MINIREVIEW / MINI-SYNTHÈSE

Regulation in the *rpoS* regulon of *Escherichia coli*

Peter C. Loewen, Bei Hu, Jeanna Strutinsky, and Richard Sparling

Abstract: In *Escherichia coli*, the transcription factor σ^S, encoded by *rpoS*, controls the expression of a large number of genes involved in cellular responses to a diverse number of stresses, including starvation, osmotic stress, acid shock, cold shock, heat shock, oxidative DNA damage, and transition to stationary phase. A list of over 50 genes under the control of *rpoS* has been compiled. The transcription factor σ^S acts predominantly as a positive effector, but it does have a negative effect on some genes. The synthesis and accumulation of σ^S are controlled by mechanisms affecting transcription, translation, proteolysis, and the formation of the holoenzyme complex. Transcriptional control of *rpoS* involves guanosine 3′,5′-bispyrophosphate (ppGpp) and polyphosphate as positive regulators and the cAMP receptor protein – cAMP complex (CRP–cAMP) as a negative regulator. Translation of *rpoS* mRNA is controlled by a cascade of interacting factors, including Hfq, H-NS, *dsrA* RNA, LeuO, and *oxyS* RNA that seem to modulate the stability of a region of secondary structure in the ribosome-binding region of the gene's mRNA. The transcription factor σ^S is sensitive to proteolysis by ClpPX in a reaction that is promoted by RssB and inhibited by the chaperone DnaK. Despite the demonstrated involvement of so many factors, arguments have been presented suggesting that sensitivity to proteolysis may be the single most important modulator of σ^S levels. The activity of σ^S may also be modulated by trehalose and glutamate, which activate holoenzyme formation and promote holoenzyme binding to certain promoters.

Key words: transcription, translation, regulation, sigma factor, starvation.

Résumé : Chez *Escherichia coli* le facteur de transcription σ^S, codé par *rpoS*, contrôle un grand nombre de gènes impliqués dans la réponse cellulaire à diverses conditions de stress comme un jeûne, un stress osmotique, un choc acide, un choc par le froid, un choc thermique, un dommage oxydatif du DNA ou un passage à la phase stationnaire. Nous avons dressé une liste de plus de 50 gènes contrôlés par *rpoS*. Le facteur σ^S agit principalement comme effecteur positif mais il a aussi un effet négatif sur certains gènes. La synthèse et l'accumulation du σ^S sont contrôlées par des mécanismes qui affectent la transcription, la traduction, la protéolyse et la formation du complexe holoenzyme. Le contrôle de la transcription par *rpoS* nécessite guanosine 3′,5′-bispyrophosphate (ppGpp) et du polyphosphate comme régulateurs positifs et protéine réceptrice d'AMPc – complexe AMPc (CRP–cAMP) comme régulateur négatif. La traduction du *rpoS* ARNm est contrôlée par une cascade de facteurs interactifs incluant Hfq, H-NS, *drsA* ARN, LeuO et le *oxyS* ARN qui semblent moduler la stabilité d'une région de la structure secondaire dans la portion de liaison du ribosome au ARNm du gène. Le facteur σ^S est sensible à la protéolyse par ClpPX conformément à une réaction favorisée par RssB et inhibée par le DnaK chaperon. Malgré le rôle confirmé d'aussi nombreux facteurs, des arguments ont été présentés pour suggérer que la sensibilité à la protéolyse pourrait être le seul plus important modulateur des niveaux de σ^S. L'activité de σ^S peut aussi être modulée par le tréhalose et le glutamate qui activent la formation de l'holoenzyme et favorisent la liaison de l'holoenzyme à certains promoteurs.

Mots clés : transcription, traduction, régulation, facteur sigma, jeûne.

[Traduit par la Rédaction]

Introduction

Gram-negative bacteria respond to different stresses with the synthesis or activation of auxiliary sigma factors that direct the transcription of regulons whose gene products mitigate the effects of the stress. In the case of *Escherichia coli*, there are six sigma factors that have evolved to respond to different stressors, including nitrogen depletion (σ^N or σ^{54}),

Received March 30, 1998. Revision received June 2, 1998. Accepted June 9, 1998.

P.C. Loewen,[1] B. Hu, J. Strutinsky, and R. Sparling. Department of Microbiology, University of Manitoba, Winnipeg, MB R3T 2N2, Canada.

[1]Author to whom all correspondence should be addressed.

heat shock (σ^H or σ^{32}), extracytoplasmic stress (σ^E or σ^{24}), the need for flagellin (σ^F or σ^{28}), starvation (σ^S or σ^{38}), and the need for citrate-dependent iron transport (FecI or σ^{19}) (Lonetto and Gross 1996). Each of these sigma factors controls a specific set of genes or the regulon that supplements the genes controlled by σ^D (σ^{70}), the vegetative or housekeeping sigma factor.

The starvation or stationary phase sigma factor, σ^S, was initially characterized as a regulatory protein controlling a diverse set of phenomena that occur when cells enter stationary phase, including near UV resistance, acid phosphatase production, and catalase production, a combination that was initially perplexing. The importance of σ^S became more obvious upon the demonstration that it directed the synthesis of more than 50 proteins during the transition from exponential phase to stationary phase (see Loewen and Hengge-Aronis 1994). Subsequently, many phenomena specific to stationary phase, starvation, osmotic shock, acid shock, heat shock, and cold shock have been ascribed to σ^S, confirming its role as one of the key transcriptional factors in *E. coli* physiology.

In some respects, σ^S has a role analogous to that of the family of sigma factors that are synthesized in response to nutrient limitation in *Bacillus subtilis*, resulting in the formation of spores to enhance survival during extended periods of starvation. In *E. coli*, the cells respond to nutrient limitation with a variety of physiological and morphological changes, controlled in part through the synthesis of σ^S, that culminate in the formation of small spherical cells, which are better adapted to extended periods of slow metabolism. There is no increase in cell number in stationary phase, but an active metabolism and turnover of cells has been revealed (Kolter et al. 1993).

Several reviews have spotlighted the role of σ^S, including one in 1993 (Hengge-Aronis 1993), one in 1994 (Loewen and Hengge-Aronis 1994), and two in 1996 (Eisenstark et al. 1996; Hengge-Aronis 1996). This has been a period of rapid development in our understanding of what σ^S regulates and how the accumulation of active σ^S is regulated. An increasingly complex picture of interactions has appeared revealing control at the translational and post-translational levels, in addition to the more conventional transcriptional level, as dominant mechanisms. This review will focus on two aspects of σ^S metabolism: the genes of the σ^S regulon and the mechanisms regulating cellular levels of σ^S.

Components of the σ^S regulon

The central role played by σ^S in the physiology of *E. coli* is best demonstrated by a consideration of the large number and diverse nature of genes that it controls. The first gene confirmed to be under the control of σ^S was *katE* (Loewen and Triggs 1984; Mulvey and Loewen 1989), but the recent literature contains a plethora of genes that are sensitive to its regulation. Table 1 contains a list of genes that fall into this category. The number of genes confirmed to be subject to σ^S control has already reached the 50 predicted by two-dimensional gel analysis of cell extracts, and it seems likely that more will be identified in the future. Difficulties in accurately assessing small changes in protein levels on two-dimensional gels and in eliciting a response from all proteins to either starvation or osmotic shock probably resulted in low initial estimates. There have also been reports linking *rpoS* to certain phenotypes, such as *rpoS* suppression of the hypersensitive phenotype of Δ*oxyR* mutations (Ivanova et al. 1997), but a specific target gene has not been identified.

Regulatory factors that have been shown to modulate σ^S activity on specific genes are also listed in Table 1. The diversity of factors is striking, with integration host factor (IHF), H-NS, Fis, and cAMP receptor protein (CRP) appearing most often. The complexity of modulation is also variable, ranging from no known regulator for *katE* to the involvement of four or more factors affecting *osmY.*

In addition to the more common motif of expression activation by *rpoS*, there are several examples of genes whose expression is reduced by σ^S (Table 1). To date, the controls surrounding these genes have not been defined, but it seems likely that a repressor protein is synthesized under the direction of σ^S, which in turn interferes with σ^D-dependent expression of the gene. It is important to realize that σ^D continues to direct the expression of many genes in stationary phase, and the components of the σ^S regulon are a supplement to this larger family of proteins that enhances survival.

