Developmental Cell, Vol. 9, 3–17, July, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.devcel.2005.06.002

Bacteria-Host-Cell Interactions at the Plasma Membrane: Stories on Actin Cytoskeleton Subversion

Review

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Summary

Exploitation of the host-cell actin cytoskeleton is pivotal for many microbial pathogens to enter cells, to disseminate within and between infected tissues, to prevent their uptake by phagocytic cells, or to promote intimate attachment to the cell surface. To accomplish this, these pathogens have evolved common as well as unique strategies to modulate actin dynamics at the plasma membrane, which will be discussed here, exemplified by a number of well-studied bacterial pathogens.

Introduction

During their long lasting coexistence with their hosts, microbial pathogens have evolved a variety of strategies to survive in the host, for instance, by avoiding or resisting various immune defense mechanisms or by replicating in protected niches such as the host-cell cytoplasm. Many bacterial pathogens are accomplishing this by employing an arsenal of highly sophisticated mechanisms that subvert the cellular actin cytoskeleton to trigger their internalization into normally nonphagocytic host cells in order to escape the humoral immune defense. One common mechanism to stimulate actin assembly in the host cell is activation of the Rho-family GTPases Rac1 and Cdc42, both of which are capable of eliciting Arp2/3-complex activation via proteins of the WASP and WAVE families (reviewed in Stradal et al. [2004]). Upon contact with host cells, some pathogens are delivering distinct effector proteins directly into the host-cell cytoplasm (e.g., *Salmonella* and *Shigella*). These effectors exert their activity either by directly affecting actin polymerization or by activating cellular upstream regulators of actin polymerization. Other pathogens like *Listeria*, *Yersinia*, or *Neisseria* can induce their uptake into nonphagocytic cells through stimulation of a unique combination of signaling pathways, which normally would bring about cell adhesion, migration, or growth-factor-induced membrane ruffling. These pathogens do not secrete virulence factors to stimulate their envelopment but instead engage host-cell receptors in an uncommon way or locally bring together an uncommon set of receptors at the sites of bacterial attachment and invasion, both of which individually or in combination exert the optimal host-cell response.

Specific manipulations of the actin cytoskeleton are not only mediating pathogen internalization into nonphagocytic cells but can also prevent uptake by professional phagocytes, as exemplified by the action of *Yersinia* species on these cells, although this aspect of *Yersinia* pathogenesis will not be discussed here.

The final group of pathogens (pathogenic *E. coli* and *Helicobacter pylori*) we will take a closer look at here do not invade their host cells but subvert the actin cytoskeleton from outside with the aim to colonize specialized niches in the stomach or gut and to gain an advantage over commensals with less sophisticated adhesion mechanisms.

Over the years, all these events have been extensively studied and, therefore, now belong to the best-understood facets of host-pathogen interaction. Because various different aspects of host-pathogen interaction accompanied by drastic actin cytoskeleton reorganization or poisoning have recently been reviewed elsewhere (Aktories and Barbieri, 2005; Gouin et al., 2005; Vogelmann et al., 2004), we are mainly aiming here at discussing recent progress in our understanding of the molecular regulation of bacteria-plasma membrane interactions driving local actin reorganizations.

Subversion of the Host-Cell Actin Cytoskeleton Leads to Triggered Invasion of *Salmonella*

Infections with *Salmonella sp.* are a common cause of food-borne gastroenteritis in humans but can also lead to severe systemic typhoid fever. Actin cytoskeleton reorganization accompanying the induced phagocytosis by epithelial cells of nontyphoidal strains of *Salmonella* species like *S. enterica* (Serovar typhimurium) is probably among the most intensely studied paradigms of a bona-fide host-pathogen interaction (Hayward and Koronakis, 2002; Patel and Galan, 2005). Ingested *Salmonellae* interact with the surfaces of epithelial cells of the small intestine after the translocation of so-called effector proteins by a type III secretory system (T3SS) (Galan, 2001), which trigger host-cell actin cytoskeleton rearrangements culminating in bacterial invagination (Figure 1). Noteworthy, the translocon, an integral part of all T3SSs that confers direct host-cell contact and forms the pore through which effectors are delivered, consists of proteins that can act as (translocated) effectors themselves (Buttner and Bonas, 2002; Coombes and Finlay, 2005). One prominent example for this is the pore forming component SipC because it also directly modifies the host cell actin cytoskeleton (see also below). Six effector proteins have so far been demonstrated to directly or indirectly target actin or regulators of the actin polymerization machinery (Figure 2A). *Salmonella* outer proteins (Sops) E, E2, and B induce actin cytoskeletal rearrangements indirectly by activation of the Rho-family GTPases Cdc42 and Rac1. Although SopE is a genuine GTPase exchange factor (GEF) for Cdc42

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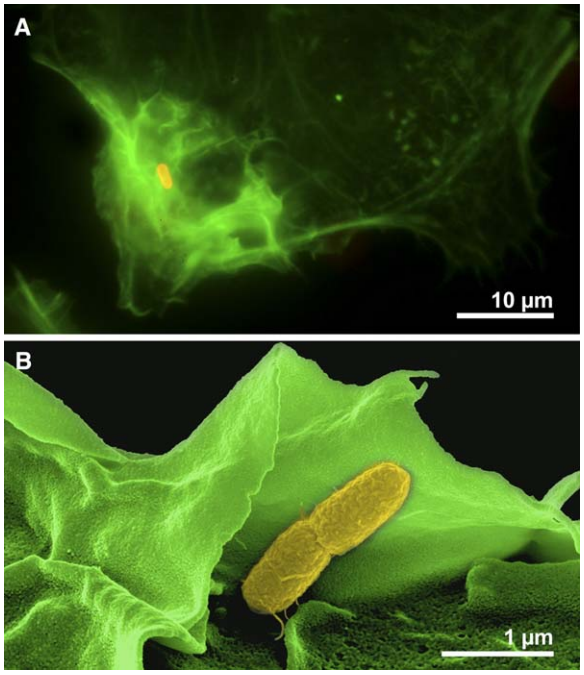

Figure 1. *Salmonella typhimurium* Invasion of Cultured Mammalian Cells

(A) HeLa cell infected with *Salmonella* fixed and stained for the actin cytoskeleton with phalloidin (green) and for *Salmonella* (yellow). The image represents a superimposition of two focal planes acquired by epifluorescence microscopy to allow for visualization of both the membrane ruffles elicited around the bacterium at the top of the cell and the cell body attached to the substrate.

(B) Cos cell infected with *Salmonella* and prepared for scanning electron microscopy. Cell surface extensions in the process of enveloping the bacterium are pseudocolored green, and the microbe is in yellow.

and Rac1, the specificity of SopE2 appears more restricted to Cdc42 (Friebel et al., 2001; Hardt et al., 1998). In contrast, SopB (also termed SigD) is an inositol polyphosphatase, which was suggested to affect invasion by indirect activation of Cdc42 (Zhou et al., 2001) but also by promoting membrane fission (Terebiznik et al., 2002). Remarkably, *S. typhimurium* has not only evolved to stimulate the activition of Rho-family GTPases but also to downregulate them. One prominent effector, termed SptP, displays GAP (GTPase-activating protein) activity toward Rac1 and Cdc42 (Fu and Galan, 1999) and was demonstrated to downregulate cytoskeletal reorganization after entry (Kubori and Galan, 2003).

