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Philippe Huber

► **To cite this version:**

Philippe Huber. Targeting of the apical junctional complex by bacterial pathogens. *Biochimica et Biophysica Acta: Biomembranes*, 2020, 1862, pp.183237. 10.1016/j.bbamem.2020.183237. hal-03489656

**HAL Id: hal-03489656**

**<https://hal.science/hal-03489656v1>**

Submitted on 7 Mar 2022

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1 **Targeting of the apical junctional complex by bacterial pathogens**

2 Philippe Huber<sup>1\*</sup>

3 <sup>1</sup>Université Grenoble-Alpes, CEA, INSERM, CNRS, Unité de Biologie Cellulaire et Infection,  
4 Grenoble, France.

5 \*Corresponding author: Philippe Huber, PhD. CEA-Grenoble, IRIG-BCI, 17 rue des Martyrs  
6 38054 Grenoble. mail: [phuber@cea.fr](mailto:phuber@cea.fr). Tel: 33 438 78 58 47

## 7 Abstract

8 Targeting the apical junctional complex during acute bacterial infections can be detrimental for the  
9 host in several aspects. First, the rupture of the epithelium or endothelium integrity is toxic in itself.  
10 In addition, extracellular bacterial pathogens or bacterial toxins can cross the body's physical barriers  
11 using the paracellular route and induce infection or intoxication of distant organs. No single strategy  
12 has been developed to disrupt junctional structures, rather each bacterium has its own method,  
13 which can be classed in one of the following three categories: (i) proteolysis/perturbation of adhesive  
14 proteins involved in tight or adherens junctions by bacterial or toxin-activated eukaryotic proteases,  
15 (ii) manipulation of host regulatory pathways leading to weakened intercellular adhesion, or (iii)  
16 delocalization of the junctional complex to open the gateway toward the subepithelial compartment.  
17 In this review, examples of each of these mechanisms are provided to illustrate how creative bacteria  
18 can be when seeking to disrupt cell-cell junctions.

19 **Keywords:** Adherens junctions, tight junctions, bacterial toxins, epithelial barriers, endothelium,  
20 bacterial transmigration, bacterial intoxication.

## 21 1. Introduction

22 During acute infections, bacterial pathogens can notably alter the body's physical barriers, which  
23 were designed to be impregnable to such assaults. Increased paracellular permeability may lead to  
24 diarrhea or pulmonary edema for example, or facilitate the uptake of a toxin so that it can affect sites  
25 beyond the colonized organ.

26 In some examples, bacteria can take advantage of the open junction to disseminate throughout the  
27 body. Intracellular pathogens can either use a transcellular route to cross epithelial barriers, or adopt  
28 a Trojan horse tactic, i.e., by allowing themselves to be internalized into professional transmitters  
29 like macrophages, and thus to shuttle across epithelia. In contrast, extracellular bacterial pathogens  
30 can cross these barriers following specific targeting of cell-cell junctions.

31 Interestingly, no common theme for weakening cell-cell junctions has emerged, rather, pathogens  
32 exploit a multitude of strategies and often deploy a combination of virulence factors which act in  
33 concert on several host targets. Exceptions to this rule are lipopolysaccharides (LPS) - released by a  
34 majority of Gram-negative bacteria, and ultimately resulting in increased barrier permeability (see  
35 5.1.). Among the specific intoxication mechanisms identified so far, many bacterial pathogens have  
36 been shown to hijack host regulatory pathways by various means to weaken cell-to-cell adhesion.

37 Epithelial and endothelial barrier integrity is ensured by two main junctional structures: adherens  
38 junctions (AJ) and tight junctions (TJ). These two types of junction form cords at the cells'  
39 circumference (1-4). AJs are composed of cadherins, which are transmembrane proteins displaying  
40 homophilic adhesive activity toward cadherins located on adjacent cells. Several cadherin types have  
41 been identified, including E-cadherin, the main epithelial cadherin, and VE-cadherin, the endothelial  
42 cadherin. The intracellular cadherin domain is linked to catenins and is associated with a number of  
43 signaling proteins regulating adhesive protein properties or sensing cell-to-cell interactions. Catenins  
44 in turn are tethered to the actin cytoskeleton, providing tissues with specific mechanical properties  
45 (5, 6).

46 The transmembrane and adhesive part of TJs is formed by several types of proteins, including claudin  
47 family members, junction adhesion molecule (JAM) family members, and occludin (7, 8). Each of  
48 these proteins has distinct properties, mainly in terms of permeability control, and their distribution  
49 is variable among the different types of epithelia and endothelia. Protein complexes are also

50 associated with the intracellular domains of TJ proteins, and include ZO-1 protein – playing the role  
51 of a molecular platform promoting the association of adhesive and regulatory proteins as well as  
52 actin fibers. TJs are also associated with a polarity complex, serving as gatekeeper to maintain the  
53 identity of the apical domain on one side and the basolateral domain on the other (9).

