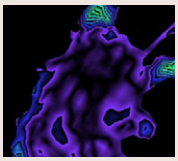
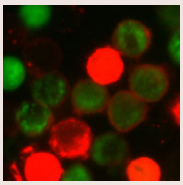
In this issue



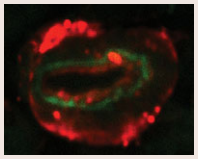
Capn4 forces the issue

When cells migrate across a substrate, they dynamically form and disassemble focal adhesions and, through these, exert traction forces on the substrate and receive physical signals from the environment. The calpains – which are Ca^{2+}-dependent cysteine proteases – are thought to regulate focal-adhesion dynamics, but do they have a role in traction-force regulation and mechanosensing? To address this question, Karen Beningo and colleagues (p. 3581) analyse the behaviour of fibroblasts that lack *Capn4* (which encodes the small regulatory subunit that is common to the ubiquitous calpains 1 and 2) on flexible polyacrylamide substrates. The authors show that *Capn4*$^{-/-}$ cells generate weaker and less-dynamic traction forces than control cells, and adhere less strongly to the substrate. Surprisingly, however, knocking down *Capn1* or *Capn2* (the catalytic subunits of calpain 1 and 2, respectively) does not cause defects in force generation or adhesion. The authors next show that *Capn4*$^{-/-}$ cells and *Capn1*- and *Capn2*-knockdown cells are all deficient in mechanosensing; they fail to respond to pushing or pulling of the substrate, or to the engagement of dorsal integrins. The *Capn4* gene product might, the authors propose, have as-yet-unknown functions that go beyond its role as a regulatory subunit.



How to grow a fusion pore

Fusion between neighbouring cells, which is a key process in the development of muscle, bone and trophoblasts, proceeds via the formation and expansion of intercellular pores. Fusion-pore initiation has been well studied, but less is known about the properties of expanded pores or the mechanism that drives enlargement. Now, Leonid Chernomordik and colleagues (p. 3619) visualise pore expansion in insect Sf9 cells that express the viral fusogen gp64. The authors show that, in this system, pore expansion occurs without the loss of membrane material in the contact zone, which suggests that membrane is displaced towards the periphery of the contact zone as pores enlarge. Moreover, cells must be metabolically active for pore expansion to occur, but plasma-membrane tension is not required. It has previously been proposed that the actin cytoskeleton drives pore expansion; the authors show, however, that pore growth is accompanied by a local disassembly of cortical actin. In addition, pore expansion is promoted and inhibited by actin-depolymerising and -polymerising agents, respectively. The authors propose an alternative model in which pore expansion is driven by factors such as membrane-bending proteins, which minimise the bending energy of the pore-rim membrane. Their results shed light on the mechanism of cell fusion and syncytium formation.

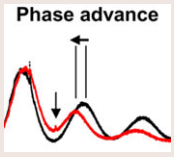


Ins(1,4,5)P_3 goes both ways

Within arterioles, endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) communicate via gap junctions at the myoendothelial junction (MEJ). This functional coordination is important for the control of blood flow, but little is known about how heterocellular communication is controlled. For instance, it is known that the transfer of the second messenger Ins(1,4,5)P_3 from VSMCs modulates Ca^{2+} stores in ECs, whereas Ins(1,4,5)P_3 that originates in ECs does not affect VSMC Ca^{2+} stores – but how is this directionality maintained? On page 3664, Brant Isakson addresses this question. Using an EC-VSMC co-culture system, the author shows that intercellular Ins(1,4,5)P_3 signalling is not affected by modulating the connexin composition of gap junctions. Notably, however, the Ins(1,4,5)P_3 receptor Ins(1,4,5)P_3-R1 is selectively localised to the EC side of the MEJ, and knocking down the expression of the receptor abolishes the response to Ins(1,4,5)P_3 in ECs. Moreover, VSMCs that are loaded with a phosphatase inhibitor respond to EC-generated Ins(1,4,5)P_3. The author concludes that Ins(1,4,5)P_3 transport at the MEJ is bidirectional but that, because of the position of the Ins(1,4,5)P_3 receptor, only ECs mount a response before Ins(1,4,5)P_3 is degraded. The MEJ might, therefore, act as a cellular signalling domain within the vasculature.

