News in brief

Targets and mechanisms

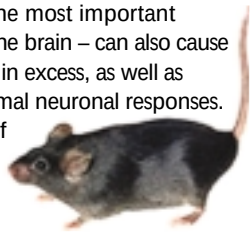
The GluR1 knock-in that couldn't remember

Phosphorylation of the GluR1 protein has been established for the first time as being crucial to mediating cellular mechanisms involved in spatial learning and memory [1] by researchers at Johns Hopkins University (http://hopkinsmedicine.org).

The phosphorylation of GluR1, the glutamate-binding subunit of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, has already been shown to be modulated during two neuronal processes: long-term depression (LTD) and long-term potentiation (LTP). LTD and LTP are mechanisms involved in excitatory synapses/neuronal communication, upon which neuronal plasticity (the ability of the brain cell to respond to external stimuli) is dependent, and thus are key cellular models of learning and memory.

Engineered knock-in mice, which were unable to phosphorylate GluR1, could not remember the position of a hidden platform in a pool of water after learning to find it eight hours previously. Wild-type mice could remember the location of the concealed resting place as long as 24 hours after originally finding it. Experiments with neurons from the hippocampus of 'phosphomutant' mice proved that phosphorylation of the protein is essential for LTP and LTD to take place.

Richard Huganir of Johns Hopkins University and corresponding author of the paper says that they have 'established [that] the two phosphorylation sites on GluR1 are crucial for retention of spatial learning, but it is likely that other sites in other subunits of this glutamate receptor will also play a role'. Glutamate – being the most important neurotransmitter in the brain – can also cause neurons to die when in excess, as well as triggering many normal neuronal responses. The understanding of how glutamate receptors are regulated could prove crucial in finding treatment for neurological diseases, such as epilepsy and stroke, to which glutamate toxicity is a major contributory factor.



1 Lee, H.-K. *et al.* (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. *Cell* 112, 631–643

Homing in on DNA hotspots

The genes responsible for inherited conditions are traditionally identified according to DNA sequences that are common to affected individuals. However, the success of this approach for traits that are caused by multiple genetic and environmental factors (e.g. obesity, heart disease and cancer) is limited. Now, it seems that by combining classical genetics with DNA chip technology, the genes behind complex traits can be identified more rapidly than by using classical genetics alone, promising to speed up the drug discovery process for many common conditions [2].

Like many disease conditions, gene expression levels can be affected by multiple factors. Eric E. Schadt of Rosetta Inpharmatics (http://www.rii.com/) and collaborators have

Genomics and proteomics

Proteomics applied to cystic fibrosis

A proteomics study of *Pseudomonas aeruginosa* has given researchers new clues about the bacterium's role in cystic fibrosis (CF). Results from one of the first whole-organism proteomics studies suggests that a cell-signalling system bolsters the bacterium's defences, allowing it to thrive in the lungs of CF sufferers [7].

P. aeruginosa is a ubiquitous organism that does not present a danger to healthy individuals. In CF sufferers, however, it can take advantage of a weakness in the airways and become infectious. Approximately 50% of CF sufferers are infected by the age of three, rising to almost 100% by teenage years. Lingering infection exacerbates the problems of CF and inflammation caused by the bacteria can eventually be fatal. Previous experiments had shown that changes within *P. aeruginosa*, soon after infection in young children, enable the bacteria to flourish and persist; but what exactly are these changes?

In search of the answer, scientists from the University of Washington (http://www.washington.edu/) and the Institute for Systems Biology (http://www.systemsbiology.org/) turned to the powerful technologies of proteomics. The team analyzed bacterial samples from the airways of CF sufferers aged 6–36 months. Using a quantitative mass spectrometric technique developed by co-author Ruedi Aebersold, more than 1000 proteins were surveyed. The results were compared with control data from bacteria grown *ex vivo*. Computer analysis showed that a quorum sensing system was activated in the clinical samples.

