News in brief

Targets and mechanisms

The life of prion

Two important questions in prion disease research might now have answers: how do prions actually kill nerve cells and how do mutated prions, implicated in disease transmission, arise? Susan Linquist of the Whitehead Institute (http://wi.mit.edu) and Jiyan Ma of the Ohio State University (http://www.ohio-state.edu) have suggested a compelling new theory that accounts for these phenomena [1,2].

Prion diseases are a growing concern. Approximately 150 young people have died from CJD and, because of the long incubation time, no one knows for sure how serious an epidemic this could become. Although prion proteins (PrPs) are known to be the causative agents of such neurodegenerative diseases, their precise role is uncertain.

PrPs are made by all mammals and are normally localized on the cell surface. A misfolded version of the protein, known as PrP^{Sc}, is found only in the cytosol of mammals with transmissible prion diseases. Once PrP^{Sc} is present, it promotes the conversion of further PrP molecules to the misfolded state. But the trigger for initial PrP^{Sc} formation is unknown and studies in mice have shown that PrP^{Sc} is not directly responsible for killing neurons.

When PrP is misfolded, it is intercepted in the cytosol by the proteasome complex, which degrades PrP to its constituents. If the proteasome is compromised, which occurs naturally in old age or during stress, misfolded PrP will accumulate in the cytosol. Using inhibitors to block the proteasome, the researchers found that if PrP accumulated fast enough, it could convert to the infectious PrP^{Sc} form. This explains, for the first time, the trigger for PrP^{Sc} formation. However, it does not explain the mechanism of cell death; the neuronal death rate was independent of the PrP^{Sc} levels and some neurons died even before PrP^{Sc} clumps formed.

Further experiments in mice showed that the toxic species is soluble PrP in the cytosol. 'We think this toxicity wasn't realized before simply because such small amounts of PrP are in the cytosol and other forms of the protein are present in much higher concentrations,' said Lindquist.

Proteasome inhibitors have been tested in recent clinical trials as anti-cancer therapies. The research of Lindquist and co-workers suggests extra caution should be taken in such studies as proteasome inhibitors could promote neurodegenerative disorders.

1 Ma, J. *et al.* (2002) Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol. *Science* DOI: 10.1126/science.1073725 (http://www.sciencemag.org)

2 Ma, J. and Lindquist, S. (2002) Conversion of PrP to a self-perpetuating PrP^{Sc}-like conformation in the cytosol. *Science* DOI: 10.1126/science.1073619 (http://www.sciencemag.org)

FTase in action

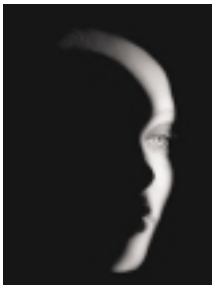
A recent study provides snapshots of the structure of a key enzyme in action [3]. The work has revealed surprising details about the way the farnesyltransferase (FTase) functions, and should give new ammunition to pharmaceutical companies developing FTase inhibitors as anti-cancer drugs.

Farnesyltransferase catalyzes the attachment of farnesyl lipid groups to numerous proteins, including Ras oncoproteins – small GTPases that have been implicated in ~30% of cancers. Because farnesylation is crucial for the correct membrane attachment and functioning of Ras proteins, FTase is being targeted in the fight against cancer, with FTase inhibitors now in clinical trials. Patrick Casey and colleagues have previously used X-ray crystallography to determine the structure of human FTase. Now, Casey's group and that of Lorena Beese, both based at Duke University (http://www.duke.edu/), have determined the structure of FTase in action. They found that FTase does not release farnesylated product as readily as expected, apparently waiting until a fresh substrate molecule is bound. Another surprise was that the FTase conformation remains essentially unchanged during the reaction cycle.

The discovery of these idiosyncrasies should help those working on FTase inhibitors. For example, the unusual product-release mechanism offers potential for the design of new drugs, and the consistent structure of FTase means that the task of designing drugs is simplified. 'Many enzymes undergo conformational changes of their protein structure as part of their reaction cycle', explained Beese. 'If the FTase protein changed its conformation every time it bound to a drug, the computational algorithms for positioning drugs into active sites wouldn't work.' Beese also envisages that their findings will be used beyond the anti-cancer field – the differences between the FTase of humans and those of *Plasmodium* and *Trypanosoma* possibly allowing the specific inhibition of FTase in disease-causing organisms.

