Impenetrable barriers or entry portals? The role of cell-cell adhesion during infection

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Host–pathogen interactions

Impenetrable barriers or entry portals? The role of cell–cell adhesion during infection

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Cell–cell adhesion plays a fundamental role in cell polarity and organogenesis. It also contributes to the formation and establishment of physical barriers against microbial infections. However, a large number of pathogens, from viruses to bacteria and parasites, have developed countless strategies to specifically target cell adhesion molecules in order to adhere to and invade epithelial cells, disrupt epithelial integrity, and access deeper tissues for dissemination. The study of all these processes has contributed to the characterization of molecular machineries at the junctions of eukaryotic cells that have been better understood by using pathogens as probes.

Introduction

The term cell adhesion encompasses a number of mechanisms that are conserved from unicellular eukaryotes to higher mammals. Unicellular organisms rely on adhesion to extracellular surfaces for movement. Multicellular organisms use cell adhesion to move, communicate, differentiate, or self-assemble to form epithelia and organs. Cell adhesion can dictate the fate of a given population of cells and is thus essential for life; it is therefore not surprising that it is subjected to tight regulation and that escaping such regulation may have extreme consequences, ranging from cell death to cell over-proliferation. Several types of cell adhesion have been characterized and can be grouped into two major classes: cell–matrix adhesion and cell–cell adhesion. Cell–matrix adhesion is mainly mediated by proteins belonging to the family of integrins, which are transmembrane mechanotransducers whose specificity for a given ligand is due to their ability to heterodimerize (Citi and Cordenonsi, 1998; Campbell and Humphries, 2011). Cell–cell adhesion represents a more complex mechanism that can be further divided into a number of different types of interactions, all characterized by specific proteins serving different purposes (Citi and Cordenonsi, 1998; Cavallaro and Christofori, 2004; Hartsock and Nelson, 2008). Common examples of cell–cell interaction sites are tight junctions (TJs), which seal the space between neighboring cells, generating an impermeable barrier between the epithelium and the extracellular environment, adherens junctions (AJs), which mediate cell polarization and organogenesis, and GAP junctions, which form channels for cell-to-cell dissemination of small molecules.

All types of cell adhesion are mediated by adhesins, transmembrane proteins with an extracellular domain involved in ligand recognition and interaction, and an intracellular domain that transduces signals downstream for the reorganization of the cell cytoskeleton and other events (Figs. 1 A and 2 A). Adhesins are mechanotransducers and their activation triggers important actin rearrangements, usually mediated by the Rho and Ras families of small GTPases (Perez-Moreno et al., 2003). This modulation of the actin cytoskeleton is controlled by accessory proteins that are generally specific for a given type of adhesion. At the level of TJs, members of the membrane-associated guanylate kinase (MAGUK) proteins link transmembrane receptors with the actin cytoskeleton, whereas at AJs, proteins of the catenin family provide this link (Citi and Cordenonsi, 1998; Perez-Moreno et al., 2003). Like for other cell-surface receptors, adhesin inactivation occurs through internalization by clathrin- and/or caveolin-mediated endocytosis, which is triggered by post-translational modifications of their cytoplasmic domains (Fujita et al., 2002; Shi and Sottile, 2008; Lobert et al., 2010). The orchestration of adhesin activation and inactivation allows cells to coordinate opposite effects such as cell migration and the establishment of tight interactions with neighboring cells.

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Abbreviations used in this paper: AJ, adherens junction; CAR, coxsackievirus and adenovirus receptor; EHEC, enterohemorragic *E. coli*; EPEC, enteropathogenic *E. coli*; JAM, junction adhesion molecule; MLCK, myosin II light chain kinase; TJ, tight junction; ZO, zona occludens.

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Despite the importance of cell–cell adhesion in providing an impermeable barrier to protect the organism from the extracellular environment, a large number of pathogens have evolved to preferentially target host proteins involved in cell adhesion (Kerr, 1999; Sousa et al., 2005a; Hauck et al., 2006; Stewart and Nemerow, 2007; Guttman and Finlay, 2009). Viruses, bacteria, and parasites adhere to host cells to avoid shear-induced clearance and most of them are subsequently internalized to find a suitable niche for survival and replication, away from immune defenses of the host. Pathogen entry mainly occurs by two strategies: either through phagocytosis by specialized cells (such as the M cells found within the Peyer's patches in the intestine and specialized in particle uptake from the lumen of the intestine across the epithelium; Sansonetti and Phalipon, 1999) or by the interaction with receptors at the surface of nonphagocytic cells (Pizarro-Cerdá and Cossart, 2006). Receptors involved in cell adhesion are often targeted by pathogens to mediate their adhesion and internalization into host cells. In addition, pathogens use cell adhesion molecules to cross or disrupt epithelia. As such, cell adhesion plays a fundamental role during infection and conversely, the study of host–pathogen interactions has given unprecedented insights into the molecular components and dynamics of such complex cell function. This review focuses on the role of cell–cell interactions during infection and on the lessons pathogens have taught us about cell–cell adhesion.

