How important is it?

Today, half a billion people worldwide are affected by this deadly infection taking millions of lives each year. To combat this pandemic, only a few antimalarial drugs are currently available and no successful vaccine has yet been developed. Threateningly, drug-resistant plasmodium strains have appeared in recent years, therefore, comprehensive understanding of *P. falciparum* developmental genetics is essential for the rational design of vaccines and better medicines in the fight against malaria.

Trenholme is confident that these findings will enable researchers to investigate new multiple targets, which they might not otherwise have been aware of. Aymami continues: 'Much work has to be done for identifying key plasmodium transcription factors and which DNA sequences they like. It is interesting to note that some antimalarial drugs are DNA intercalators and it would be useful to know which regulatory DNA sequences could be preferential drug targets.'

Elizabeth Winzeler from Scripps Institute (http://www.scripps.edu), who directed closely related research [5] published in parallel with the competitive DeRisi study, comments: 'New findings are important because the discovery of key parasite proteins for particular druggable processes, such as erythrocyte invasion or haemoglobin degradation, has occurred so far by trial and error.' However, she notes that without additional data from other plasmodium lifecycle stages, the DeRisi dataset is incomplete. Winzeler is convinced that only full-breadth analysis could realistically expand the range of therapeutic targets yielding more potential drug candidates.

No doubt these two studies are complementary. Llinás notes: 'Now, we [will] screen new drug libraries to identify potent IDC-type inhibitors.' Winzeler also plans to pursue some plasmodium targets with their in-house assays. Thinking of the future, Llinás adds: 'We intend to examine the gene-expression manifestation of parasite variability with strains differing in drug sensitivities. We are also interested in studying less characterized 'in-human' and 'in-mosquito' developmental stages.'

References

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News in brief

Targets and Mechanisms

To live or not to live? – a cell's question

Inappropriate activation or suppression of apoptosis can lead to degenerative pathologies or tumorigenesis, respectively. A newly discovered gene, which controls the life or death switch in cells, thus protecting them from dying, could help scientists identify and develop new drugs to combat degenerative diseases, such as Lou Gehrig's disease, and also the destructive effects of stroke and heart disease, autoimmune disease and cancer.

Scientists at the University of California, Santa Barbara (http://www.ucsb.edu/), have identified a gene in *Caenorhabditis elegans*, *ICD-1* (inhibitor of cell death gene 1), which prevents normal cells from committing suicide [1]. The study found that *ICD-1* is both necessary and sufficient to prevent apoptosis. *ICD-1* overexpression inhibits the apoptosis of cells that are normally programmed to die, whereas *ICD-1* loss results in inappropriate apoptosis in developing and differentiated cells in various tissues. However, the 'death pathway' is somewhat divergent from that used by cells whose normal fate is to die, suggesting that there are alternative avenues for the programmed death of cells under certain circumstances.

The protein encoded by *ICD-1* localizes to the mitochondrion, underscoring the role of mitochondria in coordinating apoptosis. '…the ICD-1 protein works in the organelle that is pivotal for the programmed suicide of cells,' says Tim Bloss, lead author of the paper.

It might sound alarming, but our cells are constantly poised on the brink of death. However, the constant death of some cells is essential for life. 'Death, that is to say cell death, is a key player in biology and medicine,' says Joel Rothman, leader of the research team. 'Cells often commit suicide so that others may live. This is the ultimate example of altruism at the cellular level.' By killing itself, an infected or malignant cells can prevent the spread of disease, however, a balance is

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essential, with excessive cell death resulting in degenerative diseases.

The initial discovery of *ICD-1* has led the research team to the discovery of other genes that similarly control the suicide of normal cells. The discovery of such genes will hopefully lead pharmaceutical companies to develop drugs for the future treatment of cancer and degenerative diseases.

1 Bloss, T.A. *et al.* (2003) Suppression of CED-3-independent apoptosis by mitochondrial βNAC in *Caenorhabditis elegans*. *Nature* 424, 1066–1071

Long life with your meal Sir?

Could a glass of red wine increase our lifespan? New research led by a team at Harvard Medical School (http://www.hardvard.edu) has brought us closer to such a possibility [2]. Molecules in everyday groceries have been identified and shown to mimic low-calorie diet and its beneficial effects of longer life in yeast. This finding could have far-reaching implications for potentially preventing age-related diseases in humans.

Calorie restriction creates cellular stress. In plants, polyphenols increase in response to stressful conditions, preparing the whole organism for imminent sub-optimal conditions by engaging in a more beneficial survival program.

When subjected to the compound resveratrol, which is a polyphenol found in red wine, *Saccharomyces cerevisiae* (bakers yeast) cells showed a 60–80% increase in lifespan. A similar, but not as pronounced, result was also obtained using another of the polyphenol group, flavones, which are found in olive oil. Similarly, cultured human cells treated with resveratrol showed increased survival when challenged with gamma radiation.

