Mechanisms of Chemotactic Migration

New PAK Gene Involved in Cytokinesis and Chemotaxis

By screening for genes with homology to yeast Ste20 and mammalian PAK, key cytoskeleton regulators, Chung and Firtel (page 559) identified and characterized a putative PAK family gene in Dictyostelium. The results are the first identification of an essential role of a putative PAK in cytokinesis, and the new gene, PAKa, also appears to regulate myosin II function during chemotaxis.

PAKα colocalizes with myosin II to the cleavage furrow of dividing cells and the posterior of chemotaxing cells, and paka null cells are defective in myosin II assembly. Cells lacking PAKα, or producing a putative dominant-negative form of the protein, produce random lateral pseudopodia during chemotaxis and also fail to undergo cytokinesis in suspension. The assembly of myosin II into the cytoskeleton is also defective in paka null cells, but PAKα does not phosphorylate myosin II.

The results suggest a model in which PAKα phosphorylates and inhibits the activity of MHC-PKC and MHCα, kinases that mediate the disassembly of myosin II fibers. Since PAKα localizes to the posterior end of the cell during chemotaxis, it would inhibit the disassembly of myosin II fibers at the posterior end while permitting disassembly at the leading edge. Other recent work suggests that PAK-family genes have a similar role in mammalian cells (Sells, M.A., J.T. Boyd, and J. Chernoff. 1999. p21-Activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts, J. Cell Biol. 145:837–849).

“Memory” in Leukocyte Chemotaxis

In leukocytes, chemotaxis must take place through a complex array of signals, often resulting in sequential movement from one chemotractant to another. But the mechanisms underlying this phenomenon remain poorly understood. Beginning on page 577, Foxman et al. describe the behavior of neutrophils in an under-agarose as new study, the team found that in SMCs treated with degraded collagen inhibits SMC proliferation, and the authors propose that degradation of collagen fibers may be necessary to promote SMC migration and proliferation. In the new study, the team found that in SMCs treated with degraded collagen fragments, calpain-mediated cleavage of pp125Fak, paxillin, and talin is rapidly induced, coinciding with focal adhesion disassembly and cell rounding. The researchers propose that this calpain-mediated cleavage pathway may regulate the activity of these proteins in the focal adhesion signaling complex in response to matrix

Protein Components of Exosomes

Beginning on page 599, Thery et al. describe the first detailed biochemical characterization of hematopoietic cell exosomes, membrane vesicles secreted by the fusion of late multivesicular endosomes with the plasma membrane. The team found several cytosolic proteins as well as integral and peripherally associated membrane proteins in the exosomes of dendritic cells (DCs), providing clues to the biogenesis and function of the vesicles.

Exosome production has been observed in several hematopoietic cell types, and exosomes produced by DCs exposed to tumor-derived antigenic peptides cause the regression of tumors in mice, but little was known about the molecular components of exosomes or the machinery involved in their production. By trypsin digestion and mass spectrometry, the researchers identified eight major exosomal proteins, including hsc73, a heat-shock protein capable of inducing antitumor responses, and MFG-E8, a soluble protein that binds integrins expressed in DCs and macrophages. “It’s clear that exosomes have molecules on their surfaces, MHC molecules for example, that in principle could induce signal transduction in other cells, like T lymphocytes,” says Sebastian Amigorena, senior author on the paper, but he adds that “we really don’t have any idea of...whether it is restricted to dendritic cells and antigens or whether this could be a more general mechanism of communication between cells.”

Degraded Collagen Fragments and Disruption of Focal Adhesions

In an effort to elucidate the molecular mechanisms involved in the formation of atherosclerotic lesions, Carragher et al. (page 619) studied the effect of degraded collagen fragments on focal adhesions in smooth muscle cells (SMCs). Their results are consistent with a model in which collagen degradation exposes cryptic motifs, which then act through integrins to induce calpain-mediated protein cleavage and focal adhesion disassembly.

The disruption of collagen fibers during atherogenesis is thought to result from increased activation of matrix metalloproteinases. Previous work has shown that fibrillar collagen inhibits SMC proliferation, and the authors propose that degradation of collagen fibers may be necessary to promote SMC migration and proliferation. In the new study, the team found that in SMCs treated with degraded collagen fragments, calpain-mediated cleavage of pp125Fak, paxillin, and talin is rapidly induced, coinciding with focal adhesion disassembly and cell rounding. The researchers propose that this calpain-mediated cleavage pathway may regulate the activity of these proteins in the focal adhesion signaling complex in response to matrix...
degradation in vivo. Elaine Raines, corresponding author on the paper, explains that “the potential for unique roles for degraded forms of the extracellular matrix are just beginning to be appreciated.” The team is now trying to identify the specific collagen fragments capable of inducing the dissolution of SMC focal adhesions.

A Novel Kinesin Motor from Dictyostelium Extracts

Whereas two reports in this issue focus on the behavior of kinesin motors in intact neurons (see Ray et al., page 507, Signor et al., page 519, and the accompanying mini-review by Cole on page 463), Pollock et al. (page 493) report the use of an in vitro assay to isolate and characterize a novel kinesin motor from Dictyostelium extracts that reconstitutes plus-end-directed membrane movement at in vivo velocities. The new system paves the way for further biochemical and genetic studies on organelle movement driven by plus-end-directed motors.

Though in vitro organelle migration assays have been developed in other systems, Ron Vale, corresponding author on the paper, emphasizes that Dictyostelium offers the advantages of “a robust in vitro assay, large starting material for biochemical purification, [and the] ability to readily knock out genes by homologous recombination.” The team began with a biochemical approach, using video microscopy and the in vitro assay to purify proteins from Dictyostelium extracts that stimulate membrane movement along microtubules. One of the purified factors is a novel dimeric kinesin motor, subsequently named DdUnc104, that is closely related to the monomeric kinesins Unc104 and KIF1A. Taking advantage of the ability to genetically manipulate their system, the researchers then disrupted the DdUnc104 gene and discovered that it is the dominant plus-end-directed organelle transport motor in Dictyostelium. They are now using this reconstituted system to dissect the regulation of organelle movement.

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