Targeting tight junctions

TJs are the most apically located cell–cell junctions and lie at the boundary between the apical and basolateral domains of epithelia (Fig. 1 A; Steed et al., 2010). The tight nature of these junctions prevents paracellular passage of fluids, electrolytes, and macromolecules. TJs are maintained by four main groups of transmembrane proteins: the occludins, the claudins, the junction adhesion molecules (JAMs), and the coxsackievirus and adenovirus receptor (CAR) proteins (Fig. 1 A; Citi and Cordenonsi, 1998). Occludins and claudins have four transmembrane domains and form homodimers via their extracellular loops. JAMs and CAR proteins have one transmembrane domain and extracellular IgG-like domains that mediate adhesion. All transmembrane components of TJs interact with cytoplasmic proteins that provide the link with the actin cytoskeleton. These proteins include zona occludens 1, 2, and 3 (ZO-1, ZO-2, and ZO-3) and the PAR family of proteins (Citi and Cordenonsi, 1998). TJs are the most apical barrier of the epithelium; therefore, many pathogens use them, and sometimes disrupt them, to infect a host and spread the infection.

Among viruses affecting TJs, Rotavirus uses the toxins NSP4 and VP4 to target and disrupt TJ integrity. These toxins depolarize host cells and allow the virus to gain access to basolaterally located integrins, which serve as the receptors for docking of the virus to the cell (Nava et al., 2004). When released in the host cytoplasm, NSP4 increases the intracellular calcium concentration, which indirectly affects the actin cytoskeleton of the host cell and inhibits the recruitment of ZO-1 to cell–cell contacts (Tafazoli et al., 2001). VP4 is cleaved to VP5 and VP8, the latter being responsible for the delocalization of claudin-3, ZO-1, and occludin (Fig. 1 B; Dickman et al., 2000; Obert et al., 2000; Nava et al., 2004; Beau et al., 2007).

Hepatitis C virus (HCV) initially contacts the host cells by interacting with the low-density lipoprotein receptors (LDL-Rs). The initial steps of viral internalization requires binding to occludin and CD81 (Ploss et al., 2009), whereas binding to the N-terminal extracellular loop of claudin-1 is involved in the later steps of internalization (Fig. 1 B; Evans et al., 2007; Yang et al., 2008). Claudin-6 and -9 are also exploited for internalization during infection, albeit with lesser efficiency (Meertens et al., 2008).

Reoviruses use the viral surface protein σ1 to specifically interact with the N-terminal domain of JAM-A and invade host cells (Fig. 1 B; Barton et al., 2001; Guglielmi et al., 2007). Adenoviruses use surface fiber proteins to bind to CAR proteins, disrupt CAR/CAR interactions at TJs (Kerr, 1999; Walters et al., 2002), and gain access to basolaterally exposed integrins for internalization (Fig. 1 B). Similarly, coxsackievirus uses the CAR proteins as a coreceptor during infections (Fig. 1 B; Bergelson et al., 1997). These viruses initially interact with CD55 (known also as the decay-accelerating factor; DAF) at the surface of host cells, inducing its clustering (Coyne and Bergelson, 2006) and triggering a downstream signaling cascade that activates the Src kinases Abl and Fyn. In turn, Abl activates Rac, which mediates the rearrangements of the actin cytoskeleton and allows coxsackie viral particles to reposition to TJs. Here, the virus binds to CAR proteins to invade host cells (Fig. 1 B; Coyne and Bergelson, 2006).

Among bacterial pathogens, enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *Escherichia coli* (EHEC) do not target host adhesins for adhesion but inject the effector Tir, which inserts into the host cell plasma membrane adopting a hairpin loop conformation and serves as the exogenous receptor for the bacterial surface protein intimin (Knodler et al., 2001; Chen and Frankel, 2005; Miyake et al., 2005; Guttman and Finlay, 2009). The intracellular domain of Tir recruits and activates the adaptor protein Nck, the actin regulators N-WASP and the Arp2/3 complex, and cytokeratin-18, thereby triggering the formation of actin-based pedestals (Gruenheid et al., 2001; Campellone et al., 2004). Formation of the pedestals is driven by the recruitment of clathrin at the bacteria–host adhesion site (Veiga et al., 2007). In addition to Tir, EPEC and EHEC secrete other T3SS (type III secretion system) effectors, including EspF, EspG, and Map, which all have an indirect effect on TJ integrity (Fig. 1 C; Dean and Kenny, 2004; Viswanathan et al., 2004; Matsuzawa et al., 2005). Although not colocalizing directly at TJs, these three effectors are able to activate the membrane-associated actin-binding protein ezrin, dephosphorylate occludin, and activate myosin II light chain kinase (MLCK). Activated MLCK can in turn activate myosin II that might have a role in TJ destabilization by pulling on actin filaments (Fig. 1 C). The precise mechanism by which these bacterial effectors are able to destabilize TJs remains uncharacterized but the related pathogen *Citrobacter rodentium*, used for in vivo modeling of EPEC and EHEC infections, induces the same loss of transepithelial resistance in mouse tissues (Guttman et al., 2006a,b).

