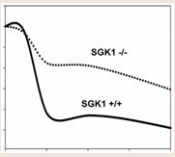
In this issue

By Jane Bradbury

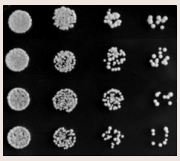
Promoting synaptic vesicle recycling

Synaptic vesicles must be efficiently recycled after neurotransmitter release to ensure sustained neural transmission. This recycling process occurs mainly through clathrin-mediated endocytosis. Jenny Hinshaw, Oleg Shupliakov and colleagues (p. 133) have been investigating the role of the interaction between the accessory protein endophilin and the GTPase dynamin in synaptic vesicle recycling. Now, using immuno-electron microscopy, the authors show that, during synaptic activity, endophilin recruits to the sites of endocytosis from the vesicle pool and colocalizes with dynamin in spirals at the neck of clathrin-coated pits (CCPs) in lamprey synapses. Blocking the interaction of the endophilin SH3 domain with dynamin, they report, reduces dynamin accumulation at the neck and inhibits the vesicle budding reaction. In vitro experiments using recombinant proteins and lipid templates confirm these observations, and show that, in the presence of endophilin, dynamin recruitment to lipid templates is increased and the diameter of the resultant protein-decorated lipid tubes is smaller than those formed in the absence of endophilin. The authors propose, therefore, that endophilin and dynamin form a 'pre-fission complex' that promotes dynamin-mediated budding of newly formed synaptic vesicles during neurotransmitter release.



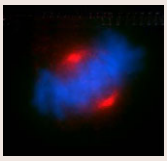
SGK1 takes signalling down a Notch

The Notch1 signalling pathway controls cell survival, death and differentiation, and its activation might be involved in tumour development. Ligand binding by the transmembrane Notch1 protein leads to release of the Notch1 intracellular domain (Notch1-IC), which moves into the nucleus where it activates Notch1 target gene transcription. Regulation of Notch1 signalling occurs at multiple levels, including through its degradation in the nucleus by the proteosome system after ubiquitylation by the E3 ligase Fbw7. Here, Hee-Sae Park and colleagues (p. 100) report that the serum- and glucocorticoid-inducible protein kinase SGK1 controls Notch1 signalling by modulating Notch1-IC stability through Fbw7 phosphorylation. The authors show that the protein level and transcriptional activity of Notch1-IC are higher in SGK1-deficient cells than in wild-type cells. Notch1-IC, Fbw7 and SGK1 form a trimeric complex, they report, and SGK1 enhances the degradation of Notch1-IC through an Fbw7-dependent proteosomal pathway. Moreover, activated SGK1 phosphorylates Fbw7, which induces Notch1-IC ubiquitylation. These new insights into the regulation of Notch1 signalling should improve our understanding of the pathogenesis of Notch1-related cancers such as T-ALL leukaemia, a cancer in which Notch1 and Fbw7 are often mutated.



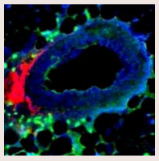
Reb1 controls G1 starvation arrest

When eukaryotic cells reach the G1–S transition, they either commit to a new round of cell division or undergo growth arrest. In the fission yeast *Schizosaccharomyces pombe*, nutritional stress induces G1 arrest and the cells sexually differentiate to form spores that germinate when conditions improve. The *ste9*$^{+}$ gene is required for these responses, but little is known about how *ste9*$^{+}$ transcription is regulated in response to nutritional stress. Here, Pablo Hernández and colleagues (p. 25) reveal that the rDNA-binding protein Reb1 upregulates *ste9*$^{+}$ transcription in response to nitrogen starvation. Reb1 is a conserved Myb-type DNA-binding protein that binds to two specific sites near the 3′ end of rRNA genes (rDNA), where it helps to terminate transcription driven by RNA polymerase 1 and arrests replication forks. The authors show that Reb1 binds in vivo and in vitro to a similar DNA sequence at the *ste9*$^{+}$ promoter, and that this binding is required for *ste9*$^{+}$ transcription and G1 arrest in response to nitrogen starvation. Consistent with these findings, the mating efficiency of Reb1-deficient cells is greatly reduced. Thus, the authors suggest, Reb1 links rDNA metabolism to cell-cycle control in response to nutritional stress in fission yeast and possibly other eukaryotic cells.