Two effectors manipulate actin directly, SipA and SipC. Mixed with purified actin, SipA decreases the critical concentration for filament assembly. Moreover, once filaments are formed, SipA binding can straighten and protect them against depolymerization (Zhou et al., 1999). More recent elegant biochemical characterizations revealed that SipA can counteract ADF/cofilin and gelsolin activities by competing with the former for filament interaction or by reannealing gelsolin-capped filaments (McGhie et al., 2004). Remarkably, SipA addition to *Xenopus* extracts could interfere with actin-based particle motility, which—among other factors—requires the actin depolymerizing activity of ADF/cofilin (Loisel et al., 1999). Last, SipA can cooperate with SipC in actin-filament nucleation and bundling (McGhie et al., 2001). Interestingly, SipC-mediated bundling and nucleation of actin filaments can be separated and are confined to the N and C terminus, respectively (Hayward and Koronakis, 1999). As an integral part of the effector translocation machinery, SipC is essential for invasion (Kaniga et al., 1995). However, the precise contribution of the actin-modulating activities of SipC to the elicitation, maintenance, and/or turnover of the membrane ruffles driving *Salmonella* entry are still controversial (Cain et al., 2004; Patel and Galan, 2005). Excitingly, Chang et al. have succeeded recently in separating the effector translocation and actin nucleation activities of SipC. Elimination of residues 201–220 left effector translocation intact but eliminated actin-nucleation activity, which reduced but did not abolish membrane ruffling and invasion (Chang et al., 2005). Similarily, isogenic mutants lacking SipA seem to induce less focal or prominent actin extrusions, but the invasive properties are comparable to wild-type strains, at least, after prolonged invasion times (Higashide et al., 2002; Zhou et al., 1999). However, are Sip proteins by themselves (i.e., in the absence of concomitant Rho-GTPase activation by Sops) capable of inducing actin rearrangements at the plasma membrane reminiscent of the membrane ruffles observed during *Salmonella* entry? In vitro analyses of entry efficiencies of panels of effector mutants suggest that this scenario is quite unlikely because ruffling elicited by SopE/E2 null strains was reduced (Stender et al., 2000), and SopE/E2/B null strains appeared entirely invasion defective (Zhou et al., 2001), at least, in epithelial-like cells (Hapfelmeier et al., 2004). Therefore, SipA and C may modulate rather than induce lamellipodial and filopodial structures, most presumably elicited by Sop-mediated activation of Rac1 and Cdc42. Interestingly, all six effectors have recently been demonstrated by mechanical fractionation to be excluded from the cytosol and to mostly associate with the plasma membrane, although overexpression in noninfected fibroblasts or T3SS-mediated translocation of FLAG-tagged variants revealed quite distinct subcellular localizations (Cain et al., 2004). Most significantly, although SopE and E2 seemed to associate with the tips or along the shafts of induced peripheral filopodia, SopB prominently targeted lamellipodia. In contrast, SipA and C appeared to accumulate on both filopodia- and lamellipodia-like structures at the cell periphery (Cain et al., 2004). The nature of these differences is currently unclear, but differential subcellular positioning of different effector proteins at the entry site may well contribute to the specific role of each of them in *Salmonella*-induced cytoskeletal rearrangements.

The detailed molecular mechanisms driving *Salmonella*-induced actin rearrangements downstream of Sop-driven Rac/Cdc42 activation are currently unclear. GTP bound Cdc42 and Rac1 interact directly and indirectly with WASP/N-WASP and WAVE proteins, respectively (Stradal et al., 2004), both capable of stimulating Arp2/3-complex-mediated actin assembly. Although Cdc42 and Rac1 have been implicated in various cellular functions, the regulation of formation and turnover of dif-

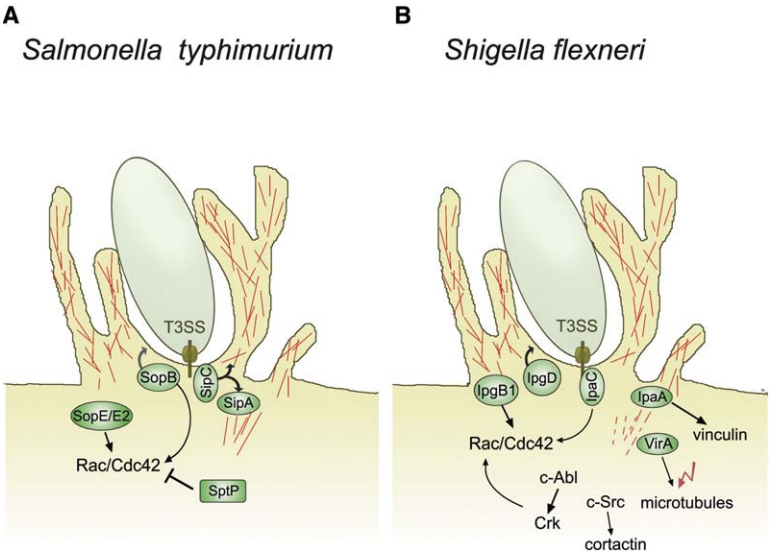

Figure 2. Assault of the Host: the Trigger Mechanism of Bacterial Invasion

Salmonella typhimurium (A) and *Shigella flexneri* (B) are two evolutionary related gram-negative pathogens, both of which induce host cell invasion by delivery of virulence factors employing a mxi/spa type III secretion system (T3SS). During uptake, both pathogens stimulate massive actin-based membrane ruffling, finally resulting in envelopment of the bacterium. Although some of the translocated virulence factors share sequence homologies, elicited signaling appears to differ clearly. (A) *Salmonella* translocates three proteins that are mimetics of small Rho GTPase regulatory enzymes, namely the Rac and Cdc42 GEFs SopE and SopE2 and the GAP SptP, which reverts cellular GTPase levels to normal after invasion. The factor SopB is an inositol polyphosphatase, which impacts indirectly on Cdc42 activation and is, furthermore, important for later steps of intracellular persistence. The effectors SipA and SipC directly interact with actin, thus inducing de novo polymerization and stabilization of F-actin. (B) *Shigella* activates the Rho-GTPases Rac and Cdc42 only indirectly in an IpaC- and IpgB1-dependent fashion. Moreover, *Shigella* invasion is accompanied by activation of Src family kinases leading to cortactin recruitment and phosphorylation. VirA induces local depolymerization of microtubules at bacterial entry sites and was described to increase Rac-dependent membrane ruffling. IpaA binds to the actin binding and focal-adhesion protein vinculin and recruits it to the *Shigella* entry focus. Although IpaA and IpaC are orthologs of the *Salmonella* effectors SipA and SipC, their specific effects on cellular signaling seem to be surprisingly divergent. Moreover, *Shigella* cross talk with the host was described to elicit activation of the tyrosine kinase c-Abl with subsequent Crk phosphorylation, an event that is required for efficient Rac and Cdc42 activation (for details see text). Once invasion is complete, intracellular behavior of *Salmonella* and *Shigella* differs significantly. *Salmonella* resides in and modifies the phagosome, whereas *Shigella* escapes from it acquiring intracellular actin-based motility mediated by the bacterial surface protein IcsA, which recruits the cellular actin polymerization machinery (see Table 1).

ferent types of cellular protrusions, i.e., filopodia and lamellipodia, respectively, are among the most prominent (Raftopoulou and Hall, 2004; Small et al., 2002). Moreover, Rac1 can also be activated downstream of Cdc42 (Hall, 1998), and explanations for how this might occur on the molecular level are now emerging (Baird et al., 2005). In partly conflicting reports, both Rho-GTPases have been ascribed essential functions in *Salmonella* engulfment (Chen et al., 1996; Criss et al., 2001; Unsworth et al., 2004). In addition, Arp2/3-complex sequestration suggested an important function of this actin nucleating machine for *Salmonella* entry (Criss and Casanova, 2003; Unsworth et al., 2004), although interference with N-WASP and/or WAVE functions by dominant-negative approaches had only modest effects on entry efficiencies (Unsworth et al., 2004). Finally, Rac/Cdc42 can interact with additional effectors implicated in actin dynamics and turnover, for instance, p65PAK driving LIM kinase-mediated ADF/cofilin inactivation (Raftopoulou and Hall, 2004). Hence, as observed with other motility processes (Disanza et al., 2005; Loisel et al., 1999), a concerted action of both actin filament nucleators and depolymerizing factors (such as ADF/cofilin) during the different stages of *Salmonella* entry may be required to allow for efficient invasion (Dai et al., 2004; Zhou, 2001).

