News in brief

Targets and Mechanisms

A new player in neural development

New research explains the severity of a brain disorder and provides important new details of the molecular mechanisms behind correct neural development [1].

During brain development, neurons migrate to particular destinations. Heterozygous mutations in the human chromosomal region 17.p13.3 lead to a severe defect in this migration known as lissencephaly (literally, 'smooth brain'). There are two lissencephaly disorders, both of which involve reduced folding of the brain surface, profound mental retardation and epilepsy. In isolated lissencephaly sequence (ILS), the severity of the lissencephaly varies but there are no associated symptoms; in Miller-Dieker syndrome (MDS), the lissencephaly tends to be more severe and there are additional defects, such as a prominent forehead, small jaw and small nose. Both disorders have been traced to mutations in *LIS1* (also known as *PAFAH1B1*) but the additional severity of MDS has remained a mystery.

Now, researchers led by Anthony Wynshaw-Boris, of the School of Medicine at the University of California, San Diego (http://medicine.ucsd.edu/), have begun to explain MDS. Toyo-oka *et al.* looked for other genes in the 17.p13.3 region and found that individuals with MDS had deletions in *YWHAE*, which encodes the phosphoserine/threonine-binding protein 14-3-3ε. The team showed that mice with mutations in the equivalent gene, *Ywhae*, had abnormally developed brains, linked to defective migration of neurons.

Wynshaw-Boris and colleagues went on to dissect the mechanics of 14-3-3ε function. They demonstrated that it interacts directly with NUDEL, a protein previously shown by the team to interact with LIS1 in neuronal migration. 14-3-3ε interacted selectively with phosphorylated NUDEL, maintaining its phosphorylation and ensuring correct localization of both NUDEL and LIS1. Their findings indicate that 14-3-3ε has a crucial role in neural development. 'Not only does our study determine a novel function for 14-3-3ε, but it also provides a molecular explanation why MDS patients have more severe lissencephaly', said Wynshaw-Boris.

1 Toyo-oka, K. *et al.* (2003) 14-3-3ε is important for neuronal migration by binding to NUDEL: a molecular explanation for Miller-Dieker syndrome. *Nat. Genet.* 10.1038/ng1169 (epub ahead of print; http://www.nature.com)

Biochemical cause for severe forms of cystic fibrosis identified

The regulatory mechanisms underlying a more severe form of cystic fibrosis (CF) have been identified by researchers at the University of California, San Diego (UCSD; http://www.ucsd.edu) [2]. CF, a chronic, progressive and often fatal disease, is caused by mutations in the CF transmembrane conductance regulator (CFTR), an anion channel in epithelial membranes. CF patients lose excessive amounts of salt when they sweat, leaving them prone to heart arrhythmias; thick mucus accumulates in their lungs and intestine, leading to difficulties breathing and lung damage, poor nutrition and health.

Previously, transport of chloride across the CFTR channel was thought to be primarily involved in CF, and a consensus developed that activation of the CFTR chloride channel requires its phosphorylation by protein kinase A and ATP hydrolysis. The severity of the disease varies widely in CF patients, however, suggesting that these requirements are not absolute – and that regulatory mechanisms underlying such differences in severity remain unidentified.

M.M. Reddy and Paul Quinton from UCSD focused on glutamate – because it has a major role in regulating neuronal channels, and because glutamate receptors are found widely in epithelial cells. The researchers showed that glutamate activates CFTR chloride channel in epithelial cells of human sweat gland tissue; and it does so in the absence of cAMP and ATP and at physiological concentrations. They also demonstrated that, in severe forms of CF, epithelial cells are unable to transport bicarbonate across their membranes. Moreover, glutamate controls this movement of bicarbonate – and chloride – across the epithelial cell membrane.

The two biochemical effects appear to be independent; however, it is thought that bicarbonate and chloride movement across the membrane draws water to the mucus that protects the epithelial cells lining the sweat glands, pancreas, lungs and intestine, which keeps the mucus from becoming sticky and thick. Indeed, the story is probably far more complicated than this.

