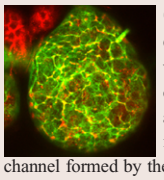
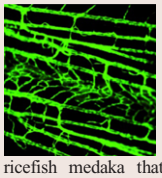
In this issue



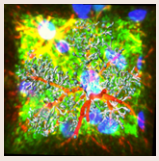
Glial cells in need of innexins

Gap junctions comprise assemblies of intercellular channels, which are formed from the products of two gene families: connexins in chordates, and innexins in prechordates. Innexins are also found in small numbers, as divergent sequences, in chordates, including humans, and are termed pannexins. Each intercellular channel formed by these gene families comprises two hemichannels, one from each of two apposed cells, which dock in the extracellular space to form a complete channel. There are eight innexin genes in *Drosophila melanogaster*, and here (p. 3823), Pauline Phelan and colleagues investigate two of these genes, *optic ganglion reduced* (*ogre*) and *innexin 2* (*Inx2*), using *in vitro* and *in vivo* approaches. They show, by expression of the proteins in *Xenopus laevis* oocytes, that Ogre, unlike Inx2, does not form homotypic intercellular channels. Coexpression of Ogre with Inx2, however, induces the formation of functional channels that have distinct properties from Inx2 homotypic channels. The authors next examine the expression pattern of these two innexins in the *Drosophila* larval central nervous system (CNS), finding partial colocalisation of Inx2 with Ogre in proliferative neuroepithelia and in glial cells. The expression of Inx2 and Ogre in glial cells suggested that the proteins are required in these cells for normal postembryonic development. To examine this, the authors use targeted expression of RNAi transgenes to selectively downregulate either *ogre* or *Inx2*, and see a significant reduction in the size of the larval CNS, as well as behavioural defects in surviving adults. Thus, this study shows that both Ogre and Inx2 are required in glial cells for normal postembryonic development of the *Drosophila* nervous system.



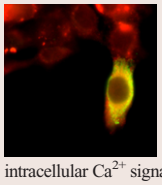
Melanoma angiogenesis by single cells

Angiogenesis is essential for the progression and metastasis of solid tumours, including melanomas. The molecular components that contribute to this process, however, are not fully understood, in part because of the limited information that current *in vivo* models can provide. Using a melanoma model in the transparent Japanese ricefish medaka that the authors developed previously, Svenja Meierjohann and colleagues (p. 3862) now establish the first transgenic fish tumour angiogenesis model. In this model, pigment cells are transformed by the use of an oncogenic receptor tyrosine kinase in fish that express GFP throughout their vasculature, thereby allowing resolution of single tumour cells and blood vessels. The authors show that angiogenesis occurs in a reactive oxygen species (ROS)- and NF-κB-dependent manner, but independently of hypoxia. Interestingly, they observe that sprouting of blood vessels can be induced by even single transformed pigment cells. Furthermore, pro-angiogenic factors, and most prominently angiogenin, are produced by transformed pigment cells of different origins as a result of NF-κB signalling. Inhibition of NF-κB prevented tumour angiogenesis and led to the regression of existing tumour blood vessels. These results show that angiogenesis can occur efficiently even in the absence of hypoxia, instead being regulated by ROS-driven NF-κB activation. Moreover, say the authors, the model of angiogenesis described here is well suited for the long-term monitoring of angiogenesis events in live fish and for evaluating the efficacy of small-molecule inhibitors in the whole organism.



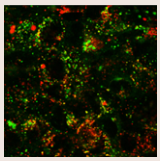
Whipped into shape: Arp2/3 activity defines astrocyte morphology

Astrocytes have a distinct complex, branched morphology. Pathological injury in the central nervous system induces a process called astrogliosis in astrocytes, in which their morphology becomes less complex, with larger cell bodies and fewer fine processes; the mechanism behind these morphological changes is unknown. On page 3873, Jonathan Hanley and colleagues ask how the actin machinery that typically promotes the outwards movement of the plasma membrane in astrocytes is involved in maintaining astrocyte complexity and in the morphological changes that occur during astrogliosis. The authors focus on the actin-nucleating complex Arp2/3, and show that its inhibition leads to rapid expansion of astrocyte cell bodies and major processes in culture and brain tissue. This expansion required functional myosin II downstream of the small GTPase RhoA and Rho-dependent kinase (ROCK). In a series of knockdown experiments to investigate which endogenous Arp2/3 regulatory proteins are required to maintain astrocyte morphology, the authors find that cell body expansion and reduced morphological complexity are induced in the absence of the Arp2/3 subunit Arp3 or the Arp2/3 activator N-WASP. Depletion of WAVE2 specifically reduced astrocyte branching complexity but, conversely, knockdown of the Arp2/3 inhibitor PICK1 increased the branching complexity. Moreover, ischaemia-induced astrocyte expansion was delayed by knockdown of PICK1 or overexpression of N-WASP. These findings show that astrocytes use a balance of Arp2/3 activation and inhibition through N-WASP, WAVE2 and PICK1 to control the complexity of their morphology, and this mechanism underlies the morphological changes to astrocytes induced by pathological insult.



