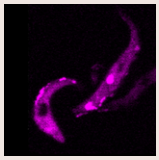
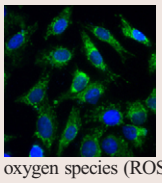
# In this issue



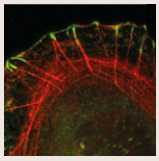
## Slow and fast trains in intraflagellar transport

The assembly of cilia and flagella relies on the bidirectional movement of protein complexes in the so-called intraflagellar transport (IFT), which is driven by kinesin II motors in the anterograde and by dynein in the retrograde direction. These complexes (IFT particles) are concentrated at the base of the flagellum, but not much in known with regard to their actual involvement in IFT. On page 327, Philippe Bastin and colleagues use the protist *Trypanosoma brucei* as a model system to investigate IFT with an automated tracking system that allows them to separate anterograde from retrograde transport. They find that the rate of retrograde transport (as visualised through GFP fused to the IFT dynein heavy chain DHC2.1 or to IFT52) is homogenous, whereas two distinct populations of IFT particles (or 'trains') moving with different speeds are engaged in anterograde transport. When a fast anterograde train catches up with a slower one, it is more likely to fuse with it than to overtake it, implying that these trains travel on a restricted set of microtubules. Interestingly, these trains split into three once they reach the tip of the flagellum and are then converted into retrograde trains. Using photobleaching and mathematical modelling, the authors show that IFT proteins that return to the flagellum base are recycled and participate in IFT again, suggesting that there are two pools of IFT particles, one that is actively involved in IFT and a second with yet unknown other function(s).



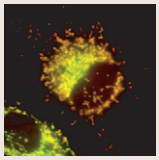
## Sirtuin-3 – a matter of life or death

The mitochondrial deacetylase sirtuin-3 (Sirt3) controls many aspects of mitochondrial function by deacetylating a number of mitochondrial matrix proteins, including anti-oxidant effectors and proteins involved in the electron-transport chain, thus acting as a tumour suppressor by limiting the production of reactive oxygen species (ROS). Consequently, depletion of Sirt3 has been shown to promote tumorigenesis owing to elevated ROS levels. Here (p. 274), John Pastorino and colleagues analyse the effects of Sirt3 on the proapoptotic Bak–Bax pathway, which is known to disrupt the integrity of the outer mitochondrial membrane and is inhibited by hexokinase II. Binding of hexokinase II to the mitochondrial membrane is regulated by cyclophilin-D, a Sirt3 target, whose deacetylation the authors have previously found to result in dissociation of hexokinase II. Here, they demonstrate that depletion of Sirt3 has an anti-apoptotic effect and promotes tumour cell survival. This is achieved though activation of cyclophilin-D and the subsequent binding of hexokinase II to the mitochondrial membrane, which prevents Bak–Bax-dependent apoptosis. By contrast, when overexpressing Sirt3, the authors find that hexokinase II detaches from the mitochondrial membrane, thus activating Bak–Bax-mediated cell death. Taken together, these data firmly establish the role of Sirt3 as a central regulator of mitochondrial metabolism, which might be exploited in the targeting of apoptotic pathways in tumour cells.



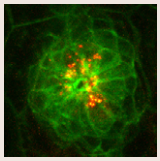
## Rac1 but no myosin – not all stress fibres are equal

Migration and spreading of cells depend on dynamic actin polymerization at their protruding edge, and on actin stress fibres providing contractility through the presence of crosslinkers, such as α-actinins or non-muscle myosins. Different types of actin fibre have been found in cells, including dorsal stress fibres, transverse arcs and ventral stress fibres. Among these, dorsal stress fibres are least understood with regard to their composition or regulation. On page 263, Tea Vallenius and co-workers set out to characterise this stress fibre subtype in human osteosarcoma cells, which have particularly well-defined stress fibres. They find that assembly of dorsal stress fibres specifically requires α-actinin-1, and that its depletion leads to decreased cell migration without affecting the activity of myosin II. This suggests that dorsal stress fibres promote migration in a myosin-II-independent manner, a theory that is further confirmed because dorsal stress fibre trunks lack myosin II and are resistant to myosin II ATPase inhibition. But how are dorsal stress fibres regulated? Rac1 – as a crucial regulator of actin polymerization – is an obvious candidate and, indeed, the authors find that Rac1 induces the assembly of dorsal stress fibres, whereas RhoA induces contractile ventral stress fibres. Taken together, these results provide evidence that α-actinin-1 and Rac1 are crucial mediators in the assembly of dorsal stress fibres in migrating and spreading cells.



