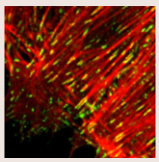
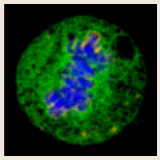
In this issue

By Jane Bradbury



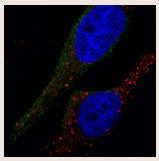
Dynamics of focal adhesion proteins

Focal adhesions (FAs) are transient membrane-associated, multi-protein structures that are involved in cell survival, proliferation and motility. They link the extracellular matrix (ECM) to the actin cytoskeleton through a network of at least 150 proteins. Although the molecular composition of FAs has been extensively described, little is known about their dynamics. Here (p. 4498), Bob van de Water and co-workers use fluorescence loss in photobleaching (FLIP) combined with fluorescence recovery after photobleaching (FRAP) to determine the dynamics of focal adhesion kinase (FAK) and of the FA-associated adaptor protein paxillin in each FA of non-migrating porcine renal epithelial cells. The authors show that FAK and paxillin exist in two states – a fast diffusing cytoplasmic pool and a transiently immobile FA-bound fraction. FAK resides in FAs for shorter times than paxillin, but the residence time for both proteins increases with increasing FA size. By contrast, increasing the ECM density increases the residence time of FAK only. These, and other results, indicate that FAK and paxillin dynamics at FAs are determined by the size, strength and life cycle status of each FA. Moreover, they suggest that a combined FLIP-FRAP approach could be used to characterise FA regulation in different biological settings.



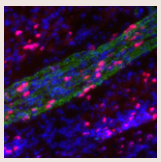
PNBs – pre-rRNA processing sites

Pre-nucleolar bodies (PNBs) are transient nuclear subdomains that form at telophase and gradually disappear during early G1 phase. They contain nucleolar proteins, small nucleolar ribonucleoproteins (snoRNPs) and pre-ribosomal RNAs (pre-RNAs) and have long been considered intermediates in the post-mitotic reconstitution of the nucleolus. Now, on page 4532, Pierre-Emmanuel Gleizes and colleagues suggest that PNBs are actually active ribosome factories where the maturation of pre-rRNAs, which is inhibited during mitosis, resumes at telophase. Using fluorescent in situ hybridization, the authors show that pre-rRNA spacers (sequences that flank the 18S, 5.8S and 28S rRNAs in the polycistronic 47S pre-rRNA) are sequentially removed in PNBs in HeLa cells when cells enter G1 phase. siRNA knockdown of proteins involved in pre-rRNA processing induces the accumulation of stalled pre-ribosomes in PNBs and prevents the gradual disappearance of PNBs during early G1 phase. Finally, electron tomography supports the presence of pre-ribosomal particles in PNBs. Given these results, the authors propose that PNBs are primarily autonomous extra-nucleolar maturation sites for mitotic pre-rRNAs and suggest that maturation and release of their pre-ribosome content drives the orderly disassembly of PNBs in G1 phase.



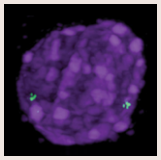
p62 FAT10-ed up for degradation

Covalent tagging of proteins with ubiquitin or ubiquitin-like modifiers is a common post-translational modification that changes the localization, function or fate of specific proteins. The ubiquitin-like modifier FAT10 (HLA-F adjacent transcript 10) is involved in apoptosis induction, cell cycle control and NF-κB activation. Treatment with the pro-inflammatory cytokines interferon γ (IFNγ) and tumour necrosis factor (TNFα) induces covalent conjugation of FAT10 to numerous, mostly unidentified, endogenous substrates that are then degraded by the proteasome. Marcus Groettrup and colleagues now use a proteomics approach to identify FAT10 substrates and interaction partners (p. 4576). The authors stimulate HEK293 cells with IFNγ and TNFα, immunopurify FAT10 conjugates and identify the conjugates using mass spectroscopy. They identify 569 interacting proteins, including 176 that are putative covalently linked substrates, and show that one of these proteins – the autophagosomal receptor p62 (SQSTM1) – is mono-FAT10ylated at several lysine residues. This modification leads to proteasomal degradation of p62, and prolonged induction of FAT10 expression by pro-inflammatory cytokines decreases the levels of p62. These findings and the validation of further FAT10 substrates should increase our understanding of FAT10 biology.



