Lamellae lead the way

Open any cell biology text and it is clear that lamellipodia, the ruffled edges of motile cells, initiate movement. So it may come as a surprise that Gupton et al. can disrupt this domain without blocking cell movement (page 619). In fact, cells move faster without lamellipodia. The driving force for movement appears to come from the lamella, the structure that lies just proximal to the lamellipodium.

Previously, the team examined the lamella and lamellipodia using quantitative fluorescent speckle microscopy (qFSM), a technique that allows for deconvolution of images of physically overlapping cell domains. The domains have distinct molecular components, filament-assembly kinetics, and motion. Moreover, although the lamellipodia moved forward and backward repeatedly in the axis of cell movement, the team saw hints that cells made forward progress only when the lamella moved forward.

To find out if lamellipodia are required for movement, the team injected skeletal muscle tropomyosin into epithelial cells in a wound-healing model. Tropomyosin normally resides in lamellae and is excluded from lamellipodia. Increasing the amount of tropomyosin pushed it into lamellipodia and disrupted resident molecules, including Arp2/3 and ADF/cofilin, which are concentrated in lamellipodia but not in lamellae. Video qFSM showed that excess tropomyosin disrupted actin treadmilling, which is a critical component of lamellipodial function. By contrast, retrograde actin flow in the lamella was not blocked under these conditions. Cells with elevated tropomyosin had more persistent leading edge movement, less retraction, and doubled their migration speed relative to untreated cells.

Adhesion proteins accumulate at the junction between the lamellipodia and the lamella in untreated cells. In treated cells the contraction of the lamella’s actin filaments appeared to be sufficient to pull the rear of the cell toward these adhesion junctions while the leading edge was pushed forward.

Gupton et al. hypothesize that lamellipodia are involved in rapid response to extracellular cues and setting up directionality. If that’s true, then cells treated with tropomyosin would be less responsive to a chemotactic gradient. The cells used in the current wound model have only one direction in which to go, which could be why they move normally without lamellipodia. JCB

A stalled fork stalls recombination

Homologous recombination does not rescue stalled replication forks during S phase, report Meister et al. on page 537. Rather they find that replication and recombination are separated in terms of when and where they occur. If inappropriate recombination does occur when replication is stalled, replication forks fall apart.

Researchers have debated whether homologous recombination, which is required for double-strand break repair, is also used to resolve stalled replication forks. However, because the S- and G2/M-phase checkpoints share molecular components in budding yeast, the question has been difficult to answer.

Now, Meister et al. test the question in fission yeast, where the checkpoints are molecularly distinct and therefore can be separated. When they added hydroxyurea to wild-type cells, thus depleting DNA precursors and stalling replication, the recombination protein Rad22 did not aggregate on the chromosomes. However, Rad22 foci did appear on chromosomes in cells deficient for the S-phase checkpoint component cds1. Furthermore, transferring the cells to fresh media rescued wild-type cells, but not cds1 mutants. 2D gel analysis of the replication forks showed that the fork structure decayed in cds1 mutant cells, but maintained normal shapes in wild-type cells. In both experiments, cells carrying mutations in the recombination protein rhp51 behaved like wild-type cells.

The researchers conclude that part of the role of the S-phase checkpoint is to prevent inappropriate—and damaging—recombination during the replication process. cds1 is a protein kinase and Meister et al. are now working to learn whether it blocks recombination by directly phosphorylating recombination proteins. JCB