Bacterial Invasion: In Vivo Veritas

A major issue is to validate, in vivo, the molecular and cellular events analyzed in vitro. If one focuses on invasion of the intestinal barrier, it is clear that *L. monocytogenes*, *Shigella*, *Salmonella*, and *Yersinia*, despite their shared capacity to invade epithelial cells in vitro, differ with regard to (i) the capacity to disrupt, invade, and eventually cause the inflammatory destruction of the epithelium; and (ii) the possibility of proceeding to systemic dissemination and possibly colonization of organs at a distance.

A major handicap to studying the respective invasive phenotypes in vivo has been the lack of a mouse model simulating the intestinal and systemic diseases observed in humans (*67*). This was particularly the case for *L. monocytogenes*, until a transgenic mouse line expressing the human E-cadherin receptor of internalin became available, thus unlocking the transintestinal route for this pathogen, i.e., via invasion of enterocytes (*68*). A relevant animal model has yet to be found for *Shigella* because, unlike infected humans, mice do not undergo extensive invasion and inflammatory destruction of their rectal and colonic mucosae. Despite these limitations, a picture is emerging (Fig. 5) concerning the various strategies used by these pathogens.

In conclusion, although current work aims to elucidate the in vivo relevance of the now well-understood mechanisms used by invasive bacteria in vitro, future efforts should focus on understanding both bacterial and host cell transcription and translation programs during infection, in various cells and tissues. This information should provide vital clues in the ongoing battle against bacterial disease and for elaborating new therapeutic strategies.

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REVIEW

Intracellular Parasite Invasion Strategies

L. D. Sibley

Intracellular parasites use various strategies to invade cells and to subvert cellular signaling pathways and, thus, to gain a foothold against host defenses. Efficient cell entry, ability to exploit intracellular niches, and persistence make these parasites treacherous pathogens. Most intracellular parasites gain entry via host-mediated processes, but apicomplexans use a system of adhesion-based motility called "gliding" to actively penetrate host cells. Actin polymerization–dependent motility facilitates parasite migration across cellular barriers, enables dissemination within tissues, and powers invasion of host cells. Efficient invasion has brought widespread success to this group, which includes *Toxoplasma*, *Plasmodium*, and *Cryptosporidium*.

Parasites exist in virtually every conceivable niche, but none is so specialized as that of the obligate intracellular parasite, which must gain entry into the cells of its host to survive. Most intracellular parasites are protozoans, many of which are responsible for lethal and debilitating diseases in animals and humans. Our defenses present an array of barriers to infection, including skin, mucosa, connective tissue, and an active surveillance system to detect and destroy foreign objects. Overcoming these defenses and breaching the final barrier imposed by the cell membrane is a formidable challenge. By entering into the confines of a host cell, the parasite assures itself of both a ready source of nutrients and a potential means to avoid immune clearance. Parasites that practice this life-style have typically given up the capacity for extracellular growth, which leaves them vulnerable if entry is impeded. Defining how parasites gain entry into their host cells is thus important for rational design of improved therapies. Parasites are among the earliest branching eukaryotes (*1*); their study expands our knowl-

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REVIEW

Bacterial Invasion: The Paradigms of Enteroinvasive Pathogens

Pascale Cossart[1]* and Philippe J. Sansonetti[2]*

Invasive bacteria actively induce their own uptake by phagocytosis in normally nonphagocytic cells and then either establish a protected niche within which they survive and replicate, or disseminate from cell to cell by means of an actin-based motility process. The mechanisms underlying bacterial entry, phagosome maturation, and dissemination reveal common strategies as well as unique tactics evolved by individual species to establish infection.

To establish and maintain a successful infection, microbial pathogens have evolved a variety of strategies to invade the host, avoid or resist the innate immune response, damage the cells, and multiply in specific and normally sterile regions. Based on their capacity to deal with these critical issues, bacteria can be grouped in different categories. Here we review the so-called invasive bacteria, i.e., bacteria that are able to induce their own phagocytosis into cells that are normally nonphagocytic. We focus on the tactics used by enteroinvasive bacteria to trigger their uptake by epithelial cells and discuss their intracellular life-styles. The mechanisms of entry and life-styles of other intracellular pathogens have been reviewed elsewhere (*1*–*4*).

During phagocytosis by phagocytes, bacteria play a passive role. In contrast, during bacterial-induced phagocytosis, the bacterium is the key and active player in the complex interplay between the invading microbe and the host cell (*5*). Another important component is the cytoskeleton, whose plasticity is critical and optimally exploited. After internalization, some bacteria remain in a vacuole, in which they replicate. They prevent the normal maturation and trafficking of the phagosome and impair its normal bacteriolytic activities. Other bacteria escape from the vacuole and replicate in the cytosol. In some cases, they also move and disseminate by means of an actin-based motility process.

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How the cell senses the bacterial intruders and adjusts its transcription and translation programs to its new life with a parasite is an important issue. Apoptosis and anti-apoptosis, as well as cell cycle– and inflammation-related signaling pathways, are reprogrammed after infection to help the cell to survive the stress induced by the infection.

The success of an infection depends on the messages that the two players—the bacterium and the cell—send to each other. At each step of the infectious process, the bacterium exploits the host cell machinery to its own profit.

Entry Mechanisms

To enter nonphagocytic cells such as intestinal epithelial cells, some microbial pathogens express a surface protein able to bind eukaryotic surface receptors often involved in cell-matrix or cell-cell adherence. Expression of this protein leads to the formation of a vacuole that engulfs the bacterium through a "zippering" process in which relatively modest cytoskeletal rearrangements and membrane extensions occur in response to engagement of the receptor. The initial interactions between the bacterial protein and its receptor trigger a cascade of signals, including protein phosphorylations and/or recruitment of adaptors and effectors, and activation of cytoskeleton components that culminate in phagocytic cup closure and bacterial internalization. Other pathogens have devised mechanisms to bind a protein that can itself act as a bridge between the bacterium and a transmembrane receptor, which then mediates the entry process. Finally, pathogens can also bypass the first step of adhesion and interact directly with the cellular machinery that regulates the actin cytoskeleton dynamics by injecting effectors through a dedicated secretory system. The effector molecules cause massive cytoskeletal changes that trigger the formation of a macropinocytic pocket, loosely bound to the bacterial body.

