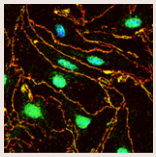
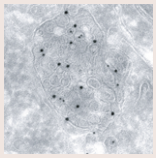
In this issue



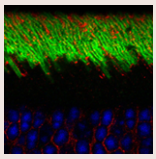
Nitric oxide at endothelial junctions

Endothelial barrier maintenance is important for the normal function of tissues, and transient disruptions of endothelial adherens junctions (AJs) and associated changes in the cytoskeleton can contribute to the pathogenesis of diseases, such as inflammation, edema, stroke and cancer. Vascular endothelial growth factor (VEGF) is one of the key factors that regulate the permeability of endothelial AJs by promoting the phosphorylation of VE-cadherin (also known as CDH5) by Src kinases, which results in junction opening. Nitric oxide (NO) that is produced by endothelial NO synthase (eNOS) has been shown to have an effect on VEGF and on endothelial permeability, but the underlying molecular mechanisms are not clear. On page 5541, William Sessa and colleagues show that loss of NO through silencing of eNOS in human endothelial cells, surprisingly, stabilises cortical actin structures. This is achieved through increased interaction of VE-cadherin with TIAM1, a guanine nucleotide exchange factor for Rac, at the cell junctions. As the authors show here, this leads to an increase in Rac activity, which in turn promotes the assembly of cortical actin at junctions. Taken together, the data presented in this work suggest that eNOS-derived NO is a key factor in regulating reversible changes in endothelial cell permeability by fine-tuning the level of small GTPases that control VE-cadherin-mediated changes in the junctional cytoskeleton.



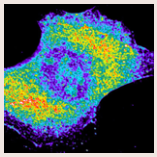
ESCRTing exosomes

Exosomes are secreted vesicles that are formed during endosome maturation in multivesicular bodies (MVBs). Some MVBs are degraded but other can fuse with the plasma membrane and, thus, secrete their internal vesicles as exosomes. Despite extensive research efforts, the exact mechanisms that underly exosome biogenesis and cargo sorting within these vesicles remain poorly understood, although components of the endosomal sorting complex required for transport (ESCRT) have been implicated in their function. In this work (p. 5553), Clotilde Théry, Graça Raposo and colleagues use RNA interference (RNAi) to target 23 different components of the four complexes (ESCRT-0, -I, II and III) as well as associated proteins in major histocompatibility complex class II (MHC II)-expressing HeLa cells, which allow the monitoring of exosome secretion. They identify five ESCRT components that appear to be involved in exosome biogenesis or secretion; knockdown of three ESCRT-0 and/or ESCRT-I factors decreases exosome secretion, whereas silencing of two accessory proteins either increases secretion or modifies the MHC II content. Interestingly, by using immuno-electron microscopy, the authors show that silencing of these factors also differentially affects size and protein composition of the secreted vesicles, suggesting that exosomes exhibit a considerable heterogeneity. Thus, the authors propose that ESCRTs have a role in the formation of exosomes from a subpopulation of vesicles within MVBs as well as in their secretion.



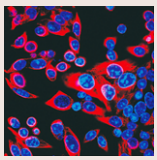
4.1G in photoreceptor organisation

In photoreceptors, the assembly of signalling molecules into macromolecular complexes is important for phototransduction and for maintaining the structural integrity of rod outer segments (ROSs). ROSs consist of a stack of plasma membrane-enclosed discs outlined by a rim structure. There is only limited information with regard to the composition of the ROS, for instance a complex between the cyclic nucleotide-gated (CNG) channel and the Na^{+}/K^{+}-Ca^{2+}exchanger is found in the plasma membrane, and one between the CNG channel and the peripherin-2/rom-1 complex on the disc rim. In this study (p. 5725), Christiana Cheng and Robert Molday set out to analyse the ROSs in greater detail by using immunoprecipitation combined with mass-spectrometry-based proteomics. They identify a short splice variant of the membrane-interacting scaffolding protein 4.1G that interacts with a subset of CNG channels in ROSs. By using a series of truncation and fusion proteins, the authors are able to narrow down the interaction site in 4.1G to its FERM and C-terminal domains. Furthermore, immunofluorescence microscopy of mouse retinal sections, demonstrates that 4.1G partially colocalises with CNG channels, indicating a role in the spatial organisation of CNG channels within the plasma membrane of ROSs. However, questions regarding the role of 4.1G in CNG activity and surface expression remain, which could be addressed by analysing the retina of 4.1G knockout-mice.



