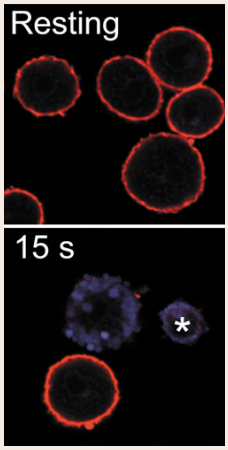
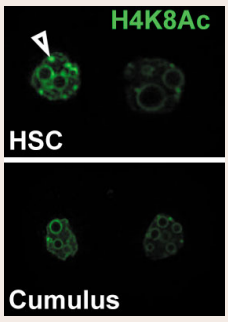
In this issue

by Jane Bradbury



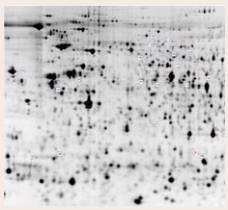
Exocytosis: removing the PIPs

Despite their low abundance in biological membranes, phosphoinositides are important cellular regulators. Phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] regulates both endocytosis and exocytosis, but its role in the latter process is unclear. On p. 2084, Giampietro Schiavo and co-workers report that elimination of PtdIns(4,5)P_2 from the plasma membrane is required for regulated exocytosis in activated mast cells. These cells release pro-inflammatory mediators by exocytosis of pre-formed granules, a process that involves granule–plasma-membrane fusion and granule–granule fusion but no compensatory endocytosis. Using quantitative immunofluorescence, the authors show that PtdIns(4,5)P_2 is present in the plasma membrane of mast cells but not their granule membranes. Upon activation of exocytosis, they report, PtdIns(4,5)P_2 is transiently depleted from the plasma membrane by phosphatidylinositol-specific phospholipase C; both removal of PtdIns(4,5)P_2 and production of its metabolite diacylglycerol (but not activation of Ca^{2+} signalling,) are required for exocytosis. The authors conclude that a cycle of PtdIns(4,5)P_2 synthesis and breakdown regulates exocytosis in mast cells and speculate that a similar cycle functions in other secretory cells.



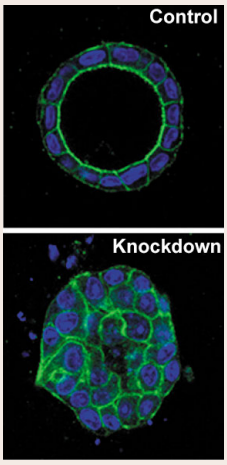
Stem cell glitch halts cloning

Cloned embryos can be produced reasonably efficiently by transferring nuclei from undifferentiated embryonic stem cells into oocytes but nuclear transfer from somatic cells is inefficient, partly because the epigenetic modifications that characterize differentiated cells must be reprogrammed. Thus, it has been proposed that adult stem cells, with their relatively undifferentiated genomes, are efficient nuclear donors. On p. 1985, Atsuo Ogura and colleagues reveal that this is not necessarily the case. They show that offspring are generated less frequently by nuclear transfer from mouse haematopoietic stem cells (HSCs) than from other somatic cells (e.g. fibroblasts). Developmental arrest occurs between the 2-cell and 4-cell stage in embryos containing HSC nuclei, and these embryos fail to activate several embryonic genes, including the chromatin-modifying enzyme histone deacetylase 1. The authors suggest that the low expression level of this enzyme is responsible for the limited developmental potency of the embryos, and conclude that the efficiency of cloning by nuclear transfer and cell differentiation status are not always reciprocally related.



