Salmonella invasion

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Abstract: *Salmonella enterica* serovar Typhimurium encodes two type Ⅲ protein secretion/translocation systems within the pathogenicity island 1 (SPI-1) and island 2 (SPI-2). These translocation systems inject a panel of bacterial effector proteins into host cells to promote bacterial entry into the host cells via the "trigger" mechanism. The translocated effectors exploit the host actin cytoskeleton leading to macropinocytosis and bacteria entry. In this review, we present a working model based on recent advances in understanding contributions from individual *Salmonella* effectors. First, activation of the type Ⅲ secretion system and the delivery of bacterial effector proteins (Ⅰ). Injection of the exchange factor SopE and the inositol polyphosphatase SopB results in the activation of CDC42 and Rac1 (Ⅱ), leading to the recruitment of ruffling-associated molecules. SipA and SipC function to lower the critical concentration of actin, stimulating the bundling activity of plastin and stabilizing fibrous actin (F-actin), and nucleating the actin assembly (Ⅲ). SopB promotes membrane fission process by decreasing the local concentration of PIP2 at the base of the membrane ruffles and by recruiting VAMP8 (Ⅳ). The combined activities of these effectors result in a localized and pronounced outward extension of the membrane ruffles, resulting in the engulfment of *Salmonella* in an enclosed membrane compartment. *Salmonella* delivers another effector protein, SptP, which reverses the activation of these small G proteins by stimulating their intrinsic GTPase activity and therefore facilitating cell recovery (Ⅴ).

Key words: *Salmonella*; Invasion; Model; Effector protein

Introduction

Microorganisms are ubiquitous and essential for human health[1,2]. Large numbers of numerous species of bacteria live in our intestine. The intestinal epithelia serve as an effective barrier to prevent live intestinal microorganisms from entering into deeper tissues while permitting the intake of nutrients into the bloodstream. Although the intestinal epithelia prevent the uptake of large particles, such as bacteria, pinocytosis and receptor-mediated endocytosis facilitate nutrients intake. Pathogenic bacteria can invade host cells by passively entering professional phagocytes. Alternatively they actively induce host cell cytoskeleton rearrangements to induce uptake in non-phagocytic cells, such as the intestinal epithelium.

Most people are familiar with the receptor-mediated bacteria entry, which uses bacterial surface components to bind host cell surface receptors to trigger the signal transduction pathways[3,4]. The other, less familiar, mechanism sends bacterial proteins directly inside the host cells to modulate the signaling components and cytoskeletal machinery[5]. The receptor-mediated mechanism involves the engagement of a bacterial ligand with a host cell receptor, leading to the gradual movement of the plasma membrane alongside the surface of the invading bacteria which eventually wraps the bacteria inside the host cell membrane. This entry process is also commonly referred as the "zipper" mechanism. The other mechanism is often termed "trigger" mechanism, which induces dramatic actin cytoskeleton rearrangements and membrane ruffling leading to macropinocytosis and bacteria entry. There are some similarities and differences between these two entry mechanisms. Multiple bacterial proteins are often involved in the "trigger" mechanism, while one bacterial factor is usually required and sufficient for inducing bacteria entry by the "zipper" mechanism. *Salmonella* and *Shigella* represent the "trigger" mechanism, and *Listeria* and *Yersinia* represent the "zipper" mechanism (Fig. 1). Here, we examine how *Salmonella* induces its own uptake into the intestinal epithelial cells.

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1 Salmonellosis

Salmonella enterica (*S. enterica*) serovar spp. are a group of Gram-negative bacteria that are usually motile and are pathogenic for humans and other warm-blooded animals[6, 7]. *Salmonella* strains cause food poisoning, gastrointestinal inflammation, typhoid fever, and septicemia in humans. Salmonellosis is a health concern not only for humans but also for the poultry industry. The most common source of human infection is through ingestion of contaminated food[8, 9]. The virulence determinants needed for *S. enterica* serovar Typhimurium are similar to those of many other intestinal pathogens. First, it needs to successfully survive the hostile acidic environment in the stomach before making its way to colonize the small intestine. In the intestine, the bacteria must breach the barrier of intestinal epithelial cells, and the bacteria have to survive inside the host cells. Pathogenic *Salmonella* spp. evolved complex systems that enable the organism to respond and survive the low pH[10] in the stomach and to reach microfold (M) cells and enterocytes in the small intestine[11-18]. *S. enterica* serovar Typhimurium has the ability to enter non-phagocytic eukaryotic cells and to exist as intracellular parasites inside enclosed vacuoles[11,12]. The intracellular environment provides a unique niche for the bacteria to multiply and evade host immune responses. In addition, *S. enterica* serovar Typhimurium is capable of surviving and replicating within macrophages[19]. Recently, *Salmonella* has been shown to be able to disseminate to extra-intestinal sites via $CD18^{+}$-expressing phagocytes[20, 21]. *S. enterica* serovar Typhimurium can enter cultured epithelial cells and macrophages, and this *in vitro* system has been found extremely useful in elucidating the molecular mechanism of interaction between *Salmonella* and host cells[5, 22]. In addition, *in vitro* cultured polarized epithelial cells have provided perhaps more appropriate experimental conditions that resemble the intestinal polarized enterocytes[23-26].

2 Pathogenicity islands and type III protein secretion systems

Several studies have led to the identification of genes that are required for *Salmonella* pathogenesis, in particular for *Salmonella* invasion into non-phagocytic cells[22, 27-31]. Many of these virulence genes and operons are located in large genetic elements of the *Salmonella* chromosome. Since these large elements are absent from the chromosome of closely related *Escherichia coli*, they are termed as pathogenicity islands. Virulence plasmids also contribute to *Salmonella*'s virulence[32-34]. At least five pathogenicity islands have been identified[22, 27-31] that contribute to virulence at defined stages of the *Salmonella* infection process. *Salmonella* pathogenicity island 1 (SPI1) is located at centisome 63 on the *Salmonella* chromosome and is 43 kb in length. SPI1 is required for *Salmonella* entry into M cells[18] and epithelial cells[22] of the intestine. This is consistent with the fact that SPI1 mutants are defective in virulence when administered orally but not if given systemically[22]. Mutants that are defective in entry into epithelial cells were found to be avirulent in studies using the mouse typhoid model[22] and in calves[35-37]. SPI2, SPI3 and SPI4 are situated at centisomes 31, 82, and 92 of the *Salmonella* chromosome. Genes in these three islands are essential for *Salmonella* survival and growth in the host[38-41]. SPI5 was originally found to be involved in inflammation and fluid secretion in the intestine[31, 42, 43]. It was recently shown that at least one gene in this island (*sopB*) is also involved in the *Salmonella* invasion process[5, 42, 44].

SPI1 and SPI2 encode specialized protein secretion and translocation systems termed as type III secretion systems. The other three islands encode no such secretion systems, but rather, appear to encode secreted proteins that feed through the type III secretion systems. Genes in SPI1 can be divided into three groups: (i) One includes genes that encode the actual secretion/translocation apparatus; (ii) A second group encodes proteins that are secreted and/or translocated into host cells; (iii) A third group is involved in gene regulation. Recently, the SPI1 secretion apparatus was shown by electron microscopy[45] to appear to constitute a 'needle complex' that is similar to the bacterial flagella system

both biochemically and structurally. Purified needle complexes consist of at least three proteins encoded in SPI1 (PrgK, PrgH, and InvG) . Mutations in *prgK*, *prgH*, or *invG* have been shown to abolish the secretion of a panel of *S. enterica* serovar Typhimurium proteins (SipA, SipB, SipC, etc.) . The subsequent translocation of these bacterial proteins into eukaryotic host cells is required for *Salmonella* invasion into non-phagocytic epithelial cells. Secretion has been reported to require host-cell contact[46] . These proteins are secreted under certain laboratory conditions in sufficient amounts to facilitate their study in the absence of host cells. These secreted proteins can be visualized by SDS-PAGE from supernatants of *S. enterica* serovar Typhimurium cultures under such inducing conditions. Among the secreted proteins in the SPI1 secretion apparatus, SipA, SipC, SopE, SopE2, and SopB (also known as SigD) were found to be responsible for promoting bacterial entry by modulating the host actin cytoskeleton[44,47-51] . SPI2 effectors are responsible for subsequent *Salmonella* survival inside the host cells by modulating bacterial trafficking[52-57] . In addition, SPI1 effectors SipA, SopA, SopB, SopD, SopE, and SopE2 are largely responsible for inducing inflammation and diarrhea in animal models[58-60] through yet undefined mechanisms.

