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Actin motors that drive formation and disassembly of epithelial apical junctions

Andrei I Ivanov

Division Gastroenterology and Hepatology, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

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1. ABSTRACT

Tight junctions (TJ) and adherens junctions (AJ) are the most characteristic morphological features of differentiated epithelia which mediate cell-cell adhesions, establishment of the paracellular barrier and development of apico-basal cell polarity. In polarized epithelial cells, TJ and AJ associate with the prominent apical actin cytoskeleton, which is known to stabilize junctional structure and to tighten the epithelial barrier. Furthermore, plasticity of the actin cytoskeleton is thought to be critical for the remodeling of epithelial junctions. Two major molecular motors such as myosin II and actin filament turnover provide driving forces for reorganizations of the actin cytoskeleton. The review focuses on the roles of these actin motors in the establishment, maintenance and disassembly of epithelial TJ and AJ during tissue morphogenesis and in pathology.

2. INTRODUCTION

Epithelia play a critical role in the survival and development of multicellular organisms. Epithelial layers establish a protective barrier that separates the body's interior from the external environment and segregate different internal compartments to preserve their unique architecture and chemical environment (1). Simple epithelial linings in the gastrointestinal, respiratory, renal and reproductive systems are composed of a single layer of specialized cells (1, 2). These cells exhibit unique morphological features, including tight lateral contacts with neighbors and prominent apico-basal cell polarity. The former feature is critical for the formation of a highly-selective paracellular barrier, whereas the latter characteristic determines the directionality of absorptive and secretory processes in differentiated epithelia (1, 2). Epithelial cell-cell adhesions and apico-basal cell polarity

are regulated by specialized plasma membrane adhesive complexes called tight junctions (TJ) and adherens junctions (AJ) (3-6).

In simple epithelia, the TJ and AJ are closely located at the apex of the lateral plasma membrane; they share similar structural design and undergo orchestrated reorganization during normal tissue morphogenesis and in pathology (2-6). Understanding the basic mechanisms of junctional remodeling is an important task of cell and developmental biology. Knowledge of these mechanisms also has implications for the pathophysiology of inflammation and cancer, reparative medicine and drug delivery. The dynamic behavior of epithelial TJ and AJ is intimately connected to fundamental regulatory mechanisms of the cell such as vesicle trafficking and cytoskeletal reorganizations (7-9). The actin cytoskeleton is one of the most studied regulators of epithelial junctions, and many molecular details of the interplay between cell-cell adhesions and actin filaments have been recently discovered. This review discusses the role of the actin cytoskeleton in the biogenesis of TJ and AJ in vertebrate epithelia. Particularly, it focuses on mechanisms of filamentous (F)-actin plasticity which are critical for the formation, maintenance, and disruption of epithelial apical junctions during normal tissue morphogenesis and in different diseases.

3. STRUCTURE AND DYNAMICS OF EPITHELIAL APICAL JUNCTIONS

Both TJ and AJ represent large complexes composed of transmembrane and cytosolic proteins (3-6, 10, 11). The transmembrane proteins physically interact with opposing partners on membranes of adjacent cells, providing a mechanical link between the two plasma membranes and establishing a physical barrier to paracellular diffusion of fluid and solutes (3, 10, 12). Proteins bound to transmembrane TJ/AJ components at the cytosolic face of the plasma membrane create so called cytosolic plaques of the TJ and the AJ. Multiple roles for these cytosolic plaques can be envisioned, including clustering and stabilization of transmembrane proteins, anchoring and accumulating a variety of signaling and trafficking molecules, and connecting intercellular adhesion sites to the cytoskeleton (3-6, 10, 11).

The most characterized transmembrane components of epithelial TJ are occludin, members of the claudin family, and immunoglobulin-like molecules such as junctional adhesion molecule-A and coxsackie adenoviral receptor (3-6). E-cadherin and members of the nectin family represent the major transmembrane proteins of epithelial AJ (10, 11, 13). The cytosolic TJ plaque has an astonishing complexity. Indeed, a recent proteomic analysis of human intestinal epithelial cells has revealed about 900 different proteins associated with TJ; most of them appeared to be cytosolic proteins (14). Generic components of the TJ cytosolic plaque are members of the *zonula occludens* (ZO) family that can form complexes with transmembrane TJ proteins and also bind to the actin cytoskeleton (15-17). The composition of the cytosolic face of the AJ is better understood and includes two homologous proteins, β-catenin and p120 catenin, interacting with the intracellular domain of E-cadherin as well as with actin-binding proteins, α-catenin, vinculin, and α-actinin (10, 11). Although a large number of actin-binding proteins are localized at epithelial junctional plaques, the physical nature and molecular details of TJ/AJ associations with underlying actin filaments remain poorly elucidated. Recent advances and controversies in understanding the interface between epithelial junctions and the actin cytoskeleton have been described elsewhere (4, 18-20) and will not be extensively discussed in this review.

It has become increasingly obvious that epithelial TJ and AJ are highly dynamic structures that undergo constant remodeling. Such a junctional dynamic has been recently visualized by live cell imaging, which shows continuous internalization of claudin from fully functional epithelial TJ (21), a large-scale rapid breakdown and reassembly of claudin-based TJ strands (22) as well as a continuous flow-like movement of E-cadherin-catenin complexes at the intercellular junctions (23). Junctional plasticity is a prerequisite for epithelial morphogenesis, which involves migration, folding, expansion, and changing the shape of epithelial sheets without compromising their integrity and barrier properties (1, 24). For example, altered cell shape during gastrulation (25), as well as changes in cell position within the epithelial sheet (so called intercalation) during organogenesis (26) are mediated by an orchestrated disruption and reassembly of E-cadherin-based intercellular junctions. In adult organisms, remodeling of TJ and AJ is a characteristic feature of constantly self-renewing tissues. Thus, reversible disruption of inter-Sertoli cell junctions is critical for translocation of germ cells through the seminiferous epithelium in the testis (27). Similarly, in intestinal epithelium, disassembly and reestablishment of apical junctions is necessary for the extrusion of apoptotic cells (28). A dynamic equilibrium between junctional disassembly and reassembly in normal epithelium is shifted toward disassembly under pathophysiological conditions, most notably in inflammation and cancer. Lists of pathogenic agents that trigger the disruption of epithelial junctions include proinflammatory cytokines, growth factors, oxidative agents, bacteria and viruses (8, 9, 29-31). These noxious stimuli increase permeability of epithelial monolayers and induce the breakdown of TJ/AJ structure. However, such disruption of epithelial barriers can be fully reversible and epithelial cells will reestablish their AJ and TJ after removal of the detrimental stimuli (32).

Various mechanisms mediate remodeling of epithelial junctions in normal and pathological conditions. They include altered expression and phosphorylation of AJ/TJ proteins, endocytosis and exocytosis of junctional components, as well as reorganization of perijunctional actin filaments and microtubules (8, 30, 33-37). These mechanisms cross-talk, synergize and antagonize in regulating steady-state and stimulated remodeling of epithelial junctions. A large body of evidence highlights the F-actin cytoskeleton as a chief regulator of TJ and AJ in

simple epithelia (12, 18, 30, 38). Actin filament dynamics is an important determinant of junctional plasticity and may act either directly via actin-binding components of AJ and TJ or indirectly by organizing intracellular vesicular trafficking and other cytoskeletal structures. Mechanisms of F-actin remodeling that are critical for disassembly and restoration of epithelial apical junctions are discussed in following sections of this review. Other regulatory mechanisms involved in junctional biogenesis such as endocytosis, microtubule cytoskeleton, kinase cascades and epithelial polarity complexes are subjects of separate reviews in this special issue of Frontiers in Bioscience.

