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The σ^S subunit of RNA polymerase as a signal integrator and network master regulator in the general stress response in *Escherichia coli*

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ABSTRACT

The σ^S (RpoS) subunit of RNA polymerase in Escherichia coli is a key master regulator which allows this bacterial model organism and important pathogen to adapt to and survive environmentally rough times. While hardly present in rapidly growing cells, σ^S strongly accumulates in response to many different stress conditions, partly replaces the vegetative sigma subunit in RNA polymerase and thereby reprograms this enzyme to transcribe σ^S-dependent genes (up to 10% of the E. coli genes). In this review, we summarize the extremely complex regulation of σ^S itself and multiple signal input at the level of this master regulator, we describe the way in which σ^S specifically recognizes "stress" promoters despite their similarity to vegetative promoters, and, while being far from comprehensive, we give a short overview of the far-reaching physiological impact of σ^S. With σ^S being a central and multiple signal integrator and master regulator of hundreds of genes organized in regulatory cascades and sub-networks or regulatory modules, this system also represents a key model system for analyzing complex cellular information processing and a starting point for understanding the complete regulatory network of an entire cell.

Keywords: *bacterial stress responses, regulatory networks, signal transduction, post-transcriptional regulation, starvation*

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Introduction

σ^S or RpoS (sometimes also named σ^{38}) is one of the seven sigma subunits of RNA polymerase in *Escherichia coli* and serves as the master regulator of the general stress response. σ^S is nearly undetectable in unstressed rapidly growing cells but is strongly induced under many stress conditions including carbon starvation, high and low temperature, low pH or high osmolarity (reviewed in[1]). Under these conditions, σ^S can replace the vegetative sigma factor σ^{70} (RpoD) at the RNA polymerase (RNAP), which reprograms RNAP to recognize promoters of σ^S–dependent genes (about 10% of the *E. coli* chromosome are under direct or indirect control of σ^S [2]). Interestingly, the promotors of σ^S- and σ^{70}-dependent genes look very similar and are often recognized by both σ^S and σ^{70} *in vitro*. Only recently the subtle differences in promoter structure and activation have been recognized that render a promoter σ^S-specific *in vivo* (reviewed in[3,4]). Due to these changes in cellular gene expression upon σ^S-induction, the cell is able to cope with the actual stress conditions; however, even more important is the

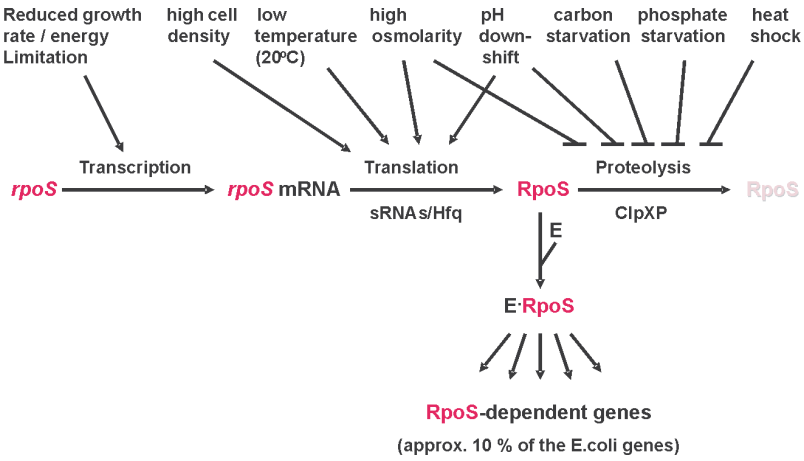

Fig. 1. *Environmental signal input in the control of* rpoS *transcription and translation as well as proteolysis of* σ^S *protein. Mechanistic details of this summary figure are explained in the text. The figure is a modified version of a previously published figure (from ref. 109 with permission).*

development of a multiple stress resistance against stress conditions not yet encountered (cross protection). This adjusts cell physiology to bad environmental conditions in general.

This review gives a short overview of what is currently known about the mechanisms involved in the regulation of the cellular σ^S content and activity, the promotor recognition determinants that make a promotor σ^S-dependent and the physiological changes after induction of the σ^S–regulon.

Regulation of the cellular σ^S content and activity

Expression and activity of σ^S have to be regulated in response to a variety of different environmental stimuli. To integrate these signals, the cell controls every level of expression, *i.e.* not only *rpoS* transcription and translation but also degradation and activity of σ^S (Figure 1). This happens in a way that different stress or environmental signals affect different levels of *rpoS* regulation. Transcription is important for the expression of a basal mRNA level, but transcriptional regulation does not contribute much to the induction of *rpoS* during acute stress. In contrast, translational induction of already existing *rpoS mRNA* and degradation of σ^S have a major impact on the rapid increase of the cellular σ^S amount promptly after exposure to stress. In addition,

recent evidence indicates the existence of stress-activated mechanisms that affect sigma factor competition in favour of σ^S and thereby activate σ^S as a transcription factor.

