

The interdependence of the Rho GTPases and apicobasal cell polarity

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Abbreviations: AB, Apicobasal; α -cat, Alpha-catenin; AJ, Adherens junction; Amot, Angiomotin; aPKC, Atypical Protein Kinase C; Arp2/3, Actin-related protein-2/3; Baz, Bazooka; β -cat, Beta-Catenin; β 2-syn, Beta-2-syntrophin; Caco2, Human colon carcinoma; *C. elegans*, *Caenorhabditis elegans*; CA, Constitutively-active; CD2AP, CD2-associated protein; Dia1, Diaphanous-related formin 1; DN, Dominant-negative; *Drosophila*, *Drosophila melanogaster*; Dys- β , Dystrobrevin- β ; Cora, Coracle; Crb, Crumbs; Dlg, Discs large; ECM, Extracellular matrix; Ect2, Epithelial cell transforming sequence 2 oncogene; Eya1, Eyes absent 1; F-actin, Filamentous actin; FRET, Fluorescence resonance energy transfer; GAP, GTPase-activating protein; GDI, Guanine nucleotide dissociation inhibitor; GEF, Guanine nucleotide exchange factor; JACOP, Junction-associated coiled-coiled protein; JAM, Junctional adhesion molecule; Lgl, Lethal giant larvae; LKB1, Liver kinase B1; MDCK, Madin-Darby canine kidney; MTOC, Microtubule-organizing center; NrxIV, Neurexin IV; Pals1, Protein associated with Lin-7 1; Par, Partitioning-defective; Patj, Pals1-associated TJ protein; Rich1, RhoGAP interacting with CIP4 homologues; ROCK, Rho-associated kinase; *S. cerevisiae*, *Saccharomyces cerevisiae*; Scrib, Scribble; SH3BP1, SH3-domain binding protein 1; *S. pombe*, *Schizosaccharomyces pombe*; Std, Stardust; Tiam1, T-cell lymphoma invasion and metastasis-inducing protein 1; TEM4, Tumor endothelial marker 4; TJ, Tight junction; WASp, Wiskott-aldrich syndrome protein; Yrt, Yurt; ZA, zonula adherens; ZO, Zonula occludens.

Signaling via the Rho GTPases provides crucial regulation of numerous cell polarization events, including apicobasal (AB) polarity, polarized cell migration, polarized cell division and neuronal polarity. Here we review the relationships between the Rho family GTPases and epithelial AB polarization events, focusing on the 3 best-characterized members: Rho, Rac and Cdc42. We discuss a multitude of processes that are important for AB polarization, including lumen formation, apical membrane specification, cell-cell junction assembly and maintenance, as well as tissue polarity. Our discussions aim to highlight the immensely complex regulatory mechanisms that encompass Rho GTPase signaling during AB polarization. More specifically, in this review we discuss several emerging common themes, that include: 1) the need for Rho GTPase activities to be carefully balanced in both a spatial and temporal manner through a multitude of mechanisms; 2) the existence of signaling feedback loops and crosstalk to create robust cellular responses; and 3) the frequent multifunctionality that exists among AB polarity regulators. Regarding this latter theme, we provide further discussion of the potential plasticity of the cell polarity machinery and as a result the possible implications for human disease.

Introduction

Cell polarity refers to the presence of asymmetry within a cell that can be generated by spatial differences in a variety of subcellular components. Cells must acquire particular types of polarity in order to perform specialized cell functions. The Rho family of GTPases are evolutionarily conserved regulators of numerous types of cell polarity. They act as molecular switches through their ability to cycle between active (GTP-bound) and inactive (GDP-bound) states.¹ Their activation status is mediated by association with one of their many guanine nucleotide exchange factors (GEFs) or GTPase activating proteins (GAPs) that promote activation or inactivation, respectively. Additionally, association with guanine dissociation inhibitors (RhoGDIs) can maintain their inactive state,² and various post-translational modifications can also control their activities.^{3,4} Furthermore, the importance of spatiotemporal regulation of their activities is becoming increasingly apparent, which often appears to be achieved through a complex regulation of both GEFs and GAPs. This review discusses the role of the Rho family GTPases in epithelial apicobasal polarity, focusing upon the 3 best-characterized members, Rho, Rac and Cdc42.

Apicobasal (AB) polarity is the generation of asymmetry along the apical-basal cell axis and is a key feature of epithelial cells. Epithelial cells arrange into multi- or mono-layered sheets in vivo and the formation and continued integrity of these sheets relies on cell-cell adhesion and the establishment and maintenance of AB polarity. This type of polarity occurs upon the generation of 2 distinct membrane domains: the apical membrane faces either the external environment or an organ lumen, while the basolateral membrane contacts neighboring cells or the underlying extracellular matrix (ECM). The acquisition of AB

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polarity is essential for normal epithelial cell shape, proliferation, organization and function, as well as for the maintenance of overall tissue architecture. Consequently, AB polarity defects have been associated with various types of human disease, particularly cancer. Loss of AB polarity is frequently observed in human tumors and is found to be associated with disease progression.⁵

The process of establishing AB polarity is highly interconnected with that of numerous cellular processes, including the establishment of intercellular adhesion protein complexes, the regulation of the actomyosin network, vesicle trafficking, and even spindle orientation during cell division. Consequently, to date, a wide array of signaling pathways have been implicated in

the regulation of AB polarity and Rho, Rac and Cdc42, are all commonplace. Hereafter, we discuss the intimate relationship between the Rho GTPases and AB polarity, highlighting the interdependent nature of their regulation and drawing upon evidence from a variety of systems and experimental approaches.

Establishing Apicobasal Polarity

The establishment of epithelia with correct AB polarity requires the generation of distinct apical and basolateral membrane domains (Fig. 1B). These domains are designated by the

