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Spectrin-adducin membrane skeleton

A missing link between epithelial junctions and the actin cytoskeletion?

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Adherens junctions (AJs) and tight junctions (TJs) represent key adhesive structures that regulate the apicobasal polarity and barrier properties of epithelial layers. AJs and TJs readily undergo disassembly and reassembly during normal tissue remodeling and disruption of epithelial barriers in diseases. Such junctional plasticity depends on the orchestrated dynamics of the plasma membrane with its underlying F-actin cytoskeleton, however the interplay between these cellular structures remains poorly understood. Recent studies highlighted the spectrin-adducin-based membrane skeleton as an emerging regulator of AJ and TJ integrity and remodeling. Here we discuss new evidences implicating adducin, spectrin and other membrane skeleton proteins in stabilization of epithelial junctions and regulation of junctional dynamics. Based on the known ability of the membrane skeleton to link cortical actin filaments to the plasma membrane, we hypothesize that the spectrin-adducin network serves as a critical signal and force transducer from the actomyosin cytoskeleton to junctions during remodeling of AJs and TJs.

Key words: adherens junctions, tight junctions, permeability, membrane skeleton, actomyosin, contractility, calcium switch

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Introduction

Integrity and barrier properties of epithelial layers depend on the assembly of adhesive contacts between adjacent epithelial cells. These adhesive contacts are composed of multiprotein complexes known as intercellular junctions. In simple polarized epithelia, several junctional complexes span the lateral plasma membrane to interact with opposing complementary junctions in the intercellular space and associate with various cytoskeletal, signaling and trafficking components at the cytosolic face of the membrane.[1,2] Among these complexes the most apically-located tight junctions (TJs) and subjacent adherens junctions (AJs) play key roles in regulating epithelial cell adhesion, polarity and differentiation.[2-5]

TJs and AJs are composed of several types of integral membrane and cytosolic scaffolding proteins. Major transmembrane components of TJs mediating intercellular adhesions include the claudin protein family, occludin and junctional adhesion molecule A.[2,3,5] 'Zonula occludens' (ZO) proteins are the most abundant molecular constituents of the cytosolic plaque of TJs.[2,3,5,6] Adhesive properties of epithelial AJs are determined by E-cadherin and nectins, which are clustered and stabilized at the plasma membrane via interactions with cytosolic scaffolds such as α, β and p120 catenins.[2,4,7]

Once considered as static, glue-like structures, TJs and AJs are now known to be very dynamic. Such dynamics includes a continuous remodeling (disassembly and reassembly) of junctional complexes in fully-differentiated epithelial layers, as well as large-scale TJ/AJ rearrangements that occur during normal epithelial morphogenesis and disruption of mucosal barriers in many diseases.[8-12] Two major mechanisms have been implicated in regulation of junctional dynamics; one is reorganizations of the perijunctional F-actin cytoskeleton and the other is remodeling of the plasma membrane.[8-13] The interplay between these mechanisms is well

appreciated. Indeed, reorganizations of the perijunctional F-actin belt are coordinated with altered endocytic and exocytic activity of the cell membrane during disassembly and reformation of epithelial contacts.[14-17] Furthermore, inhibition of such F-actin reorganizations was shown to suppress membrane dynamics and vice versa.[14,15,18,19] These findings suggest that physical attachment of the cortical F-actin cytoskeleton to the plasma membrane play an important role in transducing signals and/or forces that drive remodeling of epithelial junctions.

Several mechanisms can link actin filaments to the cytosolic face of the plasma membrane with the most abundant attachments mediated by the spectrin-based membrane skeleton.[20-22] The membrane skeleton is formed by spectrin tetramers composed of two α and β-spectrin heterodimers.[20-22] These tetramers represent flexible rods with actin-binding sites at each end. Spectrin rods are linked to the plasma membrane via specialized scaffolding proteins, such as ankyrin and protein 4.1, which have a dual affinity for spectrin and cytoplasmic domains of transmembrane transporters and channels.[20-22] Spectrin association with actin filaments is enhanced by other accessory proteins, most notably, by adducin.[21,23] Mammalian adducin has three homologous isoforms α, β and γ.[21,23] The α and γ isoforms are expressed in various tissues, whereas expression of β-adducin is limited to erythrocytes and the brain.[24,25] Adducin readily forms heterodimers and heterotetramers of either α/β or α/γ subunits.[24,26] These oligomers are thought to recruit spectrin to actin filaments and to promote assembly of the spectrin lattice at the plasma membrane.[26,27] Besides mediating spectrin-F-actin linkage, adducin is also involved in actin filament bundling and capping.[28-30] Overall, these data highlight adducin as an important regulator of both the spectrin-based membrane skeleton and the actin cytoskeleton.

