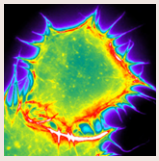
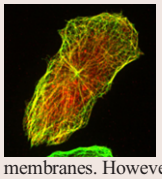
In this issue



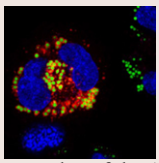
Rac: essential for protruding but not for spreading

Cell migration involves the generation of protrusions, such as membrane ruffles and lamellipodia, which help cells to explore the surrounding space and to initiate nascent adhesions. Lamellipodia consist of networks of connected actin filaments that are mediated by the actin nucleator Arp2/3. Small GTPases of the Rac family are known to stimulate lamellipodia formation, but their exact role is unclear because, thus far, only conditional Rac1-knockout cell lines have been established. In this study (p. 4572), Klemens Rottner and colleagues generate the first permanent fibroblast cell lines that are genetically deficient for Rac1, and also lack detectable levels of haematopoietic Rac2 and brain-specific Rac3. They find that these Rac-deficient cells are devoid of lamellipodia; however, these can be restored by expression of Rac, but not of RhoG or Cdc42, which had previously been implicated in driving lamellipodia formation. In addition, the Rac-deficient cells also show a strong reduction in wound closure and random cell migration. Surprisingly, however, these cells are able to spread despite the absence of lamellipodia, suggesting that Rac signalling is dispensable for actin remodelling events at the cell periphery that drive the protrusion of filopodia and the formation of nascent adhesions. These findings further highlight that the Rac-deficient cell lines presented here are useful tools in dissecting the functions of Rac in the diverse cellular processes they are involved in.



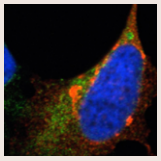
MTCL1: MT reorganiser in polarised cells

During establishment of epithelial cell polarity, microtubules (MTs) undergo a dramatic reorganization from a radial array to a vertical alignment of non-centrosomal MT bundles that run along the lateral membrane, with their minus ends toward the apical surface and a MT meshwork under the apical and basal membranes. However, the mechanisms underlying MT reorganization are not fully understood. It has been suggested that these MT bundles nucleate from the apical centrosome and are then captured by MT-minus-end-binding proteins, but it is unclear how they are bundled. On page 4671, Atsushi Suzuki and co-workers now present the identification of a new MT-binding protein they call microtubule crosslinking factor 1 (MTCL1). They show here that MTCL1 is a new MT-crosslinker that, although not required for the initial radial growth of MTs from the apical centrosome, is essential for the accumulation of MTs at the lateral cortex of polarising epithelial cells where it promotes the development of lateral MT bundles. Furthermore, the authors demonstrate that MTCL1 is a binding partner of the polarity-regulating kinase PAR-1b, which is known to positively regulate MT dynamics and to have a role in MT reorganization in polarised epithelial cells. On the basis of these results, the authors propose that MTCL1 serves as a scaffold for PAR-1b and recruits the kinase to the lateral MT bundles, which in turn maintains their dynamic state.



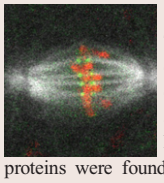
GBF1 targeting to lipid droplets

Lipid droplets (LDs) are the main energy storage depots of eukaryotic cells. They have a neutral lipid core surrounded by a phospholipid monolayer and, similar to other organelles, specific small GTPases that help to mediate their functions. ADP-ribosylation factor 1 (Arf1), a member of the Arf family of small GTPases, and its activation by guanine nucleotide exchange factors (GEFs) of the GBF and BIG families, have been shown to be required both for LD metabolism and secretory pathway trafficking. However, it is not well understood how Arf1 and its regulators are recruited to LDs. On page 4794, Catherine Jackson and colleagues now address the mechanism through which GBF1 is recruited to membranes. They find that the lipid-binding domain HDS1 in GBF1, located just downstream of the catalytic domain, mediates its association with LDs and Golgi membranes in cells, and with bilayer liposomes and artificial droplets *in vitro*. Furthermore, the authors show that an amphipathic helix within HDS1 is necessary and sufficient for LD and membrane binding, indicating that it is an LD-targeting motif. Interestingly, although HDS1 alone can bind to LDs, the routing of GBF1 to Golgi membranes requires additional sequences. Taken together, these data are, therefore, the basis for a model of how Arf1 GEFs are targeted to different membranes within the cell.



