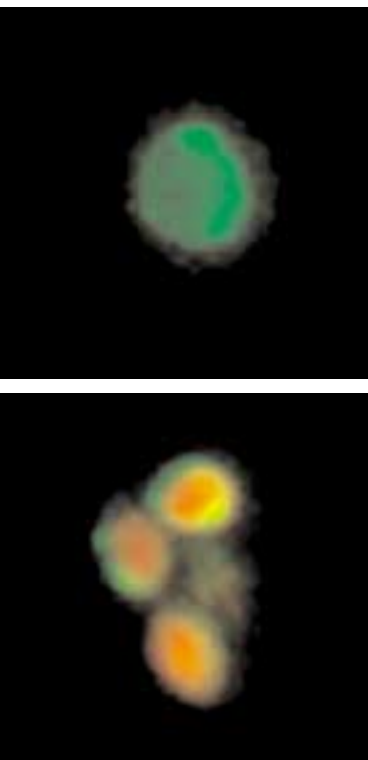


*Microbiology Today* Editor Meriel Jones takes a look at some papers in current issues of the Society's journals which highlight new and exciting developments in microbiological research.

BELOW:  
Annexin V-PI staining of *Saccharomyces cerevisiae* exponentially growing cells exposed to 20 mM (upper) or 120 mM (lower) acetic acid for 200 min. The cell in the top panel is stained only in green by annexin V, but not in red, indicating that it is in an early stage of apoptosis. On the contrary, the cells in the lower panel are stained both green and red, indicating that they are in advanced apoptosis or in necrosis.  
PHOTO COURTESY M. CORTE-REAL, BRAGA, PORTUGAL



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## Programmed to die

Dying cells have become one of the most exciting topics in biology in the last decade. Once researchers realized that the highpoint in the life of some cells was to die at exactly the right moment, they saw many phenomena with new eyes. The cells between the fingers in a human embryo have to die, neatly and on time, to form a hand, and plant cells surrounding one infected by a bacterial disease die to prevent the disease spreading. The way in which these cells die is so characteristic that the phenomenon is called programmed cell death.

In hindsight, the value to multicellular organisms of cells dying to sculpt the shape of an embryo, or to confine a pathogen, is obvious. For a single-celled organism, programmed suicide seems much less appealing. However, the sacrifice of some individuals might be advantageous to the rest of a unicellular population, if, for example, it stopped the spread of a virus. Nevertheless, the idea that everyone's favourite yeast, *Saccharomyces cerevisiae*, opts to die in particular circumstances, is still controversial. Although some researchers have recorded the characteristic signs of an organized death in yeast cells experiencing oxygen stress, not everyone is convinced.

Researchers from the Universidade do Minho and Instituto de Biologia Molecular e Celular in Portugal have now reported their studies into the death of *S. cerevisiae* in dilute acetic acid, because this also has the hallmarks of programmed cell death. Yeast is very familiar with acetic acid, because it is a normal end product of its alcoholic fermentation. The exact amount of the acid is crucial. Yeast cells certainly die when the concentration is above 80 mM, but in a messy way that has nothing to do with programmed cell death. However, at 40 mM, death happens in a very different way. Each cell's chromosome condenses and is then chopped into neat pieces as the cell's membrane subtly rearranges, in the type of events typical of programmed cell death in animal cells. As extra support, the researchers found that adding a chemical that prevents yeast making new proteins delayed death, implying that new proteins have to be made to carry out the neat, but lethal, process. This adds to the emerging picture of how even a unicellular yeast can organize its own death.

Ludovico, P., Sousa, M.J., Silva, M.T., Leao, C. & Corte-Real, M. (2001). *Saccharomyces cerevisiae* commits to a programmed cell death process in response to acetic acid. *Microbiology* **147**, 2409–2415.

## Breaking down the wall

Although the yeast *Saccharomyces cerevisiae* benefits the human race by making both bread and beer possible, other fungi with a single-celled life style are much less benign. One of them is *Candida glabrata*, an opportunistic pathogen that is now the second most common cause of systemic candidosis. It infects people who are already ill and makes a difficult situation much worse. The patients often die, partly because there is no really effective treatment. *C. glabrata* shrugs off most antifungal drugs. The hunt is therefore on for new compounds that will be lethal to this fungal pathogen.