4. ARCHITECTURE OF THE PERIJUNCTIONAL ACTIN CYTOSKELETON

Actin filaments represent double helical polymers of a 42 kDa protein actin (39). All structural subunits of filaments are assembled in identical head-to-tail fashion, resulting in filament polarity i.e. the formation of two biochemically unequal ends. The polarity of actin filaments can be visualized by decoration with myosin S1 fragments, which creates a characteristic arrowhead pattern (40). Based on this pattern, one filament end is called the barbed end, and the other − the pointed end. Such molecular polarity is important for F-actin organization and dynamics. Indeed, elongation of actin filaments occurs by the preferential addition of monomeric actin to the barbed end (41, 42). Furthermore, filament motility driven by actin motors (such as myosin II) also depends on the orientation of the filament ends (43-45). Indeed, myosin II mediates F-actin contraction by causing oppositely directed actin filaments to slide against each other. The cellular actin cytoskeleton is composed of linear, branched and bundled filaments; the latter can be formed by lateral cross-linking of several adjacent filaments (43, 46). The integrity of actin filaments can be readily disrupted by natural toxins such as cytochalasin (Cyto) D or latrunculins (Lat) A and B, which prevent actin polymerization (47, 48). Cyto D inhibits the addition of new actin monomers by occupying the filament barbed end, whereas Lat A/B directly bind to the monomers and prevent their incorporation into the filaments. These actin-binding drugs are the most popular pharmacological tools to probe F-actin-dependent processes in living cells.

4.1. Structure of the actin cytoskeleton at mature apical junctions

A functional connection between epithelial apical junctions and the actin cytoskeleton was initially suggested almost three decades ago, based on the increase in paracellular permeability observed in *Necturus* gallbladder (49) and MDCK cell monolayers (50, 51) after cytochalasin treatment. Subsequent studies in various epithelial cell lines clearly demonstrated that F-actin depolymerization induced by Cyto D or Lat A/B not only disrupts the integrity of mature apical junctions (52-54) but also inhibits rapid reorganization (assembly and disassembly) of epithelial AJ and TJ (55-58). These functional data are consistent with ultrastructural studies that show a close association of the cytosolic face of TJ and AJ complexes with actin filaments *in vivo* and *in vitro* (59-63). The architecture of the perijunctional F-actin cytoskeleton in simple polarized epithelia has been elucidated by electron microscopy (EM) analysis of chicken small intestinal and ear hair epithelia. In these systems, position and directionality of the individual actin filaments associated with AJ and TJ was visualized by using a combination of the quick freeze deep etch technique and myosin S1 fragment decoration (59, 60). In the AJ zone, F-actin is organized into a circumferential ring composed of filaments that run in parallel to the plasma membrane. According to the myosin S1 decoration, these actin filaments possess opposite polarity which highlights the contractile nature of the ring (Figure 1, zone III). Within the ring, actin filaments self-associate in tight bundles which are additionally cross-linked by non-actin filaments presumably composed of myosin II or α-actinin (59, 64). Importantly, actin filaments periodically bend or branch out their parallel bundles to be inserted into the plasma membrane at the area of the AJ cytosolic plaque (59, 60). However a more recent electron microscopic analysis of chicken retinal epithelium has suggested that actin filaments may interact with the cytosolic AJ plaque indirectly, via non-actin filamentous structures (63). Overall these ultrastructural studies suggest a physical association between AJ cytosolic plaque and the circumferential F-actin belt, although the molecular nature of such an association remains poorly defined.

The molecular architecture of actin filaments associated with normal epithelial TJ has also been investigated by EM in the epithelia of chick hair (60) and guinea pig small intestine (62). The general organization of TJ-associated actin filaments is similar to those observed in the AJ-associated F-actin belt and it is comprised of filaments of mixed polarity running in parallel to the plasma membrane (60). Interestingly, the TJ-associated actin filaments in chick hair cells appear as a loosely organized meshwork rather than a tightly packed belt (Figure 1, zone II). This appearance is consistent with the ultrastructural topography of TJ which represents a meshwork of anastomosing strands in the plasma membrane (60, 62, 65). It is tempting to speculate that each TJ strand is accompanied by its own F-actin bundle at the cytosolic face of the membrane, although such an association has not been directly demonstrated. In intestinal epithelium, TJ-associated F-actin meshwork appears to be linked to the F-actin rootlets of perijunctional microvilli (61, 62), which may indicate physical and functional connections between TJ and apical microvilli (Figure 1, zone I). Likewise, TJ-associated actin filaments and the AJ-linked F-actin belt are likely to be organized into one physical entity. Indeed, TJ- and AJ-associated actin filaments form two connected zones on EM images of cross-sections of the small intestine (61). Furthermore, visualization of F-actin by fluorescently-labeled phalloidin or GFP-actin reveals a thick circumferential F-actin belt colocalized with both TJ and AJ in confluent epithelial cell monolayers (55, 66). Contraction of the perijunctional F-actin belt triggers rapid disassembly of TJ and AJ with virtually identical kinetics (9, 66). Together, these data suggest that in simple polarized epithelia TJ and AJ complexes are associated with two distinct but

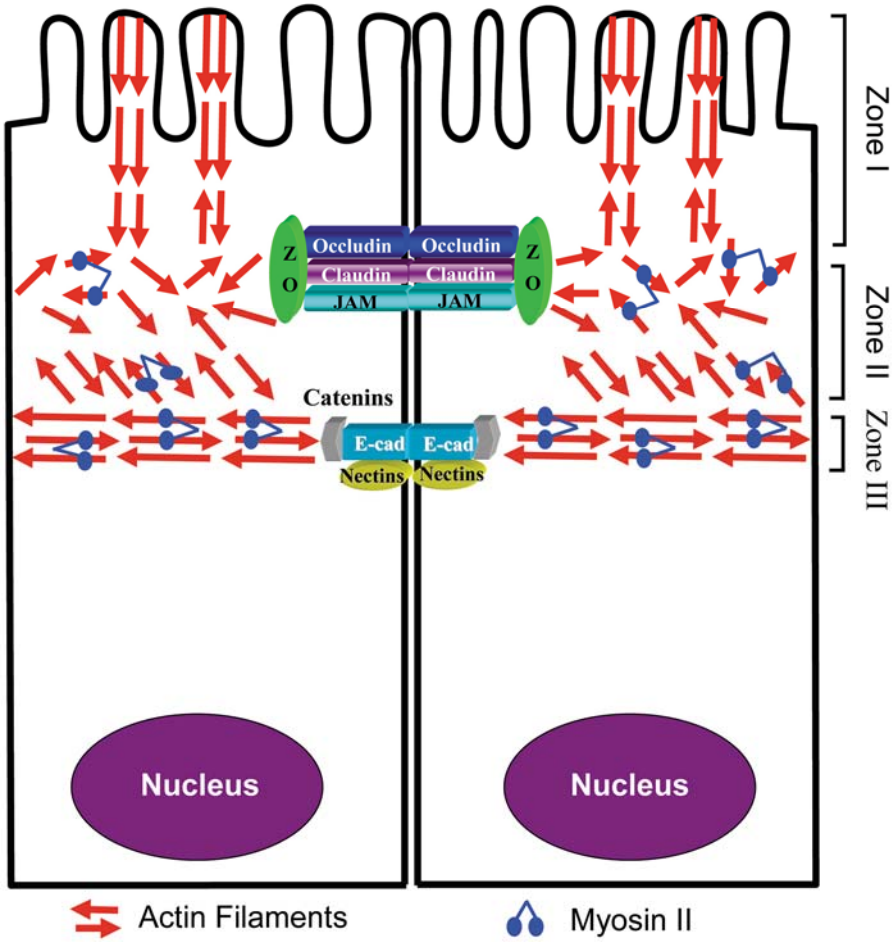

Figure 1. Organization of the apical actin cytoskeleton in simple polarized epithelia**.** The apical actin cytoskeleton in polarized epithelial cells can be divided into three different zones. Zone I represents actin filament bundles supporting apical microvilli, Zone II is composed of an actin filament meshwork associated with tight junctions, and Zone III represents a perijunctional F-actin belt, which is connected to adherens junctions. Tight junctions and adherens junctions-associated actin cytoskeleton consist of filaments with opposite polarity, and are enriched in myosin II protein.

interconnected sets of circumferential actin fibrils which have the ability to contract and are part of the large apical actin cytoskeletal machinery.

