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RhoGTPases, actomyosin signaling and regulation of the Epithelial Apical Junctional Complex

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Abstract

Epithelial cells form regulated and selective barriers between distinct tissue compartments. The Apical Junctional Complex (AJC) consisting of the tight junction (TJ) and adherens junction (AJ) control epithelial homeostasis, paracellular permeability and barrier properties. The AJC is composed of multiprotein complexes consisting of transmembrane proteins that affiliate with an underlying perijunctional F-actin myosin ring through cytoplasmic scaffold proteins. AJC protein associations with the apical actin-myosin cytoskeleton are tightly controlled by a number of signaling proteins including the Rho family of GTPases that orchestrate junctional biology, epithelial homeostasis and barrier function. This review highlights the vital relationship of Rho GTPases and AJCs in controlling the epithelial barrier. The pathophysiologic relationship of Rho GTPases, AJC, apical actomyosin cytoskeleton and epithelial barrier function is discussed.

Keywords

Epithelia; Tight Junction; Adherens Junction; Barrier Function; Rho-GTPases; actin and myosin cytoskeleton

I. Introduction

Epithelial cells form selective and dynamic barriers that control movement of fluid and solutes between distinct tissue compartments. These properties are achieved by polarized organization of proteins in intercellular junctions that mediate cell-cell adhesion and control epithelial homeostasis. The tight junction (TJ) and the subjacent adherens junction (AJ) are collectively referred to as the Apical Junctional Complex (AJC). The AJC not only plays a pivotal role in regulating intercellular adhesion, but also in controlling paracellular movement of fluid and solutes, thereby helping to maintain distinct environments across polarized epithelia [1, 2].

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Structural and signaling proteins in the AJC and the underlying apical actomyosin cytoskeleton control dynamic properties of the AJC. We provide a brief overview of proteins that constitute the AJC with a subsequent focus on signaling by Rho GTPase family members and actin-myosin dynamics that control epithelial barrier function.

II. Molecular structure and function of the Apical Junctional Complex

The cytoplasmic domains of AJC transmembrane proteins associate with underlying plaque proteins that provide a link to the actin cytoskeleton. Over a 100 proteins have been identified in the AJC that include scaffold proteins, kinases, phosphatases, small GTPases and their corresponding exchange and activating proteins, transcription factors, actin binding proteins and filamentous actin itself. Scaffold proteins containing domains such as PDZ, SH3, WW and GK that help to organize the protein complexes in these intercellular junctions [3, 4]. Such scaffold proteins include the membrane associated proteins with guanylate kinase homology (MAGUK) family of zonula occludens (ZO) proteins, ZO1, ZO2 and ZO3. The PDZ domains in ZO proteins associate with PDZ motifs in AJC transmembrane proteins including claudins, occludin, junctional adhesion molecule-A (JAM-A), E-cadherin, and nectins [5]. In addition to controlling the TJ structure, ZO proteins have been reported to traffic to the nucleus to control transcriptional events during epithelial migration and proliferation [6, 7]. Other TJ scaffold proteins, cingulin and paracingulin, have homology with non muscle myosin II NM II and regulate Rho GTPase signaling in the TJ [8].

The key integral membrane proteins in AJs include E-cadherin and nectins. [9]. These transmembrane proteins affiliate with catenin armadillo family of proteins in the cytoplasmic plaque. The term “catenin” was derived from the latin word “catena” which means chain and they were assigned this terminology as they were considered to provide a link between cadherins and the actomyosin cytoskeleton [10]. β -catenin associates with E-cadherin in the endoplasmic reticulum and is required for its effective membrane transport [11]. Once at the plasma membrane, the cadherin- β -catenin complex recruits α -catenin, which links the cadherins to the actin cytoskeleton and is essential for the AJ assembly. Another catenin, p120 catenin, stabilizes the AJ by masking a cadherin endocytic signal and provides a link to microtubules [12, 13]. In contrast, the scaffold protein afadin serves as a link between transmembrane nectins and the subjacent actomyosin cytoskeleton thereby playing an important role in the establishment of cadherin based junctions [14] (Figure 1).