Adducin Regulates Remodeling of Apical Junctions in Simple Epithelia

It has been long recognized that α and γ isoforms of adducin are enriched at intercellular junctions in cultured epithelial cell monolayers and simple mucosal epithelia in vivo.[24,31] Despite this junctional affiliation, the involvement of adducin in regulation of epithelial AJs and TJs remains poorly understood. Recently, we have examined the role of this membrane skeleton protein in the dynamics of epithelial junctions by using siRNA-mediated knockdown of α and γ adducin isoforms in SK-CO15 human intestinal epithelial cells.[32] Remodeling of epithelial junctions was induced by a so called 'calcium switch'. This model involves removal of extracellular calcium to trigger disassembly of preformed AJs and TJs followed by calcium re-addition to the culture medium (calcium repletion) to induce orchestrated recovery of junctional structure and functions.[14-16,33] We observed that knockdown of either α- or γ-adducins in SK-CO15 cells attenuated reassembly of apical junctions and development of the paracellular barrier triggered by calcium repletion. Interestingly, loss of adducin expression delayed reformation of both AJs and TJs, although α- or γ-adducins consistently colocalized with AJ, but not TJ proteins in newly-assembled and mature intercellular contacts.[32] Since AJ assembly represents an early step of epithelial differentiation that is required for the subsequent formation of TJs, we believe that adducin depletion directly impairs the establishment of epithelial AJs, which in turn attenuates TJ reassembly. Eventually, epithelial cells were able to assemble morphologically-normal cell-cell contacts even in the absence of adducin, however, such contacts appear to be less stable comparing to those of normal cells. This notion of contact instability is based on the observed collapse of the lateral plasma membrane and the increased long-range intramembrane mobility of E-cadherin in adducin-depleted cells.[27]

Given our findings that adducin promotes the establishment of epithelial AJs and TJs one can suggest that this membrane skeleton protein should antagonize junctional disassembly. Indeed, we observed such antagonisms while examining the effects of adducin isoforms knockdown on disruption of AJs and TJs in HPAF II human pancreatic epithelial cells exposed to protein kinase C (PKC)-activating phorbol ester. PKC activation is known to potently disrupt cell-cell contacts in several types of epithelia by stimulating remodeling of the perijunctional F-actin and triggering internalization of AJ/TJ proteins.[34,35] On the other hand, PKC phosphorylates adducin at several serine residues (Ser726, Ser712 and Ser660) in their C-terminal MARKS domain.[36,37] This phosphorylation has been shown to inhibit adducin functions by decreasing its associations with actin filaments and spectrin.[36,37] We found that phorbol ester induced rapid phosphorylation of α- and γ-adducins which was accompanied by their disappearance from the intercellular junctions.[32] Loss of adducin from cell-cell contacts appears to be an early event of the phorbol ester signaling that preceded AJ and TJ disassembly. Furthermore, depletion of either α- or γ-adducins significantly accelerated disruption of AJs and TJs induced by PKC activation.[32] These results suggest that PKC-dependent phosphorylation of adducin triggers its early release from complexes with spectrin and actin filaments, thereby enhancing remodeling of the cortical cytoskeleton and destabilizing epithelial junctions. Importantly, protein kinase A and Rho-dependent kinase, as well as cytokines such as pleiotropin are known to phosphorylate adducin and alter cellular distribution and activity of this scaffolding protein.[37,38] Therefore, adducin can be important down-stream effector of different signaling cascades that regulate stability and remodeling of epithelial junctions.