Regulation of rpoS transcription

Despite the fact that *rpoS* transcription is not induced by acute stress, quite a high number of factors that modulate *rpoS* transcription have been described. In *E. coli*, the cellular level of *rpoS* mRNA is relatively high during all phases of growth (even during logarithmic growth phase when σ^S levels are very low due to inefficient translation and rapid degradation) and can increase further in response to a gradual decrease in growth rate[1]. **Three σ^{70}-dependent promotors** contributing to *rpoS* transcription have been identified. Two of them are positioned in front of the *nlpD* gene, which is located directly upstream of *rpoS* and codes for a lipoprotein of unknown function[5]. The expression of a polycistronic *nlpD-rpoS* mRNA from these two promotors is not a target for transcriptional regulation and contributes to basals levels of *rpoS* expression independent of any stress conditions. In contrast, the third promotor, *rpoSp*, located within the *nlpD* coding region, is regulated by different proteins and non-proteinaceous factors[6]. *rpoSp* produces a quite long 5′-untranslated region[6], which is responsible for the translational induction of *rpoS* under different stress conditions.

Reduction of **growth rate** in minimal medium due to a competitive inhibitor of glucose uptake and metabolism (α-methyl glucoside) increases *rpoS* transcription[7]. In a global transcription profile analysis this inverse correlation between growth rate and *rpoS* transcription was shown using different carbon sources that change growth rate[8]. Although this inverse correlation has also been described in other reports[9–11], the mechanism by which the reduction of growth rate is sensed and modulates *rpoS* transcription and whether other levels of *rpoS* regulation are also affected by growth rate, is still not known.

The **cAMP-CRP** complex (cyclic adenosine monophosphate bound to cAMP receptor protein) influences *rpoS* transcription differentially, depending on the growth phase. In logarithmic growing cells, cAMP-CRP represses *rpoS* transcription[7,12]. Recent data suggest that this effect is indirect (F. Mika and R. Hengge, unpublished data) and perhaps is a consequence of the reduced growth rates of mutants defective in *cya* (encoding adenylate cyclase) or *crp* (encoding the cAMP receptor protein,

also called the catabolite activator protein). In contrast, the positive effect of cAMP-CRP on *rpoS* transcription in stationary phase cells is due to direct binding of cAMP-CRP to a class I binding site centered at −62,5 nucleotides upstream of the major transcriptional *rpoS* start site[13].

Also the **BarA/UvrY** two component system, which plays a major role in the regulation of carbon metabolism, motility, biofilm formation and virulence of uropathogenic *E. coli* strains[14–17] seems to positively modulate *rpoS* transcription, although the detailed mechanism remains to be elucidated[18,19,82].

Recently, the **ArcA** response regulator was identified as an additional molecule directly acting on the *rpoSp* promotor[13]. The ArcB/ArcA phosphorelay integrates oxygen availability and energy supply via the redox state of the respiratory chain[13,20]. Binding sites for phosphorylated ArcA were found around position −63, overlapping with the activating cAMP-CRP-binding site, and around +23, downstream of the transcriptional start site. A hypersensitive DNaseI site in the region around −15 suggests that ArcA binding at the two positions induces DNA looping. When cAMP-CRP accumulates in stationary phase, it probably competes with ArcA, and therefore may partly relieve the inhibitory effect of ArcA. Thus cAMP-CRP activation includes an anti-repression mechanisms ([13]; F. Mika and R. Hengge, unpublished). In addition, the Arc-system influences σ^S stability (see below).

The histone-like protein **Fis**, a highly abundant protein during exponential growth and nearly non detectable in stationary phase[21], inhibits *rpoSp* during vegetative growth through binding to a site around −50 [22].

Polyphosphate-free mutants have strong defects in stationary phase survival and expression of stress resistance mechanisms[23,24]. Depletion of polyphosphate in the cell leads to decreased σ^S levels, that are partly due to decreased *rpoS* transcription[25]. Since polyphosphate exerts no effect on *rpoS* transcripition *in vitro*, the observed influence may be indirect[25].

Levels of the small nucleotide **5′,3′-bis-guanosine tetraphosphate (ppGpp)**, a global regulator of gene expression (for a recent review see[26]), strongly increase in response to amino acid limitation (which triggers the stringent response) or other conditions that reduce growth rate (*e.g.* nutrient limitation)[27]. Under these conditions, the σ^S-level also increases. It has been shown that ppGpp positively influences *rpoS* transcription, but the molecular basis of this effect is unclear[6,28,29].

