia show defects at various points in the cell cycle, and the ashen hue of their delicate umbrellas is caused by the resulting failure to form black-pigmented spores.

It turns out that, in the mutants, basidia that experience problems at the beginning of meiosis (prophase I) undergo mass apoptosis, showing the classical apoptotic hallmark of DNA fragmentation. Lu and colleagues' experiments suggest that apoptosis is triggered at a single checkpoint in the mushroom cell cycle. This contrasts with the situation in the mouse, where the switch that activates apoptosis can be tripped at many steps throughout meiosis, implying that there is an almost continuous molecular assessment of the viability of the gamete-forming cells. Not surprisingly, it seems that mushrooms lack some of the developmental sophistication of mammals.

But why should apoptosis occur in mushrooms at all? Resource conservation is a likely explanation. Mushrooms disperse astonishing numbers of airborne spores, but there is an infinitesimal chance that any individual spore will reach a suitable patch of ground, survive, out-compete the resident microbes, grow and one day find a mate. So by aborting any basidia that have mishaps early in meiosis, the fungus conserves resources for healthy cells that will succeed in producing viable spores. **Nicholas P. Money**

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