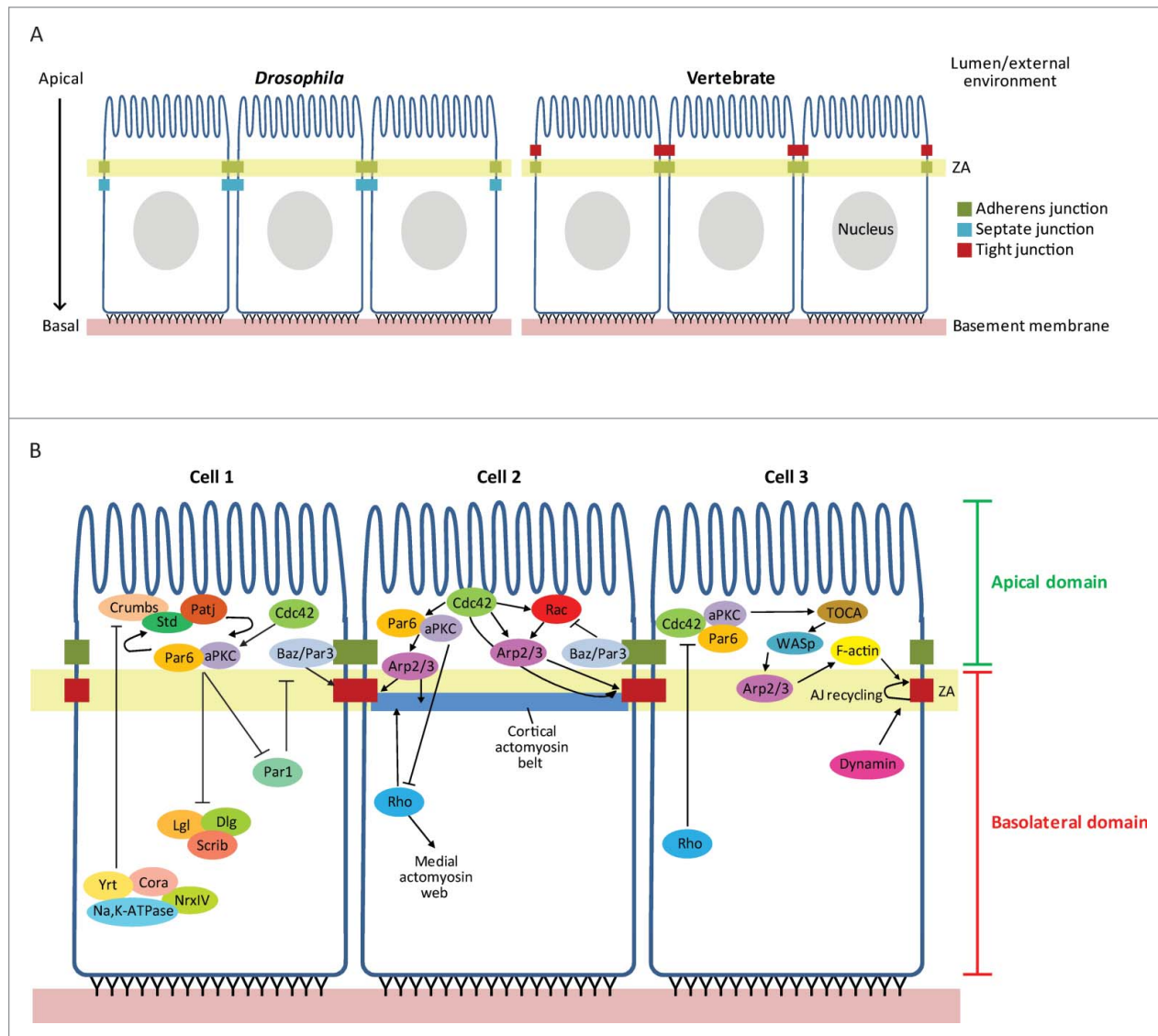


Figure 1. Schematic representations of epithelial apicobasal polarity. **(A)** Epithelial junction organization in *Drosophila* and vertebrate cells. **(B)** Epithelial apicobasal polarity is governed by numerous signaling pathways: Cell 1: conserved protein complexes are required to establish and maintain apicobasal polarity within the cell. Apical and basolateral polarity proteins act antagonistically to one another around the adherens junction (AJ), thereby forming distinct apical and basolateral domains within the cell; Cell 2: the cytoskeleton is also polarized and is regulated by several polarity proteins and Rho GTPases. This spatial regulation of the cytoskeleton is required to maintain cell shape and cell-cell junctions, and is therefore essential for epithelial integrity; Cell 3: Cdc42-Par6-aPKC is required to maintain AJ integrity by promoting the dynamin-mediated endocytosis of junction material, via TOCA proteins and Arp2/3. This allows AJ recycling, thereby promoting junction plasticity.

correct localization of particular polarity proteins and by the formation of cell-cell interactions, which mature into specialized cell-cell junctions (Fig 1A). Cell-cell interactions are particularly important for polarity establishment. It has been shown that cell-cell contact can trigger the correct segregation of apical and basal proteins to their corresponding domains in MDCK (Madin-Darby canine kidney) cells.⁶

It has long been established in a wide variety of systems that AB polarity establishment relies on the mutual exclusion of proteins that define the apical and basolateral domains of a cell (Fig. 1B, Cell 1).⁷ The apical Par proteins: Bazooka (Baz)/Par3, atypical Protein Kinase C (aPKC)/PKC ζ , Par6 (*Drosophila*/vertebrate; note that *l* distinguishes *Drosophila* from vertebrate orthologues hereafter); and the Crumbs complex: Crumbs, Stardust/Pals1, and Discs Lost/Patj, play a role in defining the apical domain. On the other hand, the Scribble complex (Igl, dlg, and scrib),⁸ and the Yurt (Yrt)/Coracle (Cora) group: Yrt/EBP41L5, Cora/EPB41, Na(+),K(+)-ATPase, Neurexin IV (NrxIV),^{9,10} together with Par1,¹¹ establish the basolateral domain (Fig. 1B, Cell 1). Interactions between these functional modules generate zones of mutual exclusion around epithelial junctions: tight junctions (TJs) in vertebrates, adherens junctions (AJs) in invertebrates, to generate an AB asymmetry (Fig. 1A and B, Cell 1). This complex process requires many concurrent events that are controlled in a spatiotemporal manner. Rho, Rac and Cdc42 have all been implicated in various stages of AB polarity generation, with substantial evidence coming from both *Drosophila* and mammalian cell culture studies, as discussed below.

Lumen Formation

When cultured in a 3-dimensional matrix, epithelial cells form spherical cyst-like structures, comprising of a single-layer epithelium surrounding a single central lumen, with their apical domains facing the lumen and their basal domains on the outer surface. This *in vitro* assay effectively recapitulates the organization of epithelial tissues found within the human body. Disruption of AB polarity perturbs this organization, resulting in lumen defects, often manifested as multiple-lumen or no-lumen cysts. Consequently, this assay has been used to identify many regulators of AB polarity, including the Rho GTPases. Here we discuss the various mechanisms by which Rho, Rac and Cdc42 regulate the establishment of AB polarization, drawing upon evidence from lumen formation assays.

Signaling through Rac is important for directing where the apical domain develops, since expression of dominant-negative (DN)-Rac causes a striking inversion of apical polarity in MDCK cell cysts.¹² Rac is thought to achieve proper apical polarity by signaling downstream of β 1-integrin to promote surface laminin assembly,¹²⁻¹⁴ and also by antagonising Rho-dependent actomyosin contractility.¹⁵

Interestingly, during AB polarization, Rac activity becomes differentially regulated along the apical-basal axis, a step that is required for proper polarization.^{16,17} Using a Rac-FRET biosensor to directly visualize Rac activity in live polarizing MDCK

cells, Mack et al. demonstrated higher Rac activity at adherens junctions (AJs) and lower activity more apically at tight junctions (TJs).¹⁶ Low Rac activity at TJs was expected since Chen and Macara had previously reported Par3-mediated inhibition of Tiam1-Rac activity and shown this to be important for TJ assembly.¹⁸ However, Mack et al. also identified β 2-syntrophin as an important activator of the Rac-GEF Tiam1 at AJs and showed that this Tiam1 activator (like Par3)¹⁹ was required for correct AB polarization, since β 2-syntrophin knockdown or the mistargeting of constitutively-active (CA)-Rac to TJs, resulted in cysts with multiple lumens. Consistent with this, Yagi et al. observed lower Rac activity at the apical membrane compared with the lateral, and found that increased apical Rac activity produced cysts with cells within the luminal space.¹⁷ Additionally, they reported that Chimaerin, a GAP for Rac, may be reducing Rac activity apically.²⁰ This differential regulation of Rac activity has also been observed in other systems. In flies, Baz/Par3 was found to inhibit Rac activity apically, via the inhibition of SIF/Tiam1,²¹ consistent with the results from Chen and Macara using mammalian cells (Fig. 1B, Cell 2).¹⁸ Gon et al. also reported a similar differential regulation of Rac activity in intestinal epithelial cells, and intriguingly also found that Rho activity is differentially regulated, but in the opposite direction to Rac.²² Additionally they suggested that Wnt5a signaling promotes polarization of Rac and Rho through Tiam1 and p190RhoGAP, respectively. Together these studies demonstrate the importance of spatiotemporal regulation of Rho GTPase signaling for correct AB polarization.