3 Long, S.B. *et al.* (2002). Reaction path of protein farnesyltransferase at atomic resolution. *Nature* 419, 645–649

From shadows into light



Researchers at Joslin Diabetes Center, Harvard Medical School (http://www.joslin.harvard.edu), and other institutions, have identified a protein function that is crucial in the recognition of native organs and tissues by immune cells, and thus prevents a self-attack [4]. The protein, dubbed aire – for autoimmune regulator – appears to function by turning on the thymus. This discovery could shed light on how the normal healthy immune system develops tolerance to its own proteins and also how this tolerance is lost, as is the case in diseases such as diabetes, rheumatoid arthritis, Crohn's disease and other autoimmune disorders.

Immune cells, in particular T cells, were thought to learn how to attack foreign bodies and spare native proteins in two places: those that widely monitor cellular or bloodstream circulating proteins were thought to be trained while still in the thymus, and those that recognize peripheral organs, such as the pancreas, were thought to have learnt the difference

  PII: S1359-6446(02)02539-4

between native and foreign molecules once having left the thymus. However, it appears that T cells might learn this training while still in the thymus.

The teams reported that a small network of thymic cells express hundreds of genes that are usually associated with peripheral organs, such as the pancreas, liver and brain. The majority of these proteins are used by these organs to prevent T cells from attacking.

Christophe Benoist, an author on the paper, said: 'No one would think you would encounter your ''big toe'' protein in the thymus but in fact, proteins from the eye, the liver, from all over the place are specifically expressed in a small population of stromal cells in the thymus.'

The scientists believe that the proteins are used in the thymus to foreshadow the self-antigens that the T cells will encounter once they travel into the body. Mathis said, 'There is a foretelling of these proteins in the thymus, which is why we call it an immunological self shadow.' The teams found that the transcription factor aire has a crucial role in producing these self-shadow proteins. By using aire-deficient mice the researchers found that in the mutant mouse thymus, only a fraction of the peripheral self-proteins were present compared with normal mice. All mutants exhibited widespread autoimmunity.

How aire controls the expression of the shadow proteins is unclear, and could be by binding to other transcription factors. Benoist said: 'It is going to be interesting to figure out what the mechanism really is.'

4 Anderson, M.S. *et al.* (2002) Projection of an immunological self-shadow within the thymus by the aire protein. *Science* 10.1126/science.1075958 (epub ahead of print; http://www.sciencemag.org)

Can't get the Staph? Now you can

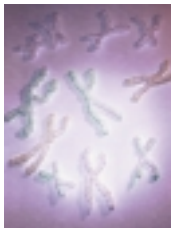
X-ray crystallographic studies of a key enzyme have revealed how the bacterium *Staphylococcus aureus* can become resistant to antibiotics [5]. Daniel Lim and Natalie Strynadka from the University of British Columbia (http://www.ubc.ca) have solved and examined the structure of penicillin-binding protein 2a (PBP2a), an enzyme that confers resistance to β-lactam antibiotics in *S. aureus*.

Drug-resistant staphylococcal infections can be a serious problem, especially in hospitals, where the bacteria are prevalent. Traditionally, β-lactam antibiotics such as methicillin have been used to combat the bacterium. These drugs work by blocking the activity of PBP, an enzyme that maintains the integrity of the bacterial cell wall. However, several methicillin-resistant strains of *S. aureus* have emerged including one that contains a version of PBP, known as PBP2a, from another bacterial species.

To investigate how PBP2a differs from PBP at a molecular level, Lim attempted to crystalize the enzyme for structural studies. This required the excision of a segment that anchored PBP2a to the cell membrane, thus solubilizing the protein. The X-ray crystal structure revealed several structural differences between PBP2a and antibiotic-sensitive PBPs. The active site of PBP2a is distorted, preventing the antibiotic from binding, and further variations occur throughout the whole structure. It is hoped that differences in the active site will lend themselves to the design of inhibitors. 'The structures we observe now allow for the rational design of specific PBP2a inhibitors that are tailored to better fit these features of the PBP2a active site', explained Strynadka. Blocking the active site would render the enzyme useless, therefore enabling β-lactam drugs to be used with this strain of *S. aureus*.