The mechanism by which these effects occur is not via the already established antioxidant qualities of polyphenols, but by stimulation of a family of enzymes called sirtuins. 'We think sirtuins buy cells time to repair damage,' said David Sinclair, co-author of the study. Whether in the round worm (SIR-2.1), yeast (Sir2) or human cells (SIRT1), the Michaelis constant of the sirtuins appears to be lowered when exposed to stress or these polyphenols. In humans, SIRT1-dependent deacetylation of the tumour suppressor gene p53 is stimulated also, thus increasing cell survival by turning this gene off. However, studies on calorie-restricted animals have shown that the corresponding increase in lifespan is not associated with higher rates of cancer.

There is still a long way to go, however. Studies using larger whole organisms, such as mice, will be the next step; and hopefully one day, an endogeneous sirtuin activator that naturally exists in humans will be found.

2 Howitz, K.T. *et al.* (2003) Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 24 August, DOI:10.1038/nature01960 [Epub ahead of print; http://www.nature.com]

Mutants to probe parasite persistence

The mystery surrounding the persistence of Leishmania major parasites in the human host after recovery could soon be uncovered. Researchers at Washington University School of Medicine (http://medschool.wustl.edu/) have engineered the parasites to lack phosphoglycans (lpg2-), finding that these mutant parasites are able to persist indefinitely, without causing disease, in genetically susceptible mice [3].

Leishmania parasites in normal persistent infections also have some safe 'niche', as the engineered mutants have. However, as Stephen Beverley, lead investigator of the study, explains, 'we don't know what these persistent parasites look like or how they interact with the immune system'. It is hoped that the mutant parasite could help to answer these questions.

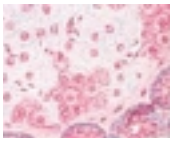
Approximately 12 million people are infected with Leishmania parasites worldwide but the development of leishmaniasis, a potentially fatal disease, is usually prevented by the immune system. However, in those with suppressed immunity, such as AIDS patients, the parasites become active and trigger the disease, hence the prevalence of leishmaniasis in AIDS endemic regions.

By comparison with normal parasites, the mutants showed a ten-times lower survival rate in sand flies, the parasite vector, and, interestingly, they were also unable to survive in macrophages, the cells that house the parasites in the mammalian host. The next step, therefore, is to identify what type of cell enabled the mutated parasites to survive in the murine hosts.

The 'L. major lpg2-' thus provides a means of studying the parasitic factors involved in persistence and their interaction with the immune system. This could potentially lead to the development of a vaccine against leishmaniasis; as Beverley concludes, 'those studies are early, but they look promising'.

3 Spath, G.F. *et al.* (2003) Persistence without pathology in phosphoglycan-deficient Leishmania major. *Science* 301, 1241–1243

The BAD side of diabetes



BAD, a protein that is known to be involved in apoptosis, might also have a role in some forms of diabetes, according to a recent report. Using mitochondria from liver cells, a research team led by Stanley Korsmeyer, of the Howard Hughes Medical Institute (http://www.hhmi.org/), have established that BAD exists within a complex alongside the enzymes that add or remove phosphate groups in the mitochondrial membrane [4].

A later stage of the study used mice with the BAD gene knocked out; Korsmeyer describes how 'the whole complex looks like it just fell apart'. More surprisingly, Nika Danial, lead author of the paper and fellow at the Dana-Farber Center Institute at Harvard Medical School (http://dfci.harvard.edu/), found the enzyme glucokinase – a key factor in the conversion of glucose to energy – within the complex. Further findings demonstrated that BAD was necessary for glucokinase to function normally, and the absence of BAD produced diabetes-like defects in glucose metabolism in the mice. Conversely, glucose deprivation resulted in dephosphorylation of BAD and BAD-dependent cell death.

It was previously believed that the two factors crucial to cell survival – metabolism and regulation of the apoptotic pathway – were separate events. However, the findings of this new study suggest that there is coordination and integration between the two pathways within the distinct multi-protein complex.

This research will continue, by exploring the question of whether BAD malfunction can actually cause diabetes, and whether defective BAD proteins might have a role in familial diabetes. As Korsmeyer explains, in the development of therapeutics targeting cell death in cancer and other diseases, 'there might be a role in regulating metabolism to alter a cell's susceptibility to apoptosis'.

4 Danial, N.N. *et al.* (2003) BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. *Nature* 424, 952–956

Penetrating the biochemistry of MALT lymphoma

By showing that Bcl10 is essential for B cell maturation, researchers are now a step closer to understanding and curing mucosa-associated lymphoid tissue (MALT) lymphoma, a relatively rare form of non-Hodgkin's lymphoma [5].