III. AJC and the perijunctional actomyosin cytoskeleton

The actin cytoskeleton consists of a dynamic network of filaments that is spatially organized to define and maintain cell morphology, regulate dynamics of cell proliferation, control cell motility and migration. The actomyosin cytoskeleton interacts with transmembrane proteins that anchor cells to the substrate and adjoining cells. Actin filaments are remodeled by regulated and reversible transition from globular monomeric actin (G-actin) to filamentous actin (F-actin). Additionally, F-actin associates with NM II that is composed of two heavy chains (230 kDa), two regulatory or light chains (20 KDa) and two essential light chains. NM II molecules form dimers through interactions between their rod domains while the

acting binding and ATPase motor domain resides in the globular head domain of the heavy chain, which also contains a rod domain. The actin-binding domain is folded until the myosin light chain (MLC) becomes phosphorylated resulting in its activation and actomyosin contraction. [15].

In polarized epithelial cells, the actomyosin cytoskeleton exhibits distinct spatial organization in the apical and basolateral compartment. AJC protein complexes associate with a circumferential belt of contractile F-actin that plays an important role in regulating paracellular permeability and cell-cell adhesion mediated by a number of signaling cascades [16]. The intimate relationship between actomyosin cytoskeleton and AJC function was highlighted in a number of studies in the early 1980's [17-20]. These studies reported organization of apical actin microfilaments in a perijunctional ring. Inhibition of actin polymerization compromised ileal epithelial TJ integrity and contraction of the perijunctional actomyosin ring [21, 22]. The perijunctional cytoskeletal anatomy that was explored by electron microscopy further confirmed its intimate association with TJs at sites of close membrane apposition between adjoining cells [23].

IV. Rho GTPases

The Ras superfamily of GTPases comprises more than 150 human proteins that are grouped in 5 families: Ras, Rho, Rab, Ran and Arf [24]. The Rho family (Ras homologous) has at least 25 protein members. The most extensively characterized family members are the classical Rho proteins: RhoA, Rac and Cdc42 that not only play a central role in controlling actin-myosin dynamics, but are also involved in other biological processes including microtubule dynamics, gene transcription, cell cycle and vesicular transport. In the broad scheme, RhoA facilitates organization of basal F-actin stress fibers and focal adhesions, while Rac1 controls membrane ruffling and lamellipodial extrusion, and Cdc42 regulates filopodia formation and cell polarity [25].

Rho GTPases function as molecular switches that integrate environmental and intracellular signaling cues. As with many GTPases, specific guanine nucleotide exchange factors (GEFs) promote the exchange of Rho GTPase(s) associated GDP with GTP thereby activating the GTPase resulting in effector binding. While Rho GTPases have an inherent capacity to hydrolyze GTP to GDP required for their inactivation, the process is promoted by a distinct family of GTPases activating proteins (GAPs). The Guanine nucleotide dissociation inhibitors (GDIs) keep Rho proteins in a suppressed state [26]. The differential functions of a number of GEFs, GAPs and GDIs, and ultimately the Rho GTPases is determined by their specific expression and localization that mediates their respective biological functions (Figure 2).

Several AJC proteins interact directly with small Rho GTPases or with GEFs and GAPs to control their activation and regulate different cellular properties. GEF-H1, the most studied Rho GEF, interacts with ZONAB, Cingulin and Paracingulin [27-29]. These proteins sequester GEFH1 at the TJ, thus inhibiting RhoA signaling. P114RhoGEF binds to cingulin, in a complex containing NM II and the Rho associated protein kinase (ROCK) [30]. This complex drives spatially restricted activation of RhoA to regulate junction

formation and epithelial morphogenesis. Paracingulin association with Tiam [28] is required for its efficient recruitment to junctions, and also provides a mechanism whereby Rac1 is activated during junction formation. PDZGEF1 interacts directly with ZO-2 and indirectly with JAM-A [31], in a complex that also binds and activates another GTPase, Rap2c to regulate epithelial permeability through the inhibition of Rho signaling. Other TJ GEFs include GEFT, RhoGEF11 and Tuba. Bves and ZO-1 bind to GEFT [32] and RhoGEF11 [33] respectively to sequester these proteins and inhibit Rho, Rac1 and Cdc42 activity. ZO-1 also associates with Tuba [34], shaping the cell junctions through the local activation of Cdc42 and its effector proteins. Two TJ GAPs that associate with paracingulin include SH3BP1 and MgcRacGAP. The latter also binds to cingulin to either inhibit Rac signaling or regulate Cdc42 to control junctional assembly and epithelial morphogenesis. [35, 36] (Figure 3A).