Regulation of rpoS translation

Translational induction of already existing *rpoS mRNA* and degradation of σ^S has a major impact on the rapid increase of the cellular σ^S amount promptly after exposure to several stress conditions. The major *rpoS* transcript with its unusually long 5′-untranslated leader folds into a secondary structure, in which the translation initiation region (TIR) is not accessible to ribosomes due to intramolecular base pairing[30–34]. Upon shift from non-inducing to inducing conditions (*e.g.* hyperosmotic shift[7,35], pH downshift[36] or during late exponential phase, when cells reach a certain density[7]), this secondary structure is believed to change into a translatable form. Several proteins and/or small RNAs are involved in this process (reviewed in[1,37]).

The **RNA-binding protein Hfq**, which facilitates RNA-RNA interactions (reviewed in[37,38]), plays an essential role in *rpoS* mRNA translation[39,40]. In a *hfq* mutant, translation of the *rpoS* mRNA is dramatically decreased and the increase of σ^S in response to the usual translation-inducing stimuli is abolished[30,39]. The Hfq protein forms a hexameric ring with a central pore, around which the RNA is bound[41,42]. Recently it became clear, that Hfq itself does not interfere with the inhibitory structure of the *rpoS* mRNA to upregulate translation, as initially thought, but is essential for the activity of at least three small RNAs involved in the regulation of *rpoS* translation, *i.e.* **DsrA**, **RprA** and **OxyS**. DsrA and RprA stimulate *rpoS* translation by base-pairing to and thereby sequestering the non-translated upstream region of *rpoS* mRNA, which in the inhibitory structure basepairs to the TIR[43–45]. Recently it was reported that the ribosomal S1 protein interacts with both DsrA and *rpoS mRNA* and that this interaction may also open up the *rpoS mRNA* secondary structure especially in the region around the initiation codon[46]. Both DsrA and RprA have the same target and mode of action but are induced under different conditions: DsrA concentration increases during growth at low temperature and is essential for *rpoS* translation in this situation[45], while RprA is under the control of the RcsC/(RcsD(YoiN)/RcsB phosphorelay system which is activated by yet undefined conditions[37,44]. The OxyS RNA, which accumulates in the cell upon exposure to oxidative stress, inhibits *rpoS* translation by a not yet clarified mechanism[47,48].

The histone-like protein **HU** has a positive effect on *rpoS* translation during late logarithmic phase[49]. Two homologue subunits, HUα (encoded by *hupA*) and HUβ (*hupB*), can form α2

homodimers or αβ heterodimers in a growth phase-dependent manner as the negatively FIS-controlled *hupB* gene is induced only in the late logarithmic growth phase. *In vitro*, binding of HU (preferentially the αβ heterodimer) to *rpoS*-mRNA has been observed in a region around the location of the TIR ([49]; D. Traulsen and R. Hengge, unpublished data). So it seems plausible that HU, perhaps only the heterodimeric form, contributes to *rpoS* translation by directly affecting the structure of *rpoS* mRNA around the TIR region.

In contrast, the abundant nucleoid associated protein **H-NS** keeps σ^S levels low by inhibiting *rpoS* translation and stimulating σ^S proteolysis[50,51] (see also below). *In vivo*, H-NS preferentially binds to bent DNA regions and acts as a repressor for more than 100 genes[52]. *In vitro*, H-NS binds to *rpoS mRNA* as well as to the sRNA DsrA, but with a quite low affinity, and appeared to enhance the cleavage of both RNAs by ribonucleases at single stranded RNA sites[53].

Some additional proteins have been shown to affect *rpoS* translation indirectly. **LeuO**, a LysR-like transcriptional regulator, negatively affects *rpoS* translation at low temperatures via repression of *dsrA*[54,55]. An additional LysR-like regulator, **LrhA**, represses *rpoS* at the level of translation in a *hfq* dependent manner, perhaps via an unidentified small RNA[56]. The **Rcs-phosphorelay system**, a cell envelope stress-sensing pathway (for recent reviews, see[57,58]), can activate *rpoS* mRNA translation either by repressing *lrhA* transcription or by directly stimulating expression of the sRNA RprA[44,56].

Finally, the **DnaK** chaperone[59,60], the PTS component **EIIA(Glc)**[61], and **ppGpp** and its "partner" protein **DksA**[62–64] all seem to affect *rpoS* translation, but the molecular mechanisms involved remain to be clarified.

Another feature in *rpoS* translational control that has remained mysterious is the nature of the primary inducing signals. How exactly do *e.g.* osmotic upshift or pH downshift affect the activities of the cis- and trans-acting components in *rpoS* translation? In addition, it is likely that additional small RNAs that affect *rpoS* translation under these or yet other conditions remain to be discovered.