3 Actin cytoskeleton rearrangements and *Salmonella* entry into host cells

To understand the molecular mechanisms of how *Salmonella* effectors modulate the host actin cytoskeleton, it is necessary to briefly discuss the components of the actin cytoskeleton and how their functions are affected by various regulators in mammalian cells. Three types of cytoskeleton components exist to provide both movement and stability in the mammalian cell: microfilaments, microtubules and intermediate filaments. Each of these cytoskeleton structures is dynamic in nature and composed of polymers of subunits. Microfilaments are assembled from monomers consisting of actin, microtubules from tubulin, and intermediate filaments from intermediate-filament proteins. In non-phagocytic epithelial cells, microfilaments project into the villi, giving shape to the cell surface. Microtubules grow out of the centrosome to the cell periphery. Intermediate filaments connect adjacent cells through desmosomes. Microfilaments are the most elastic among the three types of cytoskeleton components.

The actin cytoskeleton plays vital roles in many cellular processes, including cell movement and endocytosis[61-64] . Based on their architectural differences, there are three major classes of actin cytoskeleton structures in cells: stress fibers, lamellipodia and filopodia[65] . Stress fibers, which are primarily bundles of actin, are distributed at the bases of cultured cells. Lamellipodia and filopodia are both cellular actin extensions involved in cellular movement. Lamellipodia differ from filopodia in that they are defined by a more sheet-like actin meshwork rather than the microspike-like actin bundles found in filopodia. Actin cytoskeleton is highly dynamic and tightly regulated. Actin can exist in a monomeric globular form termed as globular actin(G-actin) , or in highly ordered multimers termed as filamentous actin or fibrous actin(F-actin) . The rate-limiting step in actin assembly is the initial formation of stable dimers, trimers and tetramers from which actin elongation occurs, a process often termed actin nucleation. Actin nucleation is facilitated by a number of actin-binding proteins and signal transduction molecules such as CDC42, N-WASP, and the Arp2/3 complex[66-69] . In addition, intracellular levels of G-actin and F-actin are maintained at a dynamic steady state by a number of actin-binding proteins. The concentration of free G-actin in the cell is kept below the level (critical concentration) that is required for actin polymerization. Although net levels of G-actin and F-actin are kept constant, actin molecules are constantly exchanging between these two states. F-actin depolymerizes from one end and polymerizes at the other end while the net length is kept constant, a process called treadmilling. This dynamic nature ensures that the cell can respond to environmental cues in a timely fashion while maintaining the integrity of the actin cytoskeleton architecture. Many actin-binding proteins have been identified that play different and yet coordinated roles in regulating the actin cytoskeleton. These proteins include: G-actin binding/sequestering proteins, such as profilin[70] ; F-actin capping proteins,

such as gelsolin[71]; F-actin severing proteins, such as ADF/cofilin[72]; and F-actin bundling proteins, such as plastin (fimbrin)[73]. Actin-binding proteins are often found to be multifunctional, especially when assayed under different experimental conditions *in vitro*. Their precise *in vivo* function is likely to depend on both their concentration and their spatial localization.

Actin dynamics are regulated by a number of factors including pH, ionic strength, phosphoinositides, Rho family GTPases CDC42 and Rac, and a number of actin-associated proteins. The small-molecular-weight Rho family GTPases have a critical role in the dynamic regulation of the actin cytoskeleton. These GTPases function as molecular switches by binding to GDP in the basal or non-activated state and GTP in the activated state. The binding of GTP results in a structure conformational change allowing the protein to bind to downstream effectors. As their names suggest, all have an intrinsic GTPase activity. The hydrolysis of GTP results in converting the protein to the basal, GDP-bound state, shifting it from active to non-active state. Likewise, GDP can be exchanged by GTP and result in the activation of these GTPases. As the intrinsic rate of hydrolysis and nucleotide exchange is slow, the hydrolysis of GTP to GDP is greatly stimulated by GTPase activating proteins (GAPs), and the exchange is facilitated by guanine nucleotide exchange factors (GEFs). All known GEFs specific for the Rho family GTPases possess the Dbl homology domain (DH). This domain binds the switch I and II regions which are responsible for the conformational change between inactive and active states of the Rho GTPases.

There are three major families of actin-nucleating proteins: the Arp2/3 complex, formins, and Spire. Biochemical, molecular modeling, and structure studies have determined that the Arp2/3 complex promotes actin nucleation by forming a dimer between the actin-related proteins Arp2 and Arp3 to bind the slow-growing "pointed" end of an actin filament[74-79]. It is proposed that the formin family nucleates actin by forming homodimers with a flexible linker to "stair-step" at the "barbed" ends of actin filaments, most likely with the help of profilin *in vivo*[80-82]. More recently, Spire has been found to nucleate actin through its four tandem WH2 domains to form a "single-stranded" actin polymer that initiates actin filament formation[83, 84].

The hallmark of *Salmonella* entry into host cells is the profuse actin cytoskeletal rearrangement at the site of *Salmonella* contact with intestinal epithelial cells[85, 86] (Fig. 2). These massive actin cytoskeleton rearrangements and subsequent entry of *Salmonella* are completely abolished when actin polymerization is inhibited by cytochalasin[87]. Effectors of the type III secretion/translocation systems of SPI1 have been found to be required for inducing such actin cytoskeleton rearrangements[22]. *Salmonella* strains have evolved to intercept and redirect the host actin dynamics to reorganize the actin cytoskeleton machinery for their own uptake. At the same time, the complex regulation also demands that any intervention by the bacteria must be precisely controlled to ensure the coexistence and viability of both the bacteria and the host cells. Increasing evidence suggests that multiple bacterial and host factors are involved in this process. In fact, it has been a hallmark of *Salmonella* effectors that mutations of individual effectors often result in only a minor defect in *Salmonella* virulence, suggesting that overlapping functions contribute to *Salmonella* pathogenesis[47, 50, 88-93].

It has been shown that cell surface membrane can be increased by fusing with vesicles recruited from endoplasmic reticulum (ER), endosomes and even lysosomes[94-97]. Both endocytosis and exocytosis processes involve regulated vesicle trafficking controlled by a number of cellular factors including the Rab GTPases, and the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. SNARE proteins present on vesicle and target membranes form complexes that are partially responsible for the specificity of vesicle targeting and to promote fusion of lipid bilayers. The vesicle-associated membrane protein (VAMP) and the syntaxin families are two major classes of SNARE proteins. VAMP8 was recently shown to play a major role in the exocytosis of zymogen granules by forming a complex with syntaxin 4 and SNAP23[98]. Exocytic vesicles have been thought to originate from the trans-Golgi network (TGN); however, recent evidence suggested that they may come

from both the endosomes[96] and lysosomes[99,100]. Ca^{2+}-dependent exocytosis of lysosomes requires synaptotagmin VII, a member of a protein family that functions as Ca^{2+} sensors, by forming a complex with VAMP7, syntaxin 4, and SNAP23[100]. SytVII/VAMP7-mediated lysosome exocytosis plays an important role in membrane repairs[99], in neurite outgrowth[101], in host defense to bacterial infections[102], and during *Trypanosoma cruzi* invasion into mammalian cells[103]. It is evident that formation of the membrane ruffles at the site of *Salmonella* entry requires addition of intracellular membranes. Coppolino *et al*. reported that expression of a dominant-negative *N*-ethylmaleimide-sensitive fusion protein (NSF) did not affect *Salmonella* invasion into epithelial cells, while it impaired the maturation of *Salmonella*-centaining vaculoes (SCVs)[104]. They also found that VAMP3, a v-SNARE in the recycling endocytic pathway that plays a role during phagocytosis by focally depositing VAMP3-containing vesicles at the site of phagosome formation, did not inhibit bacterial invasion. Thus, the intracellular compartments and the repertoire of SNARE proteins that may be involved in *Salmonella*-induced membrane ruffles remain unknown.

