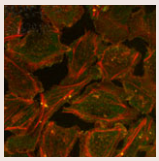
In this issue

MINIFOCUS: Ubiquitin – Part 2

Ubiquitin received its name because of its ubiquitous expression in eukaryotic cells. Since its discovery more than 40 years ago, the covalent attachment of ubiquitin to proteins has become well established as a degradative signal. However, more recently it has become clear that protein degradation is only one of many processes regulated by ubiquitylation and it has emerged that this 76 amino acid protein is also crucial for the regulation of numerous cellular processes. For example, ubiquitin is also involved in endocytosis, membrane trafficking, DNA repair, and the regulation of signalling pathways and the cell cycle. In this issue, we conclude our Ubiquitin Minifocus with three articles that provide further insight into the diverse roles of ubiquitylation. E3 ubiquitin ligases are central to the post-translational modification of proteins with ubiquitin and – in a Cell Science at a Glance article (p. 531) – Meredith Metzger, Ventzislava Hristova and Allan Weissman provide an overview of the HECT and RING finger families of E3 ubiqitin ligases and their functions. In the Commentary on page 539, Yelena Kravtsova-Ivantsiv and Aaron Ciechanover highlight the role of additional, 'noncanonical' ubiquitin-dependent degradation signals. Finally, the Commentary by Anna Schmuckle and Henning Walczak (p. 549) provides insight into how ubiquitylation affects signalling through NF-κB pathways.

Integrating the integrin network

Integrins meditate the interaction between cells and the extracellular environment. Their activity is modulated by intracellular signals that alter the ability of integrins to interact with their respective ligands. But despite more than 20 years of research on integrins, relatively few of their activators and inhibitors have been identified. Here, Johanna Ivaska and colleagues (p. 649) describe a 'druggable' genome-wide RNAi screening approach to uncover new regulators of β1 integrin. Using the recently developed cell spot microarray technology in a total of 12 cell lines, they identify 13 activators and 10 inhibitors of β1-integrin activity. They find that many of these regulators are involved in regulating inflammation, and changes in β1-integrin activity can influence invasion of cancer cell in vitro. By investigating an activating (CD9) and an inhibiting (MMP8) protein in more detail, the authors further validate their screen and define specific functions for these two regulators. CD9 associates with β1 integrin and activates ligand binding. By contrast, MMP8, a secreted metalloprotease, binds to the external region of this integrin and, thereby, negatively affects its ability to interact with ligands. Together, these findings support the fact that changes in integrin activity are associated with inflammation and tumour progression, and illustrate how complex the regulation of integrin activity is.

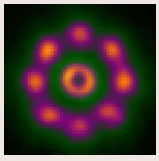


Without N-WASP cancer makes no move

Invadopodia are actin-based membrane protrusions with high proteolytic activity that are thought to have an important role in cancer-cell invasion and intravasation. However, questions have remained about the formation and precise function of these structures during metastasis. On page 724, John Condeelis and colleagues extend their previous observation that neural Wiskott–Aldrich syndrome protein (N-WASP) is an essential component of invadopodia by discovering that N-WASP-mediated formation of invadopodia is required for the process of invasion and intravasation during breast cancer metastasis. Reducing the activity or expression of N-WASP in rat mammary adenocarcinoma cells leads to a reduction in the number and proteolytic activity of invadopodia in vitro, and decreases the invasive ability of the cells in vivo. The lack of functional N-WASP does not affect tumour size, but decreases the number of circulating tumour cells and the formation of metastasis. Primary tumour cells without functional N-WASP are also more round, less polarised, form fewer protrusions and are less mobile than cancer cells that express N-WASP. In addition, cells without N-WASP lack the ability to efficiently degrade collagen I at the invasive edge and in areas enriched with blood vessels. The actin nucleator N-WASP, thus, has a crucial role in driving the formation of proteolytic invadopodia and cancer cell metastasis.

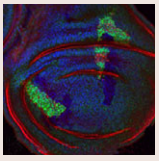
p120-catenin meets Down syndrome

The 'Down syndrome critical region' is the segment of chromosome 21 that contains the genes responsible for the pathological features of Down syndrome. One of the genes located in this region encodes the dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), which is involved in the regulation of various cellular processes. On page 561, Pierre McCrea and colleagues now report a new link between DYRK1A and several Wnt target genes. Using *Xenopus* embryos, the authors identify p120-catenin (also known as catenin-δ1), especially isoform 1, as a new DYRK1A target and show that its phosphorylation on Thr47 by the kinase stabilises this member of the catenin family. The N-terminal region of p120-catenin is required for its association with DYRK1A. Furthermore, both proteins primarily interact in the nucleus, and DYRK1A overexpression relieves Kaiso-mediated repression of the Wnt target genes *wnt11* and *siamois*. Expression of a phosphomimetic mutant p120-catenin in *Xenopus* embryos leads to increased levels of Wnt-11 and Siamois, and increases gastrulation defects. Together, these results highlight a new role for DYRK1A in p120-catenin–Kaiso signalling. In addition, they provide insight into a conceivable mechanism by which increased levels of DYRK1A that result from gene amplification in patients with Down syndrome could contribute to the pathophysiology of this disease.



NPCs in super resolution

Light microscopy is a key component of the experimental toolkit that is available to cell biologists. However, the resolution of fluorescence microscopy is limited by the wavelength of the light used to observe samples. More recently, 'super-resolution microscopy' approaches have been used to study cellular structures at much higher resolution. Markus Sauer and colleagues (p. 570) now show that super-resolution microscopy can achieve results that are comparable with those obtained by electron microscopy, while being less invasive. The authors employ direct stochastic optical reconstruction microscopy (*d*STORM) to investigate the structure of nuclear-pore complexes (NPCs) in isolated *Xenopus* oocyte nuclear envelopes. By using fluorescently labelled antibodies against the NPC component gp120 and fluorescently labelled wheat germ agglutinin bound to nucleoporins, they are able to resolve NPC structures at a resolution of ~15 nm. Additionally, they confirm the eightfold radial symmetry of gp120 dimers around the NPC and overlay more than 600 images to determine the diameter of the central NPC channel to be 41±7 nm. The researchers propose that, in the future, the high optical resolution achieved with this approach will allow functional imaging with – yet unprecedented – spatio-temporal resolution.



Lgd partners up with ESCRT-III

Loss-of-function of the tumour suppressor gene *lethal (2) giant discs* (*lgd*) in *Drosophila* results in the ligand-independent activation of the Notch signalling pathway and the overproliferation of imaginal disc cells. The Lgd protein contains a C2 domain – which can mediate binding to phospholipids or phosphorylated proteins – as well as four tandem DM14 domains of unknown function, and is involved in constitutive trafficking and degradation of Notch through the endosomal system. But how does Lgd affect endosome trafficking? Thomas Klein and co-workers (p. 763) provide an answer by using genetic and biochemical approaches to further characterise the tumour suppressor and its binding partners. They report that the C2 domain is required for stabilising Lgd and targeting it to the cytosol. The DM14 domains are required to provide specific protein function by interacting with other proteins, and largely act in a redundant manner. Specifically, the authors identify the interaction of the Lgd DM14 domains with the ESCRT-III-complex component Shrub (the *Drosophila* homologue of Snf7/CHMP4/Vps32). This interaction – which takes place in the cytosol – is required for Shrub function and shows that Lgd has an essential role in the regulation of endosomal trafficking.