Regulation of σ^S proteolysis

In rapidly growing cells, the σ^S level is very low, despite a certain basal rate of σ^S synthesis. This is due to fast degradation of the σ^S

protein in the relative absence of stress[1,7]. Within a few minutes after exposure to high osmolarity, carbon starvation, heat shock or low pH the σ^S half life increases strongly[7,35,59]. It seems that this very rapid but energetically costly regulation at the proteolysis level is especially important in emergency reactions, that threaten the cellular integrity[65].

The processive ATP-dependent **ClpXP protease** is responsible for σ^S degradation[66]. In contrast to most other known ClpXP substrates, σ^S needs a specific recognition factor, **RssB**, for being targeted to the protease[67,68]. RssB is a response regulator protein with a N-terminal receiver domain phosphorylated at D58[69] and, unlike most other response regulators, harbors an output domain without a DNA binding motif. The whole RssB protein is required for *in vivo* complementation of a *rssB* mutant, *i.e.* both domains of RssB are important for its function in σ^S degradation *in vivo* and *in vitro*[70].

The phosphorylated form of RssB binds with high affinity[71,72] to region 3.0 (also called region 2.5) of σ^S with K173 as an essential amino acid[72]. This region around K173 is also called the turnover element. The σ^S-K173E mutant does not interact with phosphorylated RssB and is completely stable *in vivo* and *in vitro* ([72] and N. Lange, E. Klauck and R. Hengge, unpublished). K173 also recognizes a cytosine at position -13 in the extended -10 promotor region, which is present in more than 80% of σ^S–dependent promotors[73]. The crystal structure of the σ^{70} homolog σ^A in *Thermus aquaticus*, which shows high similarity to σ^S, revealed that the region 3.0 (2.5) is a long α-helix and, together with two shorter α-helices right downstream, forms domain 3, which directly follows domain 2 and is connected to domain 4 via a long flexible linker buried in the holoenzyme structure[74].

Binding of phosphorylated RssB to σ^S (in a 1 : 1 stoichiometry[70]) is a prerequisite for the recognition of σ^S by ClpXP, because the N-terminal ClpX recognition site in σ^S is exposed only after binding to RssB[75]. A stable quaternary complex between σ^S, RssB and ClpXP is formed *in vitro* in the presence of acetyl phosphate (as phosphordonor for RssB, see below) and a non-hydrolyzable ATP analog (which allows hexameric $ClpX_6$ ring formation)[71]. Moreover, RssB seems to play a second role in the subsequent steps of σ^S-degradation, as an RpoS::LacZ hybrid protein which contains an incomplete σ^S moiety and can be bound by $ClpX_6$ alone, still needs the interaction with RssB to be degraded[75]. As RssB is not co-degraded with σ^S, it plays a catalytic role[70,71].