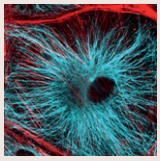
Palmitoylation a puppet master for calnexin function

The interaction of the endoplasmic reticulum (ER) with mitochondria, which occurs on the mitochondria-associated membrane (MAM), is necessary for the ER to carry out its main functions of protein and lipid production and Ca^{2+} storage. The MAM itself is the central intracellular Ca^{2+} signalling structure, and although it is known that various ER chaperones and oxidoreductases regulate ER–mitochondria Ca^{2+} exchange, the molecular machinery involved in ER–mitochondria Ca^{2+} signalling is poorly understood. Calnexin is a key ER chaperone that is found on the rough ER where it exerts a quality control function; it is also often found, however, on the MAM, suggesting a central role in regulating ER Ca^{2+} signalling. Thomas Simmen and colleagues have previously shown that palmitoylation of calnexin enriches calnexin on the MAM, and now (p. 3893), they investigate the functional significance of this palmitoylation-dependent targeting. The authors show that palmitoylated calnexin interacts with the sarcoendoplasmic reticulum Ca^{2+} transport ATPase (SERCA) 2b Ca^{2+} pump, and that this interaction determines the Ca^{2+} content of the ER and regulates ER–mitochondria Ca^{2+} crosstalk. Non-palmitoylated calnexin, however, preferentially interacted with the oxidoreductase ERp57 (also known as PDIA3) to perform its quality control function. Moreover, the authors show that palmitoylation of calnexin is also an ER-stress-dependent mechanism: short-term ER stress results in the depalmitoylation of calnexin, which shifts its function from Ca^{2+} signalling regulation to substrate chaperoning and quality control. These results, therefore, identify palmitoylation as the switch that assigns calnexin to either Ca^{2+} signalling or to protein quality control.



NDRG1 in LDLR trafficking

Receptor-mediated endocytosis of the low-density-lipoprotein receptor (LDLR) mediates the internalisation of LDL-cholesterol into cells. On page 3961, Vilja Pietiäinen, Elina Ikonen and colleagues describe a role for N-myc downstream regulated gene 1 (NDRG1) in cellular lipid homeostasis and endocytic trafficking of the LDLR. Mutations in *NDRG1* are known to cause the demyelinating neuropathy Charcot–Marie–Tooth disease type 4D, but the little is known about the cellular function of NDRG1. Using small interfering RNAs to silence *NDRG1* in A431 cells, the authors report reduced uptake of LDL-cholesterol owing to decreased levels of LDLR at the plasma membrane and diminished LDLR degradation. Moreover, LDLR accumulated in modified early endosomes and multivesicular bodies (MVBs), which had increased numbers of ceramide-enriched intraluminal vesicles. Depletion of NDRG1 also resulted in increased amounts of ubiquitylated LDLR, despite reduced levels of endosomal sorting complex required for transport (ESCRT) proteins. The authors next silenced the E3 ubiquitin ligase IDOL to prevent LDLR ubiquitylation in the NDRG1-depleted cells, and found that the LDLR seemed to be recycled to the plasma membrane, rescuing plasma membrane LDLR levels and LDL uptake. Finally, the authors studied the effects of silencing *Ndrg1* in mouse oligodendrocytes, finding that there was reduced uptake of LDL and downregulation of OLIG2, the oligodendrocyte differentiation factor – these effects were rescued by co-depletion of Idol. NDRG1 has therefore been identified as a novel regulator of MVB integrity and LDLR endosomal recycling.



Key role for EB2 in microtubule reorganisation

Microtubule plus-end-binding proteins target the microtubule plus ends, and thus control the lengths and positions of microtubules in cells. There are three microtubule end-binding (EB) proteins that are expressed in mammalian cells, and they are highly conserved core components of the microtubule plus-end-tracking protein machinery. EB1 and EB3 (also known as MAPRE1 and MAPRE3, respectively) are known to influence microtubule dynamics, but the function of EB2 in cells remains unclear. Here (p. 4000), Mette Mogensen and colleagues investigate the role of EB2 in microtubule reorganisation and apico-basal bundle formation during epithelial differentiation. The authors report inhibition of microtubule reorganisation following small interfering RNA depletion of EB2 during the early stages of apico-basal differentiation; the downregulation of EB2 at later stages, however, promoted microtubule stability and bundle formation. EB2 knockdown in undifferentiated cells was found to induce straight, less dynamic microtubules and led to EB1 lattice binding, recruitment of the microtubule–actin crosslinking spectraplakin ACF7 and co-alignment with actin filaments; this phenotype was rescued by inhibition of formins, which are downstream effectors of Rho GTPases. Finally, to confirm these *in vitro* findings *in vivo*, the authors studied *in situ* inner ear and intestinal crypt epithelial tissue, finding that a low level of EB2 expression correlated directly with the presence of apico-basal microtubule bundles, which were absent where EB2 was elevated. This is the first demonstration of a role for EB2 in microtubule reorganisation during apico-basal epithelial differentiation.