This signalling system, called *Pseudomonas* quinolone signal (PQS), might help bacteria adapt to the conditions in a CF sufferer's airways, thwarting the attempts of the host's immune system to suppress it. Commenting on the successes of the technique, Aebersold said, 'We are excited that one of the first applications of the technology we developed to a clinically important problem has yielded new insights that may ultimately help patients.'

7 Guina, T. *et al.* (2003) Quantitative proteomic analysis indicates increased synthesis of a quinolone by *Pseudomonas aeruginosa* isolates from cystic fibrosis airways. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2771–2776

Clearer picture of *C. elegans* genome

A powerful gene-mapping technique has been used to check the accuracy of the predicted map of the *Caenorhabditis elegans* genome [8].

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now shown that such complex phenotypes can be mapped to DNA 'hotspots'. They correlated variations in gene expression, detected using microarray chips, with variations in DNA sequence. In many cases, gene expression levels were linked to a section of the genome already known to affect them, confirming that the approach can pinpoint DNA responsible for complex phenotypes.

Schadt *et al.* went on to apply their approach to the complex trait of mouse obesity. They identified gene expression patterns that were inherited along with the obesity and linked them to DNA hotspots. This soon led them to putative obesity genes. The study also revealed chromosomal regions that affected the obesity of some mice but not others, reminiscent of the way that drugs do not have the same effects in all patients.

'Basically, we've created a new approach for understanding the complex molecular relationships that underlie living systems and pathological processes', says A. Jake Luis, of the David Geffen School of Medicine at UCLA (http://www.medsch.ucla.edu/), who contributed to the study. 'By combining the new kind of gene expression data with DNA sequence variability data, doctors may eventually be able to treat patients according to their precise genetic needs.'

2 Schadt, E.E. *et al.* (2003). Genetics of gene expression surveyed in maize, mouse and man. *Nature* 422, 297–302

Bacterial crystals in the battle against worms

New results from Raffi Aroian's lab (http://www.ucsd.edu) has revealed that crystal proteins from the spore-forming bacterium *Bacillus thuringiensis* (Bt) are toxic to many nematode species, including a parasitic nematode of vertebrates [3].

Bt crystal proteins have been used as natural insecticides by organic farmers for over 50 years because of their high toxicity towards insects and low toxicity towards animals. There are many variations of Bt crystal proteins, which result from the aggregation of proteins produced during the spore stage of the bacterium. When an insect consumes a Bt toxin, it dissolves in the high pH of the insect gut and damages the gut cells, killing the insect within a few days. Bt crystal proteins are also effective against crop pests when expressed in transgenic plants and such plants have been approved for use in USA since 1961.

The authors of the study questioned the target of these Bt crystal proteins: 'It is puzzling why a bacterium that is so ubiquitously found in the soil might have evolved ingestible toxins to target insects that may spend little time feeding in the soil…On the other hand, there are estimated to be more than 100,000 species of nematodes, many of which live in the soil and ingest bacteria. Could nematodes be a prime target for Bt and its crystal proteins?'

Aroian and colleagues tested the impact of seven different types of Bt crystal proteins on six different nematode species. Four of these crystal proteins were found to be toxic to six nematode species, as assessed by intoxication, developmental and gut morphology assays. More significantly, three of the crystal proteins were effective against the free-living stage of *Nippostrongylus brasiliensis*, a parasitic nematode of rats.

These results clearly demonstrated the activity of Bt crystal proteins against nematodes, indicating their potential as a chemotherapeutic agent to control parasitic nematodes of vertebrates, particularly in the light of their low toxicity to humans. Nearly 25% of the world's population is infected by parasitic nematodes and some of these infections are characterized by devastating symptoms such as gross enlargement of a limb, or areas of the trunk

This study, carried out at the Dana-Farber Cancer Institute (http://www.dana-farber.net), attempted to describe and identify all of the 19,000 predicted genes in the *C. elegans* genome in the hope that the accurate mapping technique could then be applied to the human genome.

