## 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 neuro-developmental 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**