One obvious target is the yeast's cell wall. This very dynamic structure surrounds each cell, and as well as being responsible for its shape, it mediates interactions with the environment, including any unfortunate human host. Features of the way the wall is synthesized and re-modelled are unique to fungi, and researchers hope that they can use this to design more effective antifungal drugs, or at least better diagnostic tests so that there is more time for doctors to act against infections.

*S. cerevisiae* cell walls contain a protein called Gas1, which is essential for maintaining the correct levels of sugar polymers in the cell wall. Without it, the cells lose their normal spherical shape. *S. cerevisiae*, in fact, contains five genes capable of producing a protein very similar to Gas1, but only one of them actually works. *Candida albicans*, the yeast that most commonly causes candidosis, has only two genes to produce this sort of protein, but both can work, depending on the level of acidity around the cells.

Fritz Mühlischlegel, who has recently moved to the University of Kent at Canterbury from Würzburg University, and his colleagues in Germany and Imperial College in London, wondered which system was used in *C. glabrata*. They looked for DNA sequences in this yeast that closely matched the GAS genes of the other yeasts, and found three. Two of them, which they called *CgGAS-1* and *CgGAS-2*, were used to produce proteins all the time, and in all the environments that the researchers tested. The third gene did not appear to work.

The researchers designed experiments to discover what happened if these two genes stopped working, either individually or both together. When they removed only one, the appearance of the yeast cells changed. They grew more slowly and stuck together. However, try as they might, the researchers could not knock out both of the genes. They never obtained living cells from experiments designed to make this happen. They think that a lack of both these genes is lethal and want to test this idea in more complicated experiments designed so that each gene can be switched on or off at will by the researchers. If these proteins do turn out to be essential for survival of this pathogen, they may be good targets for new antifungal drugs.

Weig, M., Haynes, K., Rogers, T.R., Kurzai, O., Frosch, M. & Mühlischlegel, F.A. (2001). A GAS-like gene family in the pathogenic fungus *Candida glabrata*. *Microbiology* **147**, 2007–2019.

## Immunobiology of TSEs

One of the many puzzles about mad cow disease (BSE) is exactly how an animal catches it. Even though a similar disease of sheep, called scrapie, has been around for centuries, the way in which it is transmitted is equally unknown. A major component of the agent that causes BSE, scrapie and several other degenerative diseases of the brain, collectively called transmissible spongiform encephalopathies (TSEs), appears to be an abnormal form of a host protein called PrP<sup>c</sup>. It is found in all animals, and an abnormal version (PrP<sup>Sc</sup>) seems responsible for TSEs, by subverting PrP<sup>c</sup> into the lethal PrP<sup>Sc</sup> form. Consumption of contaminated feed was almost certainly the way that it entered the cows' bodies, but how did it then manage to cause fatal changes in their brains? After all, every mouthful that we eat is laden with bacteria, viruses, fungi and protozoa, not to mention the complex chemicals in plants. Although most are harmless, there are sophisticated systems to eliminate any threats. One of these is the immune system, which identifies and destroys anything foreign within the body. Neil Mabbott and Moira Bruce at the Institute for Animal Health in Edinburgh have been reviewing how much we know about the way that TSEs slip past the immune system.

The key seems to lie within the lymphoid tissues. These collect and filter fluid containing a mixture of cells and dissolved solutes that accumulate within all the tissues of the body. Many of these cells play a part in the immune system in complex interactions that result in the

destruction of foreign materials. Pinning down the exact role for each cell-type in TSE disease can therefore be difficult. A further complication is that all TSEs do not seem to take the same route from gut to brain, with some studies giving directly contradictory results. The reviewers think that although this can sometimes be explained by differences in the way the experiments were carried out, there is also good evidence of real differences between different TSE diseases, and also different strains of the same TSE. For example, the lymphoid tissues associated with the gut are the first place that PrP<sup>Sc</sup> appears in animals infected with scrapie, but in BSE it seems confined to nervous tissue.