5 Lim, D. and Strynadka, N.C.J. (2002) Structural basis for the β lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat. Struct. Biol.* DOI: 10.1038/nsb858 (http://www.nature.com)

Sister act



Genes are damaged by an average of twenty thousand hits each day from sources such as cigarette smoke, ultraviolet light or viruses. Although cells have mechanisms to repair this damage, disease can follow if it the damage remains unrepaired. Most DNA repair is carried out by a process known as excision repair, but some lesions escape this mechanism and one of two 'back-up' repair mechanisms must be used. Of these, one mechanism – known as recombinant repair (RR) – had been suggested but not proved, until a recent study showed that it actually accounts for 85% DNA repair [6].

The second mechanism, translesion repair (TLR), is far more prone to incorporating mutations than RR, and researchers led by Zvi Livneh, Head of the Biological Chemistry Department at the Weizmann Institute of Science (http://wis-wander.weizmann.ac.il/), have now reported a hierarchy in which the non-mutagenic RR predominates over the mutagenic TLR to maintain the genetic stability of the cell.

RR was first hypothesized in the 1960s and relies on the help of sister-chromatids that enable repair without permitting any mutations. DNA from one identical sister chromatid can restore the damaged section of the DNA by filling in the gap. The new gap in the donor strand can then be repaired by error-free excision repair resulting in two complete DNA strands.

6 Berdichevsky, A. *et al.* (2002) Error-free recombinational repair predominates over mutagenic translesion replication in *E. coli*. *Mol. Cell* 10, 917–924

Miscellaneous

Gene therapy gets down to specifics

Another shadow was cast over gene therapy trials recently after reports that a three-year-old infant being treated for so-called 'bubble-boy' disease (severe combined immunodeficiency syndrome, x-SCID) had developed leukaemia, apparently as the result of DNA becoming randomly inserted into a neighbouring oncogene. Recent work led by Michele Calos from Stanford University School of Medicine (http://www-med.stanford.edu/) offers one way to avoid such complications in the future and provides renewed hope [7].

Current gene therapy approaches that cause genes to integrate use a viral vector to 'sneak' the therapeutic DNA into the host cell; however, the DNA inserts itself into the chromosome at random positions, which can cause problems like those seen in the x-SCID trial. Calos and co-workers at the Stanford University Medical Center have developed a new gene therapy technique that avoids the pitfalls of other approaches. This technique integrates DNA without using viral vectors, which have plagued gene therapy trials in the past, and inserts the DNA at known locations. This new method also has the advantage of being able to handle genes that are too large to fit into a viral package.

In developing their approach, Calos hijacked a mechanism used by bacteriophage to integrate its genes into bacteria. The bacteriophage uses an

integrase enzyme to insert viral genes into a specific DNA sequence on the bacterial chromosome. In this study, the integrase from phage ϕC31 was used to integrate the human Factor IX (hFIX) gene permanently into specific sites in the mouse genome. A plasmid containing an *attB* site (which acts as an 'insert me' signal to integrase) and an expression cassette for hFIX was injected into mice. When an integrase expression plasmid was co-injected, hFIX serum levels increased more than tenfold and remained stable throughout the eight months of the experiment. Further experiments confirmed that the hFIX gene had successfully integrated into the mouse DNA. Thus, the study demonstrates *in vivo* gene transfer in an animal by site-specific genomic integration.

Calos said this approach should be effective for treating diseases in several different human organs including skin, retina, blood, muscle and lung. She hopes to start human trials for the technique in a fatal childhood skin disease. "If that trial shows it is safe then that will open the door for trials in other diseases," Calos said. Collaborations are currently under way to test the technique for use in Duchenne's muscular dystrophy and cystic fibrosis.

7 Olivares, E.C. *et al.* (2002) Site-specific genomic integration produces therapeutic Factor IX levels in mice. *Nat. Biotechnol.* 10.1038/nbt753

Injection to prevent stroke brain damage

A promising drug that could reduce or stop the ischemic brain damage caused by stroke has been developed researchers in Canada [8]. When administered to rats either before or within one hour of the onset of stroke symptoms, the drug was able to stop the damage that occurs during a stroke.