54 AJ and TJ organization and functional properties have been extensively reviewed elsewhere (1, 3, 4,  
55 7, 8, 10) and will not be further discussed here.

56 Rather, this review presents examples of **bacterial factors** directly or indirectly affecting adhesive  
57 proteins making up TJs and AJs. The list of **bacterial factors** described as involved in this process  
58 (Table I and Fig. 1) is not intended to be exhaustive, but the actions discussed are examples of the  
59 many strategies employed by bacteria to achieve junction breach. Some bacteria, like *Helicobacter*  
60 *pylori* or *Pseudomonas aeruginosa*, have developed a multi-target approach, whereas others, like  
61 *Vibrio cholerae* or *Vibrio parahaemolyticus*, rely, as far as we know, on a single toxin and mechanism  
62 to affect cell-cell junctions.

63 Finally, junctional adhesive proteins can also serve as receptors for bacterial toxins, either to allow  
64 pore formation or for bacterial attachment and internalization.

65 Altogether, the effect of bacterial toxins and **virulence factors** on cellular junctions illustrates the  
66 intimate links between bacteria and their hosts, and the degree to which bacteria have evolved to  
67 exploit host functions.

## 68 **2. The most obvious action: altering adhesive proteins**

69 Some bacteria secrete **virulence factors** to specifically inhibit target junction proteins, either by  
70 proteolysis or by inactivating their adhesive properties.

### 71 **2.1. Bacterial proteases targeting junctional proteins**

72 *H. pylori* colonizes the gastric epithelium of the human stomach and causes chronic infections,  
73 leading to the formation of ulcers and eventually carcinogenesis. It secretes HtrA, a serine protease  
74 which is highly conserved among *H. pylori* strains worldwide. This protease recognizes and cleaves  
75 the [VITA][VITA]-x-x-D-[DN] motif located between E-cadherin extracellular (EC) repeats (11, 12) and  
76 is required for bacterial survival and toxicity (13). Experiments using small molecule inhibitors or  
77 substrate-derived peptides to inhibit HtrA activity blocked bacterial transmigration across epithelia  
78 (11, 12). Thus, HtrA, along with several other *H. pylori* weapons targeting either AJs or TJs (see  
79 below), plays a crucial role in junction opening during infection (14-16). HtrA homologs have been  
80 identified in other pathogens, including enteropathogenic *Escherichia coli*, *Shigella flexneri* and  
81 *Campylobacter jejuni* (17), which display similar activity toward E-cadherin, albeit with lower  
82 efficiency. In contrast, *Neisseria gonorrhoeae* HtrA does not cleave E-cadherin because of a mutation  
83 in its catalytic center (17).

84 Cadherin proteolysis is also operated by LasB, a metalloprotease secreted into the extracellular  
85 milieu by *P. aeruginosa*. LasB specifically cleaves VE-cadherin's extracellular domain, but leaves E-  
86 cadherin intact (18, 19), indicating that it may be used by bacteria to gain access to the vascular  
87 system or for bacterial extravasation in bacteremic patients. Most pathogenic Gram-negative  
88 bacteria possess a type 3 secretion system (T3SS), a syringe-like apparatus injecting toxins directly  
89 into the cytoplasm of host cells. In *P. aeruginosa*, this system has a dramatic effect on the actin  
90 cytoskeleton (see below), but *P. aeruginosa*'s T3SS can only inject through basolateral membrane  
91 domains, leaving apical domains unharmed. It is hypothesized that *P. aeruginosa* uses LasB to open

92 the route toward the basolateral membrane domain, where T3SS is effective and will eventually  
93 promote endothelial cell retraction allowing the bacterium to transit across the endothelium (19).

94 TJ proteins can also be direct targets of bacterial proteases. For example, *Vibrio cholerae* secretes a  
95 metalloprotease called HA/P, which degrades the occludin extracellular domain (20). This partial  
96 proteolysis also perturbs ZO-1, causing it to delocalize from TJs. Thus, alteration of one TJ protein  
97 may have knock-on consequences on the entire TJ complex.

## 98 **2.2. Preventing cadherin dimerization**

99 Non-protease toxins can also alter junctional adhesiveness, as is the case with some strains of  
100 *Clostridium botulinum*. This bacterium secretes a large protein complex composed of *botulinum*  
101 neurotoxin serotype A, three hemagglutinins (HAs), and a non-hemagglutinin protein. The complex,  
102 specifically through the HAs, binds to E-cadherin EC1-EC2 domains, stabilizing this protein in its  
103 monomeric form and preventing its *trans*-dimerization (21). The ability of HA to interact with E-  
104 cadherin and to disrupt cell-cell junctions plays a pivotal role in the oral toxicity of *C. botulinum* in  
105 vivo, because it increases intestinal absorption of the toxin so it can reach the neuromuscular  
106 junction.

