Signal Transduction and Regulatory Mechanisms Involved in Control of the $\sigma^S$ (RpoS) Subunit of RNA Polymerase

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INTRODUCTION ..........................................................374
THE PROBLEM OF MULTIPLE STRESS SIGNAL INTEGRATION ..........................................................374
REGULATION OF RPO S TRANSCRIPTION ..........................................................376
Promoters Contributing to rpoS Transcription ..........................................................376
Trans-Acting Factors Controlling rpoS Transcription ..........................................................376
cAMP-CRP and EIIA(Glc) ..........................................................376
The GacS-GacA two-component and Las-Rhl quorum-sensing systems in pseudomonads ..........................................................377
BarA, a histidine sensor kinase in search of a response regulator ..........................................................377
PsrA in pseudomonads: a TetR-like regulator ..........................................................377
Role for Polyphosphate in rpoS Regulation ..........................................................377
Small Molecules That Influence rpoS Transcription ..........................................................378
pppGpp ..........................................................378
Is rpoS expression controlled by quorum sensing? ..........................................................378
Homoserine lactone and homocysteine thiolactone ..........................................................378
Acetate and other weak acids ..........................................................379
Cellular NADH-to-NAD$^+$ ratio ..........................................................379
REGULATION OF rpoS mRNA Secondary Structure ..........................................................379
Role of rpoS mRNA Secondary Structure ..........................................................379
trans-Acting Factors Involved in rpoS Translation ..........................................................380
The RNA binding protein Hfq (HF-I) ..........................................................380
HU: a nucleoid protein that also stimulates rpoS translation ..........................................................381
H-NS and StpA: histone-like proteins acting as RNA chaperones? ..........................................................381
Role of small regulatory RNAs in rpoS translation: DsrA, OxyS, and RprA ..........................................................382
The LysR-like regulator LeuO: a repressor for dsrA expression ..........................................................383
DnaK and DksA: a link to heat shock and chaperones ..........................................................383
EIIA(Glc): a link to the carbon source and energy supply ..........................................................383
The cold shock domain proteins CspC and CspE ..........................................................383
A Small Molecule That Influences rpoS Translation: UDP-Glucose ..........................................................384
rpoS Translational Control Network and Stress Signal Input ..........................................................384
REGULATION OF $\sigma^S$ PROTEOLYSIS ..........................................................384
$\sigma^S$ Degradation by the Complex ATP-Dependent ClpXP Protease ..........................................................384
The Response Regulator RssB: a $\sigma^S$ Recognition Factor with Phosphorylation-Modulated Affinity ..........................................................385
The Turnover Element: the RssB Binding Site Within $\sigma^S$ ..........................................................385
Initiation of $\sigma^S$ Proteolysis: the RssB cycle ..........................................................386
Signal Integration in the Control of $\sigma^S$ Proteolysis ..........................................................387
Additional Factors with Uncharacterized Molecular Functions in $\sigma^S$ turnover ..........................................................388
RssA ..........................................................388
The histone-like protein H-NS ..........................................................388
The LysR homolog LrhA ..........................................................388
The DnaK chaperone ..........................................................388
A Small Molecule That Affects $\sigma^S$ Proteolysis: Acetyl Phosphate ..........................................................388
REGULATION OF $\sigma^S$ ACTIVITY ..........................................................389
In Vivo Evidence for Regulation of $\sigma^S$ Activity ..........................................................389
The Response Regulator RssB Can Act Like an Anti-Sigma Factor for $\sigma^S$ ..........................................................389
CONCLUSIONS AND PERSPECTIVES ..........................................................390
ACKNOWLEDGMENTS ..........................................................390
REFERENCES ..........................................................390

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373
INTRODUCTION

σ^S, or RpoS, is a sigma subunit of RNA polymerase in *Escherichia coli* that is induced and can partially replace the vegetative sigma factor σ^70 (RpoD) under many stress conditions. As a consequence, transcription of numerous σ^S-dependent genes is activated (for reviews, see references 75, 77, 79, 124, and 125). Consistent with the multiple functions of the σ^S regulon, the rpoS gene was discovered independently and named accordingly by several groups (recently summarized in references 79 and 105). It was identified as a gene involved in near-UV resistance (nuv) (216); as a regulator for the katE-encoded catalase HPII (katF) (126, 186), exonuclease III (xthA) [186], and acidic phosphatase (appR) (211); and, finally, as a starvation-inducible gene encoding a central regulator for stationary phase-inducible genes (csi-2) (113). Only then was it recognized that all the previous studies had described alleles of the same gene (113, 212), which codes for a sigma factor (152, 156, 209). Because of its crucial role in stationary phase or under stress conditions, the name σ^S or RpoS was proposed (113). In addition, the term σ^S is used sometimes (although the molecular mass of σ^S deviates from 38 kDa in various species and even in some *E. coli* strains). The rpoS gene has also been identified in other enteric and related bacteria. At present, it seems that σ^S occurs in the γ branch of the proteobacteria, i.e., in a group of gram-negative bacteria that includes many species with special importance for humans because of their pathogenic or beneficial potential. With minor variations, the general function of σ^S in these bacteria appears to be similar to that in *E. coli* (summarized in reference 79).

In more recent studies, it was demonstrated that σ^S and σ^S-dependent genes not only are induced in stationary phase but actually respond to many different stress conditions (76, 82, 121, 144, 148). Therefore, σ^S is now seen as the master regulator of the general stress response, which is triggered by many different stress signals, is often (though not always) accompanied by a reduction or cessation of growth, and provides the cells with the ability to survive the actual stress as well as additional stresses not yet encountered (cross-protection). This is in pronounced contrast to specific stress responses, which are triggered by a single stress signal and result in the induction of proteins that allow cells to cope with this specific stress situation only. While specific stress responses tend to eliminate the stress agent and/or to mediate repair of cellular damage that has already occurred, the general stress response renders cells broadly stress resistant in such a way that damage is avoided rather than needing to be repaired (for a recent review of different bacterial stress responses, see reference 203).

The major function of the general stress response is thus preventive, which is clearly reflected in the σ^S-dependent multiple stress resistance observed with starved or otherwise stressed cells (82, 113, 136) (for a recent review of the general stress response that also includes physiological aspects, see reference 79). Accordingly, the majority of the more than 70 σ^S-dependent genes known so far confer resistance against oxidative stress, near-UV irradiation, potentially lethal heat shocks, hyperosmolality, acidic pH, ethanol, and probably other stresses yet to be identified. Additional σ^S-controlled gene products generate changes in the cell envelope and overall morphology (stressed *E. coli* cells tend to become smaller and ovoid). Metabolism is also affected by σ^S-controlled genes, consistent with σ^S being important under conditions where cells switch from a metabolism directed toward maximal growth to a maintenance metabolism. σ^S also controls genes mediating programmed cell death in stationary phase, which may increase the chances for survival for a bacterial population under extreme stress by sacrificing a fraction of the population in order to provide nutrients for the remaining surviving cells (22). Finally, a number of virulence genes in pathogenic enteric bacteria have been found to be under σ^S control, consistent with the notion that host organisms provide stressful environments for invading pathogens (recently summarized in reference 80). However, even though numerous σ^S-dependent genes have been identified (see references 77, 79, and 125 for recent compilations), many more such genes will probably be found in the future. Moreover, the functions of the genes known so far are incompletely understood. Even after more than 10 years of intensive research on σ^S, much remains to be learned about the physiology of the σ^S-mediated response. The same is true for the regulatory interdependencies within the large regulatory network directed by σ^S. By contrast, the basic mechanisms of regulation of σ^S itself are now reasonably well understood and are the subject of this review (for recent minireviews on the same subject, see references 81 and 125).

Since by far most of the relevant work has been done with *E. coli* and *Salmonella*, the systems referred to in this review are those described for these enteric bacteria if not otherwise mentioned. Whereas the physiological function of σ^S is comparable in all species where it has been discovered to date, there are significant differences between enteric bacteria and pseudomonads in the regulation of σ^S that are outlined specifically.

THE PROBLEM OF MULTIPLE STRESS SIGNAL INTEGRATION

Complex and physiologically far-reaching bacterial responses often use a single master regulator at the interface of upstream signal processing and downstream regulatory mechanisms. In the general stress response of *E. coli*, σ^S plays the role of this top-level master regulator. Other examples of such central regulators are σ^H in the general stress response of various gram-positive bacteria (172) and the response regulator Spo0A in sporulation initiation of *Bacillus subtilis* (202). The master regulators serve as the decisive information processing units, which connect complex signaling networks with downstream regulatory cascades or networks that ultimately control the expression of numerous structural genes associated with a response. These regulatory networks exhibit a hierarchical and modular structure; i.e., they can be subdivided into lower-level smaller modules that are under the control of secondary regulators, which also allow specific signal input at such lower and more confined levels. A master regulator may also commit the cell to a certain complex developmental program, with specific temporal and spatial control being exerted by secondary regulators.

Depending on the type of master regulator (sigma factor, two-component response regulator, etc.) as well as on whether its cellular level or its activity (or both) is the decisive param-
FIG. 1. Various levels of $\sigma^S$ regulation are differentially affected by various stress conditions. An increase of the cellular $\sigma^S$ level can be obtained either by stimulating $\sigma^S$ synthesis at the levels of $rpoS$ transcription or $rpoS$ mRNA translation or by inhibiting $\sigma^S$ proteolysis (which under nonstress conditions is extraordinarily rapid). The most rapid and strongest reaction can be achieved by a combination of these processes (as observed, e.g., on hyperosmotic or pH shifts). For further details, see the text.
Two transcripts can be detected by Northern analysis (8). Poly-cistronic \( nlpD-rpoS \) mRNA originates from two closely spaced promoters \((nlpDp1)\) and \((nlpDp2)\) upstream of the \( nlpD \) gene (112, 115), which encodes a lipoprotein of unknown function (87, 115). Another promoter \((rpoSp)\) is located within the \( nlpD \) gene and produces a monocistronic \( rpoS \) mRNA with an unusually long nontranslated 5’ region of 567 nucleotides (112, 208). Studies with transcriptional fusions that included a 5’ deletion analysis indicated that this transcript is the major \( rpoS \) mRNA (112). Moreover, \( rpoSp \) accounts for activation of transcription in Luria broth-grown cells during entry into stationary phase (112, 208). The \( NlpD \) protein is not stationary phase induced, which indicates that the \( nlpD \) promoters are not growth phase regulated (115).

In other enteric bacteria, \textit{Vibrionaceae} members, and pseudomonads studied so far, the \( nlpD \) and \( rpoS \) genes are always linked, which suggests similar transcriptional regulation to that in \textit{E. coli}. Downstream of \( rpoS \), however, variations are quite common and even occur between different \textit{E. coli} strains (28, 37, 83). However, \( nlpD \) can occur alone in bacteria that do not possess an \( rpoS \) gene, such as \textit{Haemophilus influenzae} (55).

