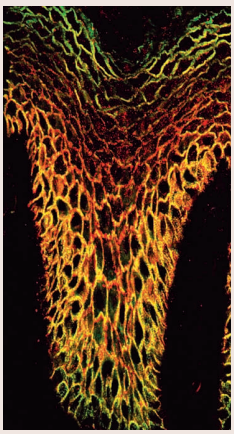
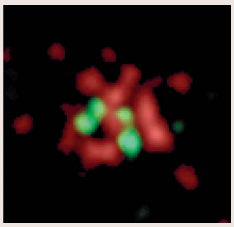
In this issue

by Jane Bradbury



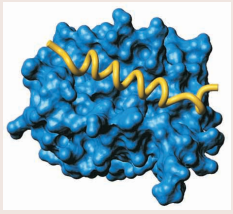
Sorting epidermal stem cells from the crowd

Despite considerable research effort, there is no widely accepted method for the purification of epidermal stem cells. On p. 4239, Hong Wan and co-workers report a new strategy for the isolation of these cells that combines selection for low levels of expression of the desmosomal cadherin desmoglein 3 (Dsg3) with high levels of β1 integrin, a previously proposed marker for epidermal stem cells. Because desmosomes become more numerous as keratinocytes differentiate, the researchers hypothesised that these intracellular junctions might be sparse in epidermal stem cells. They confirmed this by colony-formation assays of keratinocytes sorted on the basis of desmosomal protein expression. Keratinocyte populations that have high levels of β1 integrin and low levels of Dsg3 were more enriched for stem-cell-like cells than were keratinocytes selected with either marker alone. This strategy for epidermal stem-cell enrichment, they conclude, should be useful both for stem-cell studies and for the development of stem-cell-mediated replacement therapies.



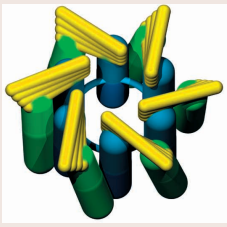
A tubulin poly-glutamylase subunit

Polyglutamylation of tubulin is common at centrioles, basal bodies and axonemes and characteristic of most neuronal microtubules. This modification is implicated in control of centriole stability, axoneme beating and neuronal differentiation, but the enzyme responsible has eluded researchers. Bernard Eddé and co-workers have now identified and characterized one of its subunits, PGs1 (see p. 4181). Starting with a partially purified preparation from mouse brain, the authors use immunoprecipitation to show that the polyglutamylase comprises three polypeptides: p32 (PGs1), p50 and p80. They then demonstrate by mass spectrometry and database searches that the PGs1 subunit is encoded by *GTRGEO22*, a gene whose mutation is associated with formation of abnormal spermatid flagella and male sterility. Eddé and co-workers confirm that PGs1 is indeed a component of the enzyme by showing that a monoclonal antibody specific for the protein pulls down polyglutamylase activity. Immunofluorescence studies using this antibody indicate that PGs1 colocalizes with highly polyglutamylated structures, including centrosomes, basal bodies and neurite microtubules. Since PGs1 has no intrinsic enzymatic activity but continues to localize correctly when overexpressed, the authors suggest it acts as a targeting subunit for the polyglutamylase complex.



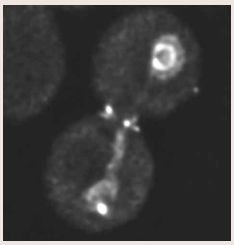
Bcl-2 in brief

Members of the Bcl-2 family are key regulators of apoptosis. Proteins such as C. elegans CED-9, Bcl-x_L and Bcl-2 itself promote cell survival, but others – for example, Bid, Bax and Bcl-G_s – are pro-apoptotic. If that wasn't confusing enough, these molecules respond to numerous different stimuli, such as DNA damage, Ca^{2+} and cytokine deprivation, and their antagonistic roles are the subject of considerable debate. Help is at hand, however. In Cell Science at a Glance (see p. 4053 + poster), David Huang and co-workers provide a survey of the Bcl-2 proteins, including the various Bcl-2 homology (BH) domains they contain and current models for how they regulate cell death and cell survival.



Uroplakin – how the umbrella works

The mammalian urothelium is one of the most effective permeability barriers evident in nature, maintaining an incredibly steep concentration gradient between the urine and plasma. Plaques comprising the transmembrane protein uroplakin on the apical surface of urothelial umbrella cells constitute the permeability barrier, and these can also act as docking sites for bacteria that cause urinary tract infections. Xiang-Peng Kong and co-workers have examined the structural basis for barrier function by performing cryoelectron microscopy of uroplakin particles, which naturally form hexagonally packed, 2D crystals (see p. 4087). This approach has allowed them to generate an ~7-Å-resolution projection map and a 20-Å-resolution 3D image of the 16-nm uroplakin particle, which reveal numerous novel structural aspects. For example, they show that each particle has a central, 6 nm, lipid-filled hole surrounded by six inverted U-shaped subunits. Uroplakin could therefore severely restrict the movement of membrane lipids, which might contribute to its barrier function. The structures also reveal that there is little contact between neighbouring subunits and thus the potential for significant conformational change, which could be important for urothelial signal transduction following bladder stretching and bacterial binding.



Cyclin B2 – Bud3 marks the spot

In budding yeast, six B-type cyclins can associate with the single cyclin-dependent kinase (CDK) that drives the cell cycle (Cdc28). These are thought to commit it to particular tasks, although the basis for this remains elusive. They could alter CDK substrate specificity, but regulation of its subcellular localization is equally likely. Marie-Noëlle Simon and co-workers have therefore investigated the subcellular localization of Clb2, a B-type cyclin particularly important for the G2/M transition (see p. 4119). Tracking GFP fusion proteins expressed at physiological levels, they observe that Clb2 localizes to the nucleus, mitotic spindle, spindle pole bodies and – in contrast to other B-type cyclins – the mother-bud neck. Localization to the mother-bud neck requires the conserved hydrophobic patch in the protein, as well as upstream Clb2-specific sequences. Moreover, it requires the axial determinant Bud3, which the authors show colocalizes with Clb2 and interacts with it in two-hybrid assays. Since the absence of Clb2 at the mother-bud neck in *bud3* mutants is associated with a delay in cytokinesis, these experiments reveal not only a novel function of Bud3 but also a possible role for neck-localized Clb2-Cdc28 in control of cytokinesis.

Development in press

Left or right: 14-3-3E makes an early move

Establishment of a left-right (LR) axis during embryogenesis underlies the asymmetric body plan of adult animals – placing the heart on the left side of the human body, for example. Writing in *Development*, Bunney et al. report that the signalling molecule 14-3-3E is involved very early in LR patterning in *Xenopus laevis*. They show that the treatment of newly fertilised eggs with fusiococcin A, a fungal toxin that interacts with 14-3-3 proteins, randomizes on which side of the body the heart, gut and gall bladder develop, as does blocking 14-3-3 function with a phosphopeptide containing a 14-3-3-interaction motif or overexpressing 14-3-3E. Their demonstration that 14-3-3E is asymmetrically localised at the first cell division of fertilised *Xenopus* eggs identifies the earliest such example of LR asymmetric localization of a molecule in any species to date.

Bunney, T. D., De Boer, A. H. and Levin, M. (2003). Fusicoccin signaling reveals 14-3-3 protein function as a novel step in left-right patterning during amphibian embryogenesis. *Development* **130**, 4847-4858.