4 *Salmonella* actin-binding effectors modulate host actin dynamics directly

Many pathogens evolved strategies to modulate the actin dynamics of the host cell cytoskeleton. *Salmonella* encodes actin-binding proteins, SipA and SipC, two type III translocated effector proteins, to modulate actin dynamics directly[47-49,105,106]. Based on *in vitro* biochemical analyses, SipA is capable of decreasing the critical concentration for actin polymerization, inhibiting depolymerization of actin filaments and increasing the bundling activity of T-plastin. These characteristics have led to the hypothesis that SipA affects actin dynamics in cells by initiating actin polymerization at the site of *Salmonella* entry by lowering the critical concentration of actin needed for polymerization. Furthermore, SipA may increase the stability of actin bundles that drive and support the growth of membrane ruffles and filopodia that ultimately engulf and internalize the bacteria by modulating the actin-bundling activity of plastin[89,106]. Studies using cryo-electron microscopy and three-dimensional image reconstruction have revealed that the actin-binding domain of SipA interacts with actin filaments by contacting subdomain 4 of one actin subunit and subdomain 1 of the other actin subunit on the opposite long-pitch helical strand[107]. The binding pattern of SipA to actin filaments share striking similarity to that of nebulin to muscle actin[107]. This suggests that SipA interacts with actin filaments by mimicking the host actin-binding protein nebulin.

SipC possesses multiple functions: translocation of effectors, actin-nucleation and F-actin bundling[49,108]. It has been technically difficult to assess the roles of individual function during *Salmonella* infection because of these multiple functional activities. One way to address it is to make a mutant that loses one activity while maintaining the other activities at the wild type level. The actin-nucleation activity was successfully separated from the effector translocation activity by Chang *et al*[48], which allowed the establishment that the nucleation-deficient mutant (*sipC* # 1) had significantly reduced ability to induce actin cytoskeleton rearrangements, resulting in lower bacterial invasion[48].

It was reported that the recombinant $SipC_{199-409}$ protein that had the actin-nucleation activity was monomeric in solution as examined by size-exclusion chromatography coupled with multiangle laser light scattering (SEC-LS) assays and analytical ultracentrifugation[48]. Previous studies have suggested that either multimer formation or tandem actin-binding domains on a single protein are essential for the actin-nucleation activity[77,78,109,110]. One possibility is that $SipC_{199-409}$ exerts its actin-nucleation activity through weak or transient multimerization. Alternatively, the presence of monomeric actin may promote SipC multimerization. As the multimeric form of $SipC_{199-409}$ protein is likely to be weak or transient, EDC, a commonly used zero-length cross-linking agent, was used to capture transient SipC multimers. When incubated with EDC, wild type $SipC_{199-409}$ protein formed dimers and to a lesser extent multimeric species, whereas the actin-nucleation-deficient $SipC_{199-409}$ #1 mutant[48] showed dramatically reduced

level of dimers and no detectable multimeric species. These data suggest that dimer and multimer formation may contribute to the actin-nucleation activity of $SipC_{199-409}$. Furthermore, a series of mixing cross-linking experiments indicated that the N-terminus, the middle, and the C-terminus of $SipC_{199-409}$ are all in close proximity, suggesting that the $SipC_{199-409}$ protein forms a parallel dimer along the entire molecule leading to close contact at both the N-terminus and the C-terminus of $SipC_{199-409}$[105]. This notion is consistent with an earlier observation that $SipC_{199-409}$ is likely folded in an extended conformation[48]. The importance of the actin-nucleation activity was further demonstrated using the streptomycin-treated mouse infection model[111]. Mice infected with wild type had small cecums and severe cecum inflammatory lesions including luminal debris, epithelial desquamation, epithelial proliferation, neutrophilic infiltration in the mucosal crypts and lamina propria, submucosal edema, and neutrophilic and mononuclear infiltration in the submucosa and occasionally, serosa. In contrast, mice inoculated with *Mt*#1 or the *invG* mutant had minimal or very small numbers of neutrophils in the mucosal lamina propria and submucosa and no or minimal edema in the submucosa.

5 *Salmonella* exploit host cell signaling pathways to regulate actin dynamics

It is evident that *Salmonella* entry into epithelial cells results from a series of highly coordinated cellular responses that are directed to the host actin cytoskeleton both directly by actin-binding proteins and indirectly by exploiting host cell signaling pathways. Type III effectors SopE and SopE activate CDC42 and Rac1 signal transduction pathways by binding to CDC42 and Rac directly and act as potent exchange factors for these small G proteins[50, 112, 113] to promote actin cytoskeleton rearrangements. The activation of CDC42 and Rac triggers a series of signal transduction events that lead to actin cytoskeleton rearrangements and the production of pro-inflammatory cytokines. Biochemical, cellular and structural studies of SopE have unraveled the molecular mechanism of CDC42 activation by SopE. SopE achieves this by binding to the switch I and II region of CDC42, the same site where the eukaryotic GEFs interact with CDC42[114]. Eukaryotic GEFs exert their GEF catalytic activity through a well-conserved DH domain. Remarkably, the catalytic domain of SopE folds into a completely different structure than the DH domain of all known eukaryotic GEFs[114].

Cells infected with *S. enterica* serovar Typhimurium quickly recover from the dramatic actin cytoskeleton rearrangements and regain their normal cellular architecture[115]. Thus, *Salmonella* has evolved the ability to induce both actin cytoskeleton reorganization and to modulate host cellular signal transduction pathways to help the recovery of the actin cytoskeleton. SptP was found to be partly responsible for this recovery by acting as a GTPase-activating protein (GAP) for CDC42 and Rac[91]. Structural analysis indicated that the GAP domain of SptP makes extensive contact with the switch I and switch II region Rac1[116]. Residues from SptP directly contact key elements in Rac1 associated with the GTP hydrolysis reaction facilitating the hydrolysis of GTP to GDP and thus inactivating Rac1[116]. SptP may exert its GAP activity toward CDC42 through a similar mechanism. Similar to the structural analysis of SopE, SptP appears to have evolved convergently from the coevolutionary selective pressures of the host – pathogen interaction.

Interestingly a *sopE* null mutant has only a mild defect in entry into epithelial cells, while a double *sopEsopB* null mutant is severely defective in entry[88]. SopB, an inositol polyphosphatase, was originally reported to have overlapping functions with SopE by mobilizing cellular inositol polyphosphate fluxes[51] and controlling levels of phosphatidylinositol-4, 5-bisphosphate at the base of *Salmonella*-induced actin rearrangements[57] to facilitate ruffle formation. Studies have suggested that SopB-dependent phosphoinositides, but not inositol polyphosphates, are responsible for the actin cytoskeleton rearrangements and bacterial invasion. PtdIns(4, 5)P_2 level was locally increased in *Salmonella* induced ruffles accompanied by a SopB-dependent PtdIns(4, 5)P_2 decrease in the invaginating regions for efficient membrane fission and the formation of SCVs[57]. SopB can also increase the level of PtdIns(3, 4)P_2 and

activates protein kinase B (Akt/PKB)[117]. More recently, SopB-dependent accumulation of PtdIns(3)P was found in the membrane ruffles and nascent SCVs[118]. The persistence of PtdIns(3)P on SCVs has been proposed to provide a niche for intracellular *Salmonella* replication. Furthermore, exogenously expressed SopB localizes to endosomes containing PtdIns(3)P and inhibits vesicle trafficking from endosomes to lysosomes[119]. RhoG was suggested to participate in SopB-induced actin cytoskeleton rearrangements[120].

More recently, it has been reported that SopB-generated PtdIns(3)P binds VAMP8/endobrevin to promote efficient bacterial phagocytosis[121]. VAMP8 is recruited to *Salmonella*-induced macropinosomes in a nocodazole-dependent, but Brefeldin A-independent, manner. VAMP8 directly binds to and colocalizes with PtdIns(3)P. The inositol phosphatase activity of SopB is required for PtdIns(3)P and VAMP8 accumulation, while wortmannin, a specific phosphatidylinositol 3 kinase inhibitor, has no effect. Knockdown of endogenous VAMP8 by small interfering RNA or expression of a truncated VAMP8 (1-79aa) reduces the invasion level of wild type *Salmonella* to that of the phosphatase-deficient SopB (C460S) mutant. This study suggests that *Salmonella* exploits host SNARE proteins and vesicle trafficking to promote bacterial entry[121]. Interestingly, it also suggested that the phosphatase activity of SopB recuits Rab5 which in turn associates with Vps34, a PI3-kinase that is responsible for PI(3)P formation on SCVs[122]. It is proposed that SopB mediates PI(3)P production on the SCVs indirectly through recruitment of Rab5 and its effector Vps34. Thus, the precise mechanism of how SopB promotes bacterial entry remains controversial.

