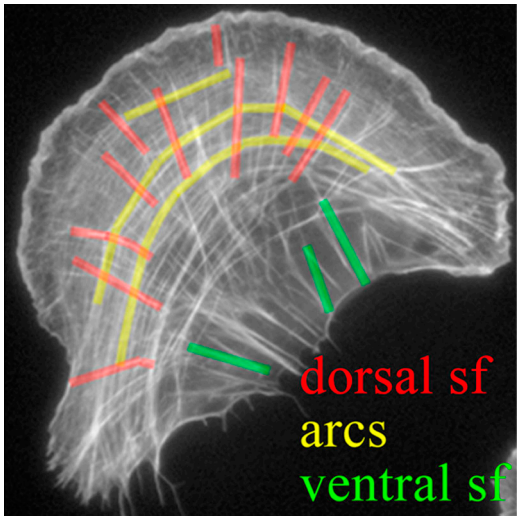
In This Issue



Stress fibers form as dorsal and ventral fibers and arcs.

Stress fibers from two sources

Stress fibers arise via two distinct pathways, according to Hotulainen and Lappalainen (page 383).

Cells contain at least three different types of contractile actin-based stress fibers. Transverse arcs do not associate with focal adhesions on either end, whereas ventral stress fibers contact focal adhesions on both ends. Dorsal stress fibers have one end tied to a focal adhesion on the ventral side of the cell and frequently attach to a transverse arc with the other end.

Using live cell microscopy and a variety of fluorescently tagged proteins, Hotulainen and Lappalainen found that dorsal stress fibers and transverse arcs underwent continuous de novo formation and disassembly. The ventral fibers, in contrast, were formed by fusion of dorsal and transverse fibers.

Dorsal fiber formation initiated at focal adhesions and was dependent on the mDia1 formin, which has been implicated previously in assembly of unbranched actin filaments. Transverse arcs arose from the fusion of short actin bundles, which themselves formed in the leading edge of the lamellipodium and then drifted back into the center of the cell. Depletion of mDia1 formin had no impact on transverse arc formation, but disruption of Arp2/3 activity prohibited assembly of the short actin bundles in the leading edge and their aggregation into transverse arcs.

Additionally, transverse arcs fell apart rapidly in response to myosin II inactivation. The dorsal stress fibers also eventually broke down in the myosin-depleted cells, but appeared to rely less on the protein for their structural integrity.

The RhoA GTPase signaling pathway is known to be involved in stress fiber assembly, activating formins and inhibiting the activity of actin depolymerization agents. The question now, say the researchers, is how RhoA and other signaling pathways control two distinct stress fiber assembly processes. JCB

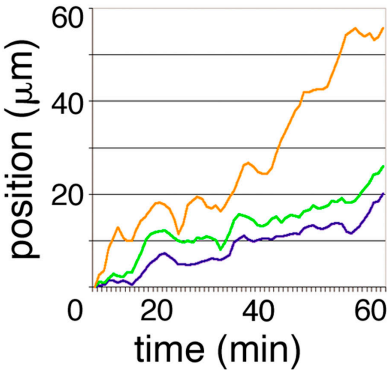
An axonal winch

Mitochondria and other cellular subunits are transported from the cell body of neurons toward the growth cone of axons via kinesin-based fast transport. On page 373, Miller and Sheetz show that mitochondria also move down the distal portion of the axon via a low-velocity transport mechanism.

Numerous models have been proposed for how axons grow, with a general consensus that the axon cytoskeleton is stationary and growth occurs at the distal tip. In the current study, Miller and Sheetz labeled mitochondria of chicken dorsal root ganglion neurons and followed their movement along the length of the axon. As expected, the mitochondria moved along the cytoskeleton at a rate consistent with kinesin-based transport. Additionally, a subset of mitochondria appeared to dock on the cytoskeleton and then move distally at a substantially slower rate. Kymographs showed that these slow-moving mitochondria moved in a correlated manner, but that the distance between them increased.

Miller and Sheetz conclude that the cytoskeleton, with its docked mitochondria, was being stretched and new material added along its length to prevent thinning. This is similar to viscoelastic stretching that has been reported in *Xenopus* axons, but in those studies there was no evidence for the addition of extra material.

The team thinks the stretching is a motor-dependent process because it continues even when the growth cone is not moving, indicating that it is not a passive response to growth cone advance. The researchers imagine that a winch-like motor pulls the cytoskeletal fibers toward the distal tip of the axon, creating tension and pulling the cytoskeletal polymers apart. New cytoskeletal subunits would then fill in the gaps, along with docked mitochondria.



Movement of mitochondria (blue and green) does not correlate with axonal advance (yellow).

Why did the group detect this low velocity transport when others have not? It might be because they looked at organelle movement along the whole length of the axon and analyzed the proximal, middle, and distal regions separately. By contrast, other groups focused on the proximal region where low-velocity movement is absent and everything seems to move by fast transport. JCB

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In-and-out signaling

Some plasma membrane sensor proteins that detect nutrients look a lot like the transporters that move those small molecules across the membrane. On page 327, Wu et al. present a model suggesting that the sensors work like them, too—minus the transport step.

