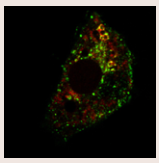
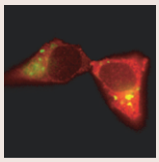
In this issue



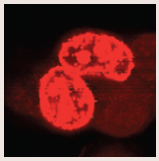
Rab14 guides GLUT4

Insulin promotes the uptake of glucose into adipocytes and muscle cells by effecting the translocation of glucose transporter isoform 4 (GLUT4) from intracellular compartments to the plasma membrane. Previous studies have implicated the Rab GTPase Rab14 in the trafficking of GLUT4 in muscle, but the intracellular site of action of Rab14 on GLUT4 trafficking is poorly understood; moreover, it is unclear whether there is any role for Rab14 in adipocytes. Here (p. 1931), Jeremy Tavaré and colleagues use a series of Rab14 constructs to show that Rab14 is involved in the endocytic trafficking of GLUT4 in adipocytes. Using confocal microscopy, the authors establish that endogenous Rab14 and GLUT4 exhibit partial colocalisation in 3T3-L1 adipocytes. Extensive colocalisation was found, however, when Rab14 was overexpressed in these cells. Interestingly, this colocalisation is restricted to enlarged 'ring-like' vesicular structures that contain markers of early endosomes; these structures were rapidly filled with GLUT4 and transferrin that were undergoing endocytosis from the plasma membrane. A constitutively active Rab14Q70L mutant reduced cell-surface GLUT4 in the basal and insulin-stimulated states, possibly by retaining GLUT4 in an insulin-insensitive early-endosomal compartment. Finally, the authors show that GLUT4 accumulates in early-endosomal compartments in the absence of Rab14. The authors, therefore, propose a role for Rab14 in adipocytes in controlling the trafficking of GLUT4 through the early endosomes to the Golgi complex.



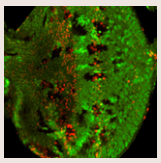
Regulation of TRIM32 by 14-3-3

Members of the tripartite motif (TRIM) protein family, including the E3 ubiquitin ligase TRIM32, have been implicated in a wide range of cellular processes, but how signalling pathways act to regulate these proteins is largely unknown. Previous studies have shown that phosphorylation by protein kinase A (PKA) negatively regulates a particular E3 ubiquitin ligase by recruiting the protein 14-3-3. On page 2014, Tohru Ichimura, Naomi Hachiya and colleagues now show that the 14-3-3 proteins have a wider repertoire in their regulation of E3 ubiquitin ligases. Using a quantitative proteomics screen of 14-3-3 interactions in PKA signalling in a cell line, the authors show that most of these interactions are located downstream of PKA. The screen identified TRIM32, together with six other E3 ubiquitin ligases, which was confirmed to be a 14-3-3-binding partner, with 14-3-3 binding TRIM32 directly when the ligase is phosphorylated by PKA at Ser651. The 14-3-3–TRIM32 interaction was then shown to prevent autoubiquitylation and the formation of TRIM32-containing cytoplasmic bodies – potential autoregulatory mechanisms that control the concentration of soluble free TRIM32. Furthermore, the negative regulation by 14-3-3 is partly a consequence of blocking TRIM32 higher-order self-association but not homodimerization. These results, say the authors, reveal a new connection between ubiquitylation and phosphorylation pathways that stimulates the formation of the 14-3-3 signalling complex and could modulate a number of cellular processes.



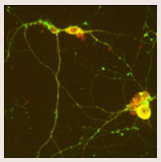
p38 MAPK helps to keep damaged DNA in check

In response to genotoxic stress, DNA damage checkpoints and repair pathways are activated that ensure DNA is transmitted intact. The kinase ataxia telangiectasia and Rad3-related protein (ATR), together with its downstream effector kinases, such as Chk1, is one of the master regulators of the DNA-damage-induced response (DDR). ATR is recruited to sites of DNA damage that are characterised by extended single-stranded (ss)DNA coated with replication protein A (RPA). Recruitment of additional proteins, such as Rad9-containing complexes, are required for activation of ATR and subsequent phosphorylation of downstream effector proteins. However, triggering a checkpoint arrest in the G2 phase of the cell cycle might involve a different mechanism, as the authors have previously shown that Rad9 does not localise to sites of UV-induced damage in G2 cells. To better understand the underlying checkpoint activation mechanism, Daniël Warmerdam, Veronique Smits and colleagues now (p. 1923) analyse the nuclear localisation of DDR factors after UV irradiation of G2 cells. They find that virtually no DDR proteins accumulate at DNA lesions but, interestingly, observe that p38 MAPK is phosphorylated and activated in these cells. However, to fully abrogate G2 arrest after UV damage, the authors needed to inhibit both p38 mitogen-activated protein kinase (MAPK) and the ATR–Chk1 pathway, suggesting that the two pathways have complementing roles in activating G2 arrest in response to UV light.