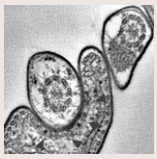
How Rac modulates actomyosin contractility

Cell migration is essential for a number of important cellular processes, and migration defects can contribute to the pathogenesis of different diseases including cancer. Cell migration involves the assembly and disassembly of cell-matrix adhesions sites, as well as associated changes in the actin cytoskeleton. Cell contractility is controlled by the actin motor protein myosin II (Myo II) and the Rho family of GTPases, but the role of other GTPases such as Rac in the process is not well understood. On page 5585, Donna Webb and co-workers set out to investigate the role of Asef2 (also known as SPATA13), a recently identified Rac guanine nucleotide exchange factor, in the modulation of cell migration. They find that activation of Rac by Asef2 inhibits cell migration on type I collagen through a mechanism that is dependent on Myo II and that enhances contractility. This is interesting because, thus far, Myo II contractility has been thought to be mediated by Rho. Furthermore, the authors show that Asef2 slows down adhesion turnover and induces large, mature adhesions sites. When they knocked down Rac or inhibited its activity, they observe reduced levels of active Myo II together with an increase in cell migration. Moreover, inhibition of Myo II by using blebbistatin abolishes the effect of Asef2 on cell migration. Taken together, these data point to a so far unknown role of Rac1 – mediated by Asef2 – in regulating actomyosin contractility and, thus, cell migration.



Wnt/β-catenin–HNF4 feedback loop in EMT

Epithelial-to-mesenchymal transition (EMT) has a crucial role in the transformation of cancer cells and the progression to an invasive phenotype. EMT is triggered by a number of signalling pathways – including Wnt/β-catenin signalling – leading to the expression of EMT-specific transcription factors and the subsequent downregulation of E-cadherin. β-catenin also functions in cell adhesion, where it links cadherins to α-catenin and the cytoskeleton. However, the molecular mechanism underlying the switch between its transcriptional and adhesion functions remains largely unclear. To address this question, Bo-An Li and co-workers (p. 5692) explore the role of Wnt/β-catenin signalling in hepatocellular carcinoma (HCC), which shows a particularly poor patient prognosis. The authors focus their attention on hepatocyte nuclear factor 4α (HNF4α) because it is a master gene of the hepatic lineage and has been implicated in regulating EMT. They now describe a double-negative feedback mechanism that controls Wnt/β-catenin signalling and HNF4α expression *in vitro* and *in vivo*; Wnt/β-catenin signalling during EMT reduces HNF4α activity, whereas HNF4α competes with β-catenin and inhibits the expression of EMT-related Wnt/β-catenin targets. Moreover, the authors suggest that HNF4α also regulates the switch between transcriptional and adhesion functions of β-catenin. These insights into the regulation of EMT might be exploited in approaches to block the invasive potential of HCC.



Getting into the groove: *Trypanosoma* flagellum inheritance

Flagella are highly conserved eukaryotic organelles that generate movement and extracellular flow, and are involved in cell signalling. Flagella are also important for the pathogenicity of a number of parasites. *Trypanosoma brucei* has a single flagellum that is required for cell motility and enables the cell to move within the vertebrate host and the insect vector. During cell division, a new flagellum is formed alongside the old one and, in the insect form, the flagella connector (FC) attaches the tip of the new flagellum to the side of the old flagellum to ensure accurate replication of the cell architecture. However, the FC is not present in the bloodstream form of the parasite and it is thus unclear cytoarchitecture faithfully inherited in this stage. To investigate the bloodstream form of the parasite further, Sue Vaughan and colleagues (p. 5748) use high-resolution imaging to closely examine the distal tip of the newly forming flagellum. They discover, what they name 'the groove', a discrete invagination of the cell body plasma membrane that contains the distal tip. They find that the groove is closely associated with the flagellum attachment zone and, thus, might have a similar role in the insect form as the FC. On the basis of these results, the authors speculate that the groove provides morphogenetic patterning to the bloodstream form of the parasite, but has been specifically adapted to withstand the hostile immune response in the mammalian blood.