

## Controlling Actin

### Kinases in Actin Patches

Many proteins bind actin, and many other proteins bind actin-binding proteins. "Sometimes I wonder why the whole cytoplasm isn't a precipitate, because it seems that everything is interacting with everything else," says David Drubin.

"What's missing is the time dimension," he says. "These interactions are probably dynamic." On page 1203, Drubin's group identifies two budding yeast proteins that may contribute to that dynamic behavior. Cope et al. isolate actin-regulating kinase 1 (Ark1p) based on its two-hybrid interaction with the actin-binding protein Sla2p. Sequence gazing and a second screen both lead to the related p53-regulating kinase 1 (Prk1p). (Yeast lacks p53; Prk1p was named based on a screen involving ectopic expression of mammalian p53 in yeast.) Ark1p, Prk1p, and related proteins appear to define a new protein kinase family.

Both proteins localize to actin patches, which in budding yeast are involved in endocytosis (actin may provide an assembly force that overcomes the osmotic forces of turgor pressure). Deletion of one kinase gene causes only a slight slowing of growth, but deletion of both genes yields cells that die at extreme temperatures and grow very slowly at 30°C. Most of the surviving cells are enlarged and have huge clumps of filamentous actin in the cytoplasm. The remaining cortical actin patches appear to be fewer in number and are no longer polarized to the growing bud.

The actin clumps may form because of a defect in the endocytic cycle, but confirming the existence and stage of any block will require conditional mutants. However, another link in the pathway is apparent from the work of Zeng and Cai (Zeng, G., and M. Cai. 1999. *J. Cell Biol.* 144:71–82). Based on a suppressor screen and kinase assays, Zeng and Cai isolated Prk1p and determined that Pan1p was a target for negative regulation by the kinase. Both Pan1p and the similar mammalian protein Eps15 have been implicated in endocytosis, which brings up the controversial question of whether actin is involved in mammalian endocytosis. The more immediate question of how all the actin-patch proteins cooperate in yeast endocytosis will probably have to wait for the development of an *in vitro* endocytosis assay.

### Localizing Actin in Cell Growth and Mating

In fission yeast, both cell wall growth and actin are somehow localized to the tips of the cell. On page 1173, Katayama et al. report that Mok1 makes 1,3- $\alpha$ -D-glucan, one of the major components of the fission yeast cell wall, and that Mok1 is required for the correct localization of actin to the growing tips.

Katayama et al. isolate *mok1* in a screen for temperature-sensitive mutants with aberrant morphology and sensitivity to the protein-kinase inhibitor staurosporine. (Protein kinase C activity is required for normal cell wall

synthesis.) Actin is not at the growing tips of *mok1<sup>ts</sup>* cells, but in randomly distributed patches in the cortex.

Overproduction of Mok1 is lethal. Dividing cells lyse and single cells swell at one end due to an excess of actin and cell wall material at the cell tip. Whereas  $\beta$ -glucan levels decline somewhat under these conditions, levels of  $\alpha$ -glucan rise threefold. In the *mok1<sup>ts</sup>* cells  $\alpha$ -glucan levels are reduced.

These data are consistent with the sequence of Mok1, which reveals a glucan synthase domain. The protein also has a glucanase domain. The cell wall must be partially degraded to allow insertion of newly synthesized material, so perhaps Mok1 can direct both synthesis and degradation.

The signal for Mok1 and actin localization is not known, but on page 1187, Nern and Arkowitz describe a landmark for another form of localized cell growth: budding yeast polarization in response to a pheromone gradient. Association of two components of this putative landmark provides a link from pheromone signaling to the cytoskeleton: the  $\beta$  subunit of a heterotrimeric G protein is involved in pheromone detection, and the guanine nucleotide exchange factor Cdc24p helps to orient and recruit the actin cytoskeleton.

Nern and Arkowitz find that Far1p is also required for the formation of this complex, functioning as an adapter. Similar interactions have been noted by Butty et al. (Butty, A.C., P.M. Pryciak, L.S. Huang, I. Herskowitz, and M. Peter. 1998. *Science*. 282:1511–1516). As these proteins are required for cell orientation to a pheromone gradient, and Cdc24p localizes to the area of cell outgrowth, Nern and Arkowitz suggest that the complex acts as a spatial marker to direct polarized growth. A competing spatial marker normally used for bud site selection, Bud1p, becomes necessary for pheromone-dependent cell polarization in the absence of this complex.

### Attaching the Bacterial Tail

The actin tail of *Listeria* propels the bacterium through the cytoplasm of one cell and into neighboring cells. The only bacterial protein required for this process is ActA. Of the necessary host proteins, the Arp2/3 complex would seem to be the perfect link to the polymerized tail, as it binds ActA and nucleates actin polymerization. But Arp2/3 creates a cloud of polymerized actin that lacks a directional focus. Laurent et al. suggest on page 1245 that VASP (vasodilator-stimulated phosphoprotein) provides another link between ActA and actin, and that this link can organize the actin growth to produce a propulsive force directed against the bacterium wall.