Helicobacter pylori uses a T4SS (type IV secretion system) to inject effectors into host cells. One of these, CagA, has been ascribed several functions that result in the disruption of epithelial polarization and tight junctions. Once injected in host cells, CagA undergoes Src-mediated phosphorylation, triggering

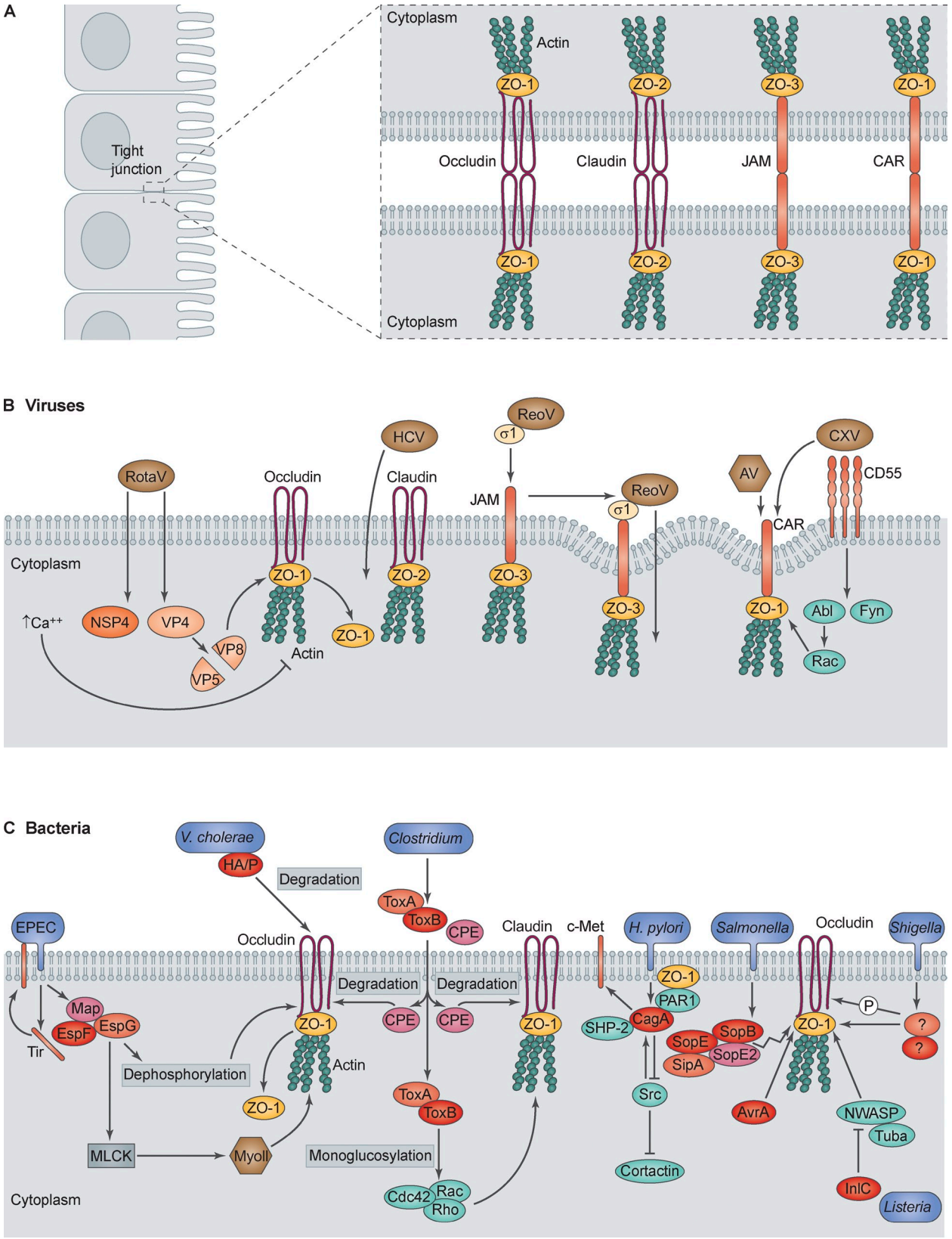

Figure 1. **Tight junctions and infection.** (A) Tight junctions (TJs) form at the border between the apical and the basolateral side of neighboring cells. Their main components are occludins, claudins, the JAM family of receptors and the CAR receptors. All of these receptors interact with the actin cytoskeleton via the members of the ZO family of proteins (ZO-1, -2, and -3). (B) The Rotavirus (RotaV) toxin NSP4 is released in the host cytoplasm where it increases the concentration of intracellular calcium and disrupts the actin cytoskeleton. VP4 is cleaved into VP5 and VP8, the latter interfering with the recruitment of ZO-1, occludin, and claudin at the plasma membrane. Upon interacting with the LDL receptor at the surface of the host cell, the hepatitis C virus (HCV) uses claudins as receptors for internalization. Reovirus (ReoV) uses the viral surface protein σ1 to interact with the JAM-1 receptors and invade host cells.

the interaction of CagA with the phosphatase oncoprotein SHP-2 and the dephosphorylation of cortactin (Fig. 1 C; Higashi et al., 2002; Selbach et al., 2002, 2003). These two events seem to be unrelated and cortactin dephosphorylation is the result of a feedback loop inhibition of Src by phosphorylated CagA (Selbach et al., 2003). In addition, CagA intracellularly activates the hepatocyte growth factor receptor Met (Fig. 1 C), which causes the infected cells to internalize E-cadherin, become motile, and assume a typical elongated morphology resulting in the disruption of the epithelial barrier (Churin et al., 2003). CagA also interacts with ZO-1 and JAM1, sequestering these proteins away from cell–cell contacts to form ectopic TJs at the bacteria–host interaction sites (Fig. 1 C; Amieva et al., 2003). Of note, it has been reported that the interaction of CagA with PAR1, a regulator of cell polarity, facilitates both SHP-2 interactions with CagA and the mislocalization of TJ proteins from cell–cell contacts (Saadat et al., 2007). At present it is unclear how these epithelial alterations may favor the infection.