Together, the exact contributions to Sop-mediated entry of Cdc42 versus Rac activity and of their common as well as distinct downstream actin regulators will require more detailed analyses, certainly including knockout or knockdown approaches in the host.

Triggered Entry of *Shigella*

Shigella flexneri, the causative agent of bacillary dysenteria in humans, invades epithelial cells in a process accompanied by actin cytoskeletal rearrangements reminiscent of those observed during *Salmonella* entry (Tran Van Nhieu et al., 2000). As with the latter pathogen, bacterial invasion also requires a functional T3SS that facilitates translocation of effector proteins (Jennison and Verma, 2004; Tran Van Nhieu et al., 2000). The most prominent translocated virulence factors include the invasion plasmid antigens IpaA, IpaB, IpaC, and IpaD as well as VirA and IpgD (Figure 2B). The detailed molecular mechanisms by which these effectors trigger *Shigella* entry are far from being understood (Tran Van Nhieu et al., 2005). IpaB and IpaC are translocated first and constitute the pore (similar to the *Salmonella* translocon), allowing delivery of additional effectors (Blocker et al., 1999; Tran Van Nhieu et al., 2000). Once secretion is activated, N-terminal IpaC interacts with IpaB, whereas the carboxyl terminus of IpaC affects actin reorganization possibly by two independent means. First, this domain has been implicated in Cdc42 and Rac1 activation, which must occur indirectly because no GEF activity has been detected for this molecule in vitro (Tran Van Nhieu et al., 1999). Strikingly, more recent experiments demonstrated the ability of this domain to directly stimulate actin polymerization, at least with purified proteins (Kueltzo et al., 2003), in analogy to what was reported for its *Salmonella* homolog SipC (see above). The relative contributions of each of these two activities, Cdc42/Rac activation versus direct actin nucleation, to *Shigella*-induced actin reorganizations

are unknown. Another effector, VirA, has been implicated in Rac1 activation by an entirely different mechanism, namely microtubule depolymerization (Yoshida et al., 2002), the repolymerization of which to the cell periphery is thought to coincide with Rac-mediated membrane ruffling (Yoshida and Sasakawa, 2003).

Until recently, IpaC and VirA had been the only effectors thought to mediate Cdc42 or Rac activation. However, a very recent study reports on a novel *Shigella* effector, IpgB1, which is not essential for, but promotes, *Shigella* invasion by activating preferentially Rac1 and more weakly Cdc42 (Ohya et al., 2005). As with *Salmonella*, the Cdc42/Rac interacting proteins driving the downstream actin rearrangments during *Shigella* invasion remain poorly defined (see below). Moreover, a number of reports suggests an involvement of elicitation of tyrosine kinase signaling pathways for efficient *Shigella* entry (Tran Van Nhieu et al., 2005), which may act upstream of or parallel to Cdc42/Rac1 activation. Significantly, cells genetically deficient for both Abl-family tyrosine kinases, Abl and Arg (Abl-related gene), have been shown to display severely impaired efficiencies of *Shigella* uptake, which is thought to result from loss of Abl-mediated phosphorylation of the adaptor protein CrkII and subsequent Rac/Cdc42 activation (Burton et al., 2003). However, Crk has also been implicated in acting independently of or parallel to Rac activation during *Shigella* invasion by interaction of its SH2 domain with tyrosine-phosphorylated cortactin (Bougneres et al., 2004). Cortactin is a major substrate for Src family kinases, also implicated in the entry process (Dumenil et al., 2000), and a well-known actin regulatory protein capable of interacting with both actin filaments and Arp2/3 complex (Selbach and Backert, 2005). Interestingly, cooverexpression of Crk and of full-length cortactin appeared to enhance *Shigella* invasion and *Shigella*-induced memrane ruffling, whereas RNAi-mediated cortactin knockdown abrogated these events. Hence, both cortactin and Crk were conluded to synergize in driving efficient actin assembly during the entry process (Bougneres et al., 2004). However, whether cortactin-driven actin filament assembly involves its interaction with Arp2/3 complex, N-WASP, or both and whether these actin assembly events might synergize with or act in parallel to Rho-GTPase-mediated Arp2/3-complex activation remains to be established (Tran Van Nhieu et al., 2005). Noteworthy, though, cortactin is most presumably not generally required for actin assembly at the plasma membrane because independent studies indicated a stimulation rather than abolishment of lamellipodia protrusion and membrane ruffling upon RNAi-mediated suppression of cortactin expression and—as opposed to *Shigella*—no interference with *Salmonella* invasion (Kempiak et al., 2005; Unsworth et al., 2004).

Finally, Src-family kinases are not only thought to orchestrate early but also late actin cytoskeletal rearrangements accompanying *Shigella* engulfment because they were also reported to be involved in downregulation of Rho, coordinating completion of the entry process (Dumenil et al., 2000; Tran Van Nhieu et al., 2000), although the molecular details accompanying these events remain to be elucidated.

Another invasion plasmid antigen directly targeting a host cytoskeletal protein is IpaA, which was demonstrated to interact with the focal adhesion component vinculin and concluded to drive the depolymerization of actin filaments, at least in vitro (Bourdet-Sicard et al., 1999). IpaA-mediated actin depolymerization was therefore suggested to further aid the localized invagination of the plasma membrane beneath bacteria during late stages of their entry (Tran Van Nhieu et al., 2000).

Yet another mechanism of interfering with actin filament dynamics during *Shigella* engulfment was proposed to be mediated by the virulence factor IpgD, a potent inositol 4-phosphatase specifically dephosphorylating PIP2 (phosphatidylinositol 4,5-biphosphate). Although IpgD is not required for *Shigella* invasion (Allaoui et al., 1993), this activity is thought to loosen the interaction of actin filaments with the plasma membrane, thereby facilitating the insertion of actin monomers onto filaments abutting the plasma membrane (Niebuhr et al., 2002).

Together, the molecular mechanisms of epithelial cell invasion by *Shigella* appear as an increasingly complex concert of activities with the most relevant features presumably being the activation of Rho-family GTPases. Further studies considering both the precise molecular activities of the distinct effector proteins and of their diverse host-cell targets will certainly shed more light on the sequence of events accompanying the intricate phenomenon of *Shigella* invasion.

Interestingly, *Shigella* has a close relative termed EIEC (enteroinvasive *E. coli*). Although most pathogenic *E. coli* strains including EPEC and EHEC (see below) remain extracellular, EIEC are true intracellular pathogens. They also trigger entry into host cells by T3SS-translocated virulence factors, which are highly homologous to those of *Shigella flexneri* such as IpaA, B, and C (Kaper et al., 2004). These and other homologies between *Shigella* and *E. coli* indicate that *Shigellae* taxonomically fall within the *E. coli* species (Escobar-Paramo et al., 2003).

Listeria monocytogenes and Two Pathways for Zippered Entry

Listeriosis caused by food-borne gram-positive pathogens such as *Listeria monocytogenes* causes gastroenteritis but, in severe manifestations, also fetoplacental and central nervous system infections.

