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# Recent Insights into the General Stress Response Regulatory Network in *Escherichia coli*

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**Abstract**

**Many bacterial species exhibit a general stress response that can be induced by numerous very different stress conditions and, phenotypically, renders the cells broadly stress resistant. In *Escherichia coli*, this response is dependent on the $\sigma^S$ (RpoS) subunit of RNA polymerase. $\sigma^S$ is a close relative of the vegetative sigma factor $\sigma^{70}$ (RpoD) and recognizes very similar promoter sequences. In recent years, significant progress has been made with respect to elucidating (i) the molecular mechanisms that control the cellular $\sigma^S$ level, which include translational regulation as well as intricate control of $\sigma^S$ proteolysis, and (ii) the molecular function of $\sigma^S$ as a transcription initiation factor, where a number of $\sigma^S$-dependent promoters have been studied in great detail, and the mechanisms that generate $\sigma^S$ selectivity are now becoming apparent.**

## Introduction

Bacteria rarely find optimal growth conditions in their natural environments, i.e. being exposed to stress is their normal life-style. Many bacteria have numerous different single stress-induced responses at their disposal, that allow them to cope with specific stress situations by eliminating the stress agent and repairing actual damage. By contrast, the general stress response can be triggered by many different stress conditions and renders bacteria broadly stress resistant, even against stresses that they have not yet experienced. This means that damage is prevented rather than repaired (Storz and Hengge-Aronis, 2000).

The general stress response is usually controlled by a single master regulator. In *E. coli* and related bacteria (i.e. the gamma-branch of proteobacteria), this master regulator is $\sigma^S$ (RpoS). $\sigma^S$ is a sigma subunit of RNA polymerase (RNAP) that is a very close relative of the vegetative $\sigma^{70}$ (RpoD). Rapidly growing cells contain very little, if any, $\sigma^S$, but a variety of quite different stresses result in rapid induction of $\sigma^S$. This is usually (but not necessarily) accompanied by a reduction of the growth rate or even entry into stationary phase. As a consequence, expression of numerous $\sigma^S$-dependent genes is stimulated, most of which have stress-protective functions. So far, more than 70 genes have been found to be under the control of $\sigma^S$ (Hengge-Aronis, 2000).

In recent years, regulation of the cellular $\sigma^S$ level, as well as the function of $\sigma^S$ as a transcription initiation factor, have been studied intensively. This minireview focusses on these recent developments, and the author apologizes in advance to all colleagues whose work in this very active field cannot be appropriately mentioned because of space constraints. For more details, a recently published compilation of reviews may be consulted (Storz and Hengge-Aronis, 2000).

## The General Stress Response of *Escherichia coli* as a Regulatory Network

Regulatory networks in living cells are characterized by their extreme connectivity and hierarchical structure. The latter implies the existence of a limited number of top-level master regulators that act as foci of information integration. Thus, a large number of signal transduction pathways often converge to determine the cellular level or activity of the master regulator. When a certain threshold is reached, a complex physiological response based on the coordinated expression of numerous genes is set in motion. Prominent examples of such responses are the Spo0A-dependent initiation of sporulation of *Bacillus subtilis*, the ComK-dependent competence development in the same species, and the $\sigma^S$-and $\sigma^B$-mediated general stress responses in *E. coli* and *B.subtilis*, respectively (Dubnau and Turgay, 2000; Hengge-Aronis, 2000; Price, 2000; Sonenshein, 2000).

In addition, complex signal integration also occurs at the lower levels of regulatory networks, in particular in the transcriptional control of the many target genes. These structural genes can have complex promoter regions with multiple binding sites, not only for the master regulator, but also for additional regulatory factors that can be subject to intricate control themselves. This complex transcriptional control often provides the connections to other regulatory circuits and establishes differential gene expression within large regulons (Hengge-Aronis, 1999a).

## Signal Integration in the Control of the Cellular $\sigma^S$ Level

During rapid growth in rich medium, $\sigma^S$ is not detectable in *E. coli*. This changes when the cells grow in minimal medium, where low $\sigma^S$ levels can be observed. In response to acute stresses, such as sudden starvation or shift to hyperosmolarity, acidic pH or non-optimally

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low or high temperature, $\sigma^S$ levels increase further between three- and 20fold. Some of these stresses stimulate $\sigma^S$ synthesis, whereas others interfere with $\sigma^S$ degradation. Some stresses (e.g. hyperosmolarity) affect both processes, and therefore $\sigma^S$ levels increase particularly rapidly and drastically (Figure 1).

