The RpoS-Mediated General Stress Response in *Escherichia coli**

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Abstract

Under conditions of nutrient deprivation or stress, or as cells enter stationary phase, *Escherichia coli* and related bacteria increase the accumulation of RpoS, a specialized sigma factor. RpoS-dependent gene expression leads to general stress resistance of cells. During rapid growth, RpoS translation is inhibited and any RpoS protein that is synthesized is rapidly degraded. The complex transition from exponential growth to stationary phase has been partially dissected by analyzing the induction of RpoS after specific stress treatments. Different stress conditions lead to induction of specific sRNAs that stimulate RpoS translation or to induction of small-protein antiadaptors that stabilize the protein. Recent progress has led to a better, but still far from complete, understanding of how stresses lead to RpoS induction and what RpoS-dependent genes help the cell deal with the stress.

Contents

INTRODUCTION

Bacteria constantly face changes in their environment, from nutrient starvation to variations in temperature, osmolarity, or pH. To adapt to or resist these changing conditions, they have developed various responses. Many of these adaptation response pathways are quite specific, involving a given regulator and a set of related genes, termed a regulon, that help the cell survive the original stress. The genes induced as a response to the stress include those necessary for repairing the damage or returning the cell to homeostasis. The ability of the bacteria to adapt to stress provides evidence of the inducible nature of the response—a pretreatment of cells with a nonlethal stress can improve resistance to a later, otherwise lethal, stress, and this adaptation requires new protein synthesis.

The alternative to these very targeted responses to specific stresses are more global changes in metabolism and gene expression that provide protection from many types of stress. Generally, these global responses have been identified under conditions of nutrient depletion, for instance, as cells enter stationary phase. In extreme cases, some bacteria, including *Bacillus subtilis* and *Myxococcus*, can differentiate into a stress-resistant spore in response to nutrient depletion.

For *Escherichia coli* and its relatives, a highly resistant state is achieved by triggering a global stress response dependent on the alternative sigma factor RpoS. This response allows cells to become more resistant not only to the stress that they first encounter but also to other stressful treatments. For instance, cells starved for carbon become resistant to hydrogen peroxide, high temperature, and low pH. This cross-protection phenomenon is typical of general stress responses and contrasts with specific stress responses that deal only with consequences of the inducing stress.

As with other sigma factors, RpoS interacts with the core RNA polymerase (RNAP) and controls the expression of a specific but large set of genes. Directly or indirectly, RpoS regulates 10% of the *E. coli* genome (approximately 500 genes) (157). The RpoS response has a number of key characteristics:

- Induction of the RpoS response is reflected in the rapid increase in levels of RpoS, which are very low in exponentially growing cells.
- Regulation of RpoS is at multiple levels [transcription, translation, degradation, and regulation of activity (see below)].
- A vast number of stresses affect RpoS accumulation, by feeding into one or more of these levels of regulation.
- Some of the genes induced as part of the RpoS response are truly RpoS specific; others are also expressed by the vegetative sigma factor, RpoD (σ^{70}), under some specific growth conditions.

In this review, we examine the complex regulatory circuits that center around the regulon controlled by RpoS, the stationary phase/stress

Regulon: a set of genes induced together in response to a signal or stress, under the control of a specific regulatory protein

Sigma factor: a protein that binds to the RNA polymerase core and allows it to initiate transcription at different classes of promoters

RNAP: RNA polymerase

sigma factor. The literature on this subject is vast and complex. We focus on work in *E. coli* and, to some extent, in *Salmonella*; we are necessarily selective even for these organisms. Among the questions we attempt to answer are the following: What are the signaling cascades leading to RpoS induction? Can the stresses that lead to induction be matched to genes in the RpoS-dependent response that help to deal with the stress? How does the RpoS-dependent response to a stress compare with the RpoS-independent, specific responses known for the same stress? Other reviews that examine the RpoS response and its regulation include References 52 and 53.

Discovering Stress Regulons and the RpoS-Dependent General Stress Response

Two general approaches were important to initially define regulons, including the RpoS regulon. Two-dimensional gels, first used for this purpose by Neidhardt and coworkers (79), made it possible to begin to define the proteins induced under a given stress treatment or affected by a given regulatory mutation. Such studies in the late 1980s and early 1990s allowed Matin and coworkers (49) to define a set of protein spots on two-dimensional gels that were common to starvation for carbon (C), phosphate (P), and nitrogen (N).

The second approach made use of transposable reporter systems—generally the *lacZ* gene, without a promoter, within an engineered transposable element such as the Mu phage. Transposition of the element containing *lacZ* led to isolation of a set of *lac* fusions to the promoters of random genes, which could then be queried on indicator plates for responses to a given treatment. These systems, developed by Casadaban & Cohen (25), were first used to define the SOS regulon (67) and then for many other stresses (reviewed in Reference 128). Studies of this sort identified genes induced by C starvation, for instance, that turned out to be induced by other stresses as well, helping to define the RpoS regulon (75). Mutations characterized by their effects on a particular phenotype or fusion were shown to be in a common gene, now called *rpoS*, encoding an alternative sigma factor referred to as RpoS (to be used here) or σ^{38} [see multiple alternative names listed in EcoCyc: *katF*, *appR*, *otsX*, *csi2*, *nur* (68)].

Untangling the Regulatory Cascades for the RpoS Regulon

Understanding any regulatory cascade requires defining the signals and mechanisms that lead to induction of the response, as well as an understanding of the outcome of induction (i.e., Which genes/functions are expressed and what do they do?) and an understanding of how the cell recovers from induction.

Induction: increasing RpoS levels and activity. Under optimal laboratory growth conditions, RpoS levels are very low and increase as cells enter stationary phase. This is achieved by a combination of regulatory mechanisms. The default situation for RpoS, during exponential growth, is for synthesis to be low, because, even if transcription occurs, translation is shut off; in addition, any RpoS that is made is rapidly degraded. Thus, the inducing signals that increase RpoS in stationary phase or in response to a variety of stresses are designed to overcome these negative locks on RpoS accumulation.