Homologues of σ^S have been found in a number of other organisms (see Eisenstark et al. 1996) including, not surprisingly, various *Salmonella* species. The role of σ^S in *Salmonella* spp. will probably be very similar to its role in *E. coli*, as reflected by the identification of certain starvation response genes that are *rpoS* dependent. However, additional specialized roles for σ^S, seemingly unique to *Salmonella* spp., have been noted, including the regulation of plasmid virulence genes (Kowarz et al. 1994; Chen et al. 1995) and the modulation of virulence in susceptible mice (Coynault et al. 1996; Nickerson and Curtiss 1997; Swords et al. 1997). These roles are also noted in Table 1. A certain degree of cross-specificity has been observed, whereby a *rpoS* homologue from *Erwinia carotovora* is functional in *E. coli* (Calcutt et al. 1998) and the transcription of genes responsible for carotenoid biosynthesis in *Erwinia herbicola* is σ^S-dependent in *E. coli* (Becker-Hapak et al. 1997).

Promoter recognition by σ^S

The extensive sequence similarity between σ^S and σ^D suggests that there should not be large differences between promoter sequences recognized by the two sigma factors. Indeed, detailed footprinting studies have confirmed very similar protection patterns for binding of the two holoenzyme complexes, with the main differences falling between the –10 and –20 regions (Nguyen and Burgess 1997). The sequences of 33 σ^S-dependent promoters have been compared revealing a possible consensus sequence in the –10 region of CTATACT, which is very similar to the corresponding σ^D sequence of TATAAT (Espinosa-Urgel et al. 1996). This sequence confirmed the TATACT sequence that was proposed earlier on the basis of fewer promoters (Loewen and Hengge-Aronis 1994). Because most other sigma factors have a –35 sequence element, it is surprising that no common –35 sequence element can be discerned in the σ^S-promoter group. However, a common physical feature

of intrinsic curvature, not necessarily present in σ^D-associated promoters, is predicted by the sequence of σ^S-dependent promoters (Espinosa-Urgel and Tormo 1993). On the basis of the hypothesis that a combination of a –10 sequence and intrinsic curvature determines a σ^S promoter, it was predicted, and successfully confirmed, that the *frd* operon promoter is σ^S-dependent (Espinosa-Urgel et al. 1996).

This picture suggests the reason some promoters are recognized by both $E\sigma^S$ and $E\sigma^D$ (E denotes the core RNA polymerase made up of 2α, β, and β′ subunits) while other promoters are recognized by either $E\sigma^S$ or $E\sigma^D$ (Tanaka et al. 1993, 1995) is based simply on whether a σ^D –35 sequence is present. $E\sigma^D$ will bind only if both the –10 and the –35 sequence elements are present, and $E\sigma^S$ will bind if an appropriate –10 sequence is supplemented by a region with intrinsic curvature. Given the very similar –10 sequences recognized by the two sigma factors, it is not surprising that there is considerable overlap in the promoters recognized by the two. This model seems to imply that the sequence in the –35 region of σ^S promoters is not important. However, evidence has been presented suggesting that sequence changes in the –35 region can affect σ^S-dependent expression of the *proU* and *osmY* promoters. Specifically, changing TT to CC in the *proU* promoter enhanced σ^S-directed transcription, whereas changing CC to TT in the *osmY* promoter enhanced σ^D-directed transcription (Wise et al. 1996). Whether these changes are modifying a region directly recognized by the sigma factor or are affecting the curvature of the region is not clear. More work is required to clarify the differences between σ^S- and σ^D-dependent promoters, and any in vitro analyses will have to be cognizant of the importance of the composition of various solutes, including salt, trehalose, and glutamate, etc., on promoter recognition by the two holoenzymes (see below; Kusano and Ishihama 1997).

The importance and number of genes regulated by σ^S through mechanisms that do not seem particularly stringent presents a paradox that is resolved only when one considers the complexity of controls affecting σ^S accumulation. It is the control of σ^S levels and activities that is the primary determinant in modulating expression in the σ^S regulon.

Regulation of cellular levels of σ^S

The discontinuous use of σ^S requires that the cell be capable of modulating the rate of synthesis and activity of the sigma factor. Indeed, the cellular levels of σ^S are modulated by a series of mechanisms that affect transcription, translation, and post-translational stability to create a complex and fascinating regulatory picture that is by no means fully resolved. The summary of these control mechanisms, presented in Fig. 1, is simply a snapshot of the current situation, and changes are inevitable as further work brings the subject to maturity. Figure 1 is based on assumptions about the most likely explanations for phenotypes and experimental data and future work will confirm, possibly change, and certainly expand the picture shown.

Transcriptional control

Protein levels of σ^S are virtually undetectable during the exponential growth phase, but during stationary phase, they increase to 30–50% of the level of σ^D, which remains relatively constant between 50 and 80 fmol/μg of protein throughout the exponential and stationary phases (Jishage and Ishihama 1995). The encoding gene, *rpoS*, is situated adjacent to, and transcribed in the same direction as *nlpD*, encoding a lipoprotein of unknown function; a majority of the low level of σ^S in exponential phase cells is a result of read through transcription from two relatively weak promoters upstream of *nlpD* (Ichikawa et al. 1994; Lange and Hengge-Aronis 1994*a*). Upon transition to stationary phase, at least four promoters are activated to transcribe *rpoS*, including *rpoS*p1, which is located 550 bp upstream from *rpoS* within *nlpD* and is responsible for most *rpoS* transcription in gene fusion systems (Takayanagi et al. 1994; Lange et al. 1995). This promoter appears to be a typical σ^D-dependent sequence with two potential weak CRP-binding domains upstream. Unfortunately, the molecular mechanisms controlling the expression of these promoters remain obscure despite the demonstrated involvement of several factors, including CRP, guanosine 3′,5′-bispyrophosphate (ppGpp), polyphosphate, *oxyS* RNA, homoserine lactone, and UDP-glucose.

The initial description of *rpoS* transcription using plasmid-based *lacZ* fusions suggested that transcription increased gradually throughout exponential phase with a very substantial increase following the transition to stationary phase (Mulvey et al. 1990). However, the segment of DNA upstream of *rpoS* did not contain *rpoS*p1 and these results reflected transcription from a minor promoter. Subsequent work using single-copy *lacZ* fusions revealed a fivefold stimulation of *rpoS* transcription from *rpoS*p1 in complex medium during entry into stationary phase but little or no stimulation in minimal medium (McCann et al. 1991; Lange and Hengge-Aronis 1991*b*, 1994*b*). Further contradictions were observed when the role of the cAMP receptor protein – cAMP complex (CRP–cAMP) was investigated. For example, with some *rpoS*::*lacZ* fusions, the exponential phase expression is increased in Δ*crp* and Δ*cya* strains, but the addition of cAMP to the Δ*cya* strains actually decreased expression. This suggested that CRP–cAMP had a negative effect on *rpoS* expression (Lange and Hengge-Aronis 1991*b*, 1994*b*), consistent with the observation that the levels of RpoS protein increase in *cya* strains and decrease with the addition of cAMP (Lange and Hengge-Aronis 1994*b*). In contrast, other transcriptional fusions suffered a decrease in expression in a *cya* mutant (McCann et al. 1993).

A positive correlation between ppGpp and σ^S levels has been observed, and the central role played by ppGpp in transcriptional control during starvation (Chesbro 1988) suggested that it influenced transcription of *rpoS* (Gentry et al. 1993). Subsequently, this was confirmed using transcriptional fusions and measurements of *rpoS* mRNA levels (Lange et al. 1995). Another common metabolite, inorganic polyphosphate, has been positively correlated with in vivo σ^S levels and increases in *rpoS* transcription, suggesting an influence of polyphosphate on *rpoS* transcription (Shiba et al. 1997). Unfortunately, polyphosphate did not have a direct effect on transcription in vitro, and a separate modulator of its effect was not identified. ppGpp, CRP–cAMP, and polyphosphate have been included in Fig. 1 as modulators of *rpoS* transcription despite the uncertainty about their precise roles.