## 107 **3. The most popular method: hijacking host signaling pathways**

108 Probably because junctional proteins are not readily accessible, or owing to the efficacy of  
109 approaches targeting pathways regulating junctional adhesion, many bacteria subvert eukaryotic  
110 signaling pathways when seeking to alter cell-cell junctions. Signaling subversion can be achieved by  
111 toxins that are injected or gain access to the cytoplasm by another means, or through their  
112 interaction with membrane-bound signaling receptors.

113 One of the most common examples of this process are toxins activating eukaryotic proteases which  
114 naturally act on host adhesive proteins. These proteases fall into two categories: (i) ADAM10, a  
115 transmembrane metalloprotease, which cleaves several receptors including the cadherins, and (ii)  
116 matrix metalloproteases (MMPs), which degrade some adhesive proteins in addition to their activity  
117 on matrix proteins. MMPs can be either secreted or membrane-associated.

### 118 **3.1. Activation of ADAM10**

119 The catalytic domain of ADAM10 is located in its extracellular domain. It cleaves the cadherin  
120 ectodomain close to the membrane, thus inducing shedding of the entire ectodomain (22-24).  
121 ADAM10-dependent cadherin cleavage is followed by a secondary proteolytic event performed by  $\gamma$ -  
122 secretase, a multi-subunit protease complex located within the plasma membrane. The resulting E-  
123 cadherin intracellular fragment is then targeted to the proteasome for degradation. Because of the  
124 potentially dramatic effects of its action on cadherins and other receptors, ADAM10 activity is tightly  
125 regulated in the cell. In physiological settings, most ADAM10 molecules are present in the cytoplasm  
126 in an inactive pro-ADAM10 form, associated with calmodulin, a high-affinity calcium-binding protein.  
127 The formation of pores in the plasma membrane by pore-forming toxins, such as *Staphylococcus*  
128 *aureus* hemolysin (HIA), *Streptococcus pneumoniae* pneumolysin (PLY), *P. aeruginosa* exolysin (ExlA)  
129 or *Serratia marcescens* hemolysin (ShIA), induces a massive influx of calcium ions into the cytoplasm  
130 (25-28). It has been hypothesized that calmodulin binding to calcium, as a result of pore formation,  
131 alters its conformation and causes it to dissociate from pro-ADAM10. The free pro-ADAM10 pool is  
132 then available for activation by furin and for export to the plasma membrane, where it can cleave E-  
133 or VE-cadherin (29-31). Cadherin cleavage and junction disruption are the earliest pathological  
134 events to have been described so far in cells following exposure to these pore-forming toxins.

135 Interestingly, ADAM10 also serves as the receptor for *S. aureus* HIA at the cell surface, but this  
136 interaction is not sufficient to trigger E-cadherin cleavage, which strictly depends on calcium influx  
137 (25). As most pore-forming toxins induce calcium influx, a similar toxicity mechanism could be  
138 induced by pore-forming toxins produced by other pathogens.

139 Interestingly, the *Bacteroides fragilis* toxin (BFT) is a metalloprotease that exhibits an intermediate  
140 mechanism of action. BFT directly activates  $\gamma$ -secretase, causing cleavage of E-cadherin  
141 transmembrane domain, and subsequent junction opening (32).

### 142 **3.2. Activation of matrix metalloproteases**

143 Matrix metalloprotease (MMP) activation has also been documented in the context of bacterial  
144 infection. For example, *Neisseria meningitidis* induces MMP-8 activation in brain microvascular  
145 endothelial cells, triggering occludin cleavage (33). In the pathogenesis of meningitis, disruption of  
146 the blood brain barrier (BBB) is a critical step, and MMP-8-dependent occludin cleavage contributes  
147 to the opening of the very sophisticated junctions in the BBB, while also delocalizing AJ components  
148 to the apical surface (see section 6).

149 Similarly, *H. pylori* induces the release of a number of MMPs targeting matrix proteins and cytokines  
150 (34). One of them, MMP-7, also cleaves E-cadherin. The *cag* pathogenicity island (*cagPAI*) identified  
151 in highly virulent *H. pylori* strains encodes a T4SS, which injects virulence factors (including CagA) into  
152 host cells. Expression of *mmp-7* is normally repressed by the transcription factor Kaiso. However,  
153 *CagPAI*-positive bacteria induce the translocation of p120-catenin to the nucleus, which alleviates  
154 Kaiso-mediated transcriptional repression of *mmp-7* (35). p120 translocation is controlled by its  
155 phosphorylation status, and p120 tyrosine phosphorylation was found to be increased in infected  
156 cells, suggesting that an effector of *cagPAI*-T4SS can induce this post-translational modification.

