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Original article

The temperature-sensing protein TlpA is repressed by PhoP and dispensable for virulence of *Salmonella enterica* serovar Typhimurium in mice

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Abstract

TlpA is a temperature-sensing, coiled-coil protein, encoded on the pSLT virulence plasmid of *Salmonella enterica* serovar Typhimurium. TlpA was previously presumed to play a role in the pathogenicity of *Salmonella*. Herein we show that TlpA is tightly regulated, differentially expressed in response to environmental and physiological signals, and can be secreted in vitro. Expression of *tlpA* was found to be repressed in modified minimal medium containing limiting concentrations of Mg^{2+} and in the stationary phase of growth, but induced in rich LB broth and in response to elevated temperatures. The response regulator PhoP was found to play a key role in the repression of *tlpA* in conjunction with two other regulators, RpoS and TlpA itself. In addition, we demonstrate that TlpA is dispensable for intracellular proliferation of *S*. Typhimurium within host cells and for virulence in mice. Based on presented homology of TlpA to the IncP plasmid encoded protein, KfrA, and to SMC family members, a potential function for TlpA is discussed. Cumulatively, our data do not support the previous hypothesis that TlpA plays a role in the pathogenicity of *Salmonella* per se, but may suggest an alternative function for TlpA unrelated to host infection.

Keywords: *Salmonella enterica* serovar Typhimurium; pSLT; Virulence plasmid; Coiled-coil protein; Pathogenicity; Temperature sensing; PhoP; KfrA; SMC

1. Introduction

Salmonella enterica is a Gram-negative facultative intracellular pathogen that infects animal and human hosts. The nature and the severity of the disease are dependent upon the bacterial serovar and the host species [1]. *S*. Typhimurium causes gastroenteritis in humans and calves, whereas in mice it leads to a typhoid-like systemic infection. While a significant number of virulence factors are clustered on the virulence plasmid or within large regions of the chromosome called *Salmonella* pathogenicity islands (SPI), they are also found scattered throughout different loci in the genome [2]. The pathogenic potential of *S. enterica* as well as many other Gram-negative bacteria is indicated by the possession of a specialized type III secretion system (TTSS) that is used to deliver virulence effector proteins directly into the cellular environment of the eukaryotic host.

TlpA is a 371-amino-acid, cytoplasmic protein, encoded on the 96-kb pSLT virulence plasmid of *S. enterica* serovar Typhimurium [3], and characterized by a remarkably long α-helical coiled-coil motif [4,5]. The N-terminus of TlpA is a sequence specific DNA-binding domain that can act as an autoregulatory repressor. It has been shown that TlpA can be found in a temperature-dependent two-state equilibrium, between unfolded monomers and highly α-helical coiled-coil oligomers. At physiological temperatures transcription of *tlpA* is kept in check by the repressing activity of TlpA, which in its dimeric and folded coiled-coil conformation is able to bind to the *tlpA* promoter and repress transcription. Elevated temperature leads to a shift in the equilibrium that favors the non-functional unfolded monomeric form, which leads to increased transcription of *tlpA* [6–8].

Entry from the ‘‘cold’’ environment into the ‘‘warm’’ host is believed to be one of the central cues triggering virulence

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factors in pathogenic bacteria [9]. TlpA was the first documented case of a temperature-sensing gene regulator and was presumed to be an ideal sensor of environmental signals. Based on that, TlpA was suggested to play a role in the pathogenicity of *S*. Typhimurium [6–8]; however, a documented function in virulence has not been reported.

2. Materials and methods

2.1. Bacterial strains and in vitro growth conditions

Bacterial strains and plasmids used in this study are listed in the supplementary data, appendix no. 1. *S*. Typhimurium SL1344 was used as the wild-type strain, and all mutants used in this study were isogenic derivatives of SL1344. Bacterial cultures were routinely maintained in Luria–Bertani (LB) medium or in modified magnesium minimal medium (MgM) containing 80 mM MES (pH 5.8), 5 mM KCl, 7.5 mM $(NH_4)SO_4$, 0.5 mM K_2SO_4, 337 μM K_2HPO_4/KH_2PO_4 (pH 7.4), 20 mM $MgCl_2$, 38 mM glycerol, and 0.1% casamino acids.