Table 1. σ^S-dependent genes and their functions in *E. coli* and *Salmonella typhimurium*.

Gene or operon	Function	Modulator	Reference
Positively affected by σ^S			
E. coli			
aidB	Methylation damage repair of DNA	Lrp	Volkert et al. 1994; Landini et al. 1996
aldB	Aldehyde dehydrogenase	CRP, Fis	Xu and Johnson 1995*b*
appY	Regulatory protein		Brøndsted and Atlung 1996
appA	Acid phosphatase	AppY	Atlung et al. 1997
bolA	Control of PBP6 synthesis		Lange and Hengge-Aronis 1991*a*
cbdAB	Cytochrome *bd* oxidase	AppY	Atlung et al. 1997
cbpA	Molecular chaperone	H-NS	Yamashino et al. 1994
cfa	Cyclopropane fatty acid synthesis		Wang and Cronan 1994
csiA-F	Six carbon starvation genes	CRP	Weichart et al. 1993; Marschall et al. 1998
csgCDEF	Curli fimbriae		Hammar et al. 1995
csgBA	Curli fimbriae	H-NS	Olsen et al. 1993; Arnqvist et al. 1994
dps (*pexB*)	DNA-binding protein	IHF	Altuvia et al. 1994
f253a	Undefined open reading frame		Van Dyk et al. 1998
ficA	Control of cell division		Utsumi et al. 1993
frd	Fumarate reductase		Espinosa-Urgel et al. 1996
ftsQ	Cell division gene		M. Vicente (see Cam et al. 1995)
ftsZ	Cell division protein		Cam et al. 1995
galEKT	*gal* operon from promoter P1	CRP	Kolb et al. 1995
glgA	Glycogen synthase		Weichart et al. 1993
glgS	Glycogen synthesis	CRP	Hengge-Aronis and Fischer 1992
glpD	Glycerol-3-phosphate dehydrogensase		Weichart et al. 1993
gor	Glutathione oxidoreductase		Becker-Hapal and Eisenstark 1995
hdeAB	Periplasmic proteins		Arnqvist et al. 1994
himA	Integration host factor	ppGpp, IHF	Aviv et al. 1994
hmp	Soluble flavoprotein	IHF, ppGpp	Membrillo-Hernandez et al. 1997*a*, 1997*b*
htrE	Pilli construction protein	IHF	Raina et al. 1993
hyaABCDEF	Hydrogenase I	AppY	Atlung et al. 1997; Brøndsted and Atlung 1994
katE	Catalase HPII		Loewen and Triggs 1984; Mulvey et al. 1990
katG	Catalase-peroxidase HPI		Ivanova et al. 1994; Mukhopadhyay and Schellhorn 1994
lacZ	*lac* operon		Kolb et al. 1995
ldcC	Lysine decarboxylase		Van Dyk et al. 1998
mcc	Microcin C7 and C51	H-NS	Diaz-Guerra et al. 1989
osmB	Outer membrane lipoprotein		Hengge-Aronis et al. 1991
osmE	Lipoprotein function		Conter et al. 1997
osmY	Periplasmic protein	Lrp, CRP, IHF, H-NS	Hengge-Aronis et al. 1993; Lange et al. 1993, Yim et al. 1994; Barth et al. 1995
otsA	Trehalose-6-phosphate synthase	H-NS	Hengge-Aronis et al. 1991; Kaasen et al. 1992; Barth et al. 1995
otsB	Trehalose-6-phosphate phosphatase	H-NS	Hengge-Aronis et al. 1991; Kaasen et al. 1992
poxB	Pyruvate oxidase		Chang et al. 1994
pqi5	Membrane protein		Koh and Roe 1996
proP	Transport protein	Fis	Mellies et al. 1995; Xu and Johnson 1997
rob	DNA-binding protein		Kakeda et al. 1995
topA	Topoisomerase I		Qi et al. 1997
treA	Trehalase		Hengge-Aronis et al. 1991
wrbA	Trp repressor binding protein		Yang et al. 1993
xthA	Exonuclease III		Sak et al. 1989
yciG	Undefined open reading frame		Van Dyk et al. 1998
yohF	Undefined open reading frame		Van Dyk et al. 1998
Salmonella spp.			
spvABCD	Plasmid virulence gene	SpvR	Heiskanen et al. 1994
spvR	Regulatory protein		Heiskanen et al. 1994; Kowarz et al. 1994; Chen et al. 1995

Table 1 (*concluded*).

Gene or operon	Function	Modulator	Reference
stiA	Starvation survival		O'Neal et al. 1994
stiC	Starvation survival		O'Neal et al. 1994
Negatively affected by σ^S			
E. coli			
fimA	Fimbiral protein		Dove et al. 1997
glnQ		Fis	Xu and Johnson 1995*a*
mglA		Fis	Xu and Johnson 1995*a*
mutH	d(GATC)-specifc endonuclease		Tsui et al. 1997
mutS	DNA mismatch binding protein		Tsui et al. 1997
sdhA		Fis	Xu and Johnson 1995*a*
xylF		Fis	Xu and Johnson 1995*a*
Salmonella sp.			
stiB	Starvation survival		O'Neal et al. 1994

Contradicting these conclusions are the assertions that the correlations between increased *rpoS* expression and cAMP or ppGpp levels are artifactual and the actual levels of *rpoS* mRNA decrease in both minimal and complex media despite an increase in the half-life of mRNA (Zgurskaya et al. 1997). Unfortunately, so far, there has been no rationalization of the extensive body of fusion expression data with the actual levels of mRNA, and it seems unreasonable to dismiss the extensive correlative and fusion expression data simply because they are inconsistent with mRNA levels. The two poles must somehow be reconciled in a common model or by further experimentation. Consequently, Fig. 1 retains the roles of ppGpp as an activator and CRP–cAMP as an inhibitor of transcription, but the reader is warned that there are issues that still must be resolved.

The search for possible metabolic signal molecules has resulted in the identification of two metabolites exhibiting a positive correlation with σ^S levels: homoserine lactone (Huisman and Kolter 1994) and UDP-glucose (Bohringer et al. 1995). Unfortunately, there has been no confirmation of their involvement, either directly or via a signal transduction pathway, nor has any mechanism been proposed for their involvement in modulating σ^S levels. Consequently, they have not been included in Fig. 1.