The Zipper Mechanism of Entry

Yersinia pseudotuberculosis and *Listeria monocytogenes* both harness transmembrane cell-adhesion proteins as receptors for entry into mammalian cells (Figs. 1A and 2A). Entry can be divided into three successive steps: (i) Contact and adherence. This step is independent of the actin cytoskeleton and involves only the bacterial ligand and its receptor. It leads to receptor clustering. (ii) Phagocytic cup formation. This step is triggered by the transient signals occurring after formation of the first ligand-receptor complexes and propagating around the invading microbe. These signals induce actin polymerization and membrane extension. (iii) Phagocytic cup closure and retraction, and actin depolymerization.

The *Yersinia* outer-membrane protein invasin binds to integrin receptors that have the β_1 chain and are normally implicated in adherence of cells to the extracellular matrix (*6*). Invasin does not possess the RGD motif present in fibronectin, but both proteins interact with integrins by a structurally similar domain. Invasin has a higher affinity for integrins and can oligomerize, inducing integrin clustering and efficient downstream signaling. The cytoplasmic tail of the β_1 chain, which normally interacts with the cytoskeleton in focal complexes of adhesion plaques, is critical for entry, but surprisingly, alterations of this domain that impair interaction with the cytoskeleton increase internalization. Thus, a lower affinity of the integrin for the cytoskeleton could allow higher mobility of the receptors in the membrane.

Activation of integrins leads to tyrosine-phosphorylation events required for entry. The tyrosine kinase FAK (focal adhesion kinase) is the most attractive candidate for transmitting a signal from clustered integrins to the cytoskeleton, because the β_1-chain cytoplasmic domain binds to FAK, and dominant-inhibitory mutations in FAK strongly impair invasin-mediated uptake (*7*). Src, phosphoinositide 3-kinase (PI 3-kinase), and

Rac are also involved in invasin-mediated uptake. Why there is a requirement for phosphoinositide 3-kinase is unknown. Efficient entry involves a Rac1-Arp2/3 pathway which may involve N-WASP (*8*–*10*). The local concentration of phosphatidylinositol 4,5-bisphosphate [(PIP_2, $PI(4,5)P_2$] is critical for entry, and Arf6 may play a role in activation of phosphoinositol-4-phosphate-5-kinase (PIP_5 kinase) and control of cytoskeleton rearrangements and membrane traffic involved in closure of the phagocytic cup (*11*).

Several surface proteins contribute to entry of *L. monocytogenes* into nonphagocytic cells in vitro (*12*). The best-characterized protein, internalin (InlA), is a surface protein that is covalently anchored to the cell wall and belongs to a large family of leucine-rich repeat (LRR) proteins. As for invasin, coating of latex beads with internalin promotes their entry, thus facilitating dissection of the specific pathway. Entry of *Listeria* into cells involves interaction between the LRR region of internalin and the first ectodomain of human E-cadherin, a transmembrane glycoprotein normally involved in homophilic E-cadherin–E-cadherin interactions at adherens junctions of polarized epithelial cells. The LRR domain surrounds the first ectodomain of E-cadherin (*13*). This weak-affinity interaction cannot take place if proline-16 is changed into glutamic acid, as in murine E cadherin (*14*). Formation and maintenance of adherens junctions require the integrity of the E-cadherin cytoplasmic domain that binds catenins (α, β, and p120 catenins), which interact with the cell actin cytoskeleton (*15*). Similarly, entry of *Listeria* into cells requires the terminal 35 amino acids of E-cadherin. The latter binds to β-catenin, which recruits α-catenin, which in turn interacts with actin. Actin polymerization during internalin-mediated entry is Rac dependent and mediated by Arp2/3, but how Arp2/3 is activated is unknown (*16*). Entry also requires an unconventional myosin, myosinVIIa, and its ligand vezatin (*17*). These two proteins probably play a role in the dynamics of the phagocytic cup. How the tension generated by the myosin motor is coupled to actin polymerization required for entry has not been established.

The second well-characterized *L. monocytogenes* invasion protein is InlB (*12*, *18*, *19*). This surface protein belongs to the LRR family of proteins and is only loosely attached by its C-terminal repeats to the bacterial surface, where it interacts with lipotechoic acids. Soluble InlB can reassociate with the bacterial surface of an InlB mutant and promote entry.

InlB interacts with three cellular ligands (*12*, *18*). The most relevant one is Met, a transmembrane receptor tyrosine kinase that upon interaction with its normal ligand, the hepatocyte growth factor (HGF), dimerizes and elicits phosphorylation on two critical residues that act as docking sites to recruit signaling and adaptor molecules (*20*). Met binding to the concave surface of the InlB LRRs also leads to its transient phosphorylation and to the recruitment and phosphorylation of the adaptor proteins Cbl, Gab1, and Shc, and activation of PI 3-kinase with the generation of PIP_3 at the plasma membrane (*21*). Optimal activity of Met requires the presence of glycosaminoglycans (GAGs) on the cell surface, probably promoting oligomerization of the growth factor and/or its protection from extracellular proteases. GAGs also increase *Listeria* InlB-dependent entry into the target cell. Heparin can detach InlB from the bacterial surface, rein-