A model for *Salmonella* invasion has emerged based on recent advances in understanding contributions from individual *Salmonella* effectors (Fig. 3). Contact with host cells activates the invasion-associated type III secretion system, resulting in the delivery of a panel of effector proteins (e.g., SipA, SipC, SopE, SopB, and SptP). Introduction of the exchange factor SopE and possibly the inositol polyphosphatase SopB results in the activation of CDC42, Rac1, and RhoG, the stimulation of downstream signaling pathways, including the Arp2/3 complex, and the recruitment of plastin and other ruffling-associated molecules initiating the actin cytoskeleton reorganization. The bacterial effector protein SipA and SipC helps this process by lowering the critical concentration of actin, stimulating the bundling activity of plastin and stabilizing F-actin, and nucleating the actin assembly. SopB helps the ruffling process by generating PtdIns(3)P to recruit VAMP8-containing vesicles. In addition, immediately following the translocation of SPI-1 type III effectors, Slingshot-mediated dephosphorylation leads to the brief activation of actin-depolymerazing factor (ADF)/cofilin. This results increased treadmilling of actin filaments at the site of bacterial entry leading to outward protrusion of membrane ruffles. ADF/cofilin activities are down-regulated through LIM kinase to increase the stability of F-actin filaments to facilitate the final engulfment of bacteria. The combined activities of these effectors result in a localized and pronounced outward extension of the membrane ruffles, resulting in the engulfment of *Salmonella* in an enclosed membrane compartment. Subsequent to the stimulation of CDC42 and Rac1, *Salmonella* also delivers another effector protein, SptP, which reverses the activation of these small G proteins by stimulating their intrinsic GTPase activity and therefore facilitating cell membrane recovery.

6 Conclusion

Although *Salmonella* and *Listeria* utilize very different molecular mechanisms to gain entry into host cells, they both employ the same fundamental strategy: functional mimicry of key host cellular factors. It is striking that primary amino acid sequences or three-dimensional structures are effective predictors of functional mimicry. These novel functional mimicry factors have most likely evolved through the long and intimate interactions between bacteria and their host cells. One of the most obvious advantages of functional mimicry is to allow the host cell to regain normal functions after bacterial entry through existing cellular regulatory pathways.

Recent advances in understanding bacterial entry have led to the identification of host cell receptors and bacterial effector targets. This provides a molecular understanding of the entry determinants from the

perspective of both the bacteria and the host. However, we still lack a comprehensive understanding of the entire entry process. For example, while more is known about how actin polymerization is initiated in *Salmonella*-induced ruffles, little is known about how the depolymerization is initiated when the actin cytoskeleton rearrangements recover. In addition, a number of *Salmonella* proteins are translocated into host cells and are responsible for bacterial survival and maintenance of the SCVs inside host cells through mechanisms that are not presently understood. We also lack any knowledge of how the activities of different effectors (some of which are antagonistic to each other) are coordinated inside the cells. Furthermore, while significant progress has been made toward understanding how *Salmonella* induces its own uptake into host cells, the fate of the translocated bacterial proteins and how host cells cope with the infusion of a large number of "foreign" proteins, many of which are cytotoxic to host cells, is unknown. A more comprehensive model for understanding *Salmonella* pathogenesis requires further investigation.

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(收稿日期: 2009-04-09)

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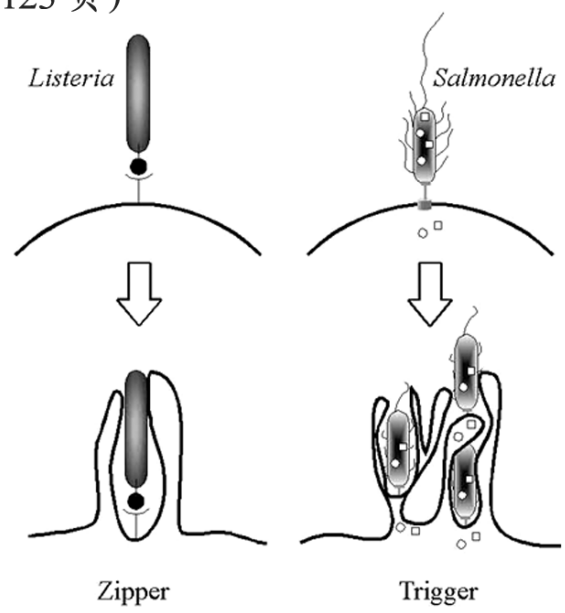
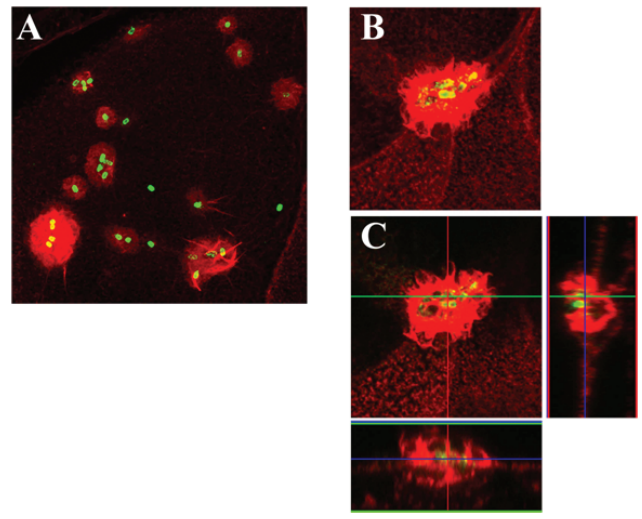


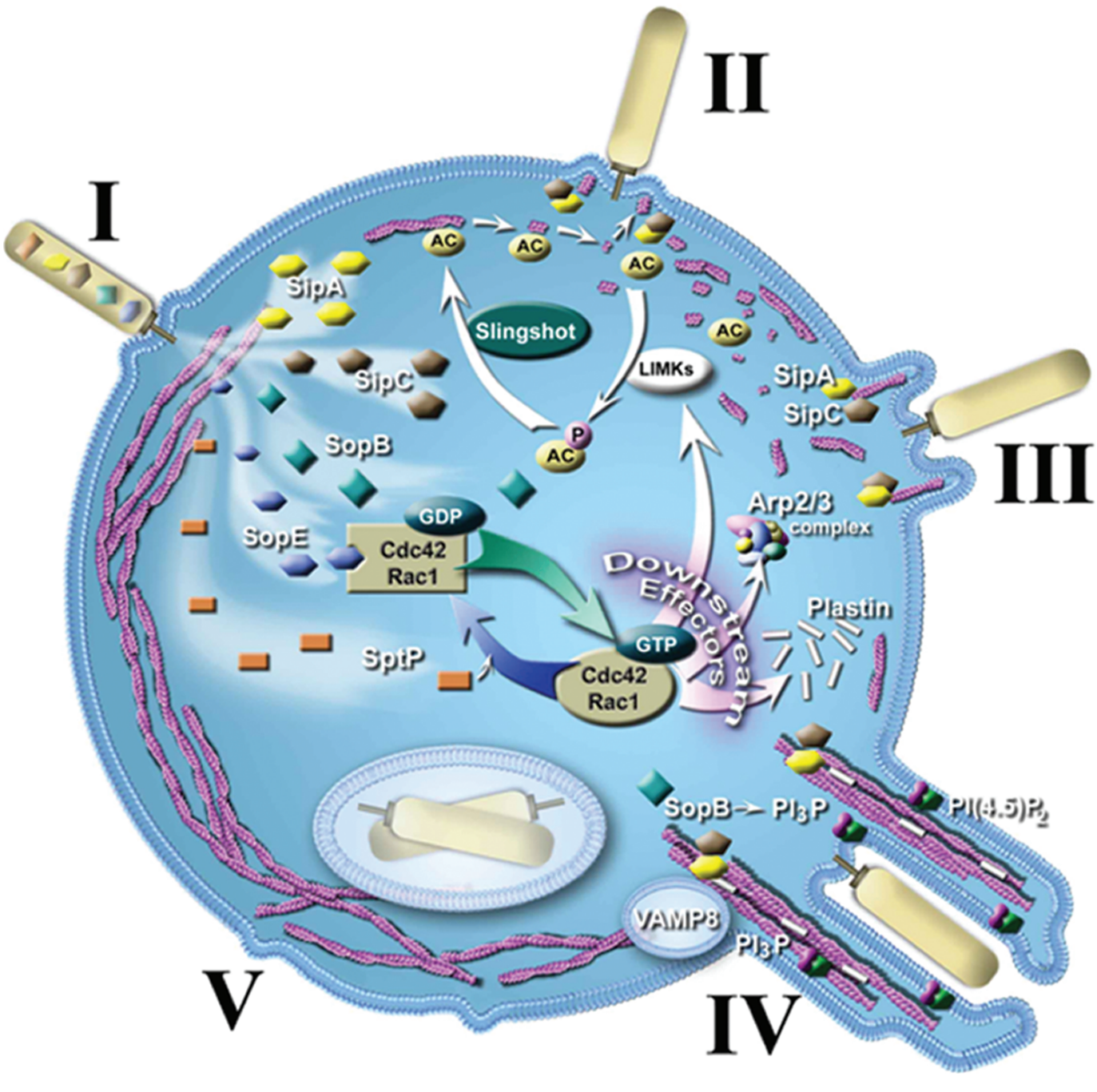
The "zipper" mechanism involves the engagement of a bacterial ligand with a host cell receptor leading to the gradual movement of the plasma membrane alongside the surface of the invading bacteria and eventually wraps the bacteria inside the host cell membrane. The "trigger" mechanism induces dramatic actin cytoskeleton rearrangements and membrane ruffling leading to macropinocytosis and bacteria entry.