Vibrio cholerae secretes a metalloprotease, called the hemagglutinin/protease (HA/P), which degrades the extracellular domain of occludin (Fig. 1 C; Wu et al., 2000). This process is also accompanied by the dissociation of ZO-1 from TJs due to the conformational change of the cytoplasmic domain of occludin that remains associated with the plasma membrane. *Clostridium perfringens* and *Clostridium difficile* also indirectly disrupt TJs via the secretion of bacterial toxins. *C. perfringens* secretes the enterotoxin CPE that binds occludin (Singh et al., 2000) and claudin-3 and -4, inducing their degradation (Fig. 1 C; Sonoda et al., 1999). *C. difficile* secretes toxin A and toxin B that monoglucosylate the small GTPases Rac, Cdc42, and Rho, thereby affecting cytoskeleton dynamics (Fig. 1 C; Nusrat et al., 2001; Voth and Ballard, 2005). In addition, these toxins are able to dissociate ZO-1, ZO-2, and occludin from TJs (Nusrat et al., 2001).

Salmonella and *Shigella* species invade nonphagocytic host cells by injecting T3SS effectors that trigger host membrane ruffling and engulf bacterial particles (Cossart and Sansonetti, 2004; Pizarro-Cerdá and Cossart, 2006). The prerequisite for T3SS effector injection requires contact with the host cell surface; the details of such initial interaction remain elusive, but it involves integrins (Watarai et al., 1996) and lipid rafts (Lafont et al., 2002). Nevertheless, both *Salmonella* and *Shigella* are known to perturb TJ integrity during infection. Although the molecular details are not known, the *Salmonella* SPI1 effectors SopB, SopE, SopE2, and SipA have been implicated in TJ alterations that include a decrease in ZO-1 expression levels as well as delocalization of occludin (Fig. 1 C; Boyle et al., 2006). The *Salmonella typhimurium* T3SS effector AvrA has been characterized as a TJ stabilizer, as cells infected with an *avrA* mutant exhibit a reduced expression of ZO-1, claudin-1, and occludins (Fig. 1 C; Liao et al., 2008). Similarly, *Shigella* infections affect the expression levels of ZO-1, claudin-1, and the phosphorylation state of occludin, leading to a severe disruption of TJs. However, the virulence factors involved in such process remain unidentified (Fig. 1 C; Sakaguchi et al., 2002).

Finally, it is interesting to note that the TJ protein ZO-1 has also been observed in association of the distal portion of actin filaments forming *Shigella* and *Listeria* comet tails as well as EPEC-induced actin pedestals (Hanajima-Ozawa et al., 2007). The function of this association is presently unknown.

Targeting adherens junctions

AJs mediate cell–cell adhesion and are localized at the basolateral surfaces of polarized epithelia (Fig. 2 A). The main components of AJs are cadherins, transmembrane proteins that form Ca^{2+} intercellular interactions. The cadherins family includes type I, type II, desmosomal, and truncated cadherins. The most extensively studied cadherins belong to type I, or classical cadherins, and include E-cadherin, which is expressed by epithelial cells, N-cadherin, first described in neuronal cells, and P-cadherin, which is expressed in the placenta (Jamora and Fuchs, 2002; Patel et al., 2003; Niessen, 2007). The extracellular domain of type I cadherins consists of five 110–amino acid immunoglobulin-like extracellular domains (EC1 to EC5) involved in E-cadherin–E-cadherin interactions and a short intracellular domain that interacts with members of the catenin family to bridge the interactions with the underlying actin cytoskeleton. β-Catenin directly binds E-cadherin and in turn recruits the actin-interacting protein α-catenin (Kobielak and Fuchs, 2004). During the formation of cell–cell contacts, different cadherins dictate the specificity of such interactions. Nectins are also transmembrane proteins that take part in AJs (Takai and Nakanishi, 2003; Niessen, 2007). Similarly to cadherins, they initiate Ca^{2+}-dependent interactions, their extracellular domain is composed of three immunoglobulin domains involved in nectin–nectin interactions, and the intracellular domain binds the scaffold protein afadin. In the case of AJs, pathogens directly or indirectly target cadherins to adhere

Adenovirus (AV) binds the coxsackievirus and adenovirus receptor (CAR) for internalization. Coxsackievirus (CXV) interaction with CD55 at the surface of host cells triggers a signaling cascade that activates the Src kinases Abl and Fyn. Abl activates Rac, inducing the rearrangements of the actin cytoskeleton that reposition coxsackie viral particles to TJs. Here the virus binds to CAR proteins to invade host cells. (C) Enteropathogenic (shown) and enterohemorragic *E. coli* (EPEC and EHEC) first adhere to host cells by bundling-forming pili (BFP, not depicted). Then T3SS effectors are secreted, including the bacterial protein Tir, which inserts into the host cell plasma membrane and serves as the bacterial receptor. The T3SS effectors EspF, EspG, and Map are able to activate ezrin, dephosphorylate occludin, and activate MLCK. Activated MLCK can in turn activate myosin II that might have a role in TJs destabilization by pulling on actin filaments. *Vibrio cholerae* secretes the metalloprotease HA/P that degrades the extracellular domain of occludin, accompanied by the dissociation of ZO-1 from TJs due to the conformational change of the cytoplasmic domain of occludin that remains associated with the plasma membrane. *Clostridium perfringens* secretes the enterotoxin CPE that binds occludin, claudin-3, and claudin-4 and induce their degradation. *Clostridium difficile* secretes toxin A and toxin B that monoglucosylate the small GTPases Rac, Cdc42, and Rho, thereby affecting cytoskeleton dynamics. *Helicobacter pylori* injects the effector protein CagA, which is phosphorylated by Src within the host cell. This triggers the inhibition of Src activity, the dephosphorylation of cortactin, and the interaction of CagA with SHP-2. In addition, CagA activates the Met receptor, triggering a motile response in infected cells, and independently recruits TJ proteins at the bacteria/cell adhesion sites. The *Salmonella* SPI1 effectors SopB, SopE, SopE2, and SipA alter TJs by decreasing ZO-1 expression levels (crooked arrow) as well as delocalizing occludin, whereas the *Salmonella* Typhimurium T3SS effector AvrA stabilizes TJ. *Shigella* infections affect the expression levels of ZO-1, claudin-1, and the phosphorylation state of occludin, leading to a severe disruption of TJs. The *Listeria monocytogenes* virulence factor InlC relieves cortical actin tension at cell–cell contacts by binding the mammalian adaptor protein Tuba, preventing its interaction with N-WASP.