4.2. Rearrangements of the actin cytoskeleton during junctional disassembly

Remodeling (disassembly and reformation) of epithelial TJ and AJ is usually accompanied by dramatic changes in architecture of perijunctional actin filaments. These changes have been extensively characterized using an *in vitro* model of junctional remodeling known as a 'calcium switch'. In this model, the removal of extracellular calcium triggers rapid and orchestrated disassembly of AJ and TJ (9, 34, 66-69). The calcium depletion-induced disruption of epithelial junctions is reversible, and the restoration of a normal calcium level (calcium repletion) results in the reformation of structurally and functionally normal AJ and TJ (55, 69-71). Reorganization of actin filaments in calcium-depleted epithelial cells involves the formation of two morphologically distinct structures, *viz.* contractile F-actin rings and radial F-actin cables (Figure 2) (56, 68, 72). F-actin rings are likely to represent a transformed perijunctional F-actin belt of normal epithelial cells. This belt undergoes a rapid centripetal contraction manifested by its progressively decreased diameter and increased thickness (56, 68, 72). Contracting F-actin rings remain attached to the plasma membrane leading to membrane invagination, and in some cases, to the formation of a cleavage furrow, which separates the apical brush border from the cell body (56, 73). In calcium depleted epithelial monolayers, contraction of the apical F-actin rings provides forces to tear apart TJ and AJ between neighboring cells (56, 68).

Radial F-actin cables are not typical for mature epithelial junctions but they become easily detectable during TJ/AJ disassembly (Figure 2). In calcium depleted epithelial cells, radial F-actin cables run perpendicularly to intercellular contacts, apparently connecting contractile rings of adjacent cells (56, 68). Each cable serves as a backbone for filopodia-like apical and subapical membrane

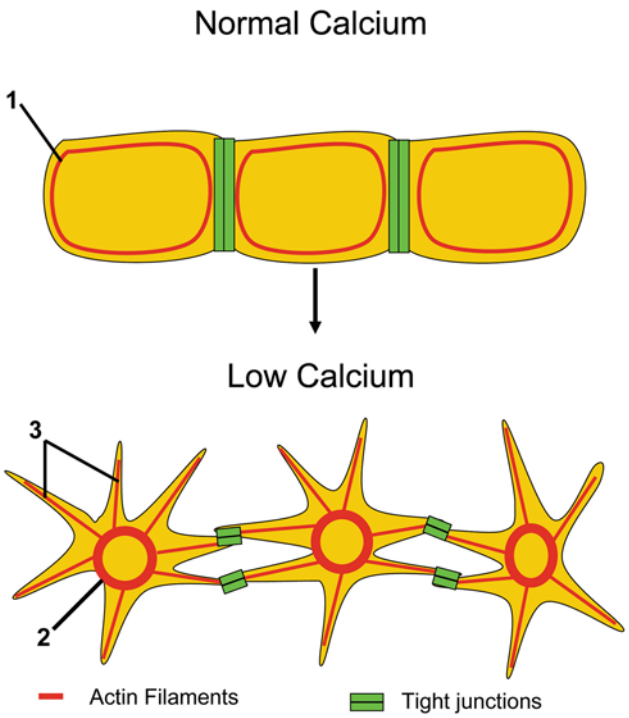

Figure 2. Reorganization of the apical F-actin during disassembly of apical junctions in calcium depleted epithelial cells. Disassembly of apical junctions in calcium-depleted epithelial cells is accompanied by a dramatic reorganization of the actin cytoskeleton. This reorganization involves the transformation of the perijunctional F-actin belt of polarized epithelial cells (1) into the contractile F-actin ring (2) and radial F-actin cables (3) of calcium-depleted cells.

protrusions (retraction fibers) through which neighboring cells remain attached during disruption of their cell-cell contacts (56, 68, 73). Unlike contractile F-actin rings, mechanisms underlying formation of radial F-actin cables are not well understood. During calcium depletion, these cables may appear as a result of excessive protrusive activity of the plasma membrane (73). A combination of membrane protrusiveness and the centripetal tension imposed by the contracting F-actin ring is likely to result in uneven retraction of different part of plasma membranes of contacting cells, leading to the formation of filopodia-like retraction fibers.

Importantly, contractile F-actin rings and radial F-actin cables are not peculiar structures of calcium-depleted epithelial cells. Indeed, contractile F-actin rings have been detected during epidermal growth factor-induced TJ disassembly in lung epithelial cells (74) and during extrusion of apoptotic cells from MDCK cell monolayers (75). Furthermore, disruption of the intestinal epithelial barrier caused by pathogenic bacteria *Salmonella typhimurium* and *Bacteroides fragilis* is accompanied by the formation of apical F-actin rings (76, 77). On the other hand, F-actin cables have been found during disassembly of epithelial junctions caused by pharmacological modulation of the plasma membrane potential (78), treatment with transforming growth factor β (79), infection with *Helicobacter pylori* (80) and simian virus (81). This data suggests that some conserved molecular mechanisms underlie reorganization of perijunctional F-actin during stimuli-induced dissociation of epithelial apical junctions.

4.3. F-actin remodeling during assembly of epithelial apical junctions

In simple epithelia, formation of mature junctional complexes occurs via at least two major steps which include the assembly of initial AJ-like junctions followed by the establishment of TJ (13, 55, 82, 83). Each step is associated with morphologically distinct F-actin structures such as F-actin cables at AJ-like junctions (55, 57, 84, 85) and circumferential F-actin bundles at assembling TJ (55, 58). F-actin cables appear at very early stages of epithelial cell-cell contact formation (55, 57, 84, 85) and they are also characteristic of naïve intercellular junctions in fibroblasts (86). These cables run perpendicularly to the plane of cell-cell contacts (Figure 3, insert I) and represent a core for thin filopodia-like membrane protrusions which mediate initial contacts between neighboring cells (57, 85). The perpendicular F-actin cables colocalize with newly-forming adhesion complexes which contain AJ proteins and certain TJ components and appear as characteristic periodical dot-like structures (‘puncta’) at the area of cell-cell contact (55, 57, 84, 85, 87). Since a high resolution ultrastructural analysis of parallel F-actin cables of contacting epithelial cells has not been performed, precise organization and orientation of the filaments in these cables remain unknown. These cables, however, resemble basal stress fibers of migrating

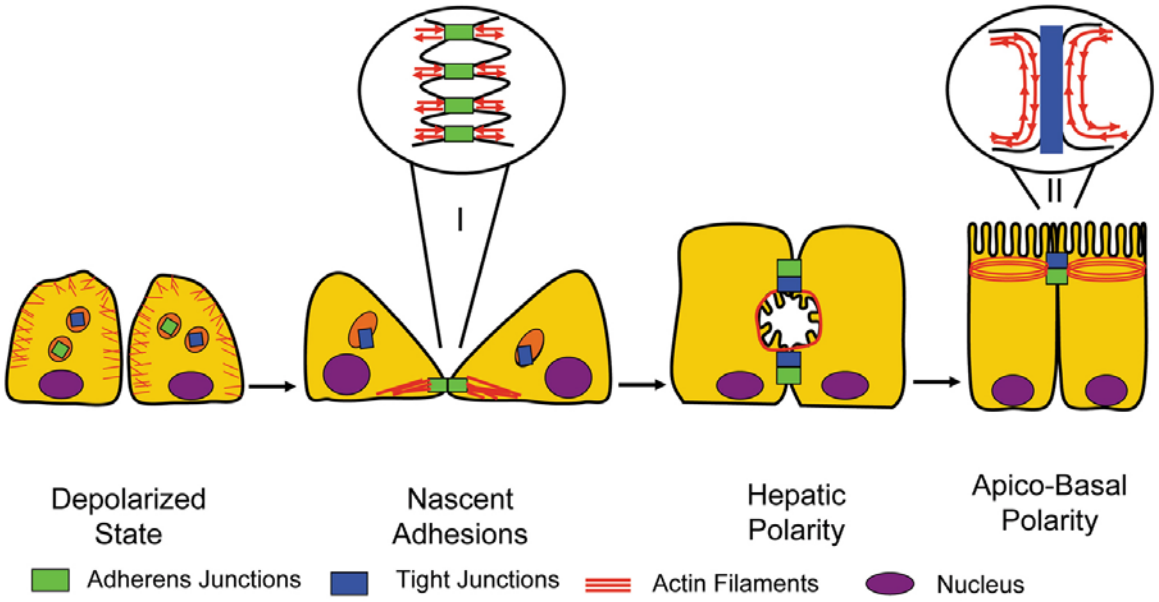

Figure 3. Reorganization of the actin cytoskeleton during the formation of apical junctions in simple epithelia. Epithelial cells transiently lose columnar cell shape and apical junctions during prolonged extracellular calcium depletion. Restoration of normal calcium level induces the orchestrated reformation of intercellular junctions and reorganization of the actin cytoskeleton in a process that can be divided into three major steps. The first step involves accumulation of parallel F-actin bundles in areas of cell-cell contacts and the assembly of nascent adherens-like junctions (insert I). The second step results in the assembly of TJ encircling a primordial apical plasma membrane that is formed in a laterally located lumen with hepatic-type polarity. The final step is the transition from the hepatic to apico-basal polarity resulting in the formation of mature AJ and TJ and the assembly of parallel perijunctional F-actin bundles (insert II).

cells (88) and, similarly to the stress fibers, may be built by bipolar contractile actin filaments.