Trans-Acting Factors Controlling \( rpoS \) Transcription

cAMP-CRP and \textit{EIIA(Glc)}. In strains carrying mutations in \textit{cya} (encoding adenylate cyclase) or \textit{crp} (encoding the cyclic AMP [cAMP] receptor protein [CRP]), also called the catabolite activator protein), \( \sigma^8 \) levels and the activities of transcriptional \( pS:\textit{lacZ} \) fusions are already high in exponential phase, indicating that cAMP-CRP is a negative regulator of \( rpoS \) transcription. In the \textit{cya} mutant, this phenotype can be reversed by the addition of external cAMP (113, 114). However, the mode of action of cAMP-CRP in \( rpoS \) transcriptional control seems to depend on the growth phase. Recent evidence indicates that during entry into stationary phase, cAMP-CRP positively controls \( rpoS \) transcription (F. Scheller and R. Hengge-Aronis, unpublished results). This may resolve an apparent contradiction between the above-mentioned observation of high log-phase levels of \( \sigma^8 \) in \textit{cya} or \textit{crp} mutants (114) and the finding that certain \( pS:\textit{lacZ} \) fusions show reduced expression in a \textit{cya} background (135).

Two putative cAMP-CRP binding sites are present upstream and downstream of \( rpoSp \) (Fig. 2), and the role of these potential binding sites is currently under investigation. Whereas the location of the upstream cAMP-CRP box is similar to that in the \textit{lac} promoter and corresponds to a classical activator position at a class I promoter (31), the location of the second cAMP-CRP box downstream of the transcriptional start site may suggest an inhibitory action. In addition, cAMP-CRP may also have an indirect effect on \( rpoS \) expression, since \( cya \) or \( crp \) mutants exhibit a reduced growth rate, which in turn can affect \( rpoS \) transcription, as mentioned above.

Adenylate cyclase activity is positively modulated by the \textit{crr}-encoded \textit{EIIA(Glc)}, which is the soluble part of the glucose-specific EII component of the phosphotransferase system for solute uptake. Consistent with this, a \textit{crr} mutation results in elevated \( \sigma^8 \) levels during exponential phase, which reflects increased \( rpoS \) transcription as well as increased \( rpoS \) translation. The former can be suppressed by cAMP addition, indicating that \textit{EIIA(Glc)} affects \( rpoS \) transcription through its...
modulation of adenylate cyclase activity (217). Phenotypically, the cya mutant thus seems to mimic the log-phase behavior of the cya mutant.

Polyamines stimulate adenylate cyclase expression at the level of translational initiation, and at the same time they lead to 2.3- and 4-fold increases of the cellular levels of σ^70 and σ^54, respectively (235). This polyamine-induced upregulation of σ^54 may mimic the positive effect of cAMP-CRP on rpoS transcription during entry into stationary phase.

The GacS-GacA two-component and Las-Rhl quorum-sensing systems in pseudomonads. GacA is a two-component response regulator in various Pseudomonas species that has long been known to positively affect the production of secondary products such as antibiotics, toxins, and lytic exoenzymes during entry into stationary phase (117, 123, 178). The cognate histidine sensor kinase is the GacS (LemA) protein (85, 178). What is actually sensed by GacS has not been clarified. Homologs of GacA are present in Salmonella (SirA, which controls certain virulence genes [93]) and E. coli (YecB or UvrY [see below]). The GacS-GacA two-component system is at the top of a regulatory cascade that controls the LasI-LasR quorum-sensing system, which in turn regulates a second quorum-sensing system, RhlII-RhlR (110, 167, 176). These quorum-sensing systems are crucial for the control of virulence factors, exoenzymes, and stress-protective proteins as well as for the formation of biofilms (62, 74, 111, 159, 232); for summaries of quorum-sensing systems, see references 61 and 231).

Mutations in gacA or gacS also result in a more than 80% reduction of σ^70 levels and equally reduced expression of a transcriptional rpoS::lacZ fusion specifically during transition into stationary phase (227). Whether this regulation of rpoS by the Gac system is direct or indirect has not been demonstrated. There are also conflicting data about how rpoS is linked to the Las-Rhl cascade. An earlier study had indicated that rpoS is under positive control of both the Las and Rhl systems (110). More recently, however, rpoS was found not to be affected by rhl mutations; in contrast, rhlII is upregulated in rpoS mutants, indicating that σ^70 is a negative regulator of the Rhl system (228). This fits with the observation that pyocyanin and pyoverdin are overproduced in rpoS mutants (205), since these virulence-associated factors are under positive control of the Rhl system (27).

In contrast to the situation in Pseudomonas, the GacA homolog YecB (UvrY) in E. coli appears not to be involved in the control of rpoS expression. Even though YecB overproduction stimulated the expression of transcriptional rpoS::lacZ fusions and resulted in a twofold-higher level of σ^54 specifically during transition into stationary phase, a yeclc::cat knockout mutation did not alter the expression of these rpoS::lacZ fusion, nor were σ^70 levels affected (Kampmann and Hengge-Aronis, unpublished). While σ^54 plays similar physiological roles in E. coli and Pseudomonas (188, 205), differential control by YecB-GacA may reflect the different environmental conditions characteristic of the natural habitats of these bacteria.

BarA, a histidine sensor kinase in search of a response regulator. The E. coli homolog of GacS is a hybrid sensor kinase called BarA (40% identity and 59% overall similarity at the amino acid level to P. aeruginosa GacS). BarA was previously found as a multicycop suppressor of an envZ mutation; i.e., when present at high levels, BarA is able to cross-phosphorylate the response regulator OmpR and thereby activate porin synthesis (88, 154). Under the name AirS, BarA has also been identified as a virulence factor in uropathogenic E. coli (241). There is evidence that BarA plays a positive role in rpoS expression. A strain with a lacZ insertion in the chromosomal copy of barA, which was originally isolated as a hydrogen peroxide-sensitive mutant, exhibits reduced levels of σ^70 (150, 151). In the mutant, rpoS mRNA levels were reduced during exponential phase but were normal in stationary phase. Therefore, BarA was suggested as a positive regulator of rpoS transcription (150). By specifically affecting rpoS mRNA levels in exponential phase, this control may determine the range within which σ^54 levels can be modulated by posttranscriptional control mechanisms in response to various stress conditions.

The homology to the GacS-GacA system, as well as recent biochemical data (166), suggests that BarA is a cognate sensor kinase for YecB. Therefore, it seems surprising that BarA but not YecB (see above) is involved in σ^54 control. However, it is possible that BarA acts on more than one response regulator with an unknown target response regulator being involved in rpoS control. BarA (GacS) belongs to the complex “built-in phosphorelay” sensor kinases in which sensor, transmitter, receiver, and histidine-containing phosphotransfer domains are combined in a single polypeptide chain. In view of their multiple interactions, phosphorelay components seem especially adequate for establishing such phosphotransfer networks.

PsrA in pseudomonads: a TetR-like regulator. A search for insertion mutations that downregulated the expression of a transcriptional rpoS::lacZ fusion in P. putida yielded a mutant defective in a gene termed psrA (for “Pseudomonas sigma regulator”), which encodes a TetR repressor-like regulatory protein. psrA is required for increased rpoS transcription during entry into stationary phase and is negatively autoregulated (104). Whether this control of rpoS is direct or indirect is currently unknown.

Role for Polyphosphate in rpoS Regulation

Inorganic polyphosphate occurs in most microorganisms and often accumulates in stationary phase or under other stress conditions (106, 107). In E. coli, the actual polyphosphate level is the result of a balance between synthesis (catalyzed by polyphosphate kinase, encoded by ppk) and degradation (catalyzed by exopolyphosphatases, encoded by ppx and gppA) (106). Polyphosphate accumulation is positively affected by the “alarmone” guanosine 3’:5’-bispyrophosphate (ppGpp; see below), which seems to inhibit the ppx-encoded exopolyphosphatase (108). Polyphosphate stimulates Lon-mediated degradation of ribosomal proteins; i.e., it may be crucial for gaining access to intracellular amino acid pools under conditions of sudden carbon or amino acid starvation (109).

Polyphosphate-free ppx mutants are multiple stress sensitive and impaired in stationary phase survival (36, 175). Consistent with these phenotypes, σ^54 levels as well as the expression of a transcriptional rpoS::lacZ fusion are reduced in a strain that overproduces yeast exopolyphosphatase and is therefore depleted of polyphosphate (195). These findings indicate that polyphosphate somehow stimulates rpoS transcription and thereby contributes to stationary-phase induction of rpoS.
(which in rich media is partly due to increased rpoS transcription). However, polyphosphate fails to stimulate rpoS transcription in vitro and therefore may exert an indirect influence in vivo (195).

Small Molecules That Influence rpoS Transcription

ppGpp. ppGpp levels in E. coli strongly increase in response to amino acid limitation (triggering the stringent response) or starvation for carbon, nitrogen, and phosphorus sources. Amino acid limitation causes a rise in the cellular level of uncharged tRNA, which is sensed by the ribosome-associated RelA protein (ppGpp synthase I). Under other starvation conditions, ppGpp synthesis is mediated by SpoT (ppGpp synthase II). SpoT is also the degrading enzyme. Only relA spoT double mutants are completely devoid of ppGpp (32).

Such ppGpp-free mutants contain strongly reduced $\sigma^5$ levels. Glucose and phosphate starvation, but not amino acid limitation, still induce $\sigma^5$ in these mutants (albeit to lower levels than in the wild type). On the other hand, $\sigma^5$ accumulation can be triggered by artificially stimulating ppGpp accumulation (64).

ppGpp affects rpoS transcription, as demonstrated with transcriptional rpoS::lacZ fusions (112). However, ppGpp does not seem to specifically target the promoters involved in rpoS transcription, since a transcriptional rpoS::lacZ fusion construct, in which these natural promoters were deleted (and basal expression was due to vector-dependent transcriptional readthrough activity), exhibited similarly reduced expression as the promoter-carrying construct in a ppGpp-free genetic background. It was therefore proposed that in the case of rpoS, ppGpp may affect transcriptional elongation or transcript stability rather than transcriptional initiation (112). In the absence of ppGpp, starvation may result in an uncoupling of transcription and translation, which may lead to increased premature termination, as demonstrated for lacZ mRNA (52, 219, 220).

It is also unclear whether this ppGpp effect is direct or indirect. An increase in the cellular ppGpp content results in the accumulation of polyphosphate, which also stimulates rpoS transcription by an unknown mechanism (see above). It is therefore conceivable that ppGpp acts indirectly via polyphosphate. The finding that a polyphosphate-depleted strain is not impaired in ppGpp accumulation but contains strongly reduced $\sigma^5$ levels is consistent with such an indirect mode of action (195). Clearly more research is required to elucidate these relationships at the molecular level.