'Of the 30,000 genes believed to be in the human genome, only about 5000 have been well defined,' says the study's senior author and Assistant Professor of Genetics at Harvard Medical School, Marc Vidal. The technique involved capturing the RNA from *C. elegans* and converting it into cDNA to gather a full set of open reading frames (ORFs) from the genome. The segments of cDNA representing the genes were then compared with sections of chromosome predicted to contain those genes.

In 56% of cases the predicted genes did not completely match the gene that was identified through gene mapping. 'This demonstrates that even in *C. elegans* – whose genome is better understood than humans – the genome map needs a great deal of refining,' says Vidal. 'The success of this technique with *C. elegans* suggests that it can be equally successful with the genomes of other creatures, including humans, and it brings us closer to a completely accurate map of the human genome.'

8 Reboul, J. *et al.* (2003) *C. elegans* ORFeome version 1.1: experimental verification of the genome annotation and resource for proteome-scale protein expression. *Nat. Genet.* 10.1038/ng1140 (http://www.nature.com)

Enzyme holds key to cocaine and heroine metabolism

The first crystal structure of human carboxylesterase 1 (hCE1) has been reported [9], along with evidence of its role in the metabolism of heroin and cocaine.

Matthew R. Redinbo, Assistant Professor of Chemistry at the University of North Carolina (http://www.northcarolina.edu) and colleagues, determined the structure of hCE1, a broad-spectrum bioscavenger found in the liver, small intestine, kidney, lungs, testes, scavenger cells and human blood plasma. They report how the enzyme was identified in complexes with analogues of cocaine and heroin, and showed that it is responsible for metabolizing the first step of cocaine breakdown and the first two steps of heroin breakdown in the body.

Redinbo commented: 'Our results can be used to generate an efficient treatment for cocaine overdose.' An injection of the enzyme in overdose patients could potentially metabolize the cocaine before it becomes toxic. Furthermore, the same enzyme could be used as a prophylactic against organophosphate chemical weapons and the US military are also looking into using hCE1 to detoxify sarin, soman, tabun and VX gases.

9 Bencharit, S. *et al.* (2003) Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme. *Nat. Struct. Biol.* 10.1038/nsb919 (http://www.nature.com)

or head in the disease lymphatic filariasis, or blindness in the case of onchocerciasis.

3 Wei, J.Z. *et al.* (2003) *Bacillus thuringiensis* crystal proteins that target nematodes. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2760–2765

The structure behind the switch

A novel, stable DNA structure in mice and humans has been shown to play a crucial role during immunoglobulin (Ig) class-switch recombination [4], a process that takes place in the nucleus of B cells and until recently was not well understood.

All Ig molecules begin life as IgM and change their class (to IgA, IgD, IgE or IgG) according to their localization in the body; for example, IgA localizes to the lungs and digestive tract. The class is 'switched' by cutting the DNA so that the code for IgM and the other undesirable Ig classes is abolished.

Kefei Yu and colleagues from the Keck School of Medicine, University of Southern California (http://www.usc.edu/schools/medicine/ksom.html), showed that during immunoglobulin class-switching the DNA is cut and transcribed by novel processes. DNA is usually cut by an enzyme at a particular nucleotide sequence, but in immunoglobulin class-switching it is the physical presence of a DNA structure called an 'R loop' that indicates where the cut is to be made. The R loop is formed when the DNA that codes for the desired class forms a stable bond with the RNA strand that is transcribing it. Only when this R loop is present can the DNA be cut and spliced to create an antibody of a different immunoglobulin class.

During normal transcription, the DNA strands separate and an RNA strand pairs with individual nucleotides on one of the DNA strands, while the leading edge of the RNA remains bonded to the DNA nucleotides being transcribed. During immunoglobulin class-switching, however, the DNA that is transcribed is a 'silent transcript' and the RNA remains attached to each DNA nucleotide, creating an 'RNA sandwich', with the RNA between the two strands of DNA, yet only attached to one of them. This is the R loop that makes immunoglobulin class-switching remarkable and unique. 'The whole process is more sophisticated that we first thought', remarked Yu, the paper's first author.