In AJs RhoGEF2, RFG and Trio Rho GEF [37-39] bind to E cadherin while Vav2 GEF associates with Nectin and p120 catenin to regulate AJ formation in kidney epithelia [40, 41]. Ect2 serves as a GEF for Cdc42 and RhoA, and it has been shown to stabilize E-cadherin in a complex with the Centralspindlin complex [42]. Interestingly, IQGAP1 has homology with GAP proteins, but does not have GAP activity and instead activates Rho GTPases. IQGAP1 in AJs, interacts with E-cadherin, β -catenin and afadin to promote the AJC morphogenesis and junctional maturation [43-45]. Additionally, p190RhoGAP [46] and DLC RhoGAP [47] regulate Rho activity and epithelial migration (Figure 3 B).

V. AJC regulation by the Rho family of GTPases

The field of Rho GTPase family member regulation of actin cytoskeletal dynamics blossomed in the early 90's after their first documentation by Alan Hall [48-50]. Subsequently in 1995 we reported that RhoGTPase control TJ function and perijunctional F-actin organization in polarized epithelial cells [51]. Many additional studies have further shed light on control of the AJC by Rho GTPases and the actomyosin cytoskeleton [52, 53]. Interestingly, a delicate balance of Rho activity is required for maintaining optimal TJ function as its inhibition as well as over activation compromises the epithelial barrier function [51, 54] [53]. Thus, finely tuned regulation of Rho GTPases and their effectors plays a pivotal role in controlling paracellular permeability in epithelial cells.

V.1. AJC assembly and maintenance

The generation of apical-basal polarity in epithelial cells requires asymmetry along an intracellular axis bearing two distinct plasma membrane domains. The apical domain faces the exterior or lumen of organs and is specialized for absorption and secretion, while the basolateral compartment mediates cell-cell and cell-matrix adhesion [55].

There are three major mechanisms by which Rho GTPases control AJC assembly: 1) Stimulation of actin polymerization in initial cell-cell contacts that is required to cluster/stabilize E-cadherin-based puncta. This is mediated by Rac-1-WAVE-Arp2/3 and Cdc42-N_WASP-Arp2/3 dependent branched actin polymerization at initial cell-cell contacts, and Rho-mDia-dependent actin polymerization that generates linear F-actin filaments, 2) Stimulation of NM IIA activity which results in expansion/linearization of initial AJ-like

junctions as well as altered cell shape, necessary for columnarization of simple epithelia, 3) Assembly of cell polarity complexes resulting in the formation of TJs and apico-basal polarity (For review see [56]).

Intercellular adhesion in epithelial cells is initiated by the recruitment of nectin to sites of nascent intercellular junction assembly. This is followed by the recruitment and subsequent Rac1 dependent stabilization of E-cadherin and assembly of AJs [43, 57, 58]. The stabilization of E-cadherin by Rac1 requires association of Rac1 with its GEF TIAM1 that in turn is dependent on β 2-syntrophin mediated accumulation of Rac1 in the lateral plasma membrane [59]. TIAM1 is recruited and maintained in AJs by paracingulin, a cingulin paralog that is distributed in both TJs and AJs [28]. Rac1 activity in this region triggers reorganization of the actin cytoskeleton thereby facilitating the recruitment of polarity protein Par3 that initiates TJ formation [59]. This signaling cascade participates in Par3 mediated inhibition of TIAM1 and Rac1 activity. Constitutive activation of Rac1 in keratinocytes results in endocytosis of E-cadherin and compromised AJs [60]. Thus, tightly regulated Rac1 activity is also needed for maintenance of AJs. Rac 1 also regulates the formation of WAVE2-Arp2/3 complex, which is necessary for AJ integrity. The complex formation occurs through direct interaction of E-cadherin with cortactin, which in turn recruits Arp 2/3 and WAVE-2 to the AJ in the presence of N-WASP [61-63]. Cdc42 interacts with N-WASP, which also activates Arp 2/3 [64] leading to actin filament elongation (Figure 4).

