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Breaking into the epithelial apical–junctional complex — news from pathogen hackers

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Abstract

The epithelial apical–junctional complex is a key regulator of cellular functions. In addition, it is an important target for microbial pathogens that manipulate the cell to survive, proliferate and sometimes persist within a host. Out of a myriad of potential molecular targets, some bacterial and viral pathogens have selected a subset of protein targets at the apical–junctional complex of epithelial cells. Studying how microbes use these targets also teaches us about the inherent physiological properties of host molecules in the context of normal junctional structure and function. Thus, we have learned that three recently uncovered components of the apical–junctional complex of the Ig superfamily — junctional adhesion molecule, Nectin and the coxsackievirus and adenovirus receptor — are important regulators of junction structure and function and represent critical targets of microbial virulence gene products.

Introduction

The apical–junctional complex (AJC) is a highly specialized structure at the apical tip of the lateral membrane of polarized epithelial cells. In addition to its multi-faceted participation in regulating cell–cell adhesion between neighboring cells, integrity of the epithelial barrier, and contractile forces during morphogenesis and wound healing, the AJC is equally important as a hub for signaling pathways controlling cell proliferation, cell differentiation and cell polarity [1]. This signaling hub consists of complex networks of interconnected proteins that dynamically interact with each other to adjust and coordinate different cellular functions. Defects in one component of the junction often lead to changes in the entire AJC [2–4].

Pathogens have exploited the AJC as a strategy for overcoming the epithelial barrier and as a site for host colonization. They have evolved mechanisms to directly break down tight junction barriers to allow entry into, or exit from, a host organism, and to co-opt nutrients from the interstitium. Remarkably, they have also been able to access receptors hidden on the basal–lateral epithelial membrane to enter cells where they may replicate or seek protection from the immune system. Pathogenic microbes have even found ways to benefit from inflammation, increased cell turnover and prevention of wound healing, all of which are controlled to some extent by the AJC [5] (Figure 1).

The AJC contains several distinct protein sub-complexes. In general, each protein sub-complex consists of a transmembrane protein bound to scaffolding proteins, each of which has multiple protein–protein binding motifs that potentially link together different membrane sub-complexes. Scaffold proteins generally bind to the actin cytoskeleton, although links to microtubules may also be present [1,6]. The cadherin/catenin and claudin/occludin/ZO (zonula occludens) protein sub-complexes form the adherens and tight junctions, respectively, and together with the cytoskeleton provide the basic structural components of the AJC [7,8]. Of course, there are many regulatory elements that control the dynamic organization of the AJC and co-ordinate its many functions (Figure 2).

Here, we review some of the strategies pathogens use to interact with the structural and regulatory components of the AJC. In particular, microbial virulence gene products point to an emerging role for a family of receptors belonging to the Ig superfamily as important regulators of the AJC. Focusing on these targets, we review what is known about the molecular regulation of the AJC by Ig superfamily receptors during junction assembly. The results of this work give us as much information about AJC structure and function as about microbial pathogenesis.

Breaking into the epithelial apical–junctional complex

Cleaving structural components

Bacterial pathogens have developed strategies to interfere with structural components of the AJC at the level of transmembrane proteins, scaffolding proteins and the cytoskeleton. Perhaps the simplest strategy used by pathogens is to secrete one or more enzymes that target the extracellular domain of AJC transmembrane proteins. This usually leads to barrier defects and disruption of the epithelial monolayer [9]. For example, *Bacteroides fragilis* secretes the toxin Fragilysin that cleaves the extracellular domain of E-cadherin [10]; *Vibrio cholerae* secretes a protease that can degrade occludin [11]; and adherent *Staphylococcus aureus* synthesizes several exfoliative toxins that cause disassembly of cell–cell contacts through cleavage of the more spatially distant transmembrane desmosome adhesion protein desmoglein 1 [12•] (Figure 3a). Proteolytic degradation by pathogens confirms the importance of their target transmembrane proteins for maintaining the structural and functional integrity at the AJC and that their disruption can have profound consequences for the host while benefiting the microbe by enabling it to establish itself, gain nutrients and replicate.

Targeting regulatory elements from inside the cell

Bacterial proteolytic toxins may be ineffective when applied to the intact apical compartment of an epithelial monolayer. Some bacterial pathogens have evolved alternative strategies that manipulate the AJC from the apical surface. One such strategy is to target regulatory elements of the actin cytoskeleton (Figure 3b). For example, cytotoxic necrotizing factor 1 (CNF-1), a toxin synthesized by certain pathogenic *Escherichia coli*, targets Rho family small GTPases to disrupt the tight-junctional seal of intact epithelial monolayer [13•]. CNF-1 applied to polarized epithelia activates RhoA, Rac1 and Cdc42 and leads to rearrangement of the actin cytoskeleton. This is followed by massive internalization of tight junctional proteins occludin, junctional adhesion molecule (JAM) and ZO-1 and concomitant disruption of the tight junction barrier. Interestingly, in CNF-1-treated epithelial cells the adherens junctions remained grossly intact. Toxin A and B from the bacterium *Clostridium* also intervene in small GTPase functions and affect tight junctions in a similar way to CNF-1 [14]. The regulation of junctions through small GTPases is highly complex and requires a balanced interaction between members of the Rho family (reviewed in detail [15,16]).

