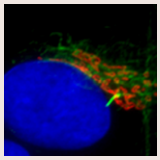
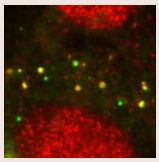
In This Issue



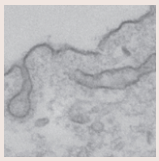
A giant(in) role in ciliogenesis

Primary cilia fulfil important roles in nearly all cells and tissues and, consequently, defects in ciliogenesis or in cilia function are associated with a number of diseases. During ciliogenesis, cilia grow by extension of a microtubule core, the axoneme, and intraflagellar transport (IFT) provides the particles for cilia assembly. Dynein-2 is known to drive retrograde IFT along the axoneme and is also important for cilia formation. Furthermore, Golgi-localised proteins have also been implicated in ciliogenesis and IFT. In this work (p. 5189), David Stephens and colleagues now further investigate the molecular mechanisms of the involvement of Golgi proteins in ciliogenesis. By using RNA interference (RNAi), they show that the transmembrane Golgi matrix protein giantin (also known as GOLGB1) is required for ciliogenesis. Interestingly, they demonstrate that giantin does not act through the Rab11–Rabin–Rab8 pathway that has previously been suggested to be important for the early steps of ciliogenesis. Instead, they find that functional suppression of giantin leads to a mislocalisation of the dynein-2 intermediate chain WDR34. Accordingly, depletion of giantin or of WDR34 results in an inability of cells to form primary cilia, whereas their partial depletion increased cilia length. Taken together, the data presented here suggest that giantin acts in ciliogenesis by controlling the localization of dynein-2.



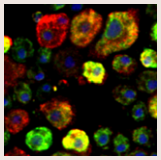
Omegasomes as autophagosome intermediates

Omegasomes are membrane extensions within the ER from which some autophagosomes, the hallmark structures of autophagy, form. The induction of autophagy also requires ULK1, a protein kinase complex, and the Vps34 lipid kinase complex. Omegasomes are thought to facilitate autophagosome formation by recruiting downstream factors, such as targets of phosphatidylinositol 3-phosphate (PtdIns3*P*) – synthesized by Vps34 – and by demarcating the outer edges of the growing autophagosomal membrane. However, it is unclear whether ULK1 has any role in omegasomes as it has been suggested to leave the pre-autophagosomal sites before omegasomes form. On page 5224, Nicholas Ktistakis and co-workers examine the interactions between the ULK1 complex and omegasomes in HEK293 cells. By using live imaging of the ULK1 component Atg13, they find that ULK1 initially colocalises to pre-autophagosomes in a manner that is dependent on PtdIns3*P* but then leaves these sites before autophagosome budding takes place. Based on their observations, the authors propose their model of autophagosome formation: first, ULK1 punctae form that associate with the ER and develop into omegasomes when PtdIns3*P* is available, then ULK1 leaves these sites and autophagosomes bud off. However, a number of questions remain – such as how does ULK1 dissociate from omegasomes – which will form the basis for additional work.



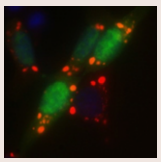
The cautionary tale of dynamin inhibitors

Dynamin is an important GTPase that has been implicated in membrane fission during clathrin-mediated endocytosis. In mammals, three dynamin isoforms can be found, with dynamin 1 and 3 mainly expressed in the brain, and dynamin 2 being ubiquitous. In accordance with a crucial role in endocytosis, knockout (KO) of dynamin 2 in mice results in embryonic lethality. To assess the impact of the lack of dynamin on cell physiology, Pietro De Camilli and colleagues had previously generated dynamin 1 and 2 double KO (DKO) mouse fibroblasts, which – unexpectedly – were viable despite a severe impairment of clathrin-mediated endocytosis. To exclude that low levels of dynamin 3 might have compensated for the loss of dynamin 1 and 2, in this work (p. 5305), the authors generate triple KO (TKO) mouse fibroblast cells that lack all dynamin isoforms. They show here that these dynamin TKO cells have the same phenotype as the DKO cells without any further defects. The authors then proceed to use the dynamin TKO cells to investigate the specificity of the commonly used small molecule dynamin inhibitor dynasore and of its structurally related compound Dyngo-4a. Surprisingly, they find that the dynamin TKO cells are sensitive to dynasore and Dyngo-4a; treatment with these drugs blocked fluid-phase endocytosis and peripheral membrane ruffling, both in TKO and wild-type cells. On the basis of these results, the authors propose that the dynamin TKO cells are a useful tool to unambiguously assess the cellular functions of dynamin and to test the specificity of dynamin inhibitors.