Cdc42 function is also critical for the polarization of MDCK cells grown in 3D. The following molecular mechanism has been elucidated, whereby PTEN-mediated generation of apically localized PIP2 recruits annexin2, which subsequently binds Cdc42 that then recruits aPKC and Par6.²³ All these components were required to establish the apical membrane and for normal lumen formation. More recently, Cdc42-mediated generation of the apical membrane and a single lumen was shown to require cooperation with membrane trafficking machineries. A Rab11a-directed network was delineated, involving Cdc42, its GEF Tuba, annexin2 and the Par complex, which ultimately directs Cdc42-dependent exocytosis and leads to the delivery of apical markers to the plasma membrane, generating the apical domain.²⁴

Cdc42 also regulates lumen formation through its control of mitotic spindle orientation, which must be tightly controlled so that cells remain in the plane of the epithelium following cell division, thereby maintaining tissue structure and polarity. Caco2 (Human colon carcinoma) cells depleted of Cdc42 produce multiple lumen cysts, as a result of misoriented mitotic spindles.²⁵ Moreover, 2 Cdc42-specific GEFs, Intersectin 2 and Tuba, have both been shown to regulate mitotic spindle orientation and are both essential for normal lumen formation in MDCK cysts.^{26,27} Furthermore, an ECM-Rho-RhoK pathway was recently described that promotes recruitment of the spindle-anchoring complex to align the spindle parallel to the apical surface in MDCK cells.²⁸ Par1B overexpression was shown to inhibit this pathway, leading to tilted spindles and lateral lumen polarity, phenotypes reminiscent of hepatocytic epithelia.

Consistent with this, Par1B depletion in hepatocytes promoted MDCK-like spindle alignment and apical polarity through changes in Rho activity.

All this work follows on from the classic experiments in *Caenorhabditis elegans* (*C. elegans*), which first identified the PAR (partitioning-defective) proteins: a set of evolutionarily conserved proteins that are necessary for establishing an anterior-posterior cortical polarity axis prior to the first zygotic division.²⁹ Subsequent studies showed how both Cdc42 and the PAR proteins regulate mitotic spindle orientation and are involved in the asymmetric positioning of the mitotic spindle during zygotic cell division.³⁰⁻³³ These findings suggest an evolutionarily conserved mechanism for determining mitotic spindle orientation, involving both the Par polarity proteins and the Rho GTPases, which play a key role in determining mitotic spindle orientation in multiple cell types.

It should also be noted that the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) and the fission yeast *Schizosaccharomyces pombe* (*S. pombe*) also rely on Cdc42 activity for their polarized cell growth and division.³⁴ In *S. cerevisiae*, the polarized localization of active Cdc42 defines the new bud site, from which Cdc42 subsequently drives polarized cell growth through its regulation of the actin cytoskeleton, the septins and polarized secretion.

In summary, the Rho family GTPases regulate lumen polarity through a variety of mechanisms, including: spatiotemporal regulation of their activities, as well as the regulation of apical identity, intracellular trafficking, and mitotic spindle orientation. These distinct, yet interconnected cellular events, work together in order to correctly generate and assemble the distinct domains that define the AB polarized state, which in the context of lumen formation, means the formation of a normal single lumen cyst. Intriguingly, close cooperation between the Rho GTPases and the Par proteins is a common theme throughout.

Cdc42-driven Apical Differentiation

The function of Cdc42 in the establishment of AB polarity is largely dependent upon its interaction with the polarity protein Par6, and we have already discussed above its role in regulating apical determination required for normal lumen formation in cultured mammalian cells.^{23,24} Importantly, the Cdc42-Par6 interaction is highly conserved as it has been found in vertebrates as well as *C. elegans* and *Drosophila* epithelia.^{33,35-39}

In agreement with the lumen formation studies,^{23,24} analysis of *Drosophila* embryogenesis has also revealed an essential role for Cdc42-Par6 in apical determination. Establishment of the epithelial apical domain occurs following cellularisation of the *Drosophila* syncytium, at which point all 3 members of the Par complex (Par6, Baz and aPKC) can be found at the apical cell cortex. *Drosophila* embryos expressing mutant Par6 that cannot bind Cdc42 fail to accumulate Par6 or Baz apically and consequently AJs are not formed.³⁹ Similarly, expression of DA- or DN-Cdc42 prevents the apical localization of Par6 and leads to loss of epithelial polarity. In addition, it has been shown that loss

of Cdc42 in mature epithelia leads to the loss of both Par6 and aPKC at the apical cortex.⁴⁰ Cdc42 and Par6 are also likely to regulate AB polarity in *Drosophila* neuroblasts. Despite Hutterer et al. reporting no effect on Par6 localization and polarity by expression of DA- or DN-Cdc42 in neuroblasts,³⁹ Atwood and colleagues later reported that neuroblasts mutant for Cdc42 or expressing mutant Par6 that cannot bind to Cdc42, both failed to localize Par6 at the apical cortex and had lost polarity.⁴¹

In addition to Cdc42's role in correctly localizing apical determinants, some evidence indicates that Cdc42 may also promote apical differentiation through enhancing aPKC activity. In vertebrates, aPKC activity was found to be increased when in a complex with Cdc42 and Par6.⁴² Additional *in vitro* and mammalian cell culture studies showed how Cdc42 binding can cause a conformational change in Par6 that may alter its affinity for aPKC substrates.⁴³⁻⁴⁵ Consequently, there is speculation that Cdc42-Par6 may promote the phosphorylation of aPKC substrates such as Baz, Crumbs and lgl, which are all important for polarity establishment. Supporting this view, *Drosophila* Cdc42 activates aPKC *in vitro* via Par6, and also the aPKC substrate, Mira, displays an unpolarised localization following loss of the Cdc42-Par6 interaction in *Drosophila* mitotic neuroblasts.⁴¹ In contrast however, lgl phosphorylation was still observed in *Drosophila* embryos expressing Par6 that is mutant for Cdc42 binding, indicating that aPKC activity is not impaired.³⁹ Also, aPKC-mediated Baz phosphorylation is reportedly retained following loss of the Cdc42-Par6 interaction during *Drosophila* photoreceptor remodelling.⁴⁶ Ultimately, the importance of Cdc42-Par6 in stimulating aPKC-mediated phosphorylation events required for AB polarization currently remains unclear.

Another mechanism by which Cdc42-Par6 may promote apical differentiation is through regulation of apical Baz/Par3 exclusion (Fig. 2). In *Drosophila* epithelial cells, both aPKC and Crumbs mediate the exclusion of Baz from the apical domain, causing it to localize basal to Par6 and aPKC, and thereby defining the apical-lateral border.⁴⁷ This requires aPKC-mediated phosphorylation of Baz at the conserved serine 980 to disrupt the Baz-aPKC interaction. In addition, it requires Crumbs-mediated prevention of the Baz-Par6 interaction. In the absence of these events, Baz is mislocalised, causing AJ components to localize apically, resulting in loss of the apical domain and expansion of the lateral. A similar mechanism has also been described to drive *Drosophila* photoreceptor remodelling, except Cdc42 was also implicated.⁴⁶ Cdc42 binding to Par6 was required for the correct apical localization of Crumbs, a step necessary for Baz exclusion and the subsequent separation of the subapical membrane from the AJ.

Furthermore, several lines of evidence suggest similar mechanisms also operate in vertebrates. In MDCK cells, Rab11a-positive vesicles have been found to deliver the Crumbs-Pals1-Par3 complex to early lumens,⁴⁸ and Cdc42 and its GEF, Tuba, are required to mediate transport of apical determinants from these vesicles.²⁴ Moreover, another Cdc42 GEF, Dbl3, was recently implicated in driving apical Cdc42 activity during apical differentiation of cultured mammalian epithelial cells.⁴⁹ Enhanced Dbl3/Cdc42 signaling increased apical aPKC and therefore also

Par3 phosphorylation, leading to repositioning of the apical-lateral border.