### 157 **3.3. Inhibiting cadherin-catenin interaction**

158 In addition to its effects on MMP-7 and p120, *cagPAI*-T4SS, and more specifically the T4SS effector  
159 CagA can bind directly to E-cadherin's intracellular domain, competing with  $\beta$ -catenin for its  
160 interaction with E-cadherin (36). Consequently,  $\beta$ -catenin is released into the cytoplasm, where it can  
161 be phosphorylated by the GSK-3/CK1/APC/axin complex, and as a result will be degraded by the  
162 proteasome. Decreased junctional  $\beta$ -catenin levels have indeed been observed in samples from *H.*  
163 *pylori*-infected patients (37). In contrast, suppression of  $\beta$ -catenin phosphorylation and degradation  
164 has also been observed in the context of *H. pylori* infection, in a CagA-independent manner (38, 39).  
165 Cytoplasmic  $\beta$ -catenin molecules that are not rapidly phosphorylated and degraded by the  
166 proteasome can translocate to the nucleus where they interact with TCF/LEF-1 transcription factors,  
167 thereby increasing the expression of genes involved in cell division. This suggests that the pool of  
168 cytoplasmic  $\beta$ -catenin, which becomes available due to the competition with CagA or another  
169 unknown mechanism, may translocate to the nucleus where it can exert a transcriptional activity and  
170 induce cell proliferation.

### 171 **4. Targeting junctions by disrupting the cytoskeleton**

172 The actin cytoskeleton is linked to both AJs and TJs, and these interactions contribute significantly to  
173 junctional adhesiveness (5, 6). Cell-to-cell adhesion is dramatically weakened by disruption of the  
174 actin cytoskeleton following exposure to pharmacological drugs, or as a result of mutations in  
175 junctional proteins affecting their interaction with actin. Oppositely, excessive contraction of actin  
176 fibers can disrupt intercellular junctions as a result of the mechanical tensions exerted by the  
177 cytoskeleton on AJs and TJs.

178 **4.1. Activation or inhibition of Rho GTPases**

179 A number of bacterial toxins affect members of the Rho-GTPase family, including RhoA, Rac1 and  
180 CDC42. Although the downstream signaling pathways from these three GTPases intersect, each has  
181 some specificities: Rho regulates actin stress fibers; Rac1 is responsible for organization of the actin  
182 network in lamellipodia; and CDC42 controls the actin filaments in filopodia (40, 41). The GTPases  
183 oscillate between an inactive GDP-linked form and an active GTP-linked form, and their GTPase  
184 activity is controlled by both positive and negative upstream regulators: guanine nucleotide  
185 exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively.

186 Bacteria have adopted two independent strategies to dismantle the cytoskeleton or to increase its  
187 tension. Bacterial toxins either mimic GAP or GEF eukaryotic proteins, or modify key residues in Rho  
188 proteins or actin.

189 Several bacterial T3SS effectors have been shown to exert a GAP activity on Rho GTPases. For  
190 example, the toxin YopE – injected by *Yersinia pestis*, *Yersinia enterocolitica* and *Yersinia*  
191 *pseudotuberculosis* – acts as a GAP with RhoA, Rac1 and CDC42 (42, 43). Similarly, two T3SS effectors  
192 produced by *P. aeruginosa*, ExoS and ExoT, inhibit all three GTPases as a result of their GAP activities  
193 (44, 45). In addition, (and even more efficiently than through its GAP activity) ExoS can inhibit Rho  
194 GTPases with differential kinetics as a result of ADP-ribosylation on Arg41 (46, 47). C3 toxin from *C.*  
195 *botulinum* and the related toxin, Edin, from *S. aureus* also ADP-ribosylate Rho on Arg41 (48-50).  
196 Additional direct modifications of Rho GTPases include (i) their glucosylation on Thr35/37 by  
197 *Clostridium difficile*'s toxins TcdA and TcdB (51), (ii) their proteolysis by the YopT cysteine protease  
198 from *Yersinia ssp* (52), and (iii) RhoA adenylylation (AMPylation) on His348 by *Vibrio parahemolyticus*  
199 VopS (53). Rho-GTPase inactivation leads to the collapse of the actin cytoskeleton as a result of  
200 ADF/cofilin activation, which has actin depolymerization and severing activities (54).

201 Alternatively, some bacterial toxins activate the Rho GTPases. SopE and SopE2 are two T3SS effectors  
202 secreted by some strains of *Salmonella typhimurium*. They are potent GEFs for Rac1 and CDC42  
203 (SopE) or for CDC42 only (SopE2) (55). Deamidation of Gln61 or 63 in Rho proteins, turning it into Glu  
204 inhibits their GTPase activity and maintains them in an active state. Several toxins use this strategy to  
205 alter intercellular junctions, such as *E. coli* toxins CNF1 and CNF2 which deamidate RhoA, Rac1 and  
206 CDC42, and *Y. pseudotuberculosis* CNFy which only targets RhoA (56-59). The same glutamine  
207 residues in Rho proteins can be altered in different ways, for example CNF1 and the *Bordetella*  
208 *bronchiseptica* toxin DNT transfer polyamines, such as putrescine, spermidine and spermine onto  
209 these residues, resulting in similar inhibition of their GTPase activity (60, 61). *Photobacterium*  
210 *luminescens* toxin TccC5 targets the same glutamine residues, catalyzing their ADP-ribosylation, and  
211 also leads to permanent activation (62).