Is rpoS expression controlled by quorum sensing? Sometimes high cell density in a bacterial population turns out to be the inducing signal for “stationary-phase-inducible” genes. The classical “quorum-sensing” system is the lux system in Vibrio fischeri, where a membrane-permeable acylated homoserine lactone (acylated HSL, the “autoinducer”) is produced by LuxI and accumulates in the medium. Beyond a certain threshold concentration, the autoinducer binds to and activates LuxR, which stimulates the expression of the luxI and luxR genes themselves and the luciferase structural genes (60, 61, 187). Numerous bacterial species contain homologs of the LuxI-LuxR pair, which control a wide variety of output functions (recently summarized in reference 231). Other types of quorum-sensing systems use different kinds of inducing molecules; e.g., gram-positive species use small peptides in general. A hallmark of all these systems is their inducibility on addition of conditioned medium, i.e., spent supernatant obtained from a culture grown to relatively high cell density, which contains the inducing molecule in sufficient concentration.

With respect to rpoS induction by conditioned medium, conflicting data have been reported. Such induction (approximately fourfold) was observed for a transcriptional rpoS::lacZ fusion, and acetate was proposed as the inducing agent (190). With a different rpoS::lacZ operon fusion present in multiple copies, fourfold induction was also found with conditioned Luria broth medium (153), but when the same fusion was present in single copy in the chromosome, induction by spent medium was reduced to a mere 1.6-fold (197). In another study, a transcriptional rpoS::lacZ fusion was found to be completely unaffected by conditioned medium (63). With a set of single-copy transcriptional and translational rpoS::lacZ fusions (114), very little if any induction was obtained, no matter whether conditioned rich or minimal medium was used, and even spent medium freshly prepared in parallel with the induction experiments (to compensate for potential instability of a putative inducer) had little effect on rpoS expression levels (D. Traulsen and R. Hengge-Aronis, unpublished results). It therefore seems that quorum sensing mediated by some excreted medium component does not play a significant role in the regulation of rpoS in E. coli. Therefore, transcriptional induction of rpoS beyond a certain cell density (114) may also be connected to some metabolic alterations rather than to quorum sensing mediated by an excreted substance (see below). The finding that rpoS itself is probably not or is only weakly controlled by quorum sensing does not preclude certain $\sigma^5$-transcribed genes from being subject to such regulation, which may affect the promoters of these genes directly (11, 197, 206).

The E. coli genome sequence (23) does not reveal obvious homologs of genes encoding known acyl-HSL synthases of the LuxI and AiiN families (17, 61, 66). There is, however, a LuxR-related protein, SdiA, which may respond to an unidentified acyl-HSL (63, 197). Expression of SdiA itself, as well as the activity of a known target promoter, $\text{ftsQ}^\text{P2}$, responds negatively to conditioned (E. coli) medium, which may mean that the potential of E. coli to respond to an acyl-HSL via SdiA (and thereby activate cell division genes) is downregulated in stationary phase$rpoS$, however, does not seem to be under the control of SdiA (63).

Homoserine lactone and homocysteine thiolactone. Nonacylated HSL has been implicated in rpoS control in E. coli. It was reported that a thrA metL hscC mutant, which is deficient early in the branched pathway that leads to biosynthesis of lysine, methionine, threonine, and isoleucine, had reduced $\sigma^5$ levels, which apparently could be suppressed by exogenously adding HSL (at concentrations up to 1 mM). This suppression was weaker in the presence of multiple copies of RspA. Such overexpression also reduced stationary-phase expression of an rpoS::lacZ fusion (which apparently was a transcriptional fusion). Therefore, it was hypothesized that HSL is an inducer for rpoS expression and that RspA may be involved in the degradation of HSL (86). At the time this work was published, this seemed to be in line with quorum-sensing studies that demonstrated the role of acylated HSLs in gene regulation. However, it is now known that free HSL is not the precursor.
for acylated HSLs that serve as autoinducers in LuxI-LuxR-like systems (72, 165) but, rather, plays the role of an intracellular metabolite. Moreover, there is good evidence against quorum sensing affecting rpoS regulation (see above).

More recently it was found that HSL (up to 1 mM) added to wild-type E. coli did not induce rpoS (197), and the RspA overproduction effect on rpoS is now considered nonphysiological (67). Nevertheless, the idea that RspA may degrade HSL is consistent with the recent observation that RspA-overproducing strains indeed seem to have increased homoserine levels (U. Sauer, personal communication). Unexpectedly, these strains actually show elevated levels, which would not seem consistent with HSL being an inducer for rpoS (226).

Another recent study (67) implicates specifically the methionine biosynthesis pathway in σ^5 control. A metE mutant, which is deficient for conversion of homocysteine to methionine and therefore accumulates homocysteine thiolactone (HCTL), exhibits increased σ^5 levels. Moreover, an asd mutant, which is deficient earlier in methionine biosynthesis (and therefore also in homocysteine and HCTL formation), shows decreased σ^5 levels, and this phenotype could be suppressed by exogenous HCTL (1 mM) (67). During entry into stationary phase in minimal glucose medium, a 2.5-fold accumulation of HCTL was observed in wild-type cells (67). Under these conditions, however, there is little if any activation of rpoS transcription, but σ^5 accumulation is due to posttranscriptional control (114; also see below). Unfortunately, HCTL effects were demonstrated by assaying for σ^5 protein only, and so the level of control affected remains open to speculation (67).

In summary, these studies (67, 86) have demonstrated that amino acid biosynthetic pathways, in particular the branch that leads to methionine (with HCTL as the putative effector), can influence the expression of rpoS. It has not been clarified, however, whether this effect is at the level of rpoS transcription, and the underlying molecular mechanism remains unknown.

**Acetate and other weak acids.** In an early study that reported the induction of a transcriptional rpoS::lacZ fusion in spent culture supernatant, the fermentation product acetate (used at 40 mM) was found to activate rpoS expression (190). In another study, however, acetate did not have an effect on rpoS expression (153). The studies agreed that benzoate (10 or 25 mM) has an inducing effect, and it was concluded that this may be so in general for weak acids with a pKa of 4.8 to 4.9 (40 mM propionate was also found to be effective) (153, 190). However, a translational rpoS::lacZ fusion did not show this induction by weak acids (190). One has to take into account, however, that these studies were performed before the advent of limitless precise PCR cloning, and therefore reporter gene fusions had to be constructed in rather complicated ways and often remained incompletely characterized. To finally settle the issue of weak acids in rpoS control, these experiments would have to be repeated and expanded with the precisely characterized systems available today.

Recent genome-wide analyses have shown that addition of acetate to buffered medium results in the activation of various rpoS-dependent genes and proteins, but unfortunately the cellular concentration and regulation of σ^5 itself were not studied under these conditions (7, 100). Similar conditions resulted in increased synthesis of σ^5 in Salmonella, but the underlying control mechanisms seemed at least in part posttranscriptional (39).

**Cellular NADH-to-NAD^+ ratio.** Experiments with a transcriptional rpoS::luxAB fusion in a nuoG mutant background (which is defective in a subunit of NADH dehydrogenase) suggested that a high NADH-to-NAD^+ ratio somehow down-regulates rpoS transcription. Consistent with this proposal, rpoS transcription is low under oxygen-limited (microaerobic) growth conditions, where NADH levels should increase due to the scarcity of oxygen as an electron acceptor for respiration (194). The mechanistic basis of this effect is unclear.

**REGULATION OF rpoS TRANSLATION**

Initial evidence for posttranscriptional regulation of rpoS was provided by clearly different patterns of expression of transcriptional and translational rpoS::lacZ fusions (114, 127, 135). Translational rpoS::lacZ fusions can actually reflect regulation of rpoS translation as well as of σ^5 proteolysis (114, 148, 192). The latter can be excluded by using translational fusions that contain fewer than 173 N-terminal codons of rpoS, since an essential proteolytic recognition element is located at and around K173 in σ^5 (20; also see below).

Translation of rpoS mRNA is stimulated by a shift to hyperosmolarity (114, 148), by low temperature (199), by a shift to acidic pH (pH 5; Kampmann and Hengge-Aronis, unpublished), or during late exponential phase when a growing culture reaches a certain cell density (114). After the onset of carbon starvation, i.e., on entry into stationary phase, rpoS translation is reduced again, and further increases in σ^5 levels are then due to inhibition of σ^5 degradation, as described below (114).

**Role of rpoS mRNA Secondary Structure**

There are two species of rpoS mRNA of clearly different lengths (the locations of relevant promoters are given in Fig. 2). Polycistronic nlpD-rpoS mRNA can have two different 5′ ends since there are two closely spaced promoters upstream of nlpD (115). Monocistronic rpoS mRNA originates from rpoSp within the nlpD gene and contains an unusually long nontranslated 5′ region of 567 nucleotides (112, 208). This leader sequence is functionally important, since 5′ deletions in it reduce rpoS expression (38).

Even under conditions where σ^5 protein is hardly detectable, cells produce fair amounts of rpoS mRNA, which seems to remain constant under the translation-inducing conditions mentioned above (8, 147). It is generally believed that control of the rate of translation of already existing complete rpoS mRNA is based on an mRNA secondary structure in which the translational initiation region (TIR) is based paired and therefore not sufficiently accessible to ribosomes (under noninducing conditions). Certain stress signals are hypothesized to trigger changes in this mRNA secondary structure that allow more frequent translational initiation. However, the actual appearance of this rpoS mRNA structure is still largely a matter of speculation.

Theoretical predictions generated with the MFOLD computer program (using complete or partial rpoS mRNA se-
quences) indicate that approximately 340 nucleotides at the 5′ end of *rpoS* mRNA fold into an very stable and complex cruciform-type structure (Traulsen and Hengge-Aronis, unpublished). Further downstream, the putative structures are somewhat less stable and the TIR has the potential to fold into two energetically almost equivalent principal structures. One is characterized by a large hairpin that includes the Shine-Dalgarno sequence. There is genetic evidence against this structure playing a role in *rpoS* translational control (S. Bouché and R. Hengge-Aronis, unpublished results). In the second putative structure, the region around the Shine-Dalgarno sequence is partially base paired to an “internal antisense” region located further upstream, with a relatively long and probably internally structured intervening sequence. There are, however, several theoretical possibilities for the exact location of the “internal upstream antisense” region (Traulsen and Hengge-Aronis, unpublished). Several variations of this second theoretical structure have been published (30, 38, 120, 131), and it is generally believed that this structure may come close to the in vivo reality under nondriving conditions. The only preliminary experimental evidence that such an “internal upstream antisense” structure is in principle correct is provided by two different complementary double point mutations, which showed wild-type expression levels (although one double mutant altered the regulatory pattern, in particular Hfq dependency [see below]) (30, 38). However, the exact details of the in vivo *rpoS* mRNA secondary structure still await experimental clarification.