During assembly of TJs, the scaffold proteins ZO-1 and cingulin recruit RhoGEF11 and p114RhoGEF respectively [30] [33]. Both of these GEFs are specific for RhoA that in turn plays an important role in actin cytoskeletal remodeling and organization of the AJC associated apical perijunctional F-actin ring [65]. Additionally, these signaling events stabilize p114RhoGEF at the AJC where it binds to NM II and ROCK, creating a complex ready for RhoA activation. ROCK, phosphorylates MLC and inhibits the myosin light chain phosphatase, MYPT [66]. As a consequence, NM II becomes active, triggering the assembly of contractile actomyosin filaments [67]. Cdc42 associates with the polarity protein Par6, thereby stimulating aPKC activity and phosphorylation of TJ proteins such as JAM-A and ZO-2, to stabilize their localization in the junction [68-71]. Ect2 has been reported to facilitate Cdc42 association with the Par3/Par6/aPKC polarity complex to influence its localization in the AJC [72, 73]. Additionally, the recruitment and activation of NM II in AJs by Ect2 stabilizes E-cadherin and influences RhoA mediated actomyosin organization [42] (Figure 4).

NM II plays a key role in epithelial junction establishment from the early formation of AJ-like structures followed by TJ assembly and establishment of apico-basal epithelial polarity [15, 74]. Depletion of NM IIA by siRNA dramatically attenuates the formation of AJs in colonic epithelial cells and promotes disassembly of primordial F-actin in early cell-cell contacts. [75]. NM IIA silencing also leads to attenuation of TJ assembly underdevelopment of the apical domain and defective perijunctional F-actin assembly [76, 77]. In initial junctions, NM IIA is a structural protein, promoting the formation of F-actin fibers that stabilize nascent E-cadherin-based intercellular junction with subsequent TJ

formation and epithelial polarization. Once the AJC is stabilized, myosin IX A and B, RhoA GAPs downregulate Rho signaling and promote AJC maturation and maintenance [78, 79].

V.2. Rho GTPases as drivers of epithelial barrier disruption and de-differentiation in inflammation, infection and cancer

V.2.1 AJC disassembly—The orchestrated disassembly of epithelial apical junctions facilitates junction remodeling required for a number of biological processes such as spermatogenesis and migration of leukocytes across the epithelium [3]. Additionally, pathogens including viruses and bacteria modulate junctional integrity in order to gain access to host tissues. Pro-inflammatory cytokines released into the epithelial milieu during mucosal inflammation modulate RhoA activity and ultimately AJC integrity [80]. Key downstream effectors of RhoA that control the tension in the apical perijunctional F-actin ring and in turn AJC function include ROCK, MLC and MYPT. In addition to these regulatory pathways, tension in the perijunctional actin-myosin ring is controlled by MLCK that has also been shown to influence AJC integrity and function [81].

NM II depletion by pharmacological inhibition using blebbistatin or siRNA knock down decreases epithelial barrier function that is observed in spite of a structurally-normal AJC, suggesting an important role of NM II in the regulating structural integrity and barrier properties of mature epithelial apical junctions [76, 82]. Several stimuli that compromise the AJC, such as calcium depletion or pro-inflammatory cytokine treatment, activate NM II [76, 82-84] triggering actomyosin contractility resulting in perturbed epithelial barrier function. Silencing of NM IIA heavy chain leads to AJC disassembly in model intestinal epithelial cells [76]. This signaling axis is in part responsible for the stimulation of NM II contractility that compromises AJC barrier function.

The structural protein ezrin activates Rac1 [85], leading to changes in AJ without influencing TJs. In addition to AJ regulation by RhoA and Rac1, change in Cdc42 activity in response to extracellular calcium depletion has also been reported to influence ubiquitination and stability of E-cadherin [60, 86]. Cdc42 promotes EGF receptor stability and Src signaling resulting in phosphorylation and stabilization of E-cadherin in AJs [87]. Furthermore, IQGAP, Rac1 and Cdc42 effector proteins associate with β -catenin to inhibit alpha catenin binding to the catenin-cadherin complex thereby destabilizing AJs [88] (Figure 5).

In summary, junctional proteins regulate Rho GTPases by interacting with their respective GEFs and GAPs, and by recruiting these regulatory proteins to cell-cell contacts. Thus, while there is a mutual dependence of Rho GTPase(s) control of AJC assembly, the AJC also controls recruitment and activation of these GTPases.

