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Transcriptional Organization and Regulation Mechanism of SPI-2 in *Salmonella enterica* serovar Typhimurium

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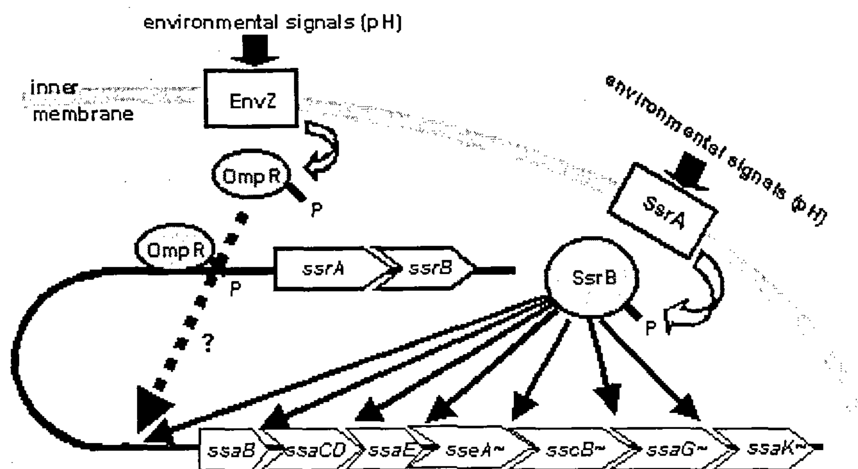
Salmonella enterica serovar Typhimurium causing a typhoid fever-like disease in mice, has provided a wide range of understanding about processes involved in *Salmonella* pathogenesis. Many virulence features of *Salmonella* are attributed to pathogenicity islands. In connection with virulence, *Salmonella* produce at least two different type Ⅲ secretion systems (TTSS), encoded by genes clusters located in pathogenicity islands designated SPI-1 and SPI-2 respectively. *Salmonella* enter and invade normally non-phagocytic cells such as intestinal epithelium cells through SPI-1-encoded system (1, 2, 3, 4, 5) and are capable of survival and proliferation within macrophages forming *Salmonella* containing vacuole (SCV) by help of system encoded by SPI-2 (6, 7, 8, 9).

A large cluster of virulence genes designated SPI-2 encodes a two-component regulator system, SsrAB, components of type Ⅲ secretion system, secreted effector proteins and their specific chaperones (6, 7, 8, 10). SsrA, a putative sensor kinase, and SsrB, a transcriptional activator, control the expression of not only all the components of SPI-2 cluster but also genes encoding effectors located elsewhere in the chromosome (11, 7, 12, 13, 14). In this study, we analyzed the transcriptional organization of SPI-2 using reverse transcriptase PCR and approved the transcripts by searching for +1 sites of respective transcripts using primer extension analysis. Total RNAs were isolated from *S. typhimurium* SL1344 grown for 3 hours and 6 hours to distinguish transcription patterns depending on growth phase. RT-PCR analysis was performed targeting on the region from *ssaB* to *ssaG* because Hensel (15) already have shown that *ssaK* to *ssaU* are transcribed simultaneously from one operon. Genes from *ssaB* to *ssaG* were transcribed organizing 5 transcriptional units and transcription pattern was differential depending on growth phase. Based on the RT-PCR results, we examined the exact location of transcriptional start sites for each transcriptional unit using primer extension analysis and found that all SPI-2 transcriptional units were regulated positively by SsrB. As reported in many other studies, SsrAB controls the expression of SPI-2 genes but it is not evident whether SsrB regulates the expression directly binding to the promoter region or indirectly through another factors. Using ChIP (Chromatin immunoprecipitation) assay, we elucidated that SsrB bound to the respective promoter regions of SPI-2 transcriptional units *in vivo* and modulated the expression directly. The binding affinity of SsrB for every transcript was generally increased entering stationary phase although the binding intensity was variable depending on genes. This fact suggests that the expression of each transcriptional unit is proportional to the level of the binding affinity of SsrB for each unit.

Several studies have analyzed SPI-2 TTSS gene expression in various conditions *in vitro*rease negative supercoiling of DNA (18), was tested to see if it can mimic effects of acidic pH on SPI2 expression. Treatment of novobiocin enhanced SPI-2 gene expression suggesting that acidic pH caused adequate DNA topological state for transcription activity and increased SPI-2 expression. OmpR, a component of OmpR-EnvZ regulatory system, was found to regulate the level of SsrAB positively by binding to the promoter regions of *ssrA* and *ssrB* directly (19, 20). In *Salmonella*, *ompR* is autoinduced by acidic shock (21) Bang *et al.*, 2002). So, it has been postulated that acidic pH encountered in macrophages might increase *ompR* expression and the increased level of OmpR subsequently induce *ssrAB* expression. As a candidate implicated in SPI-2 induction at acidic pH condition, the role of OmpR in acidic induction of *ssrB* was studied. Expression of *ssrB* was significantly enhanced by acidic pH in the presence of OmpR. But even without OmpR, *ssrB* expression was still increased in pH 4.5 although the induction ratio in the absence of OmpR was not high. And the binding affinity of SsrB for target promoters at acidic pH was not influenced significantly by the absence of OmpR. Thess results imply that at least some of *ssrB* activation mechanism was independent of OmpR. Summarizing the results, we propose a model for SPI-2 regulation inside host macrophages. The expression of SPI-2 genes is enhanced by the increased level of SsrAB through OmpR-EnvZ regulon responding to acidic pH and the transcription efficiency is accelerated by the topological changes induced by acidic pH condition regardless of OmpR. These results suggest that modulation of SsrB binding affinity for the target promoter by sensing changes in the environmental conditions plays an important role in SPI-2 gene regulation.



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