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## Commentary

# Interactions of *Salmonella* with host cells: Encounters of the closest kind

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Microbial pathogens have evolved a rich array of virulence factors to engage their hosts in very complex interactions (1). These interactions are aimed at gaining access to the host, avoiding its defense mechanisms, multiplying within it, and ultimately moving on to a new host. The complexity and fine-tuning of the strategies used by these microorganisms is particularly evident in pathogens that have sustained long-standing associations with their hosts. In these cases, the forces of evolution have shaped the terms of these encounters to secure the survival of both the host and the microbial pathogen. In fact, for many microbial pathogens the infected host constitutes their only "home" because the process of host adaptation has precluded them from the possibility of exploring other niches. Thus, it should come as no surprise that these microorganisms have "learned" very well how to manipulate the basic cellular functions of their hosts. The most recent example of such manipulation is presented in this issue of the *Proceedings* where Norris *et al.* (2) demonstrate that the *Salmonella* protein SopB is an inositol phosphate (Ins*P*) phosphatase.

*Salmonella enterica* is indeed a good example of a very well-adapted microbial pathogen. These bacteria can cause a wide variety of illnesses ranging from common food poisoning to more severe, often life-threatening, typhoid fever. Central to the pathogenesis of *S. enterica* is the function of a specialized protein secretion system, known as type III or contact dependent, which is encoded within a discrete contiguous region of its chromosome located at centisome 63 (reviewed in ref. 3). This region, which is known as a pathogenicity island, was acquired by horizontal gene transfer very early on in the evolution of *Salmonella*. This event most likely resulted in a significant niche expansion for these bacteria, perhaps marking the beginning of their long coexistence with vertebrate hosts. Type III secretion systems are composed of several proteins that form a remarkable needle-like organelle in the bacterial envelope (4). This structure resembles the flagellar basal body, which highlights the close evolutionary relationship between these two organelles. Besides the distinctive structural organization, type III secretion systems exhibit other unique features (reviewed in ref. 5). For example, proteins that travel through this pathway do not have a typical *sec*-dependent signal sequence and therefore are secreted in a *sec*-independent manner without processing their amino termini. In addition, these systems require a signal to activate their secretory function. Although certain growth conditions in the laboratory have been shown to activate type III secretion, the physiologically relevant activating signal is most likely derived from bacterial contact with host cells. Perhaps the most fascinating property of these systems is their ability to deliver bacterial effector proteins into the cytoplasm of eukaryotic host cells, therefore effectively working as "molecular syringes." Type III secretion systems are not a unique feature of *S. enterica* because they have been found in other pathogenic Gram-negative bacteria such as *Yersinia* spp., *Shigella* spp., *Escherichia coli*, *Pseudomonas aeuruginosa*, and *Bordetella* spp. and, most interestingly, in a number of plant pathogenic bacteria. Thus, this protein delivery system that has been conserved through evolution can operate not just in multiple species but also in multiple kingdoms.

When *Salmonella* comes in contact with intestinal epithelial cells, it induces dramatic changes in the actin cytoskeleton that closely resemble the membrane ruffles induced by growth factors or the activation of cellular oncogenes (reviewed in ref. 6). This activity is accompanied by profuse macropinocytosis, which ultimately directs the internalization of the bacteria into the host cell. In addition to changes in the actin cytoskeleton, *Salmonella* induces nuclear responses in the infected cell characterized by the activation of transcription factors that stimulate the production of proinflammatory cytokines (7–9). The production of cytokines plays an important role in the establishment of the inflammatory diarrhea that most often follows infection by these bacteria. The establishment of diarrhea also is helped by *Salmonella*-induced $Cl^-$ secretion and the subsequent water flux (10, 11). Stimulation of all these cellular responses requires the function of the centisome 63 type III secretion system as mutant *Salmonella* strains deficient in this system are unable to induce these responses.

Work during the last few years has identified essential host–cell signal transduction pathways and signaling molecules required for the *Salmonella*-induced cellular responses. For example, it has been established recently that the bacterial-induced nuclear responses leading to proinflammatory cytokine production are the result of the activation of the mitogen-activated protein kinases ERK, JNK, and p38 (12). Further studies have established an essential role for the small GTP binding proteins CDC42 and Rac-1 in both *Salmonella*-induced cytoskeletal and nuclear responses (13). Small molecular weight GTP-binding proteins can cycle between two states: a GDP-bound (inactive) and a GTP-bound (active) conformation capable of engaging a variety of effector molecules. Thus these molecules can function as molecular switches that control a large array of signaling events in a temporal and spatial manner (14). Calcium and inositol phosphophosphate fluxes also have been implicated in *Salmonella*-induced cellular responses (11, 15, 16). For example, *S. enterica* serovar *typhimurium* (*S. typhimurium*) was shown to induce calcium fluxes in intestinal epithelial cells, and the addition of calcium chelators blocked bacterial internalization into these cells (15, 16). Furthermore, infection of intestinal epithelial cells with *S. enterica* serovar *dublin* (*S. dublin*) resulted in a significant increase in Ins(1,4,5,6)$P_4$ levels, which promotes $Cl^-$ secretion by antagonizing PtdIns(3,4,5)$P_3$, an inhibitor of calcium-mediated $Cl^-$ secretion (see below) (11). The stimulation of both calcium and inositol polyphosphate fluxes was shown to require the function of the centisome 63 type III secretion system.



