In This Issue

Sticking to the nuclear envelope

A mutation in torsinA, an AAA ATPase protein, causes DYT1 dystonia in humans. On page 855, Goodchild and Dauer identify two protein binding partners of torsinA, one that localizes to the ER membrane and one that spans the inner membrane of the nuclear envelope. The team hypothesizes that DYT1 is a nuclear envelope disease, and that identification of such protein complexes will provide a mechanistic probe into a poorly characterized region of the cell.

In wild-type cells, the majority of torsinA protein localizes to the lumen of the ER, while a small proportion associates with the nuclear envelope. In cells carrying the disease-associated mutation, the proportion of torsinA at the envelope increases. Furthermore, a torsinA mutant that cannot hydrolyze ATP, and therefore becomes trapped on its target protein, selectively localizes to the nuclear periphery.

To find out what torsinA binds at the nuclear envelope, the team screened a series of nuclear envelope proteins in cells expressing GFP-labeled torsinA. Overexpression of LAP1, a lamin-associated protein, increased the proportion of perinuclear torsinA, and further analysis demonstrates an association between torsinA and the lumenal domain of LAP1. Based on sequence comparisons, Goodchild and Dauer identified a second torsinA binding protein, LULL1, an ER protein that has a single transmembrane domain and a long lumenal domain similar to that of LAP1. The torsinA-LULL1 association is also dependent on the lumenal region of LULL1.

The association between lamins, LAP1, and torsinA suggests that torsinA dysfunction may cause problems typical of laminopathies, including improper chromatin organization, transcriptional problems, or overall changes in nuclear architecture. Because the function of AAA ATPase proteins is known—their energy of ATP hydrolysis to disassemble multiprotein complexes or unfold individual proteins—the researchers think they can use the complex as a launching point to learn about the function of the nuclear envelope. JCB

Exocytosis in small steps

Neuroendocrine cells have two modes of exocytosis. In one mode, the vesicle fuses to the target membrane, and in the other—referred to as kiss-and-run—the vesicle touches the membrane and creates a small temporary pore. On page 929, Richards et al. show that the small synaptic vesicles in hippocampal neurons also use both mechanisms. The two modes of exocytosis differ in their kinetics and amplitude of the release, and may generate different postsynaptic responses.

To determine whether neurons have two pathways for exocytosis, the team loaded only a fraction of the synaptic vesicles with a fluorescent dye, a trick that allows them to resolve the activity of individual vesicles. They saw two types of exocytic events occurring: fast large releases of dye and slow small ones. The researchers conclude that the fast release events occur when a vesicle fuses with the plasma membrane, dumping its total dye content at a rate limited only by diffusion. On the other hand, the small release events appear to occur when a vesicle forms only a small pore in the plasma membrane. The speed of dye release in these small events is the same as that which occurs if the researchers use a toxin to poke a 1–2-nm hole in the membrane of a synthetic vesicle.

Richards et al. hypothesize that these different types of synaptic release may have a functional role in neural signaling. For example, it is known that when the vesicles of these hippocampal neurons fuse with the membrane and dump their whole load of neurotransmitter into the synaptic cleft, they excite the postsynaptic neuron. It could be that the dribbling release of neurotransmitter that occurs in the slow small exocytic events instead desensitizes the postsynaptic neuron. The data to confirm this are lacking, but several groups are now trying to figure out how to combine the electrophysiology and fluorescence experiments to find out. JCB
Beating under pressure

Ciliary beat frequencies in the respiratory and reproductive tracts are relatively constant despite changing viscosities of the mucus and fluid on their surface. Andrade et al. show that the TRPV4 calcium channel is necessary for cilia on hamster oviductal cells to maintain their beat frequency in response to increased viscosity and that the response is dependent on phospholipase A₂ activity (page 869).

Using patch-clamp electrical recordings on freshly dissociated cells, the team saw that increasing the viscosity of the medium on the cell surface caused an influx of calcium. The influx was blocked by the addition of an antibody against the TRPV4 channel. Furthermore, activation of the TRPV4 channel with a drug also induced a calcium influx and increased the ciliary beat frequency. Significantly, inhibition of phospholipase A₂ blocked ciliary beat frequency changes in response to increased viscosity. Drug-induced activation of TRPV4 was not, however, affected by inhibition of phospholipase A₂.

The team concludes that the TRPV4 channel is not the mechanosensor itself, but that something upstream in the phospholipase A₂ pathway detects a compression force of the medium on the cell, triggering activation of phospholipase A₂ and TRPV4. Just what the mechanosensor is remains a key question. JCB

Raf-1 regulates migration

Raf-1 signaling is known to be important for proliferation, differentiation, and survival. Now, Ehrenreiter et al. report that it is required for cell migration and wound healing (page 955). Unlike other Raf-1 functions, this one doesn’t require Raf-1 kinase activity, a theme the researchers think will be recurrent in future Raf-1 biology.

When Raf-1 is knocked out in the epidermis of animals carrying conditional Raf-1 alleles, the epidermal structure remains normal. However, wounds heal slowly in the absence of Raf-1, despite normal cell proliferation in the epidermis. In culture, Raf-1–deficient cells do not migrate normally and appear rounded and contracted with dense cortical actin structures.

This suggests that either Rho or its downstream effector, Rok-α kinase, is hyperactive. Biochemical experiments showed that Raf-1 was required for Rok-α inhibition in keratinocytes and fibroblasts, and that inhibition of Rok-α activity overcame Raf-1 deficiency. Kinase-dead Raf-1 mutations also rescue the defect, indicating that Raf-1 regulates Rok-α via protein–protein interaction rather than by modifying the target.

Raf-1 is a weak kinase, even when phosphorylating its favored targets such as MEK, so a kinase-independent function is novel but not entirely unexpected. Ehrenreiter et al. think Raf-1 regulates Rok-α by targeting it to proper compartments in the cell and predict that similar functions will be found in other Raf-1 pathways. They are now mapping the Raf-1 domains responsible for Rok-α regulation. JCB

Decondensation at the fork

On page 875, Alexandrow and Hamlin report that decondensation at the replication fork appears to be triggered by phosphorylation of histone H1. The phosphorylation precedes incorporation of BrdU, and is dependent on Cdk2, perhaps explaining why Cdk2 is required for S-phase progression.

To find out what happens at the fork, the team used a molecular tethering system originally designed to study higher-order chromatin remodeling and transcription (Li et al., 1998). The CHO cells used in this report contain multiple tandem copies of the lac operator stably integrated into the chromosomes. When a replication-related protein is fused to LacI protein and transfected into such cells, the replication protein is targeted to the tandem repeats because LacI binds to the lac operator.

Fusion of Cdc45, a protein associated with the replication fork itself, causes widespread decondensation of the chromatin in the system, but Cdc6, a protein required for replication initiation, does not. Moreover, Cdc45 induces phosphorylation of histone H1 by recruiting Cdk2, a protein required for entry into S phase as well as progression through it.

The team did not find evidence of acetylation or methylation changes on the core histones, which have been detected when the same experimental system was used to study transcription-induced chromatin changes. Either such changes are transient during replication—and therefore under the radar of the current experiments—or the mechanisms that underlie chromatin remodeling during replication and transcription differ. JCB

Cdc45 targeted to lacI repeats (red) leads to decondensation of the chromatin.