One particular type of lymph cell, called the follicular dendritic cell (FDC), turns out to contain high levels of the normal PrP<sup>c</sup> protein, and so researchers have suspected that this might be where the abnormal form is generated. FDCs make an ideal site for multiplication of PrP<sup>Sc</sup> because they are long-lived cells containing a high level of PrP<sup>c</sup> that are specialized to trap and retain molecules on their surface. A further indication of the crucial role of FDCs comes from experiments with mice able to, or unable to, synthesize PrP<sup>c</sup> on their FDCs. In these experiments, scrapie only accumulated in the spleen if the FDCs expressed PrP<sup>c</sup> on their surface. Scrapie did not accumulate in the spleens of mice in which PrP<sup>c</sup> was expressed on lymphocytes alone.

The only other cells with lots of PrP<sup>c</sup> are in the nervous system, where PrP<sup>c</sup> may play an important role in

neurotransmission and sleep patterns. However, the normal function of PrP<sup>c</sup> in FDCs is not obvious. One suggestion is that it helps protect them from oxidative damage during their long lives. The normal work of FDCs is as part of the system that detects and destroys any foreign molecules within an animal. Once invaders have been identified, by becoming attached to antibodies or other proteins, they can be trapped on the surface of FDCs. This is important for the development of a strong antibody response to the invaders. Recent work strongly suggests that the ability of FDCs to trap foreign molecules is hijacked by TSEs.

When a TSE infection has spread to the central nervous system it may be too late to reverse the neurodegenerative effects. However, treatments that interfere with the early stages of infection can significantly impair the spread of the disease to nervous tissue. With the accumulating information on the importance of FDCs in the amplification of PrP<sup>Sc</sup>, researchers are naturally thinking of treatments involving this early stage. One vulnerable point is the maturation of the FDCs, which is dependent on a series of signals from B lymphocytes. These are essential for maturation of FDCs and animals without B lymphocytes are particularly resistant to scrapie. Taking this to even greater detail, researchers showed that lack of one particular signal molecule sent from B lymphocytes to FDCs is enough to make an animal resistant. In another experiment, the researchers blocked a different signal from the B lymphocytes,

which resulted in the temporary disappearance of all FDCs from the animal for a time, and this simultaneously also reduced the animal's susceptibility to scrapie.

There remains the one final step in any TSE infection, which is when it begins to affect the brain. This relies on the PrP<sup>Sc</sup> moving on

from the lymphoid tissues to nerves, but the way in which this happens is only now being determined. Although researchers have made considerable progress in understanding this distressing disease, it retains many of its secrets.

**Mabbott, N.A. & Bruce, M.E. (2001).** The immunobiology of TSE diseases. *J Gen Virol* **82**, 2307–2318.

## Metal-resistant bacteria

Some bacteria are remarkably resistant to toxic metals, such as mercury, lead, cadmium and nickel. The genus *Ralstonia* appears to have more than its fair share. Scientists have been assessing them for exploitation to recycle polluted soils, treat wastewater or to simply detect the presence of excess toxic metals. However, one member of the genus is an important plant pathogen and several are opportunistic pathogens of humans. Clearly, before using any *Ralstonia* on a large scale in the environment everyone needs to be assured that it does not share these pathogenic characteristics.

One of the difficulties with bacterial classification is that as new species are identified, a genus that once had only a few, very distinctive members can change. Several new species have been added to *Ralstonia* in recent years and they suggest that the genus is much more diverse than anyone had suspected. Belgian researchers, led by Johan Goris at the University of Gent, decided that it was time to check how well the features used to identify *Ralstonia* species actually distinguish each one. They subjected 54 strains of *Ralstonia* to a battery of tests that ranged from classical biochemical ones to more modern methods that check the sequence of genes, the pattern of all the cell proteins or the type of fats within the cells. The final step was to run the information through a program that groups strains together based on the similarity of their results in each test. To make this easy to visualize, the program drew a tree-like diagram, called a dendrogram. The most similar strains appear on adjacent twigs, but were joined to the others on more distant branches.

When the dendrogram based on protein patterns was examined, the clusters of strains did not always correspond to the names already attached to them. Together with their data on the DNA of the bacteria, and the biochemical tests, the researchers felt confident that they were dealing with at least two new species, whose similarity to other *Ralstonia* species had let them remain undetected before this investigation. One of these contained many of the toxic-metal-resistant strains and so was named *Ralstonia metallidurans*. The other contained several strains from the Campine region in north-east Belgium, so they called it *Ralstonia campinensis*.