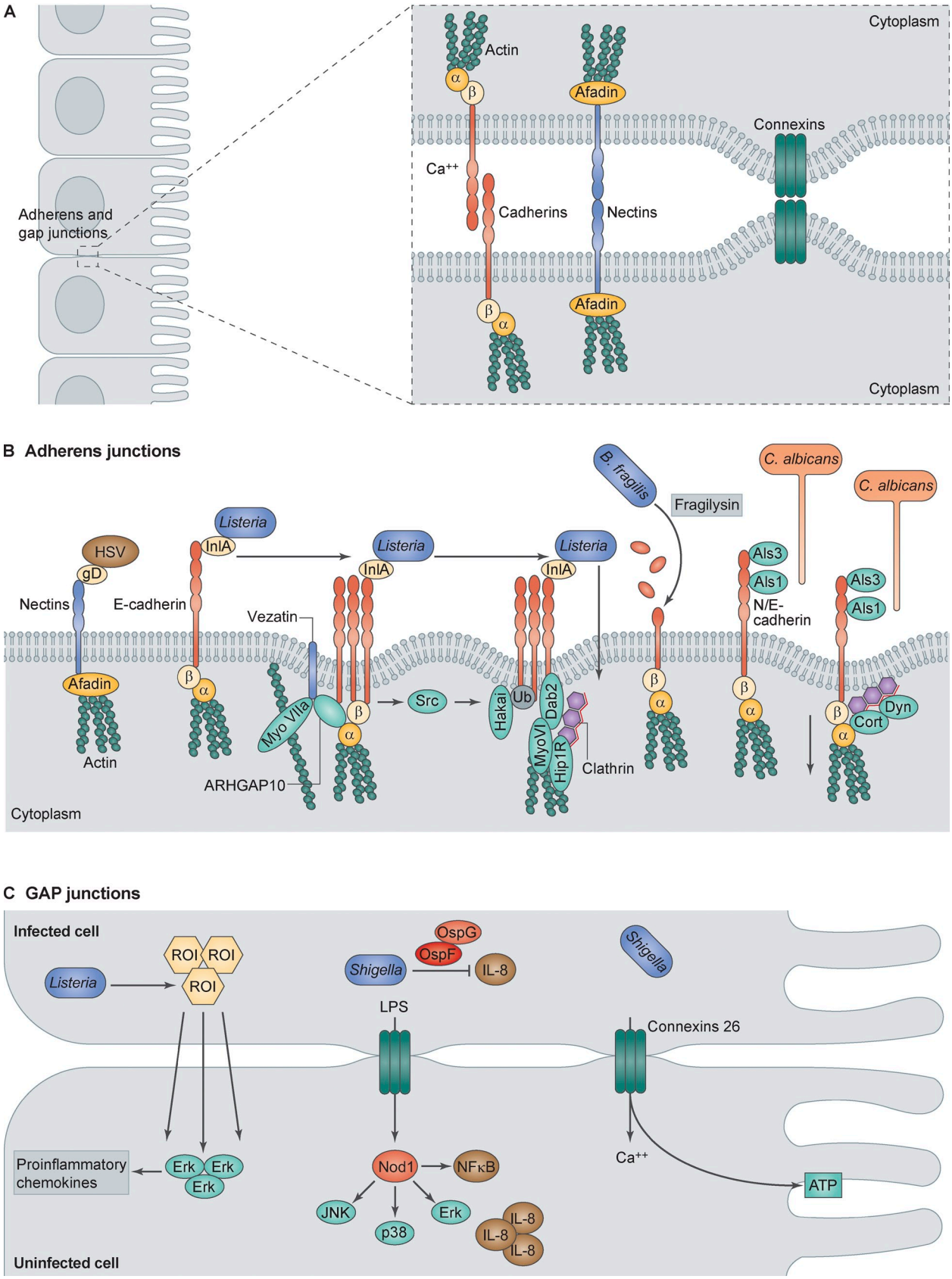

Figure 2. **Adherens junctions, gap junctions, and infection.** (A) Adherens junctions (AJs) and gap junctions (GJs) form at the lower region of cell–cell contacts. The main components of AJs are the members of the cadherin family and nectins. Both interact with the actin cytoskeleton by means of catenins (α and β) and afadin, accessory proteins of cadherins and nectins, respectively. Gap junctions are established upon interactions of connexin-based pores at the membrane of neighboring cells. (B) Herpes simplex virus (HSV) uses the glycoprotein D (gD) to interact with nectins during infection. *Listeria monocytogenes*

to host cells, promote internalization, or disrupt the integrity of epithelia to access deeper tissues.