L. monocytogenes expresses eight internalins, the function of most of which is unclear, that compose a family of proteins that share differing numbers of leucine rich repeats (LRR) (Vazquez-Boland et al., 2001). The two best-understood mechanisms, both by themselves sufficient to induce uptake into cells, are mediated through Internalin A (InlA) and Internalin B (InlB) (Figure 3A). The receptor for InlA is the cell-cell junction molecule E-cadherin (Mengaud et al., 1996), which is expressed on epithelial cells and mediates tissue cohesion through homophilic interaction with neighboring cells. The signaling complex, which is recruited by *Listeria* during InlA-mediated E-cadherin engagement, very much resembles that of a cellular-adherence junction (Vasioukhin and Fuchs, 2001) and is linked to the actin cytoskeleton via α- and β-catenins and to myosin

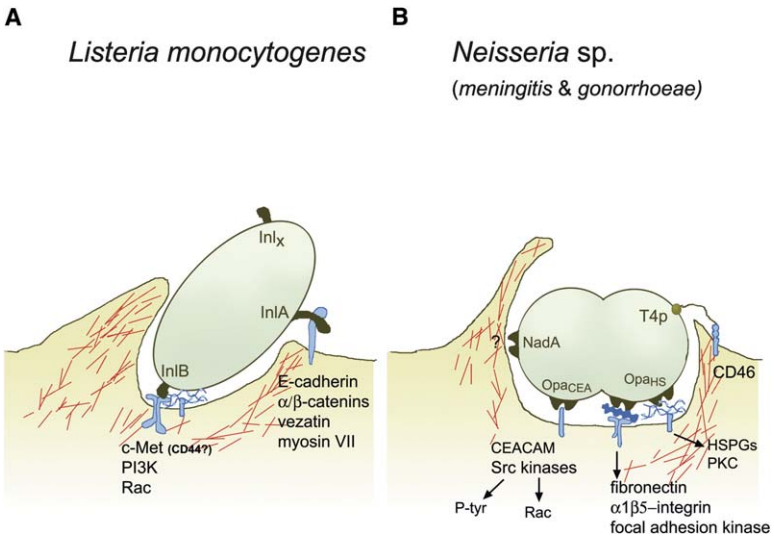

Figure 3. Sneaking into the Host: the Zipper Mechanism of Bacterial Invasion

Listeria monocytogenes (A) and pathogenic *Neisseria* species (B) both invade their target host cells by activating host-cell surface receptors finally leading to their envelopment and internalization. To do so, the pathogens exclusively rely on the signaling cascades elicited upon host cell receptor engagement, and no virulence factor delivery accompanies this type of phagocytosis. (A) *Listeria monocytogenes* can incuce uptake through nonphagocytic cells by two cooperating, albeit separable, mechanisms. The listerial surface protein InlA binds to E-cadherin and elicits signaling and the localized accumulation of proteins that are typically found during cellular-adherence-junction formation. Listerial InlB, which can also be released from the bacterial surface, interacts with the growth factor receptor tyrosine kinase c-Met and glucosaminoglycans—probably the c-Met coreceptor CD44. This induces a signaling cascade typical for growth-factor activation, including RTK-activation, PI3-kinase activation, and subsequent Cdc42/Rac activation, ultimately leading to actin assembly at the site of entry. (B) Pathogenic *Neisseria* contact host cells during early steps of adhesion employing type IV pili that bind to CD46 and elicit signaling, leading to filopodia formation, allowing more intimate contact with the host's plasma membrane. At later stages, pili disappear and *Neisseriae* become closely attached to the host-cell membrane, finally culminating in entry into the cells. This association is mediated by the neisserial surface proteins of the Opa family, which form two classes: Opa_{HS}associates with sugar residues on either cell-surface receptors such as syndecans or extracellular matrix proteins such as fibronectin, which in turn interact with their receptors, the integrins. Opa_{CS} proteins bind to the CEACAM family of cell-surface receptors. These interactions result in signaling cascades typical for the respective receptors and consequently lead to actin assembly and engulfment of the pathogen. Recently, NadA, a novel bacterial surface protein of *N. menigitis* was shown to act as an invasin and, thus, promote invasion of host cells by an unknown mechanism.

VII via vezatin (Sousa et al., 2004). Moreover, Kovacs and colleagues have described that E-cadherin engagement coincides with localized Arp2/3 complex and PI3-kinase activation (Kovacs et al., 2002). All these events may contribute to the coordination of the actin rearrangements accompanying *Listeria* engulfment (Cossart et al., 2003; Cossart and Sansonetti, 2004). InlB was identified as a ligand for c-Met, the receptor of HGF/SF (hepatocyte growth factor/scatter factor) via its N-terminal LLR portion (Shen et al., 2000) and for glycosaminoglycans (GAGs) through its unique C terminus (also compare to *Neisseria* Opa_{HS}-induced signaling, see below). Simultaneous interaction of InlB with c-Met and GAGs appears to cooperate in eliciting host-cell signaling (Jonquieres et al., 2001). Interestingly, the coreceptor of c-Met is the heparin-sulfate-modified cell-surface protein CD44 (van der Voort et al., 1999). InlB interactions with c-Met and probably CD44 lead to activation of PI3-kinase, Rho-family GTPases such as Rac1, and subsequent engagement of a number of established downstream mediators of actin assembly, finally resulting in actin rearrangements reminiscent of growth-factor-induced ruffling (Cossart et al., 2003; Cossart and Sansonetti, 2004). Notably, although soluble InlB induces membrane ruffling virtually identical to HGF, *Listeria*—when challenging the cell with surface bound InlB—induces a smooth type of entry known as the "zipper type" lacking massive cell-surface projections. Recently, employing cells devoid of the InlA receptor E-cadherin, Bierne and colleagues have concluded that WAVE-complex-mediated Arp2/3 activation downstream of Rac is the predominant actin polymerization mechanism driving InlB-mediated *Listeria* entry (Bierne et al., 2005).

How Pathogenic *Neisseria* Species Gain Access to Their Hosts

Pathogenic *Neisseria sp.* comprising *N. gonorrhoea* and *N. meningitis* are highly adapted gram-negative bacterial pathogens that initiate tissue infection by using a variety of bacterial surface proteins to interact with numerous host-cell receptors (Merz and So, 2000). Colonization begins with a multistep adhesion cascade followed by invasion, persistence within the cell, and finally exit at the basolateral surface to colonize deeper tissue layers (Plant and Jonsson, 2003). The way *Neisseria sp.* subverts the host's actin cytoskeleton is complex, part of which is due to the fact that cell-type-specific signaling cascades that are not yet well understood are activated (Figure 3B and see below). In recent years, several host-cell receptors and signaling pathways connected to them have been identified for the major bacterial adhesins and invasins (Popp et al., 2001). For instance, neisserial type IV pili are implicated in mediating initial attachment of both Meningococci and Gonococci, and this association has been shown to involve contact with the cellular receptor CD46, which also serves as a receptor for the streptococcal M protein (reviewed in Plant and Jonsson [2003]). Receptor engagement through piliated *Neisseriae* deficient for Opa proteins (see below) was shown to be sufficent for cortical actin rearrangements and clustering of tyrosine-phosphorylated proteins (Merz and So, 1997). Moreover, pathogenic *Neisseria sp.* encode a family of antigenically distinct Opa (denotes opacity associated) proteins that allow intimate bacterial attachment to almost every cell type encountered during infection. Opa proteins are integral outer membrane proteins (Omp) with a typical membrane-inserted β-barrel structure

and loops forming the extracellular interaction surface for host-cell binding. Gonococci harbor up to 12 and Meningococci up to four different *opa* genes, the expression of which can vary during infection, a phenomenon termed phase variation. Opa proteins are roughly subdivided into two classes according to their preferred receptors: some Opa variants bind cell-surface-expressed heparan sulfate proteoglycans (HSPG)—termed Opa_{HS}—including members of the syndecan family of receptors and extracellular matrix proteins such as fibronectin and vitronectin, which then interact with and activate integrins (see below). Other variants (Opa_{CS}) bind members of the carcinoembryonic antigen family of cellular adhesion molecules (CEACAM) (reviewed in Gray-Owen [2003] and Hauck and Meyer [2003]). HSPG-mediated entry can be blocked by chemical inhibitors of actin polymerization, tyrosine kinases, or protein kinase C (PKC), suggesting that this type of entry is linked to a multitude of cellular-signaling networks, resulting in efficient actin-based invasion, although the details are just beginning to emerge (reviewed in Hauck and Meyer [2003]). To emphasize further the diversity of *Neisseria* host-cell interactions, *N. meningitis* was also shown to induce Erb-B2 clustering and to elicit a Src-kinase inhibitor-sensitive phosphotyrosine-signaling cascade involving the local recruitment of cortactin (Hoffmann et al., 2001). A recent report described the identification of NadA, yet another invasion protein of *N. meningitis*. So far, its host-cell receptor has remained elusive (Capecchi et al., 2005).