Fig 1. The "zipper" and "trigger" bacterial entry mechanisms



Actin was visualized by staining with Texas Red-labeled phalloidin (red), bacteria were stained with rabbit polyclonal anti-*Salmonella* antiserum (green). A, B: Images represent black white projections of z-section slices obtained on a Zeiss AxioVert 200M deconvolution microscope. C: Represents a single section slice from the top and two sides illustrating the profuse actin cytoskeleton rearrangements and the relative position of *Salmonella*.

Fig 2. *Salmonella*-induced actin cytoskeleton rearrangements in Caco-2 intestinal epithelial cells



Activation of the type Ⅲ secretion system and the delivery of bacterial effector proteins (Ⅰ, Ⅱ). Injection of the exchange factor SopE and the inositol polyphosphatase SopB results in the activation of CDC42 and Rac1 (Ⅱ), leading to the recruitment of ruffling-associated molecules. SipA and SipC function to lower the critical concentration of actin, stimulating the bundling activity of plastin and stabilizing F-actin, and nucleating the actin assembly (Ⅲ). SopB promotes membrane fission process by decreasing the local concentration of PIP2 at the base of the membrane ruffles and by recruiting VAMP8 (Ⅳ). The combined activities of these effectors result in a localized and pronounced outward extension of the membrane ruffles, resulting in the engulfment of *Salmonella* in an enclosed membrane compartment. *Salmonella* delivers another effector protein, SptP, which reverses the activation of these small G proteins by stimulating their intrinsic GTPase activity and therefore facilitating cell recovery (Ⅴ).

Fig 3. Model for *S. typhimurium* interaction with host cells

沙门菌侵袭研究进展

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摘　要: 鼠伤寒沙门菌表达两个不同的III型分泌系统(type III secretion/translocation systems, TTSS), 分别由致病岛 1 和 2 (pathogenicity islands 1 and 2, SPI-1 and SPI-2) 编码。细菌依赖 TTSS 将效应蛋白转运至宿主细胞, 通过“触发”机制诱导细菌进入宿主细胞。这些效应蛋白可诱导细胞骨架重排, 导致“巨吞饮”, 促使细菌入侵。本综述依据多种沙门菌效应蛋白的功能, 建立沙门菌侵袭模型。TTSS 活化并转运效应蛋白进入宿主细胞发挥功能(Ⅰ)。小 G 蛋白交换因子 SopE 和肌醇磷酸酯酶 SopB 通过激活 CDC42 和 Rac1, 诱导内陷相关的蛋白聚集(Ⅱ)。SipA 和 SipC 通过降低肌动蛋白临界浓度、刺激网素成束、稳定纤维状肌动蛋白(fibrous actin, F-actin) 以及使肌动蛋白核化等功能, 促使细菌入侵 (Ⅲ)。SopB 可使膜内陷区 PIP2 的浓度降低以及 VAMP8 聚集, 促使细胞膜分裂(Ⅳ)。这些效应蛋白的联合作用, 使膜皱褶在局部向外显著延伸, 使沙门菌被细胞内形成的特殊膜结构包裹。沙门菌的另一种效应蛋白 SptP, 通过刺激小 G 蛋白内源性 GTPase 的活性, 抑制小 G 蛋白的活化, 使细胞膜恢复至原有状态(Ⅴ)。

关键词: 沙门菌; 侵袭; 模式; 效应蛋白

微生物在自然界中广泛存在, 与人类的健康密切相关。大多数的细菌寄生在人体的肠道系统, 肠上皮不仅是阻止微生物播散的有效屏障, 而且是人体吸收的营养成分进入血液循环的主要场所。它一方面阻止一些大分子物质如细菌的摄入, 另一方面通过胞饮作用和受体介导的细胞内吞作用促进营养物质的吸收。

细菌侵袭宿主细胞有 2 种机制: 一种是细菌配体与细胞相应受体结合后, 细菌被宿主细胞膜包裹而摄入, 被称为“拉链”(zipper) 机制。另一种是细菌通过其分泌的效应分子诱导细胞骨架重排和细胞膜内陷而导致了“巨吞饮”(macropinocytosis), 被称为“触发”(trigger) 机制。在“触发”机制中需要多种细菌蛋白共同发挥功能, 但在“拉链”机制中, 细菌的 1 个因子即可有效地介导侵袭。利用“触发”机制侵袭宿主细胞的典型细菌有沙门菌和志贺菌, 而以“拉链”机制侵袭宿主细胞的主要细菌为李斯特菌和耶尔森菌(图 1)。本文对沙门菌如何诱导自身侵袭肠上皮细胞的机制作一综述。

1　沙门菌病

沙门菌是一种革兰阴性肠道病原菌, 具有周身鞭毛, 能运动, 对人及多种温血动物具有致病性。在人类, 可导致食物中毒、胃肠炎、伤寒以及败血症等。沙门菌病是一种重要的人畜共患病, 感染人类的主要途径是摄入被病原菌污染的食物。与大多数肠道致病菌类似, 鼠伤寒沙门菌的致病性与其毒力因子密切相关。该菌在定植于小肠之前, 必须有效地耐受胃内的酸性(低 pH 值) 环境, 到达小肠组织, 细菌能够破坏小肠上皮的细胞屏障, 侵袭小肠上皮细胞并在细胞内存活、繁殖。致病性沙门菌在与宿主长期相互作用中进化, 形成了一种复杂的致病机制, 既能存活于胃内的酸性环境中, 又能侵袭小肠微皱褶(microfold, M) 细胞和肠上皮细胞。鼠伤寒沙门菌侵袭非吞噬细胞后, 在细胞内形成一个包裹细菌的膜性小泡, 这种特殊结构不仅有利于细菌存活, 而且可以有效地逃避宿主的免疫应答。此外, 该菌亦可在巨噬细胞中生长、繁殖。Vazquez-Torres 等学者研究发现, 沙门菌可以通过 $CD18^{+}$ 巨噬细胞的吞噬作用播散, 进而感染肠外组织器官。研究表明, 鼠伤寒沙门菌能在体外感染上皮细胞和巨噬细胞, 这一模型的建立有助于对沙门菌与宿主细胞相互作用的分子机制进行更好的研究。另外, 对类似于极性肠上皮细胞的极性上皮细胞的体外培养, 为沙门菌的研究提供了更好的实验条件。