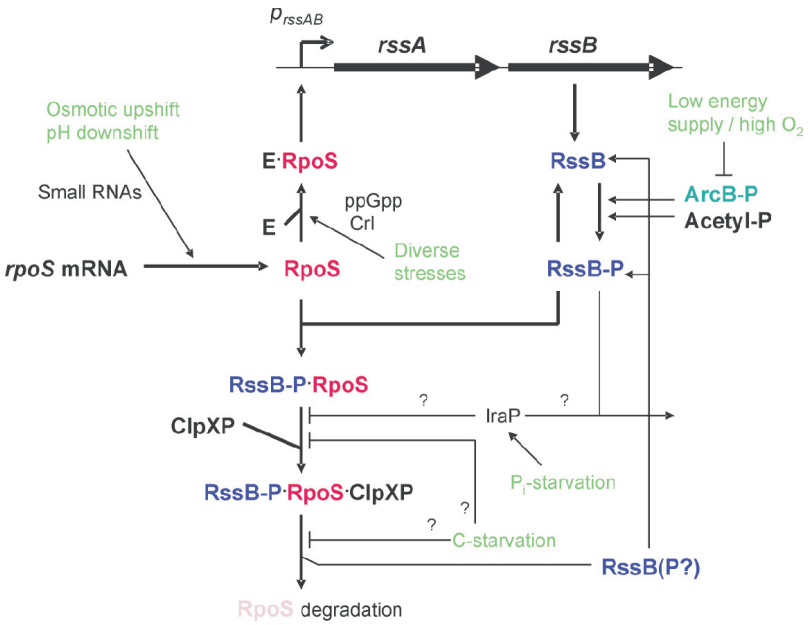

Fig. 2. *Signal integration into the proteolytic targeting cycle of* σ^S. *Phosphorylated RssB directly binds to* σ^S *and delivers it to the ClpXP protease. Alternatively,* σ^S *can bind to RNAP core enzyme (E), in which it is protected against interaction with RssB-P, and then can activate multiple genes, including the* rssAB *operon. The latter results in an adjustment of RssB levels to* σ^S *levels, and represents a negative feedback cycle that allows adaptation after* σ^S *stabilization due to RssB titration because of a sudden increase in* σ^S *expression (for details, see text). For the RssB-binding antagonist IraP, it is not yet clear whether it sequesters RssB (not allowing RssB to bind* σ^S*) or whether it interferes with RssB function also in the RssB-*σ^S *complex. Also, it has not yet been clarified, whether RssB is dephosphorylated during its release from the RssB-P-*σ^S*-ClpXP complex. The figure is a modified version of a previously published figure (from ref. 65 with permission).*

How is this basic mechanism of σ^S recognition and proteolysis regulated in response to different environmental signals? It turned out that there are quite a number of possibilities, which explains multiple signal integration in this system (Figure 2). An obvious target of regulation is phosphorylation of RssB. Although non-phosphorylated RssB can bind weakly to σ^S, phosphorylation of RssB makes recognition and degradation of σ^S much more efficient *in vivo* and *in vitro*[71,72,76]. Changes in RssB phosphorylation might change the rate of σ^S degradation in response to stress signals. A prerequisite for this is that RssB is rate-limiting for the cellular rate of proteolysis. Indeed, this is the case during log phase growth

where σ^S proteolysis occurs with a half-life of only a couple of minutes[77]. *In vitro*, RssB can easily be phosphorylated by **acetyl phosphate**[69,78] and acetyl phosphate might also contribute to RssB phosphorylation to some extent *in vivo*[69,76]. Besides acetyl phosphate, the **ArcB/ArcA** two component regulatory system contributes to RssB phosphorylation *in vivo*[13]. *In vitro*, RssB is also phosphorylated by the sensor kinase ArcB[13,79]. As this transphosphorylation is about ten times less efficient than the phosphotransfer between ArcB and ArcA, the concentration of competing ArcA can sensitively regulate RssB phosphorylation[13]. The Arc system is a global regulatory system that responds to oxygen and energy supply[13,80]. ArcB autophosphorylation is inhibited by oxidized quinones in the respiratory chain[20,81]. The redox state of the quinones is not only influenced by the availability of the electron acceptor (*i.e.* oxygen during aerobic respiration) but also by the electron input, which is reduced under starvation conditions. Energy limitation therefore should promote ArcB sensor kinase dephosphorylation which should in turn result in reduced phosphorylation of RssB by ArcB and therefore stabilization of σ^S (for a recent review see[82]). However, since an *arcB ackA-pta* mutant still shows some σ^S degradation, additional phosphodonor(s) besides acetyl phosphate and ArcB also appear to contribute to RssB phosphorylation.

In addition, σ^S degradation can also be inhibited without altering the phosphorylation status of RssB. Since the cellular RssB : σ^S ratio is very low (about 1 : 20 in non stressed cells[83]) and RssB levels are rate-limiting for overall cellular rates of σ^S degradation, it is easy to imagine that a sudden strong increase in σ^S synthesis (as *e.g.* due to translational induction upon osmotic upshift or pH downshift) may titrate the proteolysis system. Thus, under some stress conditions, stabilization of σ^S might be at least to a certain extent a consequence of this titration effect and not due to a change in the specific rate of σ^S proteolysis. Small changes in σ^S synthesis, however, can be counterbalanced by adjusting RssB levels because *rssB* transcription is under the control of a σ^S–dependent promotor[77].

Specifically under phosphate starvation conditions, RssB activity is inhibited by the specific antagonist **IraP**[84]. In addition, PhoP/Q-regulated transcription of *iraP* seems to be involved in σ^S stabilization under Mg^{2+} starvation in the closely related enteric bacterium *Salmonella enterica* (reviewed in[85,86]). In *E. coli*, however, there is only modest stabilization of σ^S under Mg^{2+} starvation conditions, and IraP is not involved in this process[86].

When σ^S is bound to RNA polymerase, it is protected against RssB-dependent degradation[71]. Thus, any factor, that improves competition of σ^S with other sigma factors for RNA polymerase should also slow σ^S degradation. This has been recently shown for the **Crl** protein[87].

In addition to its role in the regulation of translation of *rpoS mRNA* (see above), **H-NS** also acts at the level of proteolysis. A *hns* mutant shows 10-fold higher synthesis as well as a 10-fold increase in the stability of σ^S [50,51]. The reason for this stabilization of σ^S is still unclear, but could be an inactive (non-phosphorylated) RssB protein in the *hns* mutant, because the *hns* effect requires RssB to be phosphorylatable[88]. Finally, also the **DnaK** chaperone seems to play an uncharacterized role in σ^S stabilization in carbon-starved cells[59].

