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Closing the GAP between Polarity and Vesicle Transport

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How are tight junctions maintained? In this issue of *Cell*, Wells et al. (2006) provide intriguing evidence for a new pathway that links polarity proteins and vesicle transport to the maintenance of tight junctions, through the control of Cdc42 by Rich1, a GTPase-activating protein.

Cells organize themselves into a variety of forms to fulfill specific functions in higher eukaryotes. The most fundamental level of organization is the arrangement of cells into sheets, which can be flat, or folded into tubes or cysts, and can be constructed from either epithelial or endothelial cells. The sheets are asymmetric along the axis perpendicular to the plane of the sheet, having distinct apical and basolateral surfaces. The separation between these surfaces is usually maintained by barrier junctions, which in vertebrates are called tight junctions. One of the core components of tight junctions are claudins, transmembrane proteins that attach to one another both between adjacent cells and within each cell. Other transmembrane proteins, such as occludin and junctional adhesion molecules, together with a variety of peripheral membrane proteins, are recruited with the claudins to form mature junctions, the structural organization of which remains largely unknown (Macara, 2004; Margolis and Borg, 2005).

Tight junctions are also associated with a number of conserved proteins that were first identified from genetic screens in flies and worms and that are essential for cell polarization in many different contexts. For example, the polarity proteins Par-3, Par-6, and atypical protein kinase C (aPKC) were all found to be necessary for the first asymmetric cell division of the *C. elegans* zygote, but they are also required for polarization of epidermal cells and neuroblasts in the fruit fly *Drosophila*. The small GTPase Cdc42, which binds to Par-6, is also required for polarization in neuroblasts and for maintenance of the polarized state in the zygote of the nematode *C. elegans* (Macara, 2004). Moreover, several of these polarity proteins, including Par-3, aPKC, Patj, and Pals1, localize to the tight junction and are needed for it to form normally in mammalian epithelial cells. Interestingly, however, depletion of these proteins by RNA interference causes problems only with the initial assembly of tight junctions. Eventually, as if by a ratchet mechanism, junctions can slowly form and are then maintained indefinitely (Margolis and Borg, 2005). These observations suggest that polarity proteins might be required for the establishment of tight junctions but not for their maintenance.

Transmembrane proteins of the tight junctions must be delivered to the plasma membrane in vesicles, and recent studies suggest that they are constitutively recycled into endosomes and back to the plasma membrane. One key component of this recycling pathway is a Rab GTPase, Rab13. Interestingly, dominant-interfering mutants of Rab13, or of an effector protein JRAB, lead to a loss of functional tight junctions in epithelial cells, suggesting a role in their maintenance (Terai et al., 2006). A major unanswered question is how the transmembrane proteins of the tight junction are assembled at the correct location. One might imagine that they are delivered randomly to the plasma membrane and then later localize to the nascent junction; or, alternatively, delivery of vesicles containing tight junction proteins might be spatially restricted. Spatial sorting of vesicles on a large scale has, of course, been studied for many years: epithelial cells send distinct cargoes to their apical and basolateral surfaces to enhance and maintain polarity (Rodriguez-Boulan et al., 2005). However, it is possible that in addition to these superhighways there are many smaller roads that deliver cargo to more limited areas of the plasma membrane. One example of such specialized delivery occurs in the budding yeast, *S. cerevisiae*, which uses a unique class of transport vesicles to deliver the chitin synthase Chs3p only to the bud neck region of the plasma membrane (Trautwein et al., 2006). Another example is membrane insertion into the division plane during cytokinesis. And in a related process, new membrane is delivered to spatially restricted areas during cellularization of the *Drosophila* embryo. It is tempting, therefore, to speculate that specialized vesicles might also deliver tight junction proteins to restricted sites on the plasma membrane, and that polarity proteins provide the necessary spatial cues. Continued recycling of these vesicles might then be required for maintenance of functional tight junctions.

