Leading Edge
Review

Cell

Bacterial Adhesion and Entry into Host Cells

Javier Pizarro-Cerdá[1,2,3,*] and Pascale Cossart[1,2,3,*]
[1] Institut Pasteur, Unité des Interactions Bactéries-Cellules, Paris, F-75015 France
[2] INSERM, U604, Paris, F-75015 France
[3] INRA, USC2020, Paris, F-75015 France
*Contact: pizarroj@pasteur.fr (J.P.-C.); pcossart@pasteur.fr (P.C.)
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Successful establishment of infection by bacterial pathogens requires adhesion to host cells, colonization of tissues, and in certain cases, cellular invasion—followed by intracellular multiplication, dissemination to other tissues, or persistence. Bacteria use monomeric adhesins/invasins or highly sophisticated macromolecular machines such as type III secretion systems and retractile type IV pili to establish a complex host/pathogen molecular crosstalk that leads to subversion of cellular functions and establishment of disease.

Introduction

Bacteria are among the most diverse living organisms and have adapted to a great variety of ecological environments, including the human body. Pathogenic bacteria present an astounding arsenal of surface organelles and secreted toxins that allow them to conquer many different niches throughout the course of infection. Particularly fascinating is the fact that some bacterial species, by combining several of these munitions, are able to induce different diseases, as illustrated by the well-known *Escherichia coli*. Indeed, this important intestinal flora commensal can be responsible for diverse illnesses according to the expression of different combinations of pathogenic factors. For example, enteropathogenic *E. coli* (EPEC) induces the destruction of the intestinal brush border microvilli leading to diarrhea, while enterohemorrhagic *E. coli* (EHEC, which includes the famous 0157:H7 strain) is responsible for hemorrhagic colitis and sporadically hemolytic uremic syndrome. Other pathogenic *E. coli* including enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), or diffusely adhesive (DAEC) express their own specific combinations of pathogenic factors, resulting in yet other types of infections.

The complexity of the bacterial tools used for cell adhesion and invasion ranges from single monomeric proteins to intricate multimeric macromolecules that perform highly sophisticated functions and can be truly considered as nanomachines. Their huge diversity makes the task of analyzing—and comparing them—difficult. As we gain insight into the complex behavior of some pathogens, by combining information issued from tissue culture assays and in vivo studies, some concepts in the field need to be reconsidered—in particular, the strict distinction between extracellular versus intracellular bacteria (see for example Oh et al., 2005). Whatever these complex and conflicting issues, much exciting research is currently providing new information and revealing the exquisite adaptations that bacterial pathogens have evolved to subvert specific host-cell functions. In this Review, we will address some of the most remarkable strategies used by bacterial pathogens to adhere, and in some cases invade, nonphagocytic epithelial cells within their hosts.

Pili and Fimbria

Pili are adhesive hair-like organelles that protrude from the surface of bacteria. Since pili can be used as appendages for transfer of genetic material during bacterial conjugation, the term "fimbria" is more commonly used to describe pili, whose function is devoted to attach bacteria to a surface. Identified initially only in Gram-negative organisms such as *Escherichia coli*, these filamentous surface structures comprise a scaffold-like rod anchored to the bacterial outer membrane and a bacterial adherence factor or adhesin located at the tip of the scaffold, which confers the binding specificity. Some pili, such as Type IV pili, not only mediate adhesion but also perform complex functions such as force-driven contraction, providing bacteria with powerful tools to enhance their contact with target surfaces. Gram-positive bacteria have more recently been shown to also possess pili, and the mechanisms by which these organelles are assembled as well as their contribution to disease are the subject of new intense investigation.

Structures Exported by the Chaperone/Usher Pathway: P pili, Type I Pili, and Afa/Dr Adhesins

One of the first- and best-characterized fimbria is the pyelonephritis-associated (P) pilus, expressed by *E. coli* strains that colonize the urinary tract and subsequently infect the kidney, also known as uropathogenic *E. coli* or UPEC. P pili are encoded by the *pap* gene cluster, which contains regulatory as well as biosynthetic genes for fimbrial subunits, protein chaperons and outer membrane anchors. P pili biogenesis is the paradigm of the "chaperone/usher" pathway (Figure 1A), in which the periplasmic chaperone PapD transports each of the pilus subunits to the outer membrane assembly platform/usher PapC,

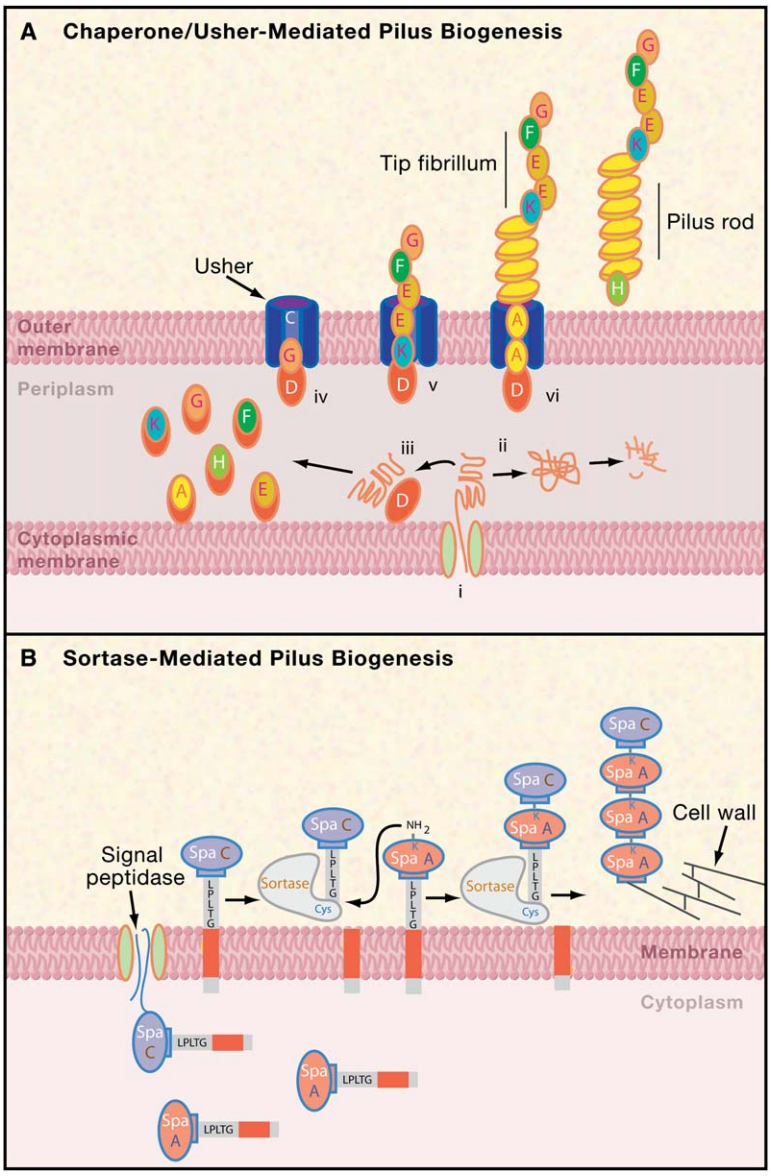

Figure 1. Pilus Biogenesis Machineries

(A) Chaperone/Usher-Mediated Pilus Biogenesis: Individual components of the pilus are transported from the bacterial cytoplasmic space to the periplasm via the type II secretion system, a general pathway for bacterial protein transport (i). In the absence of the chaperone PapD, the pilus subunits are degraded (ii). Otherwise, PapD stabilizes the subunits (iii) and transports them to the outer membrane usher PapC (iv). The pilus tip is assembled first, and then pilus grow from their base (v). The final quaternary structure is acquired outside the bacterial outer membrane (vi) (adapted from Sauer et al., 2000).

