IN THE NEWS

Loss of silencing leaves patients speechless

Children with Rett syndrome present autistic-like behaviour and specific clinical signs, such as hand wringing and loss of speech. Scientists previously showed that this X-linked neurodevelopmental disorder is caused by mutations in a gene on the X chromosome, which encodes methyl-CpG-binding protein-2 (MECP2).

As MECP2 regulates the transcriptional activity of other genes, a team led by Terumi Kohwi-Shigematsu of the Lawrence Berkeley National Laboratory, California, USA, searched for MECP2 target genes that might be dysregulated in mice with a defective *MECP2* gene. Among them was *DLX5*, the expression of which almost doubled in MECP2-knockout mice.

The maternally expressed *DLX5* gene showed loss of imprinting in lymphoblastoid cells from Rett-syndrome patients. The scientists found that, in mice, intact MECP2 was required for specifying repressive histone methylation in the region that contained the *DLX5* gene, and for organizing a transcriptionally silent chromatin loop. In MECP2-null cells, such a loop was missing.

"The findings are an important piece in a very big puzzle", according to Alan Percy of the University of Alabama, Birmingham, USA (*ScienceNow*, 21 December 2004).

In humans, DLX5 has an important role in the synthesis of γ-aminobutyric acid (GABA), which is a neurotransmitter that has been linked to other neurological disorders including epilepsy and Parkinson's disease. So the loss of imprinting might change the GABAergic neuron activity. "The next question is whether cranking up the *DLX5* gene results in some of the problems of Rett syndrome", says Kohwi-Shigematsu (*ScienceNow*, 21 December 2004).

Arianne Heinrichs

CALCIUM

A changed cameleon

Ca^{2+} is one of the most versatile signalling factors that has been identified so far, and the release of Ca^{2+} from the endoplasmic reticulum (ER) — which functions as an intracellular Ca^{2+} storehouse — has crucial roles in the regulation of processes such as apoptosis and exocytosis. In *Proceedings of the National Academy of Sciences*, Roger Tsien, John Reed and colleagues now describe the development of an improved genetically encoded fluorescent sensor that can monitor Ca^{2+}-concentration fluctuations in the ER ($[Ca^{2+}]_{ER}$), and they have used this sensor to study the role of the anti-apoptotic protein B-cell lymphoma-2 (Bcl2) in breast cancer cells.

Amy Palmer in the Tsien laboratory started with the original cameleon construct — two fluorescent proteins (cyan fluorescent protein (CFP) and citrine) separated by calmodulin (CaM) and a CaM-binding peptide. In the presence of Ca^{2+}, CaM interacts with the CaM-binding peptide, and CFP emission decreases as citrine emission increases, which is indicative of increased fluorescence resonance energy transfer (FRET). Their aim was to change this cameleon to decrease its perturbation by endogenous CaM and to vary its Ca^{2+} affinity.

Using previously obtained structural data, Palmer targeted salt-bridge interactions between CaM and the CaM-binding peptide. Compared to the wild-type peptide, a mutant peptide with four charge reversals had a 10,000-fold lower affinity for wild-type CaM. Palmer then made compensatory charge reversals in CaM with the aim of restoring its affinity for the mutant peptide. She named the mutant CaM and peptide pair Design 1 (D1), and cloned it between CFP and citrine to produce an altered cameleon.

Next, Palmer studied the properties of the D1 cameleon. In the presence of Ca^{2+}, the magnitude of the observed FRET changes was comparable to that seen for the original cameleon. In addition, the Ca^{2+} titration curve for the D1 cameleon showed that it is ideal for monitoring $[Ca^{2+}]_{ER}$ (that is, Ca^{2+} concentrations in the low micromolar to hundreds of micromolar range). Furthermore, the D1 cameleon is not perturbed by large excesses of endogenous CaM, and it has a faster k_{off} than previous cameleons, so it can monitor rapidly changing Ca^{2+} dynamics.

