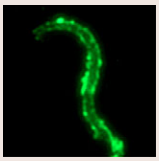
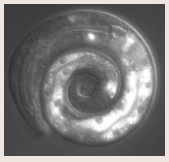
In this issue



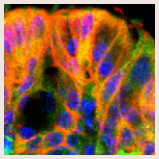
Vaults – giving us pores?

Nuclear pore complexes (NPCs) traverse the inner and outer nuclear membrane and are the sites of nuclear import and export. Although NPCs are continuously incorporated into the nuclear envelope during interphase, the mechanism has been poorly understood. Now, Marie-Christine Dabauvalle and colleagues (p. 780) show unexpectedly that major vault protein (MVP) – the main component of vaults, which are abundant ribonucleoprotein particles of uncertain function – is important for NPC incorporation. The authors first establish an in vitro assay that enables the separation of nuclear-membrane assembly and NPC incorporation; they do this by fractionating *Xenopus* egg extract to produce two membrane fractions, one of which forms nuclei that lack NPCs in the presence of chromatin. Insertion of NPCs, they show, proceeds in the presence of the second fraction. The authors next use immunoblotting and mass spectrometry to show that MVP is present in the second fraction, but not the first. In addition, recombinant MVP and purified endogenous vaults both promote NPC insertion when added to pore-free nuclei. Thus, vaults can act as NPC-incorporation factors and might, the authors speculate, help to stabilise newly formed membrane channels early in NPC incorporation. Their data advance our understanding of NPC biogenesis.



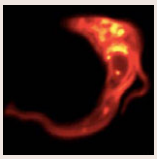
Getting to grips with GSL function

Glycosphingolipids (GSLs) are thought to be essential membrane components in mammalian cells, and have been implicated in neuronal development, protein sorting and cell adhesion and migration on the basis of cell-culture experiments. However, it has been difficult to interpret data from mice that lack GSLs; for this reason, the role of GSLs in vivo is less clear. To address this issue, Giovanni Lesa and colleagues (p. 822) now create mutants of *C. elegans* in which all three genes encoding ceramide glucosyltransferases (CGTs; enzymes that catalyse a step in GSL biosynthesis) are either knocked out or knocked down. The authors show that worms lacking CGTs fail to produce GSLs and undergo growth arrest at the first larval stage. Moreover, a subset of cells in the digestive tract of mutant worms displays defects that impair larval feeding; this leads to a starvation-like phenotype. Notably, the authors show that the reintroduction of genes encoding CGTs to this subset – but not to other cells – rescues the growth-arrest phenotype, giving rise to worms that appear to be phenotypically normal. The authors conclude that, unexpectedly, GSLs are dispensable for most tissues in *C. elegans*, including the nervous system. Their results shed light on the role of GSLs in *C. elegans* and complement data from mammalian models of GSL deficiency.



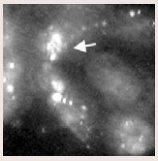
How Wnts keep Ras in check

Aberrant activation of the GTPase Ras – which regulates cell proliferation – is a major cause of human cancer, so diverse signalling mechanisms exist to regulate its activity. For instance, Kang-Yell Choi and colleagues previously showed that Wnt/β-catenin signalling regulates the degradation of the H-Ras isoform; now, they report on the underlying mechanism of this process (p. 842). The authors show that, in HEK293 cells, endogenous and overexpressed H-Ras are polyubiquitylated and degraded by the proteasome. Moreover, these processes are mediated by β-transducin-repeat-containing protein (β-TrCP) – a key subunit of the SCF E3 ubiquitin-ligase complex. Next, the authors show that Axin and APC, which are negative regulators of the Wnt/β-catenin pathway, destabilise H-Ras by enhancing its interaction with β-TrCP; by contrast, binding of β-TrCP to H-Ras, and polyubiquitylation of H-Ras, are both reduced in cells treated with Wnt3a. In colorectal cancer cells, H-Ras-dependent proliferation is reduced by β-TrCP. Importantly, mice treated intravenously with Wnt3a have higher levels of Ras and β-catenin than untreated controls. Together, these data indicate a novel mechanism for the regulation of H-Ras stability. Ras destabilisation by Wnt/β-catenin signalling might, the authors propose, act as a safeguard against tumourigenesis.