Modulation of *rpoS* translation

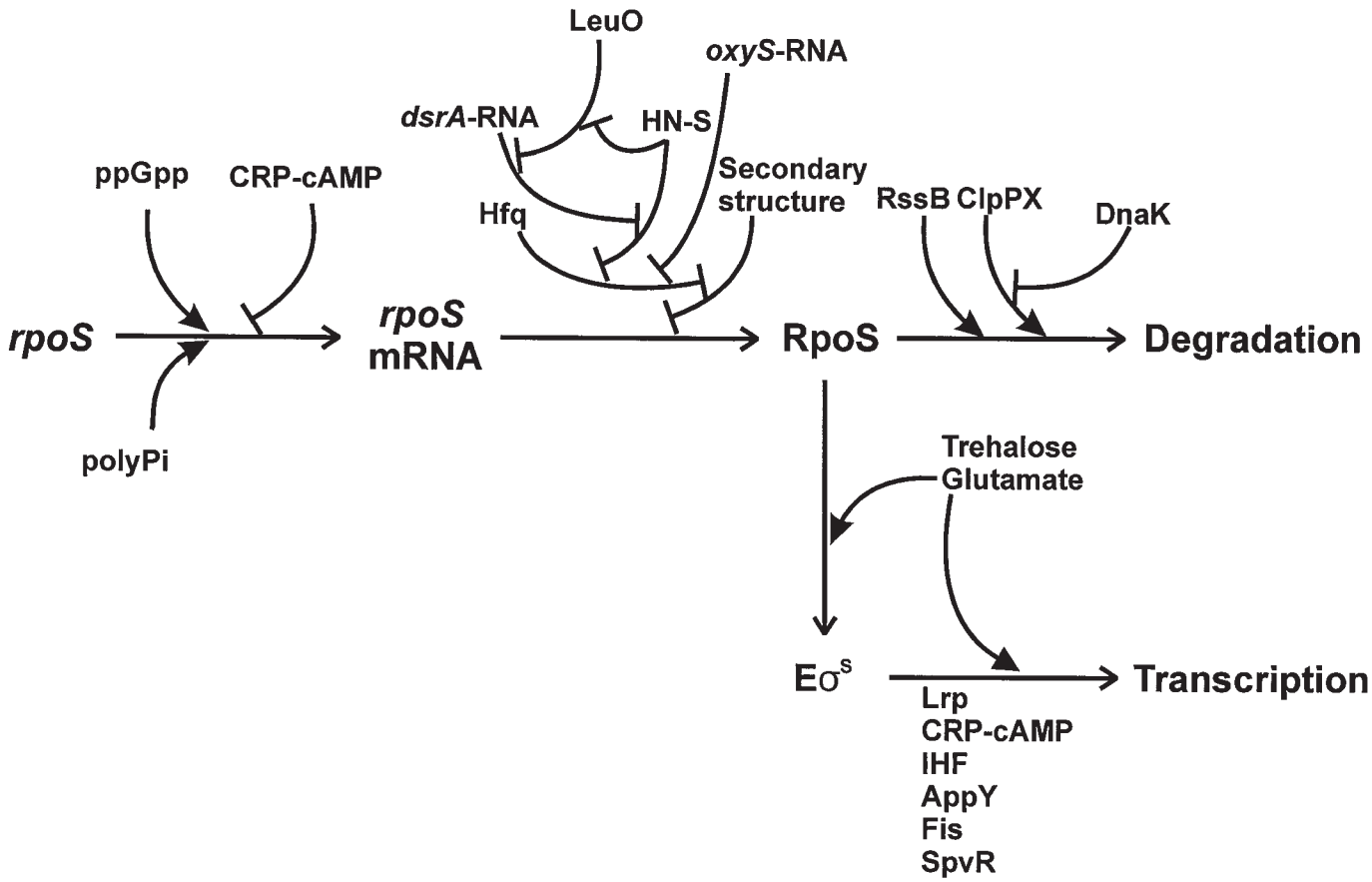
Post-transcriptional control of σ^S synthesis was first predicted by fusion expression studies (McCann et al. 1993; Loewen et al. 1993) and subsequently corroborated by the observations that high osmolarity (Lange et al. 1994*b*; Muffler et al. 1996*b*), low temperature, and the transition to stationary phase stimulated *rpoS* mRNA translation (Muffler et al. 1997*a*). The limited examples of translational control have involved controlling the access of ribosomes to the initiation codon and ribosome-binding site, either through secondary structure or protein binding. The 5′ region of *rpoS* mRNA is similar to that of *rpoH* mRNA in that it has a significant self-complementary sequence that allows the prediction of an extensive branched stem and loop structure. The ribosome-binding site and initiation codon are located in regions of secondary structure, making them inaccessible for ribosome binding (Lange and Hengge-Aronis 1994*b*). In Fig. 1, secondary structure is shown as the primary determinant controlling the translation of *rpoS* mRNA.

Disruption of this secondary structure would increase the frequency of translational initiation, and it has been proposed that a protein or proteins induced by environmental stress might cause a destabilization of the secondary structure and an increase in translation. The RNA-binding protein Hfq, originally characterized as a RNA phage host factor, has an effect on translation (Brown and Elliott 1996; Muffler et al. 1996*a*). Mutations in *hfq*, which encodes Hfq, result in significantly reduced levels of σ^S, and Hfq has been positively correlated with σ^S accumulation in both exponential and stationary growth phases (Muffler et al. 1997*b*). As a logical extension of its role as a RNA-binding protein, Hfq may promote the destabilization of secondary structure in *rpoS* mRNA to allow translational initiation. This is its role outlined in Fig. 1.

In a role that appears to be antagonistic to that of Hfq, H-NS, a nucleoid histone-like protein, has been associated with a decrease in the levels of σ^S in exponential phase and ascribed a role in the osmotic and growth phase regulation of σ^S levels (Barth et al. 1995; Yamashino et al. 1995). Mutations in *hns*, which encodes H-NS, cause significantly enhanced levels of σ^S. H-NS is shown in Fig. 1 as acting to prevent Hfq from activating translation. The cascade presented in Fig. 1 is based on our current understanding of how Hfq and H-NS function. However, if future studies show that H-NS acts to stabilize the mRNA secondary structure directly and Hfq acts to destabilize the H-NS – mRNA complex, the model in Fig. 1 would require only small changes.

In fact, the control circuit affecting translation is even more complex. The small regulatory RNA, *dsrA* RNA, has been implicated in the control of the translational expression of *rpoS* because it is essential for low temperature accumulation of σ^S. It has been proposed that *dsrA* RNA interacts with H-NS to interfere with, or antagonize, the latter's role in repressing translation (Sledjeski and Gottesman 1995; Sledjeski et al. 1996). In addition, the LysR-like protein, LeuO, represses the synthesis of *dsrA* RNA (Klauck et al. 1997), as evidenced by mutations in *leuO* causing reduced accumulation of σ^S at low temperatures. One result of the in-

Fig. 1. Schematic depicting the modulators of σ^S activity. Lines leading from an effector with arrowheads indicate an activating role or positive effect on the process indicated. Lines leading from an effector with a bar at the end indicate an inhibiting role or negative effect on the process indicated. The effectors listed under the arrow leading from $E\sigma^S$ modulate $E\sigma^S$ transcription of certain genes, as indicated in Table 1.



volvement of LeuO is that H-NS has a second role in the cascade depicted in Fig. 1: interference with LeuO accumulation. The most recent addition to the translational control cascade is *oxyS* RNA, which reduces σ^S synthesis (Altuvia et al. 1997) most likely by modulating *rpoS* translation through an interaction with Hfq (Zhang et al. 1998).

After this description of such an intricate scheme dedicated to the regulation of translation, it is paradoxical that we must return to the suggestion that none of this is necessary because an increase in σ^S stability (next section) is more than sufficient to explain the change in σ^S levels (Schweder et al. 1996; Zgurskaya et al. 1997). The evidence based on the determination of the half-life of *rpoS* mRNA and the calculation of decreased translational efficiency of *rpoS* mRNA is persuasive. However, it is unreasonable to declare the body of data arising from *rpoS*::*lacZ* translational fusion systems to be artifactual and dismissible, particularly in view of the demonstrated in vivo roles of LeuO, H-NS, Hfq, *dsrA* RNA, and *oxyS* RNA in modulating σ^S levels. At some point, the involvement of these factors will have to be rationalized.

Post-translational modulation of σ^S levels

Subsequent to its synthesis as a protein, the stability of σ^S increases substantially after the transition to stationary phase (Lange and Hengge-Aronis 1994*b*). This is in contrast to the increase in turnover of many other proteins that has been observed after entry into stationary phase (Kolter et al. 1993). The protease responsible for the instability of σ^S in exponential phase is the ClpPX protease (Schweder et al. 1996), which seems to recognize a region of the protein between residues 173 and 188 from the amino terminus. The existence of a segment of the protein between residues 23 and 247 that enhances instability had previously been noted by Lange and Hengge-Aronis (1994*b*); this was subsequently narrowed down to a sequence between residues 127 and 247 (Muffler et al. 1996*a*). Surprisingly, the decreased degradation of σ^S in stationary phase is not the result of a decrease in ClpPX levels. The phenomenon is similar to that observed for the σ^H, for which instability or sensitivity to proteolysis is enhanced by the protein being bound to one of several chaperones (Straus et al. 1990; Yura et al. 1993). Stress causes a release of σ^H from the chaperones, thereby increasing its apparent stability and allowing its accumulation. In the case of σ^S, the involvement of chaperones seems to be just the opposite. While individual deficiencies in the chaperones GroEL, DnaJ, GrpE, or CbpA have no effect on σ^S levels (Schweder et al. 1996), a deficiency in DnaK results in reduced levels of σ^S in the stationary phase (Muffler et al. 1997*a*; Rockabrand et al. 1998), and DnaK is now ascribed the role of protecting σ^S from ClpPX.