Although genetic screens in flies and worms initially failed to identify any polarity proteins that were clearly associated with vesicle traffic, several

have since turned out to have links to the machinery used in vesicle sorting. Lethal giant larvae (Lgl), a polarity protein in *Drosophila*, has homologs in yeast that associate with the exocyst complex, which delivers vesicles to the plasma membrane (Zhang et al., 2005). And mammalian Lgl interacts with syntaxins, which are also involved in vesicle delivery. Another polarity protein, Discs Large, is required for delivery of new plasma membrane during cellularization in *Drosophila* (Lee et al., 2003); and a third polarity protein, Crumbs, is a transmembrane protein that is presumably delivered by vesicles to the apical surface of epithelial cells, where it specifies apical identity. Moreover, Crumbs expression is regulated by Rab5-dependent endocytosis, and a defect in Rab5 function leads to abnormal apical expansion in *Drosophila* embryonic epithelial cells (Lu and Bilder, 2005).

Yet, is there any link between polarity proteins, vesicle transport, and tight junction proteins? A fascinating new paper by Tony Pawson and colleagues suggests that there is (Wells et al., 2006). A proteomics screen of Cdc42-specific GTPase-activating proteins (GAPs) identified Rich1 as a Cdc42-GAP that binds through a scaffold protein, angiomotin (Amot), to the Patj polarity protein. Other polarity proteins including Pals1, Par-3, and aPKC were also found to be associated with Rich1. Strikingly, a point mutation in the GAP domain, or depletion of Rich1 by RNAi, caused a profound defect in tight junctions—a permanent defect, unlike that induced by depletion of Par-3, Patj, or Pals1 but similar to the effects of Rab13 mutants. This phenotype suggests that Rich1 might play a role in maintenance rather than in the initial establishment of junctions. In support of this idea, cells lacking Rich1 began to form junctions after plating, as judged by Par-3 localization, but the protein became increasingly mislocalized over time. Moreover, when calcium was withdrawn from the cells, to disrupt junctions, the mutant Rich1 induced a more rapid disassembly of tight junctions than did the wild-type protein, consistent with a role in junction maintenance. These defects are selective for the tight junctions because the localization of E-cadherin, which is associated with adherens junctions, was normal in cells expressing the Rich1 mutant lacking GAP activity, and in cells depleted of Rich1.

The similarity between the loss-of-function phenotypes of Rich1 and Rab13 is striking. Could Rich1 be connected to membrane transport? The evidence remains circumstantial but is intriguing. First, the pulldown of Rich1 identified two associated proteins that are established players in endocytosis—CIN85 and CD2AP. These two adapters bind one another and target the EGF and HGF receptors for endocytosis. Second, Rich1 dimerizes with Amot through a C-terminal BAR domain. BAR domains form a curved structure that recognizes and can bind to convex membrane surfaces. BAR domains can also induce curvature in membranes, and most BAR proteins are implicated in some aspect of vesicle traffic. Third, both Rich1 and Amot localize in spots that are concentrated near tight junctions but are also found scattered throughout the cytoplasm. These spots might be endosomes because they partially colocalize with EEA1, which is a Rab5 effector and a component of early endosomes.

Taken together, we speculate that Rich1/Amot are components of a new sorting mechanism that decides whether tight junction transmembrane proteins are recycled back to the plasma membrane or are sent elsewhere such as lysosomes or the trans-Golgi network (Figure 1). The small GTPase, Cdc42, provides the switch. In the GTP bound state, the proteins would be directed to lysosomes, but in the GDP bound state, they would be recycled back to the plasma membrane. We speculate that an unknown GEF switches Cdc42 on, perhaps at the plasma membrane. The polarity protein Par-6 binds Cdc42-GTP and may be linked to a GEF, in a similar manner to the Rac/Pix/PAK complex, in which a GEF and a downstream effector for Rac are coupled to one another. A Rich1 mutant, defective in GAP activity, would prevent the inactivation of this Cdc42, and the tight junction proteins would be misdirected to the lysosome, resulting in the loss of tight junctions. Mutants of Rab13 produce the same phenotype because

