Meanwhile, back at the ring canal...

On page 703, Kelso et al. complete the trilogy of actin papers with a detailed biochemical analysis of the Drosophila Kelch protein. Like the Arp2/3 complex, Kelch is required for proper actin organization in ovarian ring canals. Although the structure of Kelch suggested that it might act as a dimeric actin cross-linking protein, this activity had not yet been demonstrated.

In an impressive series of biochemical experiments, the authors demonstrate that purified Kelch can bundle actin filaments through a conserved actin-binding site, and that phosphorylation of a tyrosine residue near the actin-binding site blocks Kelch from interacting with actin. In vivo, Kelch is phosphorylated by a mechanism involving the Src-family kinase src64. A loss-of-function mutation in src64 and a mutation in Kelch that removes the phosphorylation site produce identical ring canal defects.

The authors propose that ring canal growth is driven by actin polymerization and regulated actin cross-linking, in a mechanism similar to plasma membrane movement at the leading edge of motile cells. In this model, src64 phosphorylation of Kelch would be required to break cross-links, allowing rapid turnover of actin monomers. The similarity of src64 and Kelch mutant phenotypes also suggests that Kelch is the primary target of src64 activity during ring canal development.

Do cells have mouths?

Cells form clathrin-coated pits to internalize many cell surface receptors, but can these pits form de novo anywhere on the plasma membrane? On page 665, Santini et al. argue that this is not the case, and that there are particular zones of the membrane specialized for coated pit formation and endocytosis. Although the work focuses specifically on the ability of nonvisual arrestins to drive G protein coupled receptor (GPCR) internalization, the findings suggest that cells maintain defined coated pit zones on the membrane—zones that could be thought of as cellular mouths.

Nonvisual arrestins can bind to the coated pit components clathrin and AP-2, raising the possibility that arrestins induce the formation of new coated pits that internalize GPCRs. But, using fluorescently labeled proteins, the authors determined that GPCR-arrestin complexes relocate to preexisting coated pits after GPCR stimulation, rather than forming new coated pits around the receptors. Once the receptors reach the preexisting pits, the pits become more numerous and clustered. Conditions that disrupt the cortical actin membrane skeleton prevent coated pit clustering. Santini and colleagues suggest that an actin-dependent mechanism forms discrete membrane domains called coated pit zones, possibly analogous to membrane rafts, and that receptors are moved into these zones for internalization.

If you stretch it, they will come

There are two models to explain how cells convert the physical forces of substrate adhesion into biochemical signals: force may open ion channels to induce localized changes in ion concentration across the plasma membrane, or physical distortion of the cytoskeleton may affect the signaling proteins associated with it. On page 609, Sawada and Sheetz provide significant new support for the second model.

The authors grew cells on collagen-coated silicon, and then used detergent to strip the cells down to their cytoskeletons. When the cytoskeletons were stretched 10% and incubated with cytoplasmic proteins, the proteins bound at distinct spots. Relaxed cytoskeletons produced a different protein binding pattern. Biochemical analysis identified a distinct subset of proteins, including paxillin, focal adhesion kinase, and p130Cas, that bind in a stretch-dependent manner. Confirming the relevance of the system, the stretch-dependent binding pattern of GFP–paxillin appears identical in vitro and in vivo.

The absence of a cell membrane in these experiments rules out the involvement of changes in ion concentration, and suggests that matrix forces directly cause conformational changes in the cytoskeleton that can alter protein binding patterns. Although cytoskeletal binding alone could drive matrix-induced responses, the new data do not exclude the possibility that ionic changes could also influence force-dependent signaling.