**Goris, J., De Vos, P., Coenye, T., Hoste, B., Janssens, D., Brim, H., Diels, L., Mergeay, M., Kersters, K. & Vandamme, P. (2001).** Classification of metal-resistant bacteria from industrial biotopes as *Ralstonia campinensis* sp. nov., *Ralstonia metallidurans* sp. nov. and *Ralstonia basilensis* Steinle et al. 1998 emend. *Int J Syst Evol Microbiol* **51**, 1773–1782.

## Detecting pre-clinical BSE in sheep

Although there is now only very low level, or no, BSE contamination in the UK food-chain from bovine sources, the consequences of previous exposure are still being felt. For example, the UK national sheep flock may also have received BSE-contaminated feed and so, theoretically, the disease could now be present in sheep. Since BSE in sheep may well have similar clinical symptoms to scrapie, their natural TSE, it would be good to have a reliable method to differentiate the two diseases to be able to test this possibility.

James Foster and his colleagues at the Institute of Animal Health Neuropathogenesis Unit in Edinburgh have been trying to find out which tissues within sheep harbour the abnormal prion protein of BSE, called PrP<sup>Sc</sup>. They used sheep that came from other long-term experiments. These sheep had been deliberately fed BSE and symptoms have so far started to appear between 1.5 and 3 years from the start of the experiment, although some of the sheep are still healthy. One of the conditions of these experiments is that the sheep will be humanely killed if they start to show signs of the distressing symptoms of BSE.

The researchers have examined a large range of tissues from the sheep, using an immunochemical staining method that can detect very small amounts of PrP<sup>Sc</sup>. All had very extensive vacuolation in their brains, although it was most obvious in parts of the brainstem. In addition, the researchers could detect PrP<sup>Sc</sup> in central and peripheral nervous tissue, and in many lymphoid tissues throughout all of the animals. The distribution and level of PrP<sup>Sc</sup> fitted with the idea that the infectivity travelled from the digestive system to the nerves, although the intensity of the stain was much greater than in cattle with BSE. Other major organs, like the heart, lungs, liver and muscles, seemed free of the prion protein. The nictitating membrane from sheep's eyes has been proposed as a good location site for a routine test, but the researchers could only detect PrP<sup>Sc</sup> there in two of four diseased sheep.

However, the key question was whether disease caused by BSE could be reliably distinguished from scrapie. The researchers still cannot give a firm answer, because they do not have enough detailed information on the distribution of PrP<sup>Sc</sup> in sheep with natural scrapie. From the limited comparisons they have been able to make, there are some subtle, but suggestive differences, such as more intense staining in the brainstem in sheep infected with BSE. In addition, the intense staining in the sheep's lymphoid and nervous tissues may provide a way of detecting pre-clinical cases of BSE in sheep much more easily than in cattle.

**Foster, J.D., Parnham, D.W., Hunter, N. & Bruce, M. (2001).** Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission. *J Gen Virol* **82**, 2319–2326.

## Prion wasting disease in deer

Edward Hoover of Colorado State University, along with colleagues in veterinary medicine and wildlife management, and in collaboration with the Swiss company Prionics AG, has been studying the distribution of the abnormal form of a cellular protein, PrP<sup>CWD</sup>, in one of the natural TSEs of wild animals. The one they chose was chronic wasting disease (CWD) in the mule deer that roam wild in Colorado and Wyoming, USA. Even though this can affect up to 15 % of the deer in an area, relatively little is known about transmission and progress of the disease. The hypothesis was that the deer eat the abnormal PrP<sup>CWD</sup>, and this then travels from the digestive tract to the alimentary nerves, and then to the brain.