During maturation of intercellular junctions, individual puncta merge into a continuous adhesive belt and become separated into AJ and TJ (55, 57, 84, 85, 87). Simultaneously, perpendicular F-actin cables undergo a transformation into perijunctional F-actin bundles that run parallel to the area of cell-cell contacts (Figure 3, insert II) (55, 58, 85, 89). Mechanisms of such transformation remain poorly understood. One can suggest that the progressive increase in the density of junctional F-actin cables would eventually lead to their merging to form a continuous circumferential F-actin belt (90). However this mechanism does not explain the change from the perpendicular to parallel filament orientation. Another mechanism implies that parallel perijunctional bundles can be formed by a separate population of actin filaments which initially assemble as thin bundles at the distal part of the cell-cell contact zone (58, 91). As cell-cell contact mature, these bundles become thicker and they occupy the area of intercellular contact to form the perijunctional F-actin belt. Perpendicular F-actin cables supporting initial AJ-like junctions either become absorbed by the F-actin belt, or they disintegrate.

In intestinal and renal epithelial cells subjected to calcium switch, the assembly of mature TJ and AJ and transformation from perpendicular F-actin cables to the circumferential F-actin belt occurs via an intermediate step (Figure 3). This step involves the formation of a lateral lumen between two adjacent cells (55, 92, 93). The lumen accumulates components of the apical plasma membrane; it is encircled by occludin- and ZO-1-containing TJ and is supported by thick circumferential F-actin bundles. In fact, such an intermediate lateral lumen resembles a canaliculus which is normally formed between adjacent hepatocytes in the liver. However, simple epithelial cells eventually acquire apico-basal polarity with the lateral lumens becoming transformed into the apical surface, which is corralled by TJ and the apical perijunctional F-actin belt (55, 92, 93). The cytoskeletal mechanisms underlying transformation from the hepatic to apico-basal cell polarity remain to be elucidated.

The above structural studies clearly demonstrate that remodeling of epithelial AJ and TJ is accompanied by dramatic reorganizations of the apical actin cytoskeleton. Such reorganizations involve filament contraction and expansion, changes in their spatial orientation, and bundling. Furthermore, F-actin depolymerization renders epithelial cells unable to remodel their apical junctions (55-58, 89). These findings clearly indicate that reorganization of actin filaments provides driving forces for junctional remodeling. This in turn raises an important question about molecular mechanisms that govern alterations of the perijunctional actin cytoskeleton. Generally, F-actin remodeling, which includes changing the length, orientation, and intracellular location of actin filaments, can be driven by either specialized motor proteins, myosins, or

by the filament turnover (polymerization and depolymerization) (41-43, 94, 95). Subsequent sections of the review will discuss the role of these two mechanisms in maintenance, disassembly, and reestablishment of epithelial TJ and AJ.

5. ROLE OF MYOSIN II MOTOR IN REGULATION OF EPITHELIAL APICAL JUNCTIONS

5.1. Structure and functions of myosin II

A long-recognized contractile nature of the apical F-actin cytoskeleton (59, 96) suggests the role of myosin II motor in organization and regulation of perijunctional actin filaments. Myosin II, or conventional myosin, is an abundant cytoskeletal protein that converts the chemical energy of ATP into mechanical forces, thus mediating a static tension and contractility of the actin cytoskeleton (44, 45, 97, 98). This motor protein functions as a heterohexamer, which is composed of two heavy chains, two essential, and two regulatory light chains (RLC) (44, 45, 97, 98). The myosin II heavy chain consists of a globular head that binds to actin and hydrolyzes ATP and an extended tail that coils together with another heavy chain tail to form a rigid rod-like structure. The tails of multiple myosin II molecules readily self-associate, creating thick filaments. Such a high-order organization of myosin II is critical for the two major modes of its action. One mode is actin filament sliding, which mediates myosin II-dependent contractility, whereas the other is cross-linking (bundling) of actin filaments which produces thick actomyosin fibrils (44, 45, 97, 98). Epithelial cells express nonmuscle myosin (NM) II, which is characterized by three different heavy chain isoforms IIA, IIB and IIC (99-101). These isoforms possess a high degree (64-80%) of sequence identity, but have different enzymatic/biochemical properties (71, 102). Different NM II heavy chains may either play unique (71, 99, 103-106) or interchangeable roles (107, 108) in regulating cell shape, cell-cell and cell-matrix adhesions, cytokinesis and vesicular traffic.

Multiple mechanisms may regulate myosin II activity in mammalian cells. The most characterized one involves activatory phosphorylation of RLC. The myosin light chain kinase (MLCK) and Rho-associated kinase (ROCK) are primarily responsible for RLC phosphorylation and myosin II activation by various experimental and pathological stimuli (109-111). Other regulatory mechanisms target the self-assembly of NM II heavy chains. This process can be modulated by either heavy chain phosphorylation (109, 112) or by their interactions with accessory proteins such as Shroom (113), Mts1 (114) and septins (115). In contrast to RLC phosphorylation, the molecular details and biological significance of myosin heavy chain regulation in epithelial cells remain poorly investigated.

5.2. Regulation of mature epithelial junctions by myosin II motor

In polarized epithelial cells, myosin II is enriched in the perijunctional F-actin belt (55, 64, 71, 116) which may accumulate about 15% of all intracellular NM II (64). In intestinal epithelial cells co-expressing NM IIA IIB and IIC, all these heavy chain isoforms are abundant at the circumferential F-actin belt where they colocalize with TJ/AJ proteins (71). Furthermore, physical interaction of myosin II with a cytosolic TJ plaque protein cingulin has been observed in MDCK cells (117). Such close associations of myosin II and epithelial apical junctions suggest the involvement of this cytoskeletal motor in the maintenance of normal structure and barrier properties of mature TJ and/or AJ. Our recent studies have tested this suggestion using two different experimental approaches to interfere with myosin II functions in human intestinal epithelial cells. One approach involves pharmacological inhibition of myosin II activity with blebbistatin (56), and the other uses siRNA-mediated knock-down of different NM II heavy chain isoforms (71). Blebbistatin treatment causes a modest (~20%) decrease in transepithelial electrical resistance (TEER) in T84 cell monolayers without gross changes in TJ or AJ morphology (56). Furthermore, confluent SK-CO15 cells depleted of NM IIA, NM IIB, or NM IIC establish structurally-normal apical junctions. Interestingly, NM IIA-deficient cell monolayers develop a defective paracellular barrier which is manifested by a significantly (~ 4 fold) lower TEER when compared to control cells and NM IIB or NM IIC-depleted cells (71). These findings are consistent with pharmacological inhibition data and with the fact that NM IIA is the predominant myosin II isoform in human colonic epithelial cells (56). Yet they contradict with two other studies which report decreased accumulation of E-cadherin and β-catenin at intercellular contacts of mouse embryonic stem cells and COS-7 embryonic kidney epithelial cells after siRNA knock-down of NM IIA (118) and NM IIB (119) respectively. However, mouse embryonic stem cells do not express NM IIC (118), and targeted elimination of NM IIA would result in NM IIA/NM IIC-deficient cells. Likewise, COS-7 cells lack NM IIA expression (107) and the elimination of NM IIB would lead to dual NM IIA/NM IIB deficiency. Therefore, it is reasonable to suggest that a lack of two myosin II-isoforms would result in a more severe defects of apical junctions compared to the selective knock-down of NM IIA. Overall, these experiments indicate an important role for the myosin II motor in the regulation of structural integrity and barrier properties of mature epithelial apical junctions.