It should also be noted that *Drosophila* Baz and vertebrate Par3 localize to different junctional structures, with Baz localizing to AJs (Fig. 2, left-hand cell),⁵⁰ whereas Par3 localizes to TJs through binding to JAM (Fig. 2, right-hand cell).^{16,51} However, in both cases Baz/Par3 is localized to the most apical region of the cell-cell junctions, where it functions to define the apical-lateral border (Fig. 2). In summary, the evidence suggests that apical differentiation is controlled by an evolutionarily conserved mechanism involving signaling through a Cdc42-Par6-aPKC-Crumbs-Baz/Par3 pathway, resulting in destabilisation of the Par complex and subsequent segregation of Par6-aPKC into the apical domain, and Baz/Par3 to the apical-lateral border (Fig. 2).

Cell-cell Junctions

At distinct locations along the epithelial AB axis different types of cell-cell junctions can be found, including AJs, TJs (or septate junctions in *Drosophila*), gap junctions and desmosomes. AJs and TJs are important for establishing and maintaining AB polarity. AJs are particularly important for tissue integrity as they provide strong intercellular connections. However, TJs act as a “gate” by allowing selective passage of molecules through the intercellular space, and additionally act as a “fence” by preventing diffusion of molecules between the apical and basolateral domains.

Both AJs and TJs are composed of adhesion molecules with extracellular domains that form homophilic interactions with adhesion molecules on neighboring cells and with intracellular domains that associate with various cytoplasmic scaffolding and signaling complexes in a so-called cytoplasmic plaque, which subsequently associate with and regulate the Rho GTPases (Fig. 3A). In turn, signaling through the Rho GTPases regulates the junctions (Fig. 3A). These junctions also connect to the actomyosin network, forming a belt-like ring around the cell perimeter that provides junctional tension (Fig. 1B, Cell 2). AJs are comprised of cadherin- and nectin-based adhesion molecules, whereas TJs are made up of Occludin, JAM and Claudin (Fig. 3A). E-cadherin is important for the establishment of cell-cell junctions and AB polarity in MDCK cells, however intriguingly it is somewhat dispensable for the integrity of mature junctions.⁵²

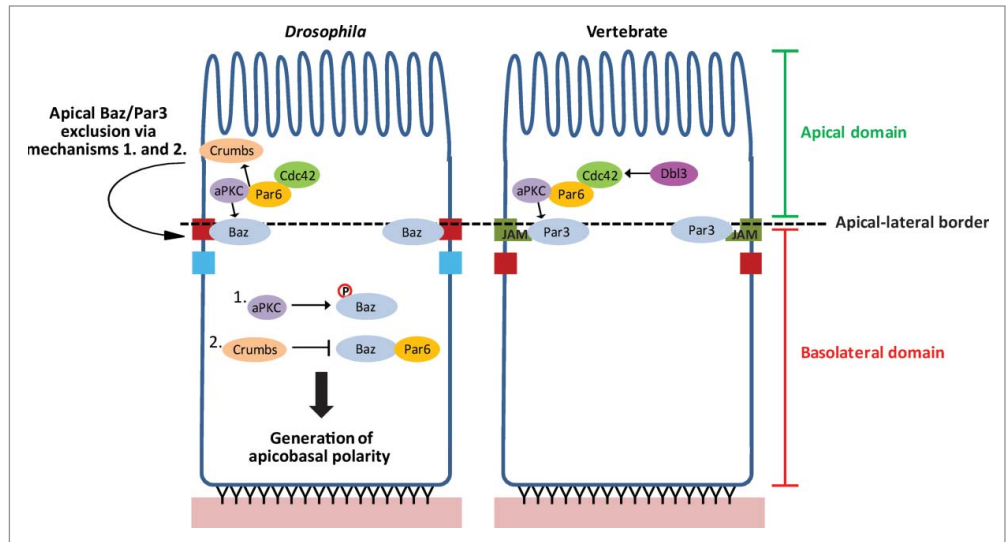


Figure 2. Apical exclusion of Baz/Par3 defines the apical-lateral border. Cdc42-mediated signaling through Par6-aPKC and Crumbs excludes Baz/Par3 from the apical domain and thereby defines the apical-lateral border.^{46,47,49} This process involves 2 mechanisms: 1. aPKC-mediated phosphorylation of Baz at Serine 980 to disrupt the Baz-aPKC interaction, and 2. Crumbs-mediated inhibition of the Baz-Par6 interaction. P = phosphorylation.

The assembly of AJs and TJs are highly interconnected, with the general consensus being that immature AJs are a prerequisite for TJ formation. Epithelial cells initiate their E-cadherin-mediated cell-cell contacts via membrane protrusions. These initial contacts require a local activation of Rac, which drives the formation of actin-based protrusions that carry E-cadherin.⁵³⁻⁵⁸ The immature cadherin-based contacts then locally induce de novo formation of both lamellipodia and filopodia^{53,54,59,60} through remodelling of the local actin cytoskeleton, thereby promoting further E-cadherin interactions. This demonstrates that the promotion of nascent cell-cell contacts requires an intimate relationship between Rho GTPase activity, and the correct localization of both E-cadherin and regulators of the actin cytoskeleton. Active Rac and Cdc42 have been shown to recruit a host of proteins to the site of cell-cell contact (including cortactin, Mena, PAK4, formin-1, and the Arp2/3 complex) all of which promote actin nucleation and remodelling, thereby promoting junction maturation.^{53-55,61,62} The importance of Rho family GTPase activities is maintained as the AJ matures, with the subsequent establishment of both TJs and AB polarity relying on Rho, Rac and Cdc42 activity, as discussed below.

Adherens Junctions

In the mid 1990s, the regulation of AJs by the Rho family of GTPases first began to be appreciated. Rho, Rac and Cdc42 were all shown to localize to and regulate E-cadherin based cell-cell adhesions.⁶³⁻⁶⁸ Subsequent studies confirmed their roles in AJ formation. In particular, Rac was found to be important during the early stages. Several reports have shown that Rac is recruited to and activated by nascent AJs^{58,69,70} and interestingly, its