212 **4.2. Preventing actin polymerization**

213 Actin filaments are constantly polymerizing at their barbed ends and depolymerizing at their pointed  
214 ends. This dynamic state makes actin an ideal target for several binary bacterial AB toxins, including  
215 *C. botulinum* C2 toxin, *Clostridium perfringens* iota toxin, *Clostridium spiroforme* CST toxin, and *C.*  
216 *difficile* CDT toxin (63-66). These toxins ADP-ribosylate the residue required for actin polymerization  
217 on globular G-actin (Arg177), but not filamentous F-actin. Thus, in the presence of these toxins, F-  
218 actin depolymerizes at the pointed end, but monomers are immediately ADP-ribosylated, and cannot  
219 be incorporated at the barbed end. Consequently, the actin cytoskeleton is rapidly broken down,  
220 leading to junction disruption and cell rounding. Other non-binary toxins have been identified that  
221 ADP-ribosylate G-actin at Arg177, including *Salmonella* SpvB, *Aeromonas salmonicida* AexT, and *P.*  
222 *luminescens* Photox (67-69).

223 *P. luminescens* TccC3 ADP-ribosylates Thr148 on both G- and F-actin, to prevent their association  
224 with thymosin- $\beta$ 4, which inhibits actin polymerization (62). Thus, in contrast to the other actin ADP-  
225 ribosylating toxins, this modification results in increased actin polymerization. This effect is  
226 consistent with the action of *P. luminescens* TccC5, which is co-injected with TccC3 (see 4.1.).

## 227 **5. Toxin binding to signaling receptors to promote junctional leakage**

### 228 **5.1. LPS effect on junctions**

229 A limited number of toxins or bacterial factors bind to and activate cell surface receptors, thereby  
230 inducing increased paracellular permeability. The most ubiquitous member of this class of factors is  
231 LPS, which binds and activates Toll-like Receptor 4 (TLR4). TLR4 signaling triggers the expression of  
232 pro-inflammatory cytokines that eventually increase paracellular permeability in both epithelia and  
233 endothelia (70). In addition, LPS binding to TLR4 at the surface of epithelia and endothelia increases  
234 cytosolic calcium levels, resulting in myosin light chain kinase (MLCK) activation. The subsequent  
235 phosphorylation of MLC induces actin fiber contraction and disrupts junctions (71, 72).

### 236 **5.2. *E. coli* toxins signal to junctions**

237 In addition to their well-known role in activation of chloride channels, two toxins secreted by  
238 enterotoxigenic *E. coli*, STa and STb, have been shown to induce a loss of TJ integrity (73). STa binds  
239 to its protein receptor GC-C, and STb binds to sulfatide, a glycosphospholipid found at the surface of  
240 intestinal epithelial cells. STb binding has been shown to trigger disruption of the actin cytoskeleton  
241 and delocalization of claudin-1, ZO-1 and occludin (74, 75). However, further work is needed to  
242 characterize the mechanisms by which these two toxins alter actin and TJ proteins.

## 243 **6. Opening the paracellular route by displacing junctional adhesive proteins**

244 A typical example of bacteria-induced adhesive protein delocalization is provided by *Neisseria*  
245 *meningitidis*, which interacts closely with the apical surface of brain endothelial cells through its  
246 type 4 pili. Recruitment and activation of the Par3/Par6/aPKC polarity complex to the site of  
247 bacterium-host interaction is sufficient to create an ectopic junction-like structure within the apical  
248 membrane domain. Both AJ (VE-cadherin,  $\beta$ -catenin and p120) and TJ proteins (ZO-1, ZO-2 and  
249 claudin-5) are re-routed to the site of bacterial adhesion, thereby inducing the formation of gaps at  
250 intercellular junctions through which bacterial transmigration can occur (76).

251 *H. pylori* also promotes junction delocalization to the apical membrane domain. CagA, once  
252 internalized, interacts with two TJ proteins, ZO-1 and JAM-1, inducing their ectopic assembly at the  
253 site of bacterium-host interaction (77). In addition, CagA binds to Par1, preventing its  
254 phosphorylation by aPKC; as a result, Par1 dissociates from the membrane (78-80). Collective  
255 delocalization of ZO-1, JAM-1 and Par1 from TJ creates junctional defects and increased permeability.  
256 Moreover, TJ protein re-routing induces a cellular reprogramming comparable to a process of  
257 epithelial mesenchymal transition (EMT), an event that has been suggested as an initiating factor in  
258 *H. pylori*-induced carcinogenesis.