CYTOSKELETON

Inspired filaments

Flies hooked on coffee? Well, *Drosophila melanogaster* does need *cappuccino* — for oocyte and embryonic polarity. It also requires *spire*. Both genes contribute to polarity by affecting the actin cytoskeleton. The *cappuccino* gene product, Capu, nucleates actin-filament formation through its formin homology (FH) domains, but Quinlan *et al.* now report that Spire (Spir) nucleates filaments by a totally new mechanism.

Spir contains four WASP-homology-2 (WH2) domains and a stretch of acidic residues, which indicated that it might activate the actin-nucleating Arp2/3 complex. But Spir induced the formation of filamentous actin clusters in the absence of Arp2/3. This was mediated by its N-terminal half, which contains the WH2 repeats and the acidic domain, and occurred by nucleating actin filaments *de novo* with growth from the barbed end of the filament.

Next, the authors compared the nucleation activities and mechanisms of Spir, Capu and Arp2/3. The FH1 and FH2 domains of Capu and the N terminus of Spir nucleated actin polymerization at similar rates — slower than that of Arp2/3. And whereas the Arp2/3 complex generated crosslinked filaments, filaments that were formed by Spir and Capu weren't crosslinked. But, similar to Arp2/3, the N terminus of Spir capped the pointed ends of filaments.

Further investigation showed that each WH2 domain and the sequences linking them have varying roles in actin nucleation. Mutating all four WH2 domains didn't completely abolish nucleation activity, and Quinlan *et al.* found that linker-3 (L-3) showed weak nucleation activity, which they propose stabilizes two actin monomers to promote actin-dimer formation.

The authors then tested their hypothesis that, to form a new filament, Spir binds several actin monomers before assembling them into a filament nucleus. Rod-like Spir–actin complexes of four actin monomers aligned along their length were seen, consistent with WH2 domains binding and aligning actin monomers end to end.

Because the last two WH2 domains connected by L-3 seemed to comprise the functional core of Spir, the authors propose that this region mediates the formation of an actin dimer — the main kinetic hurdle to nucleation. A third and fourth monomer are then added by the first two WH2 domains. Spir might then stack monomers to form a nascent filament, and rapid filament elongation from the stable

The addition of an ER signal sequence and an ER-retention tag resulted in the D1 cameleon being specifically localized to the ER, and Palmer showed that the D1 cameleon can detect both increases and decreases in $[Ca^{2+}]_{ER}$ with a much greater sensitivity than previous cameleons. Furthermore, she showed that the D1 cameleon and the fluorescent dye fura-2 can be used together to allow the simultaneous monitoring of $[Ca^{2+}]_{ER}$ and $[Ca^{2+}]_{cytosol}$, respectively.

Moving to a biological application for the improved cameleon, Palmer collaborated with Can Jin in the Reed laboratory to study the relationship between Bcl2, $[Ca^{2+}]_{ER}$ and apoptosis in a breast cancer cell line. Over-expressing Bcl2 in these cells, which is known to make them resistant to apoptosis, lowered $[Ca^{2+}]_{ER}$ under resting conditions by increasing Ca^{2+} leakage and altered the Ca^{2+} oscillations that were induced by ATP. In addition, the authors showed that a green-tea compound, epigallocatechin gallate, induced apoptosis in Bcl2-overexpressing breast cancer cells in a dose-dependent manner. Epigallocatechin gallate binds to Bcl2 and increases $[Ca^{2+}]_{ER}$ by blocking Bcl2-mediated leakage. They are now trying to elucidate the exact link between Bcl2 inhibition, increased $[Ca^{2+}]_{ER}$ and apoptosis.

