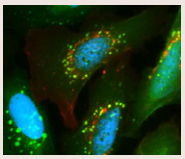
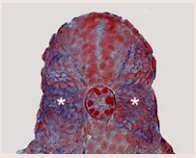
In this issue



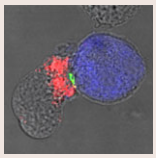
PTPσ reigns in autophagy

In addition to its role in endocytosis, the membrane lipid phosphatidylinositol-3-phosphate (PtdIns3*P*) is crucial for autophagy. Generated by the lipid kinase Vps34 (in complex with other proteins), PtdIns3*P* is concentrated on autophagic vesicles, where it recruits and tethers different effector proteins that are in turn required for the correct formation of the vesicular membrane. But – as is the case for all intracellular phosphorylation events – finding the right balance is the key to tightly controlling when autophagy is initiated. This requires the presence of phosphatases that counteract the phosphorylation by Vps34. In light of this, on page 812, Jeffrey MacKeigan and colleagues investigate enzymes that can carry out this role. Using a chimeric protein made up of an EGFP molecule fused to two tandem FYVE domains as a cellular sensor of PtdIns3*P*, the researchers visualise the intracellular localisation of PtdIns3*P* following individual siRNA knockdown of over 200 phosphatases. They reveal that the loss of the dual-domain protein tyrosine phosphatase PTPRS (PTPσ) increases both constitutive and induced autophagy. Moreover, they observed that PTPσ localisation to PtdIns3*P*-positive membranes is enhanced during the autophagic process. These results highlight a new regulatory role for this phosphatase in autophagy.



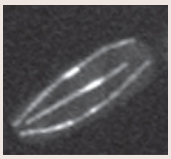
Nesprin-3 makes the connection

Members of the nesprin protein family are found in the outer membrane of the nuclear envelope. Their interaction with the inner nuclear membrane proteins SUN1 and SUN2 is crucial in maintaining nuclear structure. Outside the nucleus, nesprins are involved in linking the nuclear envelope to the cytoskeleton. Nesprin-3 is the only family member known to bind intermediate filaments (IFs). How this binding is mediated, and the in vivo effects of knocking out nesprin-3, is investigated on page 755 by Arnoud Sonnenberg and colleagues. The authors demonstrate that, despite being expressed at the nuclear envelope of epidermal and skeletal muscle cells during development, the loss of nesprin-3, rather surprisingly, does not impair zebrafish embryonic development or viability. The lack of nesprin-3 does result in the loss of the dense filamentous keratin network around the nucleus, but this association of IFs with the nuclear periphery does not seem to be crucial for development. The authors further show that amino acids R43 and L44, located in a seven-amino-acid stretch in the first spectrin homology domain of mouse nesprin-3α, are required for it to bind to plectin, which in turn associates with IFs. Nesprin-3, therefore, has an important function in establishing a chain of proteins that connect this cytoskeletal component with the nucleus.



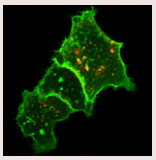
Clathrin dons a new coat

The recognition of MHC molecules loaded with antigen at the surface of antigen-presenting cells by the T-cell receptor (TCR) results in the formation of an immunological synapse (IS). Activation of the TCR not only induces the assembly of multimolecular complexes, but also induces a major accumulation of actin at the contact site. Several activators of the actin nucleator Arp2/3 have been identified at the IS, but how actin accumulation is initiated and regulated has remained unclear. On page 820, Esteban Veiga and colleagues now describe the missing link. They show that clathrin – well known as a key component in endocytosis – is required for the polymerisation of actin at the IS. Knocking down the protein using siRNA results in cells being unable to gather actin around the activated TCR. Clathrin causes these morphological changes by acting as a molecular platform for the recruitment of actin-polymerising proteins such as dynamin-2, the Arp2/3 complex and CD2AP. Intriguingly, it does so without associating with the plasma membrane. Instead, the authors show, clathrin that is associated with multivesicular bodies promotes actin polymerisation during IS formation. Based on these findings, Veiga and colleagues suggest that antigen stimulation results in rapid relocalisation of these structures to the cell–cell contact site to nucleate rapid actin remodelling.