Integrin-Mediated Invasion of Enteropathogenic *Yersinia* Species, *Streptococcus pyogenes* and *Staphylococcus aureus*

Exploitation of integrin signaling is a common strategy of enteropathogenic *Yersinia* as well as of the occasionally invasive pathogens *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Neisseria* (see above) to mediate adhesion to host tissues and their uptake into nonphagocytic cells. Enteropathogenic *Yersinia* (*Y. enterocolitica* and *Y. pseudotuberculosis*) are translocated from the intestinal lumen into local lymph nodes and, upon spreading into deep organ sites, can cause a systemic infection that can lead to enteritis, enterocolitis, or even autoimmune disorders (Naktin and Beavis, 1999). *S. aureus* causes a wide range of persistant infections and toxigenic illnesses in humans varying from arthritis to pneumonia and endocarditis, whereas *S. pyogenes* infections can result in rather mild diseases such as pharyngitis but can also cause severe complications such as necrotizing fascilitis and toxic shock-like syndrome.

During the initial infection phase, *Yersinia* adhere to specialized cells within the gut epithelium, the M cells, primarily through the outer membrane protein invasin. Noteworthy, M cells were described to be an important route of entry into the host also for other enteroinvasive pathogens such as *Salmonella*, *Shigella*, and *Listeria* (see Cossart and Sansonetti [2004]). *Yersinia* invasin was initially identified as a protein that upon expression in *E. coli*, conferred its uptake into mammalian cells and is an important early model for bacterial invasion that was investigated in great detail including atomic resolution of invasin (Hamburger et al., 1999). Members of the β_1 chain integrin family serve as mammalian cell receptors, and invasin binds $\alpha_5\beta_1$ integrin with a 100-fold higher affinity than the natural ligand, fibronectin, by using identical or closely located binding sites on integrin, thereby ensuring efficient uptake of tightly adhering bacteria (for a recent review see Wong and Isberg [2005]). At later stages of infection, *Y. pseudotuberculosis* employs another bacterial surface factor, the YadA protein (Bliska et al., 1993), which was recently reported to be the predominant adhesin in infected tissues. Fibronectin and β_1-integrin-specific antibodies could block YadA-driven invasion of *Y. pseudotubercolosis*, showing that β_1-integrins can mediate YadA-triggered cell invasion via ECM components (Eitel and Dersch, 2002).

Specific binding of ECM proteins, e.g., of fibronectin, to *S. aureus* cells was initially described 27 years ago (Kuusela, 1978) and led to the isolation of a fibronectin binding protein from *S. aureus* cells and the identification and characterization of two staphylococcal genes, *fnb*A and *fnb*B, encoding fibronectin binding proteins. Adherence of *Streptococcus pyogenes* to fibronectin led to identification of the fibronectin binding protein (Sfb1, protein F) of this organism (reviewed in Patti et al. [1994]). Both *Staphylococci* and *Streptococci* have long been considered to be extracellular pathogens, which upon binding to extracellular matrix proteins, exerted their virulence. Later, invasion of nonphagocytic cultured mammalian cells by *S. pyogenes* was observed (LaPenta et al., 1994), and this process was found to depend on fibronectin (Molinari et al., 1997) and on integrins (Ozeri et al., 1998). This was subsequently also verified for *S. aureus* (Sinha et al., 1999) explaining why certain antibiotics that do not readily penetrate the mammalian cell membrane or other specific treatments often fail to eradicate these pathogens from infected persons. Thus, these pathogens exploit integrin-mediated signaling in a highly sophisticated manner to achieve intracellular persistence.

How does integrin engagement lead to bacterial invasion? Complex biological processes such as cell migration require signaling molecules at the inner surface of the plasma membrane that can act as integrators in response to multiple extracellular stimuli. The focal adhesion kinase (FAK) fulfills such a function by linking both growth factor- and integrin-signaling to the actin cytoskeleton. FAK colocalizes with integrins at focal adhesions and, upon activation, associates with Src-family tyrosine kinases, which then promote tyrosine phosphorylation of various substrates, resulting in massive cytoskeletal reorganization, a prerequisite for cellular motility processes (reviewed in Schlaepfer and Mitra [2004]).

Therefore, it is not surprising that various microbial pathogens exploit integrin-induced cytoskeletal reorganization to achieve actin-driven microbe uptake. Overexpression of a dominant-negative form of FAK and of Src derivatives, respectively, reduced invasin-mediated uptake of *Yersinia* (Alrutz and Isberg, 1998). Moreover, the central role of FAK in integrin signaling is well reflected by FAK-deficient fibroblasts (Ilic et al., 1995), which exhibit not only an increased number and size of focal adhesions that are less dynamic but also defects in cell migration, all of which can be reconstitu-

ted by expression of wild-type FAK. FAK-null fibroblasts as well as Src-family kinase-deficient fibroblasts (SYF, deficient in Src, Yes, and Fyn) (Klinghoffer et al., 1999) have been instrumental for the analysis of integrin-mediated invasion of *S. aureus* (Agerer et al., 2003, 2005; Fowler et al., 2003) as well as of YadA-mediated *Yersinia* invasion (Eitel et al., 2005). FAK-null fibroblasts and SYF-deficient cells did not support invasion of these pathogens, defects that could be restored upon expression of wild-type FAK or c-Src, respectively.

The signaling complex that is recruited by these pathogens upon integrin engagement resembles that of focal adhesions and includes paxillin, vinculin, tensin, zyxin, and talin (Agerer et al., 2005; Ozeri et al., 2001). The exact contributions of these focal adhesion proteins to the bacterial entry process will require more detailed analyses by using knockout and/or knockdown approaches on the host side. To add another level of complexity, binding of *S. aureus* to host cells triggers recruitment of cortactin, which does not localize at focal adhesions (Wu and Parsons, 1993), and its subsequent tyrosine phosphorylation. Whereas local recruitment of cortactin still occurred in FAK null cells, its tyrosine phosphorylation was abolished, suggesting that cortactin recruitment to the bacterial attachment site does not depend on FAK. Moreover, RNAi-mediated knockdown of cortactin impaired bacterial uptake (Agerer et al., 2005). How this novel link between FAK and cortactin signaling is accomplished at the molecular level is currently unknown. Another still-unresolved issue is how integrin signaling, induced by pathogen engagement, is linked to the activation of small GTPases, which was reported to be crucial for the uptake process of *Yersinia* (Alrutz et al., 2001; Wong and Isberg, 2003). In this respect, two studies are worth mentioning that analyzed the role of N-WASP in invasin-mediated uptake of *Yersinia*. Whereas ectopic expression of N-WASP mutants reduced uptake (McGee et al., 2001), internalization was unaffected in a cell line lacking expression of WASP and N-WASP (Alrutz et al., 2001).