The induction of RpoS synthesis and downregulation of degradation in response to starvation (for C, P, or Mg) or unfavorable conditions (e.g., high or low pH, high or low temperature, high or low osmolarity, DNA damage) require that the cell has a way of sensing and transducing the starvation or stress. It is clear that induction of the RpoS system takes advantage of many of the regulatory cascades that have been studied in other contexts. Among the most prominent of these collaborating systems is the stringent response that uses (p)ppGpp as a molecule for signaling a wide variety of starvation conditions. As (p)ppGpp increases, it promotes increased *rpoS* transcription and translation, inhibits RpoS degradation, and also improves RpoS activity. The Rcs phosphorelay

(p)ppGpp (guanosine 3′,5′-bispyrophosphate): the nucleotides pppGpp and ppGpp, derived, respectively, from GTP and GDP

Rcs phosphorelay: lipoprotein RcsF transduces signals to kinase RcsC; phosphate then moves from RcsC via phosphotransfer protein RcsD to response regulator RcsB

and the PhoPQ two-component system also contribute to RpoS induction.

Some regulatory cascades, important for specific stress responses, seem to play more antagonistic roles for the RpoS regulon. Cyclic AMP (cAMP) and catabolite response protein (CRP), for instance, primarily have negative effects on RpoS. The ArcAB phosphorelay negatively regulates RpoS at multiple levels.

Cyclic AMP (cAMP): the small molecule cAMP accumulates when cells are growing on suboptimal carbon sources

Catabolite response protein (CRP): binds cAMP and binds to DNA sites, positively and negatively regulating transcription

Outcome: expression of RpoS-dependent genes. When the RpoS system is induced, the cell is resistant to a wide range of stress and starvation treatments. Cross-resistance suggests that all (or many) RpoS-dependent genes are induced in response to each of these stresses. C starvation, for instance, leads to protection from low pH, oxidative stress, high temperature, and osmotic shock. If resistance to each of these depends on different output genes, we would argue that core resistance genes must all be induced without further specific input. In fact, microarrays carried out under a variety of conditions do support the idea that there is a common core of genes for which induction of RpoS is sufficient to increase transcription (157). Presumably, the RpoS-dependent genes necessary for cross-resistance all reside in this common core of genes. Which gene(s) provide resistance to which stress are known in only a subset of cases, however.

Many other genes are induced in an RpoS-dependent manner, but only after a specific stress (157). Why are they not induced when RpoS is present? Two general mechanisms can be imagined. The simplest is that transcription of these genes requires a combination of inputs—RpoS plus something else (a small molecule, an additional transcriptional activator, or inactivation of a repressor in response to the specific inducing signal). *csiD*, for instance, requires cAMP and CRP, in addition to RpoS; Fis is necessary for the RpoS-dependent transcription of *proP* (87, 159). In both cases, binding sites for the auxiliary regulators have been defined in the promoters of the RpoS-dependent genes. The second possibility is that some genes require higher RpoS levels than others do to be transcribed and that the levels of RpoS or the ability to compete with other sigma factors varies after different inducing treatments. This would imply a nested set of gene induction, with the strongest inducing treatment leading to induction of everything and weaker ones leading to a subset of these genes. Such is not what was seen in arrays (157), but certainly some sort of hierarchy is likely to exist.

Among the genes that are induced in response to RpoS, many but not all are also transcribed by RpoD in vitro, or in vivo under certain conditions (see, for instance, References 31 and 138). During exponential growth, when RpoS is not induced, the cell responds to specific stresses with induction of genes tailored to recovery from that stress. Some genes necessary for these specific stress responses are also induced in stationary phase by RpoS; one example is *dps* (3, 48, 88). How then is specificity achieved? Although the answers vary for different genes, global regulators such as Lrp and H-NS seem to play particularly important roles in enforcing RpoS-dependent regulation by repressing RpoD-dependent transcription (10, 31).

Recovery: returning to equilibrium. For most stress responses, the process of reversing induction and returning cells to growth is as important as the induction process, but this process has been less studied. A variety of experiments as well as analysis of *E. coli* isolated from the environment suggest that there are many conditions under which the cell accumulates mutations that inactivate or downregulate *rpoS* (70, 137, 163). The isolation of these mutants certainly suggests that RpoS is not always good for the cell and supports the importance of recovery mechanisms.

REGULATING RpoS: MAJOR MECHANISMS OF REGULATION

Alternative sigma factors such as RpoS are generally under tight negative regulation, so that they do not compete for core RNAP under conditions when they are not needed.

This is certainly true for RpoS; it is barely detectable under rapid growth conditions and accumulates to high levels in response to a variety of stress treatments. We describe the general mechanisms for regulating RpoS here; some mechanisms are developed in more detail below in the context of the specific regulators and the signaling cascades in which they participate. Overexpression of RpoS in exponential phase cells is not sufficient for full induction of many RpoS-dependent genes (72), suggesting additional controls on how RpoS is able to compete effectively with RpoD.

Transcription of *rpoS*

In *E. coli* and many other species, the gene encoding RpoS is just downstream of *nlpD*, encoding an outer membrane lipoprotein of unknown function. Although a promoter upstream of *nlpD* may provide some transcription into *rpoS*, the major *rpoS* transcript initiates at a promoter internal to *nlpD*, starting 567 nucleotides (nt) upstream of the AUG for *rpoS* (**Figure 1**). This long 5′ untranslated region (UTR) is critical for translational regulation. Regulation at the level of transcription is not dramatic compared with effects on translation and protein turnover (76). In addition, interpretation of some results based on reporter fusions or mRNA levels may be complicated by effects of translational regulation on mRNA stability and should be revisited (89).

