CHAPTER 4

The Two-Component Network and the General Stress Sigma Factor RpoS ($\sigma^S$) in *Escherichia coli*

Regine Hengge*

Abstract

The general stress sigma factor RpoS ($\sigma^S$) is induced during entry into stationary phase and in response to multiple stress conditions. RpoS is regulated at the levels of transcription, translation, proteolysis and protein activity. A key factor in RpoS control is the two-component response regulator RssB, which acts as a direct recognition and targeting factor for ClpXP-mediated RpoS proteolysis. A major, but not the only phosphodonor for RssB is the complex histidine sensor kinase ArcB. ArcB coordinates RpoS proteolysis with $rpoS$ transcription by also phosphorylating the response regulator ArcA, which besides controlling a large regulon, also acts as a transcriptional repressor for $rpoS$. ArcB activity depends on the redox state of the respiratory chain, which links RpoS control to the balance between energy supply and available respiratory electron acceptor. In addition, the BarA/UvrY and Rcs phosphorelay systems can activate $rpoS$ transcription and translation, respectively. These systems are involved in the control of motility, biofilm formation and/or virulence, suggesting that further studying a potential role of RpoS in these physiological functions may be rewarding.

Introduction

The RpoS or $\sigma^S$ protein is the master regulator of the general stress response in *Escherichia coli*. RpoS, which occurs also in other $\gamma$-proteobacteria, is a sigma subunit of RNA polymerase (RNAP) present at very low levels in rapidly growing cells not experiencing any stresses. In response to a large variety of stress conditions, however, RpoS is rapidly and often rather dramatically induced (for a review of RpoS regulation, see ref. 2) When present at high levels, RpoS competes with the vegetative (RpoD or $\sigma^70$) and other sigma factors for core RNAP and reprograms this enzyme to switch to transcription at RpoS-dependent promoters. At first glance, RpoS-dependent "stress" promoters look very similar to vegetative RpoD-transcribed promoters, yet the combination of specific small deviations from the characteristics of vegetative promoters renders these promoters RpoS-specific (for reviews, see refs. 3,4). Comprehensive microarray-based transcriptome analyses performed under various conditions where RpoS is strongly induced (entry into stationary phase, shifts to high osmolarity and pH 5) have shown that almost 500 genes, i.e., approximately 10% of the genes in the *E. coli* genome are under direct or indirect RpoS control.5,7

*Regine Hengge—Institut für Biologie-Mikrobiologie, FB Biologie, Chemie und Pharmazie, Freie Universität Berlin, Königin-Luise-str.12-16, 14195 Berlin, Germany. Email: rhengge@zedat.fu-berlin.de

Regulation of RpoS

Stress conditions that induce RpoS include carbon, phosphorus, nitrogen or amino acid starvation,\textsuperscript{8–10} reductions in growth rate,\textsuperscript{9} the classical glucose/lactose diauxic lag phase,\textsuperscript{11} shifts to high osmolarity,\textsuperscript{9,12} or low pH,\textsuperscript{13,14} the classical heat shock procedure (i.e., a shift from 30° to 42°C),\textsuperscript{15} but also growth at reduced temperature (e.g., room temperature)\textsuperscript{16} (for a summary, see ref.2). Under some conditions, RpoS induction is lasting (e.g., when cells enter stationary phase due to starvation), under others it is transient (e.g., upon pH downshift or during the diauxic lag phase). As a multitude of environmental and cellular signals have to be processed in RpoS control, it is not surprising, that this control occurs at all possible levels, i.e., \textit{rpoS} transcription and translation as well as proteolysis and the activity of RpoS protein\textsuperscript{3} (Fig. 1). While \textit{rpoS} transcription can be further stimulated by reduced growth rate (by a mechanism that involves ppGpp,\textsuperscript{8,17,18}) there is always a fair amount of \textit{rpoS} mRNA present in the cell. Due to mRNA secondary structure in the translational initiation region, however, this mRNA is not efficiently translated. Yet, translation can be rapidly stimulated by small regulatory RNAs, which together with the RNA-binding protein Hfq trigger alterations in \textit{rpoS} mRNA secondary structure that allow access of ribosomes to the translational initiation site.\textsuperscript{19–24} Furthermore, RpoS, which is also synthesized at a small rate even in the absence of stress, is rapidly degraded by a proteolytic recognition factor, RssB and the ATP-dependent ClpXP protease.\textsuperscript{25–30} A rapid increase in the cellular level of RpoS can also be achieved by an instantaneous inhibition of this degradation. Finally, the activity of RpoS, i.e., its ability to successfully compete with other sigma subunits for core RNAP and thereby to activate its regulon, is controlled by factors such as ppGpp, Rsd and Crl, which interact with RNAP core, RpoD and/or RpoS.\textsuperscript{31–34}

Different stress conditions affect different levels of RpoS control, with a tendency of the more severe and potentially lethal stress conditions acting on the most rapid regulatory process in RpoS control, i.e., proteolysis. Stress conditions that most strongly induce RpoS (hyperosmotic shift or pH downshift) stimulate \textit{rpoS} mRNA translation as well as interfere with RpoS proteolysis (Fig. 1). As mentioned above, numerous factors, including small molecules, small regulatory RNAs and

![Figure 1. Environmental signal input and two-component systems controlling \textit{rpoS} transcription and translation as well as proteolysis of RpoS protein. Mechanistic details of this summary figure are explained in the text. The figure is an extended version of a previously published figure.\textsuperscript{11}](image-url)