N-WASP Exploitation Across the Plasma Membrane: EPEC and EHEC

As opposed to enteroinvasive pathogens such as *Listeria*, *Shigella*, or *Salmonella*, enteropathogenic and enterohaemorrhagic *E. coli* (EPEC and EHEC) do not usually invade their targets, epithelial cells of the intestinal mucosa, but induce focal actin filament assemblies just across the plasma membrane, so-called pedestals on which these bacteria reside (Figure 4A) (Sanger et al., 1996). EPEC and EHEC are two closely related diarrheagenic pathogens. EPEC is a major cause of diarrhea in developing countries and mainly spread through feces contamination in drinking water, whereas its Shiga-toxin-producing relative, EHEC, is common and without symptoms in cattle but highly infectious for humans.

Pathogenic *E. coli* strains possess specific adherence factors that allow them to establish at sites that *E. coli* normally do not inhabit, such as the small intestine, to instigate colonization of the target tissue. These adhesins can form distinct morphological structures such as type IV pili of EPEC or EspA filaments, which are part of the T3SS (reviewed in Kaper et al. [2004]). The diseases caused by EPEC and EHEC are prominently linked to the delivery of bacterial virulence factors again through a T3SS leading to formation of "attaching and effacing" (A/E) lesions in the gut. To do so, the bacteria build up an intimate contact to intestinal cells, which is accompanied by the effacement (eradication) of microvilli and the accumulation of actin cytoskeletal complexes at the plasma membrane beneath the bacteria (reviewed in Dean et al. [2005] and see below). Remarkably, a key feature of signaling to actin cytoskeleton reorganization as induced on the plasma membrane of their hosts by the diversity of bacterial pathogens dicussed here is the activation of members of small Rho-family GTPases. There is only one exception, pathogenic *E. coli* such as EPEC and EHEC, which despite signaling to actin assembly across the plasma membrane, have evolved mechanisms to circumvent Rho-family GTPases (Ben-Ami et al., 1998; Campellone and Leong, 2003), at least for inducing actin pedestal formation (Figure 4A). Instead, these pathogens usurp the host-cell actin-polymerization machinery differently, perhaps more directly, comparable to the intracellular actin-based motility of *Listeria* or *Shigella* (for a recent review see Gouin et al. [2005] and below).

Genes encoding the T3SS and most proteins secreted via this machine are encoded on a genomic segment termed the LEE (for "locus of enterocyte effacement") pathogenicity island.

The needle of the T3SS connected to the outer bacterial surface is composed of EscF, which, in turn, directly connects to the translocon syringe made of EspA (denotes *E. coli* secreted protein A) (Wilson et al., 2001). The pore-forming components in this case are termed EspB and EspD and mediate effector translocation to the host-cell cytosol (reviewed in Roe et al. [2003]).

The only translocated effector absolutely essential for triggering actin pedestal formation is the translocated intimin receptor Tir (Deibel et al., 1998; Kenny et al., 1997), which is quite homologous in both EPEC and EHEC, although the mechanisms evolved for signaling to cellular actin assembly have diverged (see below). Tir acts as a transmembrane receptor, with both N and C terminus facing the host-cell cytosol and the central domain remaining extracellular and constituting an interaction surface for the LEE-encoded bacterial protein intimin (Batchelor et al., 2000; Luo et al., 2000). For both EPEC and EHEC, numerous actin regulatory proteins have been found to colocalize with pedestals (Campellone and Leong, 2003; Goosney et al., 2001). Of these components, only a few such as N-WASP (for both EPEC and EHEC) or the SH2/SH3 adaptors Nck1 and 2 (for EPEC) have been demonstrated to accumulate at the pedestal tips just below the plasma membrane, indicating a critical function of them in signaling to actin assembly (Campellone and Leong, 2003; Lommel et al., 2001, 2004). Indeed, by employing elegant FRAP (fluorescent recovery after photobleaching) experiments, it was now demonstrated that actin polymerization is initiated and maintained at the pedestal tip, allowing for a continuous flow of polymerized actin filaments down the pedestal (Shaner et al., 2005).

Recently published work suggests that the number of host-cell proteins essential for actin assembly at the

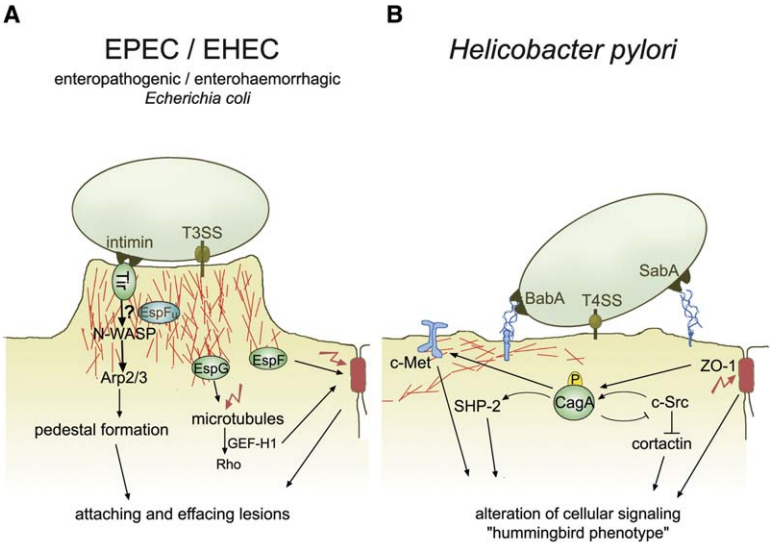

Figure 4. Siege of the Host: Modification of the Actin Cytoskeleton from Outside

Gram-negative pathogenic *E. coli*, EPEC and EHEC (A), and *Helicobacter pylory* (B) closely attach to but do not invade their host cells. (A) EPEC and EHEC inject their own receptor (called Tir) via a T3SS. The extracellular domain of Tir interacts with the bacterial surface protein intimin leading to its clustering and signaling to the actin polymerization machinery. N-WASP-mediated Arp2/3 activation is essential for pedestal formation. In the case of EPEC, this involves tyrosine phosphorylation of Tir and recruitment of SH2 domain containing adaptor proteins such as Nck. In contrast, EHEC-Tir lacks tyrosine phosphorylation sites, and EHEC translocate an additional bacterial effector ($EspF_U$) driving N-WASP activation. However, the exact mechanisms of N-WASP recruitment and/or activation in both EPEC and EHEC-mediated pedestal formation remain to be defined (for details see text). The additional bacterial effectors EspF and EspG are not directly involved in pedestal formation but affect epithelial barrier function. EspG may exert its function via microtubule depolymerization- and GEF-H1-mediated Rho activation. (B) *Helicobacter pylori* adheres to protein- and lipid-anchored sugars on the surface of gastric mucosa cells through its surface bound adhesins BabA and SabA, respectively. *H. pylori* employs its cag type IV secretion system (T4SS) to deliver CagA and probably other factors to the host cytosol. CagA becomes phosphorylated by src kinases and subsequently suppresses src activity on cellular substrates of these kinases, leading to dephosphorylation of, for example, cortactin. Moreover, phospho-CagA binds to the protein phosphatase SHP-2 and activates it, further contributing to host-cell-protein dephosphorylation. In a phosphorylation-independent manner, CagA also triggers c-Met activation and recruitment of tight junction proteins like ZO-1 and JAM, thereby disturbing epithelial barrier function. In isolated gastric epithelial cells, these actions of CagA induce the "hummingbird phenotype" characterized by cell scattering, elongation, and induction of migration.