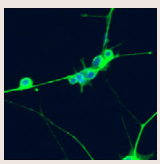
## Drp1-independent role of Fis1 in mitochondria

Mitochondrial fusion and fission in response to differentiation or pathological conditions is regulated by evolutionally conserved GTPases. One of these is the dynamin-related GTPase protein Drp1 (Dnm1 in yeast) that is important for mitochondrial fission during embryonic development as well as for normal neuronal differentiation. In yeast, the mitochondrial outer membrane protein Fis1 recruits Dnm1 to the mitochondria but the role of its mammalian homologue remains unclear, as its is dispensable for mitochondrial recruitment of Drp1. On page 176, Naotada Ishihara and colleagues describe their use of co-immunopurification assays of lysates of HeLa cells that express FLAG-tagged Fis1 in order to search for Fis1-binding proteins. They identified TBC1D15, a member of the TBC (Tre2–Bub2–Cdc16) domain-containing protein family, the domain conserved in Rab GTPase-activating proteins and discovered that Fis1 stimulates mitochondrial localization of TBC1D15, which – in the absence of Fis1 – resides in the cytoplasm. Importantly, they show that Fis1 and TBC1D15 function independently of Drp1. Furthermore, when TBC1D15 is knocked down using RNA interference (RNAi), they observe a highly connected mitochondrial network structure that displays numerous branches and is similar to that observed in Fis1 RNAi experiments, but in stark contrast to the knockdown of Drp1 using RNAi, which results in elongated mitochondria with fewer branches. These data indicate that, in mammalian cells, Fis1 acts as a mitochondrial receptor for recruitment of TBC1D15 to regulate mitochondrial morphology independently of Drp1.



## F-BAR complexes keep zebrafish swimming balanced

During development, general information regarding the body plan, such as definitions of body axes and pattern formation, need to be translated into the morphologies of individual cells and result in apical-basal and planar cell polarity, with the latter being important in sensory hair cells. Not much is known with regard to the molecular effectors that enable the required cell shapes and their changes during polarity establishment and maintenance, but the process is thought to require membrane shaping by BAR-family proteins and force generation by the cortical actin cytoskeleton. Here (p. 196), Britta Qualmann and colleagues perform side-by-side loss-of-function studies by using the zebrafish orthologues of the F-BAR protein syndapin I and of the actin nucleator Cobl. They find that lack of syndapin I or Cobl result in similar loss-of-function phenotypes. Affected are processes that rely on the accurate formation and function of motile and non-motile sensory cilia; for instance, the fish show severe swimming and balance-keeping defects. Syndapin I and Cobl both localise to the base of the forming cilia, and rescue experiments show that formation of a complex between syndapin I and Cobl is crucial for the formation of ciliary structures. Thus, the authors suggest that formation of distinct types of ciliary structure relies on the modulation of the membrane topology that is mediated by F-BAR domains and cytoskeletal components.



## LRP1 facilitates inhibition of neurite outgrowth

Neuronal regeneration in the adult CNS is limited because neuronal sprouting is inhibited by myelin, which is induced by myelin-associated inhibitory proteins (MAIs), such as Nogo-A, sialic-acid-recognizing myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMG). Detailed understanding of the mechanisms underlying MAI-mediated inhibition of neuronal growth is of considerable clinical interest and, on page 209, Steven Gonias and colleagues investigate the possible role of LDL-receptor-related protein-1 (LRP1), which – as they have reported previously – functions in phagocytosis of large particles, including degenerating myelin. Now, the authors show that LRP1 functions also as a high-affinity, endocytic MAG receptor but, interestingly, and in contrast to other MAG receptors, LRP1 binding is independent of its terminal sialic residues. The functional outcome of this interaction is the activation of RhoA, whose activation is known to be crucial for the inhibition of neuronal outgrowth. Consequently, the authors show that ablation of LRP1 function is sufficient to significantly reverse myelin-mediated inhibition of neurite outgrowth. Interestingly, they also find that LRP1 binds other MAIs apart from MAG, but additional work is necessary to elucidate whether these interactions of LRP1 are also important for myelin-mediated inhibition of neurite outgrowth.