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Despite significant advances in the understanding of *Salmonella*-induced signal transduction pathways, the identity of the bacterial effectors directly responsible for the stimulation of such responses had remained elusive until recently. The absolute requirement of the centisome 63 type III secretion system for the stimulation of these responses strongly suggested that such effectors must be substrates of this system. The identification of several substrates of this system therefore has provided several candidate proteins to examine for effector function.

In a paper published in this issue of the *Proceedings* (2) and in another paper published early this year (17), two such effector proteins have been unambiguously identified. Norris *et al.* (2) report that one of the substrates of the centisome 63 type III secretion system, termed SopB, is an Ins*P* phosphatase. The predicted amino acid sequence of SopB exhibits two regions with sequence similarity to mammalian inositol polyphosphate 4-phosphatases. One of these regions constitutes the putative catalytic site of these enzymes as it contains a highly conserved cysteine residue absolutely required for activity. Consistent with this sequence homology, purified SopB exhibited Ins*P* phosphatase activity, hydrolyzing several inositol phopholipids and polyphosphates, including PtdIns(3)*P*, PtdIns(3,4)i$P_2$, PtdIns(3,4,5)$P_3$, Ins(1,3,4)$P_3$, Ins(1,4,5)$P_3$, Ins(1,3,4,5)$P_4$, and Ins(1,3,4,5,6)$P_5$. In general, SopB was shown to be able to hydrolyze phosphates at position 3, 5, and 6 of the inositol ring as well as the 4 position of Ins(1,3,4)$P_3$ and PtdIns(3,4)$P_2$. Thus, it is clear that SopB can generate a large variety of potentially active inositol phospholipids and inositol phosphates.

Of particular significance for the pathogenesis of *Salmonella*-induced diarrhea is the ability of SopB to generate Ins(1,4,5,6)$P_4$ from Ins(1,3,4,5,6)$P_5$. As discussed above, it has been shown previously that *Salmonella* infection of intestinal epithelial cells leads to the accumulation of Ins(1,4,5,6)$P_4$ (11), which promotes $Cl^-$ secretion by antagonizing PtdIns(3,4,5)$P_3$, an inhibitor of calcium-mediated $Cl^-$ secretion. This negative regulator is responsible for the inhibition of calcium-mediated $Cl^-$ secretion that follows epidermal growth factor stimulation of intestinal epithelial cells (11, 18, 19). This finding is of particular significance because it has been shown previously that *Salmonella* infection of intestinal epithelial cells results in both calcium fluxes and the stimulation of the epidermal growth factor receptor (15, 20). An attractive model is that SopB activity leads to increased $Cl^-$ secretion by catalyzing the production of a specific inositol polyphosphate that can act as an antagonist of a negative regulator of calcium-mediated chloride secretion. Increased $Cl^-$ secretion and the subsequent water efflux is likely to play an important role in the intestinal physiopathology that follows *Salmonella* infection. Consistent with this model, a *Salmonella* mutant strain expressing a catalytically inactive mutant of SopB was significantly impaired in its ability to induce fluid accumulation in an animal model of infection.

Earlier this year, Hardt *et al.* (17) reported that SopE, another substrate of the centisome 63 type III secretion system, is capable of stimulating actin cytoskeleton rearrangements and nuclear responses that closely resemble the responses induced by *Salmonella*. This bacterial protein induces these responses by stimulating GDP/GTP nucleotide exchange on the small GTP-binding proteins CDC42 and Rac, resulting in their activation. This finding indicates that *Salmonella* has evolved mechanisms to subvert at least two independent host–cell signaling pathways through the delivery of different effector proteins via its type III secretion apparatus: the Rho GTPase pathways through the activity of SopE and the phosphoinositide and Ins*P* pathways through the activity of SopB. *Salmonella* thus serves as a remarkable example of pathogen adaptation to modulate host cellular function.

The delivery of SopB and SopE by the same type III secretion system suggests the possibility that these two effector proteins may act in conjunction with one another even though they influence seemingly unrelated signaling pathways and appear to induce different cellular responses. Considering the wide substrate specificity displayed by SopB, at least *in vitro*, this hypothesis is indeed a likely possibility. There is abundant evidence linking signaling through small GTPases, inositol phospholipids, and Ins*P*s (21–26). Inositol phospholipids have been shown to activate Rho GTPases by either directly stimulating the dissociation of GDP (e.g., in CDC42) or by binding to Pleckstrin-homology domains in exchange factors (27). Also, Ins(1,4,5)$P_4$, one of the products of SopB activity, has been shown to modulate the activity of small G-proteins by influencing the function of their GTPase-activating proteins (28). Furthermore, several inositol phospholipids have been implicated in the modulation of the host-actin cytoskeleton (22, 29), and, conversely, Rho GTPases have been implicated in the modulation of the activity of anion channels (30). Thus, both SopB and SopE have the potential to stimulate similar responses, such as actin cytoskeleton rearrangements, the activation of transcription factors, and the modulation of chloride secretion by different and yet related signaling pathways. This hypothesis recently has been substantiated by the finding that SopB can stimulate actin cytoskeleton rearrangements and nuclear responses in a CDC42-dependent manner (D. Zhou and J.E.G., unpublished work).

*Salmonella* seems to have assembled a set of effectors with diverse biochemical properties that, on delivery to the host cell, can stimulate distinct, but functionally related, signaling events that lead to the induction of a carefully orchestrated set of cellular responses for the pathogen's benefit. Remarkable as it may seem, these interactions are, after all, the result of millions of years of "molecular tinkering" by evolutionary forces aimed at securing the survival of both the host and the pathogen through long-standing, largely peaceful coexistence. In fact, it is this mutual coadaptation that makes these microorganisms such useful biological probes to explore basic cellular functions.

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