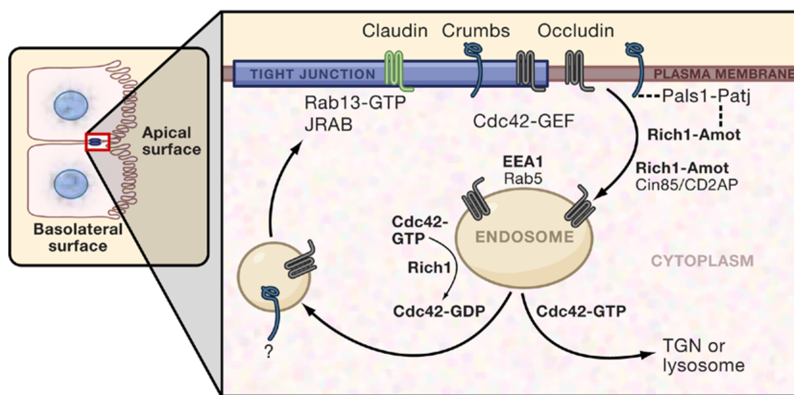

Figure 1. A Model for the Recycling of Tight Junction Components
A Rich1-Amot complex is recruited by Patj/Pals1 to the tight junction of a polarized epithelial cell, where it associates with CIN85/CD2AP or other endocytosis-specific proteins. This event triggers the endocytosis of tight junctions proteins like claudin and occludin. Crumbs might also be included in these vesicles because it would be recruited by its interaction with Pals1 to the site of endocytosis. Simultaneously, Cdc42 could be activated at the plasma membrane, or at the early endosome, by a Cdc42-GEF. Once the tight junction proteins arrive in the early endosome, their fate is determined by the GTP status of Cdc42. Rich1 has to dissociate from Amot to act as a GAP on Cdc42, which then would trigger recycling of the tight junction proteins back to the plasma membrane. However, if Cdc42 is not deactivated, the tight junction proteins will be transported to the lysosome for degradation, or back to the trans-Golgi network. Vesicles carrying the tight junction proteins are specifically targeted to the plasma membrane in the region of the tight junction via a tethering complex, which contains Rab13-GTP and its effector, JRAB.

they block the recycling step. Wells and coworkers also found that Amot can inhibit the GAP activity of Rich1, and a partial depletion of Amot stabilizes the junctions—possibly because the increased rate of Cdc42 GTP hydrolysis leads to more efficient recycling. Thus, the sorting decision might be regulated by the level of Amot that is associated with Rich1. However, as Amot, and possibly Rich1, do not arrive at the tight junctions until several hours after their initial assembly, the sorting and recycling process would not begin until the junctions were intact. Thus, establishment would be separated from maintenance.

The link to Cdc42 is particularly intriguing. Although this small GTPase was, like Rac and Rho, initially implicated in remodeling of the actin cytoskeleton, it is localized primarily to the Golgi apparatus and seems to influence vesicle traffic in numerous ways (Rodriguez-Boulan et al., 2005). It binds coatomer and regulates the interaction of COP1 with dynein (Chen et al., 2005). In addition, dominant-interfering mutants of Cdc42 inhibit basolateral vesicle sorting and stimulate apical delivery. So it is not such a stretch to imagine that it also regulates the decision as to the destination of endocytosed tight junction proteins.

Of course, many questions remain to be addressed. Clearly, the vesicles involved in endocytosis and recycling of the tight junction proteins must be distinct from those that recycle E-cadherin because manipulation of Rich1 or Amot does not interfere with adherens junction assembly or maintenance. But do these vesicles have a specialized coat? Are specific adapters involved? Do the vesicles bud from the tight junction itself, or from above or below it? Do Patj and Pals1 recruit Rich1/Amot to the vesicle exit site, or do they behave as cargo that recycles in association with Crumbs? Are Rich1 and Amot part of a signaling pathway that regulates the stability of tight junctions during epithelial-mesenchymal transitions? Is a Cdc42 switch involved in other specialized vesicle sorting decisions, for instance at the bud neck in *S. cerevisiae*? The paper by Wells and colleagues hints at an entirely new level of control for vesicle sorting that will provide a rich vein for future studies.

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Descrambling DSCAM Diversity

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Neuronal processes exhibit exquisitely complex branching patterns crucial for the formation of distinct neural circuits. In this issue of *Cell*, Chen et al. (2006) show that the isoform diversity of the Dscam protein in *Drosophila* is required to establish stereotypical axonal branching patterns, suggesting that nonrandom expression of *Dscam* alternative splice variants determines neural connectivity.

The human brain possesses around a trillion neurons that together form approximately 10^{15} synaptic connections. To achieve this extraordinary degree of connectivity, neurons must assume complex morphologies to establish specific synaptic contacts in distinct patterns that define functional circuits. The generation of precise neural connectivity patterns depends critically upon the repertoire of guidance cues