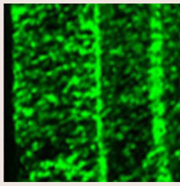
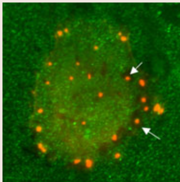
In this issue



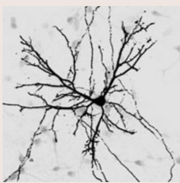
SPIRAL2 prevents a pause

Microtubules (MTs) polymerise and depolymerise dynamically, and microtubule ends exist in one of three states: growth, pause and shrinkage. Although long pauses are frequently observed in animal cells, MTs rarely remain in the pause state for longer than 30 seconds in plant cells in interphase – but what controls how long MTs spend pausing? On page 2372, Takashi Hashimoto and colleagues investigate the role of the *Arabidopsis* MT-associated protein SPIRAL2 (SPR2) and its close homologue SPIRAL2-Like (SP2L) in MT dynamics. Plants that harbour *spr2* mutations are known to display twisted (right-handed helical) growth, and the authors show that overexpressing SP2L rescues this phenotype. Moreover, MTs in mutant plants spend less time in the pause state when SPR2 is overexpressed. The authors also show that, in plants, GFP-tagged SPR2 and SP2L localise partially to the plus ends of cortical MTs and, in an in vitro assay, both proteins (expressed as trigger-factor fusion proteins) increase the rate of MT polymerisation. Crucially, SPR2 and SP2L both minimise the frequency of growth-to-pause and shrinkage-to-pause transitions in vitro, and decrease the time that MT plus ends spend in the pause state. The authors conclude that SPR2 and SP2L promote MT growth and shrinkage by suppressing the pause state.



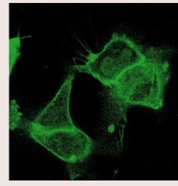
AFAP-110 – long live podosomes!

Podosomes – adhesive structures that form on the ventral surface of motile cells and actively degrade the extracellular matrix – are rich in filamentous actin. The actin-crosslinking protein AFAP-110, which promotes podosome formation, is known to be phosphorylated by PKCα, but the importance of AFAP-110 phosphorylation for podosome formation and stability is unclear. Using vascular smooth muscle (A7r5) cells, which form podosomes when treated with phorbol ester (PE), Daniel Flynn and colleagues (p. 2394) now show that AFAP-110 is phosphorylated at serine 277 (S277) in response to PE. Having generated a phosphospecific antibody against this site, the authors next show that the PH1 domain of AFAP-110 – which mediates its interactions with several PKC isoforms – is required for its phosphorylation; moreover, PKCα phosphorylates AFAP-110 at S277 in PE-stimulated COS-7 cells. In A7r5 cells, a phosphorylation-defective mutant of AFAP-110 (AFAP-110^{S277A}) and S277-phosphorylated AFAP-110 both localise to podosomes. Intriguingly, however, podosomes in AFAP-110^{S277A}-expressing cells are longer-lived than those in cells that express the wild-type protein. Thus, the PKCα-dependent phosphorylation of AFAP-110 appears to regulate podosome lifespan.



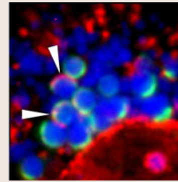
TRPC6: channelling dendrite growth

In the growing brain, dendrites extend from the cell body of neurons – a process that is crucial for the development of neuronal circuits. The influx of Ca^{2+} (via NMDA receptors and voltage-sensitive Ca^{2+} channels) is important for dendritic growth, and Yizheng Wang and colleagues (p. 2301) now explore the role of TRPC6, a Ca^{2+}-permeable nonselective cation channel, in this process. The authors show that the expression of TRPC6 in the rat hippocampus peaks between postnatal days 7 and 14 (a period of maximal dendritic outgrowth). In hippocampal cultures, they show, the overexpression of TRPC6 promotes dendritic growth and branching, both of which are impaired when TRPC6 is knocked down. Inhibiting Ca^{2+} influx blocks TRPC6-induced dendritic growth, and expressing dominant-negative mutants of CaMKIV or CREB has a similar effect; conversely, stimulating TRPC-dependent Ca^{2+} influx or expressing a constitutively active form of CamKIV both stimulate dendrite morphogenesis. Importantly, hippocampal dendrites grow longer and branch more in TRPC6 transgenic mice. TRPC6, therefore, promotes dendritic growth via the CamKIV-CREB pathway, and might have a crucial role in dendritic growth in vivo.