In conclusion, there are many different ways of how σ^S proteolysis can be regulated. As simple as it looks at first glance, the σ^S proteolytic targeting cycle in fact functions as a multiple signal-integrating machinery. Moreover, it is becoming apparent that the different levels of σ^S control do not operate independently, but may be connected as exemplified in the role of the Arc system in *rpoS* transcription (*via* ArcB/ArcA) and in σ^S proteolysis (*via* ArcB/RssB).

Regulation of σ^S activity

Upon entry into stationary phase, the level of σ^S increases strongly but nevertheless reaches only about one third of the level of the housekeeping sigma factor σ^{70} under the same conditions[89]. In addition, σ^S exhibits the lowest affinity to core polymerase of all *E. coli* sigma factors *in vitro*[90,91]. So, how can σ^S become active and even play its dominant role in stationary phase, when σ^{70} seems to have such a strong advantage in the competition for the limiting amounts of RNA polymerase? First, some factors may reduce affinity of σ^{70} to RNA polymerase in stationary phase, as discussed for the starvation-induced alarmone ppGpp together with DksA ([92], reviewed in[26]). In addition, the **Rsd** protein may sequester a fraction of the cellular σ^{70} [93,94], and 6S RNA binds and inactivates a fraction of σ^{70}-containing RNAP holoenzyme[95]. On the other hand, the **Crl** protein actively and specifically promotes the formation of a RNAP-σ^S complex during entry into stationary phase[87]. Taken together, these mechanisms may allow σ^S to act as the major sigma factor in stationary phase.

RssB acts as a targeting factor for σ^S proteolysis, but has the potential to also act as an anti-σ^S factor. Yet, the low physiological RssB : σ^S ratio (1 : 20) suggests that this is irrelevant *in vivo*. However, it seems plausible that RssB is a former anti-sigma factor that has been recruited during evolution by the proteolytic machinery as a specificity and targeting factor for σ^S proteolysis[83].

σ^S as a transcription factor and σ^S-controlled promotors

An elaborate network controlling σ^S expression, synthesis, stability and activity – described in detail above – ensures that the σ^S-containing RNA polymerase ($E\sigma^S$) assumes its role and targets a distinct set of genes only when required. During stationary phase or upon encounter of stress, $E\sigma^S$ induces the expression of a plethora of new genes (more than reported for any other alternative sigma factor in *E. coli*[2]) and at the same time takes over "house-keeping" duties of the cell from $E\sigma^{70}$ [96].

Despite the distinct physiological role of σ^S and the defined output of $E\sigma^S$, σ^{70} and σ^S show such a high degree of sequence similarity that they were found to bind optimally to nearly identical −35 and −10 elements *in vitro*[97]. This σ^S selectivity paradox has puzzled researchers for a long time, since it seemed incompatible with the σ^S specificity that many promoters exhibit *in vivo* and the large and specific σ^S regulon[3]. In the recent past, however, it could be shown that σ^S promoter selectivity is linked to a clear nucleotide preference in the extended −10 region, *i.e.* a C at position −13, which represents a hallmark of typical σ^S-dependent promoters[73]. Moreover, $E\sigma^S$ can tolerate more efficiently than $E\sigma^{70}$ various minor deviations from the optimal consensus promoter (reviewed in[4]). Thus, $E\sigma^S$-mediated promoter activity is less reduced by a degenerate −35 region or non-optimal spacer length between the −35 and −10 hexamers. $E\sigma^{70}$ has a strong preference for 17 bp spacers, but $E\sigma^S$ can nearly equally well operate with spacers that deviate from this optimum by +/− 2 bp[98]. Interestingly, apart from promoter elements recognized by the sigma factor, the UP-element configuration, which is recognized by the C-terminal domain of the α subunit of RNA polymerase, can also have a decisive impact on promoter specificity. Thus, a distal UP-element half-site alone strongly favours $E\sigma^S$-mediated activation of a promoter, whereas a full UP-element or a proximal half-site alone give an advantage to $E\sigma^{70}$ [99]. In addition to the *cis*-encoded DNA features, σ^S promoter selectivity can be introduced or further enhanced by (i) several

trans-acting factors, *i.e.* regulatory proteins (for examples see[87,100,101]), (ii) altered DNA supercoiling[102,103], and (iii) certain salts like glutamate or acetate[104,105].

In parallel with the existence of σ^S-specific promoters, the cell retains numerous genes with promoters that can be utilised by both $E\sigma^{70}$ and $E\sigma^S$, or genes with distinct but overlapping $E\sigma^S$ and $E\sigma^{70}$-specific promoters, in order to secure continuous (but differential) expression during entry into stationary phase. This target overlap by sigma factors has recently emerged as a general feature of the transcriptional logics in bacteria, even for sigma factors that target consensus promoters clearly different from those recognized by the housekeeping σ^{70} [106–108].