pedestal tips may be limited, and most additional factors may have either redundant or modulating functions (Campellone et al., 2004a). In 1999, Kalman et al. first reported an accumulation of both N-WASP and Arp2/3-complex in EPEC pedestals, and expression of dominant negative WASP constructs suggested an important function of WASP/N-WASP in pedestal formation (Kalman et al., 1999). Employment of N-WASP-null fibroblasts and complementation with a panel of N-WASP mutants proved that N-WASP is indeed essential for actin assembly and pedestal formation induced by both EPEC (Lommel et al., 2001) and EHEC (Lommel et al., 2004) and allowed the separation of N-WASP modules mediating recruitment versus stimulation of actin assembly. Not surprisingly, the C-terminal WA domain required for Arp2/3 binding and activation turned out to be absolutely essential for actin assembly at sites of EPEC/EHEC attachment. Nevertheless, these pathogens have evolved distinct strategies to accomplish N-WASP recruitment. Interestingly, the C-terminal domains of Tir proteins from EPEC and EHEC are not as homologous as the rest of the proteins, with the most striking difference being a tyrosine residue at position 474 of Tir_{EPEC}, which is absent from Tir_{EHEC}, and the phosphorylation of which appears critical for EPEC pedestal formation (Kenny, 1999). In addition, C-terminal Tir_{EPEC} was demonstrated capable of directly interacting with the SH2-domain of the SH2/SH3 adaptor proteins Nck1 and Nck2 (Gruenheid et al., 2001). Excitingly, subsequent elegant experiments employing an ectopically expressed plasma membrane targeted Tir_{EPEC} derivative revealed that experimental Tir clustering was sufficient to trigger its phosphorylation, Nck recruitment, and actin assembly, demonstrating that—besides Tir—EPEC pedestal formation does not require any additional bacterial effectors (Campellone et al., 2004a). However, because Nck-deficient cells were shown more recently still to be able to form pedestals, albeit four times less frequently than Nck-proficient cells (Campellone et al., 2004b), it's becoming clear that both Nck-dependent and -independent pathways contribute to N-WASP and Arp2/3 recruitment during EPEC pedestal formation. Notably, Nck-independent actin assembly is mostly driven by interaction of an unknown host-cell SH2 adaptor with the region around phosphorylated Tyr474 and—to a lesser extent—with residues around an additional, newly identified tyrosine residue, Tyr454 of Tir_{EPEC} (Campellone and Leong, 2005). Interestingly, the A36R protein of Vaccinia virus, which is essential for the actin-based motility of this pathogen on the cell surface, also harbors two phosphorylatable tyrosine residues (Y112 and Y132) that by direct interaction with Nck and Grb2, respectively, synergistically mediate N-WASP-recruitment and activation at the viral surface (Frischknecht et al., 1999; Scaplehorn et al., 2002). This remarkable example of convergent evolution highlights the significance of N-WASP in driving focal actin assembly at the plasma membrane. Finally, attempts to identify the host cell kinase (or kinases) responsible for Tir phosphorylation also revealed a remarkable redundancy because both Abl/Arg and Src tyrosine kinases appeared capable of independently phosphorylating Tir and of initiating EPEC pedestal formation (Phillips et al., 2004; Swimm et

al., 2004). Nck can directly stimulate N-WASP to activate Arp2/3-complex-mediated actin assembly in vitro via interaction of its SH3 domains with the polyproline domain of N-WASP (Rohatgi et al., 2001). However, the observation that N-WASP recruitment to EPEC pedestals involves its N terminus rather than the polyproline domain (Lommel et al., 2001) suggests an additional (host cell) player involved in linking Tir to N-WASP during EPEC pedestal formation whose nature remains elusive.

Remarkably, although EHEC do not appear to mimic a host-cell tyrosine kinase signaling pathway (Campellone and Leong, 2003), pedestal formation still requires N-WASP-dependent Arp2/3 recruitment and activation (Lommel et al., 2004). Importantly, Tir_{EHEC} was reported not to be functional for actin pedestal formation when expressed in EPEC strains lacking Tir_{EPEC} (DeVinney et al., 2001; Kenny, 2001), which suggested that an additional EHEC factor is required to induce actin assembly downstream of Tir_{EHEC}. Excitingly, two studies have now independently identified an EspF-like protein encoded from the cryptic prophage 933U, which was demonstrated to be crucial for N-WASP-driven actin assembly induced by EHEC (Campellone et al., 2004b; Garmendia et al., 2004). Importantly, coexpression of this protein termed either TccP (Tir-cytoskeleton coupling protein) or $EspF_U$ (referred to as $EspF_U$ below) with Tir_{EHEC} in an EPEC Tir-deletion mutant is sufficient to restore efficient actin pedestal formation. $EspF_U$ was also demonstrated to directly bind the so-called GTPase binding domain (GBD) of N-WASP (Campellone et al., 2004b), a region containing a fragment demonstrated previously to be sufficient to target N-WASP to sites of EHEC attachment (Lommel et al., 2004). Moreover, $EspF_U$ can directly stimulate N-WASP to activate Arp2/3-mediated actin assembly in vitro (Garmendia et al., 2004). Hence, this protein appears as yet another bacterial factor capable of directly usurping the prominent host-cell actin regulator N-WASP. Because of the lack of an apparent interaction of $EspF_U$ with Tir_{EHEC} (Campellone et al., 2004b; Garmendia et al., 2004), pedestal formation induced by EHEC may be accomplished by linking Tir_{EHEC} via an unkown host protein (or proteins) to EHEC-derived $EspF_U$, which in turn mediates N-WASP activation. In spite of this exciting progress, future studies are needed to unravel the missing links between Tir receptors from both EPEC and EHEC and the actin nucleation promoting factor N-WASP driving the actin reorganization events induced by these pathogens.

Infections with EPEC and EHEC are not only accompanied by pedestal formation and loss of absorbtive microvilli, but also by dysregulation of tissue permeability leading to loss of ions and liquid. This is thought to be caused by alterations of tight junction complexes as exemplified by relocalization of occludin during infection (Simonovic et al., 2000). For EPEC infections, it was established that the proline-rich EspF, probably in concert with other secreted proteins such as Map, affects occludin localization and intestinal barrier function by an unknown mechanism (Dean and Kenny, 2004; McNamara et al., 2001). Notably, involvement in epithelial barrier disruption was also found for EHEC EspF and, in addition, for its cryptic prophage, encoded EspF-related protein $EspF_U$ (Viswanathan et al., 2004) that is also critical for pedestal formation (see above). In addition, the translocated effector EspG (and its isogene orf3), which is similar to the translocated *Shigella* factor VirA (Elliott et al., 2001), does not only disrupt microtubules at sites of bacterial attachment (Matsuzawa et al., 2004) but also disturbs epithelial barrier function (Tomson et al., 2005). Moreover, EspG translocation was suggested to elicit RhoA activation via the microtubule-regulated guanine nucleotide exchange factor GEF-H1 (Matsuzawa et al., 2004). Strikingly, GEF-H1 was also shown to target to tight junctions in polarized epithelial cells, where it regulates paracellular permeability (Benais-Pont et al., 2003). Further research on the molecular mechanisms of EspF (and $EspF_U$ for EHEC) and EspG function in disturbing the intestinal barrier is expected to improve our understanding of the disease.

Global Actin Rearrangements Induced by *Helicobacter pylori*

The spiral gram-negative bacterium *Helicobacter pylori* induces actin cytoskeletal rearrangements also without invading their host cells, although these are more global than the ones observed for EPEC and EHEC. *H. pylori* manipulates actin cytoskeletal organization of its host cells by attaching through the adhesins SabA (sialic acid binding adhesin) and BabA (blood group antigen binding adhesin) followed by type IV secretion of the virulence factor CagA (Figure 4B). A very recent discussion of the secreted vacuolating cytotoxin VacA can be found elsewere (Cover and Blanke, 2005). Persistent colonization of the gastric epithelium with *H. pylori* is correlated with an increased risk to develop chronic type B gastritis, peptic ulceration, gastric carcinoma, and MALT (mucosa-associated lymphoid tissue)—lymphoma (for review see Ernst and Gold [2000]). The close association with epithelial cells and the covering mucin layer allows the bacterium to alter cellular signal transduction processes, in particular by action of the so-called cag type IV secretion system (T4SS) (reviewed in Cascales and Christie [2003]), which leads to complex actin cytoskeletal rearrangements summarized as "hummingbird phenotype" (reviewed in Hatakeyama [2004]; Rieder et al., 2005).

