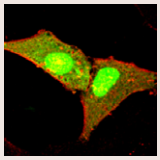
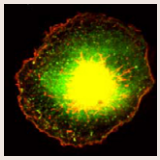
In this issue



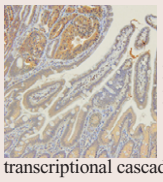
Sentinel role for DGKζ regulation of p53

In response to stress, the p53 tumour suppressor protein coordinates a number of cellular responses and, as such, requires tight regulation. Diacylglycerol kinase ζ (DGKζ) is a member of the DGK family that metabolises the lipid second messenger diacylglycerol, and contains a nuclear localisation signal (NLS). Kaoru Goto and colleagues have previously predicted that DGKζ is involved in stress responses, and indeed, they have reported that excitotoxic stress induces nucleocytoplasmic translocation of DGKζ in hippocampal neurons, but just how this kinase is involved in stress responses is unclear. Now (p. 2785), the authors examine a pathophysiological link between the cytoplasmic translocation of DGKζ and p53-mediated cytotoxicity following doxorubicin-induced DNA damage. *In vitro* experiments showed that overexpressing wild-type DGKζ (which is predominant in the nucleus) suppressed p53 induction and reduced apoptosis, but these effects were even more pronounced when overexpressing an NLS-deleted mutant DGKζΔNLS, which is predominant in the cytoplasm. Furthermore, the authors show that cytoplasmic DGKζΔNLS induces cytoplasmic localisation of p53 and enhances its degradation through the ubiquitin–proteasome system. Next, the authors provide *in vivo* evidence that p53 protein levels are upregulated in DGKζ-deficient mouse brain under both stressed (excitotoxin-induced seizures) and nonstressed conditions. These results suggest that DGKζ is a novel regulatory factor important for p53 function.



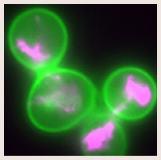
Rab GTPase trio direct MT1-MMP trafficking

Membrane type 1 matrix metalloproteinase (MT1-MMP) is a central regulator of proteolytic cell invasion and, in its surface-associated form, can cleave multiple components of the extracellular matrix. The molecular mechanisms that regulate the trafficking of MT1-MMP to and from the cell surface, therefore, are of great interest, but current knowledge is limited. Rab GTPases are of crucial importance for numerous aspects of intracellular transport, so might they impact on the trafficking of MT1-MMP? On page 2820, Stefan Linder and colleagues investigate. Using primary human macrophages, they show that RAB5A, RAB8A and RAB14, in both their endogenous and overexpressed forms, co-localise with MT1-MMP-positive vesicles. Expression of mutant constructs as well as small interfering RNA-induced knockdown of these three Rab GTPases show them to be crucial for regulating cell-surface exposure of MT1-MMP, contact of MT1-MMP-positive vesicles with podosomes, degradation of extracellular matrix at podosomes in two and three dimensions, as well as three-dimensional proteolytic invasion of macrophages. These data identify RAB5A, RAB8A and RAB14 as important regulators of MT1-MMP trafficking and invasive migration of macrophages. Because MT1-MMP is a crucial proteinase for many invasive cell types, including cancer cells, these findings could have broad translational potential.



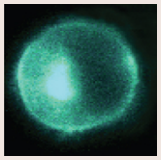
Wnt reaches out to Lin28 and let-7

The canonical Wnt–β-catenin pathway regulates stem cell pluripotency and cell fate decisions during development. This pathway can regulate mammary stem cell self-renewal and differentiation, yet despite being a key regulator of breast cancer stem cells, little is known about the downstream transcriptional cascade that is involved in this process. Here (p. 2877), Bo-An Li and colleagues use a microarray screening assay to identify novel downstream target microRNAs (miRNAs) of the Wnt–β-catenin pathway. Interestingly, the authors identify let-7 miRNAs, which have been shown to have an important function in breast cancer stem cells, as a target. Expression studies showed that whereas mature let-7 miRNAs are suppressed by the Wnt–β-catenin pathway, the primary transcripts are not, suggesting a post-transcriptional regulation of let-7 expression. The authors also identify Lin28, a negative regulator of let-7 biogenesis, as a direct downstream target. Accordingly, loss of function of Lin28 impaired inhibition of let-7 mediated by the Wnt–β-catenin pathway, and the expansion of breast cancer stem cells; moreover, this expansion was blocked by enforced expression of let-7. The authors further show that the Wnt–β-catenin pathway upregulates Lin28 and downregulates let-7 in both human breast cancer tissue samples and mouse tumour models. These findings, showing that the Wnt–β-catenin pathway, Lin28 and let-7 miRNAs connect in a signalling cascade, make an important contribution towards understanding the role of Wnt signalling in breast cancer stem cell biology.



