[Frontiers in Bioscience 14, 1129-1142, January 1, 2009]

Regulation of epithelial apical junctional complex by Rho family GTPases

Stanislav Samarin, Asma Nusrat

Epithelial Pathobiology Research Unit, Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, 30322

TABLE OF CONTENTS

1. ABSTRACT

The apical junctional complex (AJC), encompassing the tight junction (TJ) and adherens junction (AJ) plays a vital role in regulating epithelial cell differentiation and barrier function of simple epithelia. Both AJ and TJ are comprised of multiprotein complexes consisting of transmembrane proteins, which interact with the underlying cytoskeleton via cytoplasmic scaffold proteins. These interactions are tightly controlled by a number of signaling proteins that are critical for the regulation of the AJC function. Among these signaling molecules Rho family of small GTPases have been demonstrated to regulate the AJC function in diverse physiological and pathological states. In this review we will focus on experimental data addressing the role of Rho GTPase family members, Rho, Rac and Cdc42 in the regulation of epithelial AJC, and analyze Rho GTPase-mediated signaling pathways that control maintenance, disassembly and assembly of the AJC in epithelial cells.

2. INTRODUCTION

A critical function of epithelia is to form a barrier that separates tissue compartments from the external environment. Simple epithelium, such as found in the gastrointestinal and respiratory tract, represents a single layer of polarized columnar cells joined together by a series of intercellular junctions that include the apical junctional complex (AJC) and desmosomes (1, 2). The AJC represents a highly dynamic structure that plays a vital role in establishing/maintaining epithelial polarity and barrier function (3). The AJC is assembled, maintained and disassembled in diverse physiological and pathological conditions (4, 5). In fact, the AJC is currently regarded as an integration center for intracellular signaling in epithelial cells, which regulates and is regulated by a variety of signaling molecules (6-8). Although a significant progress in understanding AJC regulation has been achieved over the past years, many of the signaling cascades implicated in its regulation are not fully understood. Signaling pathways that are controlled by Rho family small GTPases (Rho, Rac

and Cdc42) stand out as the most studied and most universal in context of the AJC regulation. In this review we will focus on the role of Rho family small GTPases in the regulation of epithelial AJC by analyzing Rho GTPase-mediated signaling pathways that control assembly, integrity and disassembly of the AJC.

3. EPITHELIAL APICAL JUNCTIONAL COMPLEX

The epithelial AJC resides in the apical region of the lateral membrane, and is comprised of two morphologically distinct intercellular junctions referred to as the tight junction (TJ) (9) and adherens junction (AJ) (10). Most apically located TJs are visualized by freeze-fracture electron microscopy as a network of anastomosing strands. TJs are responsible for both gate and fence function of the epithelial monolayer, regulating selective permeability of the monolayer to ions and small molecules and preventing the diffusion of lipids and proteins between apical and basolateral membranes respectively (3). AJs, located immediately subjacent to the TJ, form a mechanical link between adjacent cells, providing the adhesiveness and mechanical strength, and stabilizing the cohesiveness of cell-cell contacts (11).

Both TJs and AJs have a common structural organization consisting of transmembrane proteins affiliated with cytoplasmic scaffolding proteins. While the transmembrane proteins mediate cell-cell adhesion and intercellular seal, cytoplasmic plaque proteins are involved in clustering and stabilization of transmembrane proteins, and in the recruitment of signaling molecules to the sites of intercellular contact. Multiprotein complexes of the TJ include transmembrane proteins, occludin, claudin family of proteins, members of the Immunoglobulin superfamily (Junctional adhesion molecule (JAM)-A and coxsackie adenovirus receptor (CAR)) and cytoplasmic plaque proteins, zonula occludens (ZO) and membrane-associated guanylyl kinase inverted (MAGI) family proteins, cingulin, partitioning-defective proteins (PAR) 3 and 6; PALS-1 (protein associated with Lin-7), PALS-1-associated tight junction (PATJ) protein, and the multi-PDZ domain protein (MUPP)-1 (3, 9, 12). AJ is comprised of key transmembrane proteins, E-cadherin (10) and nectin family proteins (13), which form Ca^{++}-dependant and Ca^{++}-independent adhesions respectively. Catenin family proteins encompassing α-, β-, and p120 catenins represent the AJ cytoplasmic plaque proteins (10). In addition, numerous signaling proteins including kinases, phosphatases and GTPases have been identified in the protein complexes that constitute the AJC (6).

Both TJs and AJs are intimately linked to underlying microfilaments organized in a perijunctional belt-like structure consisting of antiparallel actin filaments associated with conventional myosin II. The connection between the AJC and perijunctional acto-myosin ring is mediated by AJC cytoplasmic plaque proteins, several of which are known to interact with actin and myosin both directly and via a variety of actin-binding proteins (9, 14-16). The association of the AJC with underlying cytoskeletal elements is critical for the stabilization of AJC, and is believed to be important for their regulation (16-18). Given the important role of the cytoskeleton in the regulation of epithelial AJC, it is not surprising that a number of molecules known to control actin dynamics, such as Rho family small GTPases (19), have also been implicated in the regulation of AJC (20-22).

4. RHO FAMILY SMALL GTPASES

The Rho family proteins belong to Ras superfamily of small GTPases, and are defined by the presence of the so-called Rho-like insert in their GTPase domain (23). Although 22 genes encoding at least 25 Rho family GTPases have been described in mammals (24, 25), the term 'Rho family GTPases' commonly refers to the three 'classical' family members, Rho (A), Rac (1) and Cdc42.

Similar to other Ras proteins, Rho family small GTPases function as molecular switches. They cycle between inactive GDP-bound and active GTP-bound forms (26). GDP-GTP transition of Rho GTPases is regulated by a variety of upstream signaling molecules, including guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDI). GEFs catalyze the exchange of GDP for GTP, and thus activate Rho proteins. GAPs increase GTPase activity of Rho proteins, and therefore cause their transition into the inactive GDP-bound form. Finally, guanine nucleotide dissociation inhibitors (GDIs) maintain Rho family proteins in the inactive GDP-bound state (27-29).

Approximately one percent of the human genome encodes proteins that either regulate, or are regulated by a direct interaction with the members of the Rho family GTPases (30). Rho GTPases are known to induce a variety of cellular responses, namely microtubule dynamics, gene transcription, cell cycle progression and cytokinesis, vesicular transport, etc. through the regulation of multiple signaling pathways in various organisms and cell types (19, 28, 30, 31). However, the best-studied function of Rho GTPases is the regulation of actin dynamics. Indeed, in fibroblasts Rho has been linked to the assembly of stress fibers and focal adhesions (32). Rac has been implicated in the formation of lamellipodia and membrane ruffles (33), and Cdc42 has been shown to induce filopodia and microspikes ((34, 35), reviewed in (19)).

More than a decade ago Rho GTPases were also implicated in the regulation of epithelial AJC (20, 22). Below we will discuss the basic molecular mechanisms controlling assembly, maintenance and disassembly of epithelial AJC, and analyze the involvement of Rho family GTPases in each of these processes.

5. ROLE OF RHO GTPASES IN THE MAINTENANCE OF EPITHELIAL AJC

5.1. Maintenance of apical junctions

The mature AJC is a highly dynamic structure, undergoing constant remodeling (36, 37). In confluent

epithelial monolayers the integrity and normal functioning of the AJC relies on the tight control of the underlying cytoskeleton. Actin filaments are not stable, but undergo dynamic reorganizations at cell-cell contacts, and both disassembly of actin filaments (38, 39) and sequestration of G-actin (40) have a detrimental effect on the AJC integrity. Interestingly, acto-myosin contractility appears to play a less dramatic role in the stabilization of mature AJC. Indeed, pharmacological inhibition of myosin II resulted in only a moderate redistribution of E-cadherin from cell-cell contacts (41), and selective down-regulation of each of myosin II isoforms did not influence the AJC integrity (42).

5.2. Rho

The synergism between Rho activity and AJC function has been previously shown in series of early experiments, in which the inhibition of Rho either by C3 transferase, or overexpression of dominant-negative Rho resulted in the disassembly of TJs and AJs in various epithelial cell lines (20, 43, 44). Importantly, in the cells with down-regulated Rho function, the disruption of TJs correlated with the disassembly of the perijunctional F-actin ring, while the expression of a constitutively-active Rho had the opposite effect on the actin cytoskeleton, that included thickening of the perijunctional F-actin ring and aggregation of the elongated stress fibers at the basal membrane (20, 45). A detailed temporal analysis of the interplay between actin reorganization and AJC disassembly in C3-microinjected cells revealed that the earliest effect of C3 transferase microinjection in MDCK cells (15 min after microinjection) was the disappearance of stress fibers. However, no significant changes in E-cadherin and ZO-1 were observed even at 30 min after microinjection. Only at later time points (1h postmicroinjection) perijunctional F-actin appeared to be disorganized, and that disorganization was accompanied by the disruption of both TJs and AJs (46). These experiments suggest that basal Rho activity is required for normal functioning of both AJs and TJs, and the actin cytoskeleton acts as an intermediary between Rho and AJC. Another possible conclusion is that Rho-mediated regulation of basal stress fibers is not critical for the maintenance of AJC in epithelial cells.

