Ephrin-Bs: acting autonomously

Interactions between Eph receptors and their membrane-bound ephrin ligands trigger bidirectional signalling cascades that regulate cell behaviour through effects on the cytoskeleton. Catherine Nobes and colleagues (p. 1235) now investigate how ephrin B family proteins – which regulate angiogenesis in normal and pathological contexts – modulate cell morphology and migration in vitro. Using microinjection techniques, the authors show that overexpression of ephrin-B2 increases membrane ruffling, and induces rapid cycles of cell contraction and expansion specifically in endothelial cell types. Compared with control cells, ephrin-B2-overexpressing cells also migrate faster and more randomly, similar to cancer cells. When isolated cells within a monolayer are microinjected with ephrin-B2, neighbouring cells repulse them, creating more space for movement of ephrin-B2-expressing cells. Notably, these effects require the cytoplasmic PDZ-binding motif of ephrin-B2, suggesting that association with other intracellular signalling molecules is important for ephrin-B2 function. Finally, the authors show that these effects are independent of the ephrin-B2 receptor, EphB4, when ephrin-B2 is overexpressed, suggesting that ephrin-B2 can act cell autonomously. Therefore, the authors conclude, ephrin-B2 activation triggers multiple downstream events that can be EphB4-dependent and -independent.

Pinpointing p53 in the nucleolus

Controversy regarding the subnucleolar localisation of p53 has limited a full understanding of this tumour suppressor’s molecular functions. Previous studies suggested that p53 colocalises with RNA polymerase I in fibrillar centers (FCs) of the nucleolus and represses rDNA transcription at these sites; conversely, other reports found p53 in the dense fibrillar center or granular component (GC) of the nucleolus. On page 1203, Tim Krüger and Ulrich Scheer now use live-cell imaging techniques to address this issue in greater detail. In contrast to previous findings, they report that p53 does not localise to FCs or other ribosome-producing areas, but accumulates in nucleolar cavities containing proteasomes. Upon continued treatment with a proteasome inhibitor, these small p53-containing cavities expand over time to become large nucleolus-spanning regions in which p53 appears to partially colocalise with inactivated proteasomes. These regions are distinct from other ‘nonribosomal’ areas of the nucleolus, such as subdomains of the GC, perhaps providing a new example of nucleolar subcompartmentalisation. Overall, these data support the idea that p53 represses rDNA transcription indirectly, rather than within FCs, although the mechanism by which such indirect repression occurs remains an issue for future study.

Rif triggers stress-fiber formation

Rif is a Rho GTPase that regulates filopodia formation, for which it acts as an alternate trigger for other Rho GTPases such as Cdc42. On page 1247, Harry Mellor and colleagues now show that Rif can also act as an alternate trigger for another Rho-regulated process: the formation of actin stress fibers. Stress-fiber formation was thought to be induced exclusively by RhoA and the closely related proteins RhoB and RhoC. Here, the authors show that activated Rif can also induce stress-fiber formation specifically in epithelial cells. ROCK is required for stress-fiber formation induced by both RhoA and Rif; however, RhoA physically interacts with and activates ROCK, whereas Rif does not, suggesting that a basal level of ROCK is indirectly required for Rif-induced stress-fiber formation. The authors previously showed that Rif regulates filopodia formation through the Diaphanous-related formin mDia2. They show here that, like RhoA, Rif induces stress-fiber formation through directly interacting with mDia1. Intriguingly, Rif activation promotes the localisation of mDia1 to filopodia, hinting that Rif-regulated filopodia formation might involve mDia1 as well as mDia2. These data characterise a new interface at which a non-conventional GTPase regulates a highly conserved Rho-regulated cytoskeletal process.

A tale of three proteins in actin patches

Actin-filament assembly, remodeling and disassembly are dynamically regulated by many different proteins. On page 1329, John Cooper and colleagues report on the cooperative and independent roles of three such proteins – cofilin, coronin and Aip1 – by analysing cortical actin patches (which drive the formation of endocytic vesicles) in budding yeast. They analyse a variety of mutant yeast strains using live-cell microscopy to investigate the localisation, recruitment kinetics and relative importance of these three proteins in actin patches. In addition, they assess whether each protein’s localisation and functions depend on the presence of the other two proteins. The data indicate that cofilin, coronin and Aip1 are all recruited with similar kinetics late in the life of a dynamic actin patch, when actin filaments are disassembling. However, stoichiometric analyses indicate that the three proteins do not form a simple heterotrimeric complex. In addition, the localisation of the three proteins to actin patches is complex and interdependent: cofilin is required for the recruitment of coronin and Aip1, whereas coronin is not important for the recruitment of either cofilin or Aip1, and Aip1 prevents the localisation of cofilin and coronin to actin cables. The authors conclude that cofilin is by far the most important of the three proteins, and that it cooperates in some capacities with coronin and Aip1 in regulating actin-filament disassembly in actin patches.

Making waves with Gab1

The formation of dorsal membrane ruffles occurs during biological responses including cell migration and invasion. Growth factors that bind to receptor tyrosine kinases (RTKs) such as EGFR, PDGR and Met stimulate the formation of dorsal ruffles – but how RTK signalling is translated into the cytoskeletal changes that underlie dorsal-ruffle formation has been unclear. Gab1 is a scaffolding protein that is recruited to several different activated RTKs and is important for their signalling function. On page 1306, Jasmine Abella, Morag Park and colleagues now reveal that Gab1 is crucial for dorsal-ruffle formation downstream of several different RTKs. The authors first show that dorsal-ruffle formation induced by RTK activation is markedly reduced following knockdown of Gab1 expression and, conversely, promoted when Gab1 is overexpressed. Second, they demonstrate a novel interaction between Gab1 and Nck adaptor proteins that is essential for RTK-induced dorsal-ruffle formation. Notably, the Gab1-Nck interaction is required for optimal Met-mediated activation of Rac GTPases, which direct the formation of dorsal ruffles. Finally, the authors show that Gab1-dependent dorsal-ruffle formation requires the actin regulator N-WASP. The authors propose that Gab1 mediates RTK-dependent dorsal-ruffle formation by acting as a scaffold that promotes a Nck–N-WASP interaction, thereby facilitating actin polymerisation.

Development in press

Nectin and N-cadherin connect to constrict

Neural-tube formation, an important step in vertebrate development, begins with the apical constriction of neuroepithelial cells. This cell-shape change requires the apical accumulation of F-actin, which occurs through a little-known process. In a study published in Development, Naoto Ueno and colleagues now shed light on this event with their discovery that the cell-adhesion protein nectin-2 is required for apical constriction during neural-tube formation in Xenopus by associating with N-cadherin to facilitate actin accumulation. Using RNA interference and overexpression experiments, the authors show that nectin-2 is required for F-actin accumulation and apical constriction during neurulation. This activity, however, does not require the afadin-binding domain of nectin-2, with which it binds to actin. Instead, nectin-2 binds to N-cadherin through its extracellular domain. On the basis of their findings, the researchers propose a novel mechanism of neural-tube morphogenesis in which nectin-2 directs N-cadherin to the apical membrane to recruit F-actin, possibly through β-catenin, leading to apical constriction and neural-tube closure.