(B) Sortase-Mediated Pilus Biogenesis: Pilus subunits present a signal peptide that promotes their transport from the cytoplasm to the bacterial cell wall by the type II secretion system. Subunits are anchored to the bacterial membrane through their cell wall sorting signal, which are in turn cleaved by the sortase, forming a sortase-subunit acyl intermediate. In the case of *C. diphteriae*, SpaC is the first subunit to be incorporated into the pilus; a conserved lysine residue (K) at N terminus of SpaA attacks the sortase-SpaC acyl intermediate, and the remainder of the filament is assembled by a sequence of similar transpeptidation reactions. The mature pilus is attached to the cell wall envelope (adapted from Ton-That and Schneewind, 2004).

which in turn facilitates subunit translocation to the bacterial surface (Thanassi et al., 1998). PapD binds pilus subunits to prevent their aggregation at the wrong time in the periplasmic space and catalyzes subunits folding to prime them for assembly (Bann et al., 2004). P pili bind through the PapG adhesin to the α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside moiety of glycolipids of upper urinary tract cells. PapG variants recognize different but related Galα-(1-4)-Gal receptors differently distributed within host tissues and within the host population, so differential expression of PapG adhesins drives tissue and host specificity (Hultgren et al., 1991).

Type I pili are another kind of composite surface fibers present in different pathogenic *E. coli* (UPEC and DAEC). Type I pili are encoded by the *fim* gene cluster and also exported by the chaperone/usher pathway—in which FimC is the periplasmic chaperone, FimD is the outer membrane usher, and FimH is the pilus adhesin. FimH recognizes essentially monomannose- and trimannose-containing glycoprotein receptors: commensal *E. coli* isolates present FimH variants that bind with high affinity to trimannose residues, while uropathogenic bacteria present FimH molecules that exhibit a higher affinity for monomannose residues enriched within the urinary tract. Strikingly, FimH interaction with cellular receptors can induce not only the adhesion but also bacterial internalization within bladder cells, resulting in bacterial persistence and chronic urinary tract infections (discussed below).

Another family of adherent structures secreted by the chaperone/usher pathway is the extremely heterogeneous family of Afa/Dr adhesins (Servin, 2005), identified in UPEC and DAEC. Afa/Dr adhesins are typically

encoded by at least five *afa* genes (A through E), *afaE* coding for the actual adhesin. First characterized as afimbrial, several members of this family have now been shown to be assembled into true fimbrial structures: the sequence of the adhesin dictates whether or not it will be assembled into fimbriae, and genetically switching the genes encoding the adhesins switches the adhesin type (Anderson et al., 2004). Most of the Afa/Dr adhesins bind to the Dr^a-blood group antigen of the complement regulatory molecule CD55 (also known as DAF), but some are reported to interact with carcinoembryonic antigen-related adhesion molecules (CEACAMs), with type IV collagen or with $\alpha5\beta1$ integrins (see below).

Retractile Type IV Pili

Much current interest is focused in the study of type IV pili, another category of polymeric adhesive surface structures expressed by many Gram-negative bacteria including pathogens such as EPEC, EHEC, *Salmonella enterica* serovar Typhi, *Pseudomonas aeruginosa, Legionella pneumophila, Neisseria gonorrhoeae*, *Neisseria meningitidis,* and *Vibrio cholerae* (Craig et al., 2004). These organelles are essentially composed of a homopolymer of a single pilin subunit, such as PilA in *P. aeruginosa*, PilE in *Neisseria* spp., bundlin in EPEC or TcpA in *V. cholerae*. An adhesive subunit has been characterized at the tip of some pili, for example PilC in *Neisseria* spp. Besides the structural subunit(s) of the pilus, many accessory molecules including prepilin peptidases, putative nucleotide binding proteins, prepilin-like proteins, cytoplasmic membrane proteins, and outer membrane proteins are required for pilus biogenesis. In some bacterial species, such as EPEC and *V. cholerae*, type IV pili can aggregate laterally forming bundles. The structure of the EPEC bundling-forming pilus has been characterized recently, and it is demonstrated that EPEC bundlin shows significant differences in its secondary and tertiary structure compared to *V. cholerae* TcpA; nevertheless, EPEC and *V. cholerae* type IV pili have virtually similar helical organization, suggesting that evolutionary forces contribute to diversification in pilin structure while pilus structure and function are maintained (Ramboarina et al., 2005).

Unlike type I and P pili, which are assembled in the bacterial extracellular space, type IV pili are formed at the cytoplasmic membrane and the intact organelle is extruded across the outer membrane (Wolfgang et al., 2000). However, the most striking feature of type IV pili is their ability to retract through the bacterial cell wall while the pilus tip remains firmly adhered to the target surface. In *Neisseria* spp., it has been shown that the inner membrane ATPase PilT is involved in type IV pilus retraction (Wolfgang et al., 1998). PilT is also required for force-dependent pilus elongation, suggesting that bacteria can modulate interaction with surfaces by controlling tension on their pili (Maier et al., 2004). Pilus retraction is required for a specialized form of bacterial movement across semisolid surfaces (such as the mucosal epithelia) called twitching motility.

Type IV pili are also involved in bacterial adhesion to host cells. EPEC type IV bundle-forming pili, for example, are required for initial bacterial attachment to brush border cells as a prelude for the "attaching and effacing" phenotype (Tobe and Sasakawa 2002) (see below). *P. aeruginosa* type IV pili are the main adhesive organelles recognizing the disaccharide β-GalNAc(1-4)βGal on epithelial cells asialo-GM1 and -GM2 gangliosides (Suh et al., 2001). In the case of *Neisseria* spp., PilC-mediated adhesion to host cells via recognition of the complement regulator CD46 has been reported (Kallstrom et al., 1997), but recent studies suggest that the PilC ligand is a still undefined protein (Kirchner and Meyer, 2005). Nevertheless, type IV piliated *Neisseria* spp. are able to induce several activities including CD46 tyrosine phosphorylation, CD46 redistribution under signaling bacteria and release of CD46-enriched host membrane vesicles (Gill et al., 2005; Lee et al., 2005). A minor subunit of the *Neisseria* spp. type IV pili, PilV, has also been described as mediating bacterial adhesion to epithelial cells (Winther-Larsen et al., 2001). Phase and antigenic variation have been described for *Neisseria* spp. type IV pili, enabling the pathogen to express new variants during the course of infection and escape the immune system. Type IV pili have been additionally implicated in other different functions, including biofilm formation and DNA uptake by natural transformation.