A role for rafts in flagella

The parasite *Trypanosoma brucei* has a single polarised flagellum that drives cellular motility. Some plasma-membrane-associated proteins localise specifically to the flagellum, but what are the mechanisms responsible for their targeting and retention? On page 859, David Engman and colleagues propose a role for lipid rafts – which are detergent-resistant, highly ordered plasma-membrane microdomains – in targeting flagellar proteins. The authors first show that several molecules that are thought to be raft components are enriched in the flagellum. In addition, they use laurdan (an amphiphilic fluor) to show that the flagellar membrane possesses increased liquid order; together, these data indicate that flagella are enriched in lipid rafts. The authors next show that the flagellar calcium-sensing protein calflagin Tb24 concentrates in detergent-resistant membranes; in an accompanying paper (p. 867), the group shows that calflagin Tb24 must be palmitoylated to localise to flagella, and identifies the specific palmitoyl acyltransferase that modifies it. Importantly, calflagin Tb24 redistributes to other domains when lipid rafts are destabilised. Thus, association with lipid rafts directs the flagellar localisation of calflagin Tb24 and might, the authors propose, be a general mechanism for the trafficking of specific proteins to the membrane of flagella and cilia.



nAChr maturation: reining in RIC-3

The biogenesis and maturation of nicotinic acetylcholine receptors (nAChRs) in neurons is a complex multi-step process. Receptor maturation is assisted by several chaperones, including RIC-3, which is active in the early stages of receptor assembly and increases the surface expression of mature receptors. Despite its role as a chaperone, elevated RIC-3 levels can be deleterious, as Millet Treinin and colleagues (p. 807) now report. Using a yeast two-hybrid screen, the authors first identify the protein BATH-42 as an interaction partner of RIC-3. They next show that, in *C. elegans*, loss of BATH-42 function causes RIC-3 levels to increase; *bath-42* loss-of-function worms have reduced nAChR function in vulval muscle. However, the overexpression of *bath-2* in adult worms is also deleterious, causing reduced pharyngeal pumping. Notably, this effect is dependent both on RIC-3 and on the CUL-3 ubiquitin ligase complex (with which BATH-42 is known to interact). BATH-42 has a similar domain structure to other CUL-3-binding proteins, which are thought to target specific proteins for ubiquitylation and proteasomal degradation; thus, the authors propose that BATH-42 might regulate RIC-3 levels in a similar way. Their data underscore the importance of regulating chaperone levels in nAChR maturation.

Development in press

The long arm of axon guidance factors

Guided cell migration and postsynaptic membrane expansion (PME) are both important developmental processes, but much remains to be learned about their regulation. In a paper published in *Development*, Peter Roy and colleagues report that, in neuromuscular-junction formation in *C. elegans*, several previously identified cell- and axon-guidance genes also direct PME. During junction formation, muscle cells extend membrane processes known as muscle arms towards the motor axons. The authors carry out a genetic screen for mutants with fewer muscle arms and identify ten genes, including *unc-40/Dcc* (which encodes a transmembrane receptor that guides cell and axonal migration in response to UNC-6/Netrin). They find that UNC-40 is enriched in muscle arms and directs muscle-arm extension to motor axons independently of UNC-6. Among the factors that lie downstream of UNC-40, the authors report, are the guanine-nucleotide exchange factor UNC-73/Trio, members of the WAVE actin-polymerisation complex and the focal-adhesion-component homologue UNC-95. Together, these data suggest that many genes required for guided cell and growth-cone migration have related roles in directing PME.

Alexander, M., Chan, K. K. M., Byrne, A. B., Selman, G., Lee, T., Ono, J., Wong, E., Puckrin, R., Dixon, S. J. and Roy, P. J. (2009). An UNC-40 pathway directs postsynaptic membrane extension in *Caenorhabditis elegans*. *Development* **136**, 911-922.