2.2. Construction of S. Typhimurium SL1344 mutant strains

Refer to supplementary data, appendix no. 2.

2.3. Construction of TlpA::HA, CyaA, and LacZ fusion proteins

Refer to supplementary data, appendix no. 3.

2.4. Tissue culture conditions and bacterial infection

The human epithelial HeLa and the murine macrophages J774-A.1 and RAW264.7 cell lines were purchased from the American Type Culture Collection (ATCC). All cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FCS) at 37 °C in a humidified atmosphere with 5% CO_2. For additional details regarding the bacterial infection of tissue cultures refer to supplementary data, appendix no. 4.

2.5. cAMP assays

HeLa cells were seeded in 24-well plates and infected for 2, 4, or 8 h with *S*. Typhimurium SL1344 strains expressing TlpA-CyaA or SseK1-Cya. The cyclic AMP (cAMP) enzyme immunoassay (EIA) was performed according to the manufacturer's instructions for determination of intracellular cAMP with the nonacetylation EIA procedure.

2.6. Sub-cellular fractionation of infected HeLa cells

Infection of HeLa cells was performed as described in the supplementary data, appendix no. 4. At 8 h postinfection, the cells were washed, scraped into PBS, and centrifuged for 5 min at 1000 × *g* and 4 °C. The resultant pellet was resuspended in HB buffer (250 mM sucrose, 3 mM imidazole, 0.5 mM EDTA; pH 8) containing protease inhibitors, and mechanically disrupted by repeated passage through a 22-gauge needle. Ultracentrifugation fractionation was performed as previously described [10].

2.7. β-Galactosidase assays

β-Galactosidase assays were performed as described elsewhere [11]. *Salmonella* strains were grown in LB for 3 h (logarithmic phase), 16 h (stationary phase) or for 16 h in MgM medium. The substrate for β-galactosidase hydrolysis was *o*-nitrophenyl-β-D-galactopyranoside.

2.8. Bacterial infection of mice

Female BALB/c mice were purchased from Jackson Laboratories, housed at the University of British Columbia Animal Facility in sterilized filter-topped cages and given food and water ad libitum. For detailed infection procedures, refer to supplementary data, appendix no. 5.

2.9. Statistical analysis

The statistical significance between different values of the β-galactosidase assays was calculated using the Student's *t*-test. $P < 0.05$ was considered to be statistically significant. The statistical analysis that was used to evaluate the Competitive Index experiment results was the Wilcoxon rank sum test against a hypothetical value of 1.00.

3. Results

3.1. Phylogenetic distribution of TlpA among Salmonella serovars

The bacterial genus *Salmonella* is divided into two species, *S. bongori* and *S. enterica*. *S. enterica* itself is comprised of six subspecies: *enterica*, *salamae, arizona, diarizonae, indica, houtenae,* or I, II, IIIa, IIIb, IV, and VI, respectively [12]. In order to map the phylogenetic distribution of *tlpA* amongst *Salmonella* serovars, we performed a BLAST search against the currently available genome sequences of *Salmonella* strains. This analysis enabled us to identify several proteins homologous to *S*. Typhimurium SL1344 TlpA, as presented in Table 1. TlpA homologs were found in five different serovars, all of which are members of *S. enterica* subsp. *enterica* (group I) including: Typhimurium, Enteritidis, Dublin, Choleraesuis, and Gallinarum. Interestingly, no TlpA homologs were found in other *Salmonella* serovars outside of *S. enterica* subspecies I.

A common feature of these serovars is the presence of serovar-specific virulence plasmids, typically 50–100 kb in size, which share considerable homologies [13]. Correspondingly to the location of *tlpA* on pSLT, the locus of *tlpA* homologs