Available data indicate a lack of direct correlation between activation status of myosin II and tightness of the epithelial barrier. Indeed, inhibition of the NM II motor increases leakiness of the paracellular barrier in confluent epithelial cell monolayers (56, 71). Similar barrier disruption has been observed after overexpression of a dominant-negative (nonphosphorylatable) mutant of the myosin RLC (120). However, activation of NM II, by expression either of its constitutively active RLC (121) or the upstream kinase, MLCK, (122) also results in increased epithelial permeability. Therefore, it is likely that the maintenance of tight epithelial barriers requires an intermediate level of NM II activity, and that both overactivation and inhibition of this F-actin motor may lead to barrier disruption.

5.3. Role of myosin II in junctional disassembly

Several recent studies have identified NM II activation as a major driving force of epithelial TJ/AJ disassembly. Stimuli that disrupt epithelial junctions in a myosin II-dependent manner include: extracellular calcium depletion (56, 71), treatment with the proinflammatory cytokines IFNγ and members of the TNF family (123-126), and exposure to hepatocyte growth factor (127). In addition, colonization of intestinal epithelial cells with microbes such as enteropathogenic *Escherichia coli* (128), and *Giardia* (129) increase the paracellular permeability by mechanisms involving activation of NM II. The NM IIA isoform appears to play the major role in actomyosin contractility which breaks the epithelial junctions. This conclusion is based on two recent studies investigating the cellular effects of siRNA-mediated knock-down of different NM II heavy chains (71, 105). Our study has demonstrated that selective depletion of the NM IIA but not the other two heavy chain types dramatically attenuated the formation of contractile F-actin rings and TJ/AJ disassembly in calcium-depleted SK-CO15 cells (71). Another study has reported that elimination of NM IIA, but not NM IIB, inhibited thrombin-induced cell rounding in MDA-MB-231 breast cancer cells (105). Differential involvement of NM IIA and NM IIB in stimuli-induced contractility may be due to reported differences in kinetic mechanisms for these heavy chain isoforms. In particular, NM IIA is a low-duty-ratio motor, which is not attached to F-actin during most of the kinetic cycle (102). In contrast, NM IIB is an intermediate-duty-ratio motor, spending a higher proportion of its kinetic cycle firmly bound to actin (130, 131). Recent detergent fractionation data obtained in colonic epithelial cells also indicate weaker association of NM IIA with F-actin when compared to the other isoforms (71). These different biochemical properties of NM II heavy chains may determine their functional peculiarities, with NM IIB being suitable to maintain the static tension, and NM IIA being adapted to rapidly reorganize actin filaments.

Another mechanism underlying unique functional roles for NM IIA may involve its selective coupling with different activatory kinases. Indeed, NM IIA-associated RLC is preferentially phosphorylated by ROCK (105). Furthermore, ROCK and MLCK reportedly phosphorylate topographically separate RLC populations in the cell, although these RLC pools have not been linked to particular NM II heavy chains (132). It is generally believed that activation of ROCK and/or MLCK is responsible for the stimulation of myosin II contractility that disrupts epithelial AJ and TJ. For example, an increase in ROCK expression/activity induces myosin II-dependent TJ disassembly in IFNγ-treated colonic epithelial cells (125). In addition, stimulation of MLCK expression mediates the opening of the intestinal epithelial barrier upon exposure to TNFα (123, 126). The role of RLC phosphorylation in myosin II-dependent remodeling of intercellular junctions has been recently discussed in great details elsewhere (30, 133, 134) and is outside the scope of this review.

5.4. Myosin II-driven assembly of epithelial apical junctions

Besides controlling the sealing of mature epithelial barriers and driving stimuli-induced disruption of TJ and AJ, NM II plays a critical role in establishing epithelial junctions. This multistep process involves the early formation of AJ-like junctions and subsequent assembly of TJ which coincides with the establishment of apico-basal cell polarity (13, 55, 82, 83). Myosin II appears to be important for all the stages of junctional assembly (71), although conflicting results exist on its role in the formation of early AJ-like junctions. For example, inhibition of myosin II with blebbistatin has no effect on the assembly of E-cadherin-based junctions in T84 (55) and MTD-1A (119) epithelial cells, but it decreases junctional accumulation of E-cadherin in keratinocytes (58) and MCF-7 cells (116). However, blebbistatin is a relatively low affinity NM II inhibitor (135) and cells treated with 50-100 μM of the drug may retain some myosin II activity (103). Therefore, different residual levels of myosin II activity in blebbistatin-treated epithelial cells are likely to explain variable effects of this pharmacological inhibitor. Importantly, siRNA-mediated depletion of NM IIA dramatically attenuates reassembly of AJ-like junctions in colonic epithelial cells (71). NM IIA knock-down also results in complete disappearance of radial F-actin cables in the areas of initial cell-cell contacts. Similar loss of thick F-actin bundles composing basal stress fibers is observed in migrating NM IIA-deficient fibroblastic and epithelial cells (103, 105). Together these data open an intriguing possibility that at early stages of junctional assembly NM IIA works as a structural rather than a motor protein by bundling/cross-linking actin filaments. Such a myosin-mediated cross-linking results in the formation of thick F-actin cables which may stabilize nascent E-cadherin-based intercellular junctions.

In addition to its role in the assembly of early epithelial cell-cell contact, NM II is also critical for the formation of TJ. Indeed, several studies have reported attenuation of TJ reassembly after either pharmacological inhibition of myosin II with blebbistatin (55, 119, 136) or siRNA mediated knock-down of NM IIA (71). Such defects in TJ assembly appear to be a part of a broader effect of myosin II inhibition on the acquisition of the normal epithelial phenotype. During calcium repletion, epithelial cells with inhibited NM II do not acquire a columnar shape, fail to develop normal apical plasma membrane domain and do not assemble the perijunctional F-actin belt (55, 58, 71, 119). In fact, myosin II-inhibited cells appear to be locked into the hepatic cell polarity step with their TJ and the apical plasma membrane assembled within small lateral lumens (55, 93). This unusual phenotype caused by inhibition of epithelial myosin II suggests a connection between this cytoskeletal motor and intracellular machinery that regulates the establishment of apico-basal cell polarity. Although the molecular details of such a connection remain poorly elucidated, recent studies have implicated Par polarity proteins and Rho GTPases (93, 136).

6. ROLE OF F-ACTIN TURNOVER IN REORGANIZATION OF EPITHELIAL APICAL JUNCTIONS

6.1. Protein machinery that regulates actin filament turnover

Myosin IIA-dependent contractility is not solely responsible for the reorganization of the actin cytoskeleton. Turnover (polymerization and depolymerization) of the filaments represents another critical mechanism of cytoskeletal plasticity (95, 137). In simple terms, F-actin turnover can be described as removal of monomeric actin from the filament pointed end and the addition of the monomer to the filament barbed end (41, 42). This process, which is also known as F-actin treadmilling, regulates filament length and spatial localization and may work as the intracellular motor by pushing and pulling organelles and membrane structures (42, 94).

F-actin polymerization can be divided into several steps including nucleation, elongation and steady-state capping, among which nucleation is thought to be a rate-limiting step in actin polymerization (138). To date, four types of actin nucleators, namely the actin related proteins (Arp) 2/3 complex, members of the formin family and proteins called Spire and Cordon-Bleu, have been identified (138-143). Since functional roles of the last two actin nucleating proteins in mammalian epithelia have not been demonstrated, only the Arp 2/3 complex and formins will be discussed in this review.