Pili in Gram-Positive Bacteria

The presence of pili has been for many years the hallmark of Gram-negative bacteria. However, in several Gram-positive microorganisms, fimbrial structures have been described in the past, and recently the mechanisms by which Gram-positive pili are assembled have started to be uncovered (Ton-That and Schneewind, 2004). In the case of *Corynebacterium diphtheriae*, the agent of human diphtheria, pili are composed of three subunits: SpA is the major component of the pilus shaft, SpaB is spaced at regular intervals along the shaft, and SpaC is located at the pilus tip. These pili are generated by the sortase machinery, which had so far only been considered as a pathway to covalently anchor proteins presenting the LPXTG motif to the cell wall peptidoglycan. Study of the assembly mechanisms of Gram-positive pili suggests that sortases, through their transpeptidase activity, also link pili subunits (Figure 1B). A similar sortase-mediated export of pilin subunits seems to exist in other pathogenic species such as *Clostridium perfringens*, *Actinomyces naeslundii, Streptococcus agalactiae, Streptococcus mutans,* and *Streptococcus pneumoniae* (Lauer et al., 2005). However, in species such as *Ruminococcus albus*, the pilus subunit presents homology to that of Gram-negative type IV pili; moreover, pili are morphologically similar to type IV pili, suggesting that Gram-positive bacteria have also evolved multiple molecular strategies for pili formation.

As in Gram-negative pathogens, Gram-positive pili seem to play an important role in the adhesion of bacteria to host surfaces. In the case of the buccal pathogen *Streptococcus parasanguis*, the distal FimA subunit promotes adhesion to saliva-coated hydroxyl apatite; *Actinomyces* spp., another buccal pathogen, binds salivary proline-rich

molecules and mucosal tissues via type 1 and type 2 fimbria, respectively (Ton-That and Schneewind, 2004). Interestingly, the recently discovered *Streptococcus pyogenes* pili have been unwittingly used during more than five decades for serotyping; new data show that immunization of mice with recombinant *S. pyogenes* pilus proteins confers protection against challenge with virulent bacteria, highlighting the key role of these pili for infection (Mora et al., 2005).

Adhesins

Besides pili and fimbria, a plethora of different bacterial nonpolymeric adhesins exist which recognize many different elements of host-cell surfaces, including components of the extracellular matrix such as collagens, laminins, elastin, proteoglycans, and hyaluronan. Adhesive glycoproteins such as vitronectin, fibrinogen, and specially fibronectin—which can be present as secreted or plasma membrane-associated molecules—are also recognized by many different species of bacterial pathogens. Integral host membrane adhesion receptors such as integrins, cadherins, selectins, and CEACAMs are receptors of many pathogens for adhesion, and some times for cell entry (see section on invasion).

Fibronectin Binding Proteins

Impressive for its broad repertoire of adhesins is *S. pyogenes* (responsible for cutaneo-mucosal infections including necrotizing fasciitis), which presents more than twelve fibronectin and collagen binding proteins, some of them displaying additional enzymatic activities such as serine protease activity in the case of ScpA or lipoproteinase activity in the case of SfbII (Kreikemeyer et al., 2004). *Staphylococcus aureus*, agent of polymorphic cutaneo-mucosal infections and toxic shock syndromes, also express fibronectin binding proteins that present mechanico-functional properties similar to those of the streptococcal proteins, despite absence of phylogenetical relationships between them (Schwarz-Linek et al., 2003). For example, the major adhesins SfbI of *S. pyogenes* and FnBP-A of *S. aureus* bind ECM-associated fibronectin and induce the clustering of fibronectin bound integrin receptors, triggering intracellular signaling (Ozeri et al., 2001; Joh et al., 1999; see below).

Autotransporters

The so-called autotransporters are proteins located on the bacterial surface. They represent the simplest form of protein export in bacteria: molecules secreted by this system, known as type V secretion, contain an N-terminal leader peptide for secretion across the inner membrane, a C-terminal domain that forms a pore in the outer membrane, and a passenger domain that is autotransported through the outer membrane pore to be exposed on the bacterial surface, for cleavage in certain cases (Henderson et al., 2004). Originally it was proposed that a single autotransporter was required for its own transport, but it has been suggested recently that several autotransporters may associate to generate a pore that can support the transport of even folded passenger domains (Veiga et al., 2002).

Several autotransporters have been associated with adhesion in *E. coli*: antigen 43, the only autotransporter that undergoes phase variation, contains RGD motifs implicated in integrin binding and confers low levels of adhesion to cells; AIDA-A is expressed by DAEC strains and is responsible for their particular diffusively adhesive phenotype; the ETEC-expressed TibA is the first surface-localized glycoprotein described for *E. coli* and its glycosylation confers adhesive properties. In *Helicobacter pylori*, the etiological agent of several gastric diseases including gastric cancer, several adhesins have been detected and one of these adhesins, BabA, is an autotransporter. BabA recognizes the Lewis b (Le^b) blood group antigen present on red blood cells and in the gastric mucosa (Ilver et al., 1998); inflammation of the gastric tissue leads to increased expression of other Lewis antigens normally expressed at low levels such as sialylated sLe^x and sLe^a, and *H. pylori* recognizes these molecules via the sialic acid binding protein (SabA) (Mahdavi et al., 2002), suggesting that *H. pylori* infection favors the expression of its own receptors in target tissues. In the case of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (the agents of enteric yersiniosis), YadA is the prototype of the new subfamily of trimeric autotransporters, in which three autotransporter subunits associate to form the functional pore (Cotter et al., 2005); YadA mediates adhesion to collagen, laminin, and fibronectin (Nummelin et al., 2004).

Tir: The Bacterially Encoded Cellular Receptor of EPEC and EHEC

Besides their whole arsenal of pili and fimbria, EPEC and EHEC have developed an original bacterial adhesion system in which the bacteria provides both the ligand and the receptor. EPEC and EHEC induce a characteristic lesion known as the "attaching and effacing" (A/E) phenotype: after intimate attachment to intestinal epithelial cells via their adhesins and bundle-forming pili, bacteria induce the local effacement of absorptive microvilli and the accumulation of host cytoskeletal proteins, leading to the formation of pedestal-like structures on which bacteria perch. The molecules required for the induction of A/E lesions are encoded in a region known as the *l*ocus of *e*nterocyte *e*ffacement (LEE), which codes for a type III secretion system (TTSS). The TTSS gene cluster codes for structural proteins required for the formation of a needle complex or injectisome that traverses the bacterial cell wall and the host-cell plasma membrane (Figure 2); it also codes for chaperones and secreted effectors that will be delivered to the host cytoplasm through the needle complex and will modulate cellular functions favoring bacterial survival. One of the effector proteins of the EPEC/EHEC TTSS is Tir (*t*ranslocated *i*ntimin *r*eceptor), which is injected into host target cells and then is inserted into the host-cell membrane, where it functions as a receptor for another LEE encoded molecule, the outer membrane protein intimin (Kenny et al., 1997)(Figure 3A). In the case of EPEC, Tir is phosphorylated by host kinases such as Fyn and Abl (Phillips et al., 2004; Swimm et al., 2004) on tyrosine-474 and is involved in the recruitment