## Control of $\sigma^S$ Synthesis

All known acute stresses that affect $\sigma^S$ levels act at the post-transcriptional level of $\sigma^S$ control. Therefore, transcriptional regulation of the *rpoS* gene has not been studied in great detail, even though it is clear that the major *rpoS* promoter becomes activated during entry into stationary phase, when cells are grown in rich medium. *rpoS* transcription seems to be negatively controlled by cAMP-CRP (Lange and Hengge-Aronis, 1994). Positive control by a sensor kinase, BarA, has been reported (Mukhopadhyay *et al.*, 2000), although mutating an apparent cognate response regulator, YecA(UvrY), did not affect *rpoS* expression (G. Kampmann and R.H.-A., unpublished results).

Translation of existing *rpoS* mRNA is stimulated in response to hyperosmotic shift, beyond a certain cell density or during growth at low temperature (Figure 1). It is widely accepted that, under non-stress conditions, translation is inhibited by the formation of secondary structures in which the translational initiation region (TIR) is probably involved in base-pairing and, therefore, not accessible to ribosomes (although the actual secondary structure has not been demonstrated experimentally). An obvious, though still speculative, mechanism of translational activation would be a structural rearrangement somehow triggered by stress conditions. This can easily be envisioned for low temperature induction, where the small regulatory DsrA RNA is induced, which is partially complementary to an *rpoS* mRNA segment that is probably involved in intramolecular base-pairing with the TIR (Sledjeski *et al.*, 1996; Lease *et al.*, 1998; Majdalani *et al.*, 1998). How a shift to high osmolarity triggers an at least fivefold higher rate of *rpoS* translation within a few minutes (Muffler *et al.*, 1996c) is still unclear. It is clear, however, that the RNA-binding protein Hfq is crucial for *rpoS* translation (Brown and Elliott, 1996; Muffler *et al.*, 1996b) and actually binds with high affinity to *rpoS* mRNA *in vitro* (D. Traulsen and R. H.-A., unpublished results). Also, the histone-like protein HU is important for *rpoS* translation and may act directly since it can also bind to an *rpoS* mRNA fragment *in vitro* (Balandina *et al.*, 2001). By contrast, the histone-like protein H-NS represses *rpoS* translation (Barth *et al.*, 1995; Yamashino *et al.*, 1995), and so does another small regulatory RNA, OxyS, which is induced by oxidative stress and forms a complex with *rpoS* mRNA and Hfq protein (Zhang *et al.*, 1998). In conclusion, it appears that *rpoS* translational control is extremely complex and involves numerous trans-acting factors, but a clear mechanistic model that would integrate all these factors has yet to emerge.

## Control of $\sigma^S$ Proteolysis

During growth in minimal medium, $\sigma^S$ half-life is between one and several minutes. $\sigma^S$ is completely stabilized within a few minutes after osmotic upshift (Muffler *et al.*, 1996c). Also C-starvation and acid shift strongly interfere with $\sigma^S$ degradation (Lange and Hengge-Aronis, 1994; Bearson *et al.*, 1996). Heat shock results in a more moderate increase in $\sigma^S$ half-life, that takes approximately 20 minutes to develop (Muffler *et al.*, 1997).

The protease responsible for this rapid turnover is ClpXP (Schweder *et al.*, 1996), a multi-subunit ATP-dependent processive protease. In addition, the response regulator RssB is absolutely essential for $\sigma^S$ degradation (Bearson *et al.*, 1996; Muffler *et al.*, 1996a; Pratt and Silhavy, 1996). RssB acts as a direct recognition factor for $\sigma^S$, whose affinity for $\sigma^S$ and, therefore, activity in $\sigma^S$ degradation is dependent on the phosphorylation of its receiver domain (Bouché *et al.*, 1998; Becker *et al.*, 1999; Klauck *et al.*, 2001). A cis-acting region in $\sigma^S$, the turnover element, was shown to act as a binding site for RssB, with K173 in $\sigma^S$ being absolutely essential for RssB interaction *in vitro* and $\sigma^S$ proteolysis *in vivo* (Becker *et al.*, 1999). Interestingly, K173 is located just downstream of region 2.4 (known to be involved in recognition of the −10 promoter element), and has now been shown to interact directly with a cytosine present at the −13

