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Effectors of Salmonella Pathogenicity Island 2

An island crucial to the life of Salmonella

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The tug of war between a pathogen and its host has been one of the most amazing stories in the field of microbial pathogenesis for ages. The strongest known species of all living organisms is the *Homo sapiens* and yet it is incredible how a pathogen of the size of few microns is smart enough to defeat this mightiest group of survivors. It is of utmost interest to understand the mechanisms behind the successful habitation of a pathogen inside the ever-resisting and complicate human body. Numerous examples of diseases caused by such pathogens exist which intrigues us to venture in the world of host-pathogen interactions.

The Gram-negative bacteria Salmonella represent one such example of a successful pathogen. Out of the few known species, *Salmonella enterica* is the most important from the clinical point of view. There are numerous serovars of *Salmonella enterica* that cause a variety of diseases in humans as well as animals, ranging from mild diarrhea to the systemic infection of typhoid fever. Typhoid fever is an infamous disease caused by the human-adapted serovar, *Salmonella enterica* serovar Typhi, which costs thousands of lives every year across the globe.^{1,2} On the other hand, non-human-adapted serovar *Salmonella enterica* serovar Typhimurium and others are known to cause enteritis in human and typhoid-like fever in mouse.

The ability of Salmonella to survive persistently within the host, while fighting against the complex anti-bacterial immune system, is attributable to a repertoire of virulence-associated genes known as Salmonella Pathogenicity Islands (SPI). Two star pathogenicity islands (PAIs) of Salmonella are SPI1 and SPI2, acquired by horizontal transfer independently of each other. These groups of genes are pathogen specific and encode two major type 3 secretion systems (T3SS)^{3,4} that export particular virulence factors from the bacterium to the host cell. While SPI1 plays role in the invasion of host cells by the bacterium,⁵ SPI2 assists the pathogen in survival and reproduction inside the extreme intracellular conditions and hence is required for systemic infection.⁶

After invading the host system, Salmonella faces a plethora of host-adapted mechanisms that ensure the destruction of the pathogen. To prevent the inevitable, Salmonella must acquire counter-mechanisms. Here, to the dismay of the host, comes the master player SPI2, which is committed to protect Salmonella from the physiologically unfavorable environment.

What Lies Within?

The SPI2 gene-cluster is present at 30 centisome position of the Salmonella genome. The genetic organization of 44 kb long SPI2

locus reveals two major groups of genes categorized based on their function. First, a 25 kb long region essential for systemic infection comprising (1) two-component system SsrAB, regulator of SPI2 expression, representing the only two genes transcribed in anticlockwise manner, (2) secretion system apparatus (*ssa*) comprising the series *ssaJ*, K, L, M, V, N, O, P, Q, R, S, T, U that code for the components of T3SS, (3) the secretion system effectors (*sse*), also called as substrates and (4) their chaperons (*ssc*), such as SscA and SscB. Second, the remaining 15 kb region which is considered unimportant for virulence, but renders Salmonella able to use tetrathionate as electron acceptor by expressing tetrathionate reductase (*ttrABC*) and its two component regulatory system (*ttrRS*) for anaerobic respiration under certain circumstances.⁷

The Voyage

Salmonella enter the host system by means of oral ingestion as a result of consumption of contaminated food or water. The host immediately fires back with the weapons of acidic pH of the stomach, secretory antibodies, antimicrobial peptides, digestive enzymes, bile salts, etc. to get rid of the unwelcomed intruder. But Salmonella, being an adamant pathogen, beat all these strategies.⁸ Once inside the small intestine, it first attaches to the mucosal cells of the intestine and then travels across the M cells to reach the lymphoid follicles of the Peyer's patches.⁹ An alternative mechanism of invading the host system is the uptake of Salmonella by CD18⁺ phagocytes, which allows the bacteria to spread systemically to spleen and liver.¹⁰ The macrophages present inside the lymphoid follicles of the Peyer's patches engulf the bacteria by macropinocytosis and then begins the journey of the pathogen across the host body via the reticuloendothelial system. The usually inhospitable environment of macrophages for microbes is apparently a beautiful niche for the stay of Salmonella, where it resides inside a membrane-bound compartment known as Salmonella-containing vacuole (SCV)¹¹. This is the site where

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the role of SPI2 comes into play. SPI2 is equipped with several virulence proteins that are assigned to manipulate host system for intracellular multiplication and to achieve efficient systemic spread to sites such as liver, spleen and eventually gall bladder from where Salmonella can be reseeded in the host system.¹²

