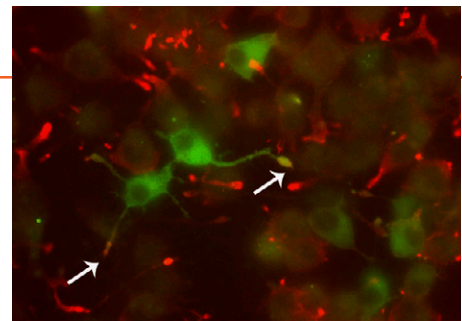
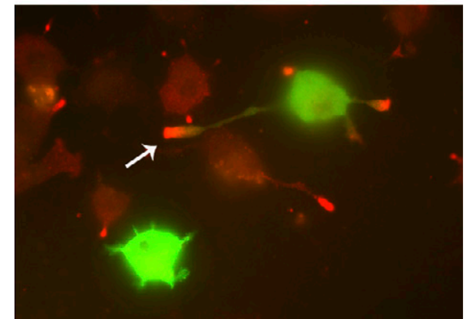
In This Issue





Kinesin-1 takes JIP1 (green) to axon tips, but not if FEZ1 (red) activity is blocked (bottom).

Partners in kinesin activation

It takes two to turn on a kinesin, say Blasius et al. on page 11. Kinesin-1 motoring on microtubules requires cargo plus a second binding partner to relieve the motor of its inhibited conformation.

On its own, Kinesin-1 is an inactive motor. Inhibition of the solitary motor probably ensures that it is not needlessly burning ATP or clogging up the microtubule roadways when cargo-loaded motors need to get through.

Truncated versions of Kinesin-1 are more active than the full-length protein, suggesting that the deleted domains are autoinhibitory. Cargo binding is thought to activate Kinesin-1 by releasing inhibitory domains from the motor. One recently identified cargo is a scaffolding protein called JIP1, but the group now finds that binding of this cargo is not enough to activate Kinesin-1.

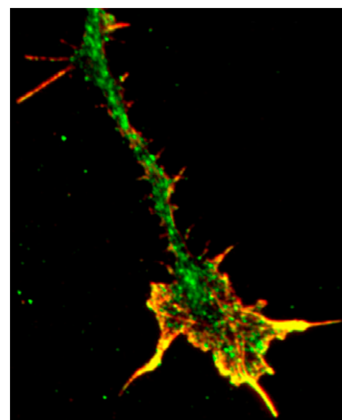
JIP1 binds to kinesin's light chain, but autoinhibition happens on the heavy chain, where the motor domain lies. The authors thus sought new binding partners for the heavy chain. A prominent partner from yeast two-hybrid assays was FEZ1—a mammalian homologue of a worm protein that supports neurite outgrowth.

Adding FEZ1 to the JIP1/Kinesin-1 mix jumpstarted motor activity. Exactly why a double whammy is needed for Kinesin-1 activation is not clear. Apparently, even a small amount of unwanted motor activity is dangerous enough to warrant a dual-layered prevention system.

In a second paper from the same group, Cai et al. (page 51) use FRET to uncover the conformational changes that accompany Kinesin-1 activation. FRET of the multiprotein mixture is technically difficult, so the group instead studied the active deletion mutants. They found two conformational differences between the structures of the full-length kinesin chains and the mutants.

In one expected change, the inhibitory C-terminal tail of the heavy chain moved away from the motor domain. This change is probably brought about by the binding of FEZ1. The second structural change moved the motor subunits of the two heavy chains closer together. The authors believe the closing of this gap—possibly upon the arrival of cargo—might be necessary for the motor to sit correctly on microtubules.

The authors are now testing whether FEZ1 is needed to move more Kinesin-1 cargoes. The next step will be to determine how FEZ1 binding to the motor is regulated. JCB



PKA (red) activates a PP1 inhibitor (green) to steer growth cones.

PKA steers growth cones

Like a pack of teenagers, the sundry proteins that steer a growth cone stick close together, Han et al. show on page 101.

Growth cones are the trailblazers at the tip of growing axons. Their paths are lit by a combination of attractive and repulsive cues in the extracellular matrix. Attraction, via growth cone turning toward a cue, is linked to high axonal levels of cAMP, but its collaborators in the process are unknown.

The authors have now identified the downstream effectors of cAMP during attraction by examining one of its favorite targets: protein kinase A (PKA). They found that the type II form of PKA is strongly enriched in growth cone filopodia. PKA activity and its filopodial localization—mediated by a member of the AKAP adaptor protein family—were required for cAMP-induced attraction.

In growth cone filopodia, PKA was found in a complex containing the PP1 phosphatase and its inhibitor, I-1. The authors show that, during cAMP-induced growth cone turning, PKA activates I-1 and thereby inhibits PP1. In the absence of I-1, attraction was blocked.

In other contexts, one of PP1's known targets is CaMKII, whose activation on one side of a growth cone is known to create turning in that direction, probably by increasing actin dynamics. PKA's inhibition of PP1 should also tip the scales in CaMKII's favor.