Bacterial pathogens often combine different strategies to change host cell functions, and have found ways to deliver their effectors to different sites of action. Several bacterial pathogens use molecular microinjection devices derived from flagellar or conjugation apparatuses (known as type III or type IV secretion systems) to introduce bacterial proteins into the host cell and target the AJC from inside. Enteropathogenic *Escherichia coli* (EPEC), for example, colonizes the apical surface of intestinal epithelial cells and becomes tightly adherent modifying the actin cytoskeleton beneath attached bacteria. This is mediated by bacterial effector proteins translocated into the host-cell membrane and cytosol through a type III secretion system. Independently of factors involved in bacterial attachment, some translocated effectors induce phosphorylation of the myosin light chain by myosin light chain kinase and concomitant contraction of the peri-junctional actomyosin ring [17]. In addition, EPEC infection leads to occludin dephosphorylation, redistribution of occludin and ZO-1, and activation of ezrin [18–20]. It was shown recently that ezrin regulates the assembly of adherens junctions in a Rac1-dependent way [21••]. These changes lead to the breakdown of tight junction barriers and thus to increased paracellular permeability in infected epithelia.

Helicobacter pylori also translocates at least one bacterial effector protein, CagA, into the host cell to unlock the AJC. CagA disrupts the epithelial AJC and forms a new protein complex with the junctional proteins JAM and ZO-1 at the bacterium–host-cell attachment site [22••] (Figure 3c). A functional consequence is that the epithelial barrier is perturbed (although gross cell–cell adhesion and integrity of the epithelial monolayer are maintained), resulting in leakage of ions and solutes from the interstitium into the apical compartment where bacteria reside [22••,23,24]. Furthermore, CagA activates the hepatocyte growth factor (HGF)/c-met pathway from within epithelial cells, which leads to changes in cell morphology and migration [25,26,27••]. HGF and other growth factors are known to be important regulators of the AJC, but the mechanism by which they interact with assembled junctions is poorly understood [28,29]. Similar to *H. pylori*, *Listeria monocytogenes* targets proteins of the AJC and growth factor pathways in order for the bacterium to enter host cells [30]. The *Listeria* surface protein InlA interacts with E-cadherin [31], and InlB activates the Met receptor tyrosine kinase for cell entry [32] (Figure 3d). Future studies on *H. pylori* CagA and *L. monocytogenes* InlA and InlB may provide new information about cellular regulation of the AJC through growth factor pathways.

Targeting regulatory elements from outside the cell

Viruses use receptors of the Ig superfamily to interact with host-cell junctions. Adenovirus interacts with the coxsackievirus and adenovirus receptor (CAR) to enter epithelial airway cells [33] (Figure 3e). CAR is an immunoglobulin-like cell–cell adhesion molecule that shares sequence similarities to JAM and is localized at the AJC in a variety of epithelial cells [34]. Disruption of CAR–CAR interactions in contacting cells causes a decrease in cell–cell adhesion and disruption of the barrier function of the epithelial monolayer [34,35••]. After adenoviruses enter epithelial cells and replicate, infected cells release complete viruses, viral particles and fiber protein. Fiber protein interacts with high affinity with CAR, which leads to disruption of cell–cell adhesion allowing viruses to exit from the space between opposing lateral membranes into the apical compartment [35••,36]. Adenovirus pathogenesis demonstrates that CAR receptors are very effective external regulators of AJC structure and function, although we know very little about the precise biochemical mechanisms involved.

Viruses such as reovirus and α -herpesviruses target JAM and nectin receptors, respectively, and use them for cell entry [37,38], but their effects on AJC structure and function have not been described [38–40]. It is not yet clear how JAM and nectin receptors regulate junctions once they are fully assembled. Glycoprotein D, a surface protein of α -herpesviruses,

interacts with nectin receptors during cell entry, disrupts cell–cell adhesion in cells expressing nectin-1 and lacking E-cadherin, and interacts with nectin receptors in polarized epithelia [39]. However, in that study and others, effects on the integrity of mature junctions were not studied [41•,42].