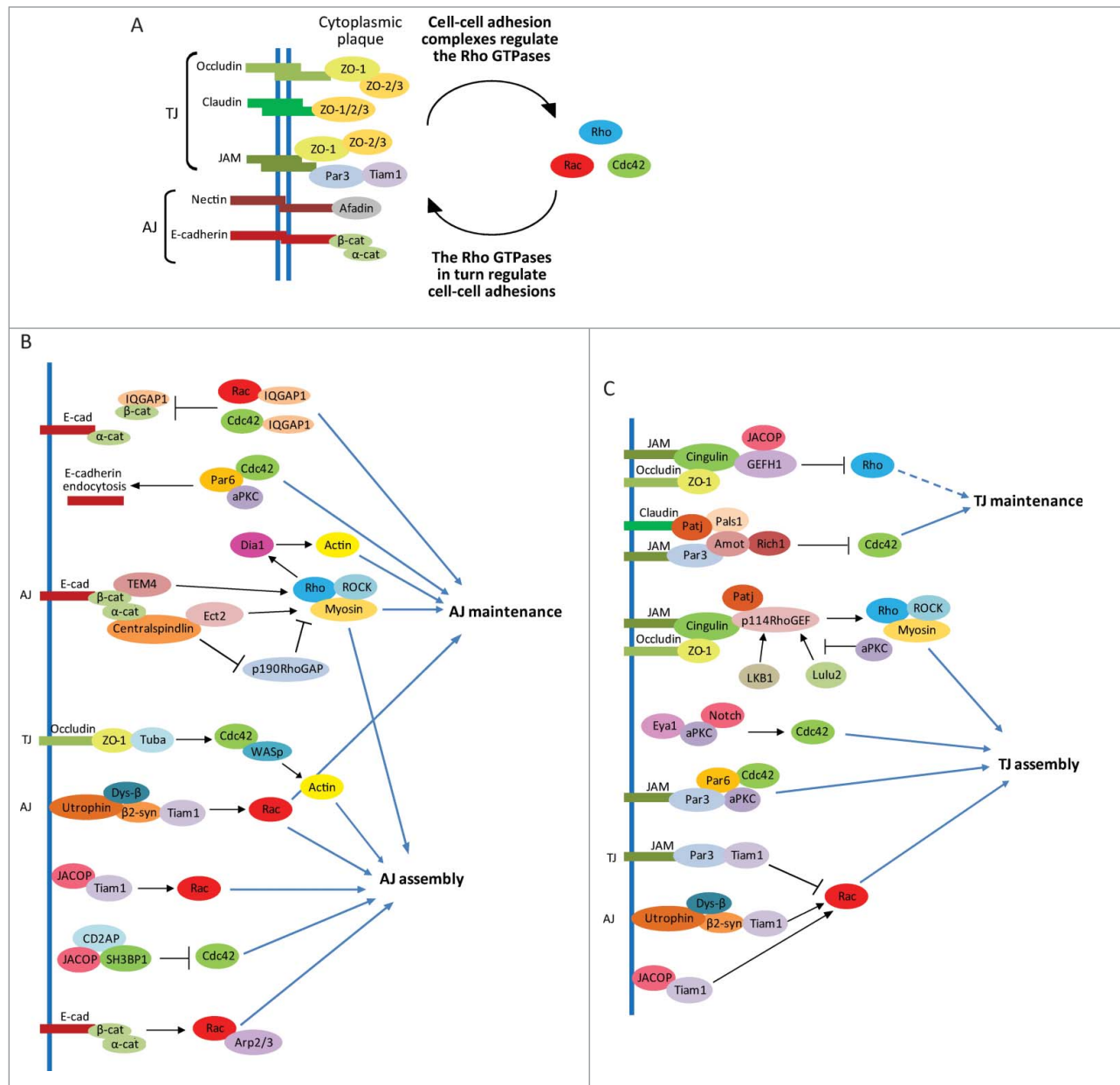


Figure 3. Epithelial cell-cell junctions are regulated by multiple signaling pathways that include the Rho family GTPases. **(A)** Shown are the main constituents of epithelial adherens junctions (AJs) and tight junctions (TJs) that connect to numerous cytoplasmic signaling and scaffolding molecules in a cytoplasmic plaque, which subsequently link the junctions with the Rho family GTPases. **(B and C)** Shown are the numerous molecular mechanisms involving the Rho GTPases that control epithelial AJs **(B)**, and TJs **(C)**. Dotted line represents a speculated link.

activation is reduced as AJs mature.⁷¹⁻⁷³ The initial burst of Rac activity stimulates lamellipodia, which as described above, help initiate cell-cell contacts. However, this burst of Rac activity, specifically localized to de novo cell-cell contacts, is short-lived. Rac activity rapidly diminishes as E-cadherin accumulates at the initial site of contact. However, since active Rac promotes lamellipodia formation, new sites of cell-cell contact form at the periphery, leading to a wave of Rac activation flowing outwards from the initial contact site and thereby driving junction expansion.⁵⁴ Further, Rho and actomyosin contractility are also activated at the contact edges, with Rho activity more peripheral than Rac.⁷⁴ Rac activity

localization correlated with that of lamellipodia and Arp2/3 activity, whereas Rho with phosphorylated myosin and its associated actomyosin contractility. Therefore, Rac and Rho activity, through their respective regulation of actin and actomyosin, are required to drive expansion of cell-cell adhesion. Earlier studies also suggest that Rac-driven actin protrusions precede or occur in concert with, E-cadherin engagement,^{59,60,75-77} and that Rho-driven actomyosin contractility is important for cell-cell adhesion regulation.⁷⁸⁻⁸¹ It is therefore believed that Rac-driven lamellipodia and Rho-driven actomyosin contractility cooperate during the initial assembly and expansion of AJs.

Filopodia may also drive AJ assembly by embedding into neighboring cells with cadherin complexes clustered at their tips.^{53,82,83} This method of cell-cell contact has been shown to be especially important when attempting to bring the free edges of epithelial sheets together, both during embryonic development in *C. elegans* and *Drosophila*,⁸²⁻⁸⁴ and during wound healing in the chick.⁸⁵ These filopodia may be Cdc42-driven, since depletion of one of its GAPs, SH3BP1, increased filopodia formation at immature cell-cell contacts, and interestingly impeded subsequent junction maturation.⁸⁶ SH3BP1 forms a complex with JACOP/paracingulin and CD2AP, which are both required for normal Cdc42 signaling and junction assembly (Fig. 3B). These findings suggest that Cdc42-driven filopodia may be important for driving initial cell-cell contact formation, but then Cdc42 activity must be down-regulated in a spatiotemporal manner for subsequent junction maturation. Consistent with a role in the early stages of AJ assembly, Cdc42 activation has been observed in response to E-cadherin-mediated adhesion in MCF-7 cells.⁸⁷ However, in contrast others have reported Cdc42 is not activated,⁷² or that reduced Cdc42 activity may be necessary for AJ assembly.^{88,89} Possibly the need for both activation and down-regulation of Cdc42 activity at different stages of AJ assembly may explain these discrepancies. Furthermore, in contrast to the SH3BP1 study, Cdc42 is reportedly crucial for AJ maturation in keratinocytes through regulation of aPKC ζ .⁹⁰ Although the role of Cdc42 activity in AJ assembly remains somewhat unclear, a common theme does seem to emerge from the plethora of experiments undertaken in multiple systems: the tight spatiotemporal regulation of Cdc42, Rac and Rho activity seems to be critical to drive the initiation and expansion of cell-cell contacts, as well as the subsequent maturation of the AJ.

In addition to regulating AJ assembly and maturation, the Rho GTPases have also been implicated in the control of AJ integrity and maintenance. Confluent monolayers of MDCK cells have higher Rac and Cdc42 activity than subconfluent cells, suggesting their activities promote junction maintenance.⁷² Moreover, Tiam1-Rac activity seems to be especially important, since it restores AJs and an epithelioid morphology in several cell types,^{68,91} and Tiam1 degradation at AJs is required for Src-induced AJ disassembly.⁹² IQGAP, an effector for Rac and Cdc42, negatively regulates AJ stability by dissociating α -catenin from the cadherin-catenin complex.⁹³ Cdc42 and Rac were found to impede the IQGAP- β -catenin interaction, thereby stabilizing AJs (Fig. 3B).⁹⁴ Furthermore, a Rac-PAK1-Ajuba feedback loop has been described, that reinforces junction-associated Rac activity and thereby promotes E-cadherin stabilization (Fig. 3B).⁹⁵ Cadherin-induced Rac-PAK1 signaling leads to phosphorylation of the Rac-interacting scaffold protein, Ajuba. Phosphorylated Ajuba preferentially binds to activated Rac, thereby helping to maintain active Rac at the junctions.