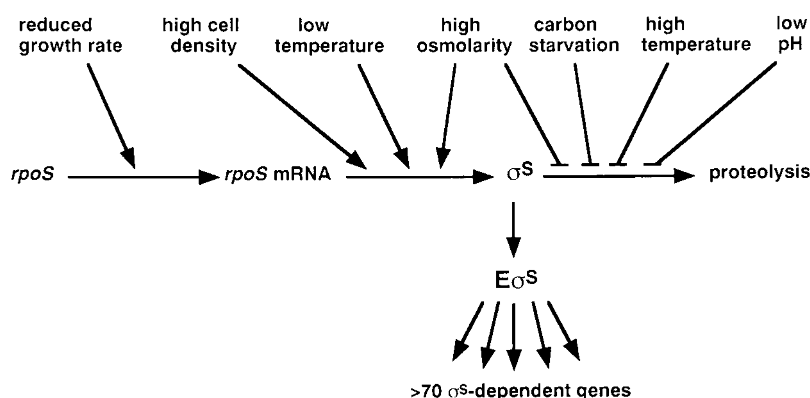


Figure 1. Various stress conditions differentially affect *rpoS* transcription and translation or $\sigma^S$ proteolysis.

position in more than 80% of known $\sigma^S$-dependent promoters (Becker and Hengge-Aronis, 2001) (see also below). All this indicates that K173 is a crucial amino acid in $\sigma^S$, especially for those functions that distinguish $\sigma^S$ from the otherwise closely related house-keeping sigma factor $\sigma^{70}$: $\sigma^{70}$ is not degraded by the RssB/ClpXP system, and, with a glutamate (E458) at the position corresponding to K173 in $\sigma^S$, $\sigma^{70}$ can contact a guanine at the $-13$ promoter position (Becker and Hengge-Aronis, 2001).

Once bound to RssB, $\sigma^S$ is transferred to the ClpXP protease, where, like other Clp protease substrates, it is unfolded and completely degraded by an ATP hydrolysis-dependent mechanism (Figure 2). RssB itself is not a substrate for ClpXP protease *in vivo*, and also its extremely low cellular level (the $\sigma^S$:RssB ratio is approximately 20:1) is consistent with a catalytic role in $\sigma^S$ delivery to ClpXP (Becker *et al.*, 2000; Klauck *et al.*, 2001; Zhou *et al.*, 2001).

Defining the entry points for stress signal pathways into the $\sigma^S$ recognition and degradation pathway (Figure 2) remains a challenge for future studies. In particular, sensor kinase(s) and/or phosphatase(s) for RssB have not yet been identified. In principle, stress signal input may result in active dephosphorylation of RssB, in inhibition of rephosphorylation of RssB (if dephosphorylation is an obligatory part of the RssB catalytic cycle), and/or in downregulation of the cellular RssB content. It is also possible that not all stress conditions affect RssB activity or cellular level. For carbon starved cells, there is indirect evidence that it is not $\sigma^S$-RssB interaction, but a process further downstream that is inhibited (Becker *et al.*, 2000). Moreover, since association with core RNA polymerase protects $\sigma^S$ against RssB binding and degradation (Zhou *et al.*, 2001), any factor that stimulates core binding of $\sigma^S$ would also contribute to $\sigma^S$ stabilization.

### What is a $\sigma^S$-dependent Promoter?

Since the first *in-vitro* transcription assays were performed with $\sigma^S$-containing RNAP holoenzyme ($E\sigma^S$), it has puzzled researchers that, *in vitro*, many genes can be transcribed by $E\sigma^S$ as well as by $E\sigma^{70}$, whereas *in vivo*, the same genes are often strongly dependent on $\sigma^S$ for expression. Therefore, the basic consensus sequence for $\sigma^{70}$- and $\sigma^S$-controlled promoters must be very similar. But, if there is little promoter-inherent $E\sigma^S/E\sigma^{70}$ selectivity *in vitro*, where does it come from *in vivo*? The currently emerging picture indicates that $E\sigma^S$ selectivity can be generated in different ways at different $\sigma^S$-controlled promoters. A subset of these promoters indeed contain sequence-based features that more or less strongly favor transcription initiation by $E\sigma^S$ (even *in vitro*), whereas at other promoters, additional regulatory factors contribute to or even generate $E\sigma^S$ selectivity (see below). Promotor sequence features that enhance $E\sigma^S$ selectivity are the following:

(i) A degenerate $-35$ region: most $\sigma^S$-controlled promoters have $-35$ regions with little similarity to the $-35(\sigma^{70})$ consensus sequence (TTGACA); an extreme case is the *fic* promoter, which is