The regulation of the actin cytoskeleton in mature AJC by Rho might be achieved via two interconnected mechanisms: the control of actin polymerization, and regulation of acto-myosin contractility. Indeed, Rho itself (47) and its two downstream effectors, diaphanous-related formins (Dia) (48) and Rho-associated kinase (ROCK) (49), involved in actin remodeling and acto-myosin contractility respectively, have been shown to localize at TJs and perijunctional acto-myosin ring. Interestingly, ROCK-mediated acto-myosin contractility appears to be important, but not critical for the integrity of epithelial junctions, as pharmacological inhibition of ROCK induced reorganization of apical F-actin and abrogated the barrier function of epithelial monolayers (49), but did not alter the distribution or detergent solubility of AJ (50) or TJ (49) proteins. In contrast, Dia-mediated actin polymerization downstream of Rho is critical for the maintenance of at least the adherence junctions. The expression of a dominant-negative mutant of Dia1 disrupted AJs in a fashion similar to a dominant-negative Rho, and overexpression of constitutively-active Dia1 rescued AJs in dominant-negative Rho-expressing cells (50).

The basal level of Rho activity appears to be maintained by both positive and negative upstream signaling. Abelson (Abl) non-receptor tyrosine kinase inhibits Rho activity, and this inhibition is critical for preserving the integrity of apical junctions. Indeed, the inhibition of Abl leads to the activation of Rho and subsequent ROCK-mediated phosphorylation of the regulatory myosin light chain (RMLC). These events induce increased acto-myosin contractility, resulting in the disruption of AJs (51). Similarly, increased Rho activation by overexpressing its constitutively-active mutants is known to disrupt epithelial AJCs (22, 45, 50). An important candidate for positive regulation of Rho activity is the Rho-specific guanidine exchange factor GEF-H1, which associates with TJs in epithelial cells (47, 52). Inhibition of GEF-H1 has been shown to increase paracellular permeability across MDCK epithelial cells, although it did not affect the AJ/TJ morphology (52), suggesting a redundancy of Rho-activating signals in the regulation of AJC integrity.

5.3. Rac

Although little is known regarding how Rac regulates stable AJCs, its role in maintaining the integrity of epithelial apical junctions is supported by the fact that both constitutively-active (22, 45) and dominant negative (45, 46) mutants of Rac induce the disruption of the AJC. The above observations suggest that a defined basal level of Rac activity is required for the proper maintenance of epithelial AJCs.

Rac inhibition studies support an intermediary role of the cytoskeleton in the regulation of AJC by Rac. Indeed, the overexpression of a dominant-negative mutant of Rac induced the disassembly of perijunctional F-actin ring and subsequently decreased the association of AJs with the actin cytoskeleton. Conversely, the overexpression of constitutively-active Rac mediated the thickening of the perijunctional F-actin ring (45, 46). Interestingly, TJs are not dramatically affected by the overexpression of dominant-negative Rac (22, 45), suggesting a differential sensitivity of mature AJs and TJs to Rac-mediated signaling. This hypothesis is further supported by the observation that Rac co-localizes with E-cadherin at AJs, but does not co-localize with the TJ protein, ZO-1 (53).

In addition to controlling actin turnover at apical junctions, Rac has been implicated in the regulation of AJs via its interaction with Rac/Cdc42 GAP, IQGAP. IQGAP (54) is an actin-interacting protein, localized at AJs (55), which directly binds to β-catenin, and thus mediates the dissociation of α-catenin from cadherin-catenin complexes (55, 56). Active Rac1 has been shown to inhibit IQGAP/β-catenin interaction, and therefore preserve the cadherin-catenin complex, thus stabilizing AJs (56).

Rac-specific GEF Tiam1 is thought to be necessary and sufficient to promote the activation of Rac required for the proper maintenance of AJCs. Indeed, both Rac (53) and Tiam1 (57, 58) are localized at AJs, and siRNA-mediated knock-down of Tiam1 results in the disassembly of cadherin-based intercellular adhesions and loss of the epithelial phenotype (59, 60).

5.4. Cdc42

Although Cdc42 is also present in mature apical junctions ((61) and our unpublished observations), its activity appears to play a less important role in the maintenance of AJCs when compared to that of Rho and Rac. Indeed, the overexpression of a dominant-negative mutant of Cdc42 does not influence the structure of mature AJs (62) and TJs (46, 63, 64). This correlates with the fact that dominant-negative Cdc42 does not dramatically alter the distribution of perijunctional F-actin filaments (62, 63). However, one study has implicated Cdc42-mediated signaling in the regulation of the tension of the perijunctional F-actin belt. Otani *et al.* recently reported that the depletion of Cdc42-specific GEF Tuba resulted in the reduction of lateral F-actin fibers. That was associated with the reduced lateral localization of E-cadherin, although its apical localization was not affected. Furthermore, the above alterations correlated with significant changes in the junctional morphology. Cell-cell boundaries appeared to be less strained, indicating that the apical junctions had acquired reduced tension. Importantly, the depletion of Tuba did not affect myosin distribution or its activity, suggesting that the reduced junctional tension was mediated by the effect of Tuba on the dynamics of the perijunctional actin, rather than the acto-myosin contractility (61).

6. ROLE OF RHO FAMILY GTPASES IN THE DISASSEMBLY OF AJC

6.1. Disassembly of apical junctions

The disassembly of epithelial apical junctions plays an important role in both the development and life cycle of multicellular organisms. The orchestrated disassembly of the AJC is observed in different physiological and pathological circumstances, such as embryonic morphogenesis (65, 66), spermatogenesis (67), tissue remodeling (65), epithelial-mesenchymal transition (68), leukocyte transmigration (69, 70), and bacterial and viral infections (71, 72). These scenarios suggest that diverse stimuli can induce AJC disassembly by a variety of signaling pathways (4, 6, 73).

Two different mechanisms have been implicated in the disruption of the epithelial AJCs. One involves endocytosis of AJ/TJ proteins ((73, 74), reviewed in (75)), while the other employs the reorganization of the acto-myosin cytoskeleton ((76, 77), reviewed in (17)). Although the relationships between these mechanisms remain poorly understood, actin dynamics appears to be critical for mediating the AJC disassembly in both processes (73, 75). Given that and the important role of Rho family GTPases in the regulation of the actin cytoskeleton, it is no surprise that many scenarios of AJC disassembly involve cytoskeleton reorganizations and Rho GTPases signaling.

6.2. Rho

Rho activity has been implicated in the regulation of the epithelial AJC disassembly induced by different physiological and non-physiological stimuli, such as proinflammatory cytokines (78) and chelation of extracellular calcium (47). Interestingly, despite the difference in basic mechanisms of the AJC disassembly in these two model systems, they are regulated by analogous Rho-mediated signaling cascades. Indeed, both IFN-γ induced macropinocytosis of TJ transmembrane proteins ((78), reviewed in (79)) and the disruption of AJC in calcium-depleted cells (47) are mediated by the acto-myosin contraction. Such contractility is induced by hyperphosphorylation of RMLC by ROCK, which is a downstream effector of Rho. The disassembly of apical junctions in both model systems was accompanied by the activation of Rho and phosphorylation of RMLC, and effectively prevented by the inhibition of myosin II and ROCK (47, 76, 78). Moreover, although the involvement of Rho signaling has not been demonstrated directly, increased acto-myosin contractility mediated by activation of myosin II has been shown to regulate the AJC disassembly induced by other stimuli, such as histamine (80), enteropathogenic *Escherichia coli* (81), and activation of Na^+/glucose co-transport (82).

Interestingly, down-regulation of acto-myosin contractility via Rho-mediated signaling appears to be critical for mediating the AJC disassembly during ATP and GTP depletion in a model of the ischemic injury. Indeed, the depletion of ATP/GTP induced a time-dependent decrease in Rho activity in renal epithelial cells (83). This correlates with a decrease in the levels of RMLC phosphorylation, resulting in the dissociation of myosin molecules from acto-myosin complexes (84). The above data are in a good agreement with the observation that further inhibition of Rho results in a more extensive loss of TJ components from epithelial junctions, and the expression of activated Rho protects TJs from the disassembly in ATP/GTP-depleted cells (44).