'Using [the knowledge presented in this paper], our future efforts will be focused on how to correct or compensate for the abnormal functions of CFTR, with the hope to find a cure or reduce the severity of cystic fibrosis,' said Reddy.

2 Reddy, M.M and Quinton, P.M. (2003) Control of dynamic CTFR selectivity by glutamate and ATP in epithelial cells. *Nature* 423, 756–760

Missing link detected in insulin mechanism

Dartmouth Medical School biochemists (http://www.dartmouth.edu/dms/) have found a missing link that could spark the connection for glucose to move into cells [3], which is an essential part in the multifaceted insulin pathway. The discovery is another strand in the web of molecular signals that regulate traffic through cells and could help to elucidate crucial aspects of how insulin regulates a membrane movement process.

Insulin maintains appropriate levels of blood glucose by accelerating the removal of glucose from the blood into muscle and fat cells. However, key aspects of this mechanism are still poorly understood. A conundrum is that muscle and fat cells have known protein transporters for ferrying glucose, but these transporters are situated within vesicles inside the cell, rather than on the cell surface where they are required. How then does insulin on the outside of the cell spur these transporter-containing vesicles to move towards and fuse with the cell surface?

This was the question addressed by Lienhard and colleagues who have identified a protein that seems to bridge insulin signalling and the protein machinery of the membrane-trafficking process. 'That was a missing link in this field. If we're

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right, this looks like a key protein that connects signalling to trafficking,' said Lienhard. The 160 kDa protein, designated AS160, is phosphorylated by an insulin-activated kinase. Six sites of *in vivo* phosphorylation have been identified, five of which show increased phosphorylation following treatment with insulin. The study strongly indicates that insulin-stimulated phosphorylation of AS160 is required for translocation of glucose transporters.

This protein could provide clues for understanding type two diabetes and could also shed light on how hormones regulate movement of membrane proteins in general; however, Lienhard stresses that studies of the protein in diabetic rodent models need to be performed.

3 Sano, H. *et al*. (2003) Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. *J. Biol. Chem*. 278, 14599–14602

Calcineurin linked to schizophrenia

Several threads of experimental evidence have been woven together to incriminate a new molecular villain at the root of schizophrenia. Defective components of the calcineurin pathway have been identified as a potential cause of the illness, and also potential targets for new antipsychotic drugs.

A leading theory of schizophrenia associates its cause with the signalling pathways of the neurotransmitter dopamine. However, a new series of experiments implicate calcineurin, an enzyme present in the brain that is unconnected with dopamine pathways.

Researchers led by Susumu Tonegawa at the Massachusetts Institute of Technology (http://www.mit.edu/) performed behavioural studies in mice that had been genetically engineered to lack calcineurin [4]. Several tests showed behaviour consistent with that observed in human schizophrenia: the mice were particularly hyperactive, yet showed signs of decreased social activity.

The calcineurin-knockout mice underwent a startle-response test used to diagnose schizophrenia in humans. In non-schizophrenics, and normal mice, a softer noise prepares the individual for a louder noise, leading to a reduced response. However, just as in schizophrenic humans, the calcineurin knockout mice were significantly more startled by the second tone. Tonegawa believes that this suggests a link between calcineurin and

Cancer Targets and Mechanisms

TBP adds another piece to the jigsaw

A new role has been suggested for the transcription protein, TATA-binding protein (TBP), in cancer development. Originally believed to be a 'housekeeping' protein because of its central role in the transcription process, TBP cellular levels were thought to remain steady at all times. However, manipulation of levels in a recent study have indicated that certain proteins are particularly acute at TBP upregulation [8]. These included proteins such as Ras, which is involved in human cancer.

Deborah Johnson of the Keck School of Medicine (USC, http://www.usc.edu) explains that, 'increased TBP alone, without Ras, still leads to tumours in nude mice', suggesting that TBP might be driving Ras to transform normal cells into malignant ones.