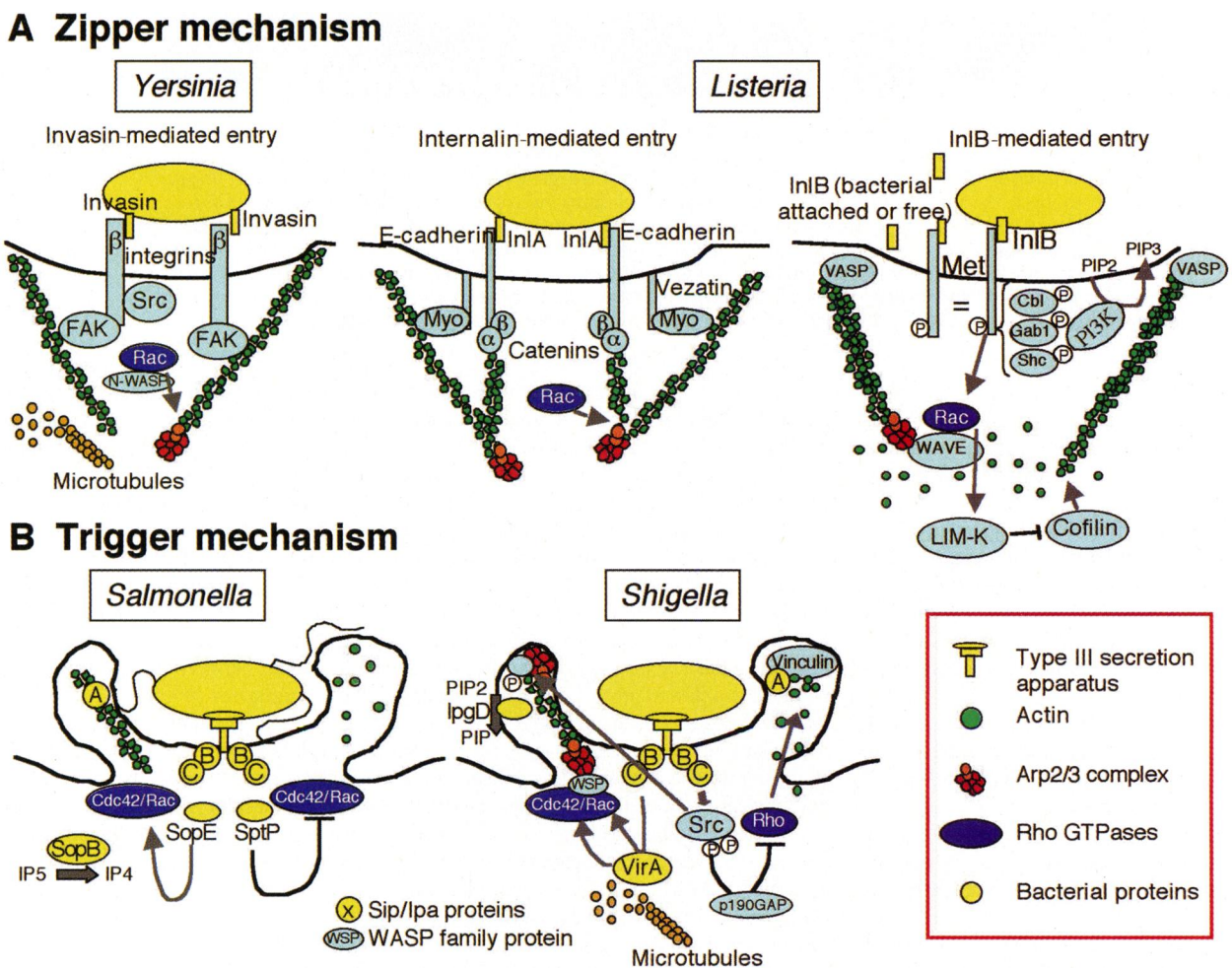

Fig. 1. Mechanisms used by bacteria to enter cells. (**A**) The zipper mechanism used by *Yersinia* and *Listeria*. (**B**) The trigger mechanism used by *Salmonella* and *Shigella*.