The response regulator RssB (Muffler et al. 1997*c*), also known as SprE (Pratt and Silhavy 1996) and as MviA in *Salmonella typhimurium* (Bearson et al. 1996), promotes the destabilization of the σ^S protein, by either increasing the rate of ClpPX proteolysis or enhancing σ^S sensitivity, in response to nutrient availability. The effect of RssB on proteolysis involves a direct interaction between σ^S and RssB, and the formation of the complex also serves to reduce the activity of σ^S (Zhou and Gottesman 1998). A possible role for acetyl phosphate, as a metabolic signal modulating RssB activity through phosphorylation, is suggested by the enhanced rate of σ^S proteolysis in its absence (Bouché et al. 1998). The interplay between RssB and DnaK in modulating the sensitivity of σ^S to ClpPX must now be determined, but it would appear that RssB enhances σ^S degradation and DnaK reduces σ^S degradation in response to environmental signals. It is clear that neither protein is the sole mediator because RssB deficiency alters, but does not completely abolish, the growth phase and osmotic regulation of σ^S (Pratt and Silhavy 1996). Similarly, DnaK deficiency enhances the turnover of σ^S, but it does not disappear completely. In Fig. 1, DnaK is shown as preventing ClpPX action on the RssB–RpoS complex, but other options are possible and further work is needed to determine the precise mechanism.

Modulation of holoenzyme formation

Because a sigma factor has to associate with the core RNA polymerase before it can influence promoter selection, there are two further stages at which σ^S activity can be modulated: the formation of the holoenzyme and the interaction of the holoenzyme with promoters. We have already noted that σ^S levels do increase as cells enter stationary phase, but they never exceed one-third of σ^D levels (Jishage and Ishihama 1995). The switch from exponential phase to stationary phase gene expression requires a change of sigma factors in the holoenzyme from σ^D to σ^S, and there should be a mechanism to enhance the ability of σ^S to bind to the core because it is always present in molar amounts lower than σ^D. Trehalose levels increase during the transition to stationary phase and, in combination with glutamate, in response to osmotic shock (Strom and Kaasen 1993). In vitro, association of σ^S with the core and binding of the Eσ^S holoenzyme to promoters, particularly on DNA with a low superhelix concentration (a feature of DNA in stationary phase cells), are both enhanced by trehalose and glutamate, resulting in up to a fivefold increase in σ^S-directed transcription (Kusano and Ishihama 1997). In vivo, the role of trehalose is not as clear because mutants lacking trehalose exhibit normal growth phase and osmotic induction of σ^S-dependent genes, suggesting that trehalose does not influence promoter recognition by Eσ^S (Germer et al. 1998). The combination of increased levels of σ^S, enhanced association of σ^S with the core polymerase, and enhanced promoter recognition by Eσ^S provides a clear mechanism to explain the change in gene expression patterns in stationary phase, but trehalose and glutamate may not be playing a direct role.

Summary of environmental stresses that increase σ^S levels

Amidst this labyrinth of regulatory mechanisms and control factors, it is easy to lose sight of which environmental factors can elicit changes in σ^S levels and activity. Carbon, phosphate, or nitrogen starvation and the corresponding onset of stationary phase were the first stresses identified that enhanced the accumulation of σ^S (Gentry et al. 1993; Lange and Hengge-Aronis 1994*b*; Jishage and Ishihama 1995; Muffler et al. 1997*a*; Zgurskaya et al. 1997). Starvation causes changes in the levels of CRP–cAMP, ppGpp, trehalose, UDP-glucose, and serine lactone, all of which have been correlated with the accumulation of active Eσ^S. Acid shock of *Salmonella typhimurium* (Bearson et al. 1996) induces the synthesis of approximately 50 σ^S-dependent proteins by a mechanism that is RssB (MviA) dependent. This is complemented by the corollary that acid resistance in both *E. coli* and *Shigella flexneri* is dependent on *rpoS* and can be induced by growth into stationary phase, as well as growth on moderately acidic medium (Gorden and Small 1993; Small et al. 1994). Heat shock induction of σ^S accumulation (Jishage and Ishihama 1995; Muffler et al. 1997*a*) and starvation-induced thermotolerance (Rockabrand et al. 1995) share the common involvement of the heat shock protein DnaK. Finally, high osmolarity causes an increase in σ^S levels (Muffler et al. 1996*b*; Pratt and Silhavy 1996) involving mechanisms focused on H-NS and RssB. It is clear that the signal transduction pathways responding to these various stresses overlap extensively and that our understanding of the pathways is far from complete. Clarification of the mechanisms will provide a focus for many more years of work.

Acknowledgements

This work was supported by grants OGP9600 (to P.C.L.) and OGP46599 (to R.S.). The authors would like to express their gratitude to G. Storz for communicating results prior to publication.

References

Altuvia, S., Aliron, M., Huisman, G., Kolter, R., and Storz, G. 1994. The *dps* promoter is activated by OxyR during growth and by IHF and σ^S in stationary phase. Mol. Microbiol. **13**: 265–272.

Altuvia, S., Weinstein-Fisher, D., Zhang, A., Postow, L., and Storz, G. 1997. A small, stable RNA induced by oxidative stress: role as a pleiotropic regulator and antimutator. Cell, **90**: 43–53.

Arnqvist, A., Olsen, A., and Normark, S. 1994. σ^S-dependent growth-phase induction of the *csgBA* promoter in *Escherichia coli* can be achieved in vivo by σ^{70} in the absence of the nucleoid-associated protien H-NS. Mol. Microbiol. **13**: 1021–1032.

Atlung, T., Knudsen, K., Heerfordt, L., and Brøndsted, L. 1997. Effects and σ^S and the transcriptional activator AppY on induction of the *Escherichia coli hya* and *cbdAB–appA* operons in response to carbon and phosphate starvation. J. Bacteriol. **179**: 2141–2146.

Aviv, M., Giladi, H., Schreiber, G., Oppenheim, A.B., and Glasser, G. 1994. Expression of the genes coding for the *Escherichia coli* integration host factor are controlled by growth phase, *rpoS*, ppGpp and by autoregulation. Mol. Microbiol. **14**: 1021–1031.

Barth, M., Marschall, C., Muffler, A., Fischer, D., and Hengge-Aronis, R. 1995. Role for the histone-like protein H-NS in growth phase-dependent and osmotic regulation of σ^S and

many σ^S-dependent genes in *Escherichia coli*. J. Bacteriol. **177**: 3455–3464.

Bearson, S.M.D., Benjamin, W.H., Jr., Swords, W.E., and Foster, J.W. 1996. Acid shock induction of RpoS is mediated by the mouse virulence gene *mviA* of *Salmonella typhimurium*. J. Bacteriol. **178**: 2572–2579.

Becker-Hapak, M., and Eisenstark, A. 1995. Role of *rpoS* in the regulation of glutathione oxidoreductase (gor) in *Escherichia coli*. FEMS Microbiol. Lett. **134**: 39–44.

Becker-Hapak, M., Troxtel, E., Hoerter, J., and Eisenstark, A. 1997. RpoS-dependent overexpression of carotenoids from *Erwinia herbicola* in OxyR deficient *Escherichia coli*. Biochem. Biophys. Res. Commun. **239**: 305–309.

Bohringer, J., Fischer, D., Mosler, G., and Hengge-Aronis, R. 1995. UDP-glucose is a potential intracellular signal molecule in the control of expression of σ^S and σ^S-dependent genes in *Escherichia coli*. J. Bacteriol. **177**: 413–422.

Bouché, S., Klauck, E., Fischer, D., Lucassen, M., Jung, K., and Hengge-Aronis, R. 1998. Regulation of RssB-dependent proteolysis in *Escherichia coli*: a role for acetyl phosphate in a response regulator-controlled process. Mol. Microbiol. **27**: 787–795.

Brøndsted, L., and Atlung, T. 1994. Anaerobic regulation of the hydrogenase 1 (*hya*) operon of *Escherichia coli*. J. Bacteriol. **176**: 5423–5428.

