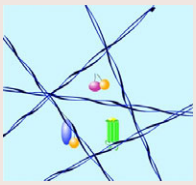
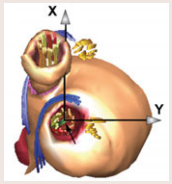
In this issue



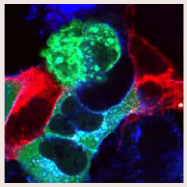
G proteins make a move

The aggregation of *Dictyostelium discoideum* in response to an extracellular cAMP gradient has been closely studied as a model of eukaryotic chemotaxis, but many important questions remain unanswered – such as how the activation of cAR1 (the cAMP-responsive GPCR at the cell surface) gives rise to directional movement. On page 2922, Thomas Schmidt and colleagues address this question by studying the mobility of the heterotrimeric G proteins downstream of cAR1. Using single-molecule epifluorescence microscopy, the authors show that Gα2-YFP and Gβ-YFP both exist in slow-diffusing (receptor-coupled) and fast-diffusing (not receptor-coupled) forms in the absence of cAMP. When cAMP is added globally, the receptor-coupled complex dissociates, and Gα2-YFP and Gβ-YFP exhibit a pattern of confined diffusion. The authors also report that a fraction of Gβ-YFP becomes immobilised in the presence of cAMP in an F-actin-dependent manner; moreover, in chemotaxing *Dictyostelium*, Gβ-YFP immobilisation occurs only at the leading edge. On the basis of their data, the authors developed a mechanistic model of the early stages of chemotactic signalling, and propose that F-actin-based membrane domains locally restrict activation signals to enable faithful chemotactic signalling.



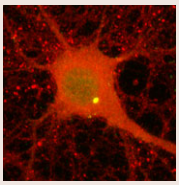
The basal body: going round in circles

To divide successfully, eukaryotic cells must duplicate organelles and segregate them accurately into daughter cells, in a complex process that has been closely studied in the protist *Trypanosoma brucei*. Now, J. Richard McIntosh, Keith Gull and colleagues (p. 2884) provide new insight into how the single *T. brucei* flagellum and flagellar pocket replicate. The authors use a combination of electron microscopy and electron tomography to investigate the duplication of the *T. brucei* basal body (BB) – which sits at the base of the flagellum – in three dimensions. They show that, early in the cell cycle, a probasal body is positioned anterior to the BB of the existing flagellum, and matures and elongates to form the new flagellar axoneme. Surprisingly, at the G1–S transition the new BB rotates anti-clockwise around the pre-existing BB, taking up a new position posterior to the pre-existing BB. A new flagellar pocket is formed as a consequence of this migration, and the authors propose a role for flagellar microtubule arrays in pocket duplication. Their results describe a striking characteristic of flagellum duplication in *T. brucei*, and shed light on how organelle replication is coordinated with the cell cycle.



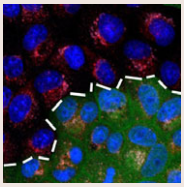
Nodder sheds light on Notch

The Notch signalling pathway – which regulates gene expression in numerous cellular processes – involves direct interactions between cell-bound ligands (such as Jagged1) and Notch receptors on juxtaposed cells. Endocytosis of Notch ligands is important for Notch activation, but its precise role is still debated. Now, Urban Lendahl and colleagues (p. 2931) use Nodder (Ndr), a missense mutant of Jagged1 in mice, to investigate the relationship between ligand endocytosis and ligand–Notch interactions. The authors show that, in cell culture, Ndr displays a normal cellular distribution in the absence of Notch signalling, but does not interact with Notch receptors. Moreover, cells expressing wild-type Jagged1 trans-endocytose the Notch extracellular domain, but Ndr-expressing cells do not. Jagged1 and Ndr both interact with the E3 ubiquitin ligase Mind bomb, but only Jagged1 shows enhanced ubiquitylation in co-culture with Notch-expressing cells. Finally, in *Jag1*$^{Ndr/Ndr}$ mice, Ndr accumulates at the cell surface. The authors' data lend support to a 'pulling-force' model, in which ligand endocytosis provides a shearing force that is important for Notch activation.