Cbl – taking on a phosphatase

Receptor tyrosine kinases (RTKs) trigger several cell signalling pathways (particularly those that regulate cell proliferation, motility and survival), so their activity must be tightly regulated. In metazoa, Cbl proteins downregulate RTKs by ubiquitinating them and targeting them for degradation, but it is not known whether they have other roles in less-complex organisms. On page 3524, Jeffrey Williams and colleagues identify CblA, the first non-metazoan Cbl protein, in the facultative multicellular organism *Dictyostelium discoideum* and characterise its function. In a *cblA*-null strain of *Dictyostelium*, they show, multicellular slugs fragment along their length and fail to form a normal basal disc. Notably, this phenotype is echoed in *Dictyostelium* strains that are deficient in signalling by DIF-1 (which normally signals through STATc to promote the differentation of *Dictyostelium* into stalk and pre-stalk cells). The authors show that DIF-inducible tyrosine phosphorylation of STATc is downregulated in *cblA*-null cells, whereas the protein tyrosine phosphatase PTP3B is present at a higher concentration. The authors conclude that, similar to DIF-1, CblA activates STATc by acting as a negative regulator of PTP3B. Thus, metazoan Cbl proteins regulate tyrosine kinases, but the *Dictyostelium* Cbl homologue acts on a tyrosine phosphatase.



REV-ERBα – time for a change

Several sleep disorders are caused by the disruption of the circadian clock, which is underpinned by the fluctuating activity of transcription factors that periodically inhibit their own expression. The nuclear receptor REV-ERBα, which is activated by heme binding, stabilises the clock by repressing *Bmal1* transcription – but could REV-ERBα be a good pharmacological target for circadian-rhythm disorders? On page 3629, Andrew Loudon and colleagues propose that it could. The authors use a FRET-based in vitro screen to identify a compound that promotes the formation of a complex between REV-ERBα and the nuclear receptor co-repressor (NCoR). In fibroblasts expressing *Bmal1* or *Rev-erba*, they show, the expression level of both genes oscillates. Importantly, the addition of the REV-ERBα ligand causes phase shifts in BMAL1 and PER2 activity that change their directionality according to the phase of the circadian clock. Moreover, phase-dependent phase resetting also occurs in lung slices treated with the REV-ERBα ligand. The authors propose that the binding of REV-ERBα to endogenous ligands, such as heme, might be a mechanism of clock resetting; moreover, pharmacological modulation of REV-ERBα might offer a novel approach to the treatment of circadian-rhythm disorders.

Development in press

Shh…autonomous axon guidance in progress

The motility of many cell types is controlled during development by Shh secreted by adjacent tissues (non-autonomous signalling). In a paper published in *Development*, Cristina Sánchez-Camacho and Paola Bovolenta unexpectedly uncover a role for autonomous Shh signalling in the growth and guidance of mouse retinal ganglion cell (RGC) axons. In mammals, the axons of contralateral RGCs (C-RGCs) cross the developing brain's midline (a source of Shh signals), whereas ipsilateral RGC (I-RGC) axons do not. The researchers first show that mouse C-RGCs express Shh, whereas I-RGCs do not. Next, they use antibodies to block Shh activity in vivo and show that midline-derived Shh funnels C-RGC axons to the contralateral side of the brain. Finally, by blocking Shh signal transduction in the RGCs themselves, they show that the outgrowth of C-RGCs is impaired well before they reach the midline, which indicates that the axons of these neurons require autonomously produced Shh for correct extension. Thus, the researchers conclude, Shh signalling influences growth-cone behaviour both autonomously and non-autonomously.

Sánchez-Camacho, C. and Bovolenta, P. (2008). Autonomous and non-autonomous Shh signalling mediate the in vivo growth and guidance of mouse retinal ganglion cell axons. *Development* **135**, 3531-3541.