Global regulation of gene expression in *Escherichia coli* -the role of ppGpp, DksA and the levels of RNA polymerase

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Abstract
Bacterial cells use global regulatory networks to respond to changes in nutrient availability and other stressful conditions. The small nucleotide ppGpp, called an alarmone, is employed to redirect transcription during such stresses but the exact mechanism for how ppGpp accomplish the task of forcing the cell to change its pattern of gene expression is still rather obscure.

The overall aim of this thesis was to increase our understanding of how ppGpp and the availability of the RNA polymerase affect global gene expression in accordance with the demands of the environment.

I show that *Escherichia coli* cells unable to produce ppGpp are virtually unresponsive to carbon starvation and fail to redirect resources from growth to maintenance upon growth arrest. Three novel phenotypes of the $ppGpp^0$ mutant were also found, namely deficiency in motility, reduced cell-cell aggregation, and increased levels of oxidized proteins. These phenotypes corroborate the notion of a general problem for $ppGpp^0$ mutants to redirect resources from growth to maintenance.

The role of DksA as a possible partner for ppGpp was investigated by comparing the phenotypes of a Δ*dksA* mutant and a $ppGpp^0$ mutant, demonstrating that their functions are not completely overlapping. In addition, overexpression of DksA can compensate for the loss of ppGpp in some, but not all, of the phenotypes exhibited by a $ppGpp^0$ strain.

The availability of RNA Polymerase (RNAP) has been suggested to be involved in gene regulation due to differential sensitivity of different promoters to changes in the levels of RNAP. This type of passive regulation has been proposed to be a part of the mechanism for the stringent response mediated by ppGpp. We analyzed the effects of changing the levels of RNAP in the cell by three different methods. Underproduction of the housekeeping sigma factor, σ^{70}, was shown to cause specific downregulation of expression of ribosomal proteins while elevated levels of $E\sigma^{70}$ (RNA polymerase programmed with σ^{70}) increased expression from the *rrnBP1* promoter as well as ribosomal protein production. Increasing the effective concentration of $E\sigma^{70}$ by expressing an RNA polymerase with a tethered σ^{70} resulted in a similar up-regulation of *rrnBP1*. In contrast, genes encoding stress defense systems and amino acid biosynthetic enzymes were markedly down-regulated by elevating $E\sigma^{70}$ levels. In other words, elevating $E\sigma^{70}$ generates a response reminiscent of the relaxed response, i.e. that of cells with no or diminished levels of ppGpp while lowered availability of $E\sigma^{70}$ caused a stringent type response. The data indicates that elevated levels of $E\sigma^{70}$ favor growth-related activities at the expense of stress defenses while underproduction of σ^{70} preferentially downregulates growth-related activities. In summary, the data suggests that changing the availability of RNAP can be a basic, and robust, regulatory mechanism on top of which more specific regulatory systems can act and that this mechanism could be one part of the stringent response elicited by ppGpp.

Keywords: Global gene regulation, Transcription, *Escherichia coli*, ppGpp, DksA, stationary phase, passive regulation, RNA polymerase, stringent response

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