

Regulating polarity by directing traffic: Cdc42 prevents adherens junctions from **Crumb**lin' aPart

Mara C. Duncan¹ and Mark Peifer^{1,2}

¹Department of Biology, and ²Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

The GTPase Cdc42 was among the original genes identified with roles in cell polarity, and interest in its cellular roles from yeast to humans remains high. Cdc42 is a well-known regulator of the actin cytoskeleton, but also plays important roles in vesicular trafficking. In this issue, Harris and Tepass (Harris, K.P. and U. Tepass. 2008. *J. Cell. Biol.* 183:1129–1143) provide new insights into how Cdc42 and Par proteins work together to modulate cell adhesion and polarity during embryonic morphogenesis by regulating the traffic of key cell junction proteins.

Nearly twenty years ago Cdc42 was recognized as an essential link between polarity cues and the machinery that generates cell polarity (Bender and Pringle, 1989). As a small membrane-associated GTPase, Cdc42 is well suited to designate where and when a particular activity should occur (Etienne-Manneville, 2004). This is illustrated by its role in polarized cell growth in the yeast *Saccharomyces cerevisiae*. At sites of polar growth, Cdc42 is activated by guanine nucleotide exchange factors. In its GTP-bound form, Cdc42 binds several effectors that help direct polarized cell growth: repolarizing actin and microtubules, directing polarized exocytosis via this reoriented cytoskeleton and by direct contact with exocytic machinery, and recruiting proteins such as septins that form a boundary restricting the region of cell growth. Cdc42 activation is limited temporally and spatially by several GTPase-activating proteins.

In addition to promoting polarity, Cdc42 has acquired additional functions in animal cells (Etienne-Manneville, 2004). Importantly, Cdc42 regulates membrane traffic at numerous sites. At cell membranes, Cdc42 recruits and stimulates activators of the Arp2/3 complex. Cdc42-dependent actin polymerization can drive cell shape change, formation of filopodia, or organelle motility. It also may drive vesicle formation or motility, modulating traffic. Cdc42 interacts with proteins that regulate endocytosis and exocytosis and mediate traffic between ER and Golgi. Inhibiting Cdc42 selectively alters membrane traffic in polarized epithelial cells, affecting some cargos at the trans-Golgi network while influencing others as they recycle between

the plasma membrane and endosomes (Kroschewski et al., 1999; Musch et al., 2001). Cdc42 also affects other aspects of cell polarity, including microtubule dynamics, centrosome positioning, and Golgi reorientation.

Cdc42 can influence polarity through the Par complex. Par proteins, like Cdc42, play conserved roles in cell polarity in many contexts, from early embryos to epithelial apical–basal polarity (Goldstein and Macara, 2007). Par3 and Par6 bind one another and atypical protein kinase C (aPKC). These interactions or localization of the complex may be regulated by Cdc42. This Par complex acts in many ways. For example, Par6 activation of aPKC is thought to stabilize microtubules, helping reorient the centrosome. Recent data suggested a new role. Par6, Par3, aPKC, and Cdc42 were all identified in a screen for proteins required for endocytosis in *Caenorhabditis elegans* oogenesis (Balklava et al., 2007); mutations reduced endocytosis of soluble trackers and changed localization of recycling endosome proteins, suggesting the Cdc42–Par–aPKC complex regulates membrane traffic.

Tepass' laboratory now expands upon the Cdc42–Par connection, exploring its biological importance in cell adhesion and mechanisms underlying it. In our current model, cell adhesion is primarily mediated by cadherin–catenin complexes at adherens junctions (AJs); cadherins mediate homophilic adhesion and catenins mediate cytoskeletal interactions. This model describes an excellent way to glue cells together and make them stiff, but animal cells are neither immobilized nor fixed in shape. A critical challenge is to expand this model to accommodate dramatic cell rearrangements and shape changes accompanying morphogenesis, for which the fruit fly *Drosophila* provides an excellent model. This model must also accommodate the fact that AJs are only one of several junctional complexes that establish and maintain cell polarity (Tepass et al., 2001; Iden and Collard, 2008). The Crumbs–Stardust complex and Par6–aPKC both localize apical to AJs. Both complexes are critical for maintaining epithelial polarity. In Crumb's absence, AJs assemble but do not coalesce into belt AJs, and polarity is lost (Tepass, 1996).

One mechanism critical in morphogenesis is assembly and disassembly of AJs by protein trafficking (Fig. 1). Experiments in cultured cells and in vivo explored how cadherins

