On the role of sigma factor competition and the alarmone, ppGpp, in global control of gene expression in *Escherichia coli*

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Abstract

The *uspA* promoter, driving production of the universal stress protein A in response to diverse stresses, is demonstrated to be under dual control. One regulatory pathway involves activation of the promoter by the alarmone guanosine 3',5'-bisphosphate, via the β-subunit of RNA polymerase while the other consists of negative control by the FadR repressor. In contrast to canonical dual control by activation and repression circuits which depends on concomitant activation and derepression for induction to occur, the ppGpp-dependent activation of the *uspA* promoter overrides repression by an active FadR during starvation. The ability of RNA polymerase during stringency to overcome repression depends, in part, on the strength of the FadR operator. This emergency derepression is operative on other FadR regulated genes induced by starvation and is argued to be an essential regulatory mechanism operating during severe stress.

The open reading frame immediately upstream *uspA* was demonstrated to encode a protein (UspB) involved in stationary phase induced resistance to ethanol. *uspB* is dependent of the stationary phase sigma factor, σ^S. A mutation in the gene encoding σ^S (*rpoS*) not only abolishes transcription of some genes (e.g. *uspB*) in stationary phase, but also causes "superinduction" of other stationary phase-induced genes, such as *uspA*. The data suggest that the superinduction of *uspA* is caused by an increased amount of σ^{70} bound to RNA polymerase in the absence of the competing σ^S. Increasing the ability of σ^{70} to compete against σ^S by overproducing σ^{70} mimics the effect of an *rpoS* mutation by causing superinduction of σ^{70}-dependent stationary phase-inducible genes (*uspA* and *fadD*) and silencing of σ^S-dependent genes (*uspB*, *bolAp1* and *fadL*). Similarly, overproduction of σ^S markedly reduce stationary phase induction of *uspA* (σ^{70}-dependent), Thus, it seems that sigma factors compete for limiting amounts of core RNA polymerase during stationary phase.

σ^S requires ppGpp for its own accumulation and it was suggested that the similar phenotypes found between ppGpp^0 and Δ*rpoS* mutants was due to this fact. However, we found that no activity from the σ^S-dependent promoters tested (P*uspB*, *bolA*P1, P*cfa* and P*fadL*) was detectable in the ppGpp^0 strain even when σ^S levels were ectopically produced to levels corresponding to wild type levels. The results suggested that ppGpp confers dual control on the RpoS regulon by i) being essential for efficient expression and accumulation of σ^S and, ii) required for σ^S function *per se*. Interestingly, the *rpoB* allele *rpoB*3449 (A532Δ) that is epistatic to defects exhibited by a ppGpp^0 strain (i.e. growth in minimal media) suppressed the lack of induction of the σ^S-dependent promoters in the Δ*relA* Δ*spoT* strain. Thus, the *rpoB*3449 allele restores both accumulation of σ^S and the function of Eσ^S. This requirement of ppGpp can be explained, in part, by the fact that alternative sigma factors (σ^S and σ^{32}) compete better against σ^{70} for core RNA polymerase in the presence of ppGpp. Underproduction of σ^{70}, specific mutations in *rpoD (rpoD40* and *rpoD35)*, or overproduction of Rsd (anti-σ^{70}) restored expression from σ^S-dependent promoters *in vivo* in the absence of ppGpp accumulation. An *in vitro* transcription/competition assay with reconstituted RNA polymerase demonstrated that addition of ppGpp reduces the ability of wild type σ^{70} to compete with σ^{32} for core binding and the mutant σ^{70} proteins, encoded by *rpoD40* and *rpoD35*, compete less efficiently than wild type σ^{70}. Similarly, an *in vivo* competition assay demonstrated that the ability of both σ^{32} and σ^S to compete with σ^{70} is diminished in cells lacking ppGpp. Consistently, the fraction of σ^S and σ^{32} bound to core was drastically reduced in ppGpp deficient cells. Thus, the stringent response encompasses a mechanism that alters the relative competitiveness of sigma factors in accordance with cellular demands during physiological stress.

Keywords: Transcription, *Escherichia coli*, *uspA*, *uspB*, sigma factors, stationary phase, stress, *rpoS*, *rpoD*, *rpoB*, FadR, ppGpp, stringent response.

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