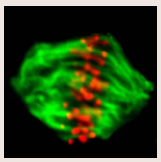
Laminin-dependent myelination wrapped up

Laminins promote peripheral nerve myelination by assembling basement membranes (BMs) on Schwann cell surfaces, but which of the many activities of laminin are required for peripheral nerve myelination? On page 4609, Peter Yurchenco and colleagues report that mice hypomorphic for expression of the laminin γ1 subunit display endoneurial BMs with reduced component density and amyelination but normal Schwann cell proliferation. To identify which laminin interactions are responsible for these characteristics, the authors treat explanted dorsal root ganglia that are unable to secrete γ1 laminin with recombinant laminins. Myelin-wrapping of axons by Schwann cells, they report, requires higher laminin concentrations than either proliferation or axonal ensheathment with BM. Moreover, laminins that are unable to polymerise or that lack their cell-adhesive laminin globular (LG) domains produce reduced BMs and little myelination, whereas laminins that bind weakly to β1 integrins through their LG domains effectively assemble BMs but do not support myelination. Finally, Schwann cell proliferation depends on both integrin binding to LG domains and laminin polymerisation. Thus, laminin concentration, polymerisation and interactions with β1 integrin differentially contribute to the promotion of Schwann cell proliferation, axonal ensheathment and myelination.



Chromatin looping upon gene activation revealed

The architecture of the chromatin in eukaryotic cells is constantly changing. Cell proliferation and differentiation, as well as changes in gene expression all alter chromatin structure. Activation of large gene loci is thought to occur through an active looping mechanism that brings promoter regions close to the transcription start of genes. Here (p. 4630), Frank Grosveld and co-workers use super-resolution imaging to reveal the three-dimensional (3D) folding dynamics of the β-globin-encoding locus upon gene activation. The authors produce images of the locus (which contains four β-globin-like genes) using 3D fluorescent in situ hybridization and 3D structured illumination microscopy, and volume render the images into 3D objects to allow geometric analysis of chromatin structures. They report that the inactive β-globin locus in mouse erythroid leukaemia cells occurs in several different conformations, which, upon cell differentiation, change their shape and surface structure to form a single more-folded and rounded structure with a substantially smaller size and volume. The authors propose, therefore, that the β-globin locus has an active 'breathing' structure before transcription that is stabilised into a single conformation, which is best explained by extra chromatin looping within the locus upon transcription of its genes.



New mitotic role for MLL5

Accurate chromosome segregation during mitosis is essential to prevent chromosome instability, which plays a causal role in tumorigenesis. The chromosomal passenger complex (CPC), which comprises the kinase Aurora B and the three non-enzymatic regulatory subunits Survivin, Borealin and INCENP, regulates chromosome segregation during mitosis, but what regulates CPC stability? On page 4676, Lih-Wen Deng and colleagues report that the nuclear protein mixed lineage leukemia 5 (MLL5) helps to regulate CPC stability in human cells. *MLL5*, which is often deleted in acute myeloid leukemia, is involved in multiple cellular events, including mitotic entry. siRNA-mediated knockdown of MLL5, the authors report, disrupts chromosome alignment at metaphase, DNA segregation and cytokinesis, and delocalizes the CPC from the inner centromere region owing to proteasome-mediated degradation. They show that the central domain of MLL5 interacts with the C-terminus of Borealin and that this interaction is required to maintain the stability of Borealin. Moreover, overexpression of wild-type MLL5, but not of an MLL5 mutant that lacks the central domain, rescues the mitotic defects of MLL5-depleted cells. Together, these results suggest that MLL5 maintains genomic integrity by regulating the stability of CPC through a functional interaction with Borealin.