In two cases, the protein regulators bind directly to the *rpoS* promoter. In *Salmonella enterica*, Fis, abundant during exponential growth and low during stationary phase, negatively regulates *rpoS* transcription ninefold during exponential growth, dependent on an upstream binding site (57). Another negative regulator of *rpoS*, ArcA-P, the response regulator of the two-component ArcB/ArcA system, had a three- to fourfold effect on *rpoS* transcription in exponential phase (92; also see Reference 69 for a review). For other regulators, direct binding remains to be shown. cAMP and CRP negatively regulate an *rpoS-lacZ* transcriptional fusion, and higher levels of RpoS are found in exponentially growing cells in rich medium in the absence of cAMP (76). The two-component BarA-UvrY system has been reported to positively regulate *rpoS* transcription, although the binding site has not been determined (100).

Levels of *rpoS* mRNA rise after (p)ppGpp induction, suggesting a stimulatory effect of (p)ppGpp on *rpoS* mRNA transcription or stability (36, 144, 145). However, studies of transcriptional fusions suggest that these effects may be at the level of mRNA stability or elongation, rather than initiation (56, 74). Some of the reported effects of (p)ppGpp on mRNA levels could be indirect, for instance, via the (p)ppGpp-dependent accumulation of polyphosphate, which is also associated with induction of *rpoS* (127).

Translational Regulation: Critical Roles for sRNAs

The long 5′ UTR of the *rpoS* transcript folds into a stem-loop that occludes the ribosome-binding site and minimizes translation of *rpoS* (21, 34, 97) (**Figure 1**). This inhibitory structure is overcome by *trans*-encoded small RNAs (sRNAs) that stimulate *rpoS* translation. The sRNAs require the RNA chaperone protein Hfq for their action (reviewed in Reference 19). Hfq both stabilizes the sRNAs in vivo and promotes pairing with mRNA targets in vitro. Although Hfq is used by many sRNAs in *E. coli*, the first observed phenotypes of *hfq* mutants in *E. coli* and *Salmonella* are attributable to very low levels of RpoS (20, 98).

Three Hfq-dependent sRNAs (DsrA, RprA, and ArcZ) stimulate *rpoS* translation. All operate by a similar mechanism, pairing between complementary nucleotides in the sRNA and the 5′ UTR of the mRNA to open the hairpin, thus freeing the ribosome-binding site (82, 84, 86). The major effect of pairing is to allow translation; an associated, and possibly indirect, effect is to stabilize the *rpoS* mRNA (89). Hfq requires an $(AAN)_4$ repeat far upstream within the *rpoS* 5′ UTR for sRNAs to activate translation effectively (131, 132). A fourth sRNA, OxyS, negatively regulates *rpoS* translation, likely by

UTR: untranslated region

ArcB/ArcA two-component system: under anaerobic conditions, the ArcB sensor kinase activates ArcA; ArcA activates some anaerobic genes and represses aerobically expressed genes

Hfq: an RNA chaperone necessary for the function of many small RNAs in *E. coli* and other bacteria

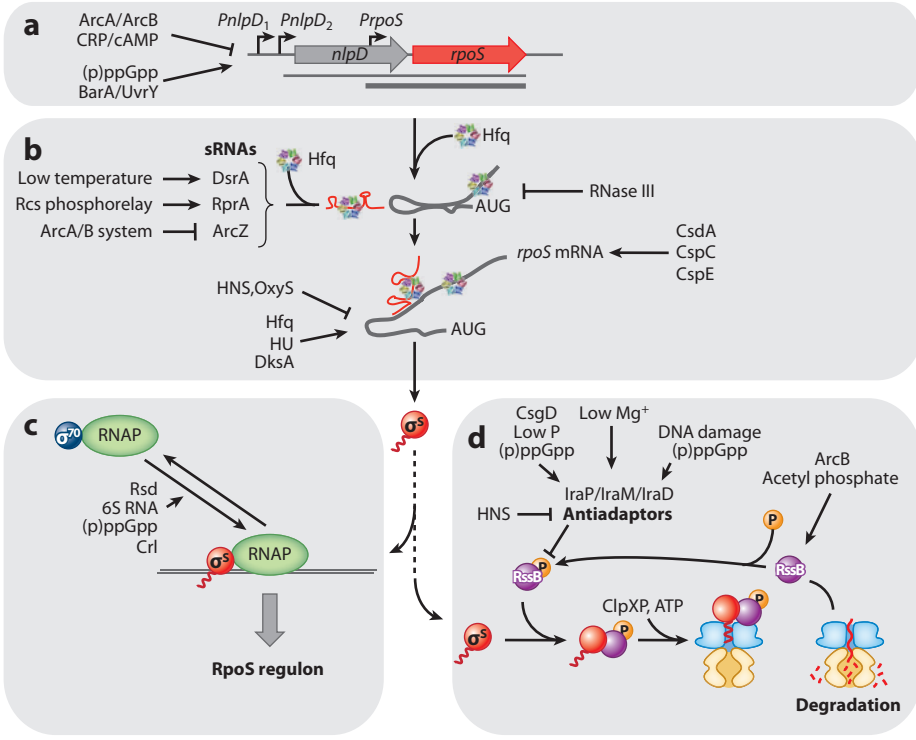

Figure 1

Regulation of RpoS expression, stability, and activity. See text for details and references relative to these regulatory mechanisms. Different levels of regulation are shown in boxes: (*a*) transcriptional regulation, (*b*) translational regulation, (*c*) regulation of RpoS activity, and (*d*) proteolytic regulation. The major transcript for *rpoS* transcription is the one initiating at *PrpoS* within the *nlpD* open reading frame. This transcript forms a repressive hairpin loop that prevents ribosome binding, but positively acting small RNAs, with the help of the Hfq chaperone, release this inhibition and promote *rpoS* translation. OxyS small RNA inhibits *rpoS* translation likely by titrating out Hfq. Other proteins also impinge positively (CsdA, CspC, CspE, HU, DksA) or negatively (RNase III, H-NS) on *rpoS* translation. Once the RpoS (shown here as σ^S) protein is synthesized, it can have two fates: In exponential phase, it is bound by the adaptor protein RssB and degraded by the ClpXP protease, unless the cell encounters some stress that leads to synthesis of one of the antiadaptors (IraP, M, or D) that then interferes with RssB and leads to stabilization of RpoS. In stationary phase and under stress conditions, stable RpoS can bind free core RNA polymerase; this protects RpoS from degradation and allows the transcription of RpoS-dependent genes. 6S RNA, Crl, Rsd, and (p)ppGpp favor RpoS in its competition with RpoD (σ^{70}) for core. Some regulators [for instance, (p)ppGpp] act at multiple levels.