In the fact, the problem of the correct in vivo *rpoS* mRNA secondary structure is more complex than, e.g., in the related case of *rpoH* mRNA, which encodes the heat shock sigma factor σ^32-*rpoH* mRNA folds into a translationally incompetent secondary structure also involving an internal antisense element, which opens up upon heat shock, resulting in a directly temperature-triggered translational induction of *rpoH* (summarized in reference 236). This process does not involve any regulatory proteins (143), and the experimentally demonstrated *rpoH* mRNA secondary structure is the one theoretically predicted (142). In *rpoS* mRNA, however, several proteins and small regulatory RNAs are positively or negatively involved in translational control, and at least some of these can directly bind to *rpoS* mRNA in vitro (see below). Therefore, theoretical calculations or in vitro structural probing based on *rpoS* mRNA alone is likely to yield incorrect or at least incomplete results. It seems that the only way of settling the issue of the correct *rpoS* mRNA secondary structure and its dynamics may be in vivo structural probing. Wild-type strains under different conditions as well as various mutants with cis- or trans-regulatory defects in *rpoS* translation will have to be tested in such experiments. However, in view of the technical difficulties of such an endeavor, especially with a large mRNA with complex and semistable secondary structure, it is not surprising that such data have yet to be reported for *rpoS* mRNA.

**trans-Acting Factors Involved in *rpoS* Translation**

The RNA binding protein Hfq (HF-I). More than 30 years ago, the Hfq protein was identified as a host factor (host factor I [HF-II]) essential for replication of phage Qβ RNA (56, 57). Hfq acts as an accessory component of Qβ replicase that binds to several sites in Qβ RNA including the 3′ end (14, 139, 193). Hfq is required for initiating replication specifically of the Qβ RNA plus strand, probably by affecting the secondary structure at its 3′ end (191). The role of the ribosome-associated (45) Hfq protein in *E. coli* physiology, however, remained enigmatic until an *hfq* mutant was observed to have a very pleiotropic phenotype (214), which resembles the phenotype of an *rpoS* mutant (147). This led to the discovery that Hfq is required for efficient *rpoS* translation (29, 146). While this can explain the pleiotropy of *hfq* mutants, Hfq also has physiological functions that are independent of σ^5^ (147). In particular, it stimulates the degradation of *ompA*, *miaA*, *mutS*, and its own mRNA (215, 221, 222). Hfq is a 11.2-kDa oligomer-forming protein (57). While this review was under revision, Hfq was reported to form hexameric rings homologous to eukaryotic Sm and Lsm proteins, which occur in the spliceosome and play various roles in mRNA processing (140, 240).

The molecular function of Hfq in *rpoS* translation is still relatively speculative. Epistasis experiments, where *hfq* mutations were combined to other mutations or overproduction constructs that affect *rpoS* translation indicated that Hfq is probably directly involved in translation initiation; i.e., it acts close to or at the level of *rpoS* mRNA (130, 146, 200, 217, 238). *rpoS* mRNA immunoprecipitates with Hfq in cellular extracts (238). Hfq also binds with high affinity to several sites in a large 5′ fragment of *rpoS* mRNA synthesized in vitro, which is predicted to fold into the same secondary structure as the wild-type mRNA (Traulsen and Hengge-Aronis, unpublished). A 5′ deletion analysis of *rpoS* mRNA indicated that regions relatively far upstream of the TIR are important for translational stimulation by Hfq (38). Thus, Hfq binds *rpoS* mRNA, just as it is able to bind Qβ RNA. However, Hfq does not show similarity to RNA helicases. This makes an active processive unfolding activity unlikely. Alternatively, by binding to a few crucial positions of *rpoS* mRNA, Hfq may affect the equilibrium between possible alternative secondary structures that are differentially productive for translational initiation. Thus, Hfq may stabilize a semistable *rpoS* mRNA secondary structure, which can easily open up when some additional stimulating factor is induced or activated (e.g., HU protein or DsrA-RNA, [Fig. 3; see below]). Yet another possibility is that Hfq does not necessarily affect *rpoS* mRNA secondary structure (although this would not be excluded) but acts like a “platform” bound to *rpoS* mRNA that recruits additional factors involved in *rpoS* translational control. The finding that single potentially base-pair-disrupting point mutations in the TIR or in the region likely to be base paired to the TIR result in increased *rpoS* translation and reduced Hfq dependence (30) appears consistent with both of these putative mechanisms of Hfq action.

It was reported that Hfq can also bind to DsrA RNA (200), which is a small regulatory RNA partially complementary to *rpoS* mRNA that stimulates *rpoS* translation above all at low temperature (see below for details). Therefore, it was suggested that Hfq may influence DsrA action by forming an active DsrA-Hfq complex and/or by altering DsrA structure (200). However, at 37°C an *hfq* mutation reduces *rpoS* translation much more than a *dsrA* mutation does (146, 199), indicating that Hfq does not act exclusively through DsrA. A hypothetical model consistent with all data available would be...
that Hfq bound to rpoS mRNA recruits DsrA into a ternary complex (in which secondary structures of rpoS mRNA and/or of DsrA could also be altered) and thereby facilitate translational stimulation by DsrA. In this complex, several Hfq molecules or oligomers bind to different sites on rpoS mRNA could be present. During revision of this review, ternary-complex formation with Hfq was also reported for flhA mRNA and OxyS RNA (240) as well as for galK mRNA and spot 42 RNA (140). Both studies came to the conclusion that the role of Hfq (and perhaps of Sm and LSm proteins in general) is to facilitate specific RNA-RNA interaction. Thus, Hfq could stimulate any process dependent on such RNA-RNA interactions.

If so, stress signal input into rpoS translational control would not necessarily be via a control of the activity or the level of Hfq itself. In fact, hfq mutants show overall reduced activity of translational rpoS: lacZ fusions or rates of σ^5 synthesis (as measured in pulse-labeling experiments), but regulation by stress signals, e.g., by hyperosmotic shift, is not abolished (146). This suggests that stress signals affect the cellular concentrations or activities of the specifically translation-activating or inhibiting components (e.g., DsrA and OxyS) that can join the rpoS mRNA-Hfq complex. These components indeed exhibit pronounced regulation (see below).

**HU: a nucleoid protein that also stimulates rpoS translation.** Protein HU is a major protein component of the bacterial nucleoid. It affects overall nucleoid structure and topology but also participates in specific gene regulation, DNA recombination, and DNA repair (155). In addition, HU is required for optimal survival during prolonged starvation (35). In members of the *Enterobacteriaceae* and *Vibrionaceae*, two homologous subunits (HUα and HUβ encoded by *hupA* and *hupB, respectively) contribute to the formation of active HU protein (158). During growth, the HUα2 homodimer is abundant, whereas during late exponential phase, HUβ is induced and HUαβ heterodimers are formed in *E. coli* (35). An HU-deficient *hu-*pAB double mutant exhibits strongly reduced σ^5 levels because of reduced rpoS translation (12).

In vitro, HU binds with high affinity to a small rpoS mRNA fragment (150 nucleotides covering the TIR and the upstream antisense region probably base-paired to the TIR) (12) as well as to a larger fragment (covering more than 700 nucleotides starting from the original mRNA 5’ end) that also binds Hfq (Traulsen and Hengge-Aronis, unpublished). As a DNA-binding protein, HU has a strong preference for nicked or cruciform DNA (94). Thus, HU may preferentially recognize secondary-structure elements, such as pronounced bends or kinks, which also occur in RNA secondary structure. HU may directly alter the rpoS mRNA secondary structure, but it is unknown how this effects relates to that of Hfq or of other components that affect rpoS translation (Fig. 3).

Since the induction of HUβ (which is under the negative control of the nucleoid protein FIS [34]) correlates with stimulation of rpoS translation during late exponential growth phase, and specifically since the HUαβ heterodimer is required for stationary phase survival (35), it is tempting to speculate that the heterodimer is the form of HU involved in rpoS translation. However, this hypothesis has yet to be tested experimentally. Nevertheless, phylogenetically, the occurrence of an HUαβ heterodimer correlates with the occurrence of σ^5 (with the exception of the *Pseudomonas* group, but regulation of σ^5 is significantly different in several aspects in this group).

**H-NS and StpA: histone-like proteins acting as RNA chaperones?** H-NS is an abundant histone-like protein with functions in nucleoid organization as well as in gene regulation, where in nearly all cases it acts as a repressor or silencer that can form large nucleoprotein complexes. StpA is a closely related paralog of H-NS with similar properties (although it seems more efficient as an RNA chaperone). Just as with HUα and HUβ, Homo- as well as heterooligomers are formed by H-NS and StpA (for reviews, see references 9, 43, and 230).
H-NS-deficient mutants exhibit strongly increased $\sigma^S$ levels, which in exponential phase are already similar to those reached by the wild-type only in stationary phase or under other stress conditions (15, 234). In these hns mutants, the rate of rpoS translation is enhanced and proteolysis of $\sigma^S$ is strongly reduced or even abolished (234). The slow growth and genetic instability typical of hns mutants are at least partially connected to these abnormally high $\sigma^S$ levels, since they can be suppressed by mutations in rpoS (15).

Mechanistically, it is still unclear how H-NS downregulates rpoS translation, but there are a number of possibilities for direct or indirect influences (Fig. 3). Since H-NS can bind to RNA (although high-affinity specific binding has not yet been demonstrated [40, 48]), it may directly interact with rpoS mRNA and affect its secondary structure, perhaps in a transient way as an RNA chaperone. H-NS may also counteract the effects of positive regulators of rpoS translation such as Hfq and/or HU. These possibilities are not mutually exclusive, since the positively acting factors and H-NS may have opposite effects on the equilibrium between two rpoS mRNA conformations that can be translated with different efficiencies (Fig. 3). Consistent with H-NS counteracting Hfq, H-NS deficiency has no effect on rpoS translation in a hfq mutant background (146). H-NS and HU in general seem to play antagonistic roles, e.g., in determining DNA supercoiling (44) or in the interaction with certain genes such as ompF (41, 164). Thus, it is also possible that H-NS inhibits rpoS translation by affecting the cellular level of HU or by directly counteracting the stimulatory effect of HU on rpoS translation.

Another candidate for promoting rpoS translation is the H-NS homolog StpA. Several studies have shown that StpA levels are significantly lower than H-NS levels (201, 239), although one report gives approximately equal numbers of H-NS and StpA molecules per cell (10). It seems clear, however, that H-NS and StpA regulate each other negatively at the level of transcription. Therefore, an hns mutant should have an increased cellular concentration of StpA (201, 239). Moreover, StpA is upregulated after a hyperosmotic shift (58). Thus, increased StpA levels appear to correlate with increased rpoS translation. Since StpA can act as a RNA chaperone (40), it was tempting to speculate that it may stimulate rpoS translation. However, high-log-phase levels of $\sigma^S$ in an hns mutant were not suppressed by introducing an stpA mutation, and also osmotic induction of rpoS translation was normal in a stpA mutant (Bouché and Hengge-Aronis, unpublished). Therefore, under these conditions, StpA does not seem to play a role in rpoS translation. This, however, does not exclude a potential involvement of StpA under different conditions or in other genetic backgrounds.