Rho signaling can also help stabilize AJs. The Rho GEF, Ect2, is specifically recruited to AJs and activated by the centralspindlin complex.⁹⁶ Subsequently, myosin IIA is recruited and activated, resulting in the stabilization of both E-cadherin and junctional tension (Fig. 3B). Moreover, centralspindlin enriches Rho activity at AJs via an interaction with α -catenin and also prevents Rho

inhibition through blocking p190RhoGAP recruitment (Fig. 3B), a process involving reduced Rac signaling. Intriguingly, this molecular ensemble also operates at the contractile furrow during cell division.⁹⁷ More recently, another Rho GEF, TEM4, was also found to regulate AJ integrity through associating with the cadherin-catenin complex (Fig. 3B).⁹⁸ Additionally, several other studies have indicated that Rho activity promotes AJs: RhoA has been found to help maintain AJs via Dial⁹⁹ and non-muscle myosin II (Fig. 3B).⁸¹ Smutny et al. highlighted the importance of myosin II-mediated contraction in promoting junction stability, with different isoforms promoting E-cadherin homophilic adhesion (myosin IIA), and supporting the integrity of the apical cortical actin ring (myosin IIB).¹⁰⁰ Numerous additional studies, carried out both *in vivo* and *in vitro*, demonstrate the importance of Rho activity and actomyosin contractility in maintaining cell-cell junction integrity in both stable and remodelling epithelia.^{74,99,101-109}

In order for epithelial integrity to be maintained, it is now known that even within a mature, stable epithelium, epithelial AJs must be plastic: they require an ability to continually form and disassemble. This is vital, as within a living epithelial sheet there are constant changes in tissue organization due to cell division, cell death and delamination. The inherent plasticity of AJs allows the epithelial sheet to accommodate these changes, as well as when required, to carry out more dramatic morphogenetic movements during tissue remodelling events. It is now known, through studies in several systems, that E-cadherin is constantly being turned over at the junction.¹¹⁰⁻¹¹⁵

Cdc42 activity and the apical Par proteins have been shown to maintain AJ integrity through their control of E-cadherin endocytosis (Figs. 1B, Cell 3, and Fig. 3B). Two studies in the pupal dorsal thorax of *Drosophila* showed that loss of Cdc42, Par6 or aPKC function led to a break-up of the AJ. This was due to disrupted endocytosis in these mutants. Cdc42, Par6 and aPKC therefore appear to promote AJ turnover.^{40,116} In contrast, in the ventral ectoderm of the *Drosophila* embryo, Cdc42 and Par proteins appear to regulate the trafficking of AJ components and apical polarity proteins by limiting endocytosis from the apical membrane.¹¹⁷ This raises the possibility of tissue specific roles for the Cdc42-Par6-aPKC module in the regulation of junction turnover.

Cdc42 is known to regulate the actin cytoskeleton through the activation of WASp, which in turn promotes actin nucleation via the Arp2/3 complex. Evidence suggests that the Cdc42-Par6-aPKC module can promote AJ endocytosis by remodelling the actin cytoskeleton through the local activation of WASp, which drives the dynamin-mediated endocytosis of junctional material (Fig. 1B, Cell 3).^{40,116} Additionally, studies in the *Drosophila* eye have identified extensive crosstalk between Cdc42 and Rho signaling for the regulation of AJs and cell shape in remodelling epithelial cells.^{118,119} Firstly, Cdc42 limits Rho-mediated apical tension by localizing Par6-aPKC to the AJs, where aPKC inhibits Rho activity (Fig. 1B, Cell 2).¹¹⁸ On the other hand, Rho signaling limits Cdc42-Par6-aPKC-driven cadherin endocytosis, which thereby helps to maintain AJs (Fig. 1B, Cell 3).¹¹⁹ The above work demonstrates a surprising level of molecular complexity to

simply maintain AJ integrity, with extensive cross-talk between the Rho GTPases, the Par proteins, regulators of the actin cytoskeleton and endocytic pathways.

The Rho GTPases may also stabilize AJs by helping them tether to the actin cytoskeleton. For many years this was believed to be achieved through actin binding directly to the cadherin-catenin complex via α -catenin. However, this model was challenged when it was revealed that actin doesn't directly bind α -catenin at AJs.¹²⁰ So exactly how AJs physically connect to the actin cytoskeleton remains unknown. The many other actin binding proteins that are known to regulate and/or localize to AJs may be responsible; such as Nectins,¹²¹ Ena/VASP proteins,¹²² formin-1,¹²³ Arp2/3, Utrophin,¹⁶ and TEM4,⁹⁸ and intriguingly all of these have been linked to Rho GTPase signaling.

There is also evidence that limiting the extent of Rho family GTPase activities is also important for AJ stability. Many studies have shown that increased Rac signaling can disrupt AJs.¹²⁴⁻¹³¹ In fact, under some physiological situations high Rac activity appears to drive AJ disassembly, for example during cell scattering^{132,133} and potentially tumorigenesis.¹³⁴ Limitation of Rho activity at AJs by various Rho-specific GAPs has also been suggested to be important for their stabilization.^{86,135} Intriguingly, Rho-ROCK mediated actomyosin contractility may also disrupt AJs.⁹⁹ Together these findings suggest that Rho and Rac must be carefully controlled, likely in a spatiotemporal manner, for proper AJ regulation.

Tight Junctions

The Rho family of GTPases have also been implicated in the control of TJs. Treatment of cultured epithelial cells with CNF-1, which activates Rho, Rac and Cdc42, was found to perturb TJ function and cause displacement of TJ proteins.¹³⁶ Moreover, expression of either DN or CA versions of Rho, Rac or Cdc42 can all perturb TJ function.¹³⁷⁻¹³⁹

Rho in particular is a key regulator of TJs. Back in 1995, inhibition of Rho signaling with the C3 toxin was found to reduce TJ function in human intestinal cells.¹⁴⁰ Since then numerous regulators of TJ-associated Rho signaling have been identified, many of which promote TJ assembly through control of the Rho-ROCK-myosin signaling pathway. For example, p114RhoGEF specifically activates Rho at TJs through its interaction with the TJ-associated adaptor cingulin.¹⁴¹ Depletion of p114RhoGEF mislocalises Rho-mediated myosin phosphorylation, and consequently disrupts TJ assembly (Fig. 3C). Moreover, other Rho GEFs also promote TJs by stimulating Rho-myosin signaling.^{142,143} Further mechanistic details of p114RhoGEF regulation at TJs have also been uncovered.^{144,145} Its localization is regulated by binding the apical polarity protein, Patj,¹⁴⁴ whereas its activity is promoted through binding the FERM-domain containing protein Lulu2,¹⁴⁴ or the polarity regulator and tumor suppressor LKB1 (Fig. 3C).¹⁴⁵ Interestingly, the Lulu2 interaction can be negatively regulated by aPKC.¹⁴⁴ Further, Rho may also be stimulated at TJs by heterotrimeric G proteins.^{146,147}

Rac signaling is also important for proper TJ assembly. Inhibition of Tiam1-Rac signaling by Par3 was found to be required for

efficient TJ assembly in MDCKII cells.¹⁸ As discussed above, Mack et al. found this occurs in concert with β 2-syntrophin-mediated activation of Tiam1-Rac at AJs, which is necessary to drive proper TJ assembly and AB polarization (Fig. 3C).¹⁶ Using epidermal keratinocytes, Mertens et al. provide additional evidence that the regulation of Tiam1-Rac by the Par polarity complex is important for TJ biogenesis.^{148,149} Moreover, the recruitment of Tiam1 to junctions through its association with JACOP/paracingulin also appears to be important for proper regulation of Tiam1-Rac signaling during AJ/TJ assembly.¹⁴⁹

Cdc42 also regulates TJs via numerous signaling complexes. In MDCK cells, the classic Cdc42-Par6-Par3 polarity complex was found to promote TJ assembly.³⁵ Additionally, Cdc42 activity induced by an Eya1-aPKC-Notch1 signaling pathway (Fig. 3C),¹⁵⁰ as well as signaling through its effectors PAK4 and Par6B,⁶² have also been implicated in TJ assembly. Moreover, the Cdc42 GEF, Tuba, localizes to TJs through its interaction with ZO-1, where it regulates junctional configuration as well as the early stages of AJ formation, through stimulation of Cdc42-WASP-driven actin polymerisation (Fig. 3B).¹⁵¹

Additionally, there is evidence to suggest that Rho family GTPase signaling can negatively regulate epithelial TJs. For example, Cdc42 activity can promote HGF-induced TJ disassembly,¹⁵² and increased Tiam1-Rac signaling can perturb TJ assembly.^{16,18} Moreover, in contrast to p114RhoGEF, another Rho GEF, GEFH1, is inhibited by its association with cingulin,¹⁵³ in addition to its association with JACOP/paracingulin,¹⁴⁹ and its activity can reportedly promote TJ disassembly,¹⁵⁴ as well as increase paracellular permeability for small tracers.¹⁵⁵ Thus, we speculate that cingulin- or JACOP/paracingulin-mediated inhibition of GEFH1 may have a role in TJ maintenance (Fig. 3C). This is in addition to its proposed role in mediating confluency-induced Rho inactivation.^{153,156} Unlike Rac and Cdc42 activities, Rho signaling is downregulated in confluent cells,⁷² which ultimately impedes cell proliferation.¹⁵⁷ Also important for TJ integrity is a complex containing the Cdc42 RhoGAP, Rich1, and the scaffolding protein Amot that associates with some key polarity proteins including Pals1, Patj and Par3 (Fig. 3C).¹⁵⁸ These studies suggest that Rho GTPase activities must also be limited for TJ maintenance.