titrating Hfq, as no pairing could be detected (59, 165; K. Moon & S. Gottesman, manuscript in preparation). These sRNAs are synthesized in response to different stresses under the control of different regulators, allowing the bacterium to integrate and respond to numerous stress signals for control of *rpoS* translation.

The essential single-stranded endonuclease RNase E appears to be a major player in degrading *rpoS* mRNA. However, inactivating RNase E is not sufficient for full translation, consistent with a critical role of sRNAs in opening up the inhibitory hairpin to allow translation (89).

Interestingly, a strain deleted for all three positively regulating sRNAs has higher expression of a translational fusion to *rpoS* than a strain deleted for *hfq*, suggesting that either Hfq

has some direct regulatory activity on the *rpoS* mRNA or that there is at least one as yet undetected sRNA that positively regulates *rpoS* translation (86). A number of reports hint at other Hfq-dependent activators. For instance, deletion of the gene for the sRNA GcvB leads to lower levels of RpoS (61), although overexpression of GcvB did not increase expression of an *rpoS-lacZ* translational fusion (86), suggesting either indirect activation or regulation of RpoS by a mechanism not detected by the reporter fusion (for instance, dependency on the 3′ end of the *rpoS* gene). The transcriptional regulator LrhA represses *rpoS* translation in an Hfq-dependent manner (108) by an as yet unknown mechanism. Constitutive expression of the *E. coli* Pho regulon owing to deletion of the *pst* genes also upregulates RpoS, dependent on both Hfq and the PhoB/R two-component system, suggesting yet another sRNA (121) possibly encoded in an intergenic region of the *pst* operon (124).

A number of other proteins have been implicated in modulating *rpoS* translational regulation or in regulating the stability of the *rpoS* message. How directly these act remains to be demonstrated. RNase III, a double-stranded endonuclease encoded by the *rnc* gene, cleaves the *rpoS* mRNA within the inhibitory hairpin. *rnc* mutants have higher levels of *rpoS* mRNA and RpoS protein, suggesting that RNase III is important to maintain low RpoS levels (118) (**Figure 1**). Binding of DsrA or RprA stimulates *rpoS* translation in a manner that appears to be additive to and independent of the effect of RNase III (89), although DsrA binding does change the RNase III cleavage site (89, 118). It is not yet clear whether specific signals modulate RNase III cleavage of the *rpoS* mRNA. One possible signal is temperature; at high temperature (43.5°C), *rnc* mutants no longer affect *rpoS* mRNA stability (89). The secondary structure elements necessary for this double-stranded endonuclease to cut may be disrupted at high temperature, preventing cleavage. If so, melting of the hairpin may contribute to the known induction of RpoS at high temperature by overcoming RNase III action (63).

The cold-shock DEAD-box protein CsdA is an RNA helicase that binds to the *rpoS* message. Deletion of *csdA* leads to a decrease in RpoS amounts at 24°C compared with those found at 37°C (119). Because *rpoS* translation at low temperature is primarily dependent on DsrA (130), CsdA may aid in unwinding the *rpoS* secondary structure to facilitate DsrA annealing (119).

Overexpression of CspC or CspE, constitutively produced members of the CspA family of RNA-binding proteins, increases RpoS levels, apparently by stabilizing *rpoS* mRNA. Deletion of *cspC* lowers expression of an *rpoS-lacZ* translational fusion modestly (30), and deletion of both *cspC* and *cspE* impairs osmotic induction of RpoS-dependent genes (109). It is not clear whether these proteins function only under conditions of high osmolarity. Similar to the models for CsdA function, one may imagine that these proteins help to open up the inhibitory hairpin at high osmolarity, contributing to the increased ability of DsrA and other sRNAs to activate translation under these conditions (81). Overall, the results suggest a possible role for CspC and CspE as alternative RNA chaperones affecting *rpoS* mRNA structure and/or stability.

Deletion of *hupA* and *hupB*, encoding the subunits of HU, significantly decreases *rpoS* translation. Because HU can bind tightly to the *rpoS* 5′ UTR in vitro, this may reflect a direct effect on *rpoS* mRNA structure (7).

Most studies of translation of *rpoS* have focused on the role of the 5′ UTR and how its inhibitory structure is overcome. The translational fusions used to study *rpoS* have also been constructed to focus on the same regions. The translation of the *rpoS* ORF, downstream of the region studied in these fusions, may be subject to additional controls that are not yet understood (56, 115, 156).

Regulated Degradation of RpoS: Critical Roles for RssB and Antiadaptors

Targeted degradation of RpoS is one of the major examples of regulated proteolysis in *E. coli*.

RpoS is rapidly degraded during exponential growth under optimal conditions; degradation stops or slows and RpoS accumulates rapidly after stress treatments (starvation for C, Mg, and P) and in stationary phase (76, 85). When cells return to rapid growth, degradation helps reduce the level of RpoS.