Role of small regulatory RNAs in rpoS translation: DsrA, OxyS, and RprA. Several small regulatory RNAs with important fine-tuning functions in complex regulatory circuits have been identified in E. coli (summarized in reference 1), and three very recently published studies suggest that small regulatory RNAs in E. coli are much more common and significant than previously thought (6, 179, 224). rpoS translation seems to be an especially prominent target for such regulation, with three regulatory RNAs having been found so far. While DsrA and RprA promote rpoS translation, OxyS has an inhibitory function.

DsrA was originally identified as a multicopy suppressor of H-NS-mediated silencing of the rcsA gene in E. coli (198) and was then found to be essential for increased rpoS translation at low temperature (199). DsrA is a stable 87-nucleotide RNA that folds into a three-stem-loop structure (119, 131). A region covering most of stem-loop 1 and the following single-stranded part of DsrA is complementary to an upstream “antisense” element in rpoS mRNA that is assumed to base pair with the TIR region, suggesting that DsrA functions by an “anti-antisense” mechanism that disrupts intramolecular basepairing in rpoS mRNA (119, 120, 131). DsrA plays only a minor role for rpoS translation in cells grown at 37 or 42°C yet becomes the major stimulating factor at 30°C and especially at 20°C (199). The basis of low-temperature translational induction of $\sigma^S$ is the clearly enhanced transcription of dsaR as well as a sixfold-increased stability of DsrA at low temperature (177). DsrA and Hfq were recently reported to interact specifically, and Hfq was suggested to stabilize DsrA as well as to alter its secondary structure in a way that promotes association with rpoS mRNA (200). Whether the formation of such a binary complex facilitates DsrA action on rpoS mRNA or whether Hfq already bound to rpoS mRNA (as described above) recruits DsrA, the result is likely to be a ternary complex (see also above). Hfq may affect the secondary structure of both RNAs such that they optimally interact, and with all partners involved interacting with each other, the complex is probably relatively stable. As a result, the formation of an “open” conformation at the TIR of rpoS mRNA that allows ribosome entry would be facilitated (Fig. 3).

Besides rpoS mRNA, DsrA has at least one other target, hns mRNA. While DsrA was initially thought to act like a conventional antisense RNA interfering with hns translation initiation (120), it now seems likely that a region corresponding to unfolded stem-loop 2 of DsrA forms a coaxial stack with two regions in hns mRNA. Negative regulation of hns expression is a consequence of more efficient degradation of hns mRNA within this complex (118). DsrA is predicted to form similar complexes with argR and ndhFH mRNAs, but an involvement of DsrA in the regulation of these genes has not yet been demonstrated (118).

Multifunctionality exerted by different regions may be common in small regulatory RNAs, since it has also been observed for OxyS. OxyS is a 109-nucleotide regulatory RNA that folds into a similar secondary structure to that of DsrA. As a member of the OxyR regulon, OxyS is induced by oxidative stress (hydrogen peroxide) and acts as a pleiotropic regulator (2). Small regions located in loops 1 and 3 of OxyS control translation of fhlA (which encodes a transcriptional activator) by forming a “kissing complex” with two sites of fhlA mRNA, one of which contains the Shine-Dalgarno sequence (3, 5). By contrast, the rather long A-rich single-stranded region between stem-loops 2 and 3 of OxyS mRNA is involved in negative regulation of rpoS translation, although this part of OxyS does not show significant sequence complementarity to rpoS mRNA. Coimmunoprecipitation experiments indicate that OxyS binds to Hfq protein. Thus, OxyS may sequester Hfq or form a translationally incompetent ternary complex with Hfq and rpoS mRNA (238) (Fig. 3). OxyS-mediated translational repression of rpoS may be a fine-tuning mechanism to avoid redundant overinduction of oxidative-stress protective genes (katG, gsrA,
and dps) that are under dual positive control of OxyR-σ^{70} and σ^{54}. It may also prevent the uneconomical induction of the large multifunctional σ^{54} regulon under conditions where the cell has to cope with oxidative stress only, i.e., a situation that can be managed by the stress-specific OxyR-mediated response alone.

The third small regulatory RNA involved in rpoS translational control, RprA, was found as a multicyclic suppressor for a dsrA mutation (130). In the dsrA mutant background, an rprA null mutation also reduces hypersomatic stimulation of rpoS translation. However, in the presence of DsrA, neither RprA overproduction nor its knockout seems to affect rpoS expression. Thus, RprA clearly has the potential to stimulate rpoS translation, but the physiological conditions under which this becomes relevant are unknown. The rprA promoter is under positive control of RcsB, a response regulator that activates capsule synthesis (unpublished evidence mentioned in reference 130). RprA exhibits some sequence complementarity to the upstream “antisense” element that basepairs with the TIR of rpoS mRNA and may thus act similarly to DsrA (M. Majdalani and S. Gottesman, personal communication).

The LysR-like regulator LeuO: a repressor for dsrA expression. LeuO is a LysR-like regulator (189), which is strongly repressed by H-NS in growing E. coli cells (101). Overproduction of LeuO (either from a multicopy plasmid or in a mutant that carries a Tn10 transposon immediately upstream of leuO with p_{out} of the transposase gene reading into leuO) reduces rpoS translation, especially at low temperature. This effect is entirely dependent on the presence of DsrA, and LeuO was shown to repress dsrA transcription (101). This regulation is direct since LeuO binding sites have recently been identified in the dsrA promoter region (177). A leuO knockout mutation, however, does not affect increased rpoS translation during late exponential phase or in response to high osmolarity or low temperature. This is not entirely surprising, since under these conditions, leuO expression is repressed or even “silenced” by H-NS (101). However, during entry into stationary phase, leuO is induced in a ppGpp-dependent manner (50). This ppGpp-mediated activation may be indirect, since leuO expression is subject to a “promoter relay” activation mechanism that involves the surrounding ihhI and leuABCD operons (33, 51). As a consequence, LeuO probably downregulates DsrA in stationary phase. While this may alter the expression of other targets of DsrA, the σ^{52} level is not affected (E. Klauck and R. Hengge-Aronis, unpublished results), probably because other σ^{54}-inducing mechanisms compensate for the reduced levels of DsrA. When all these results are taken together, the physiological role of LeuO is far from clear. However, a hint may come from the “cryptic” bgl operon, where LeuO can antagonize H-NS-mediated (and under certain conditions also σ^{54}-dependent) “silencing” (160, 218). Interestingly, the bgl operon becomes expressed in a mammalian host (97). It is thus conceivable that LeuO plays an important regulatory role in a host environment.

Dnak and DksA: a link to heat shock and chaperones. The heat shock chaperone Dnak, as well as a protein termed DksA (originally identified as a Dnak suppressor [95]), has been implicated in rpoS translation. A dnaK mutant exhibits a stationary-phase-specific multiple-stress-sensitive phenotype very similar to that observed for rpoS mutants (180, 181). This correlates with reduced σ^{54} levels in starving dnaK mutant cells (144, 181). Part of this effect is due to reduced rpoS translation, since it can also be seen with RpoS::lacZ hybrid proteins that are not subject to proteolysis. The mechanism behind this effect remains unknown, but the overproduction of the heat shock sigma factor σ^{32} in the dnaK mutant does not play a role, since a suppressor mutation that reduces the σ^{32} level and/or activity does not suppress the dnaK effect on σ^{54} (144).

DksA is a putative zinc binding protein with similarity to the transcriptional activator TraR (59) and other prokaryotic and eukaryotic regulators (103). The basis of dnaK suppression by multiple copies of dksA is still unclear, but it was suggested that production of some stress response factors might be involved (16). This is consistent with the more recent finding that dksA mutations in Salmonella exhibit reduced σ^{54} induction in stationary phase and after a shift to acidic pH. Work with rpoS::lacZ translational fusions indicated that DksA affects rpoS translation by some not yet characterized mechanism (225). In P. aeruginosa, overexpression of DksA inhibits the expression of rhl, rhlAB, and lasB (26). This would be in line with a repressing effect of σ^{54} on the rhl system (228). However, additional data suggest that this effect of DksA overproduction is not due to upregulation of σ^{54} alone (26).

EIIA(Glc): a link to the carbon source and energy supply. A crr mutant, which is defective in the glucose-specific PTS component EIIA(Glc), contains strongly elevated σ^{54} levels. Both transcriptional and posttranscriptional effects contribute to this phenotype (217). Higher expression of a transcriptional rpoS::lacZ fusion is fully suppressed by cAMP addition, indicating that the effect reflects stimulation of adenylyl cyclase by EIIA(Glc) and negative control of rpoS transcription by cAMP-CRP (see above). However, high σ^{54} levels and increased activity of a translational rpoS::lacZ fusion are not fully suppressed by cAMP addition, nor does this effect of crr disruption disappear in a rrsB mutant background, where σ^{54} is not degraded. Thus, EIIA(Glc) obviously downregulates rpoS translation by some uncharacterized and perhaps indirect mechanism. Moreover, the phosphorylated form of EIIA(Glc) is required for this activity. However, external addition of glucose, which is known to drastically decrease the level of phosphorylated EIIA(Glc) (207), does not result in σ^{54} induction (217). In the absence of phosphotransferase system-mediated glucose uptake, however, phosphorylation of EIIA(Glc) reflects the intracellular phosphoenolpyruvate-to-pyruvate ratio (84). Negative regulation of σ^{54} by EIIA(Glc) may thus be a function of this ratio, which depends on the nature of the carbon source and the energy supply in general (217).

The cold shock domain proteins CspC and CspE. CspC and CspE belong to the CspA cold shock protein family in E. coli, although they are expressed at 37°C and are not temperature regulated (169). Overproduction of these two RNA binding proteins strongly stabilizes and thereby increases the cellular level of rpoS mRNA. Whether this is a direct or indirect effect is currently unknown. Such high rpoS mRNA levels are assumed to translate into higher σ^{54} levels, since the σ^{54}-dependent genes osmY, dps, proP, and katG are significantly activated. Conversely, a cspC cspE double mutant exhibits reduced osmotic induction of osmY and dps (168). Unfortunately, rpoS mRNA levels were not determined in the osmotic shift experiment, and in general the rates of σ^{54} synthesis and the cellular σ^{54} level were not monitored directly in this CspC-CspE study.
It was previously reported that a shift to high osmolarity does not increase the rpoS mRNA level (146), which would not be consistent with rpoS mRNA-stabilizing factors playing a major role in osmotic regulation of rpoS. Therefore, it is possible that the CspC and CspE effects on osmY and dps expression are direct and do not always reflect the regulation of rpoS (168).