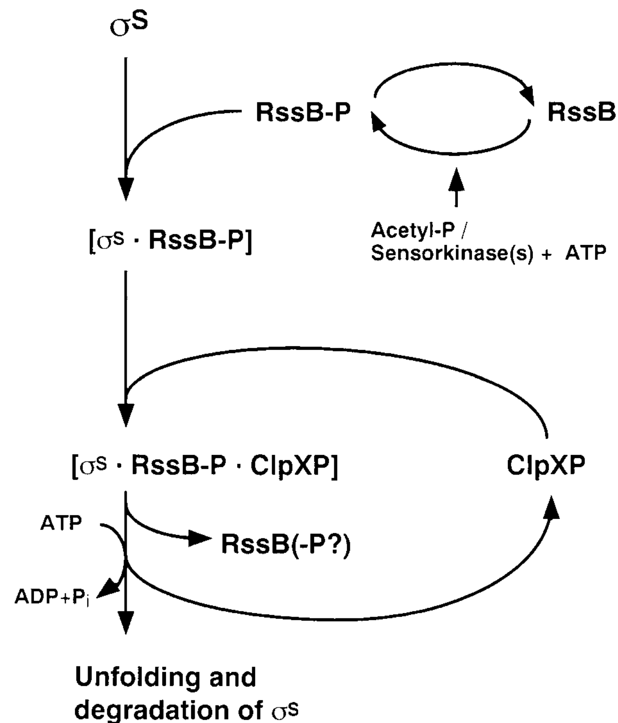


Figure 2. Pathway of $\sigma^S$ recognition, unfolding and degradation by the response regulator RssB and ClpXP protease.

completely $E\sigma^S$-dependent even *in vitro*, and completely lacks a functional −35 region (Tanaka *et al*., 1995);

(ii) An extended −10 region that includes positions (−14) to (−7) with the consensus ''KCTATACT'' (K stands for T or G): more than 85 % of *in-vivo* $E\sigma^S$-selective promoters contain C(−13); replacing C(−13) or T/G(−14) by other nucleotides reduces expression; and allele-specific suppression data indicate a direct $\sigma^S$-specific interaction of C(−13) with K173 in region 2.5 in $\sigma^S$ (Espinosa-Urgel *et al*., 1996; Bordes *et al*., 2000; Becker and Hengge-Aronis, 2001).

(iii) A subset of $\sigma^S$-controlled promoter regions feature a distal UP-element half-site located around position −50 (Ballesteros *et al*., 1998; Germer *et al*., 2001). A recent study with the *csiD* promoter indicates that a distal UP-element half-site favors transcription initiation by $E\sigma^S$, whereas a full UP-element can be equally used by $E\sigma^S$ and $E\sigma^{70}$ (Germer *et al*., 2001).

(iv) Nearly all *in-vivo* $E\sigma^S$-selective promoters are AT-rich between the −10 region and the transcriptional start site: this feature was associated with inverse growth rate-correlated expression even before $\sigma^S$ was discovered (Travers, 1984). For the stringently controlled *tyrT* promoter is was shown that GC-richness downstream of the −10 region results in instability of the open promoter complex (Pemberton *et al*., 2000). Preliminary data suggest that sequences downstream of the −10 region matter for $E\sigma^S$-selectivity (Ojangu *et al*., 2000). A $\sigma^S$-dependent promoter (*xthA*p), which is GC-rich in this region, does not exhibit stationary phase induction (Eisenstark *et al*., 1999).

## Signal Integration by Multiple Regulators at the Promoters of $\sigma^S$-Dependent Genes

Several $\sigma^S$-controlled promoters have now been studied in great molecular detail. In all these cases, it has been found that additional regulatory proteins contribute to regulation, which is reflected in quite complicated promoter architectures that involve multiple factor binding sites.

### Fine-Tuning Regulation in Response to Additional Signals

A requirement for an additional activator can efficiently limit the expression of a $\sigma^S$-dependent gene to a very specific situation. Because it has to be activated by cAMP-CRP, the *csiD* gene is specifically induced by carbon starvation, but not by other conditions that also induce $\sigma^S$ (Marschall *et al*., 1998). Simultaneous dependency on $\sigma^S$ and FIS limits *proP*p2 activity to a small time window during late exponential phase, where both factors are present (Xu and Johnson, 1997b).

In other cases, the spectrum of inducibility can be enlarged by using $E\sigma^S$ as well as $E\sigma^{70}$ for expression. The *osmE* promoter can be recognized by both holoenzymes, with $E\sigma^{70}$ being responsible for hyperosmotic induction, whereas $E\sigma^S$ mediates stationary phase induction (Conter *et al*., 1997; Bordes *et al*., 2000). The *osmC* gene features two separate but overlapping promoters p1 and p2, that are dependent on $E\sigma^{70}$ and $E\sigma^S$, respectively. Additional regulators have a modulatory role, with Lrp repressing at p1 and activating at p2, and H-NS repressing both promoters (Bouvier *et al*., 1998). Also *proP* is under dual control of a $\sigma^{70}$-controlled promoter p1 and the above mentioned $\sigma^S$-dependent promoter p2 (87 bp apart), that respond to hyperosmolarity and stationary phase, respectively. cAMP-CRP bound to a site that overlaps with the −35 region of p1 acts as a low osmolarity repressor at p1, as well as an upstream co-activator (together with FIS) at p2 (Xu and Johnson, 1997a; McLeod *et al*., 2000).