## 259 **7. Junctional proteins as surface receptors for bacteria and bacterial toxins**

260 Host cell surface receptors are frequently used for bacterial tethering or toxin interaction. This is  
261 particularly the case for pore-forming toxins, which must bind to a specific receptor to allow their  
262 oligomerization prior to pore formation (81). Some of these bacterial or toxin receptors are adhesive  
263 intercellular proteins (Table 2).

### 264 **7.1. E-cadherin as entry site for bacteria**



265 The only known example of bacterial recognition of an intercellular protein has been described for  
266 *Listeria monocytogenes*. This bacterium can enter nonphagocytic cells and cross epithelial barriers in  
267 the host. *Listeria* expresses a surface protein, InlA, that interacts directly with E-cadherin (82). Like  
268 homotypic E-cadherin interaction, InlA binding triggers the formation of an AJ complex. Furthermore,  
269 InlA-E-cadherin binding activates the protein kinase Src that phosphorylates E-cadherin, and  
270 consequently triggers E-cadherin ubiquitylation by the ubiquitin-ligase Hakai (83). This ubiquitylation  
271 promotes clathrin recruitment to the bacterium-host interaction site, initiating the process by which  
272 the bacterium will be internalized into the cells. Bacterial internalization also requires the  
273 recruitment of cortactin and Arp2/3 for actin nucleation (84). As E-cadherin is normally located  
274 below TJs in epithelia, its accessibility to bacteria has been studied, and two possible means of  
275 interaction have been proposed. During the renewing of epithelia, senescent cells are expelled from  
276 the cell monolayer, and E-cadherin is transiently exposed at the luminal surface by surrounding cells  
277 it can then be used by *Listeria* to gain entry into cells (85). Alternatively, *Listeria* may penetrate into  
278 goblet cells located in the intestinal barrier, where E-cadherin is accessible (86).

## 279 **7.2. Claudins are pore-forming receptors**

280 The claudins are receptors for *Clostridium perfringens* enterotoxin (CPE) toxin (87), although not all  
281 claudins interact with CPE, claudins-3, -4, -6, -8 and -14 are proven receptors for epithelia, and  
282 claudin-5 for endothelia (88-92). In addition, affinities for CPE vary between claudins, with claudin-4  
283 displaying the highest affinity (93). Both extracellular loops of claudins (ECL-1 and -2 domains) are  
284 required for interaction, but the ECL-2 domain provides the binding specificity (88, 93, 94). Clustering  
285 of claudin-CPE complexes allows CPE hexamerization, creating a pre-pore on the plasma membrane's  
286 surface, which rapidly inserts into the membrane where it forms a pore (95, 96). Pore formation then  
287 triggers a number of deleterious effects as a consequence of a massive calcium influx into the  
288 permeabilized cell (97).

## 289 **8. Concluding remarks**

290 Looking at the many strategies developed by bacteria to alter intercellular adhesion, it can be  
291 concluded that disruption of cell-cell interaction represents a valuable goal, but also a serious  
292 challenge, for pathogenic bacteria during the infection process. The examples given here are the  
293 most representative, but may just be the tip of the iceberg, as many bacteria are known to trigger an  
294 increase in paracellular permeability through as yet unknown mechanisms. During characterization of  
295 the mechanisms of action of bacterial toxins, several host cellular pathways were revealed, providing  
296 tools for biologists to study how eukaryotic cells function. In the future, and because of their  
297 powerful action, bacterial toxins may be exploited for therapeutic interventions, following the  
298 example of *botulinum* toxin, which is now used as part of treatment for flaccid paralysis.

## 299 **Funding sources**

300 This work was supported by grants from the French National Agency for Research (Agence Nationale  
301 de la Recherche; ANR-15-CE11-0018-01) and the Fondation pour la Recherche Médicale "Equipe FRM  
302 2017" (DEQ20170336705), as well as benefiting from institutional support from CNRS, INSERM, CEA,  
303 and University Grenoble-Alpes.