A Small Molecule That Influences rpoS Translation: UDP-Glucose

UDP-glucose has been implicated in σ^5 regulation, since several mutants with defects in central carbon metabolism that result in UDP-glucose deficiency exhibit increased σ^5 levels during exponential growth (24). These defects can be in phosphoglucomerase (encoded by pgm), with the mutant growing on fructose, as well as phosphoglucomutase (pgm) or UDP-glucose pyrophosphorylase (gamU), with the latter two mutants growing on glucose. Glucose and galactose given in trace amounts to the growing on glucose. Glucose and galactose given in trace amounts to the internal UDP-glucose pool and in parallel result in a rapid decrease of σ^5 levels (24). More recent work with transcriptional and translational rpoS::lacZ fusions and direct pulse-chase measurements of σ^5 synthesis and degradation indicate that UDP-glucose specifically affects rpoS translation. Moreover, enhanced σ^5 levels in a gamU mutation are observed only with an intact hfq gene, which suggests that UDP-glucose directly or indirectly interferes with Hfq function in rpoS translation (A. Mufller and R. Hengge-Aronis, unpublished results). However, the molecular mechanism of UDP-glucose action has yet to be clarified, and it is also unknown whether the cellular UDP-glucose level changes in response to any stress signals that affect rpoS translation.

rpoS Translational Control Network and Stress Signal Input

When all the regulatory factors involved in rpoS translation are considered together, a highly intertwined network characterized by positive and negative feedback regulation emerges (Fig. 3). The regulatory output of this network under different environmental conditions is difficult to predict, especially when changing environmental conditions affect the cellular levels of indirectly acting and multiply connected components such as H-NS or LeuO. DsrA is obviously a central player, since it affects the two global regulators H-NS or LeuO. DsrA seems to have a dual role in osmotic regulation of rpoS proteolysis. In general, it seems that relatively threatening stress conditions tend to affect σ^5 degradation, maybe because this allows the most rapid reaction. These stresses include sudden carbon starvation (114, 208), osmotic upshift (148), and shift to acidic pH (18), which result in σ^5 stabilization within a few minutes. On the other hand, the classical heat shock procedure, i.e., a shift from 30 to 42°C, results in a more moderate increase in σ^5 half-life, which takes approximately 20 min to develop (144).

σ^5 Degradation by the Complex ATP-Dependent ClpXP Protease

The ClpXP protease is responsible for σ^5 degradation. ClpXP is a barrel-shaped processive protease consisting of two
six-subunit rings of the ATP-hydrolyzing ClpX chaperone, which play the role of substrate-discriminating and unfolding gatekeepers to the inner proteolytic chamber formed by two seven-subunit rings of ClpP (69, 99, 223). Mutations in clpP as well as in clpX result in stabilization of \( \sigma^5 \) (192). Since the clpP and clpX genes constitute an operon (68, 133), the clpP phenotype could in principle have been due to polarity on clpX, but the inability to suppress \( \sigma^5 \) stability in the clpP mutant by providing clpX in trans confirmed that the entire ClpXP complex is required for \( \sigma^5 \) proteolysis (Muffler and Hengge-Aronis, unpublished). Recently, it has been possible to reconstitute \( \sigma^5 \) degradation in vitro, and these experiments have defined ClpXP as well as a specific recognition factor (see below) as essential and sufficient for the basic process of \( \sigma^5 \) proteolysis (243). \( \sigma^5 \) degradation by ClpXP is complete; i.e., no stable degradation products have been observed.

The Response Regulator RssB: a \( \sigma^5 \) Recognition Factor with Phosphorylation-Modulated Affinity

In contrast to other ClpXP substrates, \( \sigma^5 \) cannot be recognized by ClpXP alone, as demonstrated both in vivo and in vitro (145, 243). Rather, a specific recognition factor, the RssB protein (also termed SprE, MviA, or ExpM in different bacterial species), is required (4, 18, 145, 170). A mutation in \( \text{rssB} \) results in the stabilization of \( \sigma^5 \) (and also of otherwise unstable RpoS::LacZ hybrid proteins) and therefore in elevated \( \sigma^5 \) levels in exponential phase (145, 170). RssB belongs to the two-component response regulator family of proteins, whose activity is modulated by phosphorylation of a conserved aspartyl residue in the N-terminal receiver domain (DS8 in RssB). In vitro experiments have shown that phosphorylated RssB directly interacts with \( \sigma^5 \) (20). Phosphorylation as well as \( \sigma^5 \) binding in vitro is lost with RssB variants, in which DS8 is replaced by other amino acids, consistent with strains carrying the same mutations exhibiting high levels of stable \( \sigma^5 \) (21, 25, 102). RssB is essential for \( \sigma^5 \) degradation in vitro (243) and may be specific for \( \sigma^5 \), since turnover of another ClpXP substrate, \( \lambda \)O protein, does not depend on RssB (242, 243). In conclusion, the response regulator RssB is an essential, specific, and direct \( \sigma^5 \) recognition factor, whose affinity for \( \sigma^5 \) and therefore whose activity in \( \sigma^5 \) proteolysis are modulated by phosphorylation of its receiver domain.

Like most response regulators, RssB consists of at least two domains, the N-terminal receiver and a C-terminal output domain (the latter could also be more than a single domain). The unique role of RssB in proteolysis is revealed in a unique output domain(s) without similarity to any other protein of known function. In certain response regulators, the output domain alone is mechanistically responsible for the molecular function (most often in transcription initiation), with the receiver domain imposing regulation by phosphorylation-modulated intramolecular inhibition (42, 70). In other cases, phosphorylation of the receiver domain actively contributes to the output function, e.g., by stimulating oligomerization (53, 233) or by exposing an interactive surface in the receiver itself (138, 237). RssB belongs to the latter class, since the isolated N- and C-terminal domains of RssB are functionally inactive in vitro and in vivo; i.e., the N-terminal receiver domain plays an active and positive role in RssB function (102). The molecular details of the RssB-\( \sigma^5 \) interaction remain to be elucidated, but there is evidence that RssB, unlike many other response regulators, does not dimerize or oligomerize on phosphorylation and/or \( \sigma^5 \) binding and that the RssB-\( \sigma^5 \) complex exhibits 1:1 stoichiometry (102).

The cellular concentration of RssB (which in growing cells is around the limit of detection) is the limiting factor for the rate of \( \sigma^5 \) proteolysis in vivo. This means that RssB can be titrated by increased \( \sigma^5 \) synthesis (174). This mechanism can be exploited for stress-induced stabilization of \( \sigma^5 \) (see below). On the other hand, cells have to continuously adjust the expression of RssB to \( \sigma^5 \) in order to maintain \( \sigma^5 \) proteolysis during growth despite controlled or accidental variations in the rate of \( \sigma^5 \) synthesis. This is achieved by a homeostatic feedback coupling that is provided by rssB transcription being dependent on \( \sigma^5 \) (185; Pruteanu and Hengge-Aronis, submitted). These two reports, however, do not agree on the location of the \( \sigma^5 \)-dependent promoter, since Ruiz et al. (185) invoke a promoter just upstream of rssB, which was not found by Pruteanu and Hengge-Aronis (174), who provide evidence that rssB transcription is driven exclusively from the \( \sigma^5 \)-controlled rssB operon promoter. \( \sigma^5 \) control of rssB expression also results in indirect negative autoregulation of rpoS as well as of rssB, since \( \sigma^5 \) stimulates the expression of a factor, RssB, that initiates \( \sigma^5 \) disappearance (174).

The Turnover Element: the RssB Binding Site within \( \sigma^5 \)

Unlike many other proteolysis substrates, which feature recognition sequences or elements at or close to the N or C termini (96, 229), \( \sigma^5 \) was found to contain a “turnover element” somewhere in the middle of its sequence. Initial evidence for such a proteolysis-promoting element came from the analysis of RpoS::LacZ hybrid proteins carrying N-terminal \( \sigma^5 \) fragments of different lengths. Whereas relatively short hybrid proteins were stable and yielded high \( \beta \)-galactosidase activities in log phase, extending the \( \sigma^5 \) part beyond a certain region resulted in hybrid proteins that were subject to the same regulation turned over as \( \sigma^5 \) itself and yielded low \( \beta \)-galactosidase activities (148, 192). These studies roughly mapped the turnover element somewhere in or downstream of region 2.4 (which is involved in recognition of the −10 promoter element). Consistent with \( \sigma^5 \) and \( \sigma^70 \) recognizing the same −10 consensus (19, 47, 81), there is extreme amino acid similarity of these two sigmas up to the end of region 2.4. Just beyond this point, however, the sequences diverge. Reasoning that only \( \sigma^5 \) is unstable and therefore should contain the turnover element, a number of amino acids in this region of \( \sigma^5 \), which clearly differ from those in \( \sigma^70 \), were replaced by the latter ones. This identified K173 as an absolutely crucial amino acid for \( \sigma^5 \) proteolysis. A single point mutation, K173E, eliminates rapid \( \sigma^5 \) proteolysis (20). Single mutations in E174 or V177 also enhance the \( \sigma^5 \) half-life two- and threefold, respectively (20). In conclusion, K173 is a core amino acid of the turnover element. Moreover, K173 is also crucial for promoter recognition in the extended −10 part of a promoter (specifically of a C in position −13) (19), and this part of \( \sigma^5 \) or \( \sigma^70 \) is now termed region 2.5 (13, 19).

In vitro experiments with the \( \sigma^5(\text{K173E}) \) variant demonstrated...
that K173 is essential for interaction with RssB. In other words, the turnover element around K173 represents the binding site for RssB (or an essential part thereof) (20). Unfortunately, there is no experimentally determined structural information for region 2.5 of sigma factors (a known partial structure of /H9268 ends with region 2.4 [132]), but the sequence between V172 and K188 is strongly predicted to be in an -helical conformation. The double role of K173 in RssB binding and in interaction with the extended -10 promoter region also means that K173 must be surface exposed, no matter whether S is in the RNA polymerase complex or not. In an initial attempt to estimate the extension of the binding site for RssB, the amino acids predicted to form the -helix in region 2.5 were N-terminally fused to -galactosidase and were found to be sufficient for RssB phosphorylation, but it is unclear whether the catalytic cycle of RssB involves obligatory dephosphorylation during release and subsequent rephosphorylation. Stress signals may affect (i) the phosphorylation of RssB and therefore RssB- binding; (ii) the cellular level of RssB (which in growing cells is rate limiting for S proteolysis); (iii) the synthesis of S such that RssB becomes titrated on S overproduction; (iv) S association with RNA polymerase core enzyme, which protects against binding by RssB; and (v) the function of the ClpXP protease itself (see the text for details). However, the molecular details of the stress signal input pathways involved are still largely unknown.

**Initiation of S Proteolysis: the RssB Cycle**

Once bound to RssB, 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 (Fig. 4). A ternary complex between S, RssB, and ClpX and a quaternary complex also involving ClpP have been observed in vitro (243). RssB is then released from the complex, as indicated by in vitro as well as in vivo data (102, 243). Thus, RssB is recycled and plays a catalytic role in the initiation of S degradation. Taking into account a S half-life of 1.5 min (114), the cellular S-to-RssB ratio of approximately 20:1 (21), and the fact that RssB remains a monomer in the S-RssB complex (102), it has been estimated that a single mol-
ecule of RssB can initiate the degradation of at least six or seven molecules of $\sigma^5$ per minute (in cells growing in minimal glucose medium). The real number may actually be somewhat higher, since this estimation was based on the degradation of fully synthesized $\sigma^5$ molecules only (as visible in pulse-chase and immunoprecipitation experiments). In addition, however, nascent $\sigma^5$ polypeptide chains can probably enter the degradation pathway as soon as they are long enough to contain the recognition site for RssB (102).