Architecture and physiological functions of the σ^S-controlled network

Induction of σ^S in response to the many stress conditions as described above results in far-reaching physiological and morphological alterations (summarized in[109]). Cells become multiply stress-resistant, even against stress conditions that they have never experienced, a phenomenon termed cross-protection. Cell shape changes to a more ovoid morphology. Depending on the actual conditions, protective components such as trehalose and storage polymers such as glycogen or polyphosphate accumulate. Whereas metabolism, and in particular energy metabolism, is directed towards maximal growth in non-stressed cells, the metabolic balance is now shifted towards maintenance. Moreover, the cells seem to readjust to a more sessile or community life-style, as biofilm components such as certain adhesive fimbriae and the synthesis of matrix components become induced.

Physiological functions of σ^S-controlled genes

Since σ^S has been first described as a global regulator of stationary phase gene expression[110], approximately seventy genes have been identified as σ^S-controlled based on classical bacterial genetics[109,111]. More recently, using genome-wide transcriptional profiling under three different σ^S-inducing conditions (entry into stationary phase in complex medium, osmotic upshift, pH downshift), 482 genes, *i.e.* around 10% of all *E. coli* genes, have been found to be under significant σ^S control[2]. Subsets of these genes have also been identified in smaller microarray studies using only a single growth condition[112,113]. The distribution of σ^S-regulated

genes in functional categories allows to appreciate the dramatic impact of σ^S on cellular physiology (for a more detailed discussion, see[2]). Of the σ^S-dependent genes, 11% are "classical" **stress genes**, which include *e.g.* the genes encoding catalases, the DNA protection protein Dps, the DNA repair protein exonuclease III, the trehalose-synthesizing enzymes, the proton-scavenging glutamate decarboxylases, the universal stress protein UspB, the **morphogene** bolA, which induces ovoid morphology, and many others. 5% of the σ^S-controlled genes encode factors involved in **protein processing**, including **chaperones**, many of which may also contribute to stress protection. A striking number of 19% of all σ^S-dependent genes are metabolic genes (mostly belonging to **energy metabolism**) and allow the conclusion that upon σ^S induction the cells change from an aerobic respiration-driven to a more fermentative/anaerobic respiration-driven metabolism, perhaps in order to avoid the formation of oxygen radicals due to increased incomplete reduction of oxygen when energy sources become scarce. In some cases, certain enzymes in central metabolism are encoded by two isoenzymes, one of which is silent in growing cells, but is strongly activated by σ^S under stress conditions (*e.g.* the transketolases in the glyoxylate shunt encoded by *tktA* and *tktB*). Another 14% of σ^S-controlled genes code for **membrane proteins**, which include several known transport systems (*e.g.* for glutamate/γ-amino butyric acid, arginine and putrescine). In addition, this category may also contain many **exit carriers** which play a role in detoxification and thereby may also contribute to stress resistance. Around 43% of σ^S-controlled genes are "y-genes", *i.e.* encode proteins of unknown function, suggesting that a substantial part of the σ^S-regulated physiology has so far escaped our attention. There is initial evidence that some of these genes may contribute to biofilm formation (see below).

Regulatory cascades and additional signal input within the σ^S-controlled regulatory network

The perhaps most interesting category of σ^S-dependent genes are those that encode **regulators** and **signal transducing factors**, which make up 8% of all σ^S-controlled genes. This indicates that σ^S not only controls a regulon but rather a complex hierarchical and modular network which features regulatory cascades and additional signal input also at levels downstream of σ^S itself. Many of these regulators have been identified only by sequence criteria (as

they belong to known families of regulatory proteins or *e.g.* feature a clear helix-turn-helix motif) and await physiological characterization. In a few cases, however, σ^S-dependent regulatory cascades and their physiological impact are being studied in detail already.

