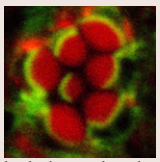
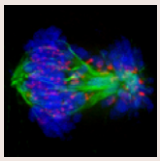
In this issue



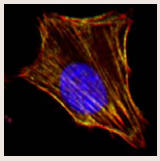
PtdIns(4,5)P_2 in retinal degradation

Members of the transient receptor potential (TRP) channel family are highly diverse ion channels that were first discovered in *Drosophila* photoreceptors, where they are the dominant light-sensitive channel. TRPs are activated by a signalling cascade that includes phospholipase C (PLC), which hydrolyses phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] in the microvillar membrane. The TRP channel in *Drosophila* is highly selective for Ca^{2+} and responsible for extensive Ca^{2+} influx during the response to light. In *trp* mutant flies this Ca^{2+} influx is reduced and results in retinal degeneration. It had previously been suggested that this is because of the resulting defects in Ca^{2+}-dependent steps of the visual pigment cycle, which lead to the accumulation of toxic hyperphosphorylated metarhodopsin–arrestin (MPP–Arr2). On page 1247, Roger Hardie and colleagues explore an alternative hypothesis, namely that degeneration in *trp* mutants is due to failure of Ca^{2+}-dependent inhibition of PLC, resulting in depletion of PtdIns(4,5)P_2. They find that retinal degeneration is not rescued when accumulation of MPP–Arr2 is prevented but rescued when PLC is mutated, and that it correlates closely with the extent of PtdIns(4,5)P_2 depletion. Furthermore, the authors demonstrate that depletion of PtdIns(4,5)P_2 leads to the dephosphorylation of the cytoskeletal component moesin, the subsequent depolymerisation of actin and the disintegration of the microvillar membrane, thus providing new insights into the molecular mechanism of degeneration.



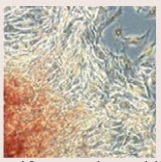
Kinase–phosphatase interplay at the mitotic spindle

The spindle assembly checkpoint (SAC) ensures that sister chromatids are accurately segregated during mitosis. In response to kinetochores being incorrectly or not at all attached to microtubules of the spindle, mitotic progression is delayed through the activity of Aurora B, which phosphorylates outer kinetochore proteins and destabilises their interaction with microtubules to allow them to establish correct attachments. Certain SAC components, such as Bub1 and BubR1 help to establish proper kinetochore–microtubule interactions, but the underlying mechanisms are unknown. The phosphatase PP2A has been implicated in dephosphorylating outer kinetochore proteins and is recruited to the kinetochore through its regulatory subunit B56, but it is unclear how B56-PP2A is targeted to kinetochores. Jakob Nilsson and colleagues (p. 1086) now show that Bub1 directly interacts with a pool of B56-PP2A and recruits it to the kinetochore. They identified the region of BubR1 that interacts with B56 as a conserved region and also contains phosphorylation sites for Cdk1 and Plk1, and show that phosphorylation by these kinases increased the binding of BubR1 to B56. This has important mechanistic implications, because phosphorylation of BubR1 at these sites only occurs at kinetochores that are not attached or without tension, thereby suggesting a means for the recruitment of PP2A through B56, in response to defects of kinetochore attachment.



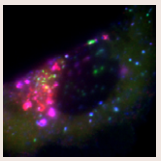
STIM1-mediated SOCE in migrating cells: may the force be with you

Cell migration is an essential process that involves several steps, including extension of the leading edge, formation of new adhesions, contraction of the cytoskeleton and detachment of the rear end of the cell. Cell migration is also required for cancer metastasis when cancer cells acquire invasive behaviour and disseminate through nearby blood and lymph vessels. However, it is not well understood how force generation is controlled during cell migration. Here (p. 1260), Meng-Ru Shen and colleagues investigate the role of stromal-interaction molecule 1 (STIM1)-dependent Ca^{2+} signalling in the regulation of cancer cell migration. The authors had previously shown that STIM1 overexpression in tumour cells promotes their migratory potential, but it was unknown whether STIM1 is also involved in exerting contractile forces. By using a micropillar assay to directly measure traction forces, the authors now provide evidence that the generation of contractile forces that are mediated by the reorganisation of the actomyosin cytoskeleton requires both the expression of STIM1 and active store-operated Ca^{2+} entry (SOCE). When they knock down STIM1 by using RNA interference or when SOCE is blocked with inhibitors, recruitment of talin and focal adhesion kinase to focal adhesions is inhibited, suggesting that force transduction from integrins is blocked in the absence of STIM1-mediated SOCE and pointing to a possible pathway for mechanotransduction in migratory cells.