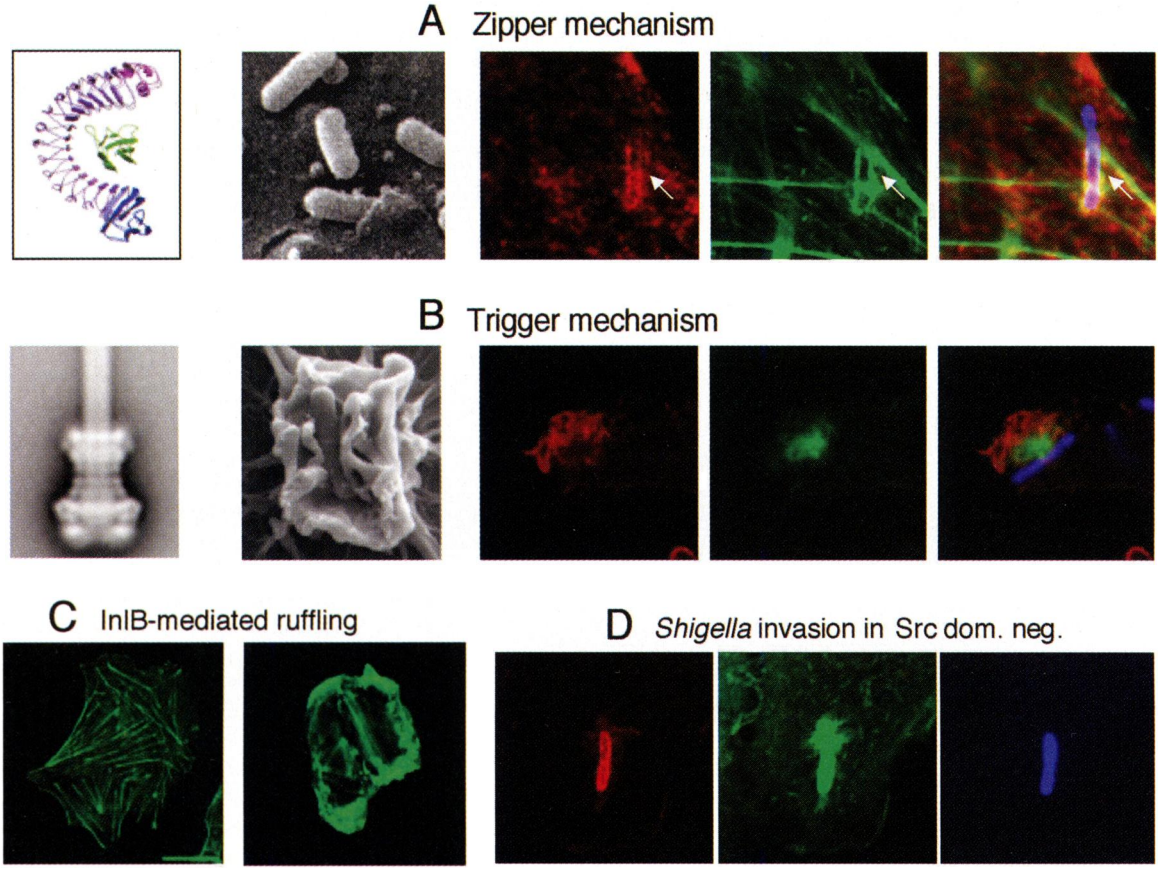

Fig. 2. The zipper and the trigger mechanisms. (**A**): Zipper mechanism. From left to right: x-ray structure of internalin interacting with E-cadherin [reprinted from (*13*) with permission from Elsevier]; scanning electron micrograph of *Listeria* entering into Caco2 cells; immunofluorescence images of *Listeria* entering into Vero cells (red: Met; green, actin; and blue: bacteria). (**B**) Trigger mechanism. From left to right: Reconstitution of the TTSS; scanning electron micrograph of *Shigella* entering into cells; immunofluorescence images of *Shigella* entering into Caco 2 cells (red: cortactin; green: actin; and blue: bacteria). (**C**) InlB-mediated ruffling. Control cells and cells ruffling upon incubation with soluble InlB (green: actin). (**D**) *Shigella* entering into Src dominant-negative cells (red: cortactin; green: actin; and blue: bacteria). Src-dependent tyrosine phosphorylation of cortactin is essential to trigger massive extension of actin filaments at a distance from the entry focus; thus, cells expressing a Src dominant-negative construct form inefficient entry foci with limited actin polymerization tightly around the entry vacuole.

forcing the hypothesis that InlB may act as a soluble protein. Thus, InlB mimics HGF, the normal Met ligand, and similarly to growth factors, soluble InlB induces actin-rich membrane ruffles (Fig. 2C).

InlB also interacts with gC1qR/p32, a ubiquitous protein first identified as the receptor for the globular part of the complement component C1q (*22*). However, the subcellular location and function of gC1qR remain controversial, and its role in cell entry remains to be clarified.

Contact between Met and InlB, present on the bacterium or released from its surface, initiates actin nucleation and polymerization via the small guanosine triphosphatase (GTPase) Rac, WAVE, and the Arp2/3 complex (*19*, *23*). Actin filament elongation, which provides the driving force for membrane extension around the bacterium, involves VASP, which may act as an anticapping protein at the barbed ends. Cofilin also participates in this process. This protein increases actin turnover by triggering actin depolymerization at pointed ends of actin filaments and by creating new free ends for polymerization by severing actin filaments. In the initial steps of cell entry, cofilin activity is modulated by LIM kinase. Then progressive accumulation of cofilin on filaments favors filament disassembly and retraction of the phagocytic cup. Thus, the InlB-Met interactions probably elicit both a Rac-WAVE-ARP2/3 and a Rac-PAK-LIM-kinase-cofilin cascade. It is still unknown how Rac is activated downstream of Met. The role of PI 3-kinase is also unknown. The working hypothesis is that, as in phagocytosis, PI 3-kinase facilitates cup closure, probably by recruiting membrane vesicles and actin regulators. It may also induce sustained activation of Rac.

InlB is thus a strong signaling protein that by itself acts as an invasin but may also potentiate other bacterial factors involved in *Listeria* entry and tissue tropism, such as internalin. Other proteins such as the autolysins Ami, Auto, and ActA contribute to *Listeria* adherence and entry (*24*). In addition, listeriolysin O (LLO), a pore-forming, cholesterol-dependent cytolysin involved mainly in escape from the internalization vacuole (*25*, *26*) and that, like other toxins, interacts with lipid rafts (*27*), allows entry of extracellular calcium and stimulates entry (*28*). Even in the absence of LLO, both internalin- and InlB-mediated entry are dependent on the presence of raft microdomains, suggesting that for entry, *Listeria* take advantage of raft microdomains, which are known to be enriched in receptors and signaling molecules. Interestingly, cholesterol depletion does not affect the internalin- and InlB-mediated pathways at the same step of the entry process (*29*).

The Trigger Mechanism of Entry

Both *Shigella* and *Salmonella* use this mechanism to enter the cell (Fig. 1B and Fig. 2B). Contact between bacteria and cells is mediated by the type III secretory system (TTSS) (Fig. 1). The TTSS allows direct activation of components of the cytoskeleton by delivery of dedicated bacterial effectors. In *Salmonella*, the TTSS is encoded by a chromosomal pathogenicity island (SPI-1) and in *Shigella* by a plasmid-located pathogenicity island (PAI). These PAIs encode the structural components of the TTSS and some of their dedicated effectors. Two of these components (i.e., SipB/C in *Salmonella*, IpaB/C in *Shigella*) form a pore, or translocator, that delivers the effectors into the cell cytoplasm, creating a continuum between the bacterial and eukaryotic cytoplasms (*30*, *31*).