The ties that bind: Num1 anchors mitochondria to cell cortex

As most membrane-bound organelles, such as mitochondria, cannot be generated *de novo* they are inherited upon cell division, and the specific cellular architecture of each eukaryotic cell type must also be re-established in daughter cells. Although there has been much investigation into many aspects of mitochondrial dynamics in recent years, little is known about how their dynamic behaviour is integrated into cellular architecture. On page 2924, Benedikt Westermann and colleagues now investigate the contribution of the cell-cortex-associated protein Num1 to mitochondrial partitioning in yeast. Using live-cell microscopy, the authors find that Num1 is required for mitochondrial attachment to the cell cortex and retention in mother cells. Next, they show that mitochondrial tips co-localise with Num1 punctae in mother cells. Further examination by electron tomography revealed points of contact between the mitochondrial outer membrane and plasma membrane invaginations, and these were not visible in the absence of Num1. The authors therefore propose that Num1 punctae represent mitochondrial cortex anchors in mother cells. Finally, the expression of chimeric plasma membrane tethers rescued mitochondrial fission defects in Δ*num1* and Δ*mdm36* mutants, allowing the authors to conclude that these defects are caused by a defect in cell cortex attachment. These findings assign a key role to Num1 in the attachment of mitochondria to the yeast cell cortex.



Tracking syntaxin conformations

The release of neurotransmitters is mediated by the Ca^{2+}-dependent exocytosis of synaptic vesicles, and various proteins, such as the soluble N-ethylmeleimide-sensitive factor attachment protein receptors (SNAREs), have been implicated in this process. Elucidating the many protein interactions and associated conformational transitions that underlie SNARE protein assembly is essential for understanding exocytosis, but current knowledge is limited. Ilana Lotan and colleagues (p. 2914) set out to investigate dynamic changes in syntaxin 1A (Syx, also known as STX1A), a plasma membrane neuronal Q-SNARE, by constructing a novel fluorescent Syx-based intramolecular reporter probe that can be incorporated within endogenous SNARE complexes. On characterising the functionality of this probe, the authors find that Syx undergoes two distinct secretion-related conformational changes in PC12 cells: a depolarisation-induced partial 'opening' in the absence of Ca^{2+} entry, and a further 'opening' following Ca^{2+} entry. They also show that the Ca^{2+}-dependent opening of Syx depends on positive charges in its juxtamembrane region, probably at the final stage of SNARE complex assembly, as neutralising this region impaired exocytosis. The probe described here is a novel and powerful molecular tool that can be used to test and validate conformational transitions of Syx that are associated with specific interactions, and to gain insight into other new *in vivo* interactions.

From *Development*

Centrosomes and cell fate: a Notch ahead

Asymmetric cell divisions (ACDs) play a crucial role in controlling cell fate and generating cell diversity during development. The centrosome is known to be involved in ACD, and recent studies have shown that centrosomes exhibit dynamic and asymmetric movements that regulate the orientation of the mitotic spindle. In *Development*, Yohanns Bellaiche and co-workers identify a novel type of centrosome movement during cytokinesis. The authors demonstrate that centrosome movements in *Drosophila* sensory organ precursors are regulated by the cell fate determinant Numb – the asymmetric localisation of Numb regulates asymmetric centrosome movements. Moreover, they report, Numb acts through the microtubule-binding protein CRMP rather than through its classical effectors. Finally, the researchers show that CRMP in turn participates in the regulation of endosome dynamics and thus probably the recycling of the Notch receptor Delta. They thereby establish a functional link between centrosome dynamics, Notch signalling and cell fate. These findings suggest a model in which asymmetric centrosome movements participate in differential Notch activation to regulate cell fate.

Jauffred, B., Llense, F., Sommer, B., Wang, Z., Martin, C. and Bellaiche, Y. (2013). Regulation of centrosome movements by Numb and the collapsin response mediator protein during *Drosophila* sensory progenitor asymmetric division. *Development* **140**, 2657-2668.