V.2 Inflammation

Mucosal inflammation is associated with release of myriad molecules that influence the composition and stability of the AJC. Cytokines in the inflammatory milieu contribute to changes in paracellular permeability by restructuring cell-cell junction [80]. In chronic inflammatory disorders such as inflammatory bowel disease, pro-inflammatory cytokines

compromise intercellular junctions and barrier function [9]. IFN γ promotes endocytosis of epithelial AJC transmembrane proteins that are internalized by macropinocytosis into a vacuolar apical compartment (VACs). Such AJC protein endocytic events are controlled by Rho-ROCK-MYPT-MLC mediated contraction of perijunctional actomyosin cytoskeleton [89]. These events are reversible with re-establishment of the AJC after withdrawal of IFN γ . In addition, the pro-inflammatory cytokine, TNF α also promotes MLC phosphorylation by MLCK thereby further contributing to the actomyosin contractile events in inflammation [81]. TGF β 1 treatment of a kidney derived epithelial cell line HK-2 has been shown to result in a RhoA/ROCK dependent decrease in ZO-1 and occludin protein thereby resulting in TJ barrier compromise [90]

V.3. Microorganisms

V.3.1. Viruses—Several viruses use AJC transmembrane proteins as receptors for host invasion. These include hepatitis C virus (occludin and claudin family of protein 1, 6 and 9), Coxsackie and adenovirus (CAR), reovirus (JAM-A) and nectins (HSV and polio virus) [91]. Other viruses with specialized proteins in their capsid that target AJC proteins to disrupt cell-cell adhesion and gain access to the blood stream include HIV (Tat protein binds occludin) and Rotavirus VP8 (binds different claudins [92, 93]). HIV-1 Tat protein increases the permeability of endothelial cells in a RhoA-ROCK dependent manner and by decreasing occludin mRNA levels resulting in its cleavage, and by promoting nuclear relocalization of ZO-1 [92, 94]. After binding to nectin, Herpes simplex virus I phagocytosis and internalization requires RhoA activity [95]. Binding of HCV to its receptor CD81 triggers RhoA, Rac and Cdc42 signaling, and actin dependent re-localization of the virus to cell-cell contacts where it encounters occludin, claudin-1 and ZO-1 that participate in the internalization of the virus [96].

V.3.II. Bacteria and bacterial toxins—Epithelial barriers inhibit microorganism access to tissue compartments, and pathogens have evolved to exploit this important barrier. *Salmonella enterica* and *Helicobacter pylori* are examples of bacteria that inject their own proteins into epithelial cells to activate RhoA signaling to compromise TJ structure and function [97, 98]. Many bacteria produce toxins that target intercellular junctions to promote their disassembly by activating Rho GTPases. *Clostridium difficile* toxins A (TcdA) and B (TcdB) monoglucosylate and inactivate Rho GTPases thereby influencing actin filaments and integrity of the TJ [99, 100]. Interestingly, increased Rho activation in response to cytotoxic necrotizing factor 1 (CNF1) *Escherichia coli* toxin also compromises TJs [101, 102]. These findings suggest that a delicate balance of Rho GTPase(s) activity is needed for maintaining TJ structure and barrier function. *Pseudomonas aeruginosa* is an opportunistic pathogen that secretes the ExoS toxin which inhibits Rho activity and alters ZO-1 and occludin localization in TJs [103]. In addition, lipopolysaccharide (LPS), a compound found in the outer membrane of gram-negative bacteria activates RhoA by increasing p115RhoGEF protein levels thereby compromising the epithelial barrier [104-106].

V.4. Cancer

Altered expression of AJC proteins has been observed in many adenocarcinomas. E cadherin and p120 catenin loss is associated with pro-invasive tumor behavior and poor prognosis

[107]. E-cadherin recruits p120 catenin, and in cancer cells that have lost E-cadherin, p120 catenin is localized in the cytoplasm thereby contributing to invasion of cancer cells through Rho signaling [108]. [109-111]. p120 catenin has been reported to both activate and inhibit RhoA in different tissues [112, 113]. In cancer cells, p120catenin has also been reported to activate Rac1 that in combination with reduced substrate adhesion due to RhoA inhibition, promotes cancer cell invasion [112]. Additionally, decreased activation of Cdc42 and Rac1 correlates with increased invasive potential of several epithelial cell lines derived from metastatic colorectal adenocarcinoma [114]. In breast epithelial cells p115RhoGEF loss leads to decreased junctional E-cadherin and enhanced migration [115], whereas ROCK inhibition disrupts the AJC and promotes cell proliferation and migration [116].