The interaction of bacteria with their epithelial cell target occurs in four successive stages:

1) A pre-interaction stage. At 37°C, the effector molecules stored in the bacterial cytoplasm are associated with dedicated chaperones, whose major role is to avoid premature association of the effector molecules and their proteolytic degradation (*32*). In exponentially growing bacteria, the TTSSs are properly assembled, but the secretion of effector proteins is repressed until the bacterium establishes contact with its cell target.

2) An interaction stage. This stage encompasses complex events leading to the formation of a signaling platform. A recognition event is likely to take place at the tip of the TTSS, activating the secretory process via a retroactive signaling, possibly involving an adenosine triphosphatase in the TTSS basal body (*33*). In *Shigella*, the high-affinity binding of IpaB to CD44—the hyaluronic acid receptor that is strongly expressed on the basolateral membrane of intestinal epithelial cells and on the surface of many other cell types, including cells of myeloid lineage—may be a key step in achieving transient adherence to the cell surface, activation of the secretory machinery, and insertion of the IpaB/C translocon into the eukaryotic cell membrane. Consistent with the association of CD44 with cholesterol and sphingolipid-rich membrane rafts, this step of the interaction is dependent on intact rafts (*34*). Cholesterol extraction disrupts binding to and entry into epithelial cells, and IpaB and CD44 segregate in these rafts. Similarly, in *Salmonella*, the protein components of the SipB/C translocon also segregate in rafts. The initial interaction may take place in these membrane subdomains because (i) the targeted receptor is enriched in rafts; (ii) the lipid composition of rafts is optimal for the formation of the pore and translocon, in a way similar to the cholesterol dependence of several hemolysins (*27*); and (iii) these domains are enriched in signaling molecules such as tyrosine kinases of the *src* family.

3) The formation of a macropinocytic pocket. This stage involves localized but massive rearrangements of the cell surface, characterized by the formation of intricate filopodial and lamellipodial structures that appear similar in *Salmonella* and *Shigella*. Rearrangements of the actin cytoskeleton largely account for the formation of the entry focus. At the early stage of *Shigella* entry, VirA, a plasmid-encoded protein secreted through the TTSS, induces local destabilization of the microtubules that results in their depolymerization (*35*). The latter affects the early events of actin rearrangement through the deactivation of RhoA, leading to Rac1 activation and formation of Rac1-IRSp53-WAVE2 complex that recruits Arp2/3. IpaC in *Shigella* (*36*) and SipC in *Salmonella* (*37*) initiate actin nucleation through their C-terminal domain, which is exposed to the cytoplasm of the eukaryotic cell, via the IpaB/C or SipB/C pore. The mechanism of initial actin nucleation, however, remains uncertain. SipC can nucleate actin alone in vitro (*37*), but IpaC requires activation of Cdc42 and Rac 1 (*36*).

Massive extension of the actin filaments that form entry foci seems to respond to different mechanisms in *Salmonella* and *Shigella*. In *Salmonella*, the translocated SopE proteins (SopE1 and SopE2) act as exchange factors for the Cdc42 and Rac-1 GTPases, thus massively boosting the initial nucleation event (*38*). Moreover, SopB/SigD, a TTSS-secreted phosphatidylinositol phosphatase (*39*), stimulates actin rearrangements and mediates bacterial entry, whereas SipA binds and stabilizes actin filaments (*40*). *Shigella* has evolved a similar process of boosting cytoskeletal rearrangements, although through different molecular mechanisms. The C-terminal domain of IpaC is central to the activation of Cdc42 and Rac-1, which is quickly followed by activation of the tyrosine kinase c-src upon contact with IpaC (*41*), recruitment of cortactin to the membrane upon its c-src–mediated tyrosine phosphorylation, and further massive actin polymerization in the vicinity of the original actin cup via the Arp2/3 complex (*42*) (Fig. 2C). This process is amplified by IpgD, a *Shigella* homolog of SopB/SigD. IpgD expresses a phospatidylinositol phosphatase activity that hydrolyzes PI(4,5)P2 into PI(5)P [phosphatidylinositol 5-phosphate], thus disconnecting the actin subcortical cytoskeleton from the membrane and favoring actin dynamics at the entry site (*43*). The Abl family of tyrosine kinases is also involved in *Shigella* entry through phosphorylation of the adaptor molecule Crk (*44*).

4) Actin depolymerization and closing of the macropinocytic pocket. This final stage is similar in *Shigella* and *Salmonella*, despite important differences between the effectors involved and the molecular mechanisms exploited. In the case of *Salmonella*, SptP, a TTSS-secreted protein, has two activities: (i) a tyrosine-phosphatase activity that regulates activity of the mitogen-activated protein kinase (MAPK) induced by entry; and (ii) a GAP (GTPase-activating protein) activity on Cdc42 and Rac that antagonizes the activity of SopE, thus leading to shrinking of the entry focus by blocking further actin polymerization (*45*). It may seem strange that proteins of opposite functions are injected simultaneously into the target cell. Recent evidence indicates that, despite equivalent amounts delivered by the TTSS, SopE is rapidly degraded through a proteasome-dependent pathway, whereas SptP is more stable (*46*). In the case of *Shigella*, IpaA, a TTSS-secreted protein, binds the N-terminal head domain of vinculin, a key protein in the formation of cell-adherence plaques, and induces actin depolymerization (*47*).

Intracellular Life-Styles

After internalization, bacteria remain in a vacuole or escape to the cytosol, where they replicate. Some intracytosolic bacteria may also move by a process of polarized actin polymerization that takes place at one pole of the bacterium and provides the force for bacterial locomotion inside the cytosol and toward neighboring cells.