Herpes simplex virus (HSV) invades cells by fusion with the plasma membrane of the host. Four glycoproteins (D, B, H, and L) are essential for this process. Glycoprotein D interacts with nectin-1, triggering a conformational change that is essential for membrane fusion (Fig. 2 B; Connolly et al., 2005).

Listeria monocytogenes is a facultative intracellular pathogen with the capacity of invading nonphagocytic cells and crossing multiple barriers of the host. *Listeria* adheres to host cells by means of the surface protein InlA that interacts with E-cadherin and triggers the same signaling cascade induced by homotypic E-cadherin interactions, including the recruitment of α- and β-catenin, myosin VIIa, and the AJ-associated protein vezatin at bacterial entry sites (Fig. 2 B; Bonazzi et al., 2009). InlA–E-cadherin interaction is characterized by a remarkable species specificity; a single amino acid in position 16 of the first EC repeat of E-cadherin is responsible for ligand-receptor specificity, totally abrogating infection in those species where a proline residue at this position is replaced by a glutamic acid (Lecuit et al., 1999). During InlA-mediated *Listeria* infections, E-cadherin clustering activates the protein kinase Src (Sousa et al., 2007; Bonazzi et al., 2008), which is upstream of post-translational modifications on E-cadherin that result in the recruitment of clathrin at the bacterial entry site (Fig. 2 B; Veiga et al., 2007; Bonazzi et al., 2008). Clathrin assembly at the bacterial entry site is required to recruit actin by means of the actin-interacting protein Hip1R and triggers bacterial internalization by recruiting the nonconventional motor protein myosin VI (Fig. 2 B; unpublished data). Of note, a second bacterial surface protein, InlB, is a ligand for the host receptor Met. Differently from InlA, InlB is loosely attached to the bacterial cell wall and as such it does not mediate bacterial adhesion. However, InlB binding to Met induces a potent downstream signaling that also results in the activation of the PI3 kinase and actin remodeling and plasma membrane ruffling (Hamon et al., 2006; Pizarro-Cerdá and Cossart, 2006). Interestingly, in in vivo infections InlB activity is not involved in the crossing of the intestinal barrier, but is essential to cross the placental barrier where possibly it exerts its function in potentiating the InlA-mediated downstream signaling (Lecuit et al., 2001, 2004; Disson et al., 2008). In tissues, E-cadherin distribution is localized below TJs and different possibilities have been proposed to understand how *Listeria* gains access to luminally accessible E-cadherin. In the intestine, apoptotic cells extrude from the tip of the intestinal villi, exposing E-cadherin at the surface of neighboring cells. Indeed, these sites seem to represent infection foci (Pentecost et al., 2010). In addition, as shown recently, goblet cells along intestinal villi present accessible E-cadherin that is targeted by *Listeria* to invade epithelial cells and transcytose across the intestinal barrier (Nikitas et al., 2011). *Listeria* also perturbs actin dynamics at cell–cell junctions by means of the virulence factor InlC, which is secreted in the cell cytoplasm upon bacterial escape from the endocytic vacuole. InlC plays a dual role: it dampens the innate immune response by binding IKKa, which has consequences on the phosphorylation and degradation of IkB (Gouin et al., 2010), and it relieves cortical actin tension at cell–cell contacts by binding the mammalian adaptor protein Tuba, thereby preventing its interaction with N-WASP (Fig. 1 C; Rajabian et al., 2009; Romero and Tran Van Nhieu, 2009). This latter effect would facilitate the typical cell-to-cell spread of *Listeria* infection.

Bacteroides fragilis is a member of the commensal intestinal microflora that becomes pathogenic when it overgrows other bacterial species. Strains of this bacterium associated with diarrheal disease (enterotoxigenic *B. fragilis*) produce the toxin fragilysin, a zinc-dependent metalloprotease that specifically cleaves the extracellular domain of E-cadherin, thereby disrupting adherens junctions and affecting TJ integrity (Fig. 2 B; Wu et al., 1998). In addition, fragilysin also has an effect on β-catenin nuclear signaling (Wu et al., 2003).

The fungal pathogen *Candida albicans* expresses two invasins, Als1 and Als3, which interact with N-cadherin on endothelial cells and E-cadherin on oral epithelial cells (Fig. 2 B; Phan et al., 2007). Both proteins are able to induce hyphae internalization, although Als1 seems to be less efficient. Of note, similarly to *Listeria* internalization, *Candida* hyphae internalization requires the rearrangements of the actin cytoskeleton and induces the recruitment of clathrin, dynamin, and cortactin at sites of hyphae interaction with the host cell (Fig. 2 B; Moreno-Ruiz et al., 2009). In the case of *Candida* infection, though, a role of the clathrin machinery in actin recruitment at sites of hyphae internalization remains to be demonstrated.