Our study also provides an important insight into the mechanisms that mediate the effects of adducin on epithelial junctions. These mechanisms involve organization of the spectrin network and assembly of actin filaments at the intercellular contacts. For example, we found that depletion of adducin isoforms decreased expression of βII-spectrin in intestinal epithelial cells and delayed recruitment of this protein to newly-forming AJs.[32] This observation suggests that loss of adducin impairs formation of the highly-ordered spectrin lattice at the plasma membrane of contacting epithelial cells, which is likely to be responsible for the attenuated junctional assembly. Another mechanism

that can mediate destabilization of apical junctions in adducin-depleted epithelia involves impaired formation of the perijunctional F-actin belt. Previous biochemical studies described the ability of α-adducin to cap[28] and cross-link[29,30] actin filaments in cell-free systems. However, we demonstrated for the first time that adducin regulates assembly of the F-actin cytoskeleton in epithelial cells. This conclusion is supported by findings that siRNA-mediated depletion of α and γ adducins increased the G/F actin ratio, which indicates either impaired polymerization or enhanced depolymerization of actin filaments. Furthermore, adducin downregulation attenuated formation of the perijunctional F-actin bundles during reestablishment of epithelial AJs.[32] Given the crucial role of the circumferential F-actin belt in supporting structure of epithelial junctions, this defective F-actin assembly should underline the impaired formation of AJs and TJs in adducin-depleted epithelial cells. What type of adducin-F-actin interactions (filament capping or cross-linking) are involved in organization of the perijunctional cytoskeleton and whether spectrin is essential for these events remain to be determined. It is also unclear whether the effects of adducin depletion on the remodeling of actin filaments and spectrin assembly at intercellular junctions represent two distinct mechanisms or if they are mutually dependent. However, based on a classical model of adducin action, it is likely that loss of this scaffolding protein breaks a physical link between spectrin oligomers and actin filaments which is important for proper organization of both cytoskeletal structures at the areas of cell-cell contacts.

Various Molecular Components of the Membrane Skeleton are Essential for the Biogenesis of Epithelial Junctions

Other molecular constituents of the membrane skeleton have been implicated in regulation of epithelial apical junctions. Spectrin itself appears to be important in invertebrate and mammalian systems. For example, removal of apical spectrin in Drosophila was shown to induce AJ disassembly in follicle epithelium,[39] whereas depletion of mammalian βII-spectrin attenuated formation of AJs in human intestinal and bronchial epithelial cells,[32,40] and early mouse embryo.[41] Two major mechanisms can be responsible for the observed spectrin-dependent regulation of epithelial junctions. The first mechanism involves βII-spectrin binding to the E-cadherin-catenin complex, which can be either direct or mediated by an accessory protein, ankyrin G. Such interactions with βII-spectrin and/or ankyrin are important for E-cadherin trafficking from the Golgi to the plasma membrane.[40,42] An alternative mechanism of spectrin actions involves regulation of cortical actin filaments. Thus, a recent study revealed a crucial role for αII-spectrin in organizing perijunctional F-actin bundles in endothelial cells, which stabilized cell-cell contacts and enhanced the endothelial barrier.[43] Furthermore, our unpublished data suggest the close interactions between spectrin oligomers and actin filaments during AJ/TJ remodeling. We found that αII-spectrin and the perijunctional F-actin belt are spatially segregated in polarized intestinal epithelial cells with intact junctions (**Fig. 1**, arrows). By contrast, spectrin became enriched in the apical contractile actin rings that are known to drive AJ/TJ disassembly in calcium-depleted epithelial cells (**Fig. 1**, arrowheads). Interestingly, adducin also accumulated at these contractile actomyosin rings where it was colocalized with fragments of disassembled AJs (data not shown). This contrasts with adducin behavior during phorbol ester-induced disruption of epithelial junctions and suggests that in some conditions, spectrin-adducin complexes can promote disruption of AJs and TJs by controlling formation and/or contraction of perijunctional actomyosin structures.