The Vacuole as an Intracellular Replication Compartment

Bacteria that replicate inside the internalization vacuole have developed an impressive array of strategies (*4*) aimed at surviving in a hostile and changing environment characterized by poor nutrient content, progressive decrease of the pH, and delivery of antibacterial peptides and lysosomal enzymes as late endosomes mature to lysosomes. In macrophages, these conditions are even more drastic and exacerbated by the delivery of reactive oxygen and nitrogen intermediates. Two major strategies can be recognized, although a given species may use a combination of both: (i) Bacteria may adapt to and eventually resist these hostile conditions, thus developing a state of metabolic adaptation to the stress imposed by these conditions; (ii) alternatively, bacteria may alter the biogenesis and dynamics of their vacuolar compartment, thus creating for themselves a less hostile niche that is permissive for their survival and growth. *Salmonella* represents a paradigm of the complex combination of these two survival and growth strategies (Fig. 3). After a few hours of invasion, bacteria reside in an atypical acidic compartment called the SCV (*Salmonella* containing vacuole), which is neither a late nor an early endosome (*48*). How bacteria redirect the fate of this compartment away from the normal phagosomal pathway involves transient acquisition of rab5, PI3-kinase, EEA1, and finally rab7 (*49*). In addition, merging of the SCV with the endoplasmic reticulum appears to contribute to early SCV maturation (*50*) and membranes of the trans-Golgi network surround the SCV at late times of infection (*51*), suggesting interactions with both the endocytic and the biosynthetic pathway. Numerous bacterial genes are required for survival and replication. A key role is played by the SPI2 effector SifA—a protein required for the formation of Sifs, filaments enriched in lysosomal glycoproteins (Lgps), and extensions of the SCV, in epithelial cells (*52*). The function of SifA may be to mediate the recruitment of vesicles and increase the SCV membrane surface area to accommodate replicating bacterial cells.

Life in the Cytosol and Actin-Based Intra- and Intercellular Motility

Some intracellular pathogens able to induce their own phagocytosis into epithelial cells escape from the internalization vacuole, replicate in the cytosol, and move by recruiting and polymerizing actin (*53*) (Fig. 4). Actin polymerization at one pole of the bacterium provides the energy for movement and enables the bacteria to reach the plasma membrane, where they form protrusions that are endocytosed by neigboring cells, allowing the formation of a two-membrane vacuole, cell to cell spread, and tissue dissemination.

For *Listeria*, escape from the vacuole is mediated by a pore-forming toxin called listeriolysin O (LLO), a potent signaling molecule that activates nuclear factor κB (NF-κB) and a variety of other pathways (*25*). Intracytosolic replication requires expression of a sugar-uptake system, which is absent in the nonpathogenic

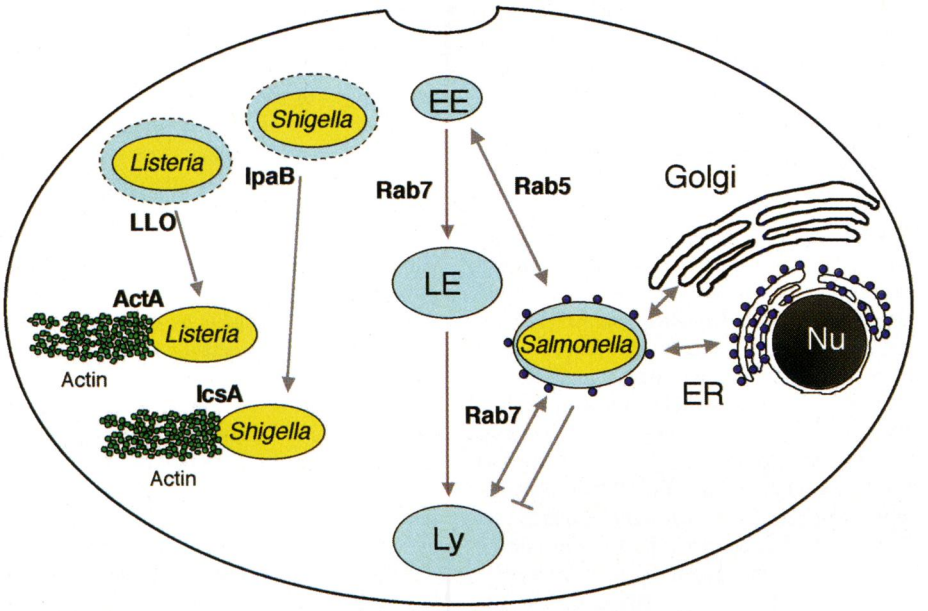

Fig. 3. Intracellular life-styles. Schematic representation of the *Salmonella*-containing vacuole (see text). *Listeria* and *Shigella* lyse the vacuole and move in the cytosol by an actin-based motility process mediated by ActA or IcsA/VirG, which interact with Arp2/3 or N-WASP and Arp2/3, respectively. EE: early endosome; LE: late endosome; Ly: lysosome; ER: endoplasmic reticulum

species *L. innocua* (*25*). Actin recruitment by *Listeria* and polymerization are triggered by the surface protein ActA, which recruits and activates the seven-protein Arp2/3 complex, hence generating a dendritic network of branched actin filaments (*54*). Modulation and control of actin-based movements involve several other proteins: (i) cofilin; (ii) capping protein, which caps the barbed ends of actin filaments; (iii) profilin, which binds to monomeric actin and, in complex with actin, to actin-filament barbed ends, hence providing actin monomers to growing barbed ends; (iv) α-actinin, which cross-links actin filaments; and (v) VASP, which binds to ActA and F-actin and modulates branch density and movement. *Shigella*, after escaping from the vacuole upon the action of IpaB, expresses on its surface an outer-membrane protein called IcsA/VirG. This protein, which is unrelated to ActA, recruits the cellular protein called N-WASP (*55*, *56*). Cellular N-WASP is functionally and structurally related to bacterial ActA and can recruit and activate the Arp2/3 complex, highlighting how bacteria may either mimic or recruit mammalian proteins to harness eukaryotic pathways (*5*).

