Regulation and Function of the Universal Stress Protein A of *Escherichia coli*

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Abstract

The expression of the *uspA* gene in *Escherichia coli* is induced by a range of stress conditions leading to stasis. The UspA protein is important during prolonged growth arrest but its exact biochemical function is obscure. The *uspA* gene is a member of the (p)ppGpp modulon. However, many genes with unrelated functions are induced by increased synthesis of (p)ppGpp and thus no obvious hints about the function of *uspA* can be derived from this type of regulation. In this work, additional pathways and mutations that modulate or affect the expression of *uspA* were investigated to obtain additional clues for the role that UspA is performing in the cell.

Binding sites for FadR, a DNA binding protein that regulates the expression of genes involved in fatty acid metabolism, were found in the promoter region of *uspA*. In addition, FadR is shown to bind *in vitro* to these sites. *In vivo*, *uspA* expression in mutants devoid of FadR is partially derepressed during exponential growth. In addition, a non-derepressible FadR mutant lowers expression of *uspA* in stationary phase and affects survival during growth arrest.

A mutation (*ftsK1::cat*) in the cell division gene *ftsK* resulted in increased levels of *uspA* expression in stationary phase. The *ftsK1::cat* mutant formed chains of cells in the culture as a result of impaired separation in the last stages of cell division. *ftsK1::cat* mutants survive poorly in stationary phase, reach stationary phase at a lower yield and are sensitive to salt (NaCl) under certain conditions. A transcriptional fusion to the *ftsK* gene was constructed and expression studies revealed that *ftsK* is induced in stationary phase in a (p)ppGpp dependent manner. The *ftsK1::cat* mutation induces the *recA-lexA* dependent SOS response and this leads to increased levels of expression of the *ftsK* gene. Abolition of the SOS signal by deleting the *recA* gene suppressed increased levels of both *ftsK* and *uspA* in the *ftsK1::cat* background in stationary phase. However, suppression could not be accomplished by introducing the non-derepressible *lexA3* allele.

The *ftsK1::cat* mutation positively affects the expression of other stationary phase induced genes. However, stationary phase induction of genes belonging to the *rpoS* regulon was attenuated. The *ftsK1::cat* mutation affects the levels of σ^S whereas transcriptional regulation of *rpoS* was not affected. Attenuation of the *rpoS* regulon was suppressed by expressing the *rpoS* gene from a multicopy vector and by deletion of *recA*. Thus, the *ftsK1::cat* mutation may affect stability of the σ^S factor or translation of its transcript under transition to stationary phase.

The *E. coli* chromosome carries five additional paralogs of UspA: *uspC, D, E, F* and *G*. Regulation of *uspC*, *D* and *E* was studied at the transcriptional and post-transcriptional levels. The results show that these are true universal stress proteins since their synthesis is increased under different stress conditions. Induction is dependent on (p)ppGpp and the *ftsK1::cat* mutation superinduces expression of these genes in stationary phase in a RecA dependent manner.

RecA dependent induction of *uspA* during DNA damaging conditions provided the impetus to test if the UspA protein itself was required to withstand such a stress condition. Indeed, cells devoid of UspA were more susceptible than the wild type to DNA damage generated by UV irradiation or the agent mitomycin C. Deletions in each of the *uspA* paralogs, *uspC, D* and *E*, were also affected by UV irradiation in a similar manner as that found for *uspA* mutants. However, the sensitivity of mutants devoid of more than one Usp paralog was more or less indistinguishable from their single mutant counterpart suggesting that the UspA paralogs in *E. coli* might co-operate in the defense against DNA damage.

Keywords: UspA, *Escherichia coli*, FtsK, *spoIIIE*, gene regulation, starvation, stationary phase, cell division, DNA damage, stress, FadR, fatty acid, *fad*, *fab*, *rpoS*, ppGpp.

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