Ig superfamily members as regulatory elements of the apical–junctional complex

Why have so many unrelated viral and bacterial factors such as CagA evolved to target the Ig superfamily at the AJC? Under physiological conditions, Ig superfamily receptors are implicated in controlling the disassembly and re-assembly of adherens and tight junctions in the AJC. For example, migration of leukocytes across cell monolayers requires the local opening of intercellular junctions. Live cell imaging of neutrophils migrating through an endothelial monolayer showed that GFP-labeled VE-cadherin, a component of the endothelial adherens junction, disappears from the plasma membrane of endothelial cells at the site of neutrophil penetration. This is followed by resealing of the local opening immediately after the neutrophil passes through the gap [43]. Presumably, tight junctions need to be opened in synchrony with the adherens junctions for this process to occur, and similar openings are required for the transmigration of leukocytes through epithelia. JAM, a member of the Ig superfamily, seems to be involved in the regulation of the AJC [44,45]. Inhibition of JAM receptor activity using antibodies directed against the JAM extracellular domain prevents transmigration of leukocytes through endothelial cell monolayers [46,47]. These results pinpoint the importance of JAM in regulating dynamic openings in the monolayer at the level of the AJC [48,49], but this effect is cell-type- and tissue-specific; for example, inhibition of JAM does not prevent leukocyte transmigration in other endothelial monolayer systems [50,51].

Studies on cell–cell junction assembly reveal how members of the Ig superfamily of cell–cell adhesion proteins regulate the AJC. Nectins, which are also members of the Ig superfamily, are linked to the actin cytoskeleton through afadin, and are closely associated with the cadherin/catenin system in adherens junctions [41•]. JAM also associates with afadin/AF-6 (AF-6 is the ALL-1 fusion partner from chromosome 6) and ZO-1 and is localized with tight-junctional proteins in polarized epithelia [45,52,53]. These protein–protein interactions highlight the interconnection between nectins, JAM and tight-junction proteins in different protein complexes.

The functions of nectin and JAM during assembly of the AJC in epithelial cells have been studied in calcium-switch experiments, in which calcium initiates cell–cell contact via the calcium-dependent cell–cell adhesion molecule E-cadherin. The nectin/afadin system seems to be closely associated with formation of adherens junctions. Nectin interacts with the cadherin/catenin system via afadin and α -catenin [41•], but the exact interaction is not completely understood. It is also thought that the nectin/afadin system is important for recruitment of JAM to sites of cell–cell adhesion. One study suggests that the recruitment of JAM occurs via afadin and ZO-1 and not through afadin alone [54••]. Although it has been shown that afadin can bind directly to ZO-1, this has not been confirmed in other studies and an indirect interaction between the two proteins is possible [55].

JAM seems to be important for the recruitment of the tight-junction transmembrane proteins occludin and claudin to sites of cell–cell adhesion. Inhibition of JAM with a functional blocking antibody prevents recruitment of JAM and occludin to cell–cell contacts and causes loss of barrier function during AJC assembly [56]. JAM may control tight junction assembly via the partitioning-defective protein (PAR) complex. JAM cytoplasmic domain binds PAR-3, thereby localizing the PAR-3/PAR-6/aPKC complex to the AJC [53,57]. In

turn, the PAR complex seems to be important for targeting junctional proteins to tight junctions (reviewed in detail in [3,4,58]). JAM may also regulate tight-junction formation via recruitment of members of the ZO protein family, ZO-1, ZO-2 and ZO-3, which bind to the tight junctional proteins occludin and claudins. Overexpression of a ZO-3 mutant in epithelial cells causes a delay in tight junction formation indicating that the protein plays an important role during assembly [59].

As discussed above, CAR receptors are regulators of the AJC in epithelial cells. One mechanism of regulation may be through their interaction with components of the adherens and tight junctions. In one study CAR was found to be in a complex with ZO-1 and localized to tight junctions [34]. Another study found CAR to be localized underneath the tight junction belt in a co-immunoprecipitated complex with β -catenin, a component of the adherens junctions [35•]. Differences in these studies could be due to the studied cell type or to the assembly stage of the junctional complex. In analogy to JAM receptors and nectin receptors regulating junctional assembly, the physical link between CAR and these adaptor protein sub-complexes implicates them in regulation of the AJC.

As epithelial and endothelial cells express different receptors of the Ig superfamily at different levels and locations relative to the AJC [42,44], CAR, JAM and nectin receptors appear to give cells the regulatory diversity to reversibly control the structural integrity and function of the AJC. Is it possible that some microorganisms unlock the AJC using the same mechanisms utilized by transmigrating leucocytes? Are the cell-type- and tissue-specific features that are characteristic of bacterial and viral entry a reflection of the nature of the AJC and its molecular components in particular tissues? Future studies in these areas should provide us with some answers.

