In This Issue

Fusion at the front

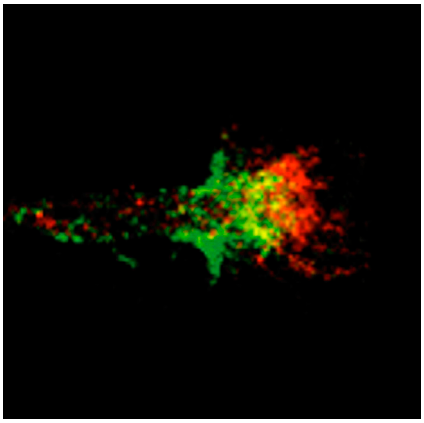
Neurite extension is dependent on the growing neurite's ability to get longer rather than fatter. Sakisaka et al. (page 17) now show that a protein called tomosyn works in a complex to help focus membrane growth to the tip of growing neurites.

As neurites grow, traveling vesicles are prevented from fusing willy-nilly to the plasma membrane first by their attachment to microtubule highways. Where those highways terminate—at the back of the growth cone—is where tomosyn takes over as a fusion inhibitor. With fusion prevented, the vesicles find their way to the actin cytoskeleton, which distributes them to the leading edge of the growth cone.

Sakisaka et al. found that, in growing neurites, tomosyn localizes at the rear of the growth cone. This area appears to be analogous to the rear of a locomoting cell. In both situations, Rho activates ROCK. The authors found that activated ROCK can, at least in vitro, phosphorylate syntaxin-1, making it a much better binding partner for tomosyn. The SNARE protein SNAP-25 also joins the complex, leading to inhibition of membrane fusion.

Consistent with this suggested function in inhibiting membrane fusion, overexpressing tomosyn in neurons resulted in stunted neurites and prevented proper transport of proteins to the cell surface. Killing tomosyn expression via RNAi caused neurites to branch out excessively.

Signals that induce neurite retraction, such as LPA, activate ROCK throughout the growth cone. This resulted in distribution of tomosyn—and presumably inhibition of fusion—throughout the growth cone. Actin contractility should then be free to reel in the existing plasma membrane as the neurite retracts. ■



Tomosyn (green) inhibits vesicle fusion so that it only occurs at the extreme ends of neurites (red, labeled with Sec8).

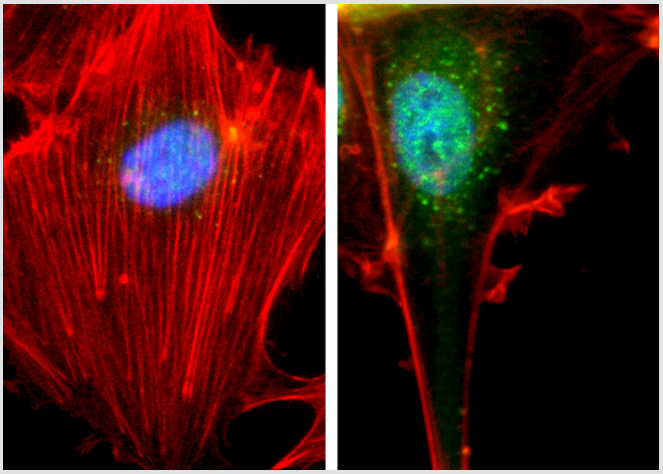
Anti-angiogenesis is anti-actin

Blood vessel growth is suppressed by several proteins—such as endorepellin—that are anti-angiogenic only after they are generated as fragments of larger proteins. Now, Bix et al. (page 97) report two surprises of endorepellin action: it exerts its effects via an integrin that collagen I uses to promote angiogenesis; and it may operate via a heat shock protein to disassemble actin structures needed for motility.

Endorepellin is a COOH-terminal fragment of perlecan, a heparan sulfate proteoglycan that acts as a cofactor for pro-angiogenic factors such as FGF. The authors found that a segment of endorepellin was enough to prevent endothelial cell migration and formation of capillaries, and that it acted by disrupting the actin cytoskeleton and attachment sites. They found that the major functional receptor for endorepellin was α2β1 integrin, one of the collagen receptors. Treating cells with endorepellin resulted in clustering of α2β1 integrin, and these integrin clusters colocalized with collapsed actin bundles. As reorganization of actin filaments is crucial to cell migration and capillary morphogenesis, the authors reason that endorepellin halts these processes by taking apart actin filaments and focal adhesions.

Collagen I binding to integrin $\alpha_2\beta_1$ decreases cAMP levels and the activity of protein kinase A, but endorepellin binding to the same integrin triggers the opposite results. Endorepellin binding also activates FAK, p38MAPK, and phosphorylation of Hsp27. FAK activation has been associated with disassembly of focal adhesions, and results with inhibitors suggest that the transient phosphorylation (or subsequent destruction) of Hsp27 may somehow prompt the disintegration of actin filaments.

Early steps in angiogenesis include proteolysis of matrix to make room for growing vessels. This proteolysis probably liberates protein fragments such as endorepellin, which damp down angiogenesis so that it does not become overactive. ■



Addition of endorepellin (right) causes activation of Hsp27 (green) and disassembly of actin stress fibers (red).