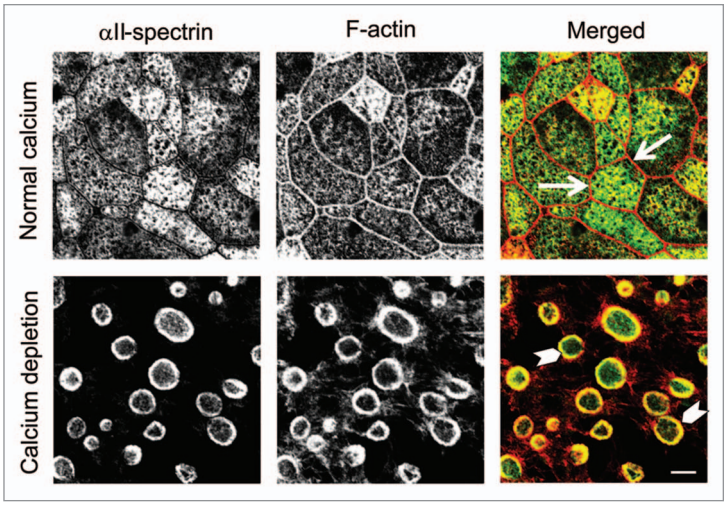

Figure 1. Spectrin is recruited to contractile F-actin rings in calcium-depleted epithelial cells. Confluent T84 human intestinal epithelial cell monolayers and cells subjected to 60 min of calcium depletion were dual immunolabeled for αII-spectrin (green color) and F-actin (red color). Confocal microscopic images show that αII-spectin does not significantly colocalize with the perijunctional F-actin belt (arrows) in control cell monolayers. By contrast, αII-spectrin is enriched in contractile apical F-actin rings assembled in calcium-depleted epithelial cells (arrowheads). Scale bar, 5 μm.

Protein 4.1 is another molecular scaffold in the membrane skeleton that was implicated in regulation of epithelial junctions. Several members of the protein 4.1 family are known to interact with spectrin and actin and to stabilize the spectrin-actin network at the plasma membrane.[44,45] In epithelial cells, protein 4.1R isoform was shown to localize at the areas of cell-cell contacts where it was able to associate with β-catenin and ZO-2.[46,47] Loss of protein 4.1 expression phenocopied major effects of other membrane skeleton proteins depletion by causing disruption of the E-cadherin-catenin complex, impairing trafficking of junctional proteins to the plasma membrane and attenuating assembly of the cortical F-actin cytoskeleton.[47,48]