A Panorama of Cinematic Events

Immediately after invasion, SCV attains the early endosomal markers, namely early-endosomal antigen 1 (EEA1) and the transferrin receptor (TfR),¹³ which are soon replaced with late endosomal markers in a Rab7-dependent manner, for instance, lysosomal glycoproteins (e.g. LAMP-1), vacuolar ATPases and Rab GTPases¹⁴ and subsequently mannose-6 phosphate receptors.¹⁵ The fusion of endosome with SCV is dependent on Rab5 and *N*-ethylmaleimide-sensitive fusion (NSF) protein. Eventually SCV escapes from endocytic pathway by SpiC-mediated vesicular trafficking inhibition¹⁶ to avoid lysosomal fusion. Also, Salmonella prefers to maintain one bacterium per SCV increasing the SCV load so that lysosome counts are comparatively insufficient to target SCVs.¹⁷

An interesting event in SCV maturation involves accumulation of cholesterol in the SCV membrane, which is originally SPI1-inducible at early stage of infection for invasion.¹⁸ The SPI2 effector SsaT is required for the incorporation of cholesterol in the lipid rafts of SCV hinting at its role in enhancing fusion of SCV with host-membrane.¹⁹

To maintain SCV within the cytoplasm and protect it from lysosomal fusion, few host cytoskeletal rearrangements are carried out by a team of SPI2 effectors. For example, the plasmid encoded SPI2 effector SpvB aids in vacuole-associated actin polymerization and destabilizes host cell cytoskeleton by ADP ribosylating actin and finally results in cytotoxicity.²⁰ SteC is a kinase that causes the assembly of F-actin meshwork around SCV.²¹ Contrastingly, SspH2 is involved in actin dissociation by binding to actin cross-linking protein filamin and G-actin binding protein, profilin.²² SseI is also found associated with filamin,²² which aids in host cell migration for systemic spread by binding to cell migration regulator IQGAP1.²³

Apart from actin, microtubule accumulation around the SCV serves as the scaffold for the synthesis of Salmonella-induced filaments (SIF) for intracellular replication. This process involves manipulation of dynein activity by SseF and SseG, to finally enable SCV to be transported to Golgi apparatus. In accordance with SifA and SseF, SseG deviates the exocytic vesicles containing nutrients, towards SCV to support the pathogen multiplication.²⁴ Formation of SIF marks multiplication of the pathogen. SIF synthesis depends on SifA²⁵ which also maintains the SCV integrity.²⁶ The other modulators of SIF formation include SpiC, SseG, SseF and SopD2.¹¹ Subsequently the acyltransferase SseJ esterifies cholesterol to remove cholesterol from SCV membrane in order to downregulate SIF formation and hence reduce the membrane rigidity at later stages.²⁷

Further, to combat the immune response targeting the pathogen, SPI2 effectors SseL and SspH1 inhibit NFκB-dependent expression, which ultimately inhibit MAPK signal pathways.²⁸

Although Salmonella possesses protection mechanisms such as, genetic pathways controlled by SoxRS, OxyR, σS, σE, SlyA, and RecA²⁹ as well as genes like hydrogen peroxide resistance gene (*hrg*)³⁰ for protection against ROS, SPI2 also confers protection against host-generated reactive oxygen (ROS) as well as reactive nitrogen species (RNS) by inhibiting the trafficking of ROS and RNS carrying vesicles in the proximity of SCV.³¹ SPI2 encoded nitrite transporter NirC is also required to suppress IFNγ-mediated nitric oxide production.³²

Hence, it is clearly evident that SPI2 is dedicated in transforming the hostile environment of host cell into quite-amiable ambience for Salmonella, by bringing about a great deal of modifications within the host cytosol.

Factors Inducing SPI2

Although SPI2 is involved in survival inside phagocytes and systemic infection, the secretion of the SPI2 is remarkably induced inside the lumen of the intestine, which is much ahead of the SPI1-mediated invasion of non-phagocytic cells.³³ This probably enables Salmonella to establish a systemic infection and also assists the successful activation of SPI1 as there is poor SPI1 induction in the absence of SPI2.³⁴ Once inside the phagosome, the environmental cues inside the phagosome milieu, like limitation of phosphate, carbon and nitrogen along with low pH and reduced level of divalent cations such as Mg²⁺ and Ca²⁺, signal the increased expression of SPI2.³⁵ The decreasing pH of SCV inside host cell stimulates SseB expression, which contributes to the needle structure of the SPI2-encoded T3SS.^{3,36} There are further more facts yet to be unfolded related to SPI2 induction inside host.

Control System

The regulation system of SPI2 expression is similar to other pathogens with few exclusive regulators. It is regulated positively by the two-component system SsrA/B where SsrA acts as the membrane-bound sensor and SsrB as the transcriptional regulator.¹¹ Other positive regulators include the global regulator PhoP/PhoQ³⁵ and a master regulator SlyA.²⁹ As in the case of all pathogens, there is a provision for negative regulation of virulence for Salmonella too, where the protein YgdT represses the expression of SPI2 associated genes.³⁷

An Insight into the Perplexing World of SPI2 Effectors

The detailed studies on SPI2 and its effectors are still not sufficient to present the complete scenario of evasion of Salmonella from host-defense. In this issue of *Virulence*, the brilliant piece of work by Buckner et al. has demonstrated the comparative study of 16 different SPI2 effectors in three standard infection models, epithelial and macrophage cell-culture infection models and animal model of mouse for infection.³⁸ This study provides a better picture of the strategies followed by Salmonella to accomplish a systemic infection inside the host by means of SPI2 effectors.