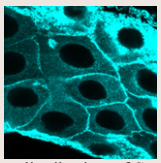
Shaping membranes for vision: curvature induction by P/rds

Vertebrate photoreceptor outer segment organelles are complex membranous structures that are derived from non-motile cilia. Outer segments utilise a stacked array of photopigment-filled membranous disks to convert light into neuronal signals. The mature rod outer segment disk is a distinct subcellular compartment with a rim structure along its periphery; however, the mechanism that distorts rim membranes into high curvature bends is not known. The photoreceptor-specific tetraspanin peripherin-2/rds (P/rds, also known as PRPH2) has been proposed to have a role in rim structure, but direct evidence for such activity has been lacking. On page 4659, Andrew Goldberg and colleagues investigate the function of P/rds in shaping outer segment membranes. They show that the cytoplasmic C-terminal domain of P/rds contains a tethered amphipathic helix that can induce membrane curvature. In addition, they find that association of this domain with liposomes requires conical phospholipids and is promoted by membrane curvature and anionic surface charge, which suggests that the amphipathic helix generates curvature through a hydrophobic insertion mechanism. In agreement with this, P/rds also induces tubulovesicular membrane foci in cultured cells. On the basis of these results, the authors propose that the membrane curvature induced by the tethered amphipathic helix contributes to outer segment disk rim structure and could explain the disrupted organelle structure in retinal diseases that are caused by genetic defects in the P/rds gene.



Cid dynamics at the centromere

The maintenance of one – and only one – functional centromere per chromosome during cell proliferation is crucial for accurate chromosome segregation. Centromeres are specified epigenetically and contain marks such as the histone 3 variant Cenp-A. In mammalian cells, more than 20 proteins were found to associate with centromeres. In *Drosophila*, centromere complexity is reduced. Maintenance of the *Drosophila* Cenp-A ortholog, centromere-identifier (Cid), might depend on only two additional centromere proteins, Cenp-C and Cal1. Therefore, *Drosophila* is an ideal model system to investigate the molecular mechanisms that underly centromere maintenance and, on page 4782, Christian Lehner and co-workers set out to analyse the dynamics of Cid, Cenp-C and Cal1 in *Drosophila* S2R+ cells. By using quantitative *in vivo* imaging and fluorescence recovery after photobleaching (FRAP), they show that the behaviour of Cid throughout the cell cycle is more complex than anticipated. It had been proposed that Cid is loaded onto centrosomes during a defined window in the cell cycle and then remains stably associated. However, the authors now demonstrate that, in these cells, there are five different centromere states, some of which include turnover or reduction in centromeric Cid. Taken together, these results provide new insights into centromere dynamics that might help to further elucidate the mechanisms that underly genome stability.



Smo activation – location is the key

The Hedgehog (Hh) signalling pathway is highly conserved and has a role in many cellular processes involved in development and disease. Hh binding to its receptor Patch (Ptc) alleviates Ptc repression of Smoothend (Smo), the key signal transductor of the Hh pathway. This leads to the redistribution of Smo to the plasma membrane, phosphorylation of its cytoplasmic tail, Smo oligomerisation and, subsequently, activation of the pathway. However, a number of questions remain with regard to the spatial and temporal regulation of these events. In this study (p. 4684), Christian Bökel and co-workers use mathematical modelling and visualisation of the phosphorylation status of Smo together with a conformation-sensitive fluorescence-based reporter to investigate the molecular events downstream of Smo phosphorylation. They find that that localization of Smo to the plasma membrane is sufficient to induce phosphorylation of its cytoplasmic tail, both in the presence and absence of Ptc. Moreover, the authors demonstrate that the inactivation of Ptc in response to Hh controls Smo clustering at the plasma membrane independently of the phosphorylation of the Smo tail. This result is surprising because Smo phosphorylation has been proposed to be required for its membrane clustering. The data presented here, therefore, support a model of Hh pathway activation, in which the inhibitory role of Ptc is primarily mediated by influencin the subcellular localization of Smo.