In summary, Rho, Rac and Cdc42 are critical regulators of epithelial cell-cell junctions, but their junction-associated activities must be carefully balanced for proper control of junction assembly, maturation and maintenance. Cells achieve this balancing act through a multitude of junction-associated Rho GTPase regulators, including many GEFs and GAPs, which cooperate over space and time (Fig. 3B and C). Additionally, many of these studies again highlight the tight relationship between Rho GTPase activities and cell polarity determinants.

Tissue Polarity

As an epithelium develops, individual cells must acquire AB polarity in a coordinated fashion in order to produce an organized, fully differentiated and functional tissue. For example, the

development of organized cell-cell junctions gives the epithelium strength and integrity. Additionally, during organism development many morphogenetic changes take place, which rely on cell-cell adhesion and changes in cell shape to determine the final size and shape of a tissue. Once again an intricate interplay between Rho GTPases, polarity proteins and regulators of the actin cytoskeleton is key in driving these morphogenetic processes to change the shape and/or position of individual cells within a tissue. The establishment and maintenance of correct AB polarity is required for correct cell shape and to form and position cell-cell junctions. Consequently, the regulation of AB polarization within single epithelial cells has major implications for the whole tissue, which therefore implicates the Rho GTPases as important regulators of tissue polarity.

In particular, Cdc42 can control a cell's height as well as its apex size. In the *Drosophila* wing, Cdc42 promotes AB elongation,⁶⁶ whereas in the *Drosophila* notum Cdc42 is required to restrict cell height as well as apical constriction.^{21,40} Many other studies also support a role for Cdc42 in restricting apical constriction,^{40,116,159} which it achieves through antagonising Rho activity at AJs to limit apical tension (Fig. 1B, Cell 2).¹¹⁸

Several other lines of evidence also show the importance of junction-associated Rho-mediated actomyosin contractility in controlling tissue polarity, particularly from the study of several developmental processes that involve extensive tissue remodelling. For example, during *Drosophila* germband extension, the tissue doubles in length but its width is halved,¹⁶⁰ which is achieved by a pattern of shrinking and growing cell-cell junctions, resulting in cell rearrangements to reorganise the tissue.^{104,106,161} Interestingly, in this intercalating ectoderm, Rho-mediated actomyosin contractility is concentrated cortically at shrinking cell-cell junctions (Fig. 1B, Cell 2), promoting their contraction, and driving tissue remodelling. Moreover, Rho-mediated actomyosin contractility is also required to promote coordinated apical constriction during *Drosophila* gastrulation.^{105,162-164} Gastrulation requires a coordinated apical constriction in the mesoderm, which is thought to be mediated by pulses of actomyosin contractility, this time driven by a medial actomyosin web-like network (Fig. 1B, Cell 2).¹⁰⁸ Therefore, mesoderm cells undergoing apical constriction utilize a different actomyosin network to the intercalating cells of the ectoderm, thereby explaining the disparity in the cell shape changes observed between the 2 cell populations.

The *Drosophila* gastrulation process involves a cell-lengthening phase, whereby individual cells elongate concomitantly with apical constriction, and a subsequent cell-shortening phase, whereby cells shorten as the tissue invaginates.¹⁶⁵ Interestingly, He et al. report that the lateral membranes of individual mesoderm cells are dispensable for the cell-lengthening process.¹⁶⁵ They present a model whereby the cytoplasm of multiple cells provides a collective force that actively promotes lengthening.

The actomyosin web-like network is also required for apical constriction in amnioserosa cells, which is necessary for *Drosophila* dorsal closure.^{166,167} Repeated cycles of assembly and disassembly of the apical actomyosin network produces pulses of contractility that ultimately drive apical constriction.¹⁶⁸ However, the involvement of

Rho activity is unclear since DN-Rho does not prevent dorsal closure.¹⁶⁹ Intriguingly though, Rac-mediated actin regulation has been implicated in this process.¹⁶⁹ Moreover, the Par complex appears to be important for the dynamics of the actomyosin pulses. Baz, Par6 and aPKC accumulate at apical puncta that transiently associate with the actomyosin network.¹⁷⁰ Baz promotes the duration of the pulses, whereas Par6-aPKC regulates the time between pulses.

In summary, the morphogenetic movement generated relies on the specific location of Rho family GTPase activities and the type of actomyosin contractility, which determines cell shape change and ultimately shapes the developing tissue.¹⁷¹ All cell shape changes fundamentally rely on asymmetry: an asymmetry of tension (mediated by Rho activity, myosin and actin filaments; Fig. 1B, Cell 2), and an asymmetry of cell-cell adhesion (mediated by cadherin and its recycling at the junction; Fig. 1B, Cell 3). Central to all this are the polarity proteins, which provide the positional information that is required to generate these asymmetries.

Discussion

Several themes are evident surrounding the function of Rho GTPases in epithelial AB polarization. The most obvious being the need for a complex spatiotemporal regulation of their activities; the large number of GEFs and GAPs within the cell is conducive to such complex regulatory mechanisms. Through their scaffolding and signaling functions they can permit activation and/or inactivation of the Rho GTPases at specific locations and times, thereby fine-tuning their activities and providing an effective control of the polarization process.

Signaling feedback loops,^{23,95} as well as crosstalk and interdependence between the Rho family GTPases are also common. In particular, Rac and Rho frequently function antagonistically,^{15,96} or their activities are distinctly separated,⁷⁴ with Rac activity often enriched basolaterally¹⁶ and Rho activity apically.²² Antagonism between Rho and Cdc42 is important for controlling apical tension,¹¹⁸ whereas on the other hand, Rac and Cdc42 frequently cooperate.^{21,94} These mechanisms provide immense robustness to AB polarization.

There is also tight cooperation between distinct cellular processes, including: intercellular adhesion, membrane protrusion dynamics, actomyosin contractility, membrane trafficking, and mitotic spindle orientation, in order to achieve and maintain a correctly AB polarized state at both the single cell and whole tissue level. Bearing all of the above in mind, it is not surprising that overstimulation as well as inhibition of Rho GTPase signaling, often through the expression of DN and CA forms of the GTPases, have been found to perturb AB polarization.^{86,137-139} These findings likely reflect the multifunctional nature of the Rho GTPases, as well as their interdependence, and also the need for their activities to be correctly balanced.

Polarity Plasticity

In addition to their roles in epithelial AB polarization, the Rho family GTPases have also been widely implicated in various

other types of cell polarity, including endothelial AB polarity,^{98,172,173} front-rear polarity,¹⁷⁴⁻¹⁷⁹ polarized cell division,^{34,180} polarized secretion,¹⁸¹ planar cell polarity,¹⁸² and neuronal polarity.^{103,143,183} Intriguingly, this is also the case for many other polarity regulators, notably the Par proteins.¹⁸⁴⁻¹⁸⁶ In fact, many of the same signaling complexes/pathways that are critical in regulating AB polarity have also been implicated in multiple cell polarization events. We would particularly like to draw attention to the analogies between AB polarization and front-rear polarity, necessary for directed cell migration (Fig. 4).