304

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534

Table I: Bacterial toxins affecting intercellular adhesive proteins or junctions

Bacterial toxins <sup>a</sup>	Effect <sup>a</sup>	Refs
<b>Modifications to adhesive proteins from outside</b>		
<b>Proteolysis</b>		
<i>H. pylori</i> , <i>S. flexneri</i> , <i>C. jejuni</i> HtrA	E-cadherin cleavage	(12, 17)
<i>P. aeruginosa</i> LasB	VE-cadherin cleavage	(19)
<i>V. cholerae</i> HA/P	Occludin cleavage	(20)
<b>Perturbed adhesiveness</b>		
<i>C. botulinum</i> BoNT/A complex	Prevents E-cadherin adhesion	(21)
<b>Modifications to adhesive proteins from inside</b>		
<b>Host protease activation, acting on AJ/TJ proteins</b>		
<i>S. aureus</i> HIA; <i>S. pneumoniae</i> PLY; <i>P. aeruginosa</i> ExlA; <i>S. marcescens</i> ShIA	ADAM10 activation	(25-28)
<i>B. fragilis</i> BFT	γ-secretase activation	(32)
<i>N. meningitidis</i> unknown toxin	MMP8 activation	(33)
<i>H. pylori</i> CagPAI	MMP7 activation	(35)
<b>Direct interaction between toxins and the AJ complex</b>		
<i>H. pylori</i> CagA	Binding to E-cadherin IC	(36)
<b>Effect on actin: Rho-GTPase inactivation</b>		
<i>P. aeruginosa</i> ExoS, ExoT; <i>Y. pestis</i> , <i>pseudotuberculosis</i> , <i>enterocolitica</i> YopE	GAP	(42-46)
<i>P. aeruginosa</i> ExoS; <i>C. botulinum</i> C3; <i>S. aureus</i> Edin	ADP-ribosylation	(46, 48-50)
<i>C. difficile</i> TcdA, TcdB	Glucosylation	(51)
<i>Y. ssp</i> YopT	Proteolysis	(52)
<i>V. parahemolyticus</i> VopS	Adenylylation	(53)
<b>Effect on actin: Rho-GTPase activation</b>		
<i>S. typhimurium</i> SopE, SopE2	GEF	(55)
<i>E. coli</i> / <i>Y. pseudotuberculosis</i> CNF1,2/CNFy	Deamidation	(56-59)
<i>Y. pseudotuberculosis</i> CNF1, <i>B. bronchiseptica</i> DNT	Polyamination	(60, 61)
<i>P. luminescens</i> TccC5	ADP-ribosylation	(62)
<b>Direct modification of actin</b>		
<i>Clostridium</i> binary toxins C2, iota, CST, CDT, <i>Salmonella</i> SpvB, <i>A. salmonicida</i> AexT, <i>P. luminescens</i> Photox and TccC3	ADP-ribosylation	(63-69)
<b>Membrane receptor activation leading to altered junctions</b>		

Gram-negative bacteria LPS	MLCK activation via TLR4	(71, 72)
<i>E. coli</i> STa	Interaction with GC-C	(73)
<i>E. coli</i> STb	Interaction with sulfatide	(74)

***Delocalization of junctional proteins***

<i>N. meningitidis</i> Type IV pili	Re-routing of endothelial AJ and TJ to the apical membrane	(76)
<i>H. pylori</i> CagA	Re-routing of epithelial TJ to the apical membrane	(77)

536 <sup>a</sup>Refer to the text for complete bacterial names and a full description of toxin action

537



538 **Table 2: Junctional proteins used as receptors for bacteria or bacterial toxins**

<b>Junctional proteins</b>	<b>Bacteria or toxin<sup>a</sup></b>	<b>Refs</b>
<i>Receptor for bacteria</i>		
E-cadherin	<i>L. monocytogenes</i>	(82)
<i>Receptors for toxins</i>		
Claudins-1,2,3,4,6,7,8,9,14,19	<i>C. perfringens</i> CPE	(87-90, 92, 93)

539 <sup>a</sup>Refer to the text for complete bacterial names and a full description of toxin or bacterial action

540

541 **Figure legend**

542 **Fig. 1: Multiple actions of bacterial toxins on intercellular junctions and the cytoskeleton**

543 A. Effects on TJs. Occludin is cleaved by HA/P toxin and also by MMP-8 following activation by *N.*  
544 *meningitidis*. This bacterium also binds to endothelial cells, thanks to its type 4 pili (T4P), causing the  
545 formation of a polarity complex at the binding site which triggers delocalization of TJ and AJ proteins. STb  
546 toxin negatively regulates actin, ZO-1 and occludin. B. Effects on AJs. BoNT/A complex prevents E-cadherin  
547 dimerization. HtrA toxin cleaves E-cadherin. Products of *H. pylori*'s *cagPAI* locus induce p120 translocation  
548 to the nucleus, where it activates *mmp-7* gene expression. MMP-7 can then cleave E-cadherin. Calcium  
549 entry promoted by pore-forming toxins (PFTs) displaces calmodulin (Cmd) from ADAM10, leading to its  
550 activation and export to the plasma membrane, where it cleaves cadherins. BFT toxin activates  $\gamma$ -  
551 secretase, which in turn cleaves E-cadherin. CagA interacts with E-cadherin, impeding  $\beta$ -catenin ( $\beta$ -cat)  
552 binding.  $\beta$ -catenin is either degraded by the proteasome or translocates to the nucleus, where it can  
553 activate genes involved in cellular proliferation. C. Effects on actin cytoskeleton. SopE and SopE2 mimic  
554 eukaryotic GEF for Rho GTPases, and ExoS, ExoT and YopE mimic GAP for Rho GTPases. ExoS, C3, Edin,  
555 TcdA, TcdB, YpT, and Vops toxins inactivate Rho GTPases by post-translational modifications, whereas  
556 CNF1, CNF2, CNFy, DNT, and TccC5 toxins *activate* the Rho GTPases by alternative post-translational  
557 modifications. C2, iota, CST, CDT, SpvB, AexT, Photox, and TccC3 toxins inhibit G-actin incorporation into  
558 F-actin. LPS binds to TLR4, triggering acto-myosin contraction following MLCK activation. Detailed  
559 information on toxin action can be found in the text.