RpoS is degraded by the ClpXP ATP-dependent protease (125). However, RpoS is not recognized directly by ClpX but needs to be delivered to the protease by an adaptor protein called RssB or SprE in *E. coli*, MviA in *S. typhimurium*, or ExpM in *Erwinia carotovora* (4, 11, 71, 96, 112, 168). RssB is not degraded, but it is recycled, promoting degradation of multiple molecules of RpoS (168).

The regulation of RpoS proteolysis during growth phase depends on the stoichiometry and activity of a number of different proteins. ClpXP expression does not change significantly with growth phase, although under C starvation, the availability of ClpXP may be limited if it is actively degrading mistranslated and misfolded proteins (44, 125) or its activity may be lower if ATP levels drop (51).

The limiting component for degradation of RpoS is RssB. The level of RssB is very low (13). If overproduced, it can lead to RpoS degradation even in stationary phase, and it can act as an antisigma factor for RpoS in cells in which RpoS degradation is artificially blocked by mutations in *clpX* or *clpP* (13, 112, 169). Whether this antisigma activity would ever be significant in wild-type cells is not clear. *rssB* is the second gene in an operon with another gene, *rssA*. Although RssA has phospholipase activity, no role for it in modulating RssB activity or synthesis has been reported (51, 114, 120).

RssB expression levels increase modestly during entry into stationary phase in an RpoS-dependent fashion (47, 114, 120). The modest induction of RssB in stationary phase has been suggested to be important for the rapid destruction of RpoS when cells exit from stationary phase, although this remains to be demonstrated. Another possibility is that RssB has another function during stationary phase as suggested by the recent demonstration that RssB promotes the association of polyA polymerase with the RNA degradosome during stationary phase, independent of RpoS (23, 24).

RssB is a response regulator that can be phosphorylated, but the role of RssB phosphorylation in RpoS degradation is not fully understood. In vitro, phosphorylation improves the interaction between RssB and RpoS and stimulates RpoS degradation (12, 14, 71, 94, 168). This suggests that phosphorylated RssB is the active form that degrades RpoS and dephosphorylation of RssB under some stress conditions might be the signal for stabilization of RpoS. However, cells carrying mutations in the site of phosphorylation on RssB still degrade RpoS and respond to starvation or stationary-phase growth signals to stabilize RpoS, suggesting that RssB phosphorylation is not essential for regulated degradation (108). Nonetheless, it remains possible that phosphorylation helps to modulate RssB activity or is important for alternative RssB activities. Although no dedicated phosphatase or histidine kinase has been found, the two-component system ArcA/B and small-molecule P donor acetyl phosphate have been implicated in RssB phosphorylation (14, 92).

Recently, three new proteins able to modulate RssB activity have been identified and termed Ira for inhibitor of RssB activity (15, 17). Each of these three proteins, IraP, IraM, and IraD, interacts with RssB and prevents RpoS degradation in vivo and in vitro; we refer to this class of proteins as antiadaptors. Although they share a similar function, they do not share any sequence similarity. The expression of these proteins under specific stress conditions provides an explanation for how RpoS degradation is regulated in response to stress (**Figure 1**).

Regulation of RpoS activity

The RpoS regulon is large and complex, including genes that are read only by RpoS, genes transcribed by both RpoS and RpoD, and genes in which auxiliary factors activate RpoS-dependent transcription. Proper expression of RpoS-dependent genes has been studied by

examining the rules for promoter recognition by RpoS as well as factors that contribute to the ability of RpoS to compete successfully for core RNAP.

Promoter recognition by RpoS. Sigma factors determine promoter recognition, and thus the sequence of the promoter will act to exclude some sigma factors and favor others. However, RpoS is a member of the same family of sigma factors as the vegetative sigma factor RpoD and shows preference for the same consensus promoter elements in vitro as RpoD (46). RpoS is more sensitive to sequences around the −10 promoter region and shows less dependency on −35 regions than does RpoD (reviewed in Reference 151). Many promoters that appear fully RpoS dependent in vivo can be read by RpoD in vitro and vice versa (139). It is difficult to make broad conclusions about RpoS specificity given that each case is different. A combination of favored promoter elements combined with differential sensitivity to repressors and activators poises promoters in vivo so that only when RpoS-containing holoenzyme levels reach a critical level will they fire.

In many genes, the same start point is used by RpoD or RpoS, under different conditions. The promoter for the DNA-binding protein *dps* is repressed by Fis and H-NS, limiting RpoD-holoenzyme access unless the activator OxyR is present. However, in stationary phase, when RpoS levels rise, *dps* is robustly expressed, dependent on RpoS. This is due both to the ability of RpoS-holoenzyme to overcome or compete successfully with H-NS for binding to the promoter and to the decrease in Fis levels in stationary phase (48). The *aidB* gene is expressed in an RpoD-dependent fashion only when the Ada activator is present; RpoS-dependent expression occurs without Ada (73, 153). The promoter for the osmotically inducible *osmY* gene can be read by both RpoD and RpoS holoenzymes but is repressed by IHF, CRP, and Lrp, all blocking access of RpoD but not RpoS (31).

For other genes, alternative promoters allow expression of the same gene dependent on either RpoS or RpoD (8, 18, 54). For instance, *proP*, encoding a transporter for the osmoprotectants proline and glycine betaine, has an osmotically induced RpoD-dependent promoter and a second RpoS-dependent promoter; recognition of the RpoS promoter is significantly helped by binding of Fis nearby (151, 159).

Competition for RNA polymerase core. All sigma factors compete for the same pool of core RNAP. Thus, anything that affects the binding of RpoD to core polymerase, for instance, necessarily also affects the ability of RpoS to bind core and recognize its promoters. The reverse is true as well; increased RpoS downregulates at least some RpoD promoters, presumably by competing for core RNAP (38).