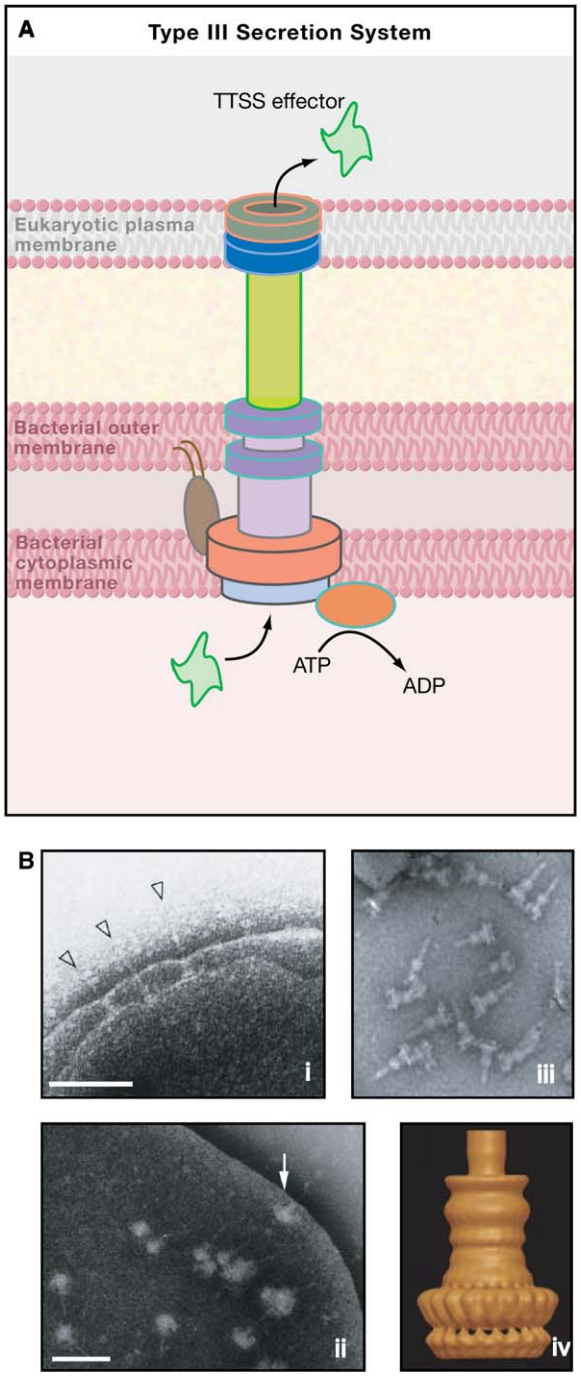

Figure 2. Type III Secretion System

Schematic figure of the Gram-negative TTSS. A cylindrical base, similar to the flagellar hook-basal body complex, spans the periplasm and is associated with the two bacterial membranes where ring-like structures are detected, ensuring stabilization of the whole structure upon the bacterial cell envelope. An elongated hollow extracellular structure called the needle extends around 50 nm outside the bacterial cell wall (it varies according to the different bacterial species) and can be inserted into eukaryotic membranes. Energy derived from ATP hydrolysis drives translocation of bacterial proteins (known as TTSS effectors) from the bacterial cytoplasm to the eukaryotic cell cytoplasm, where they can hijack host signaling pathways.

of the host adaptor molecule Nck, which in turn recruits the Wiskott-Aldrich syndrome protein (N-WASP) and the actin-related protein 2/3 (Arp2/3) complex, which nucleate actin cytoskeleton leading to the formation of the bacterial-associated pedestals (Figure 3B). Actin binding proteins such as α-actinin, talin and vinculin are also recruited to the pedestal. EPEC Tir has a GTPase activating protein (GAP) activity that has been implicated in the down-regulation of pedestals formation (Kenny et al., 2002). EPEC Tir can also trigger Nck-independent pedestal formation, which depends on tyrosine-474 and tyrosine-454 phosphorylation, recruiting a still unknown phosphotyrosine binding adaptor involved in N-WASP and Arp2/3 binding (Campellone and Leong, 2005). EHEC Tir generates pedestals in the absence of phosphotyrosines or Nck by associating with the TTSS effector $EspF_U$, which binds N-WASP and stimultates Nck-independent actin assembly (Campellone et al., 2004; Garmendia et al., 2004). Tir is so far the only example of a bacterial receptor that is encoded by the bacterium itself. The murine pathogen *Citrobacter rodentium* has also acquired by horizontal gene transfer the LEE, which enables it to form pedestals (Deng et al., 2001).

Invasive Strategies

The extracellular milieu can be a harsh environment in which pathogens are subjected, in addition to physical stresses (such as low pH or shear stress imposed by flow of mucosal secretions or blood) to many other host defense mechanisms including cellular exfoliation, complement deposition, antibody labelling and subsequent recognition by macrophages or cytotoxic T cells, etc. Several bacterial species have evolved molecular strategies to actively induce their entry into target cells for replication and/or dissemination to other host tissues. Invasion can proceed by direct engagement of surface host-cell receptors or by direct translocation of bacterial proteins into the host-cell cytosol that will promote rearrangements of the plasma membrane architecture, inducing pathogen engulfment. A growing number of bacteria that were so-far considered as extracellular have been shown to invade host cells, probably using intracellular compartments for persistence in target tissues.

Invasin and Other Ligands Involved in Integrin Engagement and Bacterial Uptake

The ability of adhesion receptors, such as integrins or cadherins, to transmit biochemical signals and mechanical

(B) (i) Transmission electron microscopy of osmotically shocked *Salmonella* exhibiting TTSS complexes on the bacterial cell envelope (open arrows). The scale bar represents 100 μm. Reprinted with permission from Kubori et al., (1998). (ii) Electron microscopy of osmotically shocked and negatively stained *Shigella* showing TTSS scattered over the bacterial cell envelope. The scale bar represents 100 μm. Reproduced with permission from Blocker et al., (2003). (iii) Electron micrographs of negatively stained *S. enterica* serovar Typhimurium TTSS and (iv) surface rendering of the TTSS base. Reprinted with permission from Marlovits et al., (2004).

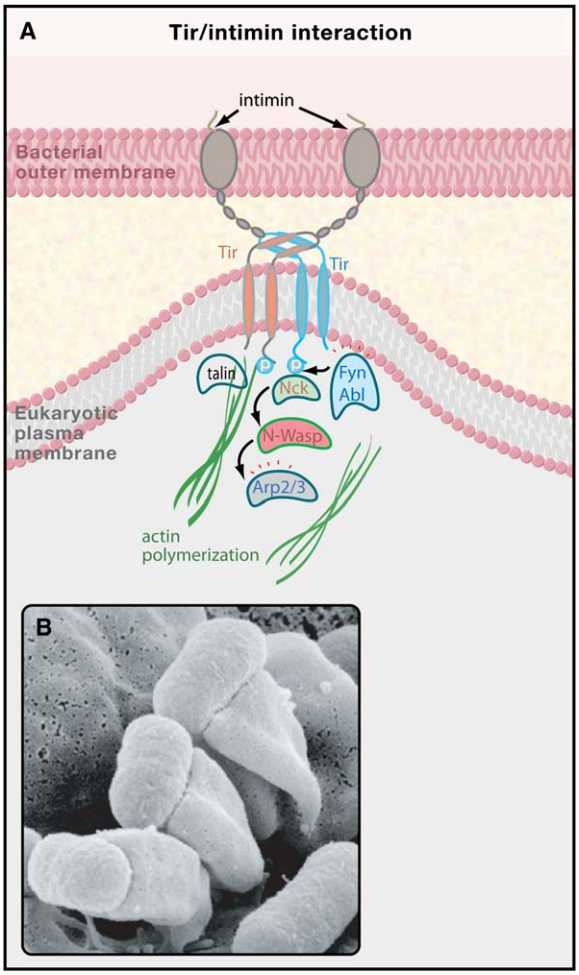

Figure 3. Tir/Intimin Interaction

(A) EPEC, via its TTSS, injects into the cytosol of target cells the protein Tir, which integrates into the host-cell plasma membrane, dimerizes, and functions as a receptor for the bacterial outer membrane intimin. Tir/intimin interaction promotes Tir phosphorylation by Fyn and Abl, inducing the recruitment of the protein adaptor Nck, which in turn recruits N-WASP and the Arp2/3 complex, leading to actin polymerization and the formation of structures known as pedestals. Actin binding proteins such as talin are recruited to the pedestal, stabilizing the structure.

