as recent studies of *Aplysia* central synapses suggest a role for the proteosome on both sides of the synapse (Zhao et al., 2003). Although preliminary evidence suggests that the role of APC/C is in synaptic plasticity (and not development, as reviewed in Kaufmann et al., 2002), it will be interesting to see what signals regulate the function of APC/C itself and how its pre- and postsynaptic functions interplay during synapse remodeling.

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Selected Reading

A New Cytoskeletal Connection for APC: Linked to Actin through IQGAP

Migrating cells reorganize their actin and microtubule cytoskeletons in response to external cues. In this issue of *Developmental Cell*, Watanabe et al. now show a molecular connection between the actin cross-linking protein IQGAP1 and the microtubule-stabilizing protein APC that impacts cells’ ability to migrate into a wound.

Multicellular life depends on cell polarity, but many of the molecular details of polarity establishment remain unknown. Polarity in living cells is created by reorganization of cytoskeletal components, in conjunction with directed intracellular transport and membrane compartmentalization. Cell polarization is turning out to be a prime instance in which two major cytoskeletal polymer systems, actin and microtubules, become physically linked. Answers to how this linkage is achieved are starting to emerge.

Cell migration into a wound is a nice system to study actin-microtubule interactions during cell polarization. It recapitulates wound healing at an organismal level, and it is highly tractable for imaging cytoskeletal dynamics. A paper in this issue of *Developmental Cell* (Watanabe et al., 2004) uses this system to describe a novel connection between actin and microtubules during cell migration. The molecular link comes from binding of the actin crosslinking protein IQGAP1 to the human colon cancer tumor suppressor Adenomatous Polyposis Coli (APC).

IQGAP1 was named for its homology to GTPase activating proteins (GAPs) and its IQ (calmodulin binding) motifs (reviewed in Briggs and Sacks, 2003; Mateer et al., 2003). GAPs inhibit GTPases by increasing their GTPase activity, cycling them to an inactive, GDP bound state. IQGAP1 binds two GTPases, Rac and Cdc42. It turns out, however, that IQGAP1 does not act as a GAP for these proteins. Rather, it appears to inhibit the GTPase activity of Cdc42, maintaining it in an active state, while increasing the amount of active Cdc42 in the cell. Notably, IQGAP1 directly binds actin filaments.
and crosslinks them (Bashour et al., 1997). Based on these and other findings, a consensus is evolving that IQGAP1 acts as a scaffold during cell migration, coordinately binding to multiple partners at the cell’s leading edge.

Kaibuchi’s group recently made the exciting observation that IQGAP1 links actin dynamics with microtubule stability (Fukata et al., 2002). It does so by binding to the microtubule plus end tracking protein (+ tip) CLIP-170 in a complex with activated Rac1 and Cdc42. Interestingly, microinjected fragments of IQGAP displaced CLIP-170 from microtubule plus ends. Their work suggested the attractive idea IQGAP might link actin to microtubules in the leading edge of migratory cells. In their current study, they begin to address this idea.

Watanabe et al. start with the novel finding that IQGAP1 binds to the tumor suppressor APC. This interaction is likely to be preserved in most colon cancer-related APC truncations. Remarkably, IQGAP1 and APC also bind activated forms of Rac1 and Cdc42, but not activated Rho or inactivated GTPases. Consistent with these protein interactions, IQGAP, APC, Rac1, and Cdc42 all accumulate at the leading edge of Vero cells (a monkey kidney epithelial line) during migration into a wound.

What are the functional consequences of these protein interactions? The authors used siRNA knockdown and dominant-negative fragments to show that IQGAP1 and APC localize to the leading edge lamella interdependently. Knockdown of either protein reduced localization of the other. Additionally, reduction of either IQGAP1 or APC prevented the accumulation of actin at the leading edge and hindered cell migration. Thus, both IQGAP1 and APC appear to be involved, directly or indirectly, in polarized actin organization.

To link these findings with their earlier work, the authors imaged GFP-CLIP-170 in cells lacking IQGAP1 or APC. In control cells migrating into a wound, CLIP-170 comets on microtubule plus ends appeared to pause or become immobilized at the leading edge. Removal of IQGAP1 or APC by siRNA eliminated this leading edge immobilization. Instead, CLIP-170 comets rapidly disappeared when microtubule ends entered the leading edge. The effects on CLIP-170 dynamics, as seen in the time-lapse movies, were subtle. Further, the experiments did not distinguish whether changes in CLIP-170 were direct or mediated through an effect on microtubule dynamics (an extremely challenging distinction to make). This is an area where confirmation by other investigators and experiments to determine the hierarchy of interactions among these proteins will be important.

There are several important implications of this study. First, it suggests that IQGAP1 contributes to local actin assembly at the leading edge of migrating cells. Second, it provides a means for recruiting APC to a region of actin crosslinking. Finally, it ties regional actin reorganization to microtubule-stabilizing activities. In addition to binding microtubule plus ends, CLIP-170 promotes microtubule rescues near the cell edge, which biases plus ends to remain near the cell periphery rather than shrinking back to the centrosome after each catastrophe (Komarova et al., 2002). APC also stabilizes microtubules, both directly and via its binding to another + tip, EB1. By binding both CLIP-170 and APC, IQGAP has the potential for complex local control of microtubule stability in the leading lamella (Figure 1).

