[S2-1]

Negative Regulation of SPI-2 by Enzyme IIA^{ntr} in *Salmonella enterica* serovar Typhimurium

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The nitrogen-metabolic phosphotransferase system (PTS) consists of enzyme I^{Ntr} (EI^{Ntr}, an EI paralog encoded by *ptsP*), NPr (an HPr paralog encoded by *ptsO*), and enzyme IIA^{Ntr} (EIIA^{Ntr}, an EIIA^{Mtl} paralog encoded by *ptsN*). Even though there have been a few reports about regulatory function of nitrogen-metabolic PTS in bacterial physiology, the potential role of nitrogen-metabolic PTS in the regulatory pathways of bacterial pathogenesis is not studied yet. Numerous regulatory proteins working in parallel or in cascade are known to be involved in the regulation of various virulence genes in *Salmonella*. The highly complex regulatory network is in place to integrate a variety of environmental signals for optimum bacterial survival in the animal host cell. Here, in order to elucidate the regulatory role of nitrogen-metabolic PTS in *Salmonella* virulence, we executed DNA microarray analysis with *Salmonella typhimurium* SL1344 deleted for the *ptsN* gene. Genes located in SPI-2 including *ssaG*, *ssaR*, *sseA*, *sseB*, and *sseE* as well as regulatory genes of SPI-2 showed higher expression levels in the *ptsN* deleted mutant compared with the wild type. Extensive transcriptional analyses of the genes confirmed ectopic expression of SPI-2 genes in the *ptsN* deleted mutant. Even though phospho-form of EIIA^{Ntr} was more effective than dephospho-form of EIIA^{Ntr}, the dephospho-form of EIIA^{Ntr} showed activity comparable to the phospho-form of EIIA^{Ntr} once it is overexpressed. The regulatory function of EIIA^{Ntr} in SPI-2 expression was mediated through OmpR and SsrB. The *Salmonella ptsN* mutant showed severe defects in survival ability within J774A.1 macrophage cells and mice infected with the *ptsN* mutant survived longer than mice infected with wild type in both oral and intraperitoneal injection. The *ptsN* mutant replicated less than wild type in spleen and liver of mice. Taken together, we demonstrate that nitrogen-metabolic PTS is another member of modulator in the complex array of *Salmonella* virulence gene regulatory network.

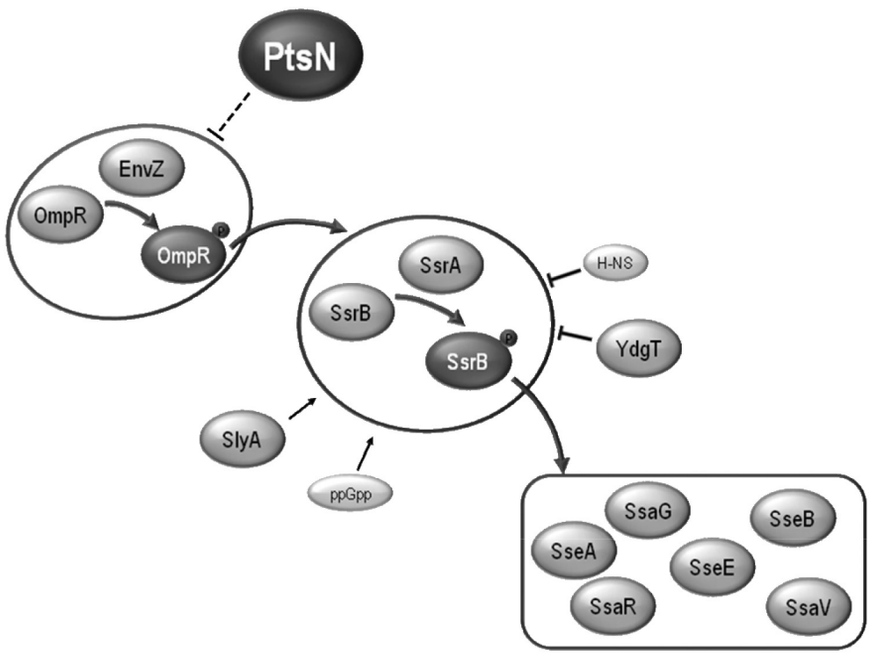

Fig. 1. Signal transduction cascade model for the negative control of SPI-2 genes by PtsN in *S. typhimurium*. The intramacrophage expression of SPI-2 genes such as *ssaG* or *sseA* requires the OmpR protein to promote transcription of the response regulator SsrB, which controls expression of SPI-2 genes. PtsN exhibited silencing effect on SPI-2 genes, which works via OmpR and SsrB. These regulatory proteins are necessary for *Salmonella* to cause a lethal infection in mice.

References

1. Brown, N. F., B. A. Vallance, B. K. Coombes, Y. Valdez, B. A. Coburn, and B. B. Finlay. 2005. *Salmonella* pathogenicity island 2 is expressed prior to penetrating the intestine. *PLoS Pathog.* 1:e32.
2. Chan, K., C. C. Kim, and S. Falkow. 2005. Microarray-based detection of *Salmonella enterica* serovar Typhimurium transposon mutants that cannot survive in macrophages and mice. *Infect. Immun.* 73:5438-5449.
3. Coombes, B. K., M. E. Wickham, M. J. Lowden, N. F. Brown, and B. B. Finlay. 2005. Negative regulation of *Salmonella* pathogenicity island 2 is required for contextual control of virulence during typhoid. *Proc. Natl. Acad. Sci. USA* 102:17460-17465.
4. Galan, J. E. 2001. *Salmonella* interactions with host cells: type III secretion at work. *Annu. Rev. Cell Dev. Biol.* 17:53-86.
5. Lee, C. R., B. M. Koo, S. H. Cho, Y. J. Kim, M. J. Yoon, A. Peterkofsky, and Y. J. Seok. 2005. Requirement of the dephospho-form of enzyme IIA^{Ntr} for derepression of *Escherichia coli* K-12 *ilvBN* expression. *Mol. Microbiol.* 58:334-344.
6. Rytkonen, A., J. Poh, J. Garmendia, C. Boyle, A. Thompson, M. Liu, P. Freemont, J. C. Hinton, and D. W. Holden. 2007. SseL, a *Salmonella* deubiquitinase required for macrophage killing and virulence. *Proc. Natl. Acad. Sci. USA* 104:3502-3507.