Correspondence to Mark Peifer: peifer@unc.edu

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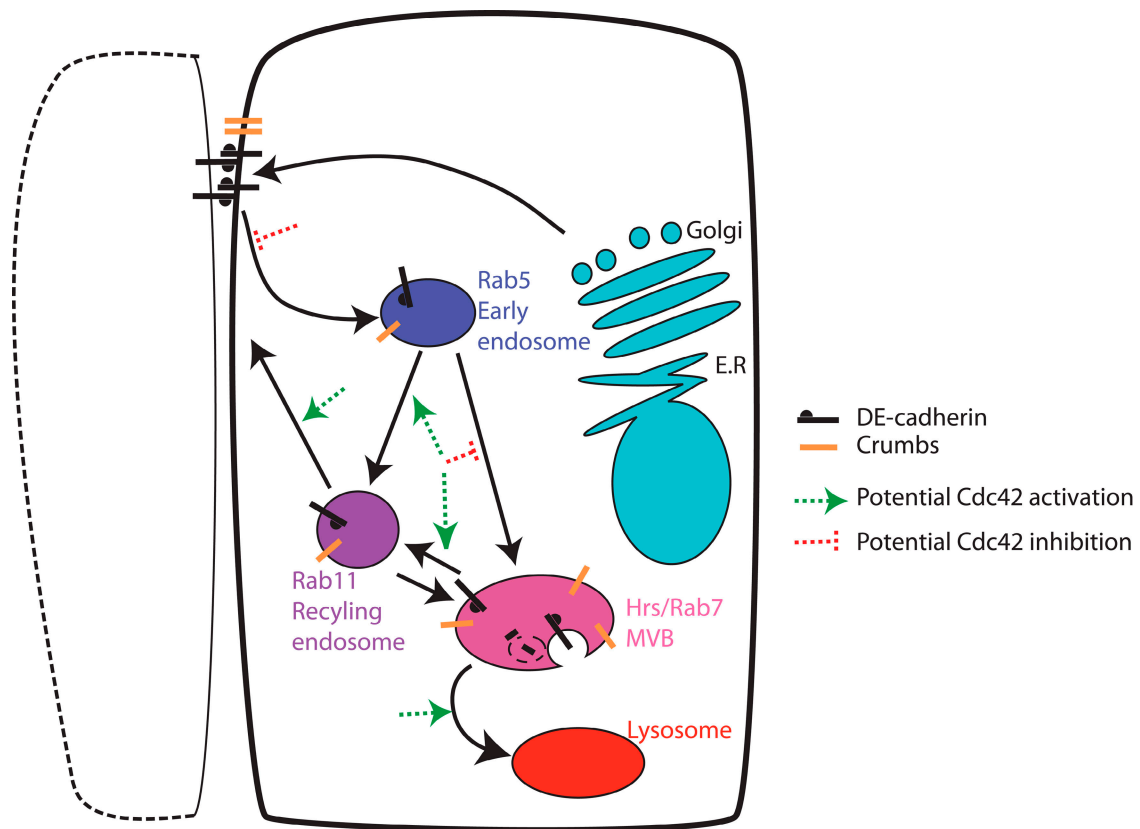


Figure 1. **Normal traffic of junctional proteins and places Cdc42 may influence this process.** Newly synthesized junctional proteins are delivered to the plasma membrane where they assemble into AJs and other apical junctions. Endocytosis, stimulated in remodeling epithelia, removes junctional proteins from the plasma membrane, and they traffic in a Rab5-dependent manner to the early endosome. Recycling from the Rab11-positive endosome returns junctional proteins to the plasma membrane where they can reassemble. Proteins that fail to recycle traffic to the Hrs-positive compartment and are sorted into multivesicular bodies and eventually degraded. Cdc42 function prevents accumulation of Crumbs in the Hrs-positive compartment and maintains steady-state levels of both cadherin and Crumbs at the cell surface, suggesting it acts in traffic before the Hrs-positive compartment, possibly by inhibiting endocytosis or promoting traffic to or recycling from the Rab11 endosome. Possible actions of Cdc42-Par complexes are illustrated by dashed lines.

are trafficked (Yap et al., 2007). Like other transmembrane proteins, cadherins are synthesized in the ER, modified in the Golgi, and trafficked to the basolateral cell surface. Once at AJs, cadherins can be endocytosed into Rab5-positive early endosomes, and either recycled back to AJs via Rab11-positive recycling endosomes, or sent via the Rab7- and Hrs-positive multivesicular body to lysosomes for destruction.

Traffic of junctional proteins may play a key role in cell polarity. Inactivating the exocyst subunit Exo84, which should reduce targeting of proteins to the plasma membrane, mimics effects of a *crumbs* mutation. Loss of Crumbs precedes AJ disassembly, suggesting Crumbs is an exocyst target (Blankenship et al., 2007). Several recent papers suggest a key role for the recycling endosome in junctional protein traffic. Cadherin accumulates in enlarged Rab11-positive recycling endosomes when exocyst function is reduced (Langevin et al., 2005; Blankenship et al., 2007). Regulating recycling of junctional proteins can modulate morphogenesis; for example, Rab11 function is turned down in some tracheal cells, to reduce cell surface cadherin and allow cell rearrangement (Shaye et al., 2008). In the male germline, Rab11 function promotes cell-cell interactions with somatic niche cells (Bogard et al., 2007).

Among the regulators of cadherin trafficking are Rho family GTPases including Cdc42 (Iden and Collard, 2008).