RB and TSC: a lethal pairing

The inactivation of the tumour suppressor retinoblastoma-associated protein (RB) is considered a hallmark of cancer. In addition to deregulating proliferation, loss of RB function also increases apoptosis, and cells that lose RB become dependent on supporting pathways for their survival. A synthetic lethal approach exploits this dependence by targeting genes that are required for the survival of cells that lack RB function. Here (p. 2004), Wei Du and colleagues build on their previous work in flies, in which they found that the tuberous sclerosis complex 2 (TSC2) fly homologue *gig* induces synergistic cell death when *rbf* (the fly homologue of RB1) is lost; now, they investigate the mechanisms underlying this synthetic lethality. They show that the deletion of *rbf* together with either *gig* or *tsc1* leads to an aberrant entry of mutant fly eye disc cells into S phase and increased levels of DNA double-strand breaks that contribute to cell death. In addition, they find that loss of *rbf* or *tsc1* induces energy stress and sensitises cells to ATP depletion. Furthermore, *rbf* or *tsc1* mutants depend on the metabolic regulator liver kinase B1 (LKB1) for their survival, as deletion of *LKB1* results in increased cell death of *rbf*/*tsc1* mutants. Taken together, these data point to multiple cellular stresses being induced and contributing to synthetic lethal interactions when RB and TSC1 or TSC2 are inactivated, which could be exploited for the treatment of tumours that have lost RB.



Kv3 channels in the driver's seat

The requirement for intracellular transport of proteins and organelles is fulfilled by molecular motors such as kinesins, which use the energy of ATP hydrolysis to move their cargo along microtubules. But can cargoes themselves regulate motor function? Chen Gu and colleagues (p. 2027) now examine this question. One of the main anterograde motors that operates in axons is conventional kinesin-1, which comprises a heavy chain (KIF5) dimer and two kinesin light chains (KLCs) that bind to the C-termini of the dimer. The axonal Kv channel Kv3.1 was the first ion channel shown to directly bind to KIF5, so the authors asked how Kv3 voltage-gated K^+ channels might regulate KIF5 function. Using biochemical assays, they report high-affinity and multimeric binding between the Kv3.1 T1 domain and the KIF5B tail domains, and show that Kv3.1 T1 competes with the KIF5B motor domain and microtubules – but not with KLC1 – for binding to the KIF5B tail. The authors next examine the roles of four known KIF5-binding proteins, including Kv3.1 and KLC1, in regulating KIF5 distribution and motility. They find that, although other binding proteins can activate the KIF5 motor, only Kv3.1 can cluster KIF5; deletion of Kv3.1 channels was shown to reduce the number of the KIF5 clusters in mouse cerebellar neurons. These results show that a Kv3.1 channel complex can bind to multiple KIF5 motors and activate them in clusters. This unique binding leads to the cargo-mediated regulation of motor function as well as the specificity of Kv3 channel transport.

From *Development*
miR-203 drives progenitor cell differentiation

MicroRNAs are crucial for the regulation of gene expression in a vast array of processes. In the skin, miR-203 has been shown to be crucial for the proper differentiation of the interfollicular progenitor cells, although the specific mechanism of this has remained elusive. In *Development*, Rui Yi and colleagues investigate the precise timing of miR-203 activation during epidermal differentiation. They show that miR-203 is transcriptionally activated in the differentiating progeny of interfollicular progenitor cells upon asymmetric cell division. Using keratinocytes derived from miR-203-inducible mice, the authors find that miR-203 functions to promote an immediate exit from the cell cycle, leading to a complete loss of self-renewal after just 72 hours. They further identify a multitude of new miR-203 targets *in vivo*, and demonstrate that co-repression by miR-203 of many of these, including *Skp2*, *Msi2* and *p63*, is necessary for the function of miR-203 in inhibiting self-renewal. These data provide new insights into the widespread role of miR-203 in differentiating interfollicular progenitor cells in the skin.

Jackson, S. J., Zhang, Z., Feng, D., Flagg, M., O'Loughlin, E., Wang, D., Stokes, N., Fuchs, E. and Yi, R. (2013). Rapid and widespread suppression of self-renewal by microRNA-203 during epidermal differentiation. *Development* **140**, 1882-1891.