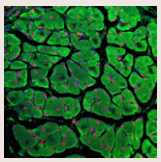
Transcription factor autoproteolysis – a mechanism borrowed from phages

Myelination in the central nervous system (CNS) has a pivotal role in vertebrate brain function, but the process is only poorly understood at the molecular level. Recently, myelin-gene regulatory factor (MRF) has been shown to control the expression of a number of mouse CNS myelin genes, and its *Dictyostelium* orthologue MrfA was found to regulate prestalk cell differentiation. To better understand the molecular mechanism of MrfA function, Jeffrey Williams and colleagues (p. 5247) set out to further characterize this factor. They show that MrfA and animal MRFs contain a predicted trans-membrane domain – a feature of other membrane-tethered transcription factors that are activated by protease-mediated cleavage and subsequent release from the membrane. However, when the authors investigate the activation mechanism of MrfA, they find that, surprisingly, MrfA undergoes a form of constitutive auto-proteolytic cleavage, which thus far has only been described for the intramolecular chaperone domains (CICMDs) of bacteriophage tail-spike proteins. Thus the MRF domain of MrfA has extensive sequence identity to the CICMDs and utilizes the same serine-lysine dyad cleavage mechanism of auto-proteolysis. Interestingly, an identical cleavage mechanism has just been reported elsewhere for the vertebrate orthologues, suggesting that the phage approach to protein activation is either remarkably conserved in evolution or there has been lateral prokaryote–eukaryote domain transfer.



A new function for SIRT6 in stress granules

The survival of cells critically depends on the sensing and response to a variety of stresses, and cells possess several control mechanisms to manage stress responses. Stress often leads to the formation of cytoplasmic RNA–protein complexes – the stress granules (SGs) – but their exact biological role remains unclear. A number of factors are involved in cellular stress responses; they include sirtuins (SIRTs), a family of conserved protein deacetylases that regulate diverse cellular processes including ageing. SIRT6 has been described to regulate the expression of a high number of stress-responsive and metabolism-related genes in the nucleus, and most of the functions of SIRT6 seem linked to its chromatin-modifying activities. On page 5166, Monika Jedrusik-Bode, Eva Bober and colleagues now investigate the role of SIRT6 and of its *C. elegans* homologue SIR-2.4 in the formation and function of stress granules. They find that, under stress, SIRT6 translocates to the cytosol, where it facilitates SG assembly through the dephosphorylation of the SG component G3BP, as well as SG disassembly during stress recovery. Similarly, the authors observe that SIR-2.4 is necessary for the efficient formation of P granules and extended survival under heat stress. Taken together, these results point to a new, evolutionary conserved, function of SIRT6 in stress protection that might also link cellular stresses and aging, because SG formation – as an important cell survival mechanism – is known to be impaired with increasing age.



M-cadherin in satellite cell activation

Satellite cells comprise adult muscle stem cells and committed myogenic procursors; they are involved in muscle growth after birth and in muscle regeneration after muscle damage. A prevalent view is that satellite cells first become activated, and then divide and differentiate before fusing to existing myofibers to initiate muscle growth. M-cadherin (Mcad), a cell-cell adhesion factor of satellite cells, is thought to be important for the fusion process, but the exact underlying molecular details are not clear. To better understand satellite cell activation and function, Juan Carlos Izpisua Belmonte and colleagues (p. 5116) follow satellite cells of newborn mice by electron and confocal microsopy, and also *in vitro* in cell culture. They find that activated satellite cells initiate fusion with myofibers while they are still in mitosis; in contrast to the proposed model of fusion of divided and differentiated satellite cells. In addition, stimulation of satellite cells *in vitro* with Mcad promotes their cell cycle progression, thereby increasing the number of activated cells and accelerating cell proliferation. Conversely, inhibition of Mcad by addition of anti M-cadherin antibodies results in fewer satellite cells with slower proliferation rates. Taken together, these results suggest that Mcad-mediated cell-cell interactions have a role in satellite cell division that could be used in strategies aimed at enhancing muscle turnover and regeneration in muscle dystrophies.