GEF-H1 has recently been implicated in the regulation of Rho during the disassembly of the AJC in calcium-depleted cells (47). Together with Rho and ROCK, GEF-H1 translocated from TJs to contractile acto-myosin rings upon the depletion of extracellular calcium, and inhibition of GEF-H1, Rho and ROCK effectively prevented the AJC disassembly (47). These results point to an important role of GEF-H1 in mediating Rho/ROCK-induced disassembly of epithelial AJCs.

6.3. Rac

Rac has long been implicated in the regulation of AJ disruption during the growth factor-induced scattering of epithelial cells (85). Indeed, Rac has been shown to mediate both lamellipodial extensions (86), and induce the disruption of AJs in the hepatocyte growth factor (HGF)-stimulated cells (85). However the interplay between these two events has not been directly analyzed. Further studies identified PI_3 kinase (85) and MEK/ERK (87) as upstream activators of Rac. Pharmacological inhibition of these kinases prevented Rac-mediated AJ disassembly. Remarkably, in those

experiments Rac activity induced selective disassembly of AJs only, while the TJs were not affected, suggesting that the disruption of TJs is regulated by a Rac-independent signaling pathway (85).

Interestingly, in some experimental systems, Rac activation has been demonstrated to protect E-cadherin-mediated adhesions, and prevent HGF-induced cell scattering (58). Additionally, the overexpression of either Tiam1 or Rac induced the reversion of the fibroblast phenotype toward the epithelial phenotype, and restored cell-cell adhesion in Ras-transformed cells (58). Such an effect might be mediated by the ability of Rac to stabilize E-cadherin adhesions similar to that observed during the formation of E-cadherin contacts, i.e. by inducing polymerization of actin at cell-cell contact sites (see below). This idea is further supported by the observation that the cells overexpressing either Tiam1 or Rac had increased polymerization of actin at AJs, and the inhibition of E-cadherin adhesive function resulted in cell scattering, even when the cells overexpressed constitutively-active Tiam1 (58). Similarly, the overexpression of constitutively-active Rac has been shown to protect AJs during ATP/GTP depletion (88). The fact that the protective effect of Rac was limited to AJs (88), whereas the protective effect of Rho was TJ-specific (44), suggests that the same stimulus can differentially influence AJs and TJs by regulating different GTPases.

6.4. Cdc42

Unlike Rho and Rac, the role of Cdc42 in the regulation of junctional disassembly is much less understood. The activation of Cdc42 has been observed during epithelial to mesenchymal transition in different epithelial cell lines (89, 90). However the role of Cdc42 in mediating the disruption of AJCs has not been shown. The overexpression of constitutively-active Cdc42 is known to induce a fibroblast-like morphology of epithelial cells with subsequent disruption of E-cadherin-based AJs (64, 91), although the physiological significance of this effect is not clear.

Recently Cdc42 has been implicated in the regulation of E-cadherin degradation following the disruption of E-cadherin adhesions. The loss of cadherin-based adhesions induced by the depletion of extracellular calcium was accompanied by the activation of Cdc42 and subsequent EGFR-dependent activation of Src downstream of Cdc42. Tyrosine-phosphorylation of E-cadherin by Src resulted in its ubiquitination and lysosomal degradation (91). This mechanism may play an important role in the disassembly of AJs following the disruption of initial cell-cell contacts by preventing the recycling of internalized E-cadherin back to apical junctions.

7. ROLE OF RHO FAMILY PROTEINS IN ASSEMBLY OF APICAL JUNCTIONS

7.1. Assembly of AJC

The formation of AJCs is a critical event in the generation of an epithelial phenotype. Orchestrated assembly of the AJC can be observed during epithelial morphogenesis (5, 92), mesenchymal to epithelial transition (5, 93) and repair of epithelial damage (94). Assembly of the AJC is a multistep process. The initial step is the formation of Ca^{++}-dependant homophilic (trans) cadherin adhesions following physical contact of the membranes between neighboring cells. This initial interaction promotes cadherin clustering at cell-cell contact sites, and leads to the recruitment of actin filaments to the cadherin-based adhesions via catenins. The engagement of actin filaments results in the stabilization of cadherin clusters and further accumulation of new microfilaments to the junctions, as well as a dramatic reorganization of microtubules, acto-myosin complexes and intermediate filaments. These reorganizations result in the formation of the defined perijunctional acto-myosin ring. Acto-myosin contractility of perijunctional cytoskeletal ring further mediates cell shape changes towards a cuboidal phenotype and serves to signal for the engagement of a variety of regulatory molecules, which target TJ proteins to the apical surface. Newly established TJs are further stabilized by the interactions with the cytoskeleton, resulting in a fully polarized epithelial phenotype with distinct apical and basolateral membrane compartments (reviewed in (21, 95, 96)). Importantly, both actin dynamics and acto-myosin contractility play critical roles in the formation of AJC. The assembly of both AJs and TJs is dependent on continuous actin polymerization. Indeed AJ/TJ assembly is accompanied by the recruitment of WASP family proteins (39, 97) and Arp2/3 complex (97) to the cell-cell contacts, and effectively prevented by sequestering of G-actin and pharmacological inhibition of N-WASP (97). The recruitment and activation of myosin II following E-cadherin homophilic ligation is thought to be important for further accumulation and straightening of E-cadherin adhesions presumably by stabilizing actin bundles at sites of cell-cell contacts (41, 42, 98). The inhibition of myosin II precludes the formation of AJs (42), as well as subsequent assembly of TJs and correct positioning of AJC within the plasma membrane (42, 97).

7.2. Rho

More than ten years ago Rho activity was demonstrated to be crucial for the formation of AJs, since the pharmacological inhibition of Rho with C3 transferase prevented Ca^{++} switch-mediated formation of cadherin-based adhesions in human keratinocytes (43) and MDCK cells (46). Similarly, the assembly of E-cadherin junctions was accompanied by Rho activation and further promoted by the expression of constitutively-active Rho (99).

One of the mechanisms by which Rho regulates the formation of AJs is thought to be the Fyn/Src kinase-mediated tyrosine phosphorylation of catenins. Phosphorylation of catenins results in their recruitment to E-cadherin, thus further stimulating cadherin clustering at cell-cell contacts ((99, 100), reviewed in (96)). Another potential mechanism responsible for the stimulation of cadherin clustering is Rho-mediated activation of myosin II through the phosphorylation of RMLC by ROCK. (41, 49). Rho has also been proposed to regulate cytoskeleton reorganizations and acto-myosin contraction at later stages of the AJC assembly following the formation of AJs (21). This hypothesis is further supported by the following observations. First, myosin II is activated upon the

formation of AJs, and acto-myosin contractility is required for further establishment of both TJs and apico-basal cell polarity (14, 97). Second, the activity of ROCK is critical for the assembly of both AJs and TJs (49).

7.3. Rac

Analogous to Rho, Rac is activated upon the homophilic cadherin interaction (53, 101), and Rac activity appears to be critical for the formation of stable cadherin adhesions ((43), reviewed in (14) and (96)). Rac is commonly accepted to be a regulator of the initial cell-cell contact development (14, 102). Rac is known to induce lamellipodia and membrane ruffles in fibroblasts (33), and is thought to regulate the development of lamellipodial extensions following the formation of primordial cadherin-based contacts in epithelial cells. These lamellipodial protrusions sweep over contacting membranes on neighboring cells thereby facilitating cadherin interactions between adjacent cells (14, 102, 103). The above model for the regulation of AJC assembly by Rac is further supported by the observations that E-cadherin transinteraction recruits activated Rac (53, 103) along with Arp2/3 complex (104), Ena/VASP (105) and WAVE (39) to the sites of cell-cell contacts. The critical roles for both Rho and Rac in actin-dependent formation of early AJs have been further confirmed by calcium switch experiments in human keratinocytes. In these experiments blocking of the cadherin function with specific antibodies or the inhibition of either Rho, or Rac prevented the recruitment of new actin subunits to the peripheral actin bundles and stress fibers after Ca^{++} repletion, and as a result, formation of stable E-cadherin-based adhesions (43).

The upstream signaling proteins implied in the activation of Rac include Rac GEF, Tiam1 (58) and PI_3 kinase (53). Indeed, the overexpression of Tiam1 has been shown to increase E-cadherin-based adhesions, resembling the effect of constitutively-active Rac (58), and the depletion of Tiam1 inhibited the formation of AJs (59). The data on the involvement of PI_3 kinase in the regulation of Rac-mediated assembly of initial cell-cell contacts are however controversial. Some studies have demonstrated the activation of PI_3 kinase upon the formation of E-cadherin-based adhesions (106). Moreover, the inhibition of PI_3 kinase has been shown to abrogate the activation of Rac following its recruitment to E-cadherin contacts (53) and further formation of cadherin-based adhesions (107). Conversely, other studies have shown that the activity of Rac is regulated in PI_3 kinase-independent manner during the formation of cell-cell adhesions (108), and although activated PI_3 kinase is enriched at primordial cell-cell contacts, the inhibition of PI_3 kinase does not affect cell-cell contact formation (103). The above discrepancies can be explained by the fact that the initial phase of cadherin-induced Rac1 activation is PI_3 kinase-independent, and only the later 'contact amplification' phase of Rac activation is PI_3 kinase-sensitive (107). Therefore in some experimental conditions the former pool of activated Rac1 may be sufficient to drive cell-cell contact growth independently of PI_3 kinase (103).

