Controlling division planes

In mammals, cells that divide with their spindle axis parallel to the surface of the epithelial sheet produce symmetric daughters, whereas a spindle perpendicular to the sheet produces asymmetric progeny. Poggi et al. (page 991) report that environmental factors can affect spindle orientation in the zebrafish retina and thus alter cell fate.

Retinal ganglion cell (RGC) formation is dependent on Ath5. Using time-lapse microscopy, the team found that as cells began to express Ath5 they migrated toward the outer surface of the cup-shaped retina. Once there, the cells divided one time, with the plane of their spindle oriented along the radial axis of the retina (i.e., perpendicular to the sheet). One daughter cell migrated back toward the center of the retina and became an RGC, while the other remained near the outer surface and differentiated into a different cell type.

When GFP-marked, wild-type cells were transplanted to embryos lacking Ath5, the cell division pattern changed. As in wild-type retinas, the cells moved to the periphery upon Ath5 expression and usually divided once. However, instead of orienting their spindle along the radial axis, the spindle was often oriented along the circumferential axis, and both daughters became RGCs.

It was known previously that the presence of a large number of RGCs decreased the likelihood that more RGCs would be formed, but how that happened was not clear. Poggi et al. hypothesize that a factor secreted by the RGCs influences the orientation of the spindle, and therefore the lineage pattern of Ath5-expressing progenitors. Thus it appears that daughter cell fate depends both on lineage and environmental cues.

A hint as to how spindle orientation can be altered may come from a recent paper (Igman et al. 2005. Neuron. 48:539–545). They found that RNAi knockdown of a mammalian homologue of Inscuteable, a key regulator of spindle orientation in flies, results in the misorientation of spindles in neuroepithelia of rat retinal explants. The spindle changes result in cell fate changes reminiscent of those reported by Poggi et al. JCB

A gas pedal for replication?

PCNA is monoubiquitylated during replication of undamaged DNA in frog eggs, report Leach and Michael on page 947, and preventing the modification slows fork progression. Thus, cells may have a mechanism for modulating the speed of replication, an idea that runs counter to long-standing dogma.

Homotrimers of unmodified PCNA clamp onto DNA during replication and increase the processivity of polymerase δ, which is the workhorse of normal replication. In response to DNA damage, PCNA is monoubiquitylated in mammalian cells and mono- and polyubiquitylated in yeast. The modified protein assists in recruitment of polymerase η to sites of damage. Leach and Michael found that Xenopus PCNA was monoubiquitylated or sumoylated during replication of undamaged DNA in egg extracts. The protein was polyubiquitylated in the presence of damaged DNA. Sumoylation was not required for replication, though inhibition of PCNA ubiquitylation slowed fork progression. Replication forks were not abandoned more frequently in the absence of ubiquitylation than in control cells.

When the polymerase slowed, the helicase also slowed, which suggests that the cells have a system in place to compensate for changes in the rate of fork movement.

The differences found in PCNA modifications in frog and mammalian cells could indicate that such regulatory changes are not conserved between species. Alternatively, the observations could reflect differences between embryonic DNA replication, which was studied here, and somatic cell replication, which was studied previously in mammalian cells.

Until now, it was thought that an increase in the number of forks fired was sufficient to account for the greater replication rate in embryonic cells relative to somatic cells. But the regulation observed here is one additional candidate for determining this difference in speed. JCB
Moving in on desmosomes

Cells rely on desmosomes to resist mechanical forces that can pull tissues apart. But how the components of these intercellular junctions arrive at the cell cortex and form the structure has been a matter of debate, with both membrane-bound and non-membrane-bound precursors implicated. On page 1045, Godsel et al. report that non-membrane-bound cytoplasmic desmoplakin motors outwards to form the structures.

The authors followed GFP-labeled desmoplakin, a key component of desmosomes, just before and after cells made contact with their neighbors. Prior to contact, GFP-desmoplakin was seen in cytoplasmic particles. Immediately after contact, the protein began to accumulate on the inner surface of the membrane at the site of contact.

Subsequently, more desmoplakin particles appeared in the cytoplasm, colocalizing with plakophilin-2 and often with intermediate filaments. These particles moved toward the site of contact, adding to the nascent structure. Inhibiting desmoplakin association with intermediate filaments or disrupting the actin cytoskeleton slowed desmosome formation.

A smaller subset of desmoplakin particles colocalized with membrane-bound Dsc2, a desmosomal cadherin. These particles were typically larger than those lacking Dsc2 and tended to be perinuclear. Because the team never saw such particles move toward sites of contact, they concluded that these were the remnants of old desmosomes, possibly headed for degradation.

The group must now determine what induces the desmoplakin–plakophilin-2 precursors to move to the membrane and how they are transported.

Anchoring under shear stress

Integrins rely on clustering and conformational changes to strengthen their attachment to substrates. On page 1073, Alon et al. report that at least one immune cell integrin must also be anchored to the cytoskeleton to resist the shear forces that affect cells in the bloodstream.

Leukocytes use integrins and L-selectins to latch onto the endothelium in response to chemokine signaling. The recent observation that L-selectins must be attached to the cytoskeleton before such binding raised the question whether there is a similar requirement in the leukocyte.

Regulating basal body replication

Cells tightly regulate the replication of centrioles, which form the heart of centrosomes. But Shang et al. (page 1035) have found a way to decouple duplication of basal bodies, which are the unicellular equivalent of eukaryotic centrioles, from the cell cycle. It appears that γ-tubulin function is necessary to repress inappropriate replication of basal bodies.

γ-Tubulin is essential for basal body and centriole assembly and maintenance, but whether it regulated their formation was unclear.

Using systematic mutagenesis in Tetrahy- mena, Shang et al. identified two point mutations in the nucleotide binding domain of γ-tubulin that caused overproduction of basal bodies. Moreover, the excess structures arose in the center of the cells, as well as at the periphery where basal bodies normally reside. Thus, the mutants may allow de novo formation of basal bodies.

Significantly, only one of the two mutant residues contacts a bound nucleotide, based on comparison to the crystal structure. Therefore, the researchers hypothesize that nucleotide binding per se is not involved in repressing basal body formation, but rather think there is a distinct inhibitor that binds to γ-tubulin and prevents replication. What that inhibitor might be is not yet clear. However, the team speculates that whatever it is, it is likely to be active in other species because the nucleotide binding domain of γ-tubulin is very highly conserved.