(B) Scanning electron micrograph of EPEC perching on top of pedestals on HeLa cells (reprinted with permission from Finlay and Cossart, 1997).

force across cell membranes depends on interactions with the actin cytoskeleton. Several pathogens seem to have exploited their interaction with host-cell receptors—and particularly with $\alpha5\beta1$ integrin—not only to adhere but also to trigger actin cytoskeleton rearrangements that can lead to cellular invasion. The first bacterial invasion protein ever described was invasin (Isberg et al., 1987), a surface protein of *Y. enterocolitica* and *Y. pseudotuberculosis*. Invasin is related to EPEC/EHEC intimins and binds with high affinity to multiple members of the $\beta1$ chain integrin family (Isberg and Leong, 1990), subverting normal interactions of these adhesion molecules with fibronectin. Invasin, in fact, binds to integrins with higher affinity than fibronectin, and crystal structure of invasin reveals that it presents an optimized surface for integrin binding, including several key residues shared with fibronectin, but in the context of different folds and surface shapes (Hamburger et al., 1999). The short cytoplasmic tail of integrins has no enzymatic activity but recruits components that serve as linking or docking proteins for cytoskeletal-associated elements. The focal adhesion kinase (FAK) or the Src family of kinases are often associated with physiological integrin-mediated signaling, and *Y. pseudotuberculosis* subverts the function of these proteins to invade nonphagocytic cells (Alrutz and Isberg, 1998; Bruce-Staskal et al., 2002). Integrin engagement by invasin leads also to activation of several small GTPases, including Rac1, a member of the Rho family of master actin regulators, and Arf6, which has been implicated in membrane trafficking; these proteins promote the recruitment to the bacterial entry site of the phosphatidylinositol-phosphate-5-kinase Iα, inducing the local production of phosphatidylinositol-4,5-biphosphate (Wong and Isberg, 2003), an important second messenger affecting the subcellular localization and activation of actin-regulating molecules (Pizarro-Cerdá and Cossart, 2004) (Figure 4A). It is important to note that the invasive behavior of *Y. enterocolitica* and *Y. pseudotuberculosis* is only required during the initial stages of disease for bacterial translocation through epithelial layers, since in vivo bacterial proliferation takes place in the extracellular space and these species have developed an important arsenal of anti-phagocytic molecules (*Yersinia* outer proteins or Yops) that are injected into target cells via a TTSS to block the potential internalization of bacteria upon adhesion.

Several bacterial pathogens that have been traditionally considered as extracellular-living microorganisms and that interact with integrins for adhesion to host tissues have been shown in recent years to be able to invade host cells; however, the in vivo relevance of many of these in vitro observations remains to be established. For example, clustering of fibronectin-bound $\beta1$ integrin receptors by streptococcal SfbI triggers the recruitment of the Rho GTPases Cdc42 and Rac, as well as recruitment/phosphorylaton of FAK, producing actin rearrangements that eventually lead to bacterial internalization (Ozeri et al., 2001). Similarly, staphylococcal FnBP-A-induced $\alpha5\beta1$ integrin clustering leads to FAK activation and Src-dependent phosphorylation of cortactin, a molecule involved in the recruitment of the Arp2/3 complex for actin polymerization; actin binding proteins such as vinculin, tensin, and zyxin are also recruited to the bacterial attachment site, promoting *S. aureus* internalization (Agerer et al., 2005). For DAEC, it has been reported that interaction between Afa/Dr members and $\alpha5\beta1$ integrins also results in bacterial internalization (Plancon et al., 2003); some Afa/Dr molecules can additionally interact with CEACAMs, triggering

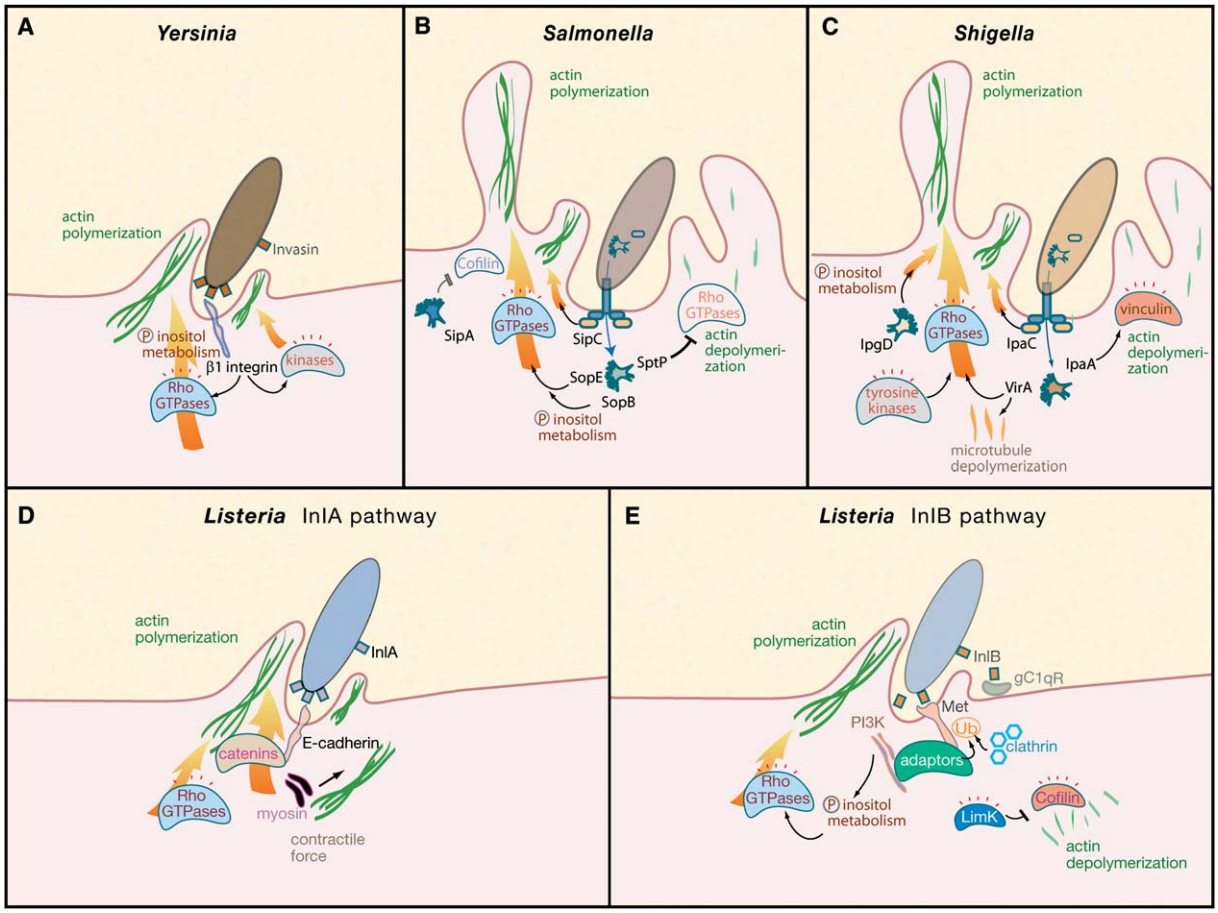

Figure 4. Invasive Molecular Strategies of *Salmonella*, *Shigella, Yersinia,* and *Listeria*

