In Brief

Integrin Crosstalk

Integrin crosstalk, in which ligation of one type of integrin changes the activity of another integrin on the same cell, has been observed in many cell types under a variety of conditions, but the molecular mechanism for this phenomenon has remained obscure. Now Blystone et al. (page 889) have found that the ligation of the integrin $\alpha_v\beta_3$ inhibits the activity of calcium-calmodulin kinase II (CamKII), an enzyme essential for transducing signals from the $\alpha_5\beta_1$ integrin on the same cell.

In cells that express only $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins, ligation of $\alpha_5\beta_1$ induces cell migration and phagocytosis, and Blystone et al. show that the activity of CamKII is required for transduction of this signal. The signal can be blocked by CamKII inhibitors or by ligation of the $\alpha_v\beta_3$ receptor, and the cytoplasmic tail of the β_3 portion of the integrin is necessary and sufficient for this crosstalk. Finally, a constitutively active form of CamKII prevents the $\alpha_v\beta_3$-mediated suppression, supporting the idea that CamKII is an essential mediator of crosstalk in this system.

Scott Blystone, first author on the study, suggests that crosstalk may have evolved as a necessary component of integrin signaling networks: "It is my belief that crosstalk provides a mechanism for integrins to know what each other are doing, and to make sure their individual functions are coordinated in some manner which results in a net cell behavior. I would imagine this trans-regulation evolved as the integrin family increased in number and complexity."

Mitochondrial Modulation of Calcium Signaling

Using fluorescent dyes to follow the propagation of Ca^{2+} waves in adult rat cortical astrocytes, Boitier et al. (page 795) have found that mitochondria in these cells can buffer calcium-mediated signals, slowing their propagation by as much as fifty percent. As astrocytes appear to have a key role in transmitting signals through the brain, the findings may have broad implications for neuroscience and pathology.

By following Ca^{2+} localization after physical or chemical stimulation of the astrocytes, Boitier et al. found that mitochondria take up and retain Ca^{2+} from the cytoplasm, acting as high-capacity Ca^{2+} buffers. Depolarizing the mitochondria blocks this activity, and causes Ca^{2+} waves to propagate significantly faster through the cell. The authors suggest that modulating the activity of astrocyte mitochondria could therefore play an important role in controlling information processing in the brain. "What we had in mind was mitochondrial depolarization which could result most obviously from anoxia but also from [nitric oxide] production," says Michael Duchen, senior author on the paper. In this model, a mitochondrial dysfunction could also contribute to the pathogenesis of CNS disorders such as ischemia and epilepsy. The team is now examining the propagation of Ca^{2+} signals between cells in astrocyte monolayers.

A Natural Apoptotic Pathway in Yeast

Apoptosis, or programmed cell death, has long been viewed as essential for the development of higher eukaryotes, but the altruistic phenomenon seemed to be limited to multicellular organisms. New work by Madeo et al. (page 757) suggests that apoptosis, mediated by reactive oxygen species (ROS), developed before the evolutionary separation between fungi and metazoans, and that a natural apoptotic pathway exists in the yeast *Saccharomyces cerevisiae*.

Artificial expression of metazoan apoptosis-inducing gene products in yeast has previously been shown to produce phenotypic changes similar to those seen in apoptosis, but the mechanism of this process, and its relevance, remained open questions. Madeo et al. show that these exogenous gene products act through intracellular ROS in yeast to cause apoptosis. Directly increasing the quantity of ROS in the cell with low doses of H_2O_2 produces the same effect. As in metazoans, yeast apoptosis requires new protein synthesis, indicating that it is an active process.

"The function of ROS at the top of the regulatory pathway in yeast may correspond to the 'downstream' function in mammals, so that only the ensuing steps (including nuclease action, membrane alteration, destruction of the nuclear skeleton) are present in yeast," says Kai-Uwe Fröhlich, senior author on the report.

In the wild, *S. cerevisiae* eliminates competitors of other species by producing ethanol, so that by the end of fermentation, the yeast is growing in a nearly pure culture. Fröhlich hypothesizes that, under these conditions, ROS may trigger apoptosis in damaged or stressed cells, increasing the overall fitness of the population.

Neurite Induction by a PKC Regulatory Domain

The molecular regulation of neurite growth is still poorly understood. Starting on page 713, Zeidman et al. describe the role of one isoform of protein kinase C (PKC) in the generation of neurite-like processes in cultured neuroblastoma cells, and come to the surprising conclusion that it is this protein's regulatory domain, not its catalytic activity, which induces neurite growth.

By overexpressing PKCα, βII, δ, and ϵ, the four isoforms of PKC found in neuroblastoma cells, Zeidman et al. determined that only PKCϵ induces neurite-like processes. A series of deletion constructs showed that the PKCϵ regulatory domain, rather than its kinase activity, is necessary and sufficient for this induction. One of the deletions also had a dominant negative phenotype and inhibited neurite induction by retinoic acid or growth factors, suggesting that the PKCϵ regulatory domain lies on the

signaling pathway used in normal development. The authors speculate that the regulatory domain may work by binding other proteins and targeting them to the plasma membrane, but Christer Larsson, senior author on the paper, emphasizes that the downstream targets in the pathway are still unknown.

Spindle Pole Body Duplication

Beginning on page 809, Adams and Kilmartin describe an extensive series of experiments that reveal some of the key components of *S. cerevisiae* spindle pole body (SPB) duplication. The SPB, functionally equivalent to the centrosome of higher eukaryotes, is duplicated on the intact nuclear envelope during yeast mitosis. A structure next to the SPB, called the half-bridge, appears to direct the assembly of a spherical satellite, which is later replaced by the new SPB, but the molecular details of this process were poorly understood.

After studying SPB duplication by EM and determining that a plaque-like structure replaces the satellite at intermediate stages of duplication, Adams and Kilmartin identified the SPB core components by mass spectrometry. Immunoelectron microscopy with GFP-tagged versions of these proteins showed that some SPB components are common to the satellite and the plaque-like structure, suggesting that the new structure is built around the satellite. Finally, temperature-sensitive mutants and overexpression of SPB components show that successful SPB duplication requires the attachment of the plaque-like intermediate to the half-bridge. The authors propose a model in which the binding of the SPB components to either end of the half-bridge ensures that the cell has exactly two separate SPBs.

Senior author John Kilmartin suggests that a newly reported in vitro system for centrosome duplication (Lacey, K.R., P.K. Jackson, and T. Stearns. 1999. *Proc. Natl. Acad. Sci. USA*. 96:2817–2822; Hinchcliffe, E.H., C. Li, E.A. Thompson, J.L. Maller, and G. Sluder. 1999. *Science*. 283: 851–854) may provide a system for testing a similar model for higher eukaryotes. Although the behavior of the centrosome is analogous to that of the SPB, Kilmartin points out that the proteins involved may have highly divergent sequences.

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