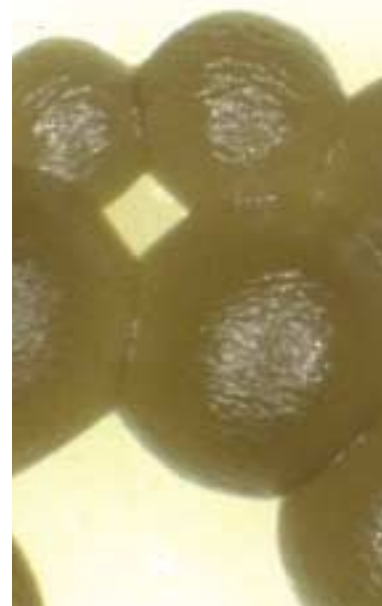
Of course, it is always good to have some data to test such assumptions, and this is now possible because there are methods to detect PrP<sup>CWD</sup> within tissues. These rely on the ability to create an immune response against the prion protein and then use these antibodies to test for PrP<sup>CWD</sup> in samples of tissue from symptomatic mule deer with naturally occurring chronic wasting disease. The brains of all the deer had the spongiform degeneration that is the hallmark of this disease, along with easily detectable PrP<sup>CWD</sup>. The researchers chose to look at nerves that were associated with the alimentary tract, and others like the sciatic nerve which have nothing to do with the digestive system. They also examined other tissues from the regions that they anticipated would be most affected by PrP<sup>CWD</sup>.

Mule deer have two major autonomic nerve tracts associated with their digestive systems. The vagosympathetic trunk includes nerves that connect with the myenteric plexus, a nerve centre within the small intestine. The splanchnic nerves reach to the oesophagus, stomach and small intestine. All the deer they tested had PrP<sup>CWD</sup> in the nerves of the vagosympathetic trunk, and also in most myenteric plexuses. The researchers were rather surprised that there was little evidence of PrP<sup>CWD</sup> in the splanchnic nerves, although they detected PrP<sup>CWD</sup> in the adrenal medulla; the best explanation for its presence there was that it had been transported via the splanchnic nerves. In contrast, as they expected, there was little sign of PrP<sup>CWD</sup> in tissues like the sciatic nerve that are distant from the digestive system or in deer from a geographic region without chronic wasting disease.

These results fit with the idea of PrP<sup>CWD</sup> travelling within nerves to reach the brain, but also PrP<sup>CWD</sup> being able to reach other organs within the deer. They are piece by piece building up a full picture of this disease.

**Sigurdson, C.J., Spraker, T.R., Miller, W., Oesch, B. & Hoover, E.A. (2001).** PrP<sup>CWD</sup> in the myenteric plexus, vagosympathetic trunk and endocrine glands of deer with chronic wasting disease. *J Gen Virol* **82**, 2327–2334.

RIGHT:  
'Pearls' (approx. 1–5 mm diam.) formed by colonies of the cyanobacterium *Nostoc commune* growing on calcium carbonate-supplemented agar. The dark-brown coloration is due to the UV-absorbing pigment scytonemin. PHOTO SUPPLIED BY MALCOLM POTTS, VIRGINIA TECH CENTER FOR GENOMICS, BLACKSBURG, USA



## Pearls of cyanobacterial wisdom

Sometimes the word microbe seems inappropriate. For example, patches on limestone rock that are blackened and crispy when dry, but green and gelatinous when wet, have been confidently identified and collected as *Nostoc commune* for over 200 years. Its visibility comes from the enormous number of individual cells in the colony in an environment where few others can compete. This conspicuous slimy organism was once considered to be a relation of seaweeds, but it is now known to be a member of the Cyanobacteria, a group of photosynthetic bacteria. It is a conspicuous component of the terrestrial microflora all over the world, especially in nutrient-poor soils.

However, some aspects of *N. commune*, and other cyanobacteria, are still a puzzle for scientists. One of these is how to identify it. Although it may be distinctive when growing on a rock in the wild, once researchers take it home to the lab, its appearance and behaviour can change so completely that it has acquired other names in culture collections. Consequently, researchers have tried the full arsenal of

molecular taxonomic methods in search of a definitive identification method. One that seems particularly useful uses the group I introns within some genes in these bacteria. Introns are regions of a gene that are clipped out and discarded from the RNA that forms a gene's working copy within a cell. They are very common in eukaryotes, but more unusual in bacteria. The acquisition, transfer and distribution of introns is both important in understanding the evolution of species, and controversial.