(A)The *Yersinia* outer membrane invasin interacts with β1 integrin receptors and favors activation of the small RhoGTPase Rac1, which will indirectly modulate the phosphatidylinositol metabolism to induce actin rearrangements at the site of bacterial entry, promoting invasion. Host kinases such as FAK or Src also participate in the process.

(B) *Salmonella* translocates several effectors into target cells, some of them allowing the initial uptake of the bacterium: SipC is part of the TTSS and drives actin polymerization and actin-filament bundling; SopE activates Rho GTPases, fostering actin polimerization and membrane ruffle formation; SopB modulates inositol-polyphosphate metabolism, activating indirectly the same Rho GTPases as SopE; and SipA blocks the actin depolymerization factor cofilin, favoring also membrane ruffle formation. SptP plays a role once the internalization has taken place, inactivating the Rho GTPases, inhibiting actin polymerization, and helping the closure of the plasma membrane over internalized bacteria.

(C) *Shigella* also translocates several TTSS effectors into target cells to induce invasion: the translocon component IpaC nucleates the formation of actin filaments; VirA indirectly stimulates the RhoGTPase Rac1 favoring actin polymerization (the host tyrosine kinases Abl/Arg also activate indirectly Cdc42 and Rac1) and inhibits microtubule polymerization; IpgD affects phosphoinositide metabolism and promotes the extension of membrane ruffles by decreasing the interactions between the plasma membrane and the actin cytoskeleton; IpaA activates the host protein vinculin, inducing actin depolymerization and recovery of the plasma membrane architecture once the bacteria are internalized.

(D) *Listeria* invades target cells combining two molecular pathways. In the InlA-dependent pathway, the sortase-anchored bacterial protein InlA interacts with the cell adhesion molecule E-cadherin and promotes the subversion of cell adherens junction machinery (including β- and α-catenins) to induce entry. The myosin VIIA probably generates the contractile force required for bacterial engulfment. Actin polymerization relies, among other molecules, on the RhoGTPase Rac1.

(E) In the InlB-dependent pathway of *Listeria*, the loosely cell-wall-attached bacterial protein InlB interacts with the molecule gC1qR, and with the signaling receptor Met, which recruits several molecular adaptors, which will perform several functions including the recruitment of a PI3K (involved in the activation of the RhoGTPase Rac1 and the polymerization of actin), and also the ubiquitination of Met and the endocytosis of the receptor via a clathrin-dependent mechanisms. A balance between actin polymerization and actin depolymerization required for efficient bacterial entry is controlled by regulation of the activities of the Lim kinase and the actin depolymerizing factor cofilin.

activation of Cdc42 and the subsequent phosphorylation of the actin binding proteins ezrin, radixin, and moesin, leading to the formation of elongated microvilli underlying the adhering DAEC (Berger et al., 2004). In the case of *Neisseria* spp., members of the opacity-associated (Opa) outer membrane proteins (which are subject to phase variation as their type IV pili) have been reported to induce invasion: in particular, Opa50 binding of α5β1

integrins induces pathogen internalization in epithelial cells in vitro (van Putten et al., 1998). Most other Opa proteins can bind CEACAMs, inducing not only bacterial invasion but also increased adhesion of infected cells to the ECM, counteracting the exfoliation of epithelial cells that normally takes place as an innate immune response against infection (Muenzner et al., 2005).

UPEC: Pili-Mediated Invasion for Persistence

As mentioned above, type I pili mediate interaction between UPEC and the urinary tract. The main characterized receptor for the adhesin FimH in bladder epithelial cells is the monomannose moiety of the tetraspanin molecule uroplakin 1a (UP1a) (Zhou et al., 2001b). UP1a forms a complex with three other uroplakin proteins to assemble into plaques that almost cover the entire luminal surface of the bladder epithelium, providing an impermeable layer that is critical for bladder function. Interestingly, FimH interaction with bladder cells triggers a signal transduction cascade that results in the uptake of bacteria (Martinez and Hultgren, 2002). The specific signaling role of UP1a in the FimH-mediated entry has not been clearly established; it has been proposed that the highly dynamic physiological endocytosis of the UP1a-containing plaques is hijacked by UPEC to produce invasion. The presence of plasma membrane microdomains known as lipid rafts, which function as platforms for recruitment of signaling molecules, are also required for invasion of target cells by the FimH-dependent pathway (Duncan et al., 2004). Entry into bladder cells needs the activity of the type I phosphatidylinositol 3-kinase (PI3K), a lipid kinase involved in the production of the important second messenger phosphatidylinositol 3,4,5-triphosphate. Several proteins are phosphorylated during UPEC entry into bladder cells including FAK, which associates with the PI3K, boosting its lipid kinase activity. Formation of complexes of the actin binding proteins α-actinin/vinculin also takes place, probably stabilizing actin filaments surrounding invading UPEC. Cdc42 and RhoA are also required for the FimH-dependent cell invasion process by UPEC (Martinez and Hultgren, 2002). FimH has been additionally reported to bind some other glycosylated and nonglycosylated receptors different from UP1a, including extracellular matrix structural proteins (laminin and fibronectin), CEACAMs, and the GPI-anchored protein CD48, which mediates FimH-dependent entry of UPEC in mast cells (Shin et al., 2000). It has been proposed that infection of bladder cells leads to the establishment of a bacterial reservoir that is probably involved in the recurrent urinary tract infections associated with UPEC (Anderson et al., 2003).

Salmonella *and* Shigella*: Paradigms of TTSS Users for Invasion*

Salmonella spp. and *Shigella* spp. were long considered as distinct species due to their characteristic associated diseases: acute gastroenteritis or systemic typhoid fever in the case of *Salmonella*, and dysentery in the case of *Shigella*. Using molecular biology tools and genomics, it has been established that these microorganisms belong to the same bacterial cluster as *E. coli*, having acquired by horizontal transfer similar genes that enable them to invade and colonize target cells in specific ways (Parsot 2005). In *Salmonella enterica* serovar Typhimurium, up to five virulence gene clusters or pathogenicity islands (SPIs) have been identified which encode different proteins involved in invasion of target cells (SPI-1) and establishment of infection within the intracellular environment (SPI-2 to 5). SPI-1 and SPI-2 code for two different TTSS. Entry of *Shigella flexneri* depends also on a TTSS encoded on a virulence plasmid pathogenicity island. The size of the *S. flexneri* LPS, regulated by its glucosylation, controls the TTSS accessibility to the cell surface (West et al., 2005).