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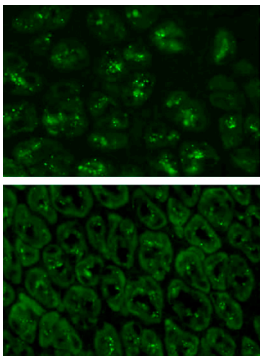
Calcium signals from outside

Calcium just got a promotion. Findings by Caroppo et al. (page 111) reveal that in addition to its many roles inside the cell, Ca^{2+} has a distinct extracellular purpose: it acts via a Ca^{2+} receptor (CaR) to regulate the function of gastric epithelial cells.



Pepsinogen stores (top) are excreted in response to an increase in extracellular Ca^{2+} (bottom).

It has been known for some time that extracellular Ca^{2+} can be sensed by the CaR. The team noted that a Ca^{2+} gradient was generated outside gastric cells after cholinergic stimulation with carbachol, which mimics a signal received during digestion processes. Ca^{2+} levels increased on the apical side and decreased on the basolateral side, and prompted secretion of pepsinogen. Proteolytic cleavage of pepsinogen yields the digestive enzyme pepsin.

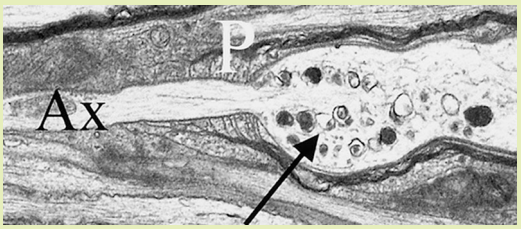
It is well known that carbachol boosts intracellular Ca^{2+} in gastric cells, and a resultant increase in extracellular Ca^{2+} is no surprise. But the authors found that extracellular Ca^{2+} was both necessary and sufficient for the induction of pepsinogen secretion.

Perhaps cells economize by using a single messenger, calcium, both inside and outside of the cell. In this way, the authors speculate, cells can use the raised Ca^{2+} levels that are present outside cells during intracellular Ca^{2+} signaling events to control necessary functions. ■

Myelin movement

Like the hard candy shell around an M&M, oligodendrocytes swathe axons with a sheath of myelin for protection. Now, Edgar et al. (page 121) show that the myelin casing may do more than just provide shelter and stability; it may also set up conditions necessary for fast axonal transport.

The team noticed multiple organelles amassed within optic nerve axons in mice with a null mutation of the *Plp* gene, which encodes two major proteins of the myelin sheath. These organelle traffic jams implied that there might be problems with transport in these cells. So Edgar et al. looked at movement of labeled tracers in fast anterograde and retrograde transport. There were minor defects in fast forward transport that took some time to accumulate, and severe defects in fast backward transport. A closer look at motor proteins revealed that levels of the retrograde motor dynein and associated dynactin were elevated, possibly due to the accumulation of proteins on stalled vesicles. Anterograde motors were unaffected.



Transport fails when oligodendrocytes are defective.

The primary function of myelin is to insulate nerves and thus speed transduction of nerve impulses. But it has also been shown to affect mitochondrial placement and cytoskeleton function in the underlying neuron. Exactly how the oligodendrocyte is communicating with the neuron to achieve such tasks, and which of these events are necessary for fast axonal transport, remains uncertain. ■

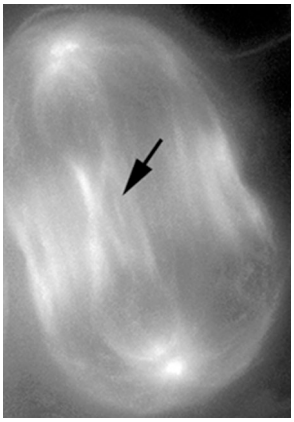
Orbit(ing) the furrow

Two distinct populations of spindle microtubules provide signals that initiate and complete cytokinesis, according to a proposition from Inoue et al. (page 49).

During interphase, microtubules radiate from centrosomes in all directions. But after chromosome segregation a structure called the central spindle forms. It provides the signals that place the actin-based cytokinetic furrow in the right place and then orchestrates its actions.

The authors take a close look at the central spindle in living fly spermatocytes and find that this structure, previously thought to be uniform, is composed of two populations of microtubules. These peripheral and interior microtubules were both geographically and biochemically distinct, with only the interior microtubules associated with the Orbit/Mast protein.

In mutants with reduced levels of Orbit protein, formation of the interior bundle of central spindle microtubules was defective. As in wild-type cells, peripheral microtubules still probed the actin-rich cortex leading to cleavage furrow initiation, but the furrows in mutants eventually snapped back. This failure in cleavage was accompanied by the mislocalization of several proteins thought to be involved in generating the cytokinesis signal.



Two populations of microtubules have different functions during cytokinesis.

Orbit helps to stabilize microtubule plus ends. This stabilization may not be favorable for the highly dynamic peripheral microtubules but may be essential for the maintenance of the more stable interior microtubules. As is clear from mutants with less Orbit, a full description of cytokinesis may have to account for the coordination not only of the actin and microtubule systems but also of two distinct microtubule populations. ■