Rachel Smallridge

References and links

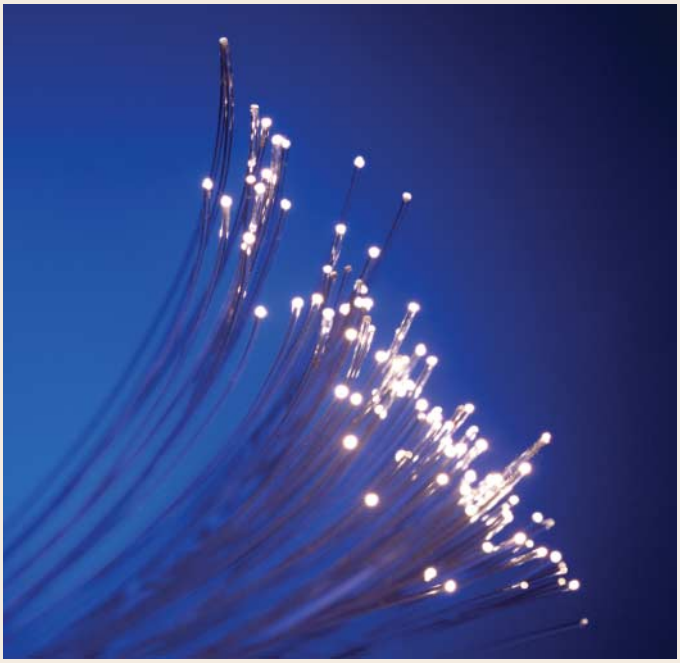
ORIGINAL RESEARCH PAPER Palmer, A. E. *et al.* Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor. *Proc. Natl Acad. Sci. USA* **101**, 17404–17409 (2004)

FURTHER READING Rudolf, R. *et al.* Looking forward to seeing calcium. *Nature Rev. Mol. Cell Biol.* **4**, 579–586 (2003)

WEB SITES

John Reed's laboratory: http://www.burnham.org/FacultyAndResearch/faculty/john_reed_bio.asp

Roger Tsien's laboratory: http://www.tsienlab.ucsd.edu/



nucleus follows. This brings the number of classes of actin-nucleation factor to three.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Quinlan, M. E. *et al.* *Drosophila* Spire is an actin nucleation factor. *Nature* 27 Jan 2005 (doi:10.1038/nature03241)

IN BRIEF

PROTEOMICS

Nucleolar proteome dynamics.

Andersen, J. S. *et al. Nature* **433**, 77–83 (2005)

Using mass-spectrometry-based organellar proteomics and stable-isotope labelling, Mann, Lamond and colleagues quantitatively analysed the proteome of human nucleoli. By monitoring 489 endogenous nucleolar proteins, they showed that the nucleolar proteome varies considerably over time in response to changes in cellular growth conditions. They therefore "…conclude that there is no unique, complete proteome for the nucleolus, or probably for any other organelle, but rather an overlapping set of proteomes that are relevant to different cell states or conditions."

MITOSIS

Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation.

Higuchi, T. & Uhlmann, F. *Nature* **433**, 171–176 (2005)

Microtubules (MTs) form the bipolar mitotic spindle, which captures chromosomes during metaphase and segregates chromosomes during anaphase. At the onset of anaphase, the highly dynamic MTs become more stable, and the authors showed that the phosphatase Cdc14, which is activated by separase at the onset of anaphase, is required for this. Without Cdc14, MTs remain dynamic, and this interferes with the transport of chromosomes towards the spindle poles and with spindle elongation.

SIGNALLING

EGF receptor signaling regulates pulses of cell delamination from the *Drosophila* ectoderm.

Brodu, V., Elstob, P. R. & Gould, A. P. *Dev. Cell* **7**, 885–895 (2004)

The authors studied the separation of oenocyte precursors from the ectoderm of developing *Drosophila melanogaster* embryos and report an example of oscillatory cell behaviour. The precursors delaminate in discrete, well-separated bursts of three. The epidermal growth factor receptor (EGFR) ligand Spitz specifies the final number of delamination pulses, but has only a permissive role in generating pulses. Before delamination, several EGFR targets, some of which might underlie pulse generation, are switched on in the three cells.

CYTOSKELETON

TSC2 modulates actin cytoskeleton and focal adhesion through TSC1-binding domain and the Rac1 GTPase.

Goncharova, E. *et al. J. Cell Biol.* **167**, 1171–1182 (2004)

Tuberosis sclerosis complex (TSC) protein-1 and -2 regulate protein translation and cell growth, but are also implicated in cell motility. Goncharova *et al.* found that TSC2 regulates stress-fibre disassembly and focal-adhesion remodelling through its TSC1-binding domain by Rac1 activation and subsequent Rho inhibition. The TSC1-binding domain, however, is not needed for TSC2 to regulate the ribosomal protein S6 or DNA synthesis.