Both pathogens trigger important cytoskeletal changes at the host-cell plasma membrane to promote invasion (Cossart and Sansonetti, 2004). The *S. enterica* SPI-1-TTSS is involved in the translocation of proteins that modulate actin rearrangements, subverting in certain cases the functions of endogenous host-cell proteins by precisely mimicking their activity. Two of these effectors, SopE and SopE2, are bona fide guanine nucleotide exchange factors (GEFs) for Cdc42 and Rac1 and mimic host-cell GEFs, catalyzing the GTP loading and activation of these small GTPases, which results in formation of membrane extensions required for bacterial engulfment (Hardt et al., 1998). A still-unknown indirect cascade of Cdc42 and Rac1 activation is mediated by another SPI-1-TTSS effector, SopB (also known as SigD), which is an inositol phosphatase that generates phosphatidylinositol-3-phosphate and inositol-1,4,5,6-tetraphosphate upon invasion (Zhou et al., 2001a). Interestingly, recovery of normal cellular architecture is observed once *S. enterica* has completely invaded target cells, highlighting that the activation of the Rho GTPases and actin polymerization must be transient. In fact, *S. enterica* translocates another SPI-1-TTSS effector, SptP, which is a GAP for both Cdc42 and Rac1, accelerating the GTP hydrolysis and the consequent inactivation of these Rho GTPases after invasion (Fu and Galan, 1999). The half-life of SptP is longer than that of SopE or SopE2, assuring in the long term the balance toward recovery of the normal cell architecture after bacterial internalization (Kubori and Galan, 2003). Two additional translocated proteins, SipC and SipA, modulate directly actin dynamics: SipC is part of the translocon-complex (together with SipB) that inserts as a pore into host membranes and allows secretion of effectors in the host-cell cytoplasm, and drives actin polymerization and actin filament bundling (Hayward and Koronakis, 1999), while SipA antagonizes the functions of the actin depolymerizing factor cofilin and of the actin filament severing protein gelsolin, preventing disassembly of formed actin fibers (McGhie et al., 2004)(Figure 4B).

In the case of *S. flexneri*, the translocon components IpaB and IpaC are homologous (although not identical in structure and function) to *S. enterica* SipB and SipC, and IpaC nucleates the formation of actin filaments beneath invading bacteria as SipC does (Kueltzo et al., 2003). IpaB interacts with the hyaluronic receptor CD44 and mobilizes this host protein in cholesterol-rich lipid rafts, allowing the

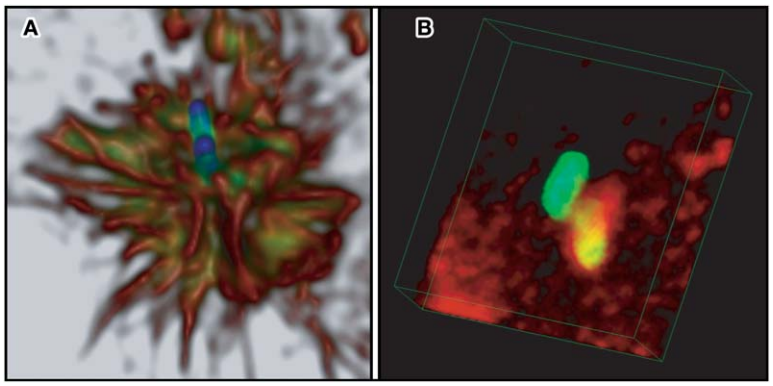

Figure 5. Imaging of Bacterial Interactions with Target Cells

(A) 3D reconstruction from confocal images depicting how translocation of IpaB (green) from *Shigella* (blue) to the cytoplasm of target cells induces the rearragement of the actin cytoskeleton (red) that will permit bacterial entry (image courtesy of Jost Enninga).

(B) 3D reconstruction from confocal images illustrating the recruitment of clathrin (red) around internalized *Listeria* (green; merge of clathrin and internal *Listeria*: yellow) during the first minutes of invasion. External *Listeria* (light blue) is located on top of host cell (image courtesy of Esteban Veiga).

potential recruitment of other signaling molecules at the site of bacterial entry (Lafont et al., 2002). The Abl/Arg tyrosine kinases phosphorylate the molecular adaptor Crk, which presumably activates a GEF for Cdc42 and Rac1 (Burton et al., 2003). Crk also recruits cortactin, which is phosphorylated by Src and is implicated in the Arp2/3-dependent actin nucleation at the bacterial internalization site (Bougneres et al., 2004). Indirect stimulation of actin polymerization via Rac1 and WAVE2 is orchestrated via the TTSS effector VirA, which also inhibits microtubule polymerization (Yoshida et al., 2002) (Figure 4C). *S. flexneri* IpgD is an homolog inositol phosphatase of *S. enterica* SopB, decreasing the plasma membrane levels of phosphatidylinositol-4,5-biphosphate and consequently limiting the membrane/cytoskeleton interactions favored by this phosphoinositide; IpgD thus promotes extension of membrane filopodia necessary for bacterial engulfment (Niebuhr et al., 2002). *S. flexneri* also induces actin depolymerization by activating the actin binding protein vinculin via the TTSS effector IpaA (Bourdet-Sicard et al., 1999), allowing recovery of the plasma membrane architecture after invasion.

Two elegant studies have recently approached the real-time visualization of TTSS effectors secretion upon contact with target cells. In the case of *S. enterica*, it was determined that the translocation process is extremely fast, and the pool of stocked intrabacterial SipA can be completely injected between 100–600 s (Schlumberger et al., 2005). Concerning *S. flexneri*, it was observed that IpaB and IpaC are synthesized and stocked within bacteria in association with chaperons before actual contact with target cells; kinetics of secretion of IpaB and IpaC are rapid and highly concurrent as revealed by live-cell imaging, indicating that quick ejection of these effectors is the rate-limiting step for the invasion process (Enninga et al., 2005) (Figure 5A).

Listeria: Engagement of E-cadherin and Met Signaling Pathways

The Gram-positive pathogen *Listeria monocytogenes* is an intracellular parasite able to traverse three human barriers—the intestinal barrier, the blood-brain barrier, and the feto-placental barrier—leading to several clinical manifestations including gastroenteritis, meningitis, or abortion (Dussurget et al., 2004). Traversal of these different barriers is mediated by InlA, the prototypic member of the internalin family of leucine-rich repeat molecules, which is present in all the *L. monocytogenes* serovars. The InlA receptor is the cell adhesion molecule E-cadherin, a protein involved in homophilic interactions required for the maintenance of cell adherens junctions. The InlA/E-cadherin interaction is species-specific, since only E-cadherins possessing a proline residue at position 16 (as is the case for human E-cadherin) are able to bind InlA (Lecuit et al., 1999). Initial interaction between InlA and E-cadherin requires integrity of lipid rafts (Seveau et al., 2004). Engagement of E-cadherin by InlA leads to recruitment of two catenins to the bacterial internalization site: β-catenin binds to the C-terminal cytoplasmic domain of E-caderin, and recruits at the same time α-catenin (Lecuit et al., 2000) (Figure 4D). Recruitment of α-catenin depends also on ARHGAP10, a molecule that exhibits GAP activity for RhoA and Cdc42 (Sousa et al., 2005). Actin polymerization in the InlA-dependent pathway relies on Rac, cortactin and Arp2/3, but the molecular events leading to the activation of this cascade remain unknown. The unconventional myosin VIIA and its ligand vezatin are also required for the InlA-mediated entry of *L. monocytogenes*, probably generating the tension required for internalization of engulfed bacteria (Sousa et al., 2004). Traversal of the intestinal barrier and of the feto-placental barrier by subversion of cellular E-cadherin function by InlA has been established (Lecuit et al., 2001, 2004), and it is suspected that traversal of the blood-brain barrier probably also requires InlA/E-cadherin interactions.