The colocalization of PKA with its downstream targets has also been seen in cardiac muscle cells. PKA is a promiscuous enzyme, so the strict positioning of PKA in signaling complexes that are tethered in place is probably necessary to ensure only localized responses. JCB

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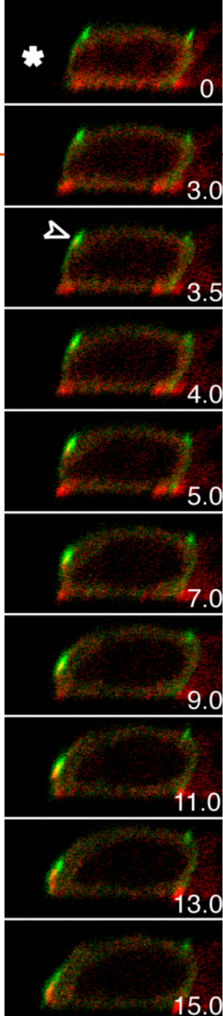

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A dual need for myosin in wounds

Myosin takes both the high road and the low road to close wounds, as revealed by Tamada et al. on page 27. The motor is found in a high contractile actin ring and in bottom-dwelling membrane protrusions.

Wound closure requires a cable of actin and myosin in the cells that border the wound. The contraction of this cable draws these border cells together to fill in the hole. In flies, however, border cells seem rather to migrate inward, dragging the cells behind them forward into the wound. Now, new videos suggest that both migration and ring contraction help to close wounds in mammalian epithelia.

The videos examine myosin localization from a unique perspective. After creating a circular injury site in an epithelial sheet, the authors imaged the wound-adjacent cells from a side view as healing progressed. They saw actin and myosin accumulate at apical tight junctions. Viewed from above, the accumulation formed a ring around the wound.

Myosin-mediated contraction of the ring was required to close the wound fully. The contractile shortening of the actomyosin ring stretched the cells thin, pulling the apical membrane down and inwards into the wound. During stretching, apical myosin moved together with a junctional protein called ZO-1, and both eventually met with the basal membrane. The authors suggest that, given this close association, tight junction proteins might provide a structural scaffold for ring assembly.

The side view revealed that the apical ring only formed on the side of the cell facing the lost neighbor. Group leader Michael Sheetz speculates that a mechanical signal, such as the loss of tension at those junctions, is the initiator of myosin assembly. Tension loss might be noted by $p130^{cas}$, which his group recently showed to be a mechanosensor.

In addition to the top ring, a basal accumulation of myosin was also seen, near lamellopodial protrusions that moved out into the wound, as seen in flies. Without this basal myosin activity, the cells formed spikey filopodium-like extensions instead of lamellipodia. Spikes were only able to close the wound partially. Mammalian cells thus seem to require two myosin rings for full closure. JCB

A ring of myosin (red) at apical junctions (green) on the wound (*) face tugs cells inwards while basal myosin spurs on cell migration.

Syt-12 for spontaneous release

On page 113, Maximov et al. show that a calcium-independent synaptotagmin (syt) enhances the spontaneous release of neurotransmitters. By linking to another syt that does respond to calcium, the calcium-independent syt might also alter evoked neurotransmitter release.

There are many synaptotagmins, but only syt-1 and syt-2 have a well-defined function, which is to trigger the release of neurotransmitters in response to calcium. Syt-12 does not bind to calcium, yet the authors found it is still localized to synaptic vesicles.

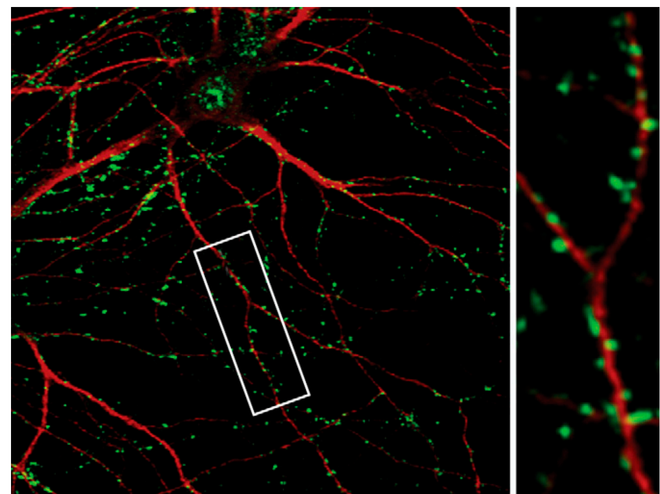
To understand its function, the authors expressed syt-12 in cultured neurons, which did not make their own endogenous version. The presence of syt-12 increased the spontaneous release of neurotransmitter in these neurons.

The function of spontaneous release is controversial: some neuroscientists believe these release events are physiologically important for neuronal structure and function, whereas others argue that they are only a meaningless byproduct of a system that is poised to fuse many vesicles so quickly. Future syt-12 studies might address this debate.

Spontaneous release might not be syt-12's only trick, however. In synaptic vesicle fractions, syt-12 interacted with syt-1, the calcium-responsive release trigger. Syt-1 must partner with SNAREs for evoked release, but this interaction was precluded by the syt-12 association.

The authors found that calcium-evoked release worked just fine in cultured neurons in the presence of syt-12, but they note that acute and long-term changes in synaptic strength, known as plasticity, cannot be studied in this system. An animal knockout should be a better model.

Syt-12 is phosphorylated by PKA, which is necessary for plasticity, but PKA's plasticity-related targets have not been identified. Given its location, Syt-12 is a promising candidate. Its late expression in mice, only after birth, also coincides well with the onset of synaptic maturation and plasticity. JCB



Syt-12 (green) on synaptic vesicles increases spontaneous release of neurotransmitters.

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