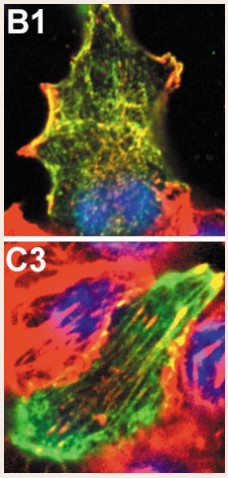
Spotting developmental regulators

Analysis of patterns of transcription has identified many genes that regulate vertebrate gastrulation, an early morphological event that forms the distinct germ layers of the embryo. But this approach misses developmentally important proteins whose activity is regulated translationally or post-translationally. To identify these, Carl-Philipp Heisenberg's team has used 2D gel electrophoresis and mass spectrometry to compare the patterns of protein synthesis and modification in zebrafish ectodermal and mesendodermal progenitor cells (see p. 2073). Using this comparative proteomics approach, the authors identified 35 differentially produced/modified proteins, most of which were not evident from a parallel transcription analysis. One protein identified by the analysis is ezrin 2, When activated by phosphorylation, this protein links actin filaments to integral plasma membrane proteins, thereby regulating cell shape, adhesion and motility. The authors find that ezrin 2 is activated by phosphorylation in zebrafish mesendodermal cells and is required for proper germ layer morphogenesis. They therefore conclude that comparative proteomics provides a powerful way to identify proteins that regulate early development.



Lgl polarizes the (t)issue

The regulation of epithelial cell polarity is essential for the maintenance and function of many tissues. In *Drosophila*, the apical PAR-3 –aPKC–PAR-6 complex and the basolateral tumour suppressor protein Lgl act antagonistically to regulate epithelial polarity. In mammals, although similar proteins regulate epithelial cell polarity, the exact role of mLgl-1 and mLgl-2, the two mammalian orthologues of *Drosophila* Lgl, is unclear. On p. 2107 Shigeo Ohno and co-workers provide new insights into this mystery by using RNAi to show that endogenous mLgl is needed for the disassembly of apical membrane domains in a mammalian kidney cell line induced to depolarize by Ca^{2+} depletion. mLgl proteins, they report, function by suppressing the activity of the apical PAR-3–aPKC–PAR-6 complex and are required in other situations where epithelial cell polarity is manipulated, such as collagen-mediated re-orientation of apical membrane polarity. Given these results, the authors speculate that mLgl helps to ensure that the polarization of individual cells is integrated with whole tissue architecture.



Actin' roles for glucose in insulin secretion

One essential role of the actin cytoskeleton is to regulate vesicle exocytosis. In pancreatic β-cells, actin filaments limit the access of insulin granules to the plasma membrane but may also facilitate regulated hormone secretion. To untangle the relationship between insulin secretion and the actin cytoskeleton, Alejandra Tomas and colleagues have studied two sublines of the mouse pancreatic β-cell line MIN6: the B1 subline responds to glucose by secreting insulin, the C3 subline does not. On p. 2156, the authors show that, whereas C3 cells have a rigid cytoskeleton that does not remodel after glucose stimulation, B1 cells have shorter actin filaments, which depolymerize readily in response to glucose. This depolymerization, they report, depends on the Ca^{2+}-dependent actin-remodelling protein gelsolin. Furthermore, the authors find that actin polymerization affects the localization of components of the MAP kinase signalling cascade, which regulates β-cell insulin secretion in response to glucose. Thus, they conclude, actin remodelling potentiates β-cell insulin secretion both by removing a physical barrier to the process and by regulating MAP kinase signalling.

Development in press

TAGging early transcription

What triggers gene expression in very early *Drosophila* embryos? Widespread expression of zygotic genes begins at the blastoderm stage, but little is known about the regulation of the small numbers of genes transcribed in the pre-cellular blastoderm, when the rest of the genome is still silent. In a paper appearing in *Drosophila*, Thomas Cline and colleagues report that the sex determination genes – which must be expressed early so that X-dosage compensation can kick in before widespread transcription begins – all possess multiple copies of the sequence CAGGTAG. Using a computational search, the authors found that this sequence, or similar degenerate sequences, is over-represented upstream of most genes expressed before the blastoderm stage; they call these sequences the TAGteam. Remarkably, eliminating TAGteam sites causes the late transcription of genes carrying these sequences, and duplication of the minimal TAGteam sequence speeds up transcription. The authors suggest that the transcriptional regulation of these very early genes involves specific regulatory proteins that are probably maternally derived.

ten Bosch, J. R., Benavides, J. A. and Cline, T. W. (2006). The TAGteam DNA motif controls the timing of *Drosophila* pre-blastoderm transcription. *Development* **133**, 1967-1977.