Invasion of epithelial cells implicates another member of the internalin family, InlB. This protein is loosely attached to the bacterial surface by GW motifs that interact noncovalently with lipoteichoic acids, as opposed to InlA, which is covalently linked to the bacterial cell wall peptidoglycan by a sortase through its C-terminal LPXTG motif. InlB can be released from the bacterial cell wall upon contact with glycosaminoglycans of the extracellular matrix (Jonquieres et al., 2001), and soluble InlB can interact through its GW motifs with a host-cell membrane molecule, the *r*eceptor for the *g*lobular head of complement *C1q* molecule (gC1q-R) (Braun et al., 2000); however, the functional

significance of this interaction is not understood yet. The main cellular receptor for InlB is the hepatocyte growth factor receptor Met, a receptor tyrosine kinase (Shen et al., 2000), which binds to the leucine rich repeats of InlB. The interaction between InlB and Met is species specific, since InlB recognizes human but not rabbit Met (Khelef et al., 2006). Activation of Met by InlB leads to recruitment of several molecular adaptors such as Gab1, Cbl, and Shc, which promote the plasma-membrane association of the PI3K (Ireton et al., 1996) (Figure 4E). PI3K then is involved in activation of Rac and induction of actin polymerization through the activity of WAVE and the Arp2/3 complex (Bierne et al., 2001, 2005). Actin rearrangements are also controlled downstream of Rac by the actin depolymerizing protein cofilin and by the cofilin-modulating enzyme LIM kinase (Bierne et al., 2001). Internalization of Met upon interaction with its natural ligand HGF is clathrin dependent and involves Cbl, which is also an ubiquitin ligase that ubiquitinates Met. As shown recently, Cbl promotes the recruitment and activity of the clathrin-dependent endocytosis machinery at the site of bacterial entry and bacterial internalization (Veiga and Cossart, 2005) (Figure 5B). InlB, thus, seems to exploit unexpectedly the normal trafficking of its receptor to promote invasion (Li et al., 2005). Whether the recruitment of the endocytosis machinery is also occurring for other bacteria is an open question. Integrity of lipids rafts is also required for the InlB-dependent pathway; however, in contrast to the InlA/E-cadherin case, it is not the initial interaction between InlB and Met that is favored by lipid rafts, but the signaling downstream of PI3K activation (Seveau et al., 2004). Potentiation of *L. monocytogenes* entry into target cells by InlA and InlB also requires other cell surface proteins such as the autolysins Ami and Auto, or the cholesterol-dependent cytolysin listeriolysin O (LLO) (Dussurget et al., 2004).

Rickettsia*: A Human Pathogen Closely Related to Plant Symbionts*

Members of the α-proteobacteria group display diverse interactions with higher eukaryotes. Species such as *Sinorhizobium melitoti* or *Agrobacterium tumefaciens* are plant symbionts or parasites, respectively. Other species such as *Rickettsia* spp. or *Brucella* spp. are animal intracellular parasites—and have been classified as ''select agents'' for bioterrorism. Interestingly, virulence/symbiosis systems are conserved between these different groups of pathogens/symbionts, indicating that an original ancestor evolved to adapt to different environments in plants or animals.

Rickettsia spp. are agents of epidemic typhus and the Mediterranean spotted fever and are strict intracellular, noncultivatable bacteria. Two outer membrane proteins, rOmpA and rOmpB, have been described as putative adhesins favoring respectively *R. conorii* and *R. japonica* attachment to epithelial cells in vitro (Li and Walker, 1998; Uchiyama 2003). rOmpB, which belongs to a family of autotransporters in Gram-negative bacteria, has been recently described as the bacterial ligand of a *R. conorii* receptor on target cells, Ku70, a component of the DNA-dependent protein kinase (Martinez et al., 2005). As described above for several bacterial receptors on host-cell plasma membranes, association of Ku70 with cholesterol-enriched lipid rafts is required for invasion. As shown for Met during the InlB-dependent internalization of *L. monocytogenes*, ubiquitination of Ku70 by Cbl is also a prerequisite for bacterial entry. Interestingly, structural motifs at the N-terminal domain of Ku70 share homology with integrin domains, and it has been already proposed that Ku70 can function as a receptor for fibronectin (Monferran et al., 2004). Activation of Src and FAK, two important events strongly associated with β1-integrin activation, are also detected during invasion of target cells by *R. conorii*; Cdc42 (but not Rac1), PI3K, cortactin and the Arp2/3 complex are also molecules required for entry (Martinez and Cossart, 2004).

Conclusions

Pathogenic bacteria have evolved an incredibly large and diverse array of adhesion and invasion molecules that enable them to exploit a variety of host-cell surface components and occupy different niches within the human body. While major advances have recently been made concerning the biogenesis, assembly and structure of previously known pili, fimbriae, invasins, and type III secretion systems, new adhesive structures have unexpectedly been discovered in Gram-positive bacteria such as streptococci, highlighting the ever growing diversity of the strategies used by pathogenic bacteria to adhere and colonize their hosts. In some cases, bacterial components mimic structurally the normal ligand as in the case of *Yersinia* invasin when binding to integrins; in other cases, the bacterial protein only exploits the specific signaling properties of a given host-cell component, as in the case of *Listeria* internalin binding to the adhesion molecule E-cadherin. The special case of EPEC/EHEC Tir protein which is first injected by the bacterium to serve as a receptor for another bacterial surface protein intimin has remained unique and not described in the other enterobacteriacae. In many cases, it is difficult to assign to a given protein an adhesin versus an invasin function, as many invasins are often working as adhesins. In the case of *Salmonella* and *Shigella*, the bacteria are not highly adherent, but their invasion machinery is particularly efficient. It seems that contact with the cell inevitably leads to entry, owing to the efficiency of the bacterial TTSS effectors injected directly into the host cytosol, which insidiously interfere with the cytoskeleton and other components critical for the entry process. How contact occurs is thus a critical issue, and in this case, as for many bacterial pathogens, membrane organization and presence of microdomains seem to govern key events in the internalization process. As demonstrated for *Listeria*, the presence of microdomains may be critical for the adhesion step. It may also be required for later stages during signaling. The events that occur at the plasma membrane are as yet far from being understood. It is clear that the challenge for the

future will be to understand how bacteria coordinate in time and space the expression of their different effectors and how the cell reacts to this aggression. Understanding how cells behave in front of a pathogen may also provide key answers to more general questions in cell biology such as signaling during adhesion or during a variety of other stress conditions.

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