In in vitro experiments, the affinity of RpoS for core is somewhat less than that of RpoD, the vegetative sigma factor (62, 64). In addition, levels of RpoS are lower than those for RpoD, even in stationary-phase cells. In one study, 7,000 molecules of RpoD were found per cell, significantly in excess of the 2,500 RNAP core complexes, in both exponential and stationary phase. RpoS, undetectable in exponential phase, rose to 1,600 molecules per cell in stationary phase (110). However, under conditions of RpoS accumulation, RpoS is clearly able to acquire core polymerase and read relevant promoters. Ferenci and coworkers (39, 70) have suggested that a balance exists between the advantages of cross-resistance (when RpoS is active) and the loss of nutritional flexibility associated with the shutdown of many RpoD-dependent pathways (SPANC, or self-preservation and nutritional competence). Not surprisingly, the cell has developed ways to regulate this balance. Crl, Rsd, and 6S RNA have been implicated in helping RpoS effectively compete for core. The pattern of expression of these factors suggests that they reinforce RpoS activity and do not themselves act as part of the signaling cascade for RpoS.

Crl protein is necessary in vivo for maximal expression of RpoS-dependent promoters but does not increase RpoS levels (113). In vitro, Crl helps to stimulate RpoS holoenzyme

formation, particularly under limiting levels of sigma. This is presumably particularly important under transition conditions when RpoS levels are first increasing or during recovery from stationary phase (45, 150). Crl is expressed constitutively, possibly so that it is available when RpoS increases. However, Crl was also identified as a substrate for the ClpXP protease (42). Whether degradation of Crl significantly affects its abundance has not been examined; it may be brought to the protease by binding to RpoS. If so, degradation of Crl could be part of the recovery mechanism when rapid growth resumes and RpoS is no longer useful. *crl* mutations also slow RpoS degradation (149); the basis for this is not known and may reflect a lower level of the RpoS-dependent expression of *rssB*. Because RNAP core protects RpoS from degradation in vitro (168), one could imagine a role for Crl as an exchange factor, promoting the ability of RpoS to both bind to and be released from core polymerase.

Unlike Crl, which acts on RpoS holoenzyme, 6S regulatory RNA negatively affects RpoD holoenzyme. 6S regulatory RNA binds to the RpoD holoenzyme and not to RpoS holoenzyme and freezes the holoenzyme in an inactive form (reviewed in Reference 154). 6S RNA is released from RNAP as growth resumes. Because 6S interacts with the holoenzyme, interaction with 6S would not increase the availability of RNAP core for RpoS, but it can lower the relative levels of free RpoD compared with cells deleted for 6S by preventing RpoD recycling.

The in vivo effect of 6S is to increase RpoS-dependent transcription at some promoters and decrease expression of a subset of RpoD-dependent promoters, characterized by extended −10 sequences and poor −35 sequences, possibly because 6S binds to the region of sigma necessary for −35 recognition (26). In competition experiments, 6S provides an advantage in late stationary and under stress conditions such as high pH (147, 148).

Rsd is a protein identified biochemically as a factor that bound RpoD in stationary-phase cells. Rsd interacts with RpoD and interferes with its ability to interact with core polymerase, thus possibly helping RpoS compete for core (162). No phenotype for an *rsd* deletion mutant has been detected in microarrays (93); only when Rsd was overproduced were some RpoS-dependent genes upregulated, consistent with competition. Thus, the physiological role of Rsd is currently unclear.

REGULATORY CASCADES: SIGNALS AND RESPONSES

As described above, many different mechanisms regulate RpoS accumulation and activity, and for each of these mechanisms, there are multiple effectors, each made under particular conditions. In addition, there are many RpoS-dependent genes, some dependent only on whether RpoS is available and some requiring additional inputs. The function and importance of only a handful of these downstream genes is known. In this section, we review a selected group of the known cascades of signaling and the downstream genes implicated in particular resistance pathways. This is not meant to be all-inclusive. In **Table 1**, a broader list of stresses and the known downstream genes implicated in the RpoS response are listed.

Starvation Responses: (p)ppGpp Unites Starvation Signaling

The small-molecule alarmone (p)ppGpp is the main effector of the stress response that takes place in *E. coli* during starvation. Its accumulation in the cell triggers the inhibition of stable RNA biosynthesis (i.e., rRNA operons and tRNA genes), growth arrest, and the activation of genes needed to resist stress conditions. This response, initially observed during amino acid starvation, was called the stringent response. Today, the term stringent response is commonly used to describe a broad set of stress responses driven by the accumulation of this nucleotide (reviewed in Reference 111).

(p)ppGpp is a unique small-molecule global transcriptional regulator that interacts directly with RNAP (6, 28, 141). In *E. coli*, synthesis of

Table 1 Stress induction of the RpoS regulon

Stress	Upstream mediator of stress signal	Level of regulation	Regulator of RpoS	Phenotype of *rpoS* mutant	Effector genes[a]	References
Late exponential phase	Multiple signals; high (p)ppGpp	Translation	Multiple; ND	ND[b]	ND	(76, 91)
		Degradation	Multiple: IraD, IraP?			
Phosphate starvation	SpoT/(p)ppGpp	Degradation	IraP	ND	ND	(16, 91)
DNA damage/UV	ND	Degradation	IraD	Sensitivity to AZT, H_2O_2	*dps*	(15, 90, 101)
Magnesium starvation	PhoQ/PhoP	Degradation	E.c: IraM S.t.: IraP	ND	ND	(15, 149)
Carbon starvation	Competition for protease	Degradation	ClpXP titration?	Loses viability faster, long-term starvation	ND	(44, 76)
Low temperature	DsrA promoter	Translation	DsrA	Slow growth, loss of viability at low temperature	*otsAB*	(66, 130)
High temperature	Secondary structure of mRNA?	Translation/ mRNA stability	RNase III resistance	Loss of viability at high temperature	*otsAB*; *dps*	(55, 89, 101)
	ND	Degradation	DnaJ, DnaK			(55, 95)
Surface	Rcs phosphorelay	Translation	RprA	Poor biofilm formation	ND	(29, 84)
Aerobic/energy status	ArcB/ArcA negative regulation	Transcription	ArcA repression	ND	ND	(92)
		Translation	ArcZ			(86)
		Degradation	RssB state			(92)
Low pH	ND	Degradation?	ND	Loss of viability at low pH	*hdeA*, *cfa*, *dps*, *gadC*	(11, 101, 155)
Constricted amino acid flux	ND	ND	ND	Acidification	ND	(152)
High pH	ND	ND	ND	Loss of viability at high pH	*dps*	(101)
High osmolarity	ND	Translation	RprA	Loss of viability at high osmolarity	*otsAB*	(55, 76, 81, 99, 134)
	ND	Degradation	ND			(99)
Oxidative stress	ND	ND	ND	H_2O_2 sensitive	*xthA*; *dps*	(2, 35, 122)
N starvation	ND	Activity	Sigma competition?	ND	ND	(65, 85, 107)

