Beating under pressure

Cilia 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

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 group 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

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