Previous research had provided a basic explanation of the causes of the disease. Marginal-zone B cells, an immune system cell predominantly located in the spleen, give rise to the cancer when they overexpress Bcl10 as a result of chromosomal translocation. Researchers led by a team from St Jude Children's Research Hospital (http://www.stjude.org/) decided to explore the relationship between Bcl10 and B cell function and maturation. They found that mice lacking the Bcl10 gene produced nearly normal numbers of precursor B cells, but these cells were unable to mature. This demonstrated an essential role for Bcl10 in cell maturation.

Delving deeper, they confirmed that Bcl10 activates a family of NF-κB proteins. These proteins are normally triggered after B cells encounter bacteria, or other invading organisms. Expression of NF-κB leads to maturation of B cells, and the release of antibodies to combat bacteria. When the system goes wrong and Bcl10 is overexpressed, NF-κB is continually activated. This causes the mature B cells to proliferate unchecked, eventually leading to MALT lymphoma.

The researchers linked this mechanism to immune response. LPS is a bacterial protein that normally stimulates B cell proliferation in the host. In the Bcl10-knockout mice, however, LPS failed to provoke this response. According to lead author Demin Wang, such insight into the workings of the Bcl10 gene 'brings us a step closer to understanding how to control its activity and cure MALT lymphoma.'

5 Xue, L. *et al.* (2003) Defective development and function of Bcl10-deficient follicular, marginal zone and B1 B cells. *Nat. Immunol.* 4, 857–865

New approach to identifying protein phosphorylation sites

A group of scientists based at University of California, San Francisco (UCSF; http://www.usfca.edu/) have revealed a novel method for mapping phosphorylation sites on proteins by using specific proteases to recognize and locate these sites on the protein themselves [6].

As Zachary Knight, lead author of the paper, explains, 'Every cellular process is controlled at some level by protein phosphorylation… It is the language by which cells communicate and make decisions'. When phosphates do not bind correctly to proteins, or not at all, life-threatening diseases can result. Insufficient phosphorylation can lead to diabetes by dampening the cellular effects of insulin, whereas aberrant phosphorylation helps cancer cells to avoid normal death.

Kevan Shokat, Professor of Cellular and Molecular Pharmacology at UCSF and Professor of Chemistry at UC Berkeley, revealed that the research group were able to chemically transform a protein by shedding phosphates from an amino acid and converting the molecule into one that resembles lysine. Lysine is the favoured amino acid target for trypsin. The protease used was then 'tricked' into cutting the transformed protein at a site that it would not normally recognize and, in doing so, pinpointed the location of the specific phosphate group.

Shokat comments: 'This finding will allow us, for the first time, to quickly identify all the changes in protein phosphorylation that occur in a cell… Since phosphorylation controls so many biological processes and is involved in so many diseases, mapping the sites where it takes place will identify new therapeutic targets'.

Indeed, if scientists can map the phosphorylation sites in a particular protein of interest, they should be able to link specific phosphate-bonding patterns with specific diseases. Drugs could be then tailored to block the specific version of protein kinase that normally carries the phosphate to that site.

6 Knight, Z.A. *et al*. (2003) Phosphospecific proteolysis for mapping sites of protein phosphorylation. *Nat. Biotechnol.* 21, 1047–1054

Viral Targets and Mechanisms

Cloak of human proteins helps HIV evade and invade

The accepted model of how HIV infects human cells is being challenged. A new theory suggests that the retrovirus hides behind a cloak of human proteins to elude the immune system [7].

Scientists normally focus on viral proteins when probing HIV mechanisms or developing vaccines. However, this standpoint does not account for several puzzling facets of HIV infection. For example, a version of the virus that is missing a key envelope protein has been shown to retain some infectivity in laboratory cell studies, despite conventional wisdom suggesting that this should be impossible.

Such anomalies led researchers from the Johns Hopkins Medical Institutions (http://www.hopkinsmedicine.org/) to revisit the model of how HIV infects human cells. They theorized that the retroviral particle behaves like an exosome – a tiny pocket derived from cell membranes that ferries molecules between cells. By carrying a cloak of human proteins similar to those found on exosomes, the virus is able to evade the immune system and readily invade new cells. The idea has been dubbed 'the Trojan exosome hypothesis' by its proponents.

Some clinical reports have already hinted in this direction. HIV is more likely to pass between people with similar blood and tissue types than those with little match, indicating human proteins are a factor. 'The Trojan exosome hypothesis explains why retroviruses carry the human proteins they do, why they are able to survive even in people with healthy immune systems, and why traditional approaches have failed to generate an effective HIV vaccine,' concluded Stephen Gould, one of the researchers in the study. If correct, the hypothesis could lead to a new wave of vaccines that target the implicated human proteins, rather than viral particles.

7 Gould, S.J. *et al.* (2003) The Trojan exosome hypothesis. *Proc. Natl. Acad. Sci. U. S. A.* 100, 10.1073/pnas.1831413100 (Epub ahead of print; http://www.pnas.org)

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