Malcolm Potts and his colleagues at the Virginia Polytechnic Institute and State University in the USA realized that *N. commune* could provide unique information on this topic. This is because there are specimens dating from the 1850s in herbaria, often accompanied by detailed records of their time and place of collection. Some even appear to have lain undisturbed since they were deposited. There are few other bacterial species where well-documented specimens are available from before the era of antibiotics and rapid world travel, and even from before

the Industrial Revolution.

The researchers were able to examine samples that had been stored in the Wien Herbarium, Austria, since the 1860s along with others collected more recently from continents as far apart as Antarctica, Australasia, Europe and the Americas.

After extracting the minute amount of DNA in these dried cells, they used the polymerase chain reaction to amplify any introns in one of the genes and finally recorded the DNA sequence of the introns. The researchers also scoured the scientific databases for any additional sequences from strains that had already been published to include in their study. Overall, 25 of the samples collected by several investigators during two centuries turned out to have extremely similar introns, fully justifying their identification as *N. commune*. Although there were small differences between the introns in many isolates, this did not seem to relate to factors like geographical distribution. For example, two isolates collected in Virginia, USA, had an identical intron to one collected in Java 118 years earlier. A more critical look at the way the isolates could be clustered using the small differences between their introns gave the impression that they formed a continuum, punctuated by clusters with ill-defined borders. This reinforces the value of morphology in identifying at least this cyanobacterial species.

**Wright, D., Prickett, T., Helm, R.F. & Potts, M. (2001).** Form species *Nostoc commune* (Cyanobacteria). *Int. J. Syst. Evol. Microbiol.* **51**, 1839–1852.

## Biofilm formation in the cystic fibrosis lung

It may be convenient for microbiologists to grow bacteria as uniform suspensions in a liquid medium, but in nature bacteria exist predominantly as communities on surfaces, called biofilms. Many bacterial infections owe their success to the production of a biofilm that shields the cells from both their unwilling host's immune system and antibiotics. The lungs of patients suffering from cystic fibrosis can become coated with a layer of bacteria embedded in their own polysaccharide matrix. One of the bacteria in these biofilms is *Burkholderia cepacia*, and understanding how it forms a biofilm may help devise new strategies for treatment.

In tests on mutants of *B. cepacia* that had lost the ability to form biofilms, a group of Danish and German researchers realized that one mutant had simultaneously developed a defect in the *cep* quorum-sensing system. This system enables a cell to detect the number of other bacterial cells in its vicinity. In *B. cepacia* this works through the production of a chemical called a homoserine lactone (involving the protein CepI), which can be detected by a sensor (called CepR) within each cell. Once the sensor detects enough homoserine lactone, it switches on a suitable response. For a pathogen, this might be something that starts an infection, because there are now enough bacteria to overwhelm the host before it can mount an efficient counter-attack.

The researchers wanted to find out how lack of either CepI or CepR stopped a biofilm being formed. So they created mutants that not only lacked either CepI or CepR, but also glowed through production of a green fluorescent protein. The way that these mutants grew on microscope slides was intriguing. Both normal and mutant cells first covered the slides with tiny microcolonies, but that was where the mutants stopped, while the normal cells went on to develop into a thick, rough layer. When the researchers repaired the malfunction in the quorum-sensing system by adding homoserine lactone to the mutant that could not synthesize it, the cells now formed a biofilm that was indistinguishable from the one made by normal cells.

The nature of the link between quorum-sensing and growth in a biofilm remained elusive, but the researchers already knew some properties that bacteria must have to form a biofilm. The ability to move is very important. *B. cepacia* has flagella that it uses for swimming, so the scientists focused on checking whether the *cep* system was also involved in controlling motility. The cells could still swim, but the researchers spotted that *B. cepacia* could also move by swarming across a surface in a thin film of biological detergent secreted by the cells themselves. The mutant cells were unable to do this, probably because they were unable to secrete the detergent. So although quorum-sensing is not essential for starting the formation of a biofilm, it may be crucial for finishing it.

**Huber, B., Riedel, K., Hentzer, M., Heydorn, A., Gotschlich, A., Givskov, M., Molin, S. & Eberl, L. (2001).** The *cep* quorum-sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. *Microbiology* **147**, 2517–2528.