Tunneling nanotubes

A particular type of cell–cell interaction with an emerging role in infection is that mediated by tunneling nanotubes. These are transient, long, cytoskeleton-rich projections that extend from one cell to another and support the intercellular transport of membranes, and even organelles, over relatively long distances (Rustom et al., 2004; Gerdes et al., 2007). The formation of these structures has been observed in vitro between several cell types, including macrophages and immune cells. A study on the morphology and function of tunneling nanotubes has identified the presence of thin and thick nanotubes, the former being

adheres to host cells by means of the surface protein InlA that interacts with E-cadherin and triggers the recruitment of α- and β-catenin, myosin VIIa, vezatin, and ARHGAP10 at bacterial entry sites. E-cadherin clustering activates Src that is upstream of E-cadherin ubiquitination mediated by the ubiquitin ligase Hakai. This triggers the recruitment of clathrin at the bacterial entry site required to recruit actin by means of Hip1R and bring about bacterial internalization by recruiting myosin VI. *Bacteroides fragilis* produces the toxin fragilysin, which cleaves the extracellular domain of E-cadherin disrupting adherens junctions and affecting TJ integrity. *Candida albicans* interacts with N-cadherin on endothelial cells and E-cadherin on oral epithelial cells by means of the surface invasins Als1 and Als3. *Candida* hyphae internalization requires the rearrangements of the actin cytoskeleton and induces the recruitment of clathrin, dynamin (Dyn), and cortactin (Cort) at the internalization site. (C) *Shigella* cell-to-cell spread is facilitated by the bacterial-mediated opening of connexin 26 hemichannels that allows the diffusion of calcium from infected to noninfected neighboring cells and the release of ATP in the medium. The *Shigella* effectors OspG and OspF attenuate IL-8 production in infected cells; however, the opening of Gap junctions favors the cell-to-cell diffusion of the bacterial peptidoglycan (LPS) that would then activate IL-8 production in bystander, noninfected cells. *Listeria monocytogenes* infections induce the synthesis of reactive oxygen intermediates (ROI) that spread to neighboring, noninfected cells by a GJ-independent mechanism and induce the activation of the MAP kinase Erk. This in turn regulates the synthesis of the proinflammatory chemokine Cxcl-2.

enriched in actin and having a diameter of less than 0.7 µm, and the latter containing F-actin and microtubules and having a diameter larger than 0.7 µm (Onfelt et al., 2006; Sowinski et al., 2008). Accordingly, it has been shown that only thicker nanotubes can support the intercellular transport of endosomal and lysosomal vesicles as well as mitochondria, which move along these channels in an ATP-dependent manner with a speed compatible with that of microtubule-based transport (Onfelt et al., 2006). Surprisingly though, thinner, and not thicker nanotubes can mediate the cell-to-cell spread of large particles, such as streptavidin-coated beads or even bacteria, as in the case of *Mycobacterium bovis*, which surf on these tubes, thereby exploiting a constant membrane turnover/dynamics to reach neighboring cells (Onfelt et al., 2006). More recently, tunneling nanotubes have been shown to play a key role for the intercellular spread of prions (Gousset et al., 2009). Prion dissemination through tunneling nanotubes has been observed between neuronal Cath.a-differentiated (CAD) cells as well as between dendritic cells and primary neurons, this latter example providing a possible mechanism for the documented retrograde transport of prions from the intestine to the central nervous system (Gousset and Zurzolo, 2009; Gousset et al., 2009). During viral infections, HIV and murine leukemia virus (MLV) trigger the formation of filopodial bridges that connect infected cells with neighboring non-infected cells, thereby spreading the infection (Sherer et al., 2007; Sowinski et al., 2008; Eugenin et al., 2009; Jin et al., 2009). However, these virus-induced structures are not open-ended (Sherer et al., 2007; Sowinski et al., 2008), suggesting the classification of these cell–cell contacts as cytonemes rather than nanotubes (Sherer et al., 2007).

Gap junctions, cell–cell communication, and propagation of the innate immune response

Gap junctions are specialized cell–cell contact sites that allow direct intercellular connections between the cytoplasm of neighboring cells (Goodenough and Paul, 2009). Each gap junction consists of two hemichannels called connexons that spans across the intercellular space and are composed of connexins, four-pass transmembrane proteins that assemble in pore-forming hexamers (Fig. 2 C). These channels switch between an open and closed conformation and allow the passage of calcium, ATP, and other second messengers with a maximum molecular mass of ∼1 kD (Goodenough and Paul, 2009). Recent reports imply gap junctions during infection with somewhat opposite long-term effects, at least in the case of *Shigella flexneri*. *Shigella* infections induce the opening of connexin 26 hemichannels, thereby facilitating the diffusion of calcium from infected cells to noninfected neighboring cells and the release of ATP into the medium (Fig. 2 C; Tran-Van-Nhieu et al., 2003). This process seems to facilitate the cell-to-cell spread of *Shigella*, although the precise mechanism that regulates this interdependency remains elusive. The dependency on the secretion of bacterial effectors is however demonstrated, as the nonsecreting *mxiD* mutant fails to reproduce the same phenotype. In addition, upon uptake by intestinal epithelial cells and escape from the internalization vacuole, *Shigella* is recognized by the pattern recognition receptor Nod1 that reacts to the bacterial peptidoglycan and activates the signaling cascade that leads to the production of interleukine-8 (IL-8). The *Shigella* effectors OspG (Kim et al., 2005) and OspF (Arbibe et al., 2007) attenuate IL-8 production by preventing IκBα degradation and blocking the activation of NF-κB–responsive genes, respectively, in infected cells (Fig. 2 C). Yet, *Shigella* infections are accompanied by abundant IL-8 secretion. It has been proposed that gap junctions could favor the cell-to-cell diffusion of the bacterial peptidoglycan that would then activate IL-8 production in bystander, noninfected cells, where the absence of bacterial effectors would allow a stronger immune response (Fig. 2 C; Kasper et al., 2010). Of note, the same study provides evidence for similar bystander IL-8 activation also during *L. monocytogenes* and *Salmonella typhimurium* infections (Kasper et al., 2010). In another report, propagation of the innate immune response upon *L. monocytogenes* infection has been shown to occur by a gap junction–independent mechanism as well (Dolowschiak et al., 2010). Indeed, studies at the single-cell level upon challenge with *L. monocytogenes* revealed that the majority of proinflammatory chemokine-positive cells were noninfected. Horizontal epithelial activation was found to be dependent on the infection-induced synthesis of reactive oxygen intermediates that spread to neighboring cells, inducing the activation of the MAP kinase Erk, which in turn regulates the synthesis of the chemokine Cxcl-2 (Fig. 2 C; Dolowschiak et al., 2010).