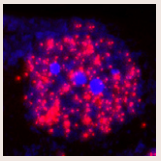
Gbx2 helps to maintain ESC self-renewal

Embryonic stem cells (ESCs) retain their ability to differentiate into any type of cell in the body and can be maintained indefinitely as self-renewing cell populations. Both extrinsic signals and intrinsic transcriptional programs govern decisions of ESC fate. Of main importance for the self-renewal capability of ESCs are leukemia inhibitory factor (LIF) and signal transducer and activator of transcription 3 (Stat3) in the LIF/Stat3 pathway. It has been proposed that the LIF–Stat3 axis triggers multiple downstream targets in order to maintain ESCs in an undifferentiated state, but the search for direct targets have been hampered by the fact that LIF also activates other pathways – including that of the mitogen-activated protein kinase (MAPK), which negatively regulates ESC self-renewal. To overcome this problem, Chih-I Tai and Qi-Long Ying (p. 1093) use a chimeric Stat3 receptor variant that selectively activates the Stat3 but not the MAPK pathway, to analyse the genes that are upregulated upon induction of Stat3 and LIF. They identify gastrulation brain homeobox 2 (Gbx2) as a direct downstream target of Stat3 and, importantly, demonstrate that overexpression of Gbx2 in the absence of LIF is sufficient to sustain ESC self-renewal. Furthermore, they show that Gbx2 expression distinguishes naïve state ESCs from primed-state epiblast stem cells (EpiSCs), and promotes the conversion of EpiSCs to ESCs, thereby presenting new insights into the molecular pathways that define and govern ESC self-renewal.



A new way to look at tubulovesicular transport

The endolysosomal trafficking pathway is responsible for the internalisation of membrane receptors and their delivery to sorting endosomes, from which they can be either recycled back to the cell surface or enter late endosomal or lysosomal compartments for degradation. Small and highly motile tubulovesicular transport carriers (TCs) have an important role in these transport processes, but very little is known with regard to their relationship with individual compartments that allow them to exchange their cargo. To address this issue, Sally Ward, Raimund Ober and colleagues (p. 1176) now present a new imaging system that combines multifocal plane microscopy (MUM) and localised activation of photoactivatable GFP-labeled proteins to study the dynamics of TCs. The key advantage of this approach, the authors argue, is the simultaneous imaging of a living cell in two focal planes to allow the tracking of TCs as they move in 3D. The authors' focus is the characterisation of the spatiotemporal dynamics of TCs that are involved in different steps of the recycling pathway of the recycling Fc receptor (FcRn). They find that – along the way – TCs associate with different combinations of Rab GTPases, SNX4 and APPL1, which could serve to mark trafficking components. Interestingly, the authors also observe a new looping pathway, in which TCs move bidirectionally to leave and return to the same endosome. These results might also be of relevance for other receptors and could help to further elucidate their cellular endolysosomal or recycling pathways.



A mighty COUP(-TFII): orchestrating endothelial cell fate

Endothelial cells line the inside of arterial, venous or lymphatic vessels, and exhibit considerable molecular and morphological heterogeneity, which in part is intrinsically predetermined owing to a subset of vessel-type-specific transcription factors they express. However, the exact mechanisms that define a particular endothelial fate remain unclear, in particular for lymphatic endothelial cells (LECs), which originate by transdifferentiation from venous endothelial cells (VECs) and thus share such fate-determining factors. One of these is NR2F2, also known as chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), which has been implicated in endothelial cell identity, as it is required for the induction of Prox1, a master-switch for LEC differentiation. Here (p. 1164), Aernout Luttun and colleagues demonstrate that NR2F2 differentially regulates gene expression in VECs compared with LECs. They show that this is achieved through the formation of NR2F2 homodimers in VECs that suppress their transdifferentiation to arterial LECs by inhibiting Notch target genes, such as *HEY1 and HEY2*. By contrast, in LECs NR2F2 is found to heterodimerise with Prox1, which alleviates inhibition of *HEY1* and *HEY2*, and regulates a distinct set of LEC genes. Taken together, these data reveal new insights into how a commonly expressed transcription factor can determine the fate of endothelial cells that could also pave the way for therapeutic interventions in disorders associated with abnormal lymphangiogenesis.