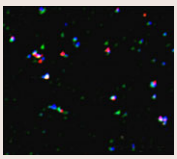
Microtubules grow with ‘flare’

Blunt or flared, sheet like or C shaped – growing microtubule plus ends have been attributed a number of different morphologies. But which of the morphologies observed in multiple systems in vitro reflect the situation in vivo? On page 693, Johanna Höög, Claude Antony, Damian Brunner and colleagues describe a new approach for assessing microtubule morphology in *Saccharomyces pombe*, and use this approach to determine what growing microtubules look like in 3D inside the cell. They combine light microscopy with electron tomography to observe the re-growth of microtubules following wash-out of the depolymerising drug methyl benzimidazol-2-yl-carbamate – with a surprising result. Whereas previous studies reported growing plus ends to be mostly blunt or in sheets, the authors show that, in vivo, 90% of them have a flared morphology. By identifying and categorising growing microtubule ends, they also demonstrate that sheets are, in fact, the rarest of the end structures found. Based on their results, the authors propose a new model for microtubule growth that challenges the way this cellular process and its regulation is currently perceived: in contrast to the previously suggested ‘closing-sheet model’, they conclude that microtubule plus-end growth in *S. pombe* occurs through closure of a funnel-like structure.



One ligand, two outcomes

Insulin not only regulates glucose metabolism, but also stimulates cell growth. But how does one hormone initiate two different cellular responses? The insulin receptor (IR) is known to exist as two splice variants. It is believed that signals initiated by splice variant A (IR-A) are more ‘mitogenic’, whereas those downstream of variant B (IR-B) are more ‘metabolic’. Just how these two receptor isoforms induce different cellular outcomes has, however, been unclear. Elizabeth Jares-Erijman and colleagues (p. 801) set out to investigate the molecular mechanisms underlying these differences by fluorescently tagging the two receptors and analysing their localisation and activation following insulin binding. They show that IR-A is more strongly and persistently phosphorylated, as well as endocytosed more readily, than IR-B. Additionally, IR-A activation leads to stronger and more prolonged Erk1/2 phosphorylation and, consequently, a higher level of AP-1 gene transcription. By contrast, IR-B remains at the plasma membrane longer and activates AKT more strongly than IR-A. Jares-Erijman and colleagues conclude that it is this difference in receptor internalisation that drives the generation of different signals: whereas internalised IRs regulate mitogenic activity inside the cell, signals resulting in metabolic changes are initiated at the cell membrane.



Rab1a gets vesicles moving

Receptor-mediated endocytosis is a complex process involving numerous proteins with different functions. Although a number of these have been identified, many more have a role in this process. On page 765, Allan Wolkoff and colleagues now identify proteins that are associated with early and late endocytic vesicles using a proteomics approach. The authors stimulate the hepatocyte-specific asialoglycoprotein receptor with the fluorescently labelled ligand asialoorosomucoid, and purify the vesicles by flow cytometry. Analysis by nano-LC ESI-MS/MS reveals the association of a number of transporters and regulatory proteins, such as annexin-2, annexin-5 and annexin-6, syntaxin-7 and various members of the Rab family. The researchers subsequently focus on the endocytic role of Rab1a, which they detect in early and late endocytic vesicles. They show that, in the absence of this protein or upon antibody-induced inhibition of its function, the minus-end-directed motility of early but not late endocytic vesicles is reduced. Furthermore, knockdown of Rab1a results in the loss of Kifc1 from early endocytic vesicles. Based on these observations, the authors conclude that Rab1a regulates the motility of early endocytic vesicles by recruiting the kinesin motor Kifc1 and, therefore, has an important role in regulating the endocytic process in vivo.