[a]Genes included here have been shown, in experiments testing deletions or inactivation, to contribute to resistance to the listed stress.
[b]ND, not determined. For some stresses, there is no specific evidence of RpoS induction, although resistance to the stress is seen, dependent on RpoS (i.e., oxidative stress).

(p)ppGpp is carried out by RelA, during amino acid starvation, and the bifunctional SpoT protein during other stress treatments. RelA, the enzyme mainly responsive for (p)ppGpp synthesis, is associated with the ribosome and is activated upon amino acid starvation (111). SpoT is also responsible for degradation of (p)ppGpp. The effect of (p)ppGpp on RNAP

SpoT: a bifunctional enzyme that synthesizes but mainly degrades (p)ppGpp; plays a role in response to many kinds of non-amino acid starvation

and on gene transcription can be enhanced by DksA, a protein also able to interact with RNAP (104). Accumulation of this nucleotide regulates many genes directly and also affects the expression of other global regulators in the cell such as RpoS. Thus, it is often difficult to discriminate direct from indirect effects of (p)ppGpp.

(p)ppGpp changes promoter activity, inhibiting some classes of promoters and activating others. During rapid growth, a major part of the available RNAP is dedicated to the transcription of stable RNA (reviewed in Reference 105). However, the presence of (p)ppGpp and DksA destabilizes the open complex formed between stable RNA promoters and RNAP holoenzyme (9). As a consequence of inhibiting rRNA transcription, RNAP becomes available for the transcription of lower affinity promoters (those that need a higher amount of available RNAP to be transcribed) (9).

In parallel, (p)ppGpp induces the expression of alternative sigma factors and of the antisigma factor Rsd (33, 36). The increased availability of RNAP core and of the alternative sigma factors, simultaneously with the titration of RpoD by Rsd, favors the transcription of genes by alternative sigma factors, including RpoS (36, 145).

In vitro studies have defined the characteristics of promoters negatively regulated by (p)ppGpp (105). This regulation depends in part on the discriminator sequence of the promoter, located between the −10 box and the transcriptional start (143). Positively regulated promoters are less well understood: In some cases, data suggest direct effects on RNAP at these promoters (see *iraP* below); in other cases, the inhibition of RNAP from transcribing the extremely active rRNA operons and other stable RNA genes may increase availability for other promoters.

Many of the experiments that tie (p)ppGpp to RpoS were done by studying the properties of strains in which (p)ppGpp cannot be synthesized, as a result of deletions of both *relA* and *spoT*. Under these conditions, very little RpoS is made, and studies have suggested that, in *E. coli*, (p)ppGpp affects RpoS at every level: transcription, translation, proteolysis, and activity. In all cases, there is a positive correlation between (p)ppGpp and RpoS levels and activity, so that these two responses act as two aspects of a more general stress response. Effects on transcription of *rpoS* have been less fully studied and are not further discussed here.

Effects of (p)ppGpp on rpoS translation. Several studies have shown a regulatory effect of (p)ppGpp on *rpoS* translation (22, 126). In fact, DksA was also implicated in *rpoS* regulation (126, 156) even before the functional link between (p)ppGpp and DksA had been established. The most compelling evidence of a specific mechanism for how (p)ppGpp may improve *rpoS* translation comes from work in *Shigella flexneri*. In *S. flexneri*, DksA has a direct positive effect on *hfq* transcription, which is highly enhanced in the presence of (p)ppGpp. The increased *hfq* transcription, in turn, activates *rpoS* translation, presumably because Hfq is in fact limiting for *rpoS* translation (59, 126; K. Moon & S. Gottesman, manuscript in preparation). It seems likely that this mechanism is conserved in *E. coli*; we have observed twofold-lower levels of Hfq protein in *dksA* mutants (K. Moon & S. Gottesman, manuscript in preparation). Consistent with an effect on Hfq-stimulated translation, the (p)ppGpp effect on *rpoS* translation is abolished in the absence of the inhibitory antisense structure (56).

Effects of (p)ppGpp in regulating RpoS stability: controlling antiadaptor synthesis. As described above, RpoS is very rapidly degraded under rapid growth conditions. Therefore, even if synthesis is high, the accumulation of RpoS will be limited by degradation. Starvation for C and P, which leads to increased (p)ppGpp, also stabilizes RpoS (76, 108, 133, 158, 164). The recent demonstration that expression of two of the three known antiadaptor proteins, inhibitors of RssB IraP and IraD, is positively regulated by (p)ppGpp provides a clear mechanism explaining at least some of the (p)ppGpp requirement for RpoS accumulation (16, 91).