PKCγ opens up in ataxia

The neurodegenerative disorder spinocerebellar ataxia type 14 (SCA14) is caused by mutations in PKCγ, which is the neuron-specific isoform of the PKC family. Twenty-three SCA14-associated mutations of PKCγ have been reported; most of these fall within the C1B regulatory subdomain, but their effect on PKCγ function is not well understood. On page 2339, Eric Reits and colleagues analyse the activity of three SCA14-associated mutants of PKCγ, each of which harbours a point mutation in the C1B subdomain. The authors show that GFP-tagged versions of all three mutant proteins localise to the cytoplasm in HeLa cells, as wild-type PKCγ does. However, when cells are treated with phorbol ester (which activates PKCγ by binding to the C1 domain), the three mutants redistribute to the plasma membrane more rapidly than the wild-type protein. In addition, the authors show that the efficiency of intramolecular FRET is reduced in the three PKCγ point mutants, which might indicate that they adopt a more open conformation in which the C1 domain is more accessible to phorbol ester. Notably, all three mutants have reduced kinase activity, and cells that express the mutant proteins exhibit reduced phosphorylation and nuclear accumulation of ERK2. These results shed light on the role of PKCγ in SCA14 pathogenesis.



A fatty route for ApoB processing

Apolipoprotein B-100 (ApoB) is the major protein component of very-low-density lipoprotein (VLDL), and its synthesis and degradation in hepatocytes is highly regulated. It is known that ApoB, which is co-translationally lipidated, undergoes ER-associated degradation (ERAD) by the proteasome when lipidation is perturbed. Mechanisms to degrade lipidated ApoB are also thought to exist, but these have been poorly understood. Toyoshi Fujimoto and colleagues (p. 2415) have previously demonstrated that ubiquitylated ApoB can accumulate in a crescent-shaped area that surrounds lipid droplets (LDs) in hepatocytes; they now show that the ApoB within the crescent is lipidated and so is likely to be processed by a mechanism other than ERAD. Using immunofluorescence microscopy, the authors show that the ApoB crescent colocalises with several ER markers; moreover, they use immunoelectron microscopy to show that the ApoB crescent is an ER subcompartment that is fused to an LD. Crescent formation is suppressed by the expression of proteins that promote LD formation and lipidated ApoB binds tightly to the LD surface, which suggests that the degradation of lipidated ApoB is linked to LD biogenesis. These results emphasise the complexity of ApoB regulation.

Development in press

Myosin IIB: a force for morphogenesis

Two tissue movements – convergence and extension – are essential for axial morphogenesis in vertebrate and invertebrate embryos. The intercalation of cells underlies both forms of movement, but what generates the tensile forces that drive intercalation? In a paper published in *Development*, Paul Skoglund and colleagues report that in *Xenopus laevis* embryos, convergence and extension at gastrulation require a myosin-IIB-dependent cortical actin network. Using morpholino knockdown, the authors show that myosin IIB (a cytoskeletal myosin that crosslinks actin filaments and acts as a molecular motor) is needed during gastrulation to maintain the cortical actin cytoskeleton. This network is polarized relative to the embryonic axis, the researchers report, and cyclically lengthens and shortens during gastrulation. Depletion of myosin IIB also results in the loss of the polarized protrusive activity that is usually seen in intercalating cells, the loss of cell-cell and cell-matrix adhesion, and failure of blastopore closure. Together, these findings reveal how a motor protein can generate the tensile forces that drive embryonic morphogenesis.

Skoglund, P., Rolo, A., Chen, X., Gumbiner, B. M. and Keller, R. (2008). Convergence and extension at gastrulation require a myosin IIB-dependent cortical actin network. *Development* **135**, 2435-2445.