N-methyl-D-aspartate receptors (NMDARs) mediate ischemic brain injury, however blocking them in animals and humans is deleterious because they also mediate essential neuronal excitation. Therefore, targeting the postsynaptic density (PSD) protein-95, which couples the NMDAR to intracellular proteins and signalling enzymes, represents an alternative therapeutic approach for diseases that involve excitotoxicity and ischemic brain damage. This latest research used neurons transduced with peptides that disrupted the interaction of NMDARs with the postsynaptic activity or calcium influx [8]. When applied either before or one hour after insult, cultured neurons were protected from excitotoxicity, focal ischemic brain damage in rats was reduced and neurological function was improved.

'The drug works by preventing the negative consequences of over stimulation of the NMDA receptors in the brain that are involved in strokes. However, it doesn't block the normal important functions of these receptors, making this a possible practical stroke therapy,' said Michael Salter, co-principal investigator of the study at the University of Toronto (http://www.utoronto.ca/).

The drug has shown no long-term adverse effects and is reportedly more effective in preventing stroke than any method currently used in animals or humans. However, it could be some time before human trials begin.

8 Aarts, M. *et al.* (2002) Treatment of ischemic brain damage by perturbing NMDA receptor-PSD-95 protein interactions. *Science* 298, 846–850

Sauerkraut is good for you!

New research suggests that chemicals found in sauerkraut could be used to fight cancer [9], adding yet another reason to the list of why greens are good for you.

Researchers led by Eeva-Liisa Ryhanen (MTT Agrifood Research; http://www.mtt.fi/english/) found that fermenting cabbage produces isothiocyanates, which have been previously identified as possible cancer-fighting agents. The compounds appear to prevent the growth of cancer, particularly in breast, liver, lung and colon, in animals models, although it is not known whether they have a similar effect in humans.

During fermentation, glucosinolate in raw cabbage is broken down by enzymes released by the fermentation process. The breakdown products are mainly isothiocyanates, thus suggesting that fermented cabbage is better for you than raw cabbage.

The researchers are now investigating how sauerkraut could be made to include even more healthy compounds, by looking at different starter cultures in the fermentation process. Fermentation leads to the production of other healthy compounds, including lactic acid, and sauerkraut is also a good source of dietary fibre and vitamin C. It is hoped that this research will help raise the profile of sauerkraut as a functional and nutritious food.

9 Tolonen, M. *et al.* (2002) Plant-derived biomolecules in fermented cabbage. *J. Agri. Food Chem.* 50, 6798–6803

Mouse model of muscular dystrophy

Often in research, an experiment designed to answer one question brings to light another unexpected result. This proved to be the case for scientists at the University of Iowa (http://www.uiowa.edu/) who developed a genetically altered mouse to investigate the role of a particular protein, dystroglycan, in the progression of muscular dystrophy. The mouse model proved that removing dystroglycan from skeletal muscle did indeed cause muscular dystrophy in the animals, but the mice appeared to be protected from the devastating, muscle-wasting consequences of the disease [10].

Following up on this surprising finding, the team discovered that a specific group of muscle cells, known as satellite cells, were making dystroglycan protein and were efficiently repairing the muscle damage caused by muscular dystrophy in these mice. The study showed that maintaining the regenerative capability of the satellite cells can prevent development of severe muscular dystrophy. This suggests that malfunctioning satellite cells may influence the severity of muscular dystrophies and could provide a therapeutic target for muscular dystrophy and other muscle diseases.

The team also discovered that the capacity of satellite cells to regenerate muscle did not diminish with age. This result challenges the idea that ongoing muscle destruction in muscular dystrophy ultimately exhausts the satellite-cell pool. Satellite cells act like stem cells in muscle. Once activated, these cells divide to form daughter cells that incorporate into damaged muscle fiber to repair the tissue. The study might therefore lend support to the possibility of using stem cell-type therapies for muscular dystrophies.

10 Cohn, R.D. *et al.* (2002) Disruption of *dag1* in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. *Cell* 110, 639–648

News in brief was written by *Matt Brown, Jayne Carey, Joanne Clough, Morag Robertson, Linsey Stapley, Carrie Viccars, Catherine Wild and Heather Yeomans*