7.4. Cdc42

As with Rho and Rac, Cdc42 is activated upon the formation of E-cadherin contacts (61, 109), and rapidly recruited to cell-cell contacts following the formation of junctions in calcium-switch experiments (61). Analogous to its ability to induce filopodia in fibroblasts (34, 35), Cdc42 has been shown to initiate E-cadherin-dependent filopodia formation at the sites of cell-cell attachments in epithelial cells (109). However, the exact role of these protrusions in the regulation of AJC assembly remains elusive. One possibility is that filopodia formation complements the function of Rac-induced lamellipodia, increasing the number of cell-cell contact sites and facilitating the recruitment of cadherin molecules to the newly formed contacts (110-112). Indeed, the inhibition of Cdc42 dramatically attenuates the recruitment of F-actin and E-cadherin to new cell-cell contacts. Conversely, the recruitment of both E-cadherin and F-actin is significantly facilitated in the cells expressing constitutively-active Cdc42, and appears to be N-WASP- and Arp2/3 complex dependent (61). Interestingly, the increase in the accumulation of F-actin and AJ proteins at cell-cell contacts is less pronounced in the cells expressing constitutively-active Cdc42, than in cells expressing constitutively-active Rac1, even when the expression level of Cdc42 is higher than that of Rac. Furthermore, co-expression of dominant-negative Rac with constitutively-active Cdc42 does not inhibit the increased accumulation of F-actin at cell-cell adhesion sites (62). This suggests that Rac and Cdc42 may regulate the accumulation of F-actin at cell-cell junctions and E-cadherin-mediated adhesions independently.

E-cadherin-mediated activation of Cdc42 is also critical for the recruitment and activation of the PAR-aPKC cassette to initial cell-cell contacts, resulting in local activation of PAR polarity complex, which is essential for the formation of TJs, maturation of the AJC and establishment of cell polarity ((113), reviewed in (114)). The important role of Cdc42 in the assembly of TJs is further supported by the experiments in MDCK cells where a partial inhibition of Cdc42 permitted the assembly of AJs, but precluded the formation of TJs (112). This indicates that differential Cdc42 activity might be required for the formation of AJs and TJs, and higher Cdc42 activation is necessary for TJ assembly.

Several signaling molecules are implicated in the regulation of Cdc42 activity during the formation of AJC. PI_3 kinase has been shown to induce E-cadherin-mediated activation of Cdc42, since pharmacological inhibition of PI_3 kinase or blocking cadherin adhesive function with specific antibodies abolishes Cdc42 activation (109). Src family kinases, activated by nectin transinteraction (115) and Cdc42-specific GEF Tuba (61) are known to regulate the formation of E-cadherin junctions upstream of Cdc42. Of note, although Tuba-depleted cells demonstrated a decreased accumulation of E-cadherin at cell-cell contacts, the recruitment of ZO-1 to cell junctions was not dramatically affected (61). This suggests that Tuba-mediated activation of Cdc42 is important for the AJ assembly, and the formation of TJs is independent of Tuba signaling.

Table 1. Upstream activators (highlighted red) and downstream effectors (highlighted blue) of Rho family GTPases that have been implicated in the regulation of maintenance, disassembly and assembly of epithelial AJCs

	Rho	Rac	Cdc42
Maintenance	Abl (51)	Tiam1 (59, 60)	Tuba (61)
	GEF-H1 (52)	IQGAP1 (56)	IQGAP1 (56)
	ROCK (49)		
	Dia1 (50)		
Disassembly	GEF-H1, ROCK II (47)	Ras, PI_3 kinase, (85)	EGFR, ERK1/2, Src (91)
		MEK1/2, ERK1/2 (85, 87)	
		Tiam1 (87)	
Assembly	ROCK (41, 49)	Abl, Crk (51)	PI_3 kinase (109)
	Fyn/Src, PRK2 (99)	Tiam1 (58, 122)	c-Src, FRG (115)
		PI_3 kinase, IQGAP1 (53)	Tuba, Arp2/3, N-WASP (61)
		WAVE2, Arp2/3 (39)	PAR6-aPKC (113)
		PAR6-aPKCζ (122)	

8. CONCLUSIONS

Three Rho GTPases, Rho, Rac and Cdc42 play a critical role in the regulation of epithelial apical junctions. Although Rho, Rac and Cdc42 control the AJC structure/function by a variety of mechanisms (Table 1, Figure 1.), a growing body of experimental evidence suggests that most of their dramatic effects on epithelial AJC are mediated through the actin cytoskeleton. Importantly, different GTPases induce their effects on AJC by influencing the cytoskeleton by different mechanisms: while Rac and Cdc42 regulate the assembly/disassembly of actin filaments, Rho regulates both actin dynamics and acto-myosin contractility. Thus, the regulation of AJC by Rac and Cdc42 is mediated through the formation of membrane protrusions and/or polymerization of perijunctional actin, while Rho regulates the AJC by controlling both actin polymerization and myosin-mediated contractility of the perijunctional actin ring.

Furthermore, all three GTPases function in an orchestrated cooperative manner in order to assemble and maintain epithelial AJCs. During the formation of AJC, Rac and Cdc42 mediate the initial E-cadherin adhesions, and target PAR polarity complex to AJCs. Rho further stimulates cadherin clustering, stabilizes AJs, and forms the perijunctional acto-myosin ring and TJs. In mature AJCs, Rac and Cdc42 mediate actin polymerization at cell-cell contacts, stabilizing AJs and regulating the tension of the apical perijunctional actin ring respectively. Rho regulates the stability of both AJs and TJs by maintaining the perijunctional acto-myosin ring. Interestingly, in many instances AJs and TJs are specifically regulated by different GTPases. Indeed, Rac and Cdc42 are preferentially involved in the regulation of AJs, while Rho regulates both AJs and TJs.

Importantly, similar Rho GTPases-mediated signaling cascades may control assembly, disassembly and stability of epithelial AJCs through analogous cytoskeleton-mediated events. Therefore, the fine-tuning of Rho GTPases activity levels is critical for mediating either assembly, or stabilization, or disassembly of the AJC. For example, ROCK-mediated phosphorylation of RMLC is critical for the formation of perijunctional acto-myosin ring and assembly of both AJs and TJs (49). In mature junctions Rho/ROCK signaling controls the gate function of TJs and maintains the integrity of perijunctional acto-myosin ring (49). Inhibition of Rho signaling in confluent epithelial cells is known to mediate the disassembly of perijunctional actin ring, resulting in destabilization and disruption of AJC (46). Similarly, downregulation of Rho in ATP/GTP-depleted cells (83) results in decreased RMLC phosphorylation (84), which induces the disassembly of AJCs (44). On the other hand, increased Rho activity influences the perijunctional acto-myosin ring, destabilizes AJs and induces the disassembly of TJs (45). This scenario mimics the disassembly of AJC in Ca^{++}-depleted cells, where upregulation of Rho/ROCK-signaling leads to the hyperphosphorylation of RMLC and increased acto-myosin contractility, thus providing the driving force for the disruption of AJCs (47). Since it is almost impossible to precisely control the activation status of GTPases in experimental conditions, it comes to no surprise that different outcomes of Rho GTPase activity on AJC structure/function have been reported in the literature.

The spatial and temporal coordination of small GTPases is another critical factor for the assembly/disassembly and maintenance of epithelial junctions. This coordination is mediated by a variety of regulators and effectors that provide signaling from the AJC to Rho GTPases and back from GTPases to the AJC (Table 1). A number of GEFs and GAPs are known to interact with AJ/TJ proteins either directly, or through the intermediary molecules such as PI_3 kinase, and thus mediate the targeting and local activation/inhibition of Rho GTPases (102, 116). Indeed, during the formation of apical AJCs, GTPases are recruited to the cell-cell contacts (53, 61, 103), and are localized in mature junctions (47, 53, 61). Activation/inhibition of Rho GTPases in the wrong place or in the wrong time eventually results in the dysregulation of the AJC. For example, during the assembly of AJCs targeting of Rac to primordial E-cadherin adhesions mediates the local formation of lamellipodia, which further facilitate cell-cell adhesions (14, 103). In contrast, Rac-mediated spontaneous induction of basal lamellipodia in HGF-treated cells results in a motile cell phenotype (57), which is antagonistic to the cadherin-mediated cell-cell adhesion (117-119).