We discussed earlier the evidence implicating Par3-Tiam1-Rac signaling in TJ assembly and AB polarization.^{16,18,148} Similarly, Par3-Tiam1-Rac signaling also promotes front-rear polarity and directed cell migration in multiple cell types (Fig. 4).^{175,176} Pegtel et al. showed that the Par-Tiam1 complex promotes persistent migration of keratinocytes by stabilizing front-rear polarity through increasing microtubule stability.¹⁷⁶ More recently, Wang et al. reported that the Par-Tiam1 complex is recruited to a subset of adhesions in a talin-dependent manner, where it mediates integrin-induced Rac activation and regulates adhesion turnover necessary for polarized migration.¹⁷⁵

Cdc42-mediated signaling via the Par complex has also been implicated in polarized cell migration (Fig. 4). In fibroblasts, Cdc42 has been shown to regulate reorientation of the microtubule organizing center (MTOC), an important event in establishing front-rear polarity, through 2 mechanisms: 1. by promoting rearward nuclear movement via an MRCK-myosin-actin pathway, and 2. by maintaining a centroid MTOC localization through a Par6-PKC ζ -Dynein pathway.¹⁷⁴ This latter mechanism likely also operates in migrating astrocytes downstream of integrin activation.¹⁸⁷ Cdc42 association with the Par complex also reinforces Par-Tiam1-Rac signaling in polarized migrating cells.¹⁷⁷

Rho-ROCK signaling also functions in both AB polarization and polarized cell migration (Fig. 4). The Rho-ROCK-myosin

pathway promotes actomyosin contractility at the rear of polarized migrating cells.^{188,189} In addition, ROCK-mediated phosphorylation of Par3 inhibits Par complex formation and consequently leads to Rac inactivation at the leading edge, as well as in central and rear regions of migrating cells.¹⁷⁷

Also noteworthy, is the specific spatiotemporal regulation of Rho, Rac and Cdc42,¹⁹⁰ which includes the distinct separation of Rac and Rho activities,¹⁹¹ as well as their interdependence;¹⁹² this tight regulation is required to control membrane protrusion dynamics at the leading edge, necessary for directed cell migration. Furthermore, like AB polarity, polarized cell migration also requires the cooperation of adhesions,¹⁷⁵ membrane protrusion dynamics,¹⁹¹ actomyosin contractility¹⁹³ and membrane trafficking.¹⁹⁴ Consequently, the regulatory mechanisms controlling the establishment and maintenance of both AB polarity and polarized cell migration are remarkably similar.

We propose the evidence suggests that there is a general “hub” of cell polarity machinery, comprised of numerous polarity regulators that are capable of promoting multiple cell polarization events. We envisage this would provide cells with “polarity plasticity,” meaning that they can efficiently reorganise their polarity in response to various stimuli or environmental changes. In fact, major changes in cell polarity are required for numerous physiological events, including cell division, various developmental events and tumor metastasis. For example, during dorsal closure of the *Drosophila* embryo, cells at the leading edge must lose AB polarity and take on front-rear polarity in order to migrate. Subsequently however, as opposing cells meet and adhere to one another to seal the gap, AB polarity must be re-established. Interestingly, the Rac and Cdc42 effector, Pak, is important for both polarized cell migration and restoration of AB polarity during this process.¹⁹⁵

We suspect that the type of polarization stimulated is context-dependent. For example, the presence of strong intercellular contacts

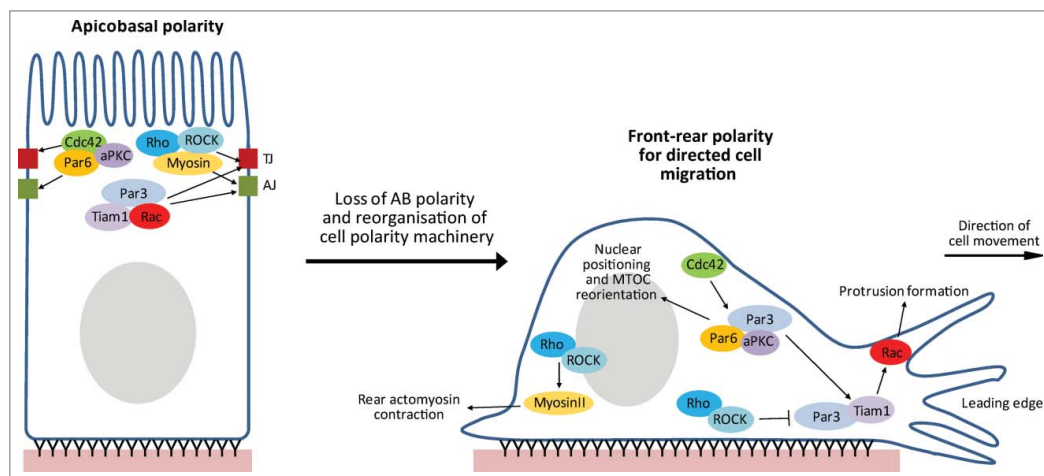


Figure 4. Schematic representation of epithelial cell polarity plasticity. Many aspects of an epithelial cells’ polarity machinery are multi-functional, potentially permitting a switch from promoting apicobasal polarity to front-rear polarity, which promotes directed cell migration. Shown are some of the Rho GTPase signaling complexes that have been implicated in the regulation of both types of polarization, and are therefore possible candidates for mediating such a polarity switch.

contacts should promote AB polarity (Fig. 4, left-hand cell), whereas in their absence, or following some perturbation of AB polarity (which frequently occurs during tumor progression),¹⁹⁶ the same polarity machinery may become free to take up alternative localisations and instead promote front-rear polarity and consequently directed cell migration (Fig. 4, right-hand cell). Therefore, this proposed phenomenon could have important implications for our understanding of tumor progression and what we consider to be oncogenes or tumor suppressors. During tumor

progression, tumor cells frequently lose AB polarity, become disorganized, divide rapidly, and may eventually invade the surrounding tissue and metastasise through polarized cell migration.¹⁹⁶ We envisage that through these events the cell's polarity machinery is rearranged, thereby encouraging drastic changes in cellular function and disease progression. We therefore speculate that some so-called tumor suppressors may also have oncogenic functions, for example, if they are capable of promoting both AB polarity and polarized cell migration.

In support of our hypothesis, several epithelial AB polarity regulators have been observed to be mislocalised in human cancers, with possible consequences for disease progression. For example, reduced membrane-localized Par3 has been found associated with potentially more aggressive invasive breast cancers.¹⁹⁷ Reduced membrane-localized β 2-syntrophin appears to be associated with more aggressive prostate cancers.¹⁶ Scribble has been found to be mislocalised away from cell-cell junctions in breast¹⁹⁸ and prostate¹⁹⁹ cancers, the latter also being associated with poor survival. A mutant version of Scribble that cannot localize to cell-cell junctions was also recently shown to promote mammary tumourigenesis in mice.²⁰⁰ Although these findings support their correct cortical localization as being tumor suppressive, they also support the possibility that once mislocalised they may actively participate in alternative cellular functions that actually encourage tumor progression.

There is also substantial evidence from a variety of human cancers that expression of the Rac activator, Tiam1, can correlate

with metastasis or poor survival.²⁰¹⁻²⁰⁵ In cancers where Tiam1 is over-expressed, these findings could reflect the reportedly negative effect of increased Tiam1-Rac signaling on cell-cell adhesions and AB polarity.^{16,18} However, they also support the possibility of a more direct function for Tiam1-Rac signaling in promoting metastasis, through its reportedly positive role in promoting polarized cell migration.^{175,176}

We propose that a detailed understanding of the extent of multifunctionality among cell polarity regulators is required. Future research investigating the potential direct oncogenic activities of mislocalised polarity regulators in human cancers could have huge implications for our understanding of tumor progression and metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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