What have pathogen infections taught us about cell–cell interactions?

The study of host–pathogen interactions has in recent years been particularly productive not only to understand the molecular mechanisms of infection, but also to address fundamental questions in cell biology, and a number of pathogens (mostly viruses and bacteria) are nowadays used as model organisms to probe specific eukaryotic pathways. The characterization of CAR as a functional component of TJ (Cohen et al., 2001) stands as the best example of how the study of viral infection has provided important insight in cell biology.

In the case of bacterial pathogens, the study of *L. monocytogenes* infection has illuminated host cell–cell interactions. To better understand how *Listeria* exploits E-cadherin during infection, we performed a yeast two-hybrid screen in search of novel α-catenin interactors. This led to the identification of the Rho GAP protein ARHGAP10 (Sousa et al., 2005b). ARHGAP10 is a protein of ∼2,000 amino acids with an N-terminal PDZ domain, a central PH domain, and GAP domain, which shows activity for RhoA and Cdc42 (Bassères et al., 2002). The C terminus of ARHGAP10 presents a binding site for α-catenin. The depletion or inhibition of ARHGAP10 prevented the recruitment of α-catenin at both AJs and *Listeria* entry sites and accordingly, E-cadherin failed to immunoprecipitate α-catenin from ARHGAP10-depleted cells. Conversely, the overexpression of the GAP domain of ARHGAP10 disrupted actin cables, enhanced α-catenin and cortical actin levels at cell–cell junctions, and inhibited *Listeria* entry (Sousa et al., 2005b). Hence, the study of bacterial infections led to the identification of a novel component of AJs that mediates α-catenin recruitment at sites of intercellular E-cadherin interactions. More recently, the post-translational modifications of E-cadherin that are triggered by InlA-mediated

Listeria infections have been characterized (Bonazzi et al., 2008). These include the Src-mediated phosphorylation and Hakai-mediated ubiquitination of E-cadherin followed by the recruitment of clathrin at bacterial entry sites (Veiga et al., 2007; Bonazzi et al., 2008). Importantly, the use of E-cadherin–coated beads showed that the same signaling cascade elicited by InlA–E-cadherin interactions is also triggered by E-cadherin–E-cadherin interactions, implying that clathrin plays a role in the formation of new AJs (Bonazzi et al., 2008). The recent characterization of the machinery that connects actin filaments with clathrin-coated pits at the plasma membrane strengthened this hypothesis (Bonazzi et al., 2011), and accordingly, the depletion of clathrin impaired the maturation of newly formed AJs (unpublished data). Indeed, Dab2, the actin-binding protein Hip1R, and myosin VI assemble at clathrin coats during bacterial infections (Bonazzi et al., 2011) and cell–cell junction formation to orchestrate the rearrangements of actin that are required for bacterial internalization and the maturation of AJs, respectively. In addition, parallels between the machinery involved in clathrin-mediated internalization and AJ formation have led us to speculate that cell–cell adhesion is similar to Fc receptor-ligand interactions during "frustrated phagocytosis" of macrophage (Takemura et al., 1986; unpublished data).

Conclusions and perspectives

Cell adhesion is an essential and extremely conserved process that allows sensing of the extracellular environment, cell–cell interactions, motility, and organogenesis. In adults, cell–cell adhesion generates a tight barrier that protects deeper tissues from external aggressions, including microbial infections. Indeed, epithelial barriers constitute the first line of defense against pathogens. However, pathogens have developed countless strategies to exploit those defenses to their advantage and seem to preferentially target host proteins implicated in cell adhesion to colonize epithelia, invade host cells, or even disrupt host barriers to facilitate access to deeper tissues. Ironically, the first line of defense against infection has become one of the most exploited gates to access and colonize the organism, which is probably why hosts have developed at these locations sophisticated immune defenses to recognize and respond accordingly to specific patterns of pathogenic proteins. Importantly, the observation that a large number of pathogens hijack endogenous host pathways has allowed the exploitation of pathogens as tools to address fundamental questions in cell biology. Thus, similarly to the fundamental contribution of the study of viruses' internalization to the characterization of endocytic mechanisms, the study of bacterial and viral infections has shed lights on novel components and dynamics of cell–cell interactions. Strikingly, proteins involved in cell adhesion are often exploited by pathogens to trigger clathrin- or caveolin-mediated internalization, which indicates that a common mechanism may regulate adhesion and endocytosis (unpublished data).

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