The complexity of small GTPase-mediated regulation of AJC is further increased by the fact that different GTPases are involved in a variety of

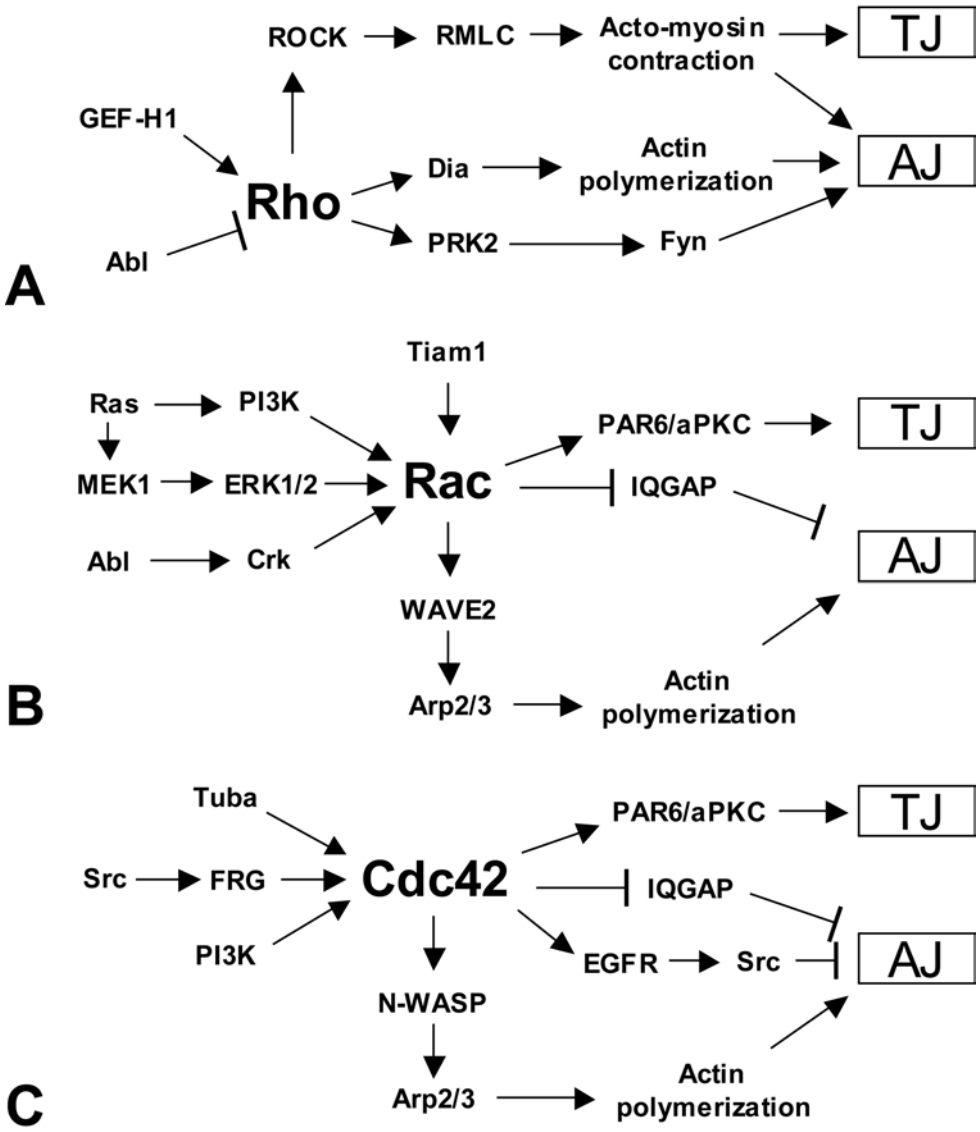

Figure 1. A schematic model for the regulation of epithelial AJC by Rho family GTPases. (A) Rho signaling pathway: The activity of Rho is controlled by GEF-H1 (47, 52) and Abl (51), which mediate the activation and inhibition of Rho respectively. Rho is known to activate Fyn/Src kinase via a Rho effector, PRK2 kinase (99). Activated Fyn/Src kinase phosphorylates catenins, resulting in their recruitment to E-cadherin, thus stabilizing AJs (99). Rho is also implicated in the regulation of actin polymerization via its downstream effector Dia1, and this signaling pathway is believed to be critical for the AJ regulation (50). ROCK regulates myosin II activity downstream of Rho by phosphorylating regulatory myosin light chain (RMLC) (41, 47, 49), eventuating in the increased acto-myosin contractility, which is critical for the regulation of both AJs and TJs. (B) Rac signaling pathway: Both Ras (85) and MAPK (85, 87) signaling have been implicated in the regulation of Rac activity. Rac activity can be modulated by Crk kinase downstream of Abl kinases (51), Rac GEF Tiam1 (58, 59, 87, 122) or PI_3 kinase (53, 85). Rac is known to influence AJs by regulating actin polymerization via WAVE2-Arp2/3 signaling (39). Additionally, Rac is a binding partner for IQGAP (53, 56), known to interact with β-catenin and thus destabilize AJs. Activated Rac has been shown to suppress binding of IQGAP to β-catenin, thereby stabilizing AJs (56). Rac has also been implicated in the recruitment and activation of the PAR-aPKC at sites of cell-cell contacts, resulting in the local activation of PAR polarity complex (122). These events play a critical role in the regulation of TJs. (C) Cdc42 signaling pathway: Cdc42 activation can be mediated by PI_3 kinase (109) and two Cdc42-specific GEFs: Tuba (61) and FRG (115). The later GEF is activated upon tyrosine phosphorylation by c-Src (115). Cdc42 can regulate AJs by controlling E-cadherin degradation through the activation of Src via EGFR signaling. Activated Src phosphorylates E-cadherin resulting in its ubiquitination and lysosomal degradation (91). Cdc42 also regulates AJs by controlling Arp2/3-dependent actin polymerization via its downstream effector N-WASP (61). Analogous to Rac, Cdc42 has been shown to stabilize AJs by binding to IQGAP (56). Cdc42 can also regulate TJs through binding to and activating PAR polarity complex (113).

interconnected signaling networks within the cell. There is an extensive crosstalk between different Rho GTPase family members, where one GTPase may act upstream to the other (s) in either an activating, or inhibiting manner (120). For example, the formation of initial cell-cell contacts has been shown to induce the activation of Rac and Cdc42 while inhibit the Rho activity (121).

In conclusion, the regulation of the epithelial AJC by Rho family small GTPases is a complex and integrated process, which requires precise control of their activity, as well as their spatial and temporal coordination. Further identification of upstream activators and downstream effectors of Rho, Rac and Cdc42, and future studies designed to elucidate their involvement in the assembly, maintenance and disassembly of AJCs will provide important insights into the regulation of epithelial AJCs in both normal and pathological conditions.

9. ACKNOWLEDGEMENTS

We thank Dr. S. Koch for valuable comments on the manuscript. The authors' research is supported by the grants from Crohn's and Colitis Foundation of America (Research Fellowship Award to S.S.), and National Institute of Health (grants DK055679 and DK59888 to A.N.).

10. REFERENCES

1. Farquhar, M. G. & G. E. Palade: Junctional complexes in various epithelia. *J Cell Biol*, 17, 375-412 (1963)2. Matter, S. Balda: Signalling to and from tight junctions. *Nat Rev Mol Cell Biol*, 4, 225-36 (2003)

3. Tsukita, S., M. Furuse & M. Itoh: Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol, 2, 285-93 (2001)

4. Hartsock, A. & W. J. Nelson: Adherens and tight junctions: Structure, function and connections to the actin cytoskeleton. Biochim Biophys Acta (2007)

5. Gumbiner, B. M.: Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell, 84, 345-57 (1996)

6. Gonzalez-Mariscal, L., R. Tapia & D. Chamorro: Crosstalk of tight junction components with signaling pathways. *Biochim Biophys Acta* (2007)

7. Perez-Moreno, M. & E. Fuchs: Catenins: keeping cells from getting their signals crossed. *Dev Cell*, 11, 601-12 (2006)

8. Paris, L., L. Tonutti, C. Vannini & G. Bazzoni: Structural organization of the tight junctions. *Biochim Biophys Acta* (2007)

9. Gonzalez-Mariscal, L., A. Betanzos, P. Nava & B. E. Jaramillo: Tight junction proteins. *Prog Biophys Mol Biol*, 81, 1-44 (2003)

10. Wheelock, M. J. & K. R. Johnson: Cadherins as modulators of cellular phenotype. *Annu Rev Cell Dev Biol*, 19, 207-35 (2003)

11. Perez-Moreno, M., C. Jamora & E. Fuchs: Sticky business: orchestrating cellular signals at adherens junctions. *Cell*, 112, 535-48 (2003)