So far, it is unknown whether RssB is dephosphorylated during its catalytic cycle. Dephosphorylation could be a convenient mechanism for RssB release. Contact with ClpXP may stimulate an RssB autophosphatase activity, which in purified RssB alone would be cryptic (spontaneous in vitro dephosphorylation occurs with a half-life of more than 1 h) (102). Alternatively, $\sigma^5$ may lose its affinity for RssB during its unfolding and initial transfer into ClpP, which would alter the conformation of $\sigma^5$ in and around region 2.5, whose integrity is required for RssB interaction (20). Dephosphorylation of RssB during its catalytic cycle would imply rephosphorylation as an obligatory part of this cycle (Fig. 4), which has interesting regulatory implications (see below).

**Signal Integration in the Control of $\sigma^5$ Proteolysis**

Defining the linkages between stress signal transduction pathways and the $\sigma^5$ recognition and degradation pathway remains a challenge for future studies, mainly because these signal transduction pathways themselves have yet to be elucidated. However, it is becoming apparent that the basic RssB-ClpXP system has the potential to act like a multiple-signal-integration machinery. Theoretically, this system provides a wide range of possibilities for downregulating the rate of $\sigma^5$ degradation by stress signal transduction pathways (Fig. 4). In the presence of preliminary data only, the existence of these mechanisms remains speculative at present, but the following theoretical discussion may provide useful hypotheses for future work.

With RssB being a response regulator, it is reasonable to expect that some stresses will affect the phosphorylation state of RssB. Unfortunately, this could not yet be demonstrated directly in vivo, since the cellular RssB level is at the limit of (and sometimes below) detection in immunoblot experiments (21, 141). So far, no cognate sensor kinase for RssB has been identified and the *E. coli* genome sequence does not provide any obvious candidate. Acetyl phosphate seems to contribute to RssB phosphorylation, since acetyl phosphate-free *pta-ackA* mutants exhibit longer $\sigma^5$ half-lives; however, since $\sigma^5$ proteolysis is not completely abolished in these mutants, at least one additional phosphoryl donor for RssB is likely to exist (25). It therefore seems possible that RssB is phosphorylated by “cross talk” from other sensor kinases, consistent with phosphorylated RssB (and therefore rapid $\sigma^5$ recognition and degradation) representing the “default” state of the system in the absence of stress. Environmental stress would then trigger some mechanism that actively dephosphorylates RssB. However, a specific RssB phosphatase (or a sensor kinase that switches to RssB phosphatase activity) still awaits identification.

An interesting variation on this theme becomes possible if RssB is obligatorily dephosphorylated during its release from the complex with $\sigma^5$ and ClpXP (Fig. 4). In this case, a specific stress-activated phosphatase may be dispensable, and the entire regulation of phosphorylation and dephosphorylation of RssB could be mediated by one (or several) cross-talking sensor kinases that would then have to be inhibited by certain stresses. However, signal input flexibility and precision would certainly be higher if the system actively controls phosphorylation as well as dephosphorylation, which could be differentially targeted by different stress conditions.

In cells growing in minimal medium, there is a finely tuned balance between $\sigma^5$ synthesis and proteolysis. There is evidence that the cellular level of RssB is the rate-limiting factor for $\sigma^5$ proteolysis in vivo (174). Accordingly, a sudden strong increase in $\sigma^5$ synthesis results in $\sigma^5$ stabilization because of titration of RssB. This is observed on artificial induction of $\sigma^5$ synthesis (174), or on osmotic upshift or pH downshift, where the rate of $\sigma^5$ synthesis increases severalfold within a few minutes (146, 148; Kampmann and Hengge-Aronis, unpublished). Therefore, osmotically triggered or pH-triggered stabilization of $\sigma^5$ may in part be a passive consequence of the stimulation of *rpoS* mRNA translation (see above).

With RssB being limiting for $\sigma^5$ proteolysis, it is theoretically also possible that some sort of stress may result in a reduction of RssB levels rather than of RssB activity. However, starvation leads to a moderate increase in the cellular RssB concentration (21, 185; Pruteanu and Hengge-Aronis, submitted), whereas osmotic upshift has no effect (21). Also, for some other stresses known to affect $\sigma^5$ proteolysis, alterations in RssB levels were not observed (M. Pruteanu and R. Hengge-Aronis, unpublished results).

Association of $\sigma^5$ with RssB or RNA polymerase core enzyme seems mutually exclusive. Core enzyme protects $\sigma^5$ against degradation in vitro and, at equimolar concentrations with RssB, can even actively displace RssB from the $\sigma^5$-RssB complex (243). This suggests that any factors that in vivo may disfavor $\sigma^5$ in its competition with $\sigma^5$ for core polymerase or that may somehow directly stimulate $\sigma^5$ holoenzyme formation would also contribute to $\sigma^5$ stabilization. Such $\sigma^5$-activating and -stabilizing factors have not yet been unequivocally identified, but there are reasons to postulate their existence (see below).

Finally, there is indirect in vivo evidence that a $\sigma^5$-RssB complex is still formed in carbon-starved cells (21), which indicates that under these conditions, inhibition of some activity in the unfolding and degradation pathway downstream from $\sigma^5$-RssB binding contributes to stabilization of $\sigma^5$. In that respect, it may be relevant that the ClpX level is likely to be reduced due to growth stage-specific *clpPX* mRNA processing in stationary-phase cells (122).

In summary, this extraordinary potential for multiple signal integration in the RssB-ClpXP system can explain why so many different stress signals can finally result in the same phenomenon, i.e., $\sigma^5$ stabilization. In the future, it will have to be worked out which stresses act by which of the mechanisms outlined above. An increased flexibility and fine-adaptive power would be achieved if certain stresses used different combinations of these mechanisms.
Additional Factors with Uncharacterized Molecular Functions in σS Turnover

It is obvious from the previous section that signal integration in the control of σS recognition and degradation is highly complex and that therefore probably several, if not many, components involved are still missing from our picture. Unfortunately, however, it may be predicted that in a system that integrates many signal input pathways (which may also be interconnected!), a mutation in a single signal-transducing component probably produces a minor or even no phenotype unless the mutant is tested under very specific conditions. This may explain why mutant searches using screens that reflect σS degradation have not yielded mutations in novel genes with clear-cut phenotypes other than in rssB, clpP, or clpX (49; F. Reindl, E. Kampmann, and R. Hengge-Aronis, unpublished results). However, there is circumstantial evidence that certain genes somehow contribute to the control of σS proteolysis.

**RssA.** The rssA gene is located upstream of rssB, and the two genes constitute an operon with a single promoter upstream of rssA (174). The N-terminal part of RssA belongs to a family of putative serine esterases of unclear physiological functions that have been conserved from bacteria to humans (128). RssA deficiency, as well as overproduction, was observed to have minor but reproducible effects on the cellular σS levels (G. Kampmann, M. Marquardt, and R. Hengge-Aronis, unpublished results). So far, it is not clear whether RssA acts directly or indirectly and what its actual biochemical function is. RssA may be involved in RssB dephosphorylation under some conditions, but alternative explanations are at present not excluded.

**The histone-like protein H-NS.** hns mutants exhibit abnormally high σS levels in exponential phase (15, 234). Although H-NS is known as an abundant nucleoid-associated protein that represses or even silences the transcription of numerous genes (230), its effect in the control of σS is posttranscriptional, with rpoS translation being stimulated and σS proteolysis being strongly reduced (15, 234). It seems likely that H-NS indirectly affects σS degradation by controlling the expression of some other regulatory factor. The stability of σS in the hns mutant is certainly not due to a lack of RssB; rather, the cellular RssB level even seems slightly increased in the mutant (102), consistent with σS control of rssB expression (174, 185). Alternatively, H-NS may regulate some component involved in the control of RssB activity, such as, phosphorylation, but the component(s) still awaits identification.

**The LysR homolog LrhA.** In stationary-phase cells, the outer membrane porin OmpF is downregulated by a component(s) still awaits identification! The LysR homolog LrhA. In stationary-phase cells, the outer membrane porin OmpF is downregulated by a component(s) still awaits identification! A homolog of LrhA, HexA, is known in Erwinia carotovora, where it controls genes involved in exoenzyme synthesis, plant virulence, and motility (73, 149).

**The DnaK chaperone.** In stationary phase, dnaK mutants show reduced σS content and exhibit a pleiotropic phenotype very similar to that of an rpoS mutant (144, 180, 181). As outlined above, part of this effect is due to reduced rpoS translation. In addition, however, various lines of evidence indicate that dnaK mutants are partially defective for σS stabilization in starved cells (144, 181). However, the mechanistic link between the DnaK chaperone and the σS recognition and degradation system (Fig. 4) has not been identified. It is interesting that the DnaK system plays opposite roles in controlling the proteolysis of σS and of the heat shock sigma factor σ32; whereas DnaK stabilizes σS, it is crucial for the degradation of σ32. DnaK may also play a role in heat shock stabilization of σS, which is a relatively slow process (taking up to 20 min) that more or less correlates with the accumulation of the heat shock protein DnaK (144). By contrast, the extremely rapid and transient heat shock stabilization of σ32 is due to titration of the DnaK chaperone by suddenly accumulating denatured proteins (236). It is possible that DnaK plays a role in starvation sensing. Sudden carbon source starvation is almost immediately followed by a strong reduction in overall protein biosynthesis and consequently in reduced levels of newly synthesized but not yet natively folded polypeptides, some of which are DnaK substrates. Thus, more DnaK may be available to somehow protect σS from degradation (144). At present it is unknown whether only DnaK or the entire DnaK chaperone machine (also including DnaJ and GrpE) is involved in σS protection from proteolysis.

**A Small Molecule That Affects σ32 Proteolysis: Acetyl Phosphate**

During growth on glucose as a carbon source, acetyl phosphate is produced by phosphotransacetylase (encoded by the pta gene) from acetyl coenzyme A. Acetate kinase (ackA) then uses acetyl phosphate and ADP to produce ATP and acetate, which is excreted (46, 173). An acetyl phosphate-free pta-ackA mutant exhibits an approximately twofold-increased σS half-life, and since acetyl phosphate is an excellent phosphoryl donor for RssB in vitro, a similar in vivo function has been postulated (25). With a few exceptions (98, 173, 196), this effect is in contrast to findings with most other response regulators, where in vivo phosphate transfer from acetyl phosphate usually cannot be detected unless the cognate sensor kinase (which in the absence of its specific stimulus often acts as the response regulator phosphatase) is eliminated by mutation (137). Thus, for most response regulators, phosphorylation with acetyl phosphate is possible in vitro but does not play a physiological role. For σS proteolysis, however, it seems to be physiologically relevant. Nevertheless, acetyl phosphate cannot be the only phosphodonor for RssB, because σS turnover is not completely abolished in the pta-ackA mutant. Also, the observation that the pta-ackA mutation has a minor (and under some conditions
no) effect on the $\sigma^5$ level (25, 39) indicates that acetyl phosphate plays a rather subtle role in the combination of all the influences that together determine the actual $\sigma^5$ level (in practice this means that in order to see such an effect, it is not sufficient to measure the $\sigma^5$ level, an all-integrative parameter, but that $\sigma^5$ degradation has to be directly assayed, e.g., by pulse-chase labeling).