Conclusions

The AJC is a signaling hub regulating many important cellular functions. It comprises basic structural components, including the cadherin/catenin and claudin/occludin complexes that form the adherens and tight junctions, respectively, and the cytoskeleton, which connects these junctions into an integrated network. Regulatory sub-complexes comprising transmembrane receptors of the Ig superfamily resemble a software operating system, and may control the organization of the structural components during assembly and maintenance of the AJC. Pathogens have evolved mechanisms to target both the structural and regulatory components of the AJC. In some cases, pathogens, like hackers, have developed ways to finely manipulate the software that subtly adjusts AJC structure and function. Future studies based upon what work on pathogens has identified as key components of the AJC, should provide new insights into how the software controls the hardware to adapt the AJC signaling hub for different functions. Moreover, devising a 'firewall' to prevent disruptive hacking of the AJC hardware and software may provide us with novel preventive measures against microbial incursion.

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Abbreviations

AJC	apical–junctional complex
CAR	coxsackievirus and adenovirus receptor
CNF-1	cytotoxic necrotizing factor 1
EPEC	enteropathogenic <i>Escherichia coli</i>
HGF	hepatocyte growth factor
JAM	junctional adhesion molecule
PAR	partitioning-defective protein
ZO	zonula occludens

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Figure 1.

Bacteria and viruses interact with, and disrupt, apical junctions of polarized epithelia. From left to right, examples of different AJC targets of pathogens. Clostridia (yellow) and *Vibrio cholerae* (blue-green) secrete enzymes that cleave tight junction membrane proteins. Pathogenic *E. coli* (blue) deliver bacterial toxins to the host cell, and affect tight junction function by disrupting the peri-junctional actin cytoskeleton. *Listeria monocytogenes* (brown) exemplifies a bacterium that disrupts junctions to enter epithelial cells and uses AJC components as receptors. *Helicobacter pylori* (purple) adheres to gastric epithelial cells near apical junctions and injects the bacterial protein CagA into the host cell. CagA interacts with scaffolding components, growth factor receptors and transmembrane molecules of the AJC leading to defects in tight-junction barrier functions and changes in cell polarity. Various unrelated viruses (green) such as adenovirus, coxsackievirus, reovirus, and herpesvirus interact with transmembrane AJC components of the immunoglobulin superfamily. This is important for both viral entry into cells and exit from the basal-lateral surface to reach the apical surface.

Figure 2.

The apical-junctional complex is organized into structural and regulatory domains by protein sub-complexes. Junctions are held together by transmembrane molecules of the occludin, claudin, cadherin and immunoglobulin superfamilies (red shading denotes proteins with a transmembrane role). Each transmembrane protein is linked via its cytoplasmic tail to scaffolding proteins of the ZO, afadin or catenin families (blue shading denotes proteins with a scaffolding role). Scaffolding proteins then form complexes with multiple signaling and adaptor proteins. For example, the Par3/Par6/aPKC/Rho-GTPase protein complex (purple shading), which is involved in regulating epithelial polarity, is linked to JAM. Scaffolding proteins also link transmembrane proteins to the peri-junctional actin cytoskeleton and its associated proteins and connect different sub-complexes with each other. The apical junctions are traditionally divided into tight and adherens junctions. The tight junctions form the epithelial barrier via their transmembrane proteins claudins and occludin. These are linked to the actin cytoskeleton through scaffolding proteins of the ZO family. At the adherens junction, E-cadherin is linked to the peri-junctional actin via β and α -catenins. These structural domains of the junctions (yellow shading) interact with regulatory protein sub-complexes that are involved in the control of cell polarity, cell division, cell movement and junction assembly. Several regulatory sub-complexes use transmembrane proteins of the immunoglobulin superfamily, such as JAM, CAR and nectin. Other regulatory systems include receptor tyrosine kinases such as c-met. (The key shows examples of the types of molecules at the AJC and is not meant to be exhaustive.)

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Figure 3.

Structural and regulatory sub-complexes of the AJC are targets for pathogens. **(a)** Bacterial enzymes can cleave structural transmembrane molecules of tight junctions; for example, the *V. cholerae* HA-protease cleaves the extracellular loops of occludin. **(b)** Several bacterial toxins and effector molecules target the peri-junctional actin cytoskeleton; for example, *E. coli*'s CNF-1 activates Rac1 and Cdc42 small GTPases at junctions. **(c)** Bacteria that translocate effector molecules into the host cell may target the AJC from the inside; for example, *H. pylori*'s CagA targets JAM and ZO-1 at the junctions as well as activating the c-met growth factor pathway. **(d)** Some bacteria target AJC components from the outside; for example, *Listeria monocytogenes* uses E-cadherin as a receptor for cell invasion, and activates the c-met receptor growth factor pathway. **(e)** Multiple viruses target the immunoglobulin superfamily at the AJC; for example, reovirus uses JAM as a receptor, adenovirus and coxsackievirus use CAR, and α -herpesviruses and poliovirus use nectins.