12. Schneeberger, E. E. & R. D. Lynch: The tight junction: a multifunctional complex. *Am J Physiol Cell Physiol*, 286, C1213-28 (2004)

13. Ogita, H. & Y. Takai: Nectins and nectin-like molecules: roles in cell adhesion, polarization, movement, and proliferation. *IUBMB Life*, 58, 334-43 (2006)

14. Mege, R. M., J. Gavard & M. Lambert: Regulation of cell-cell junctions by the cytoskeleton. *Curr Opin Cell Biol*, 18, 541-8 (2006)

15. Cordenonsi, M., F. D'Atri, E. Hammar, D. A. Parry, J. Kendrick-Jones, D. Shore & S. Citi: Cingulin contains globular and coiled-coil domains and interacts with ZO-1, ZO-2, ZO-3, and myosin. *J Cell Biol*, 147, 1569-82 (1999)

16. Weis, W. I. & W. J. Nelson: Re-solving the cadherin-catenin-actin conundrum. *J Biol Chem*, 281, 35593-7 (2006)

17. Turner, J. R.: 'Putting the squeeze' on the tight junction: understanding cytoskeletal regulation. *Semin Cell Dev Biol*, 11, 301-8 (2000)

18. Goodwin, M. & A. S. Yap: Classical cadherin adhesion molecules: coordinating cell adhesion, signaling and the cytoskeleton. *J Mol Histol*, 35, 839-44 (2004)

19. Hall, A.: Rho GTPases and the actin cytoskeleton. *Science*, 279, 509-14 (1998)

20. Nusrat, A., M. Giry, J. R. Turner, S. P. Colgan, C. A. Parkos, D. Carnes, E. Lemichez, P. Boquet & J. L. Madara: Rho protein regulates tight junctions and perijunctional actin organization in polarized epithelia. *Proc Natl Acad Sci U S A*, 92, 10629-33 (1995)

21. Braga, V. M.: Cell-cell adhesion and signalling. *Curr Opin Cell Biol*, 14, 546-56 (2002)

22. Jou, T. S., E. E. Schneeberger & W. J. Nelson: Structural and functional regulation of tight junctions by RhoA and Rac1 small GTPases. *J Cell Biol*, 142, 101-15 (1998)

23. Valencia, A., P. Chardin, A. Wittinghofer & C. Sander: The ras protein family: evolutionary tree and role of conserved amino acids. *Biochemistry*, 30, 4637-48 (1991)

24. Aspenstrom, P., A. Fransson & J. Saras: Rho GTPases have diverse effects on the organization of the actin filament system. *Biochem J*, 377, 327-37 (2004)

25. Wennerberg, K. & C. J. Der: Rho-family GTPases: it's not only Rac and Rho (and I like it). *J Cell Sci*, 117, 1301-12 (2004)

26. Bishop, A. L. & A. Hall: Rho GTPases and their effector proteins. *Biochem J*, 348 Pt 2, 241-55 (2000)

27. Mackay, D. J. & A. Hall: Rho GTPases. *J Biol Chem*, 273, 20685-8 (1998)

28. Van Aelst, L. & C. D'Souza-Schorey: Rho GTPases and signaling networks. *Genes Dev*, 11, 2295-322 (1997)

29. Bos, J. L., H. Rehmann & A. Wittinghofer: GEFs and GAPs: critical elements in the control of small G proteins. *Cell*, 129, 865-77 (2007)

30. Jaffe, A. B. & A. Hall: Rho GTPases: biochemistry and biology. *Annu Rev Cell Dev Biol*, 21, 247-69 (2005)

31. Etienne-Manneville, S. & A. Hall: Rho GTPases in cell biology. *Nature*, 420, 629-35 (2002)

32. Ridley, A. J. & A. Hall: The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell*, 70, 389-99 (1992)

33. Ridley, A. J., H. F. Paterson, C. L. Johnston, D. Diekmann & A. Hall: The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell*, 70, 401-10 (1992)

34. Kozma, R., S. Ahmed, A. Best & L. Lim: The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol Cell Biol*, 15, 1942-52 (1995)

35. Nobes, C. D. & A. Hall: Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell*, 81, 53-62 (1995)

36. Sasaki, H., C. Matsui, K. Furuse, Y. Mimori-Kiyosue, M. Furuse & S. Tsukita: Dynamic behavior of paired claudin strands within apposing plasma membranes. *Proc Natl Acad Sci U S A*, 100, 3971-6 (2003)

37. Kametani, Y. & M. Takeichi: Basal-to-apical cadherin flow at cell junctions. *Nat Cell Biol*, 9, 92-8 (2007)

38. Stevenson, B. R. & D. A. Begg: Concentration-dependent effects of cytochalasin D on tight junctions and actin filaments in MDCK epithelial cells. *J Cell Sci*, 107 (Pt 3), 367-75 (1994)

39. Yamazaki, D., T. Oikawa & T. Takenawa: Rac-WAVE-mediated actin reorganization is required for organization and maintenance of cell-cell adhesion. *J Cell Sci*, 120, 86-100 (2007)

40. Shen, L. & J. R. Turner: Actin depolymerization disrupts tight junctions via caveolae-mediated endocytosis. *Mol Biol Cell*, 16, 3919-36 (2005)

41. Shewan, A. M., M. Maddugoda, A. Kraemer, S. J. Stehbens, S. Verma, E. M. Kovacs & A. S. Yap: Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts. *Mol Biol Cell*, 16, 4531-42 (2005)

42. Ivanov, A. I., M. Bachar, B. A. Babbin, R. S. Adelstein, A. Nusrat & C. A. Parkos: A unique role for nonmuscle myosin heavy chain IIA in regulation of epithelial apical junctions. *PLoS ONE*, 2, e658 (2007)

43. Braga, V. M., L. M. Machesky, A. Hall & N. A. Hotchin: The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. *J Cell Biol*, 137, 1421-31 (1997)

44. Gopalakrishnan, S., N. Raman, S. J. Atkinson & J. A. Marrs: Rho GTPase signaling regulates tight junction assembly and protects tight junctions during ATP depletion. *Am J Physiol*, 275, C798-809 (1998)

45. Bruewer, M., A. M. Hopkins, M. E. Hobert, A. Nusrat & J. L. Madara: RhoA, Rac1, and Cdc42 exert distinct effects on epithelial barrier via selective structural and biochemical modulation of junctional proteins and F-actin. *Am J Physiol Cell Physiol*, 287, C327-35 (2004)

46. Takaishi, K., T. Sasaki, H. Kotani, H. Nishioka & Y. Takai: Regulation of cell-cell adhesion by rac and rho small G proteins in MDCK cells. *J Cell Biol*, 139, 1047-59 (1997)

47. Samarin, S. N., A. I. Ivanov, G. Flatau, C. A. Parkos & A. Nusrat: Rho/Rho-associated kinase-II signaling mediates disassembly of epithelial apical junctions. *Mol Biol Cell*, 18, 3429-39 (2007)

48. Grosshans, J., C. Wenzl, H. M. Herz, S. Bartoszewski, F. Schnorrer, N. Vogt, H. Schwarz & H. A. Muller: RhoGEF2 and the formin Dia control the formation of the furrow canal by directed actin assembly during Drosophila cellularisation. *Development*, 132, 1009-20 (2005)

49. Walsh, S. V., A. M. Hopkins, J. Chen, S. Narumiya, C. A. Parkos & A. Nusrat: Rho kinase regulates tight junction function and is necessary for tight junction assembly in polarized intestinal epithelia. *Gastroenterology*, 121, 566-79 (2001)

50. Sahai, E. & C. J. Marshall: ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat Cell Biol*, 4, 408-15 (2002)

51. Zandy, N. L., M. Playford & A. M. Pendergast: Abl tyrosine kinases regulate cell-cell adhesion through Rho GTPases. *Proc Natl Acad Sci U S A*, 104, 17686-91 (2007)

52. Benais-Pont, G., A. Punn, C. Flores-Maldonado, J. Eckert, G. Raposo, T. P. Fleming, M. Cereijido, M. S. Balda & K. Matter: Identification of a tight junction-associated guanine nucleotide exchange factor that activates Rho and regulates paracellular permeability. *J Cell Biol*, 160, 729-40 (2003)

53. Nakagawa, M., M. Fukata, M. Yamaga, N. Itoh & K. Kaibuchi: Recruitment and activation of Rac1 by the formation of E-cadherin-mediated cell-cell adhesion sites. *J Cell Sci*, 114, 1829-38 (2001)

54. Brandt, D. T. & R. Grosse: Get to grips: steering local actin dynamics with IQGAPs. *EMBO Rep*, 8, 1019-23 (2007)

55. Kuroda, S., M. Fukata, M. Nakagawa, K. Fujii, T. Nakamura, T. Ookubo, I. Izawa, T. Nagase, N. Nomura, H. Tani, I. Shoji, Y. Matsuura, S. Yonehara & K. Kaibuchi: Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin- mediated cell-cell adhesion. *Science*, 281, 832-5 (1998)