Cdc42 has several roles in *Drosophila* morphogenesis. In early embryos it activates Par6-aPKC, helping establish polarity (Hutterer et al., 2004). Studies of Cdc42 function in later embryogenesis with activated and dominant-negative constructs suggested roles in nervous system and muscle development (Luo et al., 1994), and in cell shape changes during dorsal closure (Riesgo-Escovar et al., 1996). Loss-of-function *Cdc42* mutants (Genova et al., 2000) disrupt integrity of the ventral (but not dorsal) epidermis, but do not affect axon outgrowth or dorsal closure, highlighting the need to use both dominant-negative and loss-of-function alleles.

In this issue, Harris and Tepass (see p. 1129) examine Cdc42's roles in morphogenesis. Reducing Cdc42 function by either dominant-negative or loss-of-function mutants disrupts cadherin-based AJs specifically in the ventral ectoderm. Intriguingly, this tissue is very sensitive to reduced cadherin function (Tepass et al., 1996). AJ loss occurred during dynamic cell rearrangements in the ventral ectoderm, due to mitosis and to invagination of cells to form the nervous system. Strikingly, if neuroblast invagination was blocked, ventral epidermal integrity was restored, suggesting that its differential sensitivity is due to the more dynamic nature of adhesion there. Reducing Cdc42 function also disrupted localization of other junctional and apical membrane proteins, including Crumbs, once again specifically

in the ventral ectoderm. In contrast, basolateral junctional proteins were relatively unaffected. Genetic interaction studies suggested that Crumbs is the key target, with effects on cadherin localization a possible secondary consequence of Crumbs loss.

Reducing Cdc42 function dramatically altered traffic of apical proteins. Crumbs accumulated in an enlarged Hrs-positive endosome. Inhibiting Rab5 in Cdc42 dominant-negatives, thus blocking early endocytosis, increased epithelial integrity and partially restored Crumbs and cadherin to the plasma membrane. This suggests that endocytosis is either the target of or upstream of Cdc42 function; in contrast, Cdc42 reduction had no apparent effects on biosynthesis or exocytosis of junctional proteins. Blocking Rab5 function did not eliminate enlarged Crumbs-positive endosomes, suggesting Cdc42 also has targets after endocytosis. Crumbs-positive enlarged endosomes were visible in both dorsal and ventral ectoderm, suggesting that Cdc42 regulates traffic throughout the ectoderm but that the dynamic ventral ectoderm is more sensitive to defective traffic.

Harris and Tepass (2008) next explored whether the Par complex was the key effector. Strikingly, Par3, Par6, and aPKC are all lost from apical junctions in embryos with reduced Cdc42 function, and accumulate at enlarged apical endosomes. To see whether Par proteins were passive targets or active players in the process, they examined loss-of-function in each gene. In all cases, apical proteins accumulated in enlarged endosomes. Reducing levels of these Par proteins also enhanced effects of reducing Cdc42 function. Importantly, constitutively active aPKC partially suppressed effects of Cdc42 reduction, strongly implicating the Par complex as the key effector regulating apical endocytosis.

Harris and Tepass (2008) propose a model in which Cdc42 plays dual roles in regulating traffic of apical proteins. They suggest that it works with the Par complex to slow entry of apical proteins into the endocytic pathway, and also accelerates protein traffic from early to late endosomes (Fig. 1). In its absence, apical proteins are internalized more rapidly, but become trapped in an enlarged Hrs-positive endosome rather than being sent to the lysosome. An alternate explanation is that Cdc42 promotes recycling of proteins back to the cell surface, consistent with the localization of dominant-negative Cdc42 to the enlarged endosome. In remodeling tissue, AJs must be inactivated to allow cells to change partners, but epithelial integrity must not be lost during remodeling. AJ proteins may treadmill, constantly being endocytosed and then recycled back to the plasma membrane, allowing cells to slide in relationship to one another. In *Cdc42* mutants, reduced apical protein recycling would disrupt epithelial organization, with tissues undergoing extensive remodeling most sensitive to this reduction. Because traffic itineraries are complex even in yeast, it will be challenging to definitively assign Cdc42 function to a particular compartment. For example, Cdc42 could help direct cargo into recycling endosomes, with its loss resulting in default traffic into the Hrs-positive compartment. Alternatively, Cdc42 could directly promote recycling, or, as the authors suggest, Cdc42 may act at multiple steps in the process.

This paper raises many exciting new questions. If Cdc42 acts through the Par–aPKC complex, what are its phosphorylation

targets? They could include cargo, trafficking machinery, or regulators of actin polymerization that in turn block or facilitate traffic. The differential effects of Cdc42 on Crumbs versus cadherin are also intriguing. They could reflect different itineraries of the two proteins, with Cdc42 directing the traffic of one or both, or could reflect different sensitivity to lytic features of the endosome. If Crumbs is the direct target, this raises the question of how reducing Crumbs affects AJ stability. Ultimately, cell fate cues must control protein traffic differentially, and identifying how protein traffic is regulated in developing tissues is the next major challenge.

Note added in proof: After submission of this review, two additional papers (Georgiou et al., 2008; Leibfried et al., 2008) were published that also reveal a role for *cdc42* and the Par proteins in endocytosis of junctional proteins, and that further suggest that the Arp2/3 complex is one target of their action.

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