Even though *Rickettsia* is not an enteroinvasive microorganism, it is worth mentioning that after its escape into the cytoplasm, it forms actin tails made of long, unbranched actin filaments, which differ from those generated by ActA or IcsA/N-WASP (Fig. 4). Similar to proteins of the WASP family, the bacterial surface protein involved, RickA (*57*), is composed of three regions, with a central proline-rich region and a C-terminal part that recruits Arp2/3. Because Arp2/3 generates a network of branched actin filaments, the discovery that RickA activates Arp2/3 in vitro and is recruited on the McRettsial surface was unexpected, providing a new tool to address Arp2/3 regulation.

Cell Responses to Intracellular Pathogens

In addition to the transient posttranslational modifications occurring upon entry, intracellular bacteria induce drastic changes in the pattern of transcription and translation of infected cells. This is particularly true for intestinal epithelial cells that, upon invasion by *Salmonella* or *Shigella*, behave as sentinels by inducing a transcriptional program whose major function is to up-regulate innate immune defense mechanisms (*58*). This program occurs largely in response to the induction of NF-κB that regulates a large portion of the pro-inflammatory genes. The pro-inflammatory program of epithelial cells—in contrast to the outside-in signaling pathway that Toll-like receptors mediate in phagocytic cells, in the presence of bacterial PAMPs (pathogen-associated molecular patterns)—appears to be mediated by an intracellular sensing system involving cytosolic proteins of the Nod family (*59*). Nod1 is prevalent in intestinal epithelial cells and shows specific recognition for muropeptides originating from the peptidoglycan of Gram-negative microorganisms (*60*, *61*). Another cytosolic protein, Nod2, recognizes peptidoglycans from any bacterial species, essentially because it is able to recognize muramyl-dipeptide, a structure common to all peptidoglycans.

Through their capacity to regulate gene transcription and by other pathways, intracellular bacteria can take over the fate of their host cell. Among the most striking paradigms are bacteria that manipulate cell apoptotic processes. Three major pathways have so far been identified: (i) Intracellular *Shigella* and *Salmonella*, respectively, secrete IpaB and SipB through their TTSS. These two proteins activate the pro-apoptotic cysteine protease caspase-1, which causes apoptotic death of infected macrophages while also initiating an inflammatory response through processing or maturation of two potent pro-inflammatory cytokines, interleukin-1β (IL-1β) and IL-18 (*62*, *63*). (ii) *Yersinia* translocate plasmid-encoded Yop proteins, one of which, YopP/YopJ, binds to and neutralizes the activity of a MAPK kinase, thereby blocking the activation of NF-κB, an essential system supporting cell survival (*64*). (iii) The third pathway, although not yet clearly described in enteroinvasive bacteria, is worth mentioning. Upon interaction of *Neisseria gonorrhoeae* with epithelial cells, the secreted protein PorB causes Ca^{2+} fluxes that activate caspases, and consequently cell apoptosis (*65*). PorB creates mitochondrial pores, thus inducing apoptosis through the release of cytochrome c. Finally, epithelial cells infected by *Shigella* undergo activation of their connexin-constituted hexameric hemichannels. The infected cells release ATP, which acts as a paracrine mediator activating Ca^{2+} fluxes in neighboring cells, thus increasing their competence for bacterial invasion and cell-to-cell spread (*66*).

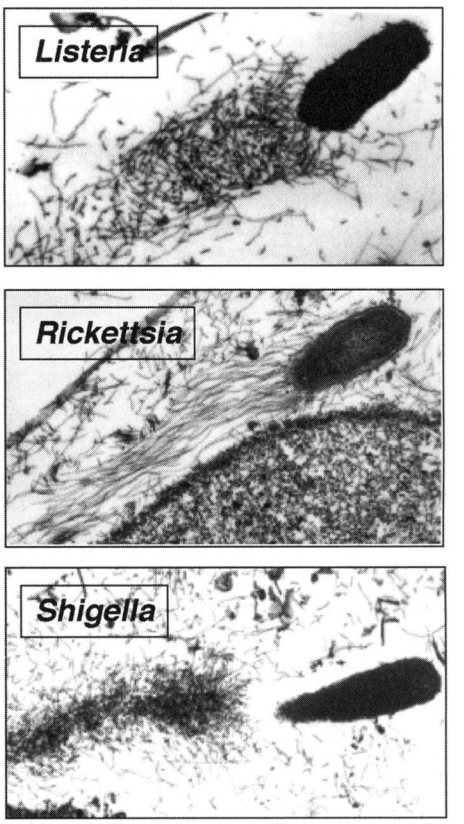

Fig. 4. Actin-based motility of *Listeria*, *Rickettsia*, and *Shigella*. Electron micrographs of actin tails labeled with fragment S1 of myosin (*69*) [reprinted with permission from *Journal of Cell Science*].