56. Fukata, M., S. Kuroda, M. Nakagawa, A. Kawajiri, N. Itoh, I. Shoji, Y. Matsuura, S. Yonehara, H. Fujisawa, A. Kikuchi & K. Kaibuchi: Cdc42 and Rac1 regulate the interaction of IQGAP1 with beta-catenin. *J Biol Chem*, 274, 26044-50 (1999)

57. Sander, E. E., S. van Delft, J. P. ten Klooster, T. Reid, R. A. van der Kammen, F. Michiels & J. G. Collard: Matrix-dependent Tiam1/Rac signaling in epithelial cells promotes either cell-cell adhesion or cell migration and is regulated by phosphatidylinositol 3-kinase. *J Cell Biol*, 143, 1385-98 (1998)

58. Hordijk, P. L., J. P. ten Klooster, R. A. van der Kammen, F. Michiels, L. C. Oomen & J. G. Collard: Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. *Science*, 278, 1464-6 (1997)

59. Malliri, A., S. van Es, S. Huveneers & J. G. Collard: The Rac exchange factor Tiam1 is required for the establishment and maintenance of cadherin-based adhesions. *J Biol Chem*, 279, 30092-8 (2004)

60. Mertens, A. E., R. C. Roovers & J. G. Collard: Regulation of Tiam1-Rac signalling. *FEBS Lett*, 546, 11-6 (2003)

61. Otani, T., T. Ichii, S. Aono & M. Takeichi: Cdc42 GEF Tuba regulates the junctional configuration of simple epithelial cells. *J Cell Biol*, 175, 135-46 (2006)

62. Kodama, A., K. Takaishi, K. Nakano, H. Nishioka & Y. Takai: Involvement of Cdc42 small G protein in cell-cell adhesion, migration and morphology of MDCK cells. *Oncogene*, 18, 3996-4006 (1999)

63. Rojas, R., W. G. Ruiz, S. M. Leung, T. S. Jou & G. Apodaca: Cdc42-dependent modulation of tight junctions and membrane protein traffic in polarized Madin-Darby canine kidney cells. *Mol Biol Cell*, 12, 2257-74 (2001)

64. Kroschewski, R., A. Hall & I. Mellman: Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. *Nat Cell Biol*, 1, 8-13 (1999)

65. Pilot, F. & T. Lecuit: Compartmentalized morphogenesis in epithelia: from cell to tissue shape. *Dev Dyn*, 232, 685-94 (2005)

66. Shook, D. & R. Keller: Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. *Mech Dev*, 120, 1351-83 (2003)

67. Lui, W. Y. & W. M. Lee: Regulation of junction dynamics in the testis--transcriptional and post-translational regulations of cell junction proteins. *Mol Cell Endocrinol*, 250, 25-35 (2006)

68. Grunert, S., M. Jechlinger & H. Beug: Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. *Nat Rev Mol Cell Biol*, 4, 657-65 (2003)

69. Edens, H. A. & C. A. Parkos: Modulation of epithelial and endothelial paracellular permeability by leukocytes. *Adv Drug Deliv Rev*, 41, 315-28 (2000)

70. Huber, D., M. S. Balda & K. Matter: Transepithelial migration of neutrophils. *Invasion Metastasis*, 18, 70-80 (1998)

71. Hecht, G., C. Pothoulakis, J. T. LaMont & J. L. Madara: Clostridium difficile toxin A perturbs cytoskeletal structure and tight junction permeability of cultured human intestinal epithelial monolayers. *J Clin Invest*, 82, 1516-24 (1988)

72. Spitz, J., R. Yuhan, A. Koutsouris, C. Blatt, J. Alverdy & G. Hecht: Enteropathogenic Escherichia coli adherence to intestinal epithelial monolayers diminishes barrier function. *Am J Physiol*, 268, G374-9 (1995)

73. Ivanov, A. I., A. Nusrat & C. A. Parkos: Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. *Mol Biol Cell*, 15, 176-88 (2004)

74. Kartenbeck, J., M. Schmelz, W. W. Franke & B. Geiger: Endocytosis of junctional cadherins in bovine kidney epithelial (MDBK) cells cultured in low Ca2+ ion medium. *J Cell Biol*, 113, 881-92 (1991)

75. Ivanov, A. I., A. Nusrat & C. A. Parkos: Endocytosis of the apical junctional complex: mechanisms and possible roles in regulation of epithelial barriers. *Bioessays*, 27, 356-65 (2005)

76. Ivanov, A. I., I. C. McCall, C. A. Parkos & A. Nusrat: Role for actin filament turnover and a myosin II motor in

cytoskeleton-driven disassembly of the epithelial apical junctional complex. *Mol Biol Cell*, 15, 2639-51 (2004)
77. de Rooij, J., A. Kerstens, G. Danuser, M. A. Schwartz & C. M. Waterman-Storer: Integrin-dependent actomyosin contraction regulates epithelial cell scattering. *J Cell Biol*, 171, 153-64 (2005)

78. Utech, M., A. I. Ivanov, S. N. Samarin, M. Bruewer, J. R. Turner, R. J. Mrsny, C. A. Parkos & A. Nusrat: Mechanism of IFN-{gamma}-induced Endocytosis of Tight Junction Proteins: Myosin II-dependent Vacuolarization of the Apical Plasma Membrane. *Mol Biol Cell*, 16, 5040-52 (2005)

79. Bruewer, M., S. Samarin & A. Nusrat: Inflammatory bowel disease and the apical junctional complex. *Ann N Y Acad Sci*, 1072, 242-52 (2006)

80. Guo, Y., C. Ramachandran, M. Satpathy & S. P. Srinivas: Histamine-induced myosin light chain phosphorylation breaks down the barrier integrity of cultured corneal epithelial cells. *Pharm Res*, 24, 1824-33 (2007)

81. Zolotarevsky, Y., G. Hecht, A. Koutsouris, D. E. Gonzalez, C. Quan, J. Tom, R. J. Mrsny & J. R. Turner: A membrane-permeant peptide that inhibits MLC kinase restores barrier function in *in vitro* models of intestinal disease. *Gastroenterology*, 123, 163-72 (2002)

82. Turner, J. R., B. K. Rill, S. L. Carlson, D. Carnes, R. Kerner, R. J. Mrsny & J. L. Madara: Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. *Am J Physiol*, 273, C1378-85 (1997)

83. Hallett, M. A., P. C. Dagher & S. J. Atkinson: Rho GTPases show differential sensitivity to nucleotide triphosphate depletion in a model of ischemic cell injury. *Am J Physiol Cell Physiol*, 285, C129-38 (2003)

84. Sutton, T. A., H. E. Mang & S. J. Atkinson: Rho-kinase regulates myosin II activation in MDCK cells during recovery after ATP depletion. *Am J Physiol Renal Physiol*, 281, F810-8 (2001)

85. Potempa, S. & A. J. Ridley: Activation of both MAP kinase and phosphatidylinositide 3-kinase by Ras is required for hepatocyte growth factor/scatter factor-induced adherens junction disassembly. *Mol Biol Cell*, 9, 2185-200 (1998)

86. Ridley, A. J., P. M. Comoglio & A. Hall: Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac, and Rho in MDCK cells. *Mol Cell Biol*, 15, 1110-22 (1995)

87. Ray, R. M., R. J. Vaidya & L. R. Johnson: MEK/ERK regulates adherens junctions and migration through Rac1. *Cell Motil Cytoskeleton*, 64, 143-56 (2007)

88. Gopalakrishnan, S., K. W. Dunn & J. A. Marrs: Rac1, but not RhoA, signaling protects epithelial adherens junction assembly during ATP depletion. *Am J Physiol Cell Physiol*, 283, C261-72 (2002)

89. Chen, L., B. M. Necela, W. Su, M. Yanagisawa, P. Z. Anastasiadis, A. P. Fields & E. A. Thompson: Peroxisome proliferator-activated receptor gamma promotes epithelial to mesenchymal transformation by Rho GTPase-dependent activation of ERK1/2. *J Biol Chem*, 281, 24575-87 (2006)

90. Patel, S., K. I. Takagi, J. Suzuki, A. Imaizumi, T. Kimura, R. M. Mason, T. Kamimura & Z. Zhang: RhoGTPase activation is a key step in renal epithelial mesenchymal transdifferentiation. *J Am Soc Nephrol*, 16, 1977-84 (2005)

91. Shen, Y., D. S. Hirsch, C. A. Sasiela & W. J. Wu: CDC42 regulates E-cadherin ubiquitination and degradation through an EGF receptor to SRC-mediated pathway. *J Biol Chem* (2007)