The Deleted in Liver Cancer (DLC) proteins are small GTPase GAPs that have tumor-suppressor properties. When DLC-1 is expressed in metastatic prostate carcinoma, RhoA is inhibited leading to an increase in E-cadherin expression and cell aggregation [117]. DLC-3 silencing in breast epithelial cells leads to mislocalization of E-cadherin and catenins, resulting in impaired cell aggregation and increased migration. This phenotype reverts when ROCK is inhibited [118].

In summary, RhoGTPases play a pivotal role in controlling actomyosin cytoskeletal and AJC dynamics. Perturbation in the steady state activity of these proteins influences cytoskeletal mediated junctional assembly or disassembly. Pathologic events associated with inflammation and cancer perturb the balance of Rho GTPase(s) activity in the AJC thereby contributing to compromised epithelial barrier function.

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Figure 1. Molecular structure of the Apical Junctional Complex

The Apical Junctional complex (AJC) consisting of the tight junction (TJ) and adherens junction (AJ) resides at the interface between the apical and basolateral plasmamembrane. An apical perijunctional actomyosin ring associates with AJC proteins that include the following:

- Transmembrane proteins; **CTX proteins** (JAM-A), CAR, ESAM, CRTAM, CLMP, claudins, TMPs, Bves, LSR, E-cadherin, Nectin.
- Scaffold proteins; paracingulin (**Pcing**), PLEKHA7 (**PA7**), catenins including α catenin; β catenin and **p120**.

Figure 2. GEFs, GAPs and GDIs control Rho GTPases

The function of RhoGTPases (**SGP**) is regulated by proteins that either facilitate or inhibit their activation. While GEFs promote the GTP-bound state, GAPs inhibit GTP association. Additionally, GDIs sequesters RhoGTPases in an inactive state.

Figure 3. AJC associated GEFs and GAPs

GEFs and GAPs interact with Tight Junction (A) and Adherens Junction (B) proteins to modulate RhoGTPase(s) signaling and actomyosin cytoskeleton dynamics. While some GEFs are sequestered in the TJ to inhibit function of AJC proteins such as GEFH1, GEFT and RhoGEF11, other GEFs and GAPs form complexes with AJC proteins leading to the local activation or inactivation of specific RhoGTPases. GEFs are highlighted in green color while GAPs are represented in red color. Paracingulin (**Pcingulin**) and MgRacGAP

(MgRac): The protein affiliations represented in this figure include confirmed and hypothetical proteins complexes reported in the literature.

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Figure 4. RhoGTPases regulation of AJC assembly and maintenance

Rho, Rac and Cdc42 signaling promotes AJC and perijunctional actomyosin cytoskeletal assembly. Rac1 stabilizes E-cadherin at the AJ in a complex that includes $\beta 2$ syntrophin, Tiam and paracingulin. Rac1 promotes assembly of the WAVE2-Arp2/3 complex, which interacts with E cadherin through cortactin. Rac1 also facilitates the junctional recruitment of Par3. Cdc42 interacts with N-WASP and activates Arp 2/3. Cdc42 also binds Par6 and promotes the formation of Par3-Par6-aPKC polarity complex. This protein complex is stabilized by Ect2. Cdc42 activates aPKC, which phosphorylates TJ proteins to stabilize

their localization in the AJC. Rho GEFs p114RhoGEF and RhoGEF11 are recruited at the TJ by cingulin and ZO-1 respectively. p114 RhoGEF binds to NM II and ROCK, creating a RhoA activation complex. These signaling events facilitate assembly of contractile actomyosin filaments that lead to the establishment of the perijunctional actomyosin ring and AJC maturation. T containing red colored circles represent active GTPases.

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Figure 5. RhoGTPases control of AJC disassembly

Diverse stimuli can influence function of Rho GTPases and consequent junctional disassembly. Depletion of extracellular calcium activates Cdc42 that which stabilizes EGF receptor and activates Src signaling that promotes E-cadherin ubiquitination and lysosomal degradation. An apical actin binding protein, ezrin activates Rac1 resulting in E-cadherin endocytosis and epithelial mesenchymal transition (**EMT**). IQGAP1, binds to Rac1 and Cdc42 in their active state. When Rac1 and Cdc42 are inactive, IQGAP1 associates with β -catenin and inhibits AJ disassembly.