Brøndsted, L., and Atlung, T. 1996. Effect of growth conditions on expression of the acid phosphatase (*cyx-appA*) operon and the *appY* gene, which encodes a transcriptional activator of *Escherichia coli*. J. Bacteriol. **178**: 1556–1564.

Brown, L., and Elliott, T. 1996. Efficient translation of the RpoS sigma factor in *Salmonella typhimurium* requires host factor I, an RNA-binding protein encoded by the *hfq* gene. J. Bacteriol. **178**: 3763–3770.

Calcutt, M.J., Becker-Hapak, M., Gaut, M., Hoerter, J., and Eisenstark, A. 1998. The IrpoSI gene of *Erwinia carotovora*: gene organization and functional expression in *E. coli*. FEMS Microbiol. Lett. **159**: 275–281.

Cam, K., Cusange, A., and Bouche, J.P. 1995. Sigma S dependent overexpression of *ftsZ* in an *Escherichia coli* K-12 *rpoB* mutant that is resistant to the division inhibitors *DicB* and *DicF* RNA. Molec. Gen. Genet. **248**: 190–194.

Chang, Y.Y., Wang, A.Y., and Cronan, J.E. 1994. Expression of *Escherichia coli* pyruvate oxidase (PoxB) depends on the sigma factor encoded by the *rpoS* (*katF*) gene. Mol. Microbiol. **11**: 1019–1028.

Chen, C.Y., Buchmeier, N.A., Libby, S., Fang, F.C., Krause, M., and Guiney, D.G. 1995. Central regulatory role for the RpoS sigma factor in expression of *Salmonella dublin* plasmid virulence genes. J. Bacteriol. **177**: 5303–5309.

Chesbro, W. 1988. The domains of slow bacterial growth. Can. J. Microbiol. **34**: 427–435.

Conter, A., Menchon, C., and Gutierrez, C. 1997. Role of DNA supercoiling and RpoS sigma factor in the osmotic and growth phase-dependent induction of the gene *osmE* of *Escherichia coli* K12. J. Mol. Biol. **273**: 75–83.

Coynault, C., Robbe-Saule, V., and Norel, F. 1996. Virulence and vaccine potential of *Salmonella typhimurium* mutants deficient in the expression of RpoS (σ^S) regulon. Mol. Microbiol. **22**: 149–160.

Diaz-Guerra, L., Moreno, F., and San Millan, J.L. 1989. *appR* gene product activates transcription of microcin C7 plasmid genes. J. Bacteriol. **171**: 2906–2908.

Dove, S.L., Smith, S.G.J., and Dorman, C.J. 1997. Control of *Escherichia coli* type 1 fimbrial gene expression in stationary phase: a negative role for RpoS. Mol. Gen. Genet. **254**: 13–20.

Eisenstark, A., Calcutt, M.J., Becher-Hapak, M., and Ivanova, A. 1996. Role of *Escherichia coli rpoS* and associated genes in defense against oxidative damage. Free Radical Biol. Med. **21**: 975–993.

Espinosa-Urgel, M., and Tormo, A. 1993. σ^S-dependent promoters in *Escherichia coli* are located in DNA regions with intrinsic curvature. Nucleic Acids Res. **21**: 3667–3670.

Espinosa-Urgel, M., Chamizo, C., and Tormo, A. 1996. A consensus structure for σ^S-dependent promoters. Mol. Microbiol. **21**: 657–659.

Gentry, D.R., Hernandez, V.J., Nguyen, L.H., Jensen, D.B., and Cashel, M. 1993. Synthesis of the stationary phase sigma factor σ^S is positively regulated by ppGpp. J. Bacteriol. **175**: 7982–7989.

Germer, J., Muffler, A., and Hengge-Aronis, R. 1998. Trehalose is not relevant for in vivo activity of σ^S-containing RNA polymerase in *Escherichia coli*. J. Bacteriol. **180**: 1603–1606.

Gorden, J., and Small, P.L.C. 1993. Acid resistance in enteric bacteria. Infect. Immun. **61**: 364–367.

Hammar, M., Arnqvist, A., Bian, Z., Olsen, A., and Normark, S. 1995. Expression of two *csg* operons is required for production of fibronectin- and Congo red-binding curli polymers in *Escherichia coli*. Mol. Microbiol. **18**: 661–670.

Heiskanen, P., Taira, S., and Rhen, M. 1994. Role of *rpoS* in the regulation of *Salmonella* plasmid virulence (*spv*) genes. FEMS Microbiol. Lett. **123**: 125–130.

Hengge-Aronis, R. 1993. Survival of hunger and stress: the role of *rpoS* in early staionary phase gene regulation in *E. coli*. Cell, **72**: 165–168.

Hengge-Aronis, R. 1996. Back to log phase: σ^S as a global regulator in the osmotic control of gene expression in *Escherichia coli*. Mol. Microbiol. **21**: 887–893.

Hengge-Aronis, R., and Fischer, D. 1992. Identification and molecular analysis of *glgS*, a novel growth-phase-regulated and *rpoS*-dependent gene involved in glycogen synthesis in *Escherichia coli*. Mol. Microbiol. **6**: 1877–1886.

Hengge-Aronis, R., Klein, W., Lange, R., Rimmele, M., and Boos, W. 1991. Trehalose synthesis genes are controlled by the putative sigma factor encoded by *rpoS* and are involved in stationary-phase thermotolerance in *Escherichia coli*. J. Bacteriol. **173**: 7918–7924.

Hengge-Aronis, R., Lange, R., Henneberg, N., and Fischer, D. 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. J. Bacteriol. **175**: 259–265.

Huisman, G.W., and Kolter, R. 1994. Sensing starvation: a homoserine lactone-dependent signaling pathway in *Escherichia coli*. Science (Washington, D.C.), **265**: 537–539.

Ichikawa, J.K., Li, C., Fu, J., and Clarke, S. 1994. A gene at 59 minutes on the *Escherichia coli* chromosome encodes a lipoprotein with unusual amino acid repeat sequences. J. Bacteriol. **176**: 1630–1638.

Ivanova, A., Miller, C., Glinsky, G., and Eisenstark, A. 1994. Role of *rpoS* (*katF*) in *oxyR*-independent regulation of hydroperoxidase I in *Escherichia coli*. Mol. Microbiol. **12**: 571–578.

Ivanova, A.B., Glinsky, G.V., and Eisenstark, A. 1997. Role of *rpoS* regulon in resistance to oxidative stress and near-UV radiation in Δ*oxyR* suppressor mutants of *Escherichia coli*. Free Radical Biol. Med. **23**: 627–636.

Jishage, M., and Ishihama, A. 1995. Regulation of RNA polymerase sigma subunit synthesis in *Escherichia coli*: intracellular levels of σ^{70} and σ^{38}. J. Bacteriol. **177**: 6832–6835.

Kaasen, I., Falkenberg, P., Styrvold, O.B., and Strøm, A. 1992. Molecular cloning and physical mapping of the *otsBA* genes, which encode the osmoregulatory trehalose pathway of *Esche-*

richia coli: evidence that transcription is activated by KatF (AppR). J. Bacteriol. **174**: 889–898.

Kakeda, M., Ueguchi, C., Yamada, H., and Mizuno, T. 1995. An *Escherichia coli* curved DNA-binding protein whose expression is affected by the stationary phase-specific sigma factor σ^S. Molec. Gen. Genet. **248**: 629–634.

Klauck, E., Bohringer, J., and Hengge-Aronis, R. 1997. The LysR-like regulator LeuO in *Escherichia coli* is involved in the translational regulation of *rpoS* by affecting the expression of the small regulatory DsrA-RNA. Mol. Microbiol. **25**: 559–569.

Koh, Y.S., and Roe, J.H. 1996. Dual regulation of the paraquat-inducible gene *pqi-5* by SoxS and RpoS in *Escherichia coli*. Mol. Microbiol. **22**: 53–61.

