Interdependence in Cytokinesis

On page 1391, Jantsch-Plunger et al. describe a protein that regulates both the structure of the late mitotic spindle and the function of the contractile ring. CYK-4, therefore, conceptually links microtubules to actin, and mitosis to cytokinesis.

Worms with a cyk4<sup>ts</sup> mutation were isolated in an embryonic-lethal screen. In the first embryonic division, the mutant cells form a cleavage furrow that ingresses but then regresses. The central spindle, a set of microtubule bundles that normally lies between the separating genomes, is reduced and disorganized. In wild-type cells, both CYK-4 and the kinesin-like protein ZEN-4 localize to the central spindle, and they rely on each other for their localization. CYK-4 may help to cluster multiple copies of ZEN-4 so the ZEN-4 can assemble anti-parallel microtubules into a central spindle.

Although CYK-4 is required for organizing the central spindle, the protein contains a Rho family GTPase activating protein (GAP) domain and has GAP activity in vitro. The central spindle, which is required for a late step in cytokinesis, could act as a localized source of CYK-4. The CYK-4 should decrease RhoA activity, perhaps helping to disassemble the contractile ring and complete cytokinesis.

Cdc28 Activates Mitotic Exit

Early cell cycle experiments implied that cyclins plus Cdc2 (Cdc28 in budding yeast) induced not only the entry into but also the exit out of mitosis. Later results have shown, however, that active Cdc28 inhibits the anaphase to G1 transition by phosphorylating a protein called Hct1. The phosphorylated Hct1 fails to partner with the anaphase promoting complex (APC) to achieve the destruction of mitotic cyclins.

Rudner et al. (page 1361) and Rudner and Murray (page 1377) explain this paradox by showing that Cdc28 first activates the binding of Cdc20 to the APC (either Hct1 or Cdc20 binding is needed to activate the APC). This complex initiates three events: the destruction of Pds1 (and therefore the separation of chromosomes), a first phase of cyclin destruction, and the activation of the Hct1-containing APC that will take the cell out of mitosis.

Rudner et al. start with Cdc28-VF, a mutant form of the protein that lacks two negative regulatory phosphorylation sites. These sites were thought to help in turning off Cdc28 at the end of mitosis, particularly after an arrest caused by spindle defects, but Rudner et al. show that Cdc28-VF cells are slow to exit mitosis even in a normal cell cycle. The delay is not seen when other non-phosphorylatable residues are substituted in the same positions, or when the responsible kinase is mutated. Thus, the delay with Cdc28-VF appears to be a result of structural changes to the protein, and indeed the kinase activity of Cdc28-VF/cyclin complexes is half that of wild-type.

A Plant Vacuolar Sorting Receptor

Vacuolar targeting is more complex in plants than in other organisms as there are both lytic and storage vacuoles in the same cell. On page 1335, Ahmed et al. show that AtELP, an Arabidopsis thaliana protein, is a vacuolar sorting receptor specific for the NH<sub>2</sub>-terminal propeptide (NTPP) signal that probably directs proteins to the lytic vacuole.

A tELP interacts with NTPP sorting signals of both heterologous and endogenous vacuolar proteins, and colocalizes by immunogold staining with an NTPP-containing sweet potato.
protein. In contrast, AtELP does not bind to COOH-terminal propeptide (CTPP) signals (which are thought to direct proteins to storage vacuoles) and does not colocalize with a CTPP-containing barley protein. The sequence-specific binding of AtELP to NTPP is pH dependent, and should, therefore, be disrupted by the acidity of vacuolar compartments. If true, this postulated acid-dependent dissociation would mimic the dissociation seen during vacuolar transport in other species, although the receptors and recognition systems are entirely different.

GMC Proteins and PIP$_2$

GAP43, MARCKS, and CAP23 (the GMC proteins) have been implicated in actin-based motility, nerve growth, and synaptic plasticity, but their mechanism of action has remained obscure. On page 1455, Laux et al. suggest that one key activity of GMC proteins may be their masking of the phosphatidylinositol lipid PI(4,5)P$_2$, thus freeing up various actin-interacting proteins so that they can promote protrusion.

Laux et al. find that GMC proteins and P1(4,5)P$_2$ are codistributed on the plasma membrane and can be isolated in a raft fraction. (These rafts lie in the inner leaflet of the plasma membrane and should not be confused with sphingolipid-based signaling rafts in the outer leaflet.) Cells overexpressing GMC proteins show larger P1(4,5)P$_2$ clusters even in the absence of actin fibers, and increased formation of actin-based filopodia and spikes. Sequestration of P1(4,5)P$_2$ by neomycin also results in recruitment of actin to the periphery, leading Laux et al. to suggest that the GMC proteins normally carry out this sequestering function. How additional signals then regulate GMC function to produce localized outgrowths is still unknown.

Frey et al. suggest that the GMC proteins may have similar functions despite their complete lack of homology (page 1443). The proteins share only expression patterns, colocalization, reports of actin-structure induction, and the presence of a stretch of basic and hydrophobic residues (the effector domain). Frey et al. find, however, that the low survival rate, reduced body weight and at least some of the defects in nerve sprouting seen in mice lacking CA P23 can be alleviated by knockin replacement with GAP43.

Neurons Regulate Astrocytic Communication

Glia, such as astrocytes, used to be thought of as structural filler. Gradually their active modulation of neuronal function and signaling has come to light. On page 1513, Rouach et al. report on the other half of this conversation: active neurons increase the amount of gap junction communication (GJC) between astrocytes and, thus, the extent of the calcium waves that propagate through astrocytes. Another means of modifying GJC is reported by Lampe et al., who find that phosphorylation on a specific site on the gap junction protein connexin 43 (Cx43) by protein kinase C changes single channel behavior and decreases cellular communication (page 1503).

Rouach et al. find that the increase in astrocytic GJC coincides with elevated expression of Cx43, although a decline in GJC after neuronal activity is blocked does not coincide with a drop in Cx43 levels. The GJC increase is not caused by diffusible messengers and takes more than a week of coculture to manifest itself. Pharmacological treatments that prevent communication across synapses, but not necessarily spontaneous spiking activity within a neuron, abolish the neuronal-induced increase in GJC. Thus, synaptic communication may lead to a change in a membrane protein (such as an adhesion molecule) which then alters GJC in astrocytes. Further work will focus on identifying these putative membrane proteins, and determining a function for the mysterious astrocytic calcium waves.

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