560

561

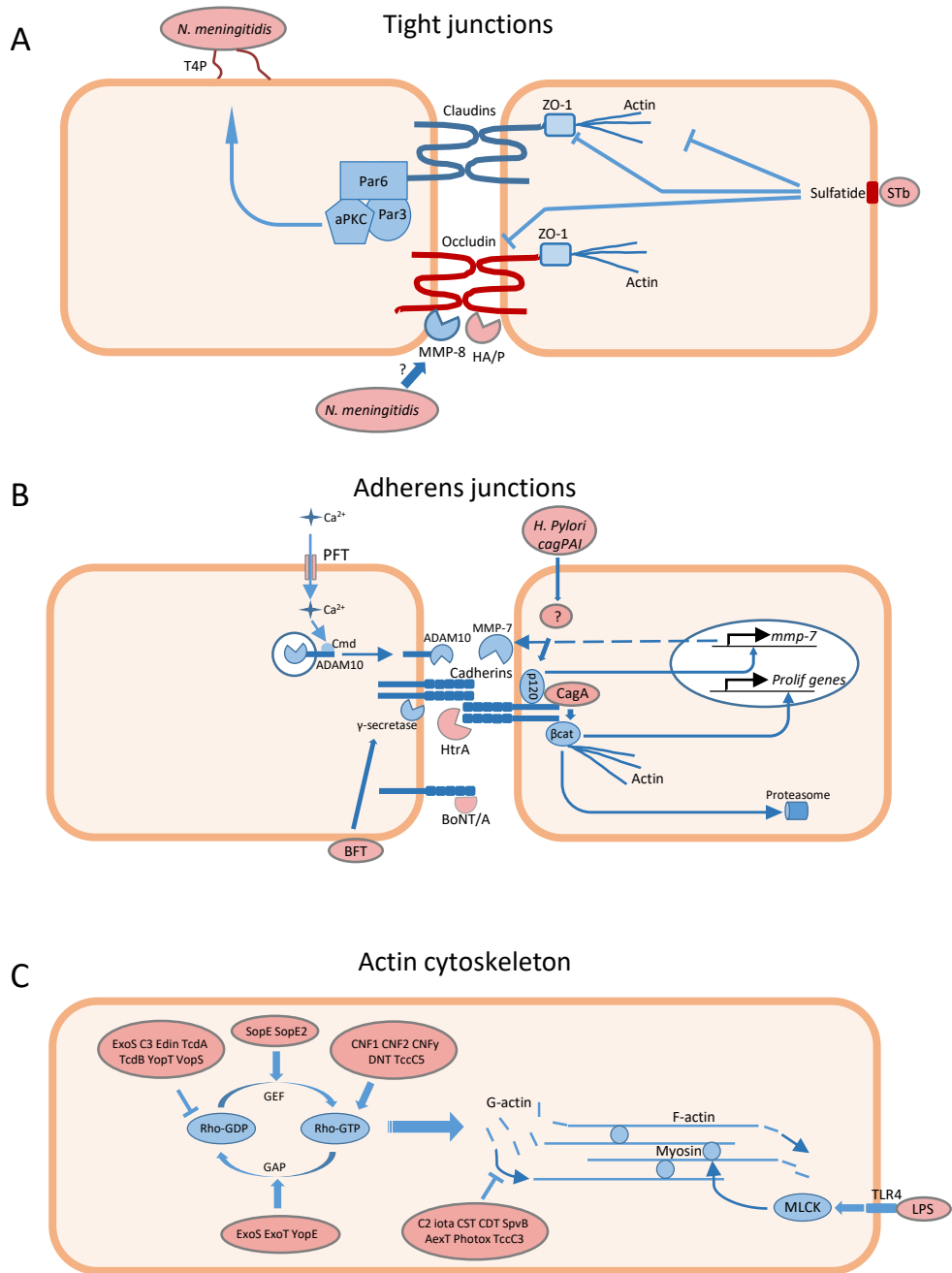


Fig. 1