Recently, the effects of acetate addition (at neutral pH) on genome-wide gene expression have been investigated. More than two dozen acetate-inducible genes (7) or proteins (100) have been identified, many of which are $\sigma^5$ controlled. This effect is specific for acetate since formate produced largely opposite effects (100). In one of these studies, acetate phosphatase was excluded as the direct inducer, since a rsa-ackA mutant exhibited constitutively high levels of the same proteins. Therefore, a high acetyl coenzyme A level was proposed to be the inducing signal (100). Unfortunately, $\sigma^5$ itself and its different levels of control were not studied in those acetate-treated cells (see above for discussion of a putative effect of acetate on $rpoS$ transcription).

### REGULATION OF $\sigma^5$ ACTIVITY

$\sigma^5$ activity in transcriptional initiation requires its association with RNA polymerase core enzyme. However, of all the sigma factors of *E. coli*, $\sigma^5$ is the one with the lowest affinity for the core enzyme in vitro (129). Moreover, even in stationary phase, the cellular level of $\sigma^5$ does not exceed approximately one-third of the cellular level of $\sigma^70$ (89, 92). Given these basic data and the general competition of sigma factors for core, one wonders how $\sigma^5$ can recruit RNA polymerase core to any significant extent in vivo and activate the expression of genes at all. It may be that the putative anti-$\sigma^70$ factor Rsd (90, 91) shifts the balance somewhat in favor of $\sigma^5$, but, given the relatively low cellular level of Rsd (91), this effect cannot be expected to quantitatively eliminate $\sigma^70$ activity. Moreover, some stationary-phase-induced genes are expressed by $\sigma^70$, containing RNA polymerase (77). It is conceivable that covalent modification (163) or ppGpp binding (213) of core polymerase may improve the interaction with $\sigma^5$. In addition, it is tempting to speculate that at least under stress conditions, where $\sigma^5$ is induced, some unidentified factor(s) may exist that stimulates its interaction with core polymerase.

### In Vivo Evidence for Regulation of $\sigma^5$ Activity

The crl gene product stimulates the expression of curli fimbriae (162), which are involved in cell-cell aggregation (182, 183) and adhesion to eukaryotic cells (71, 162). Synthesis of curli (with the subunits encoded by csgAB) is also dependent on $\sigma^5$ (8, 161). More recently, it was found that the role of Crl is not curli specific but that Crl has a stimulatory effect on the expression of a number of $\sigma^5$-activated genes. Also, for negative effects of $\sigma^5$ (e.g., on the expression of OmpF or in a not further clarified negative autoregulation of $\sigma^5$ itself), Crl seems to play a synergistic role (171). However, Crl does not downregulate $\sigma^5$ itself (if anything, $\sigma^5$ levels increase in a crl mutant, because the above-mentioned negative feedback in $\sigma^5$ control is relieved). Moreover, Crl does not seem to be a DNA binding protein since it does not contain any known DNA binding motif, nor could (nonspecific) binding to DNA cellulose be observed. Therefore, it was proposed that Crl may activate $\sigma^5$, perhaps by modulating the $\sigma^5$ association with RNA polymerase core (171). This is certainly an attractive hypothesis, but a direct demonstration of such a function is still missing. If Crl stimulates $\sigma^5$-core interaction in stationary phase, it could be expected to directly interact with $\sigma^5$ and/or core, and it should also contribute to $\sigma^5$ stabilization (since association with core protects $\sigma^5$ from RssB binding, as outlined above), but these specific hypotheses have not yet been tested.

There are specific conditions where high cellular levels of $\sigma^5$ do not result in high expression of $\sigma^5$-dependent genes, i.e., where $\sigma^5$ levels and activities do not appear to correlate. This seems to be the case when $\sigma^5$ is artificially overproduced during exponential growth (e.g., from an isopropyl-$\beta$-thiogalactopyranoside [IPTG]-inducible promoter [R. Lange and R. Hengge-Aronis, unpublished results]). Another such situation is provided by the classic glucose-lactose diauxie experiment (54). $\sigma^5$ is degraded with a half-life of 2 min during the first growth phase on glucose. During the lag phase, $\sigma^5$ is completely stabilized, resulting in the accumulation of $\sigma^5$ as well as mRNA of the $\sigma^5$-dependent osmY gene. When the cells then start to grow on lactose, the $\sigma^5$ half-life remains relatively high (more than 20 min) and $\sigma^5$ levels therefore decrease only slowly. However, no osmY mRNA can be detected during the end of the lag phase and during growth on lactose (54). This observation is formally reminiscent of the inactivation of $\sigma^{32}$ during temperature downshift, where $\sigma^{32}$ levels decrease much more slowly than expression levels of $\sigma^{32}$-dependent heat shock genes (204).

It is tempting to speculate that under such conditions, some factor(s) necessary for $\sigma^5$ activation may be missing or inactive (or some inactivating factor may be abundant). However, such effects have to be interpreted with caution, because some $\sigma^5$-dependent genes require additional regulatory factors besides $\sigma^5$ (78), which may not be present or active under the specific conditions studied.

In summary, there is initial although not conclusive evidence in vivo that $\sigma^5$ activity of *E. coli*, i.e., probably $\sigma^5$ association with RNA polymerase core in competition with other sigma factors, is regulated. At present, it can only be speculated that core enzyme modification (e.g., by ppGpp binding) or additional proteins (e.g., Crl) could be involved. One of the problems, however, is to experimentally distinguish this activation of $\sigma^5$ from activation of $\sigma^5$-dependent transcription by some conventional regulatory mechanism. A clear answer probably requires in vitro transcription experiments that would allow the effects on sigma factor competition to be separated from “normal” activation of transcription.

### The Response Regulator RssB Can Act Like an Anti-Sigma Factor for $\sigma^5$

In wild-type cells, RssB binding to $\sigma^5$ is the first step in $\sigma^5$ delivery to the ClpXP protease. However, in clp mutants as well as in stationary-phase cells engineered to contain a slightly increased RssB-to-$\sigma^5$ ratio, RssB binding to $\sigma^5$ results in $\sigma^5$ inhibition as a transcription initiation factor; i.e., reduced expression of $\sigma^5$-dependent genes can be observed (21, 141, 242).
This suggested that in the absence of ClpXP, or under conditions where σ^5 degradation is inhibited at the protease level, RssB can in principle act like an anti-sigma factor, e.g., by interfering with σ^5-core polymerase association (21). Recent in vitro data indicate that the association of σ^5 with RssB is indeed mutually exclusive with σ^5-core association (243).

Thus, in principle, RssB has the potential to function as an anti-sigma factor for σ^5. Are there any conditions where this is physiologically relevant? Under the best-studied conditions (growth in minimal medium with various carbon sources), any binding of σ^5 to RssB results in rapid degradation of σ^5. Moreover, RssB is present at clearly substoichiometric concentrations (21). However, two scenarios where σ^5 inhibition by RssB could be physiologically relevant are at least conceivable (21). First, earlier in evolution, RssB may have been a stress-regulated anti-σ^5 factor (originally produced in stoichiometric amounts with σ^5) before it was recruited by the proteolysis machinery to serve as a specific recognition factor with a catalytic function. Second, there may be unidentified conditions where (i) significant levels of σ^5 are present, (ii) RssB may be upregulated, and (iii) ClpXP may be less active or downregulated. Although RssB is moderately upregulated in stationary phase (21, 174, 185), further studies of rssB regulation have so far not produced evidence that there are any strongly RssB-inducing conditions under which RssB could function as an anti-σ^5 factor (Pruteanu and Hengge-Aronis, unpublished).

Thus, the evolutionary scenario of a change in RssB function from anti-σ^5 factor to σ^5 proteolysis recognition factor seems more likely.

CONCLUSIONS AND PERSPECTIVES

With an ever-increasing number of factors that contribute to rpoS transcription and translation as well as to σ^5 proteolysis, σ^5 now appears to be an E. coli protein with one of the most complex regulation systems. Nevertheless, a relatively clear picture of the basic regulatory mechanisms, at least in post-transcriptional regulation, has emerged recently. The basic control of rpoS translation uses rpoS mRNA secondary structure, the Hfq and HU proteins, and small RNAs such as the DsrA mRNA. The core σ^5 degradation machinery clearly consists of the ClpXP protease and the phosphorylation-modulated σ^5 recognition factor RssB. Beyond this, however, numerous questions have yet to be answered.

Above all, the way in which the multiple signals that control σ^5 are integrated remains largely unexplored. Translational control of rpoS involves a plethora of components (Fig. 3), but what are their molecular functions and interplay? How does late log phase, high osmolality, or shift to acid pH affect the rate of rpoS translation? In σ^5 proteolysis, the way in which the RssB-ClpXP system functions allows us to predict the overall functions of the “missing” components that have yet to be identified (Fig. 4). These include factors that, in response to certain stress conditions, affect (i) RssB phosphorylation, (ii) ClpXP activity in general or its ability to specifically degrade σ^5, or (iii) σ^5 association with core RNA polymerase. The latter also indicates a link between the control of σ^5 proteolysis and activity. In general, the different levels of σ^5 control do not operate independently from each other, but components like H-NS or EIIA(Glc), which affect more than one level of control, may play a coordinating role.

There is growing evidence for complex connections between σ^5 regulation and other regulatory circuits. These include a linkage to oxidative stress that operates via OxyS RNA; to the CRP regulon, catabolite repression, and inducer exclusion that uses cAMP-CRP and EIIA(Glc); or to the heat shock response which involves the DnaK chaperone. These connections are certainly relevant under multiple simultaneous stress conditions, which is probably a more natural situation than the carefully controlled single-stress situations usually studied in the laboratory.

With all the currently available information taken together, we appear to be approaching a situation where the σ^5 regulatory network is becoming so complex that quantitative (mathematical) analysis and simulation may become helpful in really understanding its inherent overall potential and actual behavior under different conditions. With many regulatory components and their basic biochemical functions now identified, such analysis seems feasible. Since σ^5 is connected to many other crucial regulatory modules in the cell, it may even provide a good starting point for a future quantitative analysis of the entire cellular regulatory network.

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Vol. 66, 2002

REGULATION OF THE α SUBUNIT OF RNAP

393


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