Oscillating Actin

The existence and possible function of actin at the tip of growing pollen tubes has been controversial, with results differing depending on the fixation or visualization techniques. Now Fu et al. have used the actin-binding protein talin, linked to an enhanced green fluorescent protein (GFP), to detect sparse and transient short actin bundles (SABs) in pollen tips (page 1019). They also use mutant and overproduced Rop1At to implicate this pollen-specific Rho GTPase in the control of SAB formation and polarized tip growth.

The SABs oscillate in abundance every 60–75 s, with SABs disappearing at the peak of a tube’s calcium transient and growth spurt. Fu et al. suggest that phosphatidylinositol-4,5-bisphosphate [PIP(2)] production stimulated by Rop1At might control all of these variables. First, a direct inhibition of profilin by PIP(2) would permit actin polymerization, allowing actin-based delivery of vesicles to a site near the plasma membrane. Then, slower production of inositol trisphosphate (IP3) from PIP(2) would induce calcium release, leading both to exocytosis of the vesicles (and thus visible growth) and actin disassembly via calcium activation of profilin.

Pulsatile growth of pollen tubes is postulated to be important so that cell growth can alternate with cell wall consolidation. The sparse and oscillating nature of the SAB may be necessary to accommodate this pattern of growth.

A Cargo for Kinesin

In the fifteen years since kinesin was identified in squid axoplasm, the cargoes that directly bind to this microtubule-based motor have eluded researchers, perhaps because most kinesin is not bound to cargo at any one time. Now, Verhey et al. have used two hybrid analysis to identify JIP-1, JIP-2, and JIP-3, three scaffolding proteins for the JNK signaling pathway, as one set of cargoes (page 959).

The bait for the two-hybrid was a collection of the TPR motifs from kinesin light chain (KLC). The JIP–KLC interaction was confirmed by coimmunoprecipitation experiments, and shown to be necessary for JIP-1 localization to neurite tips.

The JIPs are also associated with both an upstream JNK-pathway kinase and the reelin receptor, ApoER2. Reelin is produced in the brain where it aids in neuronal process maturation, axonal branching, and synaptogenesis, so the transport of its receptor to growth cones makes sense.

Verhey et al. propose that ApoER2 helps anchor kinesin to vesicle membranes. In a recent paper, Bowman et al. suggested that JIP-3 was a transmembrane protein that provided a direct link from kinesin to the membrane (Bowman, A.B., A. Kamal, B.W. Ritchings, A.V. Philp, M. McGrail, J.G. Gindhart, and L.S.B. Goldstein. 2000. Cell. 103:583–594). But Verhey et al. find no evidence that JIP-3 is a transmembrane protein, and suggest instead that it helps assemble a signaling complex, ready for transport. Future work will focus on whether the activity of this signaling complex regulates cargo binding or kinesin activity, and whether other cargo molecules attach to other regions of the kinesin chains.

Microtubules in the End-Zone

Protein kinase C (PKC) enhances (although it is not absolutely necessary for) neurite extension. Based on known effects of PKC on actin-associated proteins, this phenomenon had been attributed to changes in actin dynamics at the growth cone. However, on page 1033 Kabir et al. report that PKC has only minor effects on neurite actin dynamics, but dramatically stimulates microtubule growth into the peripheral (P) zone of growth cones, from which microtubules are normally excluded. Dynamic microtubules are necessary for membrane delivery to growth cones, and may also deliver signaling proteins (see previous summary).

Kabir et al. find that PKC increases microtubule growth lifetimes by increasing rescue frequency and decreasing catastrophe frequency, so rescue and catastrophe factors are amongst the candidate PKC targets. If physiological PKC activation is focal, and if this focal activation stimulates directional microtubule growth, the result may be growth cone turning.

Linking without Signaling

Integrins are involved not only in adhesion to the extracellular matrix (ECM), but also in many signaling events. Integrin-linked kinase (ILK) is
one possible participant in these signaling events. On page 1007, Zervas et al. report that Drosophila melanogaster ILK helps connect the actin cytoskeleton to the plasma membrane, but is not necessary for the activity of the integrin, protein kinase B (PKB), or Wnt signaling pathways. Furthermore, ILK’s essential function does not require an active kinase domain, suggesting that ILK is acting primarily as a linker and not as a signal transducer.

ILK was first isolated as a protein that interacts with the cytoplasmic tail of a β-integrin. Overexpression phenotypes in tissue culture had implicated ILK in PKB and Wnt signaling, so Zervas et al. set out to evaluate ILK’s mutant phenotype. Flies with a stop mutation in ILK do not show the reduced cuticle characteristic of PKB mutants, the pattern defects seen in Wnt mutants, or even the early muscle detachment seen in certain integrin mutants. Although the integrin–ECM link remains intact, a lack of ILK eventually leads to a clumping of actin inside muscle cells as the linkage from actin to plasma membrane is lost.

The mutant phenotype can be rescued by various kinase-dead versions of ILK, raising the question of why any kinase homology has been conserved. Zervas et al. suggest that ILK’s kinase domain may have retained the ability to bind to certain key proteins, even if it no longer phosphorylates them.

A Stretchy Structure

From sea cucumber dermis to lobster aorta, fibrillins help give connective tissues elasticity. Various models have been advanced to explain how fibrillins can fluctuate between periodicities of 56–160 nm. Now, Baldock et al. propose their own model based on a three-dimensional reconstruction from automatic electron tomography (AET) data (page 1045).

Fibrillin-rich microfibrils look like beads on a string, with two arms extending out from each bead. Baldock et al. confirm this basic structure, and then use antibodies and mass mapping to make sense of it. They predict that microfibrils are composed of eight fibrillin molecules that are initially produced as extended rods ~160 nm long. The rods then fold back on themselves to produce repeating structures ~90 nm in length thanks to an approximately one third overlap. This unstable structure readily folds back on itself again to form repeats ~56 nm in length.

The 56-nm mature form of the protein is a large bead with two arms (each with loops from four fibrillin molecules) extending out from it. The mature form can be reversibly extended by unfolding of interbead material to yield the 100-nm form. Further extension of the 100-nm form reduces the size of the bead and appears to be irreversible, probably because it breaks a covalent transglutaminase cross-link.

This model fits the available data, but Baldock et al. hope to test the model further by using atomic force microscopy to see if protein hinges are in the predicted locations.

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