Kolb, A., Kotlarz, D., Kusano, S., and Ishihama, A. 1995. Selectivity of the *Escherichia coli* RNA polymerase $E\sigma^{38}$ for overlapping promoters and ability to support CRP activation. Nucleic Acids Res. **23**: 819–826.

Kolter, R., Siegele, D.A., and Tormo, A. 1993. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47**: 855–874.

Kowarz, L.C., Coynault, C., Robbe-Saule, V., and Norel, F. 1994. Role of *rpoS* in the regulation of *Salmonella* plasmid virulence (*spv*) genes. FEMS Microbiol. Lett. **123**: 125–130.

Kusano, S., and Ishihama, A. 1997. Stimulatory effect of trehalose on formation and activity of *Escherichia coli* RNA polymerase $E\sigma^{38}$ holoenzyme. J. Bacteriol. **179**: 3649–3654.

Landini, P., Hajec, L.I., Nguyen, L.H., Burgess, R.R., and Volkert, M.R. 1996. The leucine-responsive regulatory protein (Lrp) acts as a specific repressor for σ^S-dependent transcription of the *Escherichia coli aidB* gene. Mol. Microbiol. **20**: 947–955.

Lange, R., and Hengge-Aronis, R. 1991*a*. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^S. J. Bacteriol. **173**: 4474–4481.

Lange, R., and Hengge-Aronis, R. 1991*b*. Identification of a central regulator of stationary-phase expression in *Escherichia coli*. Mol. Microbiol. **5**: 49–59.

Lange, R., and Hengge-Aronis, R. 1994*a*. The *nlpD* gene is located in an operon with *rpoS* on the *Escherichia coli* chromosome and encodes a novel lipoprotein with a potential function in cell wall formation. Mol. Microbiol. **13**: 733–743.

Lange, R., and Hengge-Aronis, R. 1994*b*. The cellular concentration of the subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation, and protein stability. Genes Dev. **8**: 1600–1612.

Lange, R., Barth, M., and Hengge-Aronis, R. 1993. Complex transcriptional control of the σ^S-dependent stationary-phase-induced and osmotically regulated *osmY* (*csi-5*) suggests novel roles for Lrp, cyclic AMP (cAMP) receptor protein – cAMP complex and integration host factor in the stationary-phase response of *Escherichia coli*. J. Bacteriol. **175**: 7910–7917.

Lange, R., Fischer, D., and Hengge-Aronis, R. 1995. Identification of transcriptional start sites and the role of ppGpp in the expression of *rpoS*, the structural gene for the σ^S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **177**: 4676–4680.

Loewen, P.C., and Hengge-Aronis, R. 1994. The role of the sigma factor σ^S(KatF) in bacterial global regulation. Annu. Rev. Microbiol. **48**: 53–80.

Loewen, P.C., and Triggs, B.L. 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. **160**: 668–675.

Loewen, P.C., von Ossowski, I., Switala, J., and Mulvey, M.R. 1993. KatF (σ^S) synthesis in *Escherichia coli* is subject to posttranscriptional regulation. J. Bacteriol. **175**: 2150–2153.

Lonetto, M.A., and Gross, C.A. 1996. Nomenclature of sigma factors from *Escherichia coli* and *Salmonella typhimurium* and relationships to sigma factors from other organisms. *In* *Escherichia coli* and *Salmonella*: cellular and molecular biology. *Edited by* F.C. Neidhardt. ASM Press, Washington, D.C. p. 821.

Marschall, C., Labrousse, V., Kreimer, M., Weichart, D., Kolb, A., and Hengge-Aronis, R. 1998. Molecular analysis of the regulation of *csiD*, a carbon starvation-inducible gene in *Escherichia coli* that is exclusively dependent on σ^S and requires activation by cAMP–CRP. J. Mol. Biol. **276**: 339–353.

McCann, M.P., Kidwell, J.P., and Matin, A. 1991. The putative σ factor KatF has a central role in development of starvation-mediated general resistance in *Escherichia coli*. J. Bacteriol. **173**: 4188–4194.

McCann, M.P., Fraley, C.D., and Matin, A. 1993. The putative σ factor KatF is regulated posttranscriptionally during carbon starvation. J. Bacteriol. **175**: 2143–2149.

Mellies, J., Wise, A., and Villarejo, M. 1995. Two different *Escherichia coli porP* promoters respond to osmotic and growth phase signals. J. Bacteriol. **177**: 144–151.

Membrillo-Hernandez, J., Cook, G.M., and Poole, R.K. 1997*a*. Roles of RpoS (σ^S), IHF and ppGpp in the expression of the *hmp* gene encoding the flavohemoglobin (Hmp) of *Escherichia coli* K-12. Mol. Gen. Genet. **254**: 599–603.

Membrillo-Hernandez, J., Kim, S.O., Cook, G.M., and Poole, R.K. 1997*b*. Paraquat regulation of *hmp* (flavohemoglobin) gene expression in *Escherichia coli* K-12 is SoxRS independent but modulated by σ^S. J. Bacteriol. **179**: 3164–3170.

Muffler, A., Fischer, D., and Hengge-Aronis, R. 1996*a*. The RNA binding protein HF-1, known as a host factor for phage Qβ RNA replication, is essential for *rpoS* translation in *Escherichia coli* Genes Dev. **10**: 1143–1151.

Muffler, A., Traulsen, D.D., Lange, R., and Hengge-Aronis, R. 1996*b*. Posttranscriptional osmotic regulation of the σ^S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **178**: 1607–1613.

Muffler, A., Barth, M., Marschall, C., and Hengge-Aronis, R. 1997*a*. Heat shock regulation of σ^S turnover: a role for DnaK and relationship between stress responses mediated by σ^S and σ^{32} in *Escherichia coli*. J. Bacteriol. **179**: 445–452.

Muffler, A., Traulsen, D.D., Fischer, D., Lange, R., and Hengge-Aronis, R. 1997*b*. The RNA-binding protein HF-1 plays a global regulatory role which is largely, but not exclusively, due to its role in expression of the σ^S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **179**: 297–300.

Muffler, A., Fischer, D., Altuvia, S., Storz, G., and Hengge-Aronis, R. 1997*c*. The response regulator RssB controls stability of the σ^S subunit of RNA polymerase in *Escherichia coli*. EMBO J. **15**: 1333–1339.

Mukhopadhyay, S., and Schellhorn, H.E. 1994. Induction of *Escherichia coli* hydroperoxidase I by acetate and other weak acids. J. Bacteriol. **176**: 2300–2307.

Mulvey, M.R., and Loewen, P.C. 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ transcription factor. Nucleic Acids Res. **23**: 9979–9991.

Mulvey, M.R., Switala, J., Borys, A., and Loewen, P.C. 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. **172**: 6713–6720.

Nickerson, C.A., and Curtiss, R., III. 1997. Role of sigma factor RpoS in initial stages of *Salmonella typhimurium* infection. Infect. Immun. **65**: 1814–1823.

Nguyen, L.H., and Burgess, R.R. 1997. Comparative analysis of the interactions of *Escherichia coli* σ^S and σ^{70} RNA polymerase

holoenzyme with the stationary-phase sepcific *bol*A*p1* promoter. Biochemistry, **36**: 1748–1754.

Olsen, A., Arnqvist, A., Hammar, M., Sukupolvi, S., and Normark, S. 1993. The RpoS sigma factor relieves H-NS-mediated transcriptional repression of *csgA*, the subunit of fibronectin-binding curli in *Escherichia coli*. Mol. Microbiol. **7**: 523–536.

O'Neal, C.R., Gabriel, W.M., Turk, A.K., Libby, S.J., Fang, F.C., and Spector, M.P. 1994. RpoS is necessary for both the positive and negative regulation of starvation survival genes during phosphate, carbon, and nitrogen starvation in *Salmonella typhimurium*. J. Bacteriol. **176**: 4610–4616.

Pratt, L.A., and Silhavy, T.J. 1996. The response regulator SprE controls the stability of RpoS. Proc. Natl. Acad. Sci. U.S.A. **93**: 2488–2492.

Qi, H., Menzel, R., and Tse-Dinh, Y.C. 1997. Regulation of *Escherichia coli topA* gene transcription: involvement of a σ^S-dependent promoter. J. Mol. Biol. **267**: 481–489.