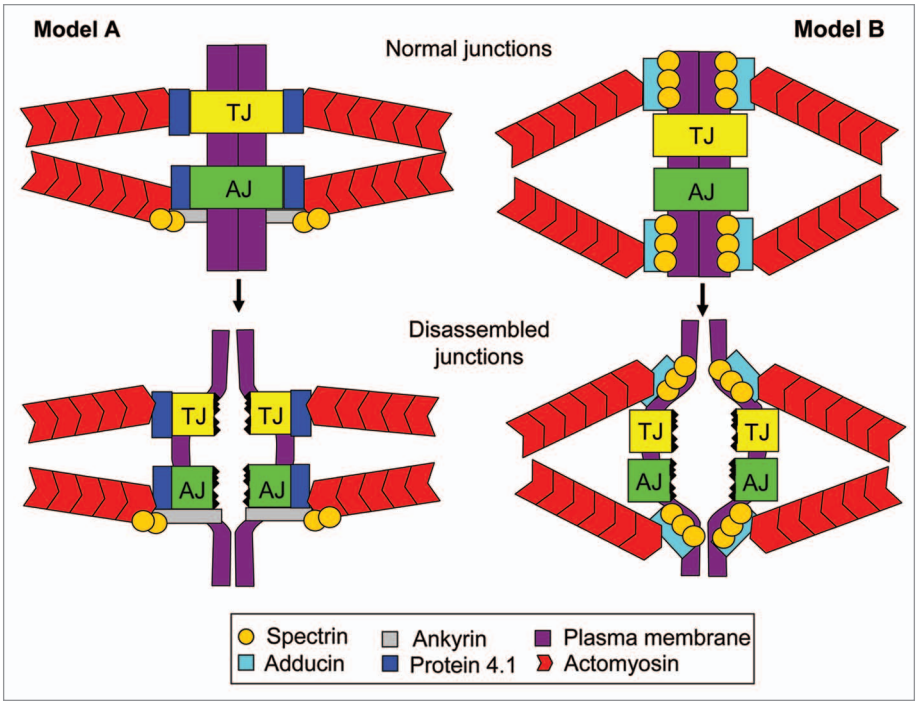

Figure 2. Hypothetical mechanisms by which the membrane skeleton can mediate F-actin dependent disassembly of epithelial junctions. The scheme depicts two different models proposed to explain how the spectrin-adducin membrane skeleton can mediate epithelial AJ/TJ disassembly driven by perijunctional actomyosin contractility. Model A implies that the membrane skeleton physically links actin filaments to cytosolic plaques of AJs and TJs, whereas Model B proposes indirect force transduction via adducin-spectrin-mediated actin attachment to the plasma membrane in a close vicinity of apical junctions. See detailed explanation in the text.

Possible Mechanisms of the Membrane Skeleton-Dependent Regulation of AJ/TJ Structure and Remodeling

Recent studies that unravel close associations between the spectrin-adducin-based membrane skeleton and epithelial junctions not only add another level of complexity to AJ and TJ regulation but may also help to solve a puzzle about associations of epithelial junctions and the underlying actin filaments. A critical role of F-actin cytoskeleton in regulating structure and barrier properties of apical junctions is well appreciated and is supported by a large amount of morphological, pharmacologic and genetic data.[9,10,12,13,49,50] Furthermore, it is known that perijunctional actomyosin bundles regulate junctional structure and remodeling by generating tension or contractile forces.[9,10,49,50] The force transduction from actin filaments to epithelial junctions implies that these structures are physically-connected, however molecular organization of the cytoskeletal/junctional interface remains enigmatic. Particularly, it is unclear which cytosolic scaffolds link AJs and TJs to the underlying actin filaments in living cells.[51,52] We believe that the spectrin-adducin membrane skeleton can serve as a linker/force transducer between the actin cytoskeleton on epithelial junctions. **Figure 2** depicts two hypothetic mechanisms by which membrane skeleton can mediate AJ/TJ disassembly driven by contractile actomyosin structures, as it happens in calcium-depleted epithelial cells. The canonical mechanism presented in Model A implies that membrane skeleton proteins physically link epithelial junctions to underlying actin filaments. For example, protein 4.1 can mediate cytoskeletal attachments of both AJs and TJs due to its known interactions with actin and junctional plaque components ZO-2 and β-catenin.[46,47] Additionally, AJs can be linked to actin filaments by a scaffolding complex composed of ankyrin G, spectrin and adducin. Such membrane skeleton mediated linkage between apical junctions and actin filaments will ensure disassembly of AJs and TJs upon synchronized contraction of perijunctional actin bundles in two contacting epithelial cells (**Fig. 2**). However, the membrane skeleton can transduce actomyosin-generated forces to epithelial junctions even without direct attachments of AJs and/or TJs to actin filaments. This may explain recent live cell imaging data that demonstrated different turnover rates of junctional proteins and cortical actin thereby arguing against their direct physical associations.[51,52] Model B depicted in **Figure 2** illustrates how this indirect mechanism can mediate junctional disassembly. Spectrin-adducin complexes are known to attach actin filaments to the cytosolic domains of integral plasma membrane proteins such as Na/K ATPase or ammonium transporter.[20,21,44] Because of this attachment, synchronized contraction of perimembrane actomyosin bundles should result in pulling apart two opposing plasma membranes of contacting epithelial cells. If such membrane retraction occurs in a close vicinity to apical junctions, it will create sufficient pulling forces to disrupt trans-interactions between

adhesive AJ/TJ proteins thereby triggering junctional disassembly. Similar mechanism can contribute to junctional reassembly and to the organization of mature AJ and TJ in polarized epithelial cells. In the last scenario, spectrin-adducin-mediated membrane attachments of the perijunctional F-actin belt may stabilize junctional structures by corralling AJ/TJ proteins at the cell apex and limiting their diffusion within the plasma membrane.

In summary, several recent studies shed a new light on a long forgotten association between epithelial junctions and the membrane skeleton by demonstrating crucial involvement of adducin and spectrin in regulating AJ/TJ dynamics. We hope that future works will unravel important molecular mechanisms that underlie membrane skeleton-dependent remodeling of apical junctions during normal epithelial morphogenesis and/or disruption of mucosal barriers in different diseases.

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