Although the studies on individual SPI2 effectors conducted previously have explained the importance of each effector separately, this study links all of them and suggests how these effectors may work in coordination with each other.

Since SPI2 is involved in localization of bacteria in systemic sites and aids in the replication of the pathogen, the authors constructed mutants of almost all SPI2 effectors known and examined their localization and replication in intestine as well as in systemic organs like the liver, spleen and mesenteric lymph nodes (MLN). The highlights of the study can be compiled under the following points:

Majority of SPI2 genes are committed to the intracellular survival and systemic infection. By using the model epithelial cell lines HeLa and CaCo2, the authors demonstrated that the invasion as well as the replication within epithelial cells was not hindered in the absence of SPI2. The breaching of epithelial barrier is followed by the uptake of bacteria by macrophages and dendritic cells wherein the bacteria replicates and gets transported to other organs.⁸ As this phenomenon is controlled by SPI2,⁶ the authors proceeded to determine the role of SPI2 effectors by infecting the macrophage model cell line RAW264.7 with various SPI2 effector mutants. The results obtained indicated that the effectors SifA, SifB, SseK1, SseK2, SseK3, SteC, SpiC and SpvB are required for intracellular replication within macrophages. As earlier studies³⁹ have shown that SseK1, SseK2 and SseK3 are required for intracellular replication and not for systemic infection, these results re-establish the previously known facts. However, a few SPI2 effectors are not essential for intracellular multiplication, which may give the possibility that these effectors have subtle roles as compared to other comrades.

SPI2 required in intestine as well as systemic sites. *Salmonella enterica* serovar Typhimurium infection in mouse is similar to typhoid fever in human by *Salmonella enterica* serovar Typhi. Hence this serves as an ideal model system for typhoid fever in human.⁴⁰ On infecting C57BL/6 mice with wild-type *Salmonella* and other SPI2 mutants, the authors observed that the effector proteins SpiC and SpvB were important for colonization of *Salmonella* in the colon, cecum and ileum as well as systemic sites of liver, spleen and mesenteric lymph nodes. Similarly an additional effector protein SseI was shown to be required for colonization in ileum and so was SseF in case of spleen and MLN. This again indicates towards the importance of SPI2 induction in the initial stage of infection.

SPI2 facilitates dissemination of bacteria. The authors demonstrated that SpvB and SseF were not as essential for the early

stage of infection as for the later stage, which confirms the role of SpvB⁴¹ and SseF in systemic infection. Thus, for the first time, the importance of the effector SseF in systemic disease was highlighted in this study. Examination of extracellular bacterial load in bile from gall bladder of mouse model, another favorable site for *Salmonella* replication,¹² indicated a possible role of SsaR-, SseF- and SpvB-mediated systemic distribution of *Salmonella* in gall bladder but lacked significant evidence.

Collectively these studies depict differential behavior of *Salmonella* in a site-specific manner inside the host. The avenues for the action of SPI2 are open shortly after the entry of the pathogen inside the host where it assists the uptake of bacteria by the circulating phagocytes for systemic infection. But the major occupation of SPI2 is executed once *Salmonella* breach the epithelial barrier and is engulfed by macrophages. Thereon, the players of SPI2 take over the host system to support intracellular survival and multiplication of *Salmonella* residing inside SCV and finally to disseminate them to various organs. Clearly, SPI2 is dispensable for survival inside the nourishing environment of epithelial cells available in the intestine and systemic sites like gall bladder, where the nutrients are abundant, but is essential for dwelling under stressful conditions inside macrophages of liver and spleen and systemic dissemination.

Future Ahead

The biological functions associated with SPI2 are poorly understood and the regulatory mechanisms behind the induction of SPI2 under various environmental cues are yet to be addressed. The work by Buckner et al.³⁸ has generated valuable information regarding the interplay of *Salmonella* with the host mediated by the pleiotropic effects of SPI2 in different tissues. This may serve as a prerequisite for the better understanding of intricate host-system manipulative mechanisms adopted by *Salmonella*. The major finding of this work is that various SPI2 effectors are essential for localization in intestinal and systemic sites including gall bladder. Also, recent findings suggest that extracellular survival in spleen (a systemic site) is more desirable for *Salmonella* than cellular invasion.⁴² This leads to many queries regarding the role of SPI2 in extracellular survival of *Salmonella* in these sites. So it can be speculated that SPI2-mediated functions may not be limited to intracellular survival and multiplication of *Salmonella* anymore, throwing light on the future platforms to work on the newly implicated roles of SPI2 in *Salmonella* pathogenesis.

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