Connections between APC and the actin cytoskeleton go beyond its connection to IQGAP (reviewed in Nathke, 2004). For example, APC can bind to and stimulate the activity of the Rac GTPase exchange factor (GEF) Asef. Interestingly, another model for selective microtubule stabilization in migrating cells involving Rho, mDia, APC, and EB1 was recently proposed (Wen et al., 2004). Together, these studies motivate additional questions. Do actin-microtubule linkages modulate microtubule dynamics, end-on attachments, or both? Are there really several separate modules for actin-microtubule linkages in the leading edge? Do they act concurrently or sequentially? More likely, is there promiscuous crosstalk among multiple connections to actin and microtubules, and both may act as scaffolds to coordinate actin-microtubule interactions during cell migration?

These studies also raise the question of relevance to APC’s role as a tumor suppressor. Expression of truncated APC fragments in tissue culture cells increased cell migration in Transwell chambers (Kawasaki et al., 2003), and overexpression of full-length APC in the mouse intestine caused increased, disordered cell migration (Wong et al., 1996). APC’s profound effects on cell proliferation, migration and to draw our increasing attention.

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Selected Reading


Nuclear Pore Complex Structure: Unplugged and Dynamic Pores

Nuclear pore complexes (NPCs) are large protein assemblies embedded in the nuclear envelope that act as passageways for transport of molecules into and out of the nucleus. Two new studies, one in *Nature Cell Biology* (Rabut et al., 2004) and one in *Science* (Beck et al., 2004), offer direct evidence that the NPC is a highly dynamic structure.

All molecular traffic between the nucleus and the cytoplasm travels through the nuclear pore complex (NPC). Extensive studies have revealed the protein composition and structure of the NPC. The mammalian NPC is composed of 30 different proteins (nucleoporins) that are arranged in an octagonal structure consisting of a massive membrane embedded ring (the lumenal spoke ring) sandwiched between two thin rings (cytoplasmic and nuclear rings). Eight short filaments protrude from the cytoplasmic ring into the cytosol, and a basket-like structure is attached to the nuclear ring. The lumenal spoke ring contains a central channel of ~50 nm in diameter through which molecular transport occurs. This central channel is often plugged with a particle called the central plug or transporter, which is a controversial component of the NPC. The central plug has been hypothesized to represent material in transit rather than a permanent component of the NPC.

Thanks to the improvements in electron microscopy (EM) methods and instrumentation, the structure of the NPC has been refined over the years. Five different three-dimensional (3D) reconstructions have revealed the fine structure of the NPC (Hinshaw et al., 1992; Akey and Radermacher, 1993; Yang et al., 1998; Stoffler et al., 2003; Beck et al., 2004). While previous 3D reconstructions have been calculated from isolated, detergent-extracted NPCs or from isolated nuclear envelopes, the new 3D reconstruction by Baumeister and coworkers (Beck et al., 2004) has been generated from electron tomography of frozen-hydrated intact Dictyostelium nuclei. These isolated nuclei were shown to be transport competent, and they have thereby opened the exciting possibility of determining the structure of the NPC as it is transporting a cargo. In addition, the steps of nuclear transport can be blocked, and cryo-electron tomographic reconstructions can be conducted to reveal transport-related configurations. Dictyostelium also has the advantage of allowing one to combine genetic, structural, and functional analyses.

The novel 3D reconstruction by Beck et al. (2004) is consistent with previous reconstructions (Yang et al., 1998; Stoffler et al., 2003) and reveals similar features of the NPC structure. However, due to further improvements of cryo-electron tomography pioneered by the Baumeister laboratory, a higher resolution of 8–9 nm was achieved. These improvements include increasing the angular range and decreasing the tilt increments for the collection of images. They also used anisotropic algorithms and procedures for alignments and averaging that accounted for the missing cone (i.e., images cannot be collected by tilting the specimen more than 63° clockwise or counterclockwise). In addition, Beck et al. (2004) took only identical images for their calculations. This yielded a 3D model of the NPC that is less elongated than previous models. Thus, to go from the tip of the cytoplasmic filament to the distal ring of the nuclear basket, a cargo has to travel only ~120 nm instead of the ~200 nm it has been assumed to travel before.

The new 3D reconstruction revealed the cytoplasmic filaments for the first time. They look extremely kinked but shorter (35 nm instead of 50 nm) and more delicate than it had been assumed before. In addition, Beck et al. (2004) could identify two distinct structural states of the NPC. One is with the cytoplasmic filaments bent toward the central channel of the NPC and with a central particle in the same plane as the tip of the filaments. Thus, the filaments appear to be interacting with this central particle (similar to images that showed filaments ushering cargo into the central channel; Panté and Aebi, 1996). In the second structural state depicted by Beck et al. (2004), the cytoplasmic filaments were more disorganized and probably in an extended configuration. The central particle was located inside the central channel.

Based on these two models and the fact that the size, shape, and position of the central particle was highly variable, Beck et al. (2004) concluded that the central particle most likely represents cargo complexes in transit. Based on their 3D reconstruction and their data using atomic force microscopy, a similar conclusion was draw by Stoffler et al. (2003). NPCs without central plugs will account for images in which large cargo (for example, hepatitis B capsids) is seen within the central channel (Panté and Kann, 2002; Rabe et al., 2003). Thus, we now