In This Issue

**Wnt separate ways, met later**

Members of the Wnt family of secreted signaling proteins control a wide range of developmental and pathological processes, with each Wnt protein signaling through either the “canonical” or “noncanonical” pathway. Two papers in this issue (and a Comment on page 753) now bring these two pathways together, showing that the noncanonical can directly antagonize the canonical to regulate signals critical for vertebrate body axis determination, limb development, and possibly oncogenesis.

The canonical Wnt pathway stabilizes the signaling protein β-catenin against degradation, whereas the noncanonical pathway has been considered largely β-catenin independent, operating instead through a network of calcium-dependent intermediates.

Westfall et al. (page 889) identified the noncanonical Wnt family members in zebrafish and found that a loss of function in one of them, Wnt-5, leads to an increase in β-catenin activity and activation of genes downstream of the canonical pathway. When Wnt-5 is absent from both the mother and the zygote, embryos become hyperdorsalized, showing that this noncanonical Wnt signal is required for proper embryonic axis formation.

Topol et al. (page 899) found that in mice the loss of a homologous gene, Wnt-5a, leads to an increase in canonical Wnt signaling in the distal limb bud. The unchecked canonical signal then inhibits chondrogenesis, causing defects in limb development. Analysis of cultured mammalian cells confirms that Wnt-5a signaling decreases β-catenin activity.

In both systems, the noncanonical signal increases β-catenin degradation, thereby inhibiting the canonical pathway and allowing development to proceed normally. Topol et al. also show that this activity of Wnt-5a requires APC, suggesting that Wnt-5a could also be an oncosuppressor. The authors are now trying to determine whether Wnt-5a is mutated in any human tumors. Meanwhile, Westfall et al. hope to use the zebrafish model to identify intermediates in the noncanonical Wnt pathway.

**Linking formins to Arp2/3**

F-actin filaments can be induced to form by either of two pathways, but the elaborate cytoskeletal rearrangements seen in live cells imply that these mechanisms must somehow be coordinated. Now, Carnahan and Gould (page 851) provide the first evidence that a single, highly conserved protein links these two pathways during cytokinesis.

Previous work has shown that both the Arp2/3 complex and the formin family of proteins can induce F-actin nucleation, the rate-limiting step in filament formation. The authors show that in the yeast *Schizosaccharomyces pombe*, the protein Cdc15p interacts directly with both the formin Cdc12p and an Arp2/3 complex regulator. Both Cdc12p and the Arp2/3 complex are essential for forming the cytokinetic actomyosin ring, a structure required for cell cleavage. The Cdc15p–Cdc12p complex appears in a medial structure in cells before ring formation, and Cdc15p is also required for the medial localization of Arp2/3 complex regulators. Cdc15p is highly phosphorylated in interphase, but becomes dephosphorylated early in mitosis.

The authors propose that dephosphorylation of Cdc15p allows it to associate with Cdc12p and initiate formation of a primary F-actin ring during metaphase. Arp2/3 could then join the complex, driving the maturation of the ring in late anaphase. In interphase, Cdc15p is distributed in a pattern very similar to that of actin patches, raising the possibility that it also coordinates cytoskeletal rearrangements at other times in the cell cycle.

As Cdc15p is the founding member of a highly conserved family of proteins, similar mechanisms are likely to be at work in many types of eukaryotic cells. Cdc15p may simply be a scaffold that brings Cdc12p and Arp2/3 together, or it could also have a catalytic function.
A prolific nucleator program

In studies of Drosophila cells, aurora-A kinase and centrosomin (CNN) are sometimes used as markers for centrosomes, but, other than their localization, relatively little is known about these proteins. On page 757, Terada et al. show that aurora-A and CNN must physically interact to be translocated to the centrosome, and, once there, CNN acts as a powerful microtubule nucleator, placing these two convenient markers at the center of centrosomal function.

Using a two-hybrid screen, the authors discovered that aurora-A specifically binds to a COOH-terminal domain of CNN, and RNAi inhibition of either protein prevents the translocation of the other to the centrosome. The NH$_2$-terminal half of CNN interacts with the $\gamma$-tubulin complex. When overexpressed in fly or mammalian cells, or in an in vitro system, CNN induces microtubule nucleation with striking efficiency.

Terada et al. speculate that the two functionally distinct domains of CNN connect the $\gamma$-tubulin complex to aurora-A and the centrosome. Although the natural CNN analogues in mammalian cells have so far eluded detection, the ability of Drosophila CNN to induce microtubule nucleation in mammalian cells underscores the evolutionary conservation of this mechanism. The authors hope to use their new in vitro system to determine how the nucleating site is constructed.

Overexpressed CNN (yellow) nucleates microtubules (green).

Bcl-w buries its tail, lets cell die

Cells contemplate suicide by monitoring the opposing signals of pro-apoptotic and pro-survival members of the Bcl-2 protein family, but how are the pro-survival signals turned off when a cell initiates apoptosis? On page 877, Wilson-Annan et al. report that one pro-apoptotic signal causes Bcl-w, a pro-survival Bcl-2 protein, to stick itself into a membrane and become inert.

Bcl-2 is an integral membrane protein, so it has long been assumed that its close relatives are also membrane integrated. However, the authors found that in healthy cells Bcl-w only associates loosely with membranes. Inducing apoptosis, however, causes Bcl-w to integrate into the mitochondrial membrane, an effect that is mimicked in cell lysates treated with a peptide from a BH3-only pro-apoptotic protein. Chimeric proteins with a BH3 domain attached to the NH$_2$ terminus of Bcl-w also integrate into membranes. Membrane-bound Bcl-w does not become pro-apoptotic, but it loses its pro-survival activity.

These results, and the recently solved structure of Bcl-w, suggest that Bcl-w normally keeps its hydrophobic COOH terminus folded into a groove on its surface. Binding to a BH3-only protein releases the COOH terminus, which then inserts into a membrane and neutralizes the pro-survival activity of Bcl-w.

Unpublished data support a similar type of regulation for the pro-apoptotic protein Bcl-xL.

Some clathrin spots (red) remain in cells lacking AP-2.

AP-2 gets demoted

The conventional view of clathrin-mediated endocytosis holds that the adaptor complex AP-2 is uniquely able to recruit clathrin to the plasma membrane to form coated pits. Using two different approaches, Motley et al. (page 909) and Conner and Schmid (page 773) now disprove this model. Instead of being an irreplaceable component of clathrin-coated pits and vesicles, AP-2 appears to be just one of several adaptors, and not all endocytic cargoes require it.

Using siRNA, Motley et al. knocked expression of an AP-2 subunit down to undetectable levels and found that this inhibits the endocytosis of transferrin receptors, but surprisingly does not abolish coated pit formation or inhibit the internalization of EGF receptors or an LDL receptor chimera. Conner and Schmid found that overexpression of the adaptor associated kinase AAK1 in HeLa cells interferes with AP-2 function, apparently by sequestering AP2 complexes and preventing them from clustering on the plasma membrane. This also blocks transferrin endocytosis but does not stop coated pit formation or internalization of the EGF receptor.

The results show that although some proteins require AP-2 for internalization, others do not, suggesting that alternative adaptors can drive coated pit formation and endocytosis.