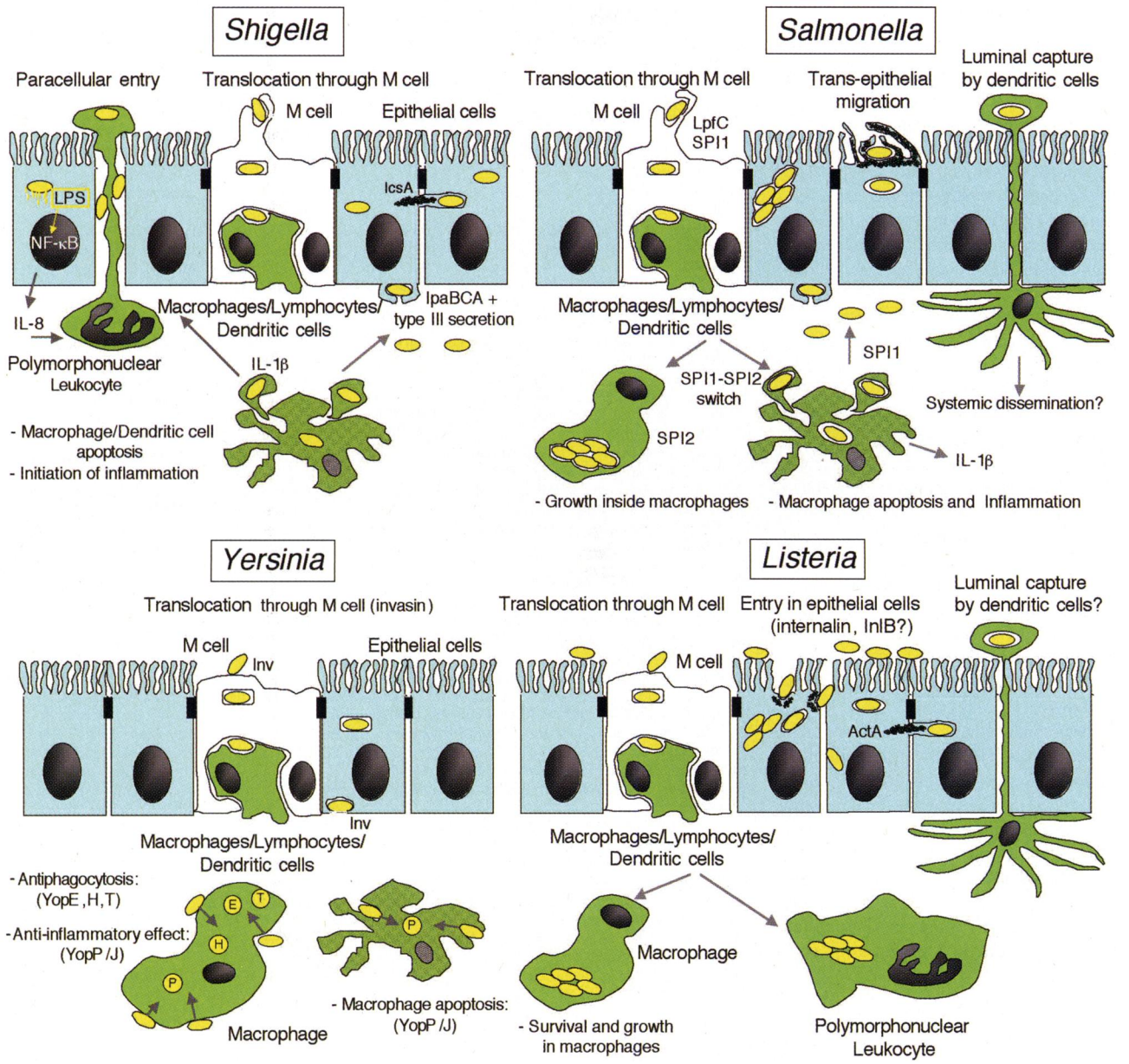

Fig. 5. The invasive strategies of enteroinvasive pathogens. Intestinal epithelial cells (IECs) maintain a physical barrier against commensal flora, although specialized sites such as the follicle-associated epithelium (FAE) allow constant sampling of the luminal flora through M cells. Translocated bacteria thus exposed to macrophages, dendritic cells (DCs), and B lymphocytes are captured, killed, processed, and presented to the immune system. Invasive pathogens take advantage of this route to cross the epithelial barrier. Once translocated, bacteria must survive attack by macrophages. The four bacterial species considered have solved this issue differently: *L. monocytogenes* are phagocytosed but escape into the cytoplasm, and thus avoid being killed in lysosomal compartments. *Yersinia* adopt an antiphagocytic strategy by intracellular injection of YopE, H, and T that inactivate the actin cytoskeleton. In addition, they adopt an anti-inflammatory strategy, with YopP/J blocking tumor necrosis factor–α production, which prevents further local recruitment of predators such as monocytes and polymorphonuclear leukocytes. Alternatively, phagocytosed *Yersinia* may cause YopP/J-dependent apoptosis of their host cell. *Shigella* not only cause apoptosis of macrophages and monocytes, thus ensuring their own survival, but also trigger early mucosal inflammation through the release of mature IL-1β and IL-18, which disrupts epithelial impermeability and facilitates bacterial spread at a distance. Finally, *Salmonella* remodel their phagosomes, thus avoiding its transition to a lysosome and creating an intracellular niche that allows their efficient replication; this Spi2-dependent process is an alternative to the Spi1-dependent apoptotic killing of macrophages similar to that caused by *Shigella*. Having crossed the epithelial barrier and circumvented the threat of phagocytosis, the bacterial species considered here proceed along different pathways. *L. monocytogenes* disseminate systemically, possibly inside circulating monocytes and DCs. *Yersinia* may invade IECs through their basolateral pole, a process mediated by invasin; they also cause local and mesenteric abscesses in local and loco-regional lymphoid structures. *Shigella* proceeds to TTSS/Ipa-dependent entry into epithelial cells followed by escape into the cytoplasm, intracellular motility, and cell-to-cell spread, thus establishing the infectious process at the mucosal level, without extensive systemic dissemination. *Salmonella* may, like *Shigella*, enter IECs through their basolateral pole in a TTSS/Sop-dependent manner. Alternative routes of invasion involve IECs directly, away from the FAE. In particular, invasion by *L. monocytogenes* is mediated by internalin (InlA) and possibly InlB. In addition, *Salmonella* are able to dislocate the brush border cytoskeleton and cause an apical entry ruffle. *Shigella* and *Yersinia* seem unable to disrupt the epithelial barrier from a luminal position unless massive inocula are used. A third process of translocation may involve DCs crawling between IECs or sending pseudopods to capture luminal bacteria and retract in a subepithelial position. *Salmonella* are able to translocate in this way, possibly followed by systemic diffusion of *Salmonella*-loaded DCs. It is not yet clear whether this type of translocation occurs in the other invasive species.