The Arp2/3 complex consists of seven polypeptides: two actin-related subunits Arp2, Arp3 and five novel proteins, p40, p34, p21, p20 and p16. This complex is especially enriched at the leading edge of migrating cells where it organizes actin in relatively short, Y-shaped filaments. The Arp2/3 complex is thought to bind to the side of the preexisting actin filament and to initiate a new filament growth at a 70º angle, which is called 'dendritic actin nucleation' (138, 142, 144). It is noteworthy that the Arp2/3 complex alone was shown to be unable to trigger actin polymerization *in vitro*, and it had to interact with 'nucleation promoting factors' (NPF) to initiate filament branching. Several NPF were identified in eukaryotic cells, including members of the WASP (Wiskott-Aldrich syndrome protein), the WAVE (WASP family verprolin-homologous) protein families and cortactin (142, 145-147). All WASP and WAVE proteins display similar modular organization, with a conserved C-terminal domain that binds and brings together actin and the Arp2/3 complex, and a more diverse N-terminal region that interacts with various regulators. The C- and N-terminal parts of WASP are able to self-associate resulting in a closed auto-inhibited conformation of the protein, whereas a trans-inhibition by association with other proteins is characteristic for members of the WAVE family (142, 145-147). Both auto-inhibition of WASP and trans-inhibition of WAVE can be overcome by binding to small GTPases, Cdc42 and Rac-1 respectively. Therefore, WASP and WAVE proteins are important down-stream effectors that mediate Cdc42 and Rac-1-induced reorganizations of the actin cytoskeleton (142, 145-147). Although Arp2/3 activation by another NPF, cortactin, is much less efficient compared to WASP proteins (148), cortactin acts synergistically with WASP to stimulate Arp2/3-dependent actin polymerization (142). Furthermore, the activation of cortactin by phosphorylation on tyrosine residues (149) is likely to provide a mechanistic link between tyrosine kinase signaling and actin polymerization in various physiological and pathophysiological conditions.

The alternative mechanism of actin nucleation is regulated by formins. In contrast to the Arp2/3 complex, formin proteins nucleate actin filaments at their barbed ends thereby producing linear unbranched filaments (140, 141, 150). As a result, these actin-nucleating factors are important for the formation of cytoskeletal structures, which are composed of linear actin cables including stress fibers, filopodia and contractile F-actin rings (140, 141). There are 15 different mammalian formins, all possessing the characteristic 400-amino-acid FH2 domain, which is critical for F-actin assembly. The mechanism of formin-mediated F-actin assembly is likely to involve stabilization of F-actin polymerization intermediates, actin dimers and trimers. Two subgroups of this large protein family may be particularly important for the organization and functions of perijunctional cytoskeleton in epithelial cells (90, 151). One of the subgroups contains the originally discovered formins (formin-1 and -2) and the other subgroup − the mammalian Diaphanous (mDia) formins, consisting of mDia1, mDia2 and mDia3. It is noteworthy that mDia formins are activated by RhoA GTPase and therefore may mediate the effects of Rho on the perijunctional F-actin cytoskeleton (152).

Upon nucleation, actin filaments undergo an elongation step which is regulated by the interplay between different accessory proteins (39, 150). Among them, profilin, elongating factors and capping proteins represent the major regulators of actin filament growth. Profilin promotes actin polymerization by forming a 1:1 complex with monomeric actin and accelerating actin monomers binding to the filament barbed end (153, 154). Furthermore, profilin is known to stimulate ADP-ATP exchange on an actin monomer thereby increasing the level of ATP-actin available for incorporation into filaments. On the other hand, F-actin elongating factors such as vasodilator-stimulated phosphoprotein (VASP) and mammalian Ena (Mena) promote filament growth by acting as anti-capping proteins (155) or by bundling and stabilizing actin filament barbed ends (156). Either way, VASP/Mena antagonizes filament termination, thereby increasing the length of actin filaments.

Depolymerization represents the opposite side of the coin in actin filament turnover. Only a handful of accessory proteins accelerate F-actin depolymerization *in vivo* (157) and members of the actin-depolymerizing factor (ADF)/cofilin family are the most extensively characterized (158, 159). ADF/cofilin are small (15-21 kDa) proteins found in all eukaryotic organisms. These proteins disassemble actin filaments by either enhancing the dissociation of monomers from the pointed end or by severing the filament. It is noteworthy that only

Table 1. Localization of actin-polymerizing proteins at epithelial apical junctions

Protein	Junctional type	Cell type	Detection method	Reference
The Arp2/3 complex	AJ, TJ	MDCK, T84, Caco-2	Arp3 and p34 antibodies	(55, 162, 166, 167)
Formin-1	AJ	Keratinocytes	Antibody	(168)
mDia-1	AJ	MCF-7	Antibody, GFP-mDia-1	(169)
N-WASP	AJ, TJ	T84	Antibody	(55)
WAVE-2	AJ	MDCK	Antibody, GFP-WAVE-2	(170)
Cortactin	AJ, TJ	MDCK	Antibody, GFP-Cortactin	(171, 172)
VASP	TJ	T84	Antibody	(173)
Mena	AJ	MCF-7	Antibody	(174)

ADF/cofilin possess physiologically significant pointed-end depolymerizing activity, and according to *in vitro* estimations, they alone cause more than a 20 fold increase on the rate of F-actin turnover (158, 159). ADF/cofilin are phosphorylated at a conserved N-terminal serine Ser-3, which makes these proteins unable to bind to and depolymerize F-actin. Hence, phosphorylation/dephosphorylation of ADF/cofilin acts as a simple on and off switch for the F-actin disassembly. This phosphorylation is catalyzed by several kinases (158) with a predominant role for LIM kinases (LIMK; (160)). It is noteworthy that LIMK activity is regulated by Rho GTPases such as RhoA, Rac1 and Cdc42. Therefore, LIMK and ADF/cofilin provide another important link between Rho GTPase signaling and remodeling of the F-actin cytoskeleton.

6.2. F-actin treadmilling at mature epithelial junctions

It has become increasingly obvious that the perijunctional F-actin belt experiences rapid treadmilling, which is important for maintenance of the normal structure and functions of epithelial apical junctions. The turnover of the perijunctional F-actin belt can be directly visualized by incorporation of fluorescently-labeled monomeric actin which is introduced into live cells by either microinjection (161) or after gentle cell permeabilization with saponin (55, 162). PtK2, MDCK or T84 epithelial cells exposed to exogenous actin show a rapid incorporation of a labeled monomer into the F-actin bundles associated with mature AJ and TJ (55, 161, 162). A recent analysis of protein dynamics by fluorescence recovery after photobleaching (FRAP), demonstrates that virtually all F-actin associated with cell-cell contact sites is highly mobile and its recovery after photobleaching occurs even faster when compared to the recovery of AJ components, E-cadherin, and catenins (163). Furthermore, actin-binding drugs Cyto D and jasplakinolide, which dramatically change the organization and dynamics of the perijunctional actin filaments, have no effect on the dynamics of E-cadherin and α-catenin according to FRAP measurements (163). These data suggest a lack of stable physical association between actin filaments and E-cadherin-catenin complexes at epithelial AJ (163, 164). Yet sequestration of monomeric actin by Lat A, which induces rapid disassembly of the perijunctional F-actin belt in confluent MDCK cells, triggers a simultaneous increase in paracellular permeability, disruption of TJ structure and endocytosis of TJ proteins (53). One can speculate that actin filaments organize epithelial junctions even without stable physical interactions by corralling AJ/TJ proteins at the cell-cell contact zone and preventing their endocytosis. Interestingly, not only disassembly of actin filaments but also their excessive stabilization by jasplakinolide induces the disruption of AJ and the formation of intercellular gaps in endothelial cells (165). This suggests that treadmilling of the perijunctional F-actin is a delicately balanced process and both the acceleration and inhibition of F-actin dynamics may have detrimental effects on the integrity and functions of epithelial junctions.

The importance of F-actin treadmilling in the maintenance of normal epithelial apical junctions is also supported by a number of studies that show the association between different actin-binding proteins and AJ/TJ complexes in confluent epithelial cell monolayers. Indeed, virtually all major F-actin nucleating and elongating factors are enriched at mature AJ and/or TJ (Table 1). The physical association of several actin-binding proteins and junctional components has also been reported. For example, the Arp 2/3 complex and cortactin interact with E-cadherin (171, 175), while formin-1 binds a cytosolic AJ scaffold protein, α-catenin (168). At TJ, VASP associates with ZO-1 (173) and profilin interacts with another junctional scaffold, afadin (176). Nevertheless, the physiological significance of these physical interactions remains unknown.

