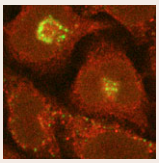
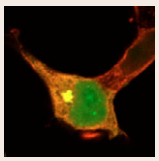
In this issue



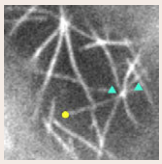
AP-2 looks beyond clathrin

In addition to clathrin-mediated endocytosis (CME), cells take up extracellular contents and cell-surface proteins through several routes that are independent of clathrin. The relationship between the various endocytic pathways is not well understood, although it has been suggested that CME components have broader roles than previously thought. Now, Alan Lau and Margaret Chou (p. 4008) provide evidence that the adaptor AP-2 – which is a key mediator of CME – also has a role in a clathrin-independent endocytic pathway. Using HeLa cells, the authors investigate the uptake of β1 integrin and MHC class I, which occurs through the non-clathrin, Arf6-regulated endocytic pathway in this system. Unexpectedly, the intracellular distribution of both proteins is altered if clathrin or an AP-2 subunit is knocked down; moreover, uptake of Arf6 cargo proteins is delayed when AP-2 is depleted. The authors go on to show that depletion of AP-2 promotes the lysosomal degradation of MHC class I, and decreases its half-life. In addition, depleting AP-2 (but not clathrin) enhances the colocalisation of AP-2 with late endosomes and lysosomes. The authors conclude that AP-2 has trafficking functions beyond its role in CME, underscoring the complexity and interdependence of endosomal membrane systems.



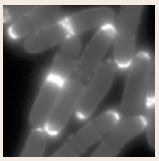
FAT10 takes on aggresomes

When proteasomal degradation is impaired, poly-ubiquitylated proteins are instead transported along microtubules to aggresomes – pericentriolar protein aggregates that store proteins for later removal. Transport to aggresomes is mediated by the cytosolic deacetylase HDAC6, which links polyubiquitin to the microtubule motor dynein. Now, Marcus Groettrup and colleagues (p. 4079) show that the ubiquitin-like protein FAT10, which usually targets proteins for proteasomal degradation, also acts in the aggresome pathway. Using a yeast two-hybrid screen, the authors show that FAT10 interacts with HDAC6; in cells, this only occurs when the proteasome is inhibited. They next identify the domains of each protein that mediate the HDAC6-FAT10 interaction, and go on to show that FAT10 and GFP-FAT10 both localise to aggresomes when a proteasome inhibitor is added; notably, this localisation requires an intact microtubule network. In *Hdac6*$^{-/-}$ cells treated with a proteasome inhibitor, FAT10- and ubiquitin-containing aggresomes are smaller and less numerous than in wild-type cells. The authors conclude that FAT10 reroutes its target proteins to the aggresome when proteasomal degradation cannot take place. These results enhance our understanding of the diverse cellular strategies for protein degradation.



Making microtubules move with MOR1

The dynamic behaviour of microtubules – which undergo frequent transitions between growth, shrinkage and pause states – is regulated by an array of microtubule-associated proteins (MAPs). For instance, the MAP215 proteins (including XMAP215 in *Xenopus* and CKAP5 in humans) have been proposed to promote both growth and shrinkage of microtubules, but aspects of their function remain unclear. On page 4114, Eiko Kawamura and Geoffrey O. Wasteneys investigate the in vivo role of MOR1, the *Arabidopsis* homologue of XMAP215. The authors have previously shown that, in the cells of a temperature-sensitive *mor1* mutant, microtubule arrays are disrupted at the restrictive temperature; now, they investigate the dynamics of individual microtubules in *mor1* cells. They first demonstrate that microtubule dynamics are reduced in the *mor1* mutant, even at the permissive temperature. Moreover, both the growth and shrinkage rates of microtubules are dramatically reduced at the restrictive temperature, and the incidence and duration of pause events increases. The authors go on to show that the interaction of microtubules with the MAP EB1 is reduced at the restrictive temperature in the *mor1* mutant. Thus, the role of MOR1 in microtubule organisation echoes that of its animal homologues.



How to cope with (osmotic) stress

In the fission yeast *S. pombe*, the actin and microtubule cytoskeletons are crucial for polarised growth. Conditions of osmotic stress perturb growth in several ways (for instance, F-actin dissociates from growing tips and microtubules become static) – but what are the signalling pathways that mediate cell recovery? To address this question, Alasdair Robertson and Iain Hagan (p. 4055) investigate the impact of the stress-response MAP kinase pathway (SRP) and the actin-modulating kinase Ssp1 on the recovery of osmotically stressed *S. pombe*. The authors demonstrate that SRP signalling promotes the resumption of microtubule dynamics by activating the transcription factor Atf1. They show that the timely recovery of tip growth requires the activity of the SRP-pathway MAP kinase Sty1, whereas Ssp1 maintains correct polarity of tip growth. Moreover, Ssp1 and Wsh3 (an SRP-associated polarity factor) are required to select the correct site for polarised tip extension. Notably, the authors show that the cell-cycle kinase Polo – which is phosphorylated to promote recovery of tip growth in response to other environmental stresses – is not required for growth recovery after osmotic stress. These results identify key features of stress-response signalling in *S. pombe*, and highlight the specificity of cellular responses to distinct stresses.

Old age brings changes for hMSCs

Adult human mesenchymal stem cells (hMSCs) can differentiate into several cell lineages – consequently, they have promise as therapies in regenerative medicine. When grown in culture, however, hMSCs rapidly undergo senescence, which could impact on their use as therapeutic agents. Nuclear structural alteration is a key feature of senescence in many cell types; now, Vered Raz and colleagues (p. 4018) use newly developed quantitative image-processing tools to analyse how the nuclear architecture of hMSCs changes during senescence. The authors show that, early in senescence, the nuclear lamina of hMSCs becomes deformed and gives rise to intranuclear structures; in addition, centromeres and telomeres of chromosomes relocalise to a peripheral nuclear position that spatially overlaps with these structures. The authors observe that, during senescence, telomeres form large aggregates that colocalise with the DNA-damage marker γ-H2AX but not with the telomerase component TERT. Notably, the binding of telomere aggregates to lamina structures is enhanced when the lamina is distorted through the expression of mutant lamins. Thus, the nuclear lamina of senescent hMSCs regulates the spatial positioning of heterochromatic chromosome regions. These data shed light on how nuclear architecture changes during hMSC senescence.

Development in press

Neurons escape death with γ-protocadherins

During development of the central nervous system, excessive numbers of neurons are initially generated before approximately half of them undergo programmed cell death – often during the formation of synapses. The factors that regulate central neuron survival and synaptic specificity have remained largely unknown. Now, in a paper published in *Development*, Joshua Sanes and colleagues report that the protocadherin-γ (Pcdh-γ) gene cluster – which encodes a family of 22 adhesion proteins – is important for the survival of developing neurons, but not for the specificity of their synaptic connections. By selectively inactivating the Pcdh-γ cluster in the mouse retina, the authors demonstrate that the programmed cell death of certain retinal cell types is accentuated by the loss of γ-protocadherins, whereas the formation of appropriate synapses and of complex functional circuits remains unaffected. Importantly, these findings suggest that Pcdh-γ-mediated regulation of neuronal survival is independent of synaptic connectivity.

Lefebvre, J. L., Zhang, Y., Meister, M., Wang, X. and Sanes, J. R. (2008). γ-Protocadherins regulate neuronal survival but are dispensable for circuit formation in retina. *Development* **135**, 4141-4151.