Contact is initiated by binding of *H. pylori* to carbohydrate structures on the surface of gastric epithelial cells, such as the fucosylated Lewis b (Leb) histo-blood group antigen and the sialyl Lewis x (s-Lex) glycosphingolipid, which act as receptors for the adhesins BabA (Aspholm-Hurtig et al., 2004; Ilver et al., 1998) and SabA (Mahdavi et al., 2002), respectively (reviewed in Rieder et al. [2005]). The translocated CagA is a 120–145 kDa (depending on the variable C terminus in different strains) protein and the major antigen of *H. pylori* associated with the development of disease, particularly of gastric cancer (Blaser et al., 1995; Crabtree et al., 1991). The *CagA* gene is localized on a segment of the *H. pylori* genome termed the cag pathogenicity island, which additionally encodes the components of the cag T4SS.

T4SS in *H. pylori* serves as a molecular syringe to deliver CagA and presumably other factors to the host-cell cytosol (Odenbreit et al., 2000). The hummingbird phenotype is developed upon translocation of CagA to the cytosol of gastric epithelial cells and characterized

Table 1. Pathogens Capable of Actin Comet Tail/Pedestal Formation

Pathogen	Virulence Factor	Host-Cell Factors Driving Actin Assemby	References
Shigella flexneri	IcsA	N-WASP, Arp2/3	Reviewed in Gouin et al. [2005] and Rottner et al. [2004]
Listeria monocytogenes	ActA	Arp2/3, VASP[b]	Reviewed in Gouin et al. [2005] and Rottner et al. [2004]
EPEC	Tir[c]	Nck1/2[d], WASP/N-WASP, Arp2/3	(Campellone and Leong, 2005; Lommel et al., 2001)
EHEC	Tir[c], $EspF_U$	WASP/N-WASP, Arp2/3	(Campellone et al., 2004b; Garmendia et al., 2004; Lommel et al., 2004)
Rickettsia conorii	RickA[a]	Arp2/3[a]	Reviewed in Gouin et al. [2005]
Mycobacterium marinum	?	WASP[a], Arp2/3[a]	(Stamm et al., 2003)
Burkholderia pseudomallei	BimA	actin[e]	(Stevens et al., 2005)

List of virulence factors and essential host cell factors recruited by the respective pathogens to induce actin-tail formation. Note, that in vitro reconstitution of *Listeria* and *Shigella* motility revealed that capping protein and ADF/cofilin are also essential for actin-tail formation but are not recruited by the pathogen.
[a] Not formally proven to be essential.
[b] Not essential but important factor recruited by the ActA.
[c] Pathogen resides extracellular and factor acts through the plasma membrane.
[d] Can be substituted by other host-cell factors as demonstrated in Nck 1/2 knockout cells.
[e] Not formally proven to be sufficient.

by cell scattering, cell elongation, and increased motility (reviewed in Cascales and Christie [2003]; Rieder et al., 2005). Upon translocation, CagA is localized at the plasma membrane and becomes tyrosine phosphorylated by cellular Src-family kinases (Selbach et al., 2002; Stein et al., 2002) at residues in the variable C terminus of CagA, known as EPIYA-motifs (reviewed in Hatakeyama [2004]). Phospho-CagA binds to the tyrosine-phosphatase SHP-2 (Higashi et al., 2002) via the SHP-2 SH2 domains and activates it. Prevention of both, CagA phosphorylation and phospho-CagA/SHP-2 complex formation, prevents development of the hummingbird phenotype (reviewed in Hatakeyama [2004]; Higashi et al., 2002). Notably, deregulation of SHP-2 also plays a role in the development of haematological malignancies (Tartaglia et al., 2003), providing a potential link to MALT-lymphoma development. Another mechanism by which CagA could contribute to cellular transformation was suggested by Mimuro and colleagues, who found that phospho-CagA can associate to the SH2-domain of the Grb2 adaptor, leading to activation of SOS and, thus, of Ras, inducing growth factor-like downstream signaling (Mimuro et al., 2002). In addition, CagA phosphorylation results in subsequent dephosphorylation of the actin-associated protein cortactin, which is then translocated to cellular processes, the formation of which typically accompanies the morphological changes of the hummingbird phenotype (Selbach et al., 2003). Moreover, CagA was described to interact with the protooncogene c-Met, the hepatocyte growth factor receptor, and, thus, to contribute to the onset of motogenic signaling (Churin et al., 2003). Finally, CagA was found to interact with proteins of the epithelial tight-junction complex ZO-1 and JAM in a phosphorylation-independent manner, leading to alteration and disruption of this subcellular compartment (Amieva et al., 2003). The list of signaling alterations caused by CagA could be continued here (reviewed in Hatakeyama [2004]; Rieder et al., 2005) and is likely to grow further.

Together, CagA interacts with a panoply of host-cell signal transducers in phospho tyrosine-dependent and -independent fashions, thereby simultaneously perturbing numerous signaling pathways that ultimately promote gastric carcinogenesis. Finally, although no factor other than CagA was characterized so far to be secreted by the T4SS or to have an impact on *H. pylori* virulence, Churin and colleagues have found activation of the Rho-family GTPases Rac1 and Cdc42 in gastric epithelial cells, which depended on an intact T4SS but was independent of CagA translocation (Churin et al., 2001).

Epilog: Intracellular Motility of Bacterial Pathogens

Another common theme in bacterial usurpation of the host's actin cytoskeleton is the onset of intracellular motility. This phenomenon has so far been observed for diverse pathogens including *Listeria monocytogenes*, *Rickettsia conorii*, *Shigella flexneri* (and EIEC), *Burkholderia pseudomallei*, or *Mycobacterium marinum*. For most of these pathogens, bacterial and/or cellular factors have been identified that are essential for or at least contribute to polarized actin polymerization leading to rocketing motility. So far, the overwhelming majority of bacterial factors identified utilize Arp2/3 complex activation to promote actin tail formation (see Table 1). However, an in depth discussion on the different strategies employed to allow for Arp2/3-complex recruitment and/or activation or on other cellular factors that aid actin-based motility and efficient vectorial spreading can be found elsewhere (for recent reviews see Gouin et al. [2005] and Rottner et al. [2004]).

Concluding Perspectives

The availability of extensive new genomic information and the rapid technological advance in molecular and cell biology will continue to drive the analysis of host-pathogen interaction. The rapid progress in sequencing of the complete genomes of microbial pathogens already enables identification of new potential effector proteins, the function of which can be analyzed not only in vivo by site-directed mutagenesis but also in in vitro systems by using heterologous expression. Screening of microbial genomes from less well-characterized or-

ganisms for gene products with homologies to already-known effector proteins will allow for more-detailed comparative analyses of related effector proteins ultimately leading to an understanding of the common and diverse molecular mechanisms of virulence. Moreover, systematic interference with constituents of complex host-cell-signaling cascades by RNAi or genetic inactivation can be converged now with the exploitation of microbial libraries in which every potential virulence factor has been inactivated. Together, these approaches are expected to increase our knowledge not only on pathologic manifestation but also on the regulation of cell signaling.

Acknowledgments

We would like to thank all former and present members of our lab who have contributed to the work dicussed here and Manfred Rohde for kindly providing the scanning electron micrograph used in Figure 1B. This work was supported in part by the Deutsche Forschungsgemeinschaft (SFB 621 to K.R. and J.W. and SPP1150 to K.R. and T.E.B.S.) and the Fonds der Chemischen Industrie (to J.W.).

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