A particularly complex case is the large **acid resistance module** within the general stress response. This module is induced in a σ^S-dependent manner during entry into stationary phase and is then responsible for the strong acid resistance of stationary phase cells or upon pH downshift[2,114]. Moreover, acid resistance genes are also expressed in a σ^S-independent manner when cells are grown continously at low pH, illustrating the principle that modules or functional sub-networks can be recruited conditionally into other regulatory networks (in this case here, the acid resistance genes get under control of the EvgS/A – YdeO pathway;[115]). Many of the acid resistance genes, including those whose gene products execute the response (*e.g. gadA* encoding a glutamate decarboxylase) as well as regulatory genes, are clustered on an ''island'' in the chromosome. Here, σ^S controls the expression not only of the response-executing genes but also of at least three module-specific but nevertheless global regulatory genes, *gadX*, *gadW* and *gadE*. The GadE regulator is essential for the expression of the many acid resistance genes[116], but at least during entry into stationary phase, *gadE* is itself under GadX control[2], but GadX also regulates additional genes independently of GadE (H. Weber, J. Heuveling and R. Hengge, unpublished results). GadW is a minor regulator that under certain conditions can antagonize GadX[114]. In addition, a σ^S-regulated small RNA, GadY, seems to modulate GadX expression[117]. Finally, recent evidence suggests that upon pH downshift, the kinetics of acid resistance gene activation are very complex, with σ^S and GadE serving as rapidly and transiently induced emergency regulators, whereas GadX seems to be induced more lastingly (J. Heuveling and R. Hengge, unpublished results).

Another highly sophisticated cascade module within the σ^S network is involved in the formation of **biofilms**. It has been known for a long time that the expression of adhesive curli fimbriae and the matrix component cellulose, which are expressed only at reduced temperatures (below 30°C) and are required for the ''rdar'' morphotype, *i.e.* a dry colony-type biofilm on a solid medium/air interface, are under σ^S control (reviewed in[118]). While this has been regarded as a specific peculiarity, it has only recently become clear, that many enzymes that synthesize and degrade the

signal molecule **cyclic-di-GMP** (c-di-GMP), which coordinates the transition between the motile single cell and the sessile biofilm/community life-styles, are under σ^S control[119]. c-di-GMP is synthesized from GTP by diguanylate cyclases, which carry the GGDEF domain, and is degraded by specific phosphodiesterases, characterized by EAL domains (a second domain, HD-GYP, can also hydrolyze c-di-GMP, but occurs more rarely). High cellular levels of c-di-GMP (which can be generated by overproducing certain GGDEF proteins) inhibit motility and induce biofilm formation, overproduction of EAL proteins produces the opposite phenotype (for recent reviews on the biochemistry and function of c-di-GMP, see[120–122]). Strikingly, most bacterial species have dozens of GGDEF and EAL proteins (sometimes also combined in single polypeptides), which has lead to the suggestion that not all of these proteins contribute to controlling the overall cellular level of c-di-GMP, but that some may act in specific modules consisting of a GGDEF domain, a EAL domain and an effector protein in a specific complex, which would control only a specific target[119]. Such microcompartimentation may allow to use specific GGDEF/EAL/effector/target modules at many different points in the celllular regulatory network. Unfortunately, no such minimal module has so far been characterized completely, as either effectors or direct targets are still mostly elusive. A c-di-GMP-binding effector domain recently identified is the PilZ domain[123,124]. One of the two *E. coli* PilZ proteins, BcsA, is a regulatory subunit of cellulose synthase[124], the other one, YcgR, interferes with motility by an anknown mechanism[125]. Among the 13 GGDEF domain proteins in *E. coli* with an intact GGDEF motif (a prerequisite for diguanylate cyclase activity), six are under σ^S control and several others are not expressed under any conditions tested so far, *i.e.* their putative σ^S dependence could not yet be assessed ([119]and H. Weber, N. Sommerfeldt and R. Hengge, unpublished results). This indicates that c-di-GMP signaling largely occurs within the context of the σ^S-controlled stress response network. A well-characterized target of this signaling is the synthesis of the adhesive curli fimbriae and cellulose biosynthesis. Here, the σ^S-dependent GGDEF protein YdaM and the σ^S-dependent GGDEF + EAL protein YciR act as diguanylate cyclase and phosphordiesterase, respectively, and are specifically involved in the transcription of the crucial curli and cellulose regulator CsgD, but the direct c-di-GMP effector in this module still awaits conclusive identification[119].

Conclusions and perspectives

In this review, we have tried to give an overview of the regulation of σ^S itself and multiple signal input at the level of this master regulator, we have summarized the way in which σ^S specifically recognizes ''stress'' promoters despite their similarity to vegetative promoters, and we have tried to give the reader at least a glance of the physiological impact of σ^S. With σ^S probably being the protein with the most complex regulation ever analyzed in the model organism *E. coli*, and with σ^S controlling the expression of at least 10% of the genes in the *E. coli* genome, we had to restrict our presentation to representative examples of these features of regulation of and by σ^S. It is clear, however, that σ^S with its central position as a multiple signal integrator and master regulator of hundreds of genes organized in regulatory cascades and subnetworks or regulatory modules represents a key model system for analyzing complex cellular information processing and a starting point for understanding the complete regulatory network of an entire cell. From this perspective, we may predict that the σ^S network will remain at the forefront of molecular microbiology research for many years to come.

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