Previous work showed that a single mutation in the Ssy1p amino acid sensor increased its basal signaling level and made it hyperresponsive to extracellular ligand. Starting from those data, the team developed a model of how the sensor might control transcriptional activation of amino acid transporters.

According to the model, the sensor could sit in the membrane with its ligand-binding site facing the intracellular or extracellular space. In its unbound state, the sensor freely flips between inside- and outside-facing conformations. But ligand freezes the sensor in one conformation or the other so it cannot readily shift between the two sides of the membrane. Transporters, by contrast, do their job by flipping their ligand binding site from one side of the membrane to the other when ligand is bound.

When the sensor is facing the outside of the cell, it initiates signaling. If the model were correct, then increased levels of intracellular amino acids should inhibit signaling. When the team tested this by raising the levels of intracellular leucine, they found that Ssy1p signaling was inhibited.

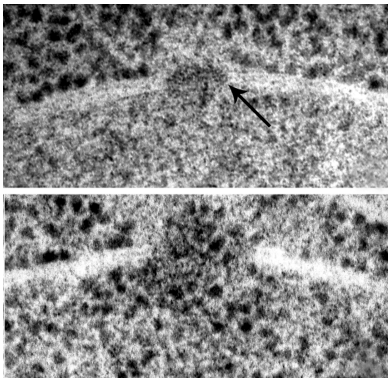
By converting the extracellular and intracellular nutrient concentrations into a regulated signaling pathway, Ssy1p may better control nutrient homeostasis. Now the question is whether the model can be generalized to other sensors, such as those that detect glucose. JCB

Assembling pores

Despite the prominence of the nuclear pore structure, its assembly mechanism remains largely undescribed. On page 361, Madrid et al. show that one of the three transmembrane pore proteins is required for interphase assembly in yeast. The data suggest that insertion of nascent pores into the interphase nuclear envelope proceeds in a manner similar to that of postmitotic pore formation.

In budding yeast lacking Pom34 or Pom152, two of the transmembrane proteins, soluble pore components localized normally to the nuclear envelope. However, nuclear pore components were significantly mislocalized in cells depleted only for the third transmembrane protein, Ndc1. The Ndc1 defect was exaggerated in cells lacking Pom152.

Electron microscopy showed that double mutants had wide openings in the nuclear envelope, but little associated protein. These gaps allowed indiscriminant movement through the envelope, with the occasional mislocalized ribosome showing up in the nucleus.

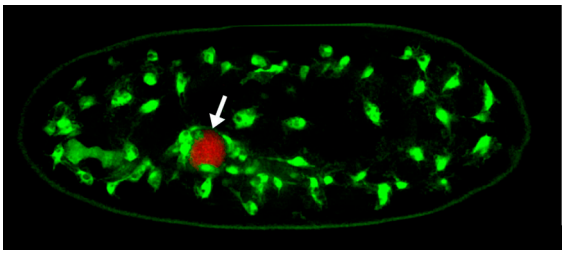


A normal nuclear pore (top) loses its structure in the absence of Ndc1 and Pom152 (bottom).

Comparison of these results to previously published work from *Xenopus* suggests that the assembly of both interphase and postmitotic pores rely on the initial localization of these transmembrane proteins. Significantly, Mansfeld et al. recently identified the mammalian homologue of Ndc1 and found that it too was essential for pore assembly (*Mol. Cell.* 2006. 22:92–103).

Madrid et al. are now working on the development of in vivo assays to monitor the formation of new pores and learn just how Ndc1 controls the process. JCB

One cell, two chemotaxis systems



PI3K is needed for hemocytes (green) to migrate to a wound.

During fly development, macrophage-like cells called hemocytes move along a stereotyped migration route relying on PDGF chemotactic ligands. Yet, in response to injury in embryos, hemocytes require phosphoinositide-3 kinase (PI3K) signaling for chemotaxis, report Wood et al. on page 405. The reliance on PI3K suggests that hemocytes are a better model system than previously thought for studying mammalian cell chemotactic behavior.

During embryogenesis, GFP-labeled hemocytes migrated normally in wild-type embryos, but failed to distribute properly in mutants lacking PDGF ligands. However, the cells moved normally toward wounds in both types of flies, indicating that PDGF signaling was not required for the immune response.

By contrast, hemocytes in flies lacking a functional catalytic subunit of PI3K migrated normally during development but failed to infiltrate a wound. Moreover, localized administration of a PI3K inhibitor at a wound blocked hemocyte migration.

The use of two different mechanisms for chemotaxis by hemocytes may partially explain why the cells move much more quickly in response to wounding than during development. As fly hemocytes and mammalian neutrophils share the use of PI3K during migration, Wood et al. are planning to combine fly genetics and whole organism imaging to uncover other molecular components involved in chemotaxis and wound responses. JCB