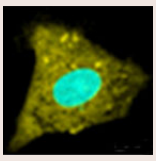
TPX2 regulates Aurora-A stability

The Aurora-A serine/threonine kinase is an important regulator of cell division that acts in spindle assembly and function, and is often overexpressed in tumour cells. It is most abundant during the G2 and M phases of the cell cycle, and is downregulated through APC/C–Cdh1-dependent proteosome-regulated proteolysis at the end of mitosis. The microtubule-binding protein TPX2 controls both the activity and the localisation of Aurora-A, but now Giulia Guarguaglini and colleagues (p.113) report that TPX2 also regulates the stability of Aurora-A in human cells. The authors show that RNAi silencing of TPX2 decreases Aurora-A levels in G2 and prometaphase cells, whereas overexpression of the Aurora-A-binding region of TPX2 impairs the degradation of Aurora-A in telophase cells. Moreover, Aurora-A degradation in TPX2-silenced cells requires proteosome activity, and the reintroduction of full-length TPX2 or the Aurora-A-binding region of TPX2 restores Aurora-A levels in TPX2-silenced prometaphase cells. Together, these findings reveal a new role for TPX2 in protecting Aurora-A from degradation, underscore the importance of the regulated stability of Aurora-A in mitotic control, and provide new insights into the origin of Aurora-A deregulation in tumour cells.



Nuclear actin halts epithelial growth

The establishment and maintenance of differentiation is tightly coordinated with growth arrest, which is controlled by cues from the microenvironment. Signals from the extracellular matrix protein laminin 111 (LN1), for example, decrease transcription and suppress mammary epithelial growth in culture and, although an LN1-rich basement membrane envelops mammary ducts in vivo, LN1 is absent around the proliferating end buds of these otherwise quiescent structures. But how do LN1 and other cues induce quiescence? On page 123, Virginia Spencer, Mina Bissell and colleagues report that depletion of nuclear β-actin mediates quiescence in mouse mammary epithelial cells. Serum deprivation or LN1 addition, the authors show, decreases nuclear β-actin levels and reduces transcription and DNA synthesis in mammary epithelial cells in culture. Constitutive overexpression of nuclear β-actin reverses these effects and prevents the cells from becoming quiescent in the presence of LN1. Importantly, high levels of β-actin and transcription are localised to regions of growth in developing mammary end buds where there is little or no LN1. Together, these results identify LN1 as a physiological regulator of nuclear β-actin levels in mammary epithelial cells and implicate the loss of β-actin as a key mediator of epithelial cell quiescence.



RCAN1 role switch explained

The calcineurin–NFAT (nuclear factor of activated T cells) signalling pathway controls many physiological processes, including T-cell activation and cardiac cell growth. Regulator of calcineurin 1 (RCAN1) is a conserved regulator of this signalling network, but its functional role is hotly debated because, although it inhibits calcineurin–NFAT signalling in some experimental systems, it facilitates signalling in others. To resolve this debate, Kwang-Hyun Cho, Won Do Heo and colleagues (p. 82) have used a new approach in which they combine in silico simulations and single-cell live imaging experimentation. The authors' approach reveals a hidden incoherent regulation switch that is formed by cross-talk signals mediated through extracellular signal-regulated protein kinase and glycogen synthase kinase-3. This switch, they report, diverts negative regulation by RCAN1 to positive regulation, which leads to a dose-dependent biphasic response. Specifically, RCAN1 functions as an inhibitor when its levels are low, but as a facilitator when its levels are high. The authors conclude that the discovery of this incoherent regulation switch, which can induce apparently opposite responses depending on conditions, provides an explanation for the different roles of RCAN1 in calcineurin–NFAT signalling that have been suggested in previous studies.