Several experiments and collaborations have accompanied these findings, the latest focussing on the flipside of the coin: do tumour suppressors reduce TBP levels? One such suppressor, p53 (which is mutated in many cancers), was manipulated, and the subsequent effect on TBP levels was observed. The cellular concentration of TBP remained unchanged, but the 'effective' concentration was reduced; functioning was suppressed, preventing TBP from binding to an RNA polymerase and, thus, diminishing the role of TBP in gene transcription [9].

The two different findings are mere pieces of a larger jigsaw but as Johnson points out, 'together, they contribute to the ability of the cell to transform from normal to malignant'.

8 Johnson, S.A. *et al.* (2003) Increased expression of TATA-binding protein, the central transcription factor, can contribute to oncogenesis. *Mol. Cell. Biol*. 23, 3043–3051

9 Crighton, D. *et al.* (2003) p53 represses RNA polymerase III transcription by targeting TBP and inhibiting promoter occupancy by TFIIIB. *EMBO J*. 22, 2810–2820

A gene to halt ovarian cancer

A gene that has the power to stop the development of epithelial ovarian cancer (EOC) has been discovered. A recent study reports that the 'OPCML' gene has characteristics that are consistent with tumour-suppressor genes (TSGs) and can dramatically suppress tumour growth when inserted into human ovarian cancer cells [10].

Hani Gabra, who led the research at the Edinburgh oncology unit of Cancer Research UK (http://www.cancerresearchuk.org), explains, 'this is a very important discovery in identifying what seems to be a key tumour suppressor gene in ovarian cancer'.

Known as the 'silent killer', ovarian cancer affects 1 in 48 women in England and Wales and is the leading cause of death from gynaecological malignancy. There are no obvious symptoms in the early stages and, with the lack of an effective screening procedure, EOC is difficult to detect.

The onset and development of EOC is surrounded by mystery, but it is known that genetic alterations manifest as activation of oncogenes and inactivation of TSGs. Findings indicate that OPCML is a prime candidate for such a TSG; in normal ovarian tissue it seems to prevent cells from being cancerous but defects could open the way for disease development.

Drugs to mimic the effects of this gene and, thus, block the growth of ovarian cancer cells, could be potential in the future but the picture is by no means complete – the full mechanism of OPCML, and what makes it switch off, remain unclear. As John Toy, Medical Director of Cancer Research UK concludes, 'this work still has a long way to go in the laboratory before patients could benefit but results so far are promising'.

10 Sellar, G.C. *et al.* (2003) OPCML at 11q25 is epigenetically inactivated and has tumour-suppressor function in epithelial ovarian cancer. *Nat. Genet*. 34, 337–343

schizophrenia. 'This is the first knockout mouse to show such a comprehensive array of behavioural abnormalities that mirrors the abnormal behaviour of human schizophrenic patients,' he said.

In a second paper from MIT [5], researchers led by David Gerber searched for calcineurin-related genes to strengthen the link with schizophrenia. The disease is complicated, with many implicated genes scattered at various loci in the genome. Interestingly, many genes in the calcineurin pathway map to these loci, and the team homed in on those. They examined these genes in families containing schizophrenic members. A variant form of one calcineurin gene, *PPP3CC*, was found to be inherited at significantly higher frequency than expected in schizophrenics.

Taken together, all these results suggest that calcineurin defects are linked to schizophrenia and therefore offer a completely new target for therapeutic intervention.

4 Miyakawa, T. *et al.* (2003) Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1432926100 (http://www.pnas.org)

5 Gerber, D.J. et al. (2003) Evidence for association of schizophrenia with genetic variation in the 8p21.3 gene, PPP3CC, encoding the calcineurin gamma subunit. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1432927100 (http://www.pnas.org)

Viral Targets and Mechanisms

Blame the virus, not the bacteria

Until now, it was believed that disease-causing bacteria transferred between individuals by contact was the culprit in transferring disease. However, new research at the Rockefeller University (http://www.rockefeller.edu/) shows that sometimes a virus, which infects and destroys the bacteria, could be to blame [6]. The bacteriophage ('bacteria-eating' virus) causes disease by transferring toxins and other disease causing genes between bacteria.