In previous experiments, the VASP-binding site on ActA has been deleted, but this work was always open to the interpretation that the interaction of other proteins with ActA had been disturbed. In any case, VASP was seen primarily as a profilin-recruiting protein.

Laurent et al. show that immunodepletion of VASP from an extract eliminates bacterial movement. Although

actin polymerization remains, there are no directional tails. Only pure VASP is needed to restore movement, thus disposing of the profilin-recruitment hypothesis. Homologous proteins of the same family can also substitute for VASP.

Arp2/3 provides seeds for actin polymerization. It does not, however, remain bound to ActA, but to the filaments that it has nucleated, which form the actin tail. In contrast, VASP binds tightly to ActA and less tightly to F-actin, which should permit insertional polymerization by frequent attachment–detachment steps.

### *Doing without Acetylcholinesterase*

The acute inhibition of acetylcholinesterase (AChE) by nerve gas causes prolonged nerve activation by acetylcholine, which leads quickly to respiratory paralysis and death. But on page 1349, Feng et al. find that mice can adapt to a chronic lack of AChE in the neuromuscular junction.

AChE is anchored in the synaptic cleft of the neuromuscular junction by a collagen tail dubbed collagen Q, whereas it is cytoplasmic or membrane-bound at other sites. To determine the function of this synaptic AChE, and whether Q-less AChE is also present at synapses, Feng et al. create a collagen Q knockout mouse.

The homozygous knockout mice are born in normal numbers, but by day 5 they develop a tremor when moving. The tremor persists for the rest of their lives. Although the mice are active and responsive, they fail to thrive, half die by the age of weaning (day 21), and <20% survive to adulthood. Still, any level of survival is remarkable, especially given that there is no detectable AChE activity at mutant neuromuscular junctions. (Even some forms of acetylcholine that normally lack a collagen modification are missing, perhaps because the collagen is needed for their assembly.) Consistent with the lack of AChE activity at mutant neuromuscular junctions, inhibitors of AChE cause tremor and paralysis in normal mice, but only minor effects in the mutants.

“These animals have an incredible ability to compensate,” says first author Guoping Feng. The compensation is not based on the activity of the related butyrylcholinesterase, as this activity is also lost in the knockout, so perhaps the nerve cells are adapting by releasing less acetylcholine. A precedent for such synaptic feedback exists in *Drosophila*, as shown by Graeme Davis and Corey Goodman.

Feng et al. do not measure the size of acetylcholine quanta, but they do observe that the nerve terminals of the knockout mice are partially enwrapped by Schwann cell processes. This physical block to acetylcholine transmission is seen more often in older animals, even as the signs of post-synaptic muscle damage are decreasing.

Another component of the synaptic cleft, laminin-11, was previously shown by this group to prevent the entry of Schwann cells into the synaptic cleft. Although laminin-11 is present in the mutants, collagen Q may have a similar activity, or postsynaptic damage may somehow induce Schwann cell invasion.

### *Multiple Regulators of One Kinesin Motor*

On page 1219, Manning et al. report that two proteins can independently associate with the same kinesin, Kar3p, with different functional consequences.

The Snyder group previously characterized Cik1p as a Kar3p-associated protein necessary for the microtubule–cross-linking and -sliding activity of Kar3p in mating cells. Cik1p is induced by mating pheromone, and targets Kar3p to cytoplasmic microtubules where Kar3p brings together the two nuclei of a zygote. But Cik1p does not help with all of Kar3p’s duties. Manning et al. looked for proteins similar to Cik1p and found Vik1p (vegetative interaction with Kar3p), which takes up where Cik1p leaves off. Kar3p is dependent on the new protein Vik1p, but not Cik1p, for spindle pole body localization in vegetative cells. The loss of Vik1p (or Kar3p, but not Cik1p) can suppress the growth defect caused by lack of the kinesin motors Cin8p and Kip1p, which are involved in spindle pole separation.

Kar3p–Vik1p may act antagonistically to Cin8p and Kip1p by depolymerizing microtubules near the spindle pole, as cells without Vik1p are resistant to a microtubule-depolymerizing agent. The vegetative role of Kar3p–Cik1p is even less clear, although some reports have placed Kar3p at the kinetochore, and strains lacking Cik1p frequently missegregate chromosomes. In vitro motility assays and ectopic expression experiments should show whether Cik1p and Vik1p impart different activities to Kar3p, or whether they differ in their actions merely because they differ in when and where they are present.

By William A. Wells, 1095 Market St. #516, San Francisco, CA 94103.  
E-mail: wells@biotext.com