Recent studies provide direct evidence for the role of actin-polymerizing machinery in organization of epithelial AJ. Thus, siRNA-mediated knockdown of WAVE-2 (170), mDia-1 (169), or profilin (177) decrease E-cadherin accumulation at intercellular junctions in confluent MDCK and human mammary epithelial cell monolayers. Similar distortion of E-cadherin-based AJ is observed after overexpression of a dominant-negative mDia-1 mutant in HCT116 colonic epithelial cells (152). It is noteworthy that functional inhibition of individual actin-nucleating proteins such as WAVE-2 or mDia-1 does not result in gross changes in morphology of AJ or redistribution of junctional proteins. Such mild effects contrast with bold actions of F-actin-depolymerizing drugs, which induce complete disassembly of TJ and AJ and translocation of junctional proteins from the plasma membrane into the cytoplasm (53-55). These differences suggest that the architecture of perijunctional F-actin which is important for AJ/TJ integrity may be regulated by synergistic actions of different actin-nucleating proteins. In addition, F-actin-elongating factors are likely to be important for this process. A simultaneous knockdown of all members of the VASP/Mena family in mice results in a dramatic increase of vascular permeability (178), whereas expression of a dominant negative VASP mutant induces

gross abnormalities of intercellular adhesion in the skin of transgenic mice (57). Similar defects of the endothelial barrier can be observed after interfering with VASP/Mena functions *in vitro* (178, 179). However, functional roles and molecular details of the VASP/Mena-dependent actin filament growth during reorganization of epithelial junctions are not well understood.

6.3. Role of F-actin treadmilling motor in junctional disassembly

The F-actin treadmilling motor cooperates with myosin II-mediated contraction to drive disassembly of epithelial AJ and TJ. Indeed, rapidly turning over actin filaments compose contractile F-actin rings in calcium-depleted colonic epithelial cells, since these rings readily incorporate exogenous monomeric actin and disappear after Lat A treatment (56). Furthermore, apical contractile rings are enriched with the Arp2/3 complex and cortactin, and their formation is attenuated by inhibition of F-actin turnover with jasplakinolide (56). Attenuated TJ disassembly in calcium-depleted MDCK cells has also been found after overexpression of EFA6 protein which is known to stabilize the actin cytoskeleton (180). Beside calcium depletion, F-actin turnover is involved in disassembly and internalization of AJ triggered by phorbol ester (181), thus suggesting a common role for this mechanism in disintegrating epithelial apical junctions.

Stimuli that induce TJ/AJ disassembly not only increase the rate of F-actin treadmilling, but may also shift the F-actin turnover toward filament depolymerization. Indeed, a dramatic F-actin breakdown occurs during oxidant-induced disassembly of TJ in intestinal and airway epithelia (182-184). Furthermore, contact-naïve calcium-depleted epithelial cells have significantly higher monomeric actin level compared to cell monolayers with abundant intercellular junctions (55). What is the mechanism of rapid and dramatic increase in F-actin depolymerization during disruption of epithelial cell-cell contacts? The most likely scenario involves activation of ADF/cofilin proteins. Although the role of ADF/cofilin in disassembly of epithelial TJ and AJ has not been yet directly tested, it is supported by several indirect evidences. For example, calcium depletion in T84 cells induces rapid and dramatic activation (dephosphorylation) of ADF/cofilin and accumulation of these activated proteins at apical contractile rings (56). Similar activation of ADF/cofilin accompanies capsaicin-induced disruption of the paracellular barrier in Caco-2 cell monolayers (185) and disassembly of TJ in brain endothelial cells caused by pathogenic fungi (186). Furthermore, overexpression of an inactive ADF/cofilin mutant attenuates dramatic reorganization of the apical actin filaments in renal epithelial cells subjected to ATP depletion (187). Overall, these data suggest that destabilization of the apical actin cytoskeleton by either accelerated treadmilling or increased actin filament disassembly is critical for stimuli-induced disruption of epithelial cell-cell adhesions.

The interplay between myosin II-driven contractility and F-actin treadmilling is intriguing but a poorly understood process that mediates formation of apical actomyosin rings during junctional disassembly (56). Similar interplay regulates other biological processes, most notably the healing of epithelial wounds and the formation of the cleavage furrow during cytokinesis (188-190). These observations indicate that well-conserved cytosolic and membrane mechanisms may mediate different types of plasma membrane invagination and that the proteins involved in the formation of contractile rings during wound closure and cytokinesis might also be important for the breakdown of epithelial junctions. How myosin II activity and F-actin treadmilling cooperate in the formation of apical contractile rings remains unknown. One can suggest that the mature perijunctional F-actin belt is too rigid to allow a deep contraction and it needs to be destabilized or partially disassembled by increased F-actin turnover. F-actin treadmilling might be also important for the proper filament orientation within the ring to allow more efficient myosin II-driven contraction. Moreover, additional regulatory mechanisms are likely to be involved, since recent studies have demonstrated actin-depolymerizing activity of myosin II in a cell-free system (191) and Diaphanous formin-dependent activation of perijunctional myosin II during *Drosophila* morphogenesis (192). A cross-talk between myosin II contraction, F-actin treadmilling and other cytoskeletal and membrane processes is a new and rapidly expanding area of research which will result in better understanding the regulation of epithelial junctions.

6.4. F-actin treadmilling-driven assembly of epithelial apical junctions

Since depolymerization of actin filaments is thought to accelerate disassembly of epithelial apical junctions, one can expect that the assembly of AJ and/or TJ is driven by actin polymerization. Indeed, a critical role of actin polymerization in the establishment of epithelial junctions has been initially demonstrated in experiments with human keratinocytes (57) and was subsequently confirmed in renal, intestinal and mammary epithelia (55, 162, 174). Evidence supporting this mechanism include the increase in the amount of total (55) or perijunctional F-actin (58) during the formation of AJ and TJ, as well as pharmacological modulation of junctional assembly by latrunculins and jasplakinolide. The former F-actin depolymerizing drugs prevent the establishment of AJ and TJ (55, 58), whereas the later agent, which promotes actin-polymerization, accelerates junctional assembly (55).

Several recent studies have identified components of the actin-polymerization machinery that regulate the formation of epithelial AJ. Both Arp2/3-mediated (162, 170, 171, 175) and formin-mediated actin nucleation (168) appear to be important for AJ assembly. This role of the Arp2/3 complex is based on two lines of evidence. First, the Arp2/3 complex or Arp2/3-related NPFs colocalize and/or physically associate with E-cadherin during initial assembly of AJ-like junctions. Second, interfering with the functions of Arp2/3-related NPFs attenuates formation of nascent cell-cell adhesions. In particular, the establishment of E-cadherin-based adhesions in different epithelial cells can be inhibited by siRNA-mediated knockdown or dominant-negative mutants of N-WASP, WAVE-2 and

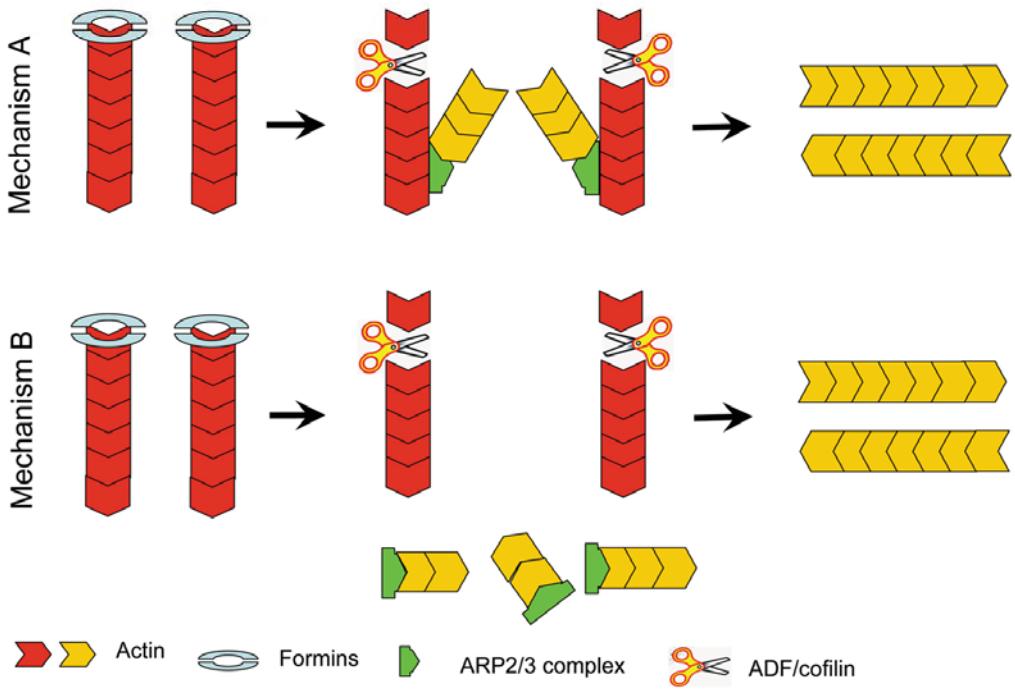

Figure 4. Hypothetical mechanisms which mediate reorganization of perijunctional actin filaments during the formation of epithelial junctions. The scheme depicts two possible scenarios of the interplay between formin- and Arp2/3-dependent F-actin nucleation, which mediate the formation of perpendicular F-actin cables (red color) and parallel F-actin bundles (orange color) at assembling epithelial junctions. See explanation in the text.

cortactin (162, 170, 171). Furthermore, pharmacological inhibition of N-WASP with wiskostatin blocks the formation of TJ (55), although this data should be interpreted with caution due to a possibility of nonspecific wiskostatin-induced energy depletion of the cell (193).