FTD: the spines have it?

Frontotemporal dementia (FTD) is caused by dominant mutations in CHMP2B, a subunit of the ESCRT-III complex (which is important for the restructuring of membranes during diverse cellular activities). The mechanism of slow cortical degeneration in FTD is not well understood, but impaired neuronal autophagy has been thought to be important. Now, Yves Goldberg and colleagues (p. 2943) propose instead that mutant CHMP2B inhibits dendritic-spine maturation. Using confocal microscopy and 3D reconstruction, the authors show that cultured hippocampal neurons that express FTD-linked CHMP2B mutants have strikingly fewer large, mushroom-shaped dendritic spines than wild-type cells; moreover, depleting endogenous CHMP2B has a similar effect on spine shape. They next show that neurons expressing an FTD-linked CHMP2B mutant exhibit a drop in the frequency and amplitude of spontaneous excitatory postsynaptic currents. In contrast to studies that have used other cell types, the authors find no evidence that autophagy is perturbed in these cells. They conclude that CHMP2B is important for normal dendritic-spine development, and propose that gradual neurodegeneration through impaired spine formation explain the slow progression of FTD.



Scattering cells with PKC

In tumours, impaired blood supply leads to a shortage of oxygen (hypoxia), nutrients and growth factors. Hypoxia stimulates invasion and metastasis in tumour cells, but it is less clear how they adapt to a shortage of other blood-borne compounds. Now, Hong-Chen Chen and colleagues (p. 2901) investigate the role of protein kinase Cδ (PKCδ) – which is activated in response to a wide variety of stressors – in adapting to growth-factor deprivation. The authors grow MDCK cells under serum-depleted conditions to mimic a lack of growth factor and show that, when expression of PKCδ is elevated, cell–cell contacts are lost and cells scatter. Additionally, serum starvation stimulates the catalytic activity of PKCδ through the action of the kinase Src and reactive oxygen species (ROS; treatment with the ROS scavenger N-acetylcysteine blocks PKCδ activation). Notably, activated PKCd also stimulates ROS production. The authors next show that JNK is important for PKCd-induced cell scattering. Finally, they report that, in human bladder carcinoma cells, depletion of PKCδ reverses scattering and restores cell–cell contacts. These and other results indicate that PKCδ is important in the cellular response to growth-factor deprivation, and highlight the signalling pathways involved.

Development in press

Hematopoietic differentiation: lessons from development

Efficient production of hematopoietic stem (HPS) cells from embryonic stem (ES) cells or from induced pluripotent stem (iPS) cells requires a thorough understanding of hematopoietic differentiation pathways. In *Development*, Gordon Keller and colleagues show that the generation of HPS cells from ES and iPS cells follows the same steps as hematopoietic development in the embryo. They induced ES cells with known agonists of embryonic hematopoiesis (activin A, BMP4 and VEGF) and identified two temporally distinct populations of cells that express the hematopoetic marker Flk1. The gene expression profiles, cell-surface markers and lymphoid potential of the early Flk1-positive population resembled those of the first site of embryonic hematopoiesis, whereas the cells that expressed Flk1 at a later stage corresponded to a later stage of hematopoiesis. The ability to identify and isolate different stages in the progression from ES or iPS cells to HPS cells will facilitate the study of the generation of blood-cell lineages, and may improve the efficiency of the production of transplantable cells.

Irion, S., Clarke, R. L., Luche, H., Kim, I., Morrison, S. J., Fehling, H.-J. and Gordon M. Keller (2010). Temporal specification of blood progenitors from mouse embryonic stem cells and induced pluripotent stem cells. *Development* **137**, 2829-2839.