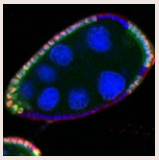
In this issue

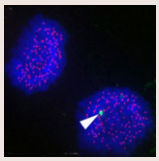
MINIFOCUS: Ubiquitin – Part 1

There is no doubt that protein phosphorylation is the most extensively studied post-translational modification, and it is well established that a plethora of cellular activities are regulated by the interplay between kinases and phosphatases. However, in recent years, ubiquitylation has emerged as a close runner-up. Since its discovery as a degradation signal, numerous studies have shown that the covalent attachment of ubiquitin moieties to proteins regulates various cellular processes, including DNA repair, the cell cycle and receptor endocytosis. In this Minifocus, we present a collection of articles that examine a number of these functions in detail. The first part of this collection begins with a comprehensive overview of the roles that ubiquitin and SUMO have in the regulation of DNA repair in Helle Ulrich's Cell Science at a Glance poster article (p. 249). In their Commentary, Annamaria Mocciaro and Michael Rape (p. 255) highlight the emerging mechanisms in ubiquitin-dependent cell cycle control. Kaisa Haglund and Ivan Dikic (p. 265) summarise the roles that ubiquitylation has in regulating the endocytic pathways of receptor tyrosine kinases in a Commentary. Finally, in their Commentary on page 277, Michael Clague, Judy Coulson, and Sylvie Urbé discuss what is currently known about the enzymes that reverse this post-translational modification, the deubiquitylases.



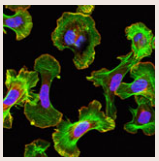
CoREST turns signal up a Notch

The Notch signalling pathway regulates a variety of cellular events, including differentiation, proliferation and apoptosis. Compared with many other signalling events, this pathway is relatively simple; however, the context-dependent activity of numerous proteins that can influence Notch signalling increases its complexity and specificity. Here, Elena Domanitskaya and Trudi Schüpbach (p. 399) identify the transcriptional cofactor CoREST as a new positive regulator that mediates epigenetic regulation through the Notch signalling pathway in *Drosophila*. Ovarian follicle cells lacking CoREST show defects in the Notch-dependent mitotic-to-endocycle switch and in the expression of Notch target genes. The authors find, that CoREST acts downstream of Notch proteolytic cleavage, but upstream of the Notch target gene Hindsight, which regulates the mitotic-to-endocycle switch. Furthermore, CoREST antagonises the activity of components of the Notch repressor complex, such as Hairless, C-terminal Binding Protein and Groucho, and the absence of CoREST results in increased levels of H3K27 trimethylation and H4K16 acetylation. Taken together, these results highlight that CoREST is a positive regulator of Notch signalling in *Drosophila*.



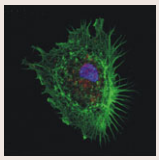
Transcription disrupts kinetochores

In eukaryotes, kinetochore assembly takes place on the centromere. The histone H3 variant CENP-A is important for this process and for maintenance of centromere epigenetic identity. In addition, the centromeric alpha-satellite DNA contains a specific set of post-translational modifications and is transcribed at low levels. Removing the H3K4 dimethylation mark on kinetochore chromatin results in a decrease in transcription and prevents the loading of CENP-A, thereby disrupting kinetochores. But is it the chromatin environment or the level of transcription that is important for CENP-A recruitment? By using human artificial chromosome rich in alpha-satellite sequences, Bill Earnshaw and co-workers (p. 411) provide an answer to this question. They show that the C-terminal transactivation domains from the NF-κB p65 subunit and the herpes virus VP16 upregulate H3K9 acetylation to a similar level, and increase transcription ~10- and ~150-fold, respectively. The changes induced by p65 do not affect kinetochore structure or function. By contrast, the greater increase in transcriptional activity induced by VP16 is accompanied by a loss of pre-assembled CENP-A from the kinetochore as well as defective CENP-A loading. From these results, the authors conclude that transcriptional activity from centromeric chromatin has to be carefully balanced to maintain kinetochore integrity.