2　致病岛和III型蛋白分泌系统

大多数与沙门菌侵袭力相关的毒力基因及操纵

子聚集成簇分布于细菌染色体的某些特定区域, 而这些毒力基因在染色体组成相似的大肠埃希菌中并不存在, 因而被命名为沙门菌致病岛(*Salmonella* pathogenicity island, SPI) 。毒力质粒与沙门菌的致病性亦有关。沙门菌至少存在 5 个 SPI 与其感染过程的特定阶段相关。SPI1 位于沙门菌染色体的 63 位点处, 全长 43 kb。SPI1 在沙门菌侵袭 M 细胞和肠上皮细胞的过程中发挥至关重要的作用。研究证实, SPI1 的突变株经口服感染时毒力缺失, 但经腹腔注射感染时毒力并没有明显变化, 进一步验证了 SPI1 的功能。对小鼠伤寒模型和小牛感染模型的研究中发现, 上皮细胞侵袭能力缺陷的细菌突变株是无致病性的。SPI2、SPI3 和 SPI4 分别位于染色体 31、82 和 92 位点处, 均与细菌在宿主细胞内的存活、繁殖密切相关。SPI5 初发现时与炎症和腹泻有关。最近研究表明, 至少有 1 种 SPI5 编码的蛋白 (SopB) 也参与沙门菌侵袭宿主细胞的过程。

SPI1 和 SPI2 编码 1 种特异性的蛋白分泌和转运系统, 称之为Ⅲ型分泌系统(type Ⅲ secretion system) 。另 3 种致病岛虽然并不编码Ⅲ型分泌系统, 但编码分泌蛋白, 并经 SPI1 和 SPI2 编码的Ⅲ型分泌系统分泌。SPI1 基因根据其编码的蛋白可分为 3 类: ①编码分泌/转运装置的蛋白; ② 编码分泌/转运进入宿主细胞的蛋白(效应蛋白) ; ③编码具有调节作用蛋白。电子显微镜分析表明, SPI1 编码的Ⅲ型分泌系统是一种在生化和结构上均类似于细菌鞭毛系统的“针状复合物”, 这种针状复合物至少包括 SPI1 编码的 3 种蛋白(PrgK, PrgH 和 InvG) 。*prgK*、*prgH* 或 *invG* 的突变可导致一系列的鼠伤寒沙门菌效应蛋白(SipA, SipB, SipC 等) 分泌受阻, 而这些蛋白是细菌入侵非吞噬的上皮细胞所必需的。一些研究表明蛋白的分泌需要宿主-细胞的接触。在特定的实验条件下, 亦可诱导分泌这些蛋白, 这将有利于在缺乏宿主细胞的情况下对其进行研究, 这些分泌于鼠伤寒沙门菌培养上清液中的蛋白, 可通过十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE) 进行观察。SPI1 分泌的蛋白中, SipA、SipC、SopE、SopE2 和 SopB (亦称 SigD) 通过调节宿主肌动蛋白细胞骨架, 促使细菌入侵宿主细胞。SPI2 效应蛋白通过调节细菌在细胞内的移动, 关系到细菌在宿主细胞内的存活。在动物模型的研究中发现, SPI1 的效应蛋白 SipA、SopA、SopB、SopD、SopE 以及 SopE2 主要与炎症和腹泻相关, 但其机制目前尚未明确。

3 肌动蛋白细胞骨架重排和沙门菌入侵宿主细胞

为了理解沙门菌效应蛋白调节宿主细胞细胞骨架的分子机制, 我们首先对哺乳动物细胞骨架的组分及其功能的调节作一简单介绍。哺乳动物细胞骨架由微丝(microfilament, MF) 、微管(microtubule, MT) 和中间纤维(intermediate filament, IF) 3 种组分构成, 它们与细胞的运动和稳定性有关。细胞骨架的 3 种组分在结构上是一个聚合和解聚的动态过程, 均由单体蛋白聚合而成。MF、MT 以及 IF 分别由单体肌动蛋白(actin) 、微管蛋白(tubulin) 和中间丝蛋白(intermediate-filament protein) 聚合而成。在上皮细胞, MF 同向平行排列, 构成绒毛, 维持细胞表面的形状。MT 自中心体生长至细胞外周。IF 通过桥粒与邻近细胞连接。在构成细胞骨架的所有组分中, MF 的可塑性最强。

肌动蛋白细胞骨架在很多细胞活动中起重要作用, 包括细胞的运动和内吞。根据结构的不同, 细胞内的肌动蛋白细胞骨架主要分为 3 种类型: 应力纤维(stress fibers) , 板状伪足(lamellipodia) 和丝状伪足(filopodia) 。肌动蛋白纤维束构成的应力纤维, 主要分布在培养细胞的基质。板状伪足和丝状伪足均是细胞运动过程中肌动蛋白的延伸形成的。尖刺状的丝状伪足包含长束状的肌动蛋白纤维, 而扁平的板状伪足则是由网状的肌动蛋白纤维构成。肌动蛋白细胞骨架的形成是一个高度动态过程, 并且受精密的调节控制。肌动蛋白的单体为球形分子, 称为球形肌动蛋白(globular actin, G-actin) , 其聚合形成的高度有序的多聚体称为纤维状肌动蛋白 (fibrous actin, F-actin) 。肌动蛋白组装过程的限速步骤是稳定二聚体、三聚体和四聚体的“种子”形成阶段, 通常也称为肌动蛋白核化。一些肌动蛋白结合蛋白以及信号转导因子如 CDC42、N-WASP 和 Arp2/3 复合体等均可促进肌动蛋白核化过程。此外, 细胞内多种肌动蛋白结合蛋白可调节细胞内 G-actin 和 F-actin 水平, 使其达到一种动态平衡状态。细胞内 G-actin 单体浓度低于临界浓度, 即肌动蛋白开始聚合的浓度。尽管细胞内的 G-actin 和 F-actin 浓度是恒定的, 但在细胞内两者持续处于一种相互转换的动态平衡中。F-actin 一端解聚与另一端聚合同步发生, 其纤维长度不变, 称为踏车 (treadmilling) 。肌动蛋白维持这种动态转换平衡状

态, 有利于细胞及时对环境的变化作出反应, 从而维持细胞骨架结构的完整性。肌动蛋白细胞骨架结构受到不同肌动蛋白结合蛋白(actin-binding protein) 的协同调节, 这些结合蛋白种类繁多, 包括: G-actin 结合/隐蔽蛋白, 如前纤维蛋白(profilin) ; F-actin 封端蛋白, 如凝溶胶蛋白(gelsolin) ; F-actin 截断蛋白, 如肌动蛋白解聚因子(actin depolymerazing factor, ADF) /丝切蛋白(cofilin) ; F-actin 成束蛋白, 如网素(丝束蛋白) 。利用体外不同的实验条件, 对肌动蛋白结合蛋白的研究发现, 这些蛋白功能复杂。据此推测, 肌动蛋白结合蛋白在生物体内的功能发挥可能依赖于其浓度和空间定位。

肌动蛋白的动态组装过程受很多因素调节, 包括 pH 值、离子强度、磷酸肌醇、Rho 家族的鸟苷三磷酸酶(Rho family GTPases, Rho GTPases) CDC42 和 Rac、肌动蛋白关联蛋白等。小相对分子质量 Rho 蛋白家族的 GTPases 是肌动蛋白细胞骨架的重要调节因子。Rho GTPases 作为一种“分子开关”, 在无活性的 GDP 结合形式和有活性的 GTP 结合形式之间转换。正如命名所示, Rho GTPases 均有内源性 GTPase 的活性, 活化时蛋白构象发生变化, 从而与下游的效应分子结合而发挥相应的作用。GTP 水解导致蛋白基底部与 GDP 结合, 使其从活性状态转变为去活性状态; 同样, GTP 亦可交换 GDP 从而激活 GTPases。由于 GTPases 内源性的水解和核酸交换发生效率较低, 因此, GTP 水解成 GDP 的过程主要受 GTPase 激活蛋白(GTPase activating protein, GAP) 的激发作用, 且鸟苷酸交换因子(guanine nucleotide exchange factor, GEF) 可促进 GDP/GTP 交换。所有已知的 Rho GTPases 特异性 GEF 均具有 Dbl 同源结构域(Dbl homology domain, DH) , DH 可与分子开关 I 区和 II 区相互作用, 从而促进 Rho GTPases 在无活性状态与活性状态间的转变。