92. Takeichi, M.: Cadherin cell adhesion receptors as a morphogenetic regulator. *Science*, 251, 1451-5 (1991)

93. Auersperg, N., J. Pan, B. D. Grove, T. Peterson, J. Fisher, S. Maines-Bandiera, A. Somasiri & C. D. Roskelley: E-cadherin induces mesenchymal-to-epithelial transition in human ovarian surface epithelium. *Proc Natl Acad Sci U S A*, 96, 6249-54 (1999)

94. Blikslager, A. T., A. J. Moeser, J. L. Gookin, S. L. Jones & J. Odle: Restoration of barrier function in injured intestinal mucosa. *Physiol Rev*, 87, 545-64 (2007)

95. Braga, V.: Epithelial cell shape: cadherins and small GTPases. Exp Cell Res, 261, 83-90 (2000)

96. Van Aelst, L. & M. Symons: Role of Rho family GTPases in epithelial morphogenesis. Genes Dev, 16, 1032-54 (2002)

97. Ivanov, A. I., D. Hunt, M. Utech, A. Nusrat & C. A. Parkos: Differential roles for actin polymerization and a myosin II motor in assembly of the epithelial apical junctional complex. Mol Biol Cell, 16, 2636-50 (2005)

98. Krendel, M. F. & E. M. Bonder: Analysis of actin filament bundle dynamics during contact formation in live epithelial cells. *Cell Motil Cytoskeleton*, 43, 296-309 (1999)

99. Calautti, E., M. Grossi, C. Mammucari, Y. Aoyama, M. Pirro, Y. Ono, J. Li & G. P. Dotto: Fyn tyrosine kinase is a downstream mediator of Rho/PRK2 function in keratinocyte cell-cell adhesion. *J Cell Biol*, 156, 137-48 (2002)

100. Calautti, E., S. Cabodi, P. L. Stein, M. Hatzfeld, N. Kedersha & G. Paolo Dotto: Tyrosine phosphorylation and src family kinases control keratinocyte cell-cell adhesion. *J Cell Biol*, 141, 1449-65 (1998)

101. Noren, N. K., C. M. Niessen, B. M. Gumbiner & K. Burridge: Cadherin engagement regulates Rho family GTPases. *J Biol Chem*, 276, 33305-8 (2001)

102. Yap, A. S. & E. M. Kovacs: Direct cadherin-activated cell signaling: a view from the plasma membrane. *J Cell Biol*, 160, 11-6 (2003)

103. Ehrlich, J. S., M. D. Hansen & W. J. Nelson: Spatio-temporal regulation of Rac1 localization and lamellipodia dynamics during epithelial cell-cell adhesion. *Dev Cell*, 3, 259-70 (2002)

104. Verma, S., A. M. Shewan, J. A. Scott, F. M. Helwani, N. R. den Elzen, H. Miki, T. Takenawa & A. S. Yap: Arp2/3 activity is necessary for efficient formation of E-cadherin adhesive contacts. *J Biol Chem*, 279, 34062-70 (2004)

105. Scott, J. A., A. M. Shewan, N. R. den Elzen, J. J. Loureiro, F. B. Gertler & A. S. Yap: Ena/VASP proteins can regulate distinct modes of actin organization at cadherin-adhesive contacts. *Mol Biol Cell*, 17, 1085-95 (2006)

106. Pece, S., M. Chiariello, C. Murga & J. S. Gutkind: Activation of the protein kinase Akt/PKB by the formation of E-cadherin-mediated cell-cell junctions. Evidence for the association of phosphatidylinositol 3-kinase with the E-cadherin adhesion complex. *J Biol Chem*, 274, 19347-51 (1999)

107. Kovacs, E. M., R. G. Ali, A. J. McCormack & A. S. Yap: E-cadherin homophilic ligation directly signals through Rac and phosphatidylinositol 3-kinase to regulate adhesive contacts. *J Biol Chem*, 277, 6708-18 (2002)

108. Goodwin, M., E. M. Kovacs, M. A. Thoreson, A. B. Reynolds & A. S. Yap: Minimal mutation of the cytoplasmic tail inhibits the ability of E-cadherin to activate Rac but not phosphatidylinositol 3-kinase: direct evidence of a role for cadherin-activated Rac signaling in adhesion and contact formation. *J Biol Chem*, 278, 20533-9 (2003)

109. Kim, S. H., Z. Li & D. B. Sacks: E-cadherin-mediated cell-cell attachment activates Cdc42. *J Biol Chem*, 275, 36999-7005 (2000)

110. Vasioukhin, V., C. Bauer, M. Yin & E. Fuchs: Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell*, 100, 209-19 (2000)

111. Vasioukhin, V. & E. Fuchs: Actin dynamics and cell-cell adhesion in epithelia. *Curr Opin Cell Biol*, 13, 76-84 (2001)

112. Fukuhara, A., K. Shimizu, T. Kawakatsu, T. Fukuhara & Y. Takai: Involvement of nectin-activated Cdc42 small G protein in organization of adherens and tight junctions in Madin-Darby canine kidney cells. *J Biol Chem*, 278, 51885-93 (2003)

113. Yamanaka, T., Y. Horikoshi, A. Suzuki, Y. Sugiyama, K. Kitamura, R. Maniwa, Y. Nagai, A. Yamashita, T. Hirose, H. Ishikawa & S. Ohno: PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. *Genes Cells*, 6, 721-31 (2001)

114. Ohno, S.: Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. *Curr Opin Cell Biol*, 13, 641-8 (2001)

115. Fukuhara, T., K. Shimizu, T. Kawakatsu, T. Fukuyama, Y. Minami, T. Honda, T. Hoshino, T. Yamada, H. Ogita, M. Okada & Y. Takai: Activation of Cdc42 by trans interactions of the cell adhesion molecules nectins through c-Src and Cdc42-GEF FRG. *J Cell Biol*, 166, 393-405 (2004)

116. Guillemot, L., S. Paschoud, P. Pulimeno, A. Foglia & S. Citi: The cytoplasmic plaque of tight junctions: A scaffolding and signalling center. *Biochim Biophys Acta*, 1778, 601-13 (2008)

117. Behrens, J., M. M. Mareel, F. M. Van Roy & W. Birchmeier: Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *J Cell Biol*, 108, 2435-47 (1989)

118. Shiozaki, H., H. Oka, M. Inoue, S. Tamura & M. Monden: E-cadherin mediated adhesion system in cancer cells. *Cancer*, 77, 1605-13 (1996)

119. Vleminckx, K., L. Vakaet, Jr., M. Mareel, W. Fiers & F. van Roy: Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell*, 66, 107-19 (1991)

120. Matozaki, T., H. Nakanishi & Y. Takai: Small G-protein networks: their crosstalk and signal cascades. *Cell Signal*, 12, 515-24 (2000)

121. Fukata, M. & K. Kaibuchi: Rho-family GTPases in cadherin-mediated cell-cell adhesion. *Nat Rev Mol Cell Biol*, 2, 887-97 (2001)

122. Mertens, A. E., T. P. Rygiel, C. Olivo, R. van der Kammen & J. G. Collard: The Rac activator Tiam1 controls tight junction biogenesis in keratinocytes through binding to and activation of the Par polarity complex. *J Cell Biol*, 170, 1029-37 (2005)

Abbreviations: AJC, apical junctional complex; TJ, tight junction; AJ, adherens junction; ZO, zonula occludens; Rho, Rac-homologous; Rac, ras-related C3 botulinum toxin substrate; Cdc42, cell division cycle 42; PAR, partitioning-defective protein; RMLC, regulatory myosin light chain; ROCK, Rho-associated kinase; GEF, guanine nucleotide exchange factor; GAP, GTPase activating protein; Dia, diaphanous-related forming; ROCK, Rho-associated kinase; Abl, Abelson tyrosine kinase; Tiam1, T-cell lymphoma invasion and metastasis-inducing protein 1; HGF, hepatocyte growth factor, EGFR epidermal growth factor receptor; FRG, FGD1-related Cdc42-GEF; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAP-ERK kinase; Arp, actin-related protein; WASP, Wiscott-Aldrich syndrome protein; WAVE, WASP/verprolin homologous protein; VASP vasodilator-stimulated phosphoprotein; PKC,

protein kinase C; PI3K, phosphoinositide 3-kinase; PRK proliferation-related kinase.

Key Words: Rho, Rac Cdc42, Tight Junctions, Adherens Junctions, Actin, Myosin, Review

Send correspondence to: Asma Nusrat, Department of Pathology and Laboratory Medicine, Emory University, Room 105E 615 Michael Street, Atlanta, GA 30322; Tel: 404-727 8543, Fax: 404-727 3321, E-mail: anusrat@emory.edu

http://www.bioscience.org/current/vol14.htm