Temperate bacteriophage can transfer toxin-encoding genes between bacteria because they encode toxins and virulence factors in addition to their essential viral proteins. This transfer can result in acquired pathogenicity. However, little is known about the eukaryotic host effects on the phage–pathogen interaction. This new research demonstrates that the eukaryotic host acts as an essential component in the phage-mediated evolution of virulence within the microbial population [6].

Scientists were unsure where the bacteria picked up the phage; however, a factor in human saliva has been identified, which is secreted by cells in the pharynx and causes the phage to activate and burst from the bacterium. 'It is possible that phage present in the saliva of an individual can cause the conversion of an existing non-toxigenic organism to a toxigenic one', says Vincent A. Fischetti, lead author of the paper.

The question raised is whether a non-toxigenic organism that is found in the oral cavity can become toxigenic if a toxin-encoded phage-carrying bacterium is added? This new research found that phage could 'jump' from a toxigenic *Streptococcus* strain, causing a strain that was not carrying the phage to produce toxins.

The research is the first to show that bacteriophage (or phage) could be a new target for fighting certain bacteria that produce toxins. 'Controlling the phage may be as important as controlling the bacteria', Fischetti adds.

6 Broudy, T.B. and Fischetti, V.A. (2003) *In vivo* lysogenic conversion of Tox(-) Streptococcus pyogenes to Tox(+) with lysogenic Streptococci or free phage. *Infect. Immun.* 71, 3782–3786

Genomics and Proteomics

RNAi unleashed on HIV

A new development involving RNA interference (RNAi) looks particularly encouraging in the battle against HIV. By using transfected stem cells, researchers might have found a way to permanently inhibit HIV replication in the cells the virus attacks [7].

Previous experiments had demonstrated that RNAi can be used to inhibit HIV-1 replication in cultured cells. Building on this, researchers from Colorado State University (http://welcome.colostate.edu/) and the City of Hope Cancer Center (http://www.cityofhope.org/) have demonstrated how to block HIV replication in the immune system cells that the virus attacks. Small interfering RNA (siRNA) was designed to target the HIV Rev gene, which is crucial for replication of the virus and highly conserved amongst viral strains. The gene encoding the siRNA was inserted into a lentiviral vector, which then delivered the gene to purified CD34+ stem cells. These cells are the progenitors of T-cells and macrophages, and the researchers hoped that the siRNA would continue to be expressed in the mature immune system cells. A portion of the transfected stem cells was cultured in the presence of growth factors; they matured into macrophages, which expressed the siRNA and showed resistance to HIV infection. Other stem cells were injected into SCID-hu mice, which have impaired immune systems and implanted human thymus and liver tissue. The cells matured into T-cells with resistance to HIV.

These findings demonstrated the power of the technique both *in vitro* and *in vivo*. Because stem cells are self-renewing, anti-HIV siRNA will continue to be produced indefinitely. This development could therefore lead to a method of managing HIV throughout a patient's lifetime, by preserving the efficiency of the immune system. Results so far also indicate that the siRNA is non-toxic and that the stem cells are able to differentiate without hindrance.

'We are now poised for human clinical trials, but I don't yet see siRNA as a sole treatment for HIV,' said Ramesh Akkina, lead researcher. But he added that, in the longer term, siRNAs have great potential as anti-HIV therapeutics.

7 Banerjea, A. *et al.* (2003) Inhibition of HIV-1 by lentiviral vector-transduced siRNAs in T-lymphocytes differentiated in SCID-hu mice and CD34+ progenitor cell-derived macrophages. *Mol. Ther.* 8, 62-71

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