肌动蛋白核化蛋白主要有三大家族: Arp2/3 复合体、形成素(formin) 和 Spire。对 Arp2/3 复合体生化、分子模型及其结构的研究发现, 肌动蛋白关联蛋白 Arp2 和 Arp3 形成的二聚体结合到微丝慢速生长末端, 促进肌动蛋白核化。形成素家族成员促进肌动蛋白核化的机制可能是因其形成的同源二聚体结合到微丝的倒刺端而发生, 在体内需前纤维蛋白的协同发挥功能。近来研究发现, Spire 的 4 个纵向排列的 WH2 结构域可形成 1 条单链肌动蛋白聚合体, 可诱导微丝合成, 促进肌动蛋白核化。

沙门菌进入宿主细胞的显著标志是在细菌和肠上皮细胞接触位点处发生大范围的肌动蛋白细胞骨架重排(图 2) 。肌动蛋白聚合抑制剂——细胞松弛素可完全抑制该细胞骨架重排过程, 并阻止细菌侵袭宿主细胞。研究表明, SPI1 编码的Ⅲ型分泌系统的效应蛋白在诱导主细胞骨架重排过程中是必需的。在与宿主细胞相互作用的长期进化过程中, 沙门菌已形成一种精密的调控系统, 一方面诱导细胞骨架重排以利于细菌侵袭宿主细胞, 另一方面可保证细菌与宿主细胞共存。越来越多的资料表明, 该过程受多种细菌和宿主细胞因子的共同调节。单一效应蛋白突变对沙门菌毒力并没有明显的影响, 表明沙门菌致病性是多种因素偶联产生的。

细胞膜表面可与内质网(endoplasmic reticulum, ER) 、内体甚至溶酶体的小泡融合而增大。小泡运输过程受很多细胞因子的影响, 如 Rab GTPases、可溶性 *N*-乙基顺丁烯二酰亚胺-敏感因子附着蛋白受体 (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor, SNARE) 蛋白, 细胞内吞和胞吐过程均与细胞内小泡运输过程有关。SNARE 蛋白位于小泡表面(v-SNARE) , 与目标膜形成复合体, 保证识别的特异性以及促进脂质双层的融合。SNARE 蛋白有两大类, 即小泡相关的膜蛋白(vesicle-associated membrane protein, VAMP) 和突触融合蛋白。VAMP8 通过与突触融合蛋白 4 与 SNAP23 形成复合体, 在酶原的胞吐过程中起重要作用。研究表明 VAMP8 和胞泌小泡均来自内体和溶酶体。突触结合蛋白Ⅶ参与依赖 Ca^{2+} 的溶酶体胞吐过程, 突触结合蛋白Ⅶ与 VAMP7、突触融合蛋白 4 和 SNAP23 形成复合体, 调节 Ca^{2+} 浓度。Syt Ⅶ/VAMP7 调节的溶酶体胞吐过程主要与膜修复、轴突生长、宿主对细菌感染的防御以及克氏锥虫侵袭哺乳动物细胞过程有关。可见, 沙门菌在侵袭细胞过程中, 与宿主细胞接触位点处形成的细胞膜内陷需要额外的胞内膜参与。Coppolino 等报道显性负突变的 *N*-乙基顺丁烯二酰亚胺-敏感融合蛋白(*N*-ethylmaleimide-sensitive fusion protein, NSF) 的表达并不影响沙门菌侵袭上皮细胞, 但会影响细胞内包裹沙门菌的膜性小泡(*Salmonella*-containing vacuole, SCV) 的成熟过程。他们研究还发现 VAMP3, 一种 v-SNARE, 通过在吞噬体形成部位沉积形成富含 VAMP3 的小泡, 与细胞吞噬过程密切相关, 亦不会影响细菌侵袭。因此, 参与沙门菌诱导的膜内陷过程中所需的细胞内组分以及 SNARE 蛋白, 目前尚未明确。

4 沙门菌效应蛋白对宿主肌动蛋白动态装配的直接调节

很多病原体在长期进化中形成多种机制, 从而调节宿主细胞骨架肌动蛋白的动态装配过程。沙门菌编码的 2 种效应蛋白 SipA 和 SipC 与肌动蛋白结合, 直接调节肌动蛋白的动态装配过程。体外研究表明, SipA 能降低肌动蛋白聚合的临界浓度, 从而阻止微丝的解聚合作用, 并增加 T-丝束蛋白的交联活性。据此推测, SipA 通过降低肌动蛋白聚合所需的临界浓度, 诱导沙门菌侵袭部位肌动蛋白聚合, 干扰细胞肌动蛋白的动态平衡。此外, SipA 可增加肌动蛋白纤维束的稳定性, 促进膜内陷的发生和丝状伪足形成, 最终通过网素的调节促使细菌内吞而进入宿主细胞。冷冻电子显微镜术以及三维立体成像技术的研究已经证实了 SipA 的肌动蛋白结合域, 在反向平行的螺旋链上分别与肌动蛋白亚结构域 4 和另一种肌动蛋白亚结构域 1 结合, 从而与微丝相互作用。SipA 与微丝的这种结合模式同 nebulin 与肌肉肌动蛋白相互作用模式高度相似, 表明 SipA 模仿宿主 nebulin 与微丝相互作用而发挥功能。

SipC 具有多种功能, 包括转运效应蛋白、使肌动蛋白核化以及促使 F-actin 成束。由于其在沙门菌感染过程中功能复杂, 在技术上很难对其单一功能进行研究。构建缺乏其中一种功能, 但其他功能不受影响的突变株是研究方法之一。Chang 等在不影响其效应蛋白转运功能的前提下, 构建了 SipC 肌动蛋白核化功能缺陷的突变株(*sipC*#1) , 成功地对其肌动蛋白核化功能进行了研究, 这种突变株不能诱导产生明显的细胞骨架重排, 从而降低了细菌入侵宿主细胞的能力。

多角度激光散射法(multiangle laser light scattering assays, SEC-LS) 和分析超速离心法检测发现, 具有肌动蛋白核化活性的可溶性 $SipC_{199\text{-}409}$ 重组蛋白以单体形式存在。以往的研究表明, 多聚体或单个蛋白上串联的肌动蛋白结合域是肌动蛋白核化所必需的。因此, $SipC_{199\text{-}409}$ 可能是通过弱聚合作用或暂时的多聚化发挥其核化活性。或者, 单体的肌动蛋白可促进 SipC 多聚化。鉴于 $SipC_{199\text{-}409}$ 蛋白这种弱聚合或暂时的多聚化, 一种常用的交联剂 EDC 被用于捕获 SipC 的短暂聚合状态。与 EDC 共同孵育的野生型 $SipC_{199\text{-}409}$ 蛋白形成二聚体和较少的多聚体, 而缺乏这种核化功能的 $SipC_{199\text{-}409}$#1 突变株与 EDC 共孵育时, 蛋白形成的二聚体明显降低, 而且几乎检测不到多聚体的形成。这些结果表明, 二聚体和多聚体的形成与 $SipC_{199\text{-}409}$ 的核化功能活性密切相关。进一步经 1 组混合的交联实验证实, $SipC_{199\text{-}409}$ 的 N 端、中间部分和 C 端是紧密相连的, 表明该蛋白沿着整个分子形成平行二聚体, 导致蛋白 N 端与 C 端紧密接触。这一观点与早期观察结果是一致的, 即 $SipC_{199\text{-}409}$ 可能以一种扩展的方式进行折叠。对经链霉素处理的小鼠感染模型研究进一步证实了肌动蛋白成核活性的重要性。野生株感染的小鼠表现严重的盲肠炎症损伤, 包括腔内坏死组织、上皮细胞脱落、上皮增生、黏膜腔中性粒细胞浸润、固有层和黏膜下层水肿、黏膜下层中性粒细胞和单核细胞浸润, 浆膜偶见炎性细胞。相比较而言, 接种 *Mt*#1 或 *invG* 突变株的小鼠黏膜、固有层以及黏膜下层中性粒细胞少见, 黏膜下层伴有轻微水肿或无水肿。