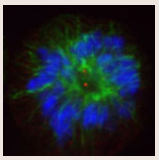
Integrins and EGFR meet at ruffles

Circular dorsal ruffles are dynamic actin-based structures on the plasma membrane. They form in response to growth factor stimulation, which induces activation of the Src kinase and the transient rearrangement of the actin cytoskeleton. In their study on page 435, Reinhard Fässler and colleagues now discover that signalling crosstalk between the epidermal growth factor (EGF) receptor and integrins is required to mediate dorsal ruffle formation. The researchers show that integrin-α5β1-mediated adhesion to fibronectin and integrin-linked kinase (ILK) are required for EGF-induced ruffling in fibroblasts. Furthermore, they find that the localisation of active Src to focal adhesions is impaired in ILK$^{-/-}$ cells stimulated with EGF, suggesting that ILK regulates ruffle formation by controlling Src activity at adhesions. SILAC-based mass spectrometry and siRNA-mediated protein knockdown experiments reveal the tumour suppressor cylindromatosis (CYLD) as a target protein for integrin–ILK and EGFR signal crosstalk. Tyrosine phosphorylation of CYLD in response to EGF stimulation requires the presence of ILK and Src and is necessary for dorsal ruffling. These data thus highlight how the interplay between growth factor receptor and integrin signalling mediates fast actin cytoskeletal rearrangements during circular dorsal ruffle formation.



MSCs mind the (adhesive) gap

Cells not only sense and respond to soluble stimuli, but also receive various cues from the physical extracellular environment. Stimuli such as changes in substrate elasticity and extracellular matrix (ECM) composition have been shown to influence mesenchymal stem cell (MSC) fate determination. On page 317, Justin Cooper-White and colleagues now show that the spatial arrangement of cell adhesion ligands also affects MSC behaviour. Using adhesive substrates containing arginine-glycine-aspartate peptides tethered to self-assembled polymers of defined spacing, they show that an increase in the lateral spacing between peptides (from 34 or 44 nm to 50 or 62 nm) results in morphological changes. The cells become less well-spread, extend multiple filopodia and display a disorganised actin cytoskeleton. Closely spaced peptides also induce the formation of larger, fully mature focal adhesions, whereas nascent adhesions are more commonly found when the peptides are spaced further apart. A greater distance between adhesive ligands also results in a higher migration rate, and, depending on the spacing between peptides, they can induce opposing differentiation programmes. Together, these observations not only indicate that the lateral spacing of adhesion peptides can influence stem cell behaviour but also highlight a new aspect of the cellular environment that can be exploited for tissue engineering.



Centriole duplication: GCP6 joins in

The centrosome is not only important for the regulation of cell division, but also forms the main microtubule-organising centre in the cell. The γ-tubulin ring complex (γ-TuRC) is a key component of the centrosome and is required for the nucleation of microtubules. The γ-TuRC is an assembly of several γ-tubulin small complexes (γ-TuSCs) and several other proteins, including GCP4, GCP5, GCP6 and NEDD1, whose functions have not been identified so far. Here, Ingrid Hoffmann and co-workers (p. 486) describe a role for GCP6 in γ-TuRC assembly, mitotic spindle formation and centriole duplication downstream of the serine/threonine kinase PLK4. Cells depleted of GCP6 by siRNA form monopolar mitotic spindles and are unable to recruit γ-tubulin to the centrosome. Immunoelectron microscopy reveals that GCP6 localises to both the PCM at the proximal end of the centriole and the distal part of the centriolar lumen, thereby mirroring the localisation pattern of γ-tubulin. Furthermore, GCP6 has an important role in centriole duplication, as depletion of the protein results in a decrease in centriole number. This role depends on PLK4-mediated serine phosphorylation of GCP6. Disruption of the phosphorylation event does not abolish recruitment of GCP6 to the γ-TuRC. However, it does result in defects in centriole duplication, which suggests that GCP is an essential component of PLK4-mediated centriole biogenesis.