Raina, S., Missiakas, D., Baird, L., Kumar, S., and Georgopoulos, C. 1993. Identification and transcriptional analysis of the *Escherichia coli htrE* operon which is homologous to *pap* and related pilin operons. J. Bacteriol. **175**: 5009–5021.

Rockabrand, D., Arthur, T., Korinek, G., Livers, K., and Blum, P. 1995. An essential role for *Escherichia coli* DnaK protein in starvation-induced thermotolerance, H_2O_2 resistance, and reductive division. J. Bacteriol. **177**: 3695–3703.

Rockabrand, D., Livers, K., Austin, T., Kaiser, R., Jensen, D., Burgess, R., and Blum, P. 1998. Roles of DnaK and RpoS in starvation-induced thermotolerance of *Escherichia coli*. J. Bacteriol. **180**: 846–854.

Sak, B.D., Eisenstark, A., and Touati, D. 1989. Exonuclease III and the catalase hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* gene product. Proc. Natl. Acad. Sci. U.S.A. **86**: 3271–3275.

Schweder, T., Lee, K.-H., Lomovskaya, O., and Matin, A. 1996. Regulation of *Escherichia coli* starvation sigma factor (σ^S) by ClpXP protease. J. Bacteriol. **178**: 470–476.

Shiba, T., Tsutsumi, K., Yano, H., Ihara, Y., Kameda, A., Tanaka, K., Takahashi, H., Munekata, M., Rao, N.N., and Kornberg, A. 1997. Inorganic polyphosphate and the induction of *rpoS* expression. Proc. Natl. Acad. Sci. U.S.A. **94**: 11 210 – 11 215.

Sledjeski, D., and Gottesman, S. 1995. A small RNA acts as an antisilencer of the H-NS-silenced rcsA gene of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **92**: 2003–2007.

Sledjeski, D.D., Gupta, A., and Gottesman, S. 1996. The small RNA, DsrA, is essential for the low temperature expression of RpoS during exponential growth in *Escherichia coli*. EMBO J. **15**: 3993–4000.

Small, P., Blankenhorn, D., Welty, D., Zinser, E., and Slonczewski, J.L. 1994. Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: role of *rpoS* and growth pH. J. Bacteriol. **176**: 1729–1737.

Straus, D., Walter, W., and Gross, C.A. 1990. DnaK, DnaJ and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of sigma 32. Genes Dev. **4**: 2202–2209.

Strøm, A.R., and Kaasen, I. 1993. Trehalose metabolism in *Escherichia coli*: stress protection and stress regulation of gene expression. Mol. Microbiol. **8**: 205–210.

Swords, W.E., Cannon, B.M., and Benjamin, W.H., Jr. 1997. Avirulence of LT2 strains of *Salmonella typhimurium* results from a defective *rpoS* gene. Infect. Immun. **65**: 2451–2453.

Takayanagi, Y., Tanaka, K., and Takahashi, H. 1994. Structure of the 5′ upstream region and the regulation of the *rpoS* gene of *Escherichia coli*. Mol. Gen. Genet. **243**: 525–531.

Tanaka, K., Takayanagi, Y., Fujita, N., Ishihama, A., and Takahashi, H. 1993. Heterogeneity of the principal σ factor in *Escherichia coli*: the *rpoS* gene product, σ^{38}, is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **90**: 3511–3515.

Tanaka, K., Kusano, S., Fujita, N., Ishihama, A., and Takahashi, H. 1995. Promoter determinants for *Escherichia coli* RNA polymerase holoenzyme containing σ^{38} (the *rpoS* gene product). Nucleic Acids Res. **23**: 827–834.

Tsui, H.C.T., Feng, G., and Winkler, M.E. 1997. Negative regulation of *mutS* and *mutH* repair gene expression by Hfq and RpoS global regulators of *Escherichia coli*. J. Bacteriol. **179**: 7476–7487.

Utsumi, R., Kusafuka, S., Nakayama, T., Tanaka, K., Takayanagi, Y., Takahashi, H., Noda, M., and Kawamukai, M. 1993. Stationary phase-specific expression of the *fic* gene in *Escherichia coli* K-12 is controlled by the *rpoS* gene product (σ^S). FEMS Microbiol. Lett. **113**: 273–278.

Van Dyk, T.K., Ayers, B.L., Morgan, R.W., and LaRossa, R.A. 1998. Constricted flux through the branched-chain amino acid biosynthetic enzyme acetolactate synthase triggers elevated expression of genes regulated by *rpoS* and internal acidification. J. Bacteriol. **180**: 785–792.

Volkert, M.R., Hajec, L.I., Matijasevic, Z., Fang, F.C., and Prince, R. 1994. Induction of the *Escherichia coli aidB* gene under oxygen-limiting conditions requires a functional *rpoS* (*katF*) gene. J. Bacteriol. **176**: 7638–7645.

Wang, A.Y., and Cronan, J.E., Jr. 1994. The growth phase-dependent synthesis of cyclopropane fatty acids in *Escherichia coli* is the result of an RpoS (KatF)-dependent promoter plus enzyme instability. Mol. Microbiol. **11**: 1009–1017.

Weichart, D., Lange, R., Henneberg, N., and Hengge-Aronis, R. 1993. Identification and characterization of stationary phase inducible genes in *Escherichia coli*. Mol. Microbiol. **10**: 407–420.

Wise, A., Brems, R., Ramakrishnan, V., and Villarejo, M. 1996. Sequences in the –35 region of *Escherichia coli rpoS*-dependent genes promote transcription by Eσ^S. J. Bacteriol. **178**: 2785–2793.

Xu, J., and Johnson, R.C. 1995*a*. Identification of genes negatively regulated by Fis: Fis and RpoS co-modulate growth-phase-dependent gene expression in *Escherichia coli*. J. Bacteriol. **177**: 938–947.

Xu, J., and Johnson, R.C. 1995*b*. *aldB*, an RpoS-dependent gene in *Escherichia coli* encoding an aldehyde dehydrogenase that is repressed by Fis and activated by Crp. J. Bacteriol. **177**: 3166–3175.

Xu, J., and Johnson, R.C. 1997. Activation of RpoS-dependent *proP* P2 transcription by the Fis protein in vitro. J. Mol. Biol. **270**: 346–359.

Yamashino, T., Kakeda, M., Ueguchi, C., and Mizuno, T. 1994. An analogue of the DnaJ molecular chaperone whose expression is controlled by σ^S during stationary phase and phosphate starvation in *Escherichia coli*. Mol. Microbiol. **13**: 475–483.

Yamashino, T., Ueguchi, C., and Mizuno, T. 1995. Quantitative control of the stationary phase-specific sigma factor, σ^s, in *Escherichia coli*: involvement of the nucleoid protein H-NS. EMBO J. **14**: 594–602.

Yang, W., Ni, L., and Somerville, R.L. 1993. A stationary-phase protein of *Escherichia coli* that affects the mode of association between the *trp* repressor protein and operator-bearing DNA. Proc. Natl. Acad. Sci. U.S.A. **90**: 5796–5800.

Yim, H.H., Brems, R.L., and Villarejo, M. 1994. Molecular characterization of the promoter of *osmY*, an *rpoS*-dependent gene. J. Bacteriol. **176**: 100–107.

Yura, T., Nagai, H., and Mori, H. 1993. Regulation of the heat-shock response in bacteria. Annu. Rev. Microbiol. **47**: 321–350.

Zgurskaya, H.I., Keyhan, M., and Matin, A. 1997. The σ^S level in starving *Escherichia coli* cells increases solely as a result of its increased stability, despite decreased synthesis. Mol. Microbiol. **24**: 643–651.

Zhang, A., Altuvia, S., Tiwari, A., Hengge-Aronis, R., and Storz, G. 1998. The *oxyS* regulatory RNA represses *rpoS* translation by binding Hfq (HF-I). EMBO J.

Zhou, Y., and Gottesman, S. 1998. Regulation of proteolysis of the staionary-phase sigma factor RpoS. J. Bacteriol. **180**: 1154–1158.