5 沙门菌利用宿主细胞信号转导通路调节肌动蛋白动态装配

沙门菌通过一系列高度精密的细胞反应调节宿主肌动蛋白细胞骨架排列, 导致细菌侵袭上皮细胞, 这些细胞反应或由肌动蛋白结合蛋白直接诱发, 或通过宿主细胞信号通路间接产生。III型分泌系统的效应蛋白 SopE 和 SopE2 不仅可直接结合于 CDC42 和 Rac, 活化其信号转导通路, 而且可作为小 G 蛋白有效的交换因子, 诱导细胞骨架重排。CDC42 和 Rac 的活化触发的细胞信号转导通路可导致细胞骨架重排以及促炎细胞因子的活化。对 SopE 的生化、细胞水平以及结构研究阐明了 SopE 活化 CDC42 的分子机制。SopE 结合到 CDC42 交换 I 区和 II 区, 即真核细胞 GEF 的作用位点, 活化 CDC42。真核细胞 GEF 是通过其高度保守的 DH 结构域发挥催化活性, 而 SopE 的催化结构域与所有已知 GEF 的 DH 结构域是完全不同的。

鼠伤寒沙门菌感染的细胞在发生细胞骨架重排后, 会快速恢复其原有的结构。因此, 沙门菌既可诱导细胞骨架的重排, 又可调节宿主细胞信号转导通路使细胞恢复原有形态。沙门菌效应蛋白 SptP 与细胞骨架恢复部分有关, 研究发现, SptP 是作为 GAP 发挥其功能的。对该蛋白的结构分析发现, SptP 的 GAP 功能域可与 Rac1 的转换 I 区和 II 区相互作用。SptP 的残基可直接与 Rac1 中促使 GTP 水解的关键区域结合, 使 Rac1 去活性。SptP 对 CDC42 的激活机制可能与 Rac1 类似。与 SopE 结

构进化过程类似, 在宿主-病原体长期共进化过程的选择压力下, SptP 蛋白结构趋同性进化。

sopE 无效突变株对沙门菌侵袭上皮细胞并无明显影响, 但 *sopE* 与 *sopB* 双突变株明显干扰细菌侵袭过程。最初研究认为, 肌醇磷酸酯酶 SopB 与 SopE 功能重叠, 一方面动员细胞内肌醇聚磷酸聚集, 另一方面抑制细胞内磷脂酰肌醇-4, 5-二磷酸〔phosphatidylinositol-4, 5-bisphosphate, PtdIns(4, 5)P_2〕的水平, 使其有助于沙门菌诱导的细胞骨架重排时膜内陷的发生。研究表明, 依赖 SopB 的磷酸肌醇, 而非肌醇磷酸酯, 与肌动蛋白细胞骨架重排和细菌侵袭有关。沙门菌诱导产生膜内陷, 形成有效的细胞膜分裂和形成 SCV, 局部 PtdIns(4, 5)P_2 增加, 而内陷区 SopB 依赖性 PtdIns(4, 5)P_2 降低。SopB 亦可使 PtdIns(3, 4)P_2 聚集并激活蛋白激酶 B (protein kinase B, Akt/PKB)。最近研究发现, 在膜内陷处以及新生 SCV 上可出现 SopB 依赖性 PtdIns(3)P 的聚集。SCV 上聚集的 PtdIns(3)P 可能与小泡内沙门菌的繁殖有关。进一步研究表明, 外源性的 SopB 定位于含有 PtdIns(3)P 的内体, 并可阻止小泡从内体至溶酶体的运输。RhoG 亦参与 SopB 诱导的肌动蛋白细胞骨架的重排过程。

据最新报道, SopB 催化生成的 PtdIns(3)P 与 VAMP8/endobrevin 结合, 可有效地促进细菌吞噬。沙门菌在 VAMP8 的协同作用下诱导产生“巨吞饮”, 这种方式依赖于 nocodazole, 而与 Brefeldin A 无关。VAMP8 与 PtdIns(3)P 直接结合并共定位于胞内, 其聚集依赖于 SopB 的肌醇磷酸酯酶活性, 与磷脂酰肌醇-3-激酶(phosphatidylinositol 3 kinase, PI3 kinase) 抑制剂 wortmannin 无关。通过 RNA 干扰技术敲除体内内源性 VAMP8 或截短的 VAMP8 (1 ～ 79 氨基酸) 蛋白后, 沙门菌野生株侵袭力与 SopB (C460S) 突变株的侵袭力相当。这些表明沙门菌可利用宿主 SNARE 蛋白和小泡运输过程促进细菌入侵。Mallo 等研究发现 Rab5 参与调节 SopB 的肌醇磷酸酯酶活性, Rab5 与 SCV 上 PI(3)P 生成所需的 PI(3)P 激酶 Vps34 有关。据此推测, SopB 通过 Rab5 及其效应蛋白 Vps34 间接调节 SCV 上 PI(3)P 的生成。SopB 如何促进细菌侵袭进入宿主细胞, 其机制有待于进一步研究。

依据不同沙门菌效应蛋白的功能, 建立了沙门菌侵袭模型(图 3)。通过沙门菌与宿主细胞接触活化Ⅲ型分泌系统, 从而转运一组效应蛋白(如 SipA、SipC、SopE、SopB、SptP) 进入宿主细胞。交换因子 SopE 以及肌醇磷酸酯酶 SopB 可活化 CDC42、Rac1 和 RhoG, 刺激下游的信号转导通路, 包括 Arp2/3 复合体、网素和其他膜内陷相关因子的聚集, 最终诱导细胞骨架重排。细菌效应蛋白 SipA 和 SipC 通过降低肌动蛋白临界浓度、刺激网素成束、稳定 F-actin 以及使肌动蛋白核化等, 促进细菌侵袭过程的发生。SopB 催化生成的 PtdIns(3)P 聚集在富含 VAMP8 的小泡, 引发膜内陷。此外, 一旦 SPI1 Ⅲ型分泌系统效应蛋白被转运入宿主细胞后, Slingshot 调节的去磷酸化即会暂时活化 ADF/cofilin, 增强细菌侵袭部位的微丝踏车行为, 导致膜皱褶的延伸。ADF/cofilin 在 LIM 激酶的作用下活性下调, 增加 F-actin 的稳定性, 最终促进细菌入侵。这些效应蛋白的联合作用, 使膜皱褶在局部向外显著延伸, 使沙门菌被细胞内形成的特殊膜结构 SCV 包裹。随后, 沙门菌的另一种效应蛋白 SptP, 通过刺激小 G 蛋白内源性 GTPase 的活性, 抑制 CDC42 和 Rac1 的活化, 使细胞膜恢复至原有状态。

6 结语

尽管沙门菌和李斯特菌通过不同的分子机制侵袭宿主细胞, 但它们所用的基本策略是相同的, 即模仿起主要作用的宿主细胞因子的功能。主要的氨基酸序列及其三维结构可有效预测其功能模仿, 这些新的功能模仿因子很有可能是在细菌与宿主细胞长期相互作用中因细菌进化而生成的。这些模仿因子最大的优势在于: 在细菌侵袭宿主细胞后, 通过已有的细胞调节通路使宿主细胞恢复其原有的功能。

近年来对细菌侵袭的研究明确了宿主细胞受体以及细菌效应蛋白所作用的靶点, 分别从细菌和宿主两方面阐明了细菌侵袭宿主细胞的决定因素。尽管如此, 我们仍不能从整体上把握细菌的整个侵袭过程。例如, 肌动蛋白聚合诱发膜内陷, 但细胞骨架恢复原有结构时的解聚合是如何发生的? 大量转运进入宿主细胞的沙门菌效应蛋白是通过何种机制促使细菌在细胞内存活, 并维持 SCV 的完整性? 在宿主细胞内, 不同的效应蛋白(一些蛋白在功能上是相互拮抗的) 之间是如何相互协同发挥作用? 此外, 在沙门菌诱导其自身进入宿主细胞后, 那些被转运的细菌蛋白命运如何? 宿主细胞又会如何处理大量对其自身有害的外源性蛋白? 因此, 更全面的沙门菌致病机制的模式有待于进一步研究。

(刘倩 译)

(收稿日期: 2009-04-07)