It is noteworthy that the formation of initial cell-cell adhesions is mediated by long linear F-actin cables (Figure 3). Assembly of these cables is unlikely to be driven by the Arp2/3 dependent polymerization, which is known to produce short and branched actin filaments (142, 144). This suggests the involvement of an alternative actin polymerization mode. Indeed, one study in human keratinocytes has demonstrated a critical role of formin-1 for the assembly of linear F-actin cables at nascent cell-cell junctions (168). Although it has not been directly proved yet, Arp2/3- and formin-dependent actin polymerization are likely to cooperate in building the perijunctional F-actin cytoskeleton (90). Two different modes of such cooperation can be envisioned. One implies that formins are responsible for the assembly of radial F-actin cables which run perpendicularly to cell-cell contacts. Afterwards, Arp2/3-dependent polymerization induces asymmetric branching of these cables, which in a combination with ADF/cofilin-mediated depolymerization, will lead to filament reorientation and transformation of radial F-actin cables into circumferential F-actin bundles (Figure 4, Mechanism A). Another mechanism is that while formins mediate the assembly of radial F-actin cables, the Arp2/3 complex is involved in the creation of spatially-different population of actin filaments, which move to cell-cell contacts to become circumferential F-actin bundles (Figure 4, Mechanism B). The latter mechanism resembles the formation of different types of stress fibers in motile cells, where formins were shown to build dorsal stress fibers and the Arp2/3 complex to assemble transverse arcs (194). Future experiments are needed to clarify which actin-polymerization machinery is involved in the assembly of particular F-actin-based structures at epithelial junctions.

Molecular triggers that initiate polymerization of perijunctional F-actin remain elusive, although current opinion favors the role of E-cadherin-catenin complexes (18, 19, 195). Indeed, E-cadherin adhesive clusters recruit and activate Rac1 and phosphadidylinositol-3 kinase (196-201), which are powerful stimulators of actin polymerization. Additionally, E-cadherin-catenin complexes may directly affect F-actin assembly through their physical interactions with the Arp2/3 complex (162, 164, 175) and formin-1 (168). However, other studies suggest that perijunctional F-actin bundles can be assembled prior to plasma membrane accumulation of E-cadherin or catenins (89) and even in the absence of E-cadherin (202, 203). Furthermore, a recent high temporal and spatial resolution imaging shows that E-cadherin clustering, while rapidly recruiting/activating Rac1 and PI3-kinase, does not induce F-actin assembly in close vicinity of adhesive clusters (201). Quite oppositely, E-cadherin transinteractions depend on an intact F-actin cytoskeleton. Together, these data suggest complex mutual relationships between E-cadherin adhesions and actin filaments, where actin filaments at the edges of contacting

cells promote the formation of adhesive E-cadherin clusters which in turn stimulate reorganization of F-actin in the area of cell-cell contacts.

The F-actin treadmilling motor appears to be critical for both initiation of intercellular adhesions and disassembly of epithelial AJ and TJ. This is consistent with morphological data that show the formation of perpendicular F-actin cables and filopodial membrane protrusions during initiation and disassembly of cell-cell contacts (55-57, 73, 82). Such a dual role for F-actin dynamics explains seemingly confusing data that the same signaling cascades (involving for example Rho family GTPases and Src tyrosine kinase) can mediate the opposite aspects of junctional biogenesis: TJ/AJ formation and disassembly (33, 204, 205). Since the F-actin dynamics represents a common downstream effector for many signaling molecules, it is logical to expect the same signaling events to happen at the initiation of cell-cell assembly and junctional disassembly which are driven by fast actin filament turnover.

It should be noted that initiation and maturation of epithelial apical junctions is accompanied by a decrease in the turnover rate of cortical F-actin. This conclusion is based on two lines of evidence. First is a live cell imaging of contacting epitheliocytes that demonstrate inhibition of membrane protrusive activity and the perimembrane flow of actin filaments during transformation from free lamellipodial edge of spreading/migrating cells to the contact zone between neighboring cells (206, 207). Second is differential effects of pharmacological inhibition of F-actin treadmilling on immature and mature junctions (55, 87). Indeed, inhibition of *de novo* actin polymerization with Cyto D and Lat B causes rapid disappearance of F-actin bundles associated with newly-formed, but not mature, AJ and TJ. These results indicate a significantly higher turnover rate of actin filaments at nascent junctions, when compared to the mature perijunctinal F-actin belt. Furthermore, newly-formed AJ and TJ become rapidly disrupted after inhibition of actin polymerization, whereas mature epithelial junctions appear to be more resistant to Cyto D or Lat B treatment (55, 87). These observations suggest the important role of the decreased F-actin turnover in stabilizing epithelial apical junctions and establishing the tight paracellular barrier.

7. CONCLUSIONS

Although many junctional proteins are known to readily oligomerize, their self-association is not sufficient to form structurally intact and functionally competent AJ and TJ which are characteristic for well-differentiated epithelia. The actin cytoskeleton appears to be a critical organizer of junctional architecture and a regulator of epithelial barrier function. Furthermore, intrinsic plasticity of actin filaments is likely to determine the dynamics of epithelial AJ and TJ during normal tissue morphogenesis and in pathology. Studies in the last decades have shown that the molecular machinery, which builds and moves actin filaments, is also involved in the formation and disassembly of epithelial junctions. Future research in this field faces a formidable task of integrating the enormous complexity of epithelial junctions *per se* and the complexity of regulatory mechanisms that determine the structure and dynamics of the actin cytoskeleton. The unresolved issues include the molecular nature of interactions between AJ/TJ components and actin filaments, functional roles of the different actin-polymerizing and depolymerizing proteins in the formation and disassembly of epithelial junctions, signaling pathways which regulate the remodeling of perijunctional F-actin, as well as the relationships between F-actin dynamics, and vesicular trafficking in regulation of junctional biogenesis. These big challenges are expected to lead to exciting future discoveries, which will improve our understanding of the basic biology of epithelial barriers and will help to prevent disruption of these barriers in human diseases.

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Abbreviations: ADF, actin-depolymerizing factor; AJ, adherens junctions; Arp2/3, actin-related proteins 2/3; Cyto D, cytochalasin D; EM, electron microscopy; F-actin, filamentous actin; IFNγ, interferon γ; Lat, latrunculin;

mDia, mammalian Diaphanous; Mena, mammalian Ena; MLCK, myosin light chain kinase; NM II, nonmuscle myosin II; NPF, nucleation-promoting factors; N-WASP, neuronal Wiskott-Aldrich syndrome protein; RLC, regulatory light chain; ROCK, Rho-associated kinase; TEER, transepithelial electrical resistance; TJ, tight junctions; TNF, tumor necrosis factor; VASP, vasodilator-stimulated phosphoprotein; WAVE, WASP family verprolin homologous; ZO, *zonula occludens*.

Key Words: Tight Junctions, Adherens Junctions, Barrier, Permeability, Actin, Myosin, Polymerization, Depolymerization, Turnover, Review

Send correspondence to: Andrei I. Ivanov, Division Gastroenterology and Hepatology, Department of Medicine, University of Rochester School of Medicine and Dentistry; 601 Elmwood Avenue, Box 646, Rochester, NY 14642, Tel: 585-276-4067, Fax: 585-275-8118, E-mail: Andrei_Ivanov@urmc.rochester.edu

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