### Generation of $\sigma^S$ Selectivity

*In vivo* $\sigma^S$-dependent promoters, that do not have a strong sequence-inherent preference for $E\sigma^S$ (and therefore show expression by $E\sigma^S$ and $E\sigma^{70}$ in standard *in-vitro* transcription assays), must use other means to establish $E\sigma^S$-selectivity *in vivo*. High concentration of salt (especially potassium glutamate, which *in vivo* transiently accumulates upon osmotic upshift) and reduced negative supercoiling of the template have been shown to stimulate (or less inhibit) $E\sigma^S$-mediated *in-vitro* transcription (Ding *et al*., 1995; Kusano *et al*., 1996). Promoter-specific enhancement of $E\sigma^S$ selectivity, however, can be due to the effect of additional regulatory proteins, in particular certain abundant nucleoid-associated or histone-like proteins (Hengge-Aronis, 1999b).

This has first been recognized for H-NS, which has a repressing effect on many $\sigma^S$-dependent genes (partly, this is due to negative control of $\sigma^S$ itself). A number of $\sigma^S$-controlled genes exhibit higher expression in *hns rpoS* double mutants than in a strain that is defective in *rpoS* alone, i.e. $E\sigma^{70}$ can take over expression only in the absence of H-NS (Olsén *et al*., 1993; Barth *et al*., 1995; Yamashino *et al*., 1995).

The *osmY* promoter is negatively modulated by cAMP-CRP, Lrp and IHF (Lange *et al*., 1993). For all three factors it could be demonstrated *in vitro* that they interfere more strongly with $E\sigma^{70}$-mediated than with $E\sigma^S$-mediated transcriptional initiation. By adding these factors to the *in-vitro* assays for open complex formation and run-off transcription, $E\sigma^S$ selectivity could thus be generated (Colland *et al*., 2000). At the *osmC*p1 promoter, Lrp selectively interferes with $E\sigma^S$-initiated transcription, thus making this promoter $\sigma^{70}$-dependent *in vivo* (Bouvier *et al*., 1998).

The *csiD* promoter exhibits sequence-inherent $E\sigma^S$ preference, which is apparent when basal transcription in the absence of the activator cAMP-CRP is tested *in vitro* (Marschall *et al*., 1998; Germer *et al*., 2001). However, also cAMP-CRP contributes to $E\sigma^S$ selectivity, since it is bound at a position (−68.5), which in the context of the *csiD* promoter region, is unproductive for $E\sigma^{70}$-mediated expression, but which can activate $E\sigma^S$-initiated transcription. Moreover,

H-NS activates this promoter by a novel mechanism (Germer *et al.*, 2001).

In all these cases, the RNAP holoenzymes have to deal with complex nucleoprotein structures formed in the promoter regions. Recent reports suggest subtle differences in the modes of DNA binding and open complex formation by $E\sigma^S$ and $E\sigma^{70}$ (Bown *et al.*, 1999; Colland *et al.*, 1999). These properties may differentially affect the ability of the two holoenzymes to interact productively with a specific promoter-protein complex and thereby strongly contribute to or even generate sigma factor selectivity (Hengge-Aronis, 1999b).

## Perspectives

Taken together, significant progress has been made in recent years with respect to the regulation and the mechanistic and physiological functions of the $\sigma^S$ subunit of RNAP. Many important questions, however, remain to be solved. How do the signal transduction pathways function that relay so many stress conditions to alterations in the rates of *rpoS* transcription or translation or $\sigma^S$ proteolysis? What is the molecular role of the abundant nucleoid-associated protein H-NS in $\sigma^S$ translation and proteolysis as well as at many $\sigma^S$-dependent promoters? No sigma factor crystal structure has yet been reported – what are the exact molecular similarities and differences between $\sigma^S$ and $\sigma^{70}$ alone and in the complex with RNAP core? What is the physiological function of all $\sigma^S$-controlled genes (many of which are just being identified now)? How is the $\sigma^S$-mediated stress response interconnected with other stress responses? With $\sigma^S$ and the general stress response now being under intense investigation, we can be confident to get the answers before long.

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