4 Yu, K. *et al.* (2003) R-loops at immunoglobulin class switch regions in the chromosomes of stimulated B cells. *Nat. Immunol.* 10.1038/ni919 (http://www.nature.com)

Miscellaneous

'New paradigm' for coronary artery disease

A key component of blockages in the blood vessels of the heart seems to originate in the bone marrow (BM) and not in the vessel walls, according to a recent report [5].

'This study establishes a new paradigm for coronary artery disease, and opens a whole new set of approaches for treatment of heart disease and prevention of heart attacks,' said Noel Caplice, a cardiologist at the Mayo Clinic, Rochester, MN (http://www.mayo.edu/).

Atherosclerosis, or blockages in arteries, is a major cause of mortality in the Western world. Blockages are caused by the formation of plaques, which comprise mainly fats, including cholesterol, inflammatory cells and smooth muscle (SM) cells. Fats and inflammatory cells derive from the blood, but there is controversy over the origin of the SM cells, which are thought by some to form from multiplication of the SM cells of the vessel wall. Caplice *et al.* have shown, however, that progenitor cells produced by BM can become SM cells and home in on sites of plaque formation.

Postmortem specimens from 13 atherosclerosis patients who had undergone BM transplants were studied; eight had received marrow from donors of the opposite sex, five were sex-matched. Coronary artery samples were analyzed using a staining technique that could distinguish between male- and female-derived SM cells. Sex-mismatched recipients had high levels of SM cells from the opposite sex in their plaques (often greater than 10%); sex-matched recipients had only same-sex cells. In undiseased vessels, the recruitment of sex-mismatched SM cells was markedly reduced.

The survival time for patients ranged from 41 days to 41 months; however, plaques are thought to develop over many years. Recruitment of BM-derived SM cells over such a short time could suggest that the BM is not an incidental source of SM cells.

These data have broad implications for our understanding of the cellular events leading to atherosclerotic plaque formation and could lead to novel treatments and diagnostic tools for coronary artery disease.

5 Caplice, N.M. *et al.* (2003) Smooth muscle cells in human coronary atherosclerosis can originate form cells administered at marrow transplantation. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4754–4759

A real turn-off for epigenetic regulation

Human DNA contains the information for roughly 35,000 different proteins, but not all of these genes are active all of the time. Like switches, epigenetic modifications to proteins surrounding the DNA regulate a given gene's activity, such that only those that are required in a particular cell are switched on. Incorrect epigenetic modifications have been implicated in many human disorders.

A new study from scientists at the University of North Carolina at Chapel Hill (http://www.unc.edu/) [6] has identified a gene that may be critical for proper epigenetic changes. Using expression analyses the gene *Eed* (embryonic ectoderm development) was shown to be required for the proper epigenetic regulation of a subset of genes that normally show parent of origin expression, known as genome imprinting.

Genome imprinting is a phenomenon in which only one copy of specific genes are active, or switched on. Which copy is active depends upon whether they are inherited from the mother or the father. When *Eed* is mutated or its function impaired, loss of imprinting can occur — both the maternal and paternal copy become active.

'Basically, *Eed* forms a complex of proteins and alters those chromosomal proteins that affect the configuration of the chromosome so as to allow or not allow expression. If this gene, *Eed*, isn't functioning properly, the imprint is lost resulting in incorrect activity of specific genes,' said Magnuson, senior author of the study.

The Human Genome Project has highlighted the need to understand more about the regulation of gene expression and how genes are turned on and off. This study opens up new possibilities for looking into mechanisms responsible for epigenetic alterations in human disorders.

6 Magner, J. *et al.* (2003) Genome imprinting regulated by the mouse Polycomb group protein Eed. *Nat. Genet.* 10.1038/ng1125 (http://www.nature.com)

News in Brief was written by *Matt Brown, Jayne Carey, Lisa Deakin, Clare Rathbone, Morag Robertson, Catherine Wild* and *Heather Yeomans.*