IraP was named for its role in stabilizing RpoS after P starvation (17); *iraP* is transcriptionally induced after P starvation, and this induction depends on SpoT activity and (p)ppGpp. Because the discriminator region of the *iraP* promoter is important for (p)ppGpp-dependent regulation, it seems likely expression is under the direct control of (p)ppGpp (16). Interestingly, under conditions other than P starvation [amino acid starvation or artificial overproduction of (p)ppGpp], the level of *iraP* mRNA increases but not the level of the protein, suggesting high levels of (p)ppGpp may inhibit IraP translation (16). This further suggests that *iraP* induction would be triggered by a moderate accumulation of (p)ppGpp, which is consistent with SpoT-dependent *iraP* expression, and not under conditions that induce higher levels of (p)ppGpp.

IraD, identified in a multicopy screen for proteins that stabilize RpoS, stabilizes RpoS after DNA damage; deletions of *iraD* are more sensitive to some types of DNA damage, in an RpoS-dependent fashion (15, 90). IraD also plays a role in stabilizing RpoS as cells enter stationary phase (91). The major *iraD* promoter, P1, is induced during stationary phase, dependent on (p)ppGpp (91). The induction of *iraD* by DNA damage is also dependent on P1; the mechanism of induction is undefined but is not dependent on the SOS regulator LexA (91).

The role of (p)ppGpp in the synthesis of these two antiadaptors provides an explanation for some of the effects of (p)ppGpp on RpoS accumulation. P starvation leads to induction of *iraP*. The end of exponential phase may be the inducing signal for *iraD*; as (p)ppGpp accumulates, *iraP* may also be induced. This leaves a number of aspects of this system unexplained. If the same signal induces both *iraP* and *iraD*, are they really expressed at the same time? For instance, is *iraD* induced under P starvation? If so, why does it not contribute more to stabilization of RpoS (because a deletion of *iraP* eliminates most of the stabilization after P starvation)? If it is not induced, why not? Does it require higher or more sustained levels of (p)ppGpp, or does some other regulator contribute to induction for one or the other of these proteins? A requirement for relatively high levels of (p)ppGpp for RpoS induction was recently shown, although the contributions of IraP and IraD were not specifically tested (146).

Specific Stresses and the Role of sRNAs

In addition to the global role of (p)ppGpp in sensing and transducing starvation signals, RpoS is induced under a large variety of stress treatments that appear to be independent of (p)ppGpp and, in many cases, dependent on expression of sRNAs. In this section, for a selected subset of stress treatments, we try to link what is known about the inducing pathways to the downstream genes that help to protect from the stress. Other stresses known to induce RpoS are listed in **Table 1**.

DsrA, the RpoS low-temperature response, H-NS, and osmoprotection. One definition of a stress, as seen by the cell, may come from identifying the downstream genes that provide protection from the stress. For instance, stationary-phase cells may perceive the stress of low temperature similarly to the stress of high osmolarity, as evidenced by the observation that increasing trehalose synthesis provides protection for both.

RpoS accumulates and is active at low temperature, even in exponential phase, and this accumulation reflects increased translation, dependent on the sRNA DsrA (130). No specific low-temperature activator of DsrA is known, and thermocontrol seems to be a combined function of the suboptimal spacer and −10 sequences of the *dsrA* promoter as well as differential stability of the sRNA at different temperatures (116, 117). What is the physiological role of RpoS at low temperature? At least one set of RpoS-dependent genes, *otsAB*, is important at low temperature. The products of *otsA* and *otsB* are necessary for synthesis of internal trehalose, an osmoprotectant. *E. coli* cells subjected to cold shock accumulate high levels of trehalose but die quickly in the absence of *otsA* (66).

In other experiments, the expression of the *otsAB* operon has been shown to be necessary and sufficient for resistance to high osmolarity in stationary-phase cells. Mutants in *otsAB* are hypersensitive to high osmolarity. Cells deleted for *rpoS* and evolved to grow at high osmolarity in chemostats accumulate insertions that activate the *ots* operon (134). Combined with the observations above, this suggests that adaptation to cold shock is akin to adaptation to high osmolarity and provides a rationale for induction of RpoS at low temperatures as well as at high osmolarity. When cells are exposed to high osmolarity, rapid translational induction of RpoS is primarily dependent on DsrA (81). *otsA* mutants also partially impair the thermotolerance of stationary-phase cells (55). Thus, high levels of internal trehalose may serve to help protect proteins from denaturation at high temperature, low temperature, and high osmolarity.

DsrA also directly negatively regulates the translation of the histone-like protein H-NS, a global regulator of gene expression (77). H-NS has broad effects on transcription. H-NS directly represses RpoD-dependent transcription of many genes with both RpoD- and RpoS-dependent promoters, and RpoS is less sensitive to H-NS repression than RpoD, at least in one tested case (5, 10, 48). The net effect is that H-NS helps make expression of these genes fully RpoS dependent.

In parallel to increasing the RpoS dependency of many promoters, H-NS and related proteins negatively regulate *rpoS* translation by an unknown mechanism (10, 160) and promote its degradation (**Figure 1**). RpoS is stable in *hns* mutants, suggesting that H-NS may negatively regulate one or more antiadaptors, although which antiadaptors is not yet known (167). In *Salmonella*, an H-NS homolog, StpA, acts to repress the RpoS regulon in exponential growth by repressing expression of the *rssC* antiadaptor. RssC is most similar to the *E. coli* IraM antiadaptor (80). Whether RpoS-inducing signals other than DsrA also negatively regulate H-NS synthesis or activity is not yet known but would not be surprising.

hns mutants are impaired for growth under many conditions; this growth defect is overcome by a second mutation in *rpoS* (10). Because RpoS levels are high in *hns* mutants, this may reflect poor growth due to RNAP core competition, coupled with the higher requirement for RpoD owing to high transcription of genes usually silenced by H-NS.

Acid stress, H-NS, and PhoPQ. One of the most striking phenotypes of stationary-phase cells is their resistance to low pH. In particular, *E. coli* and *Shigella* become resistant to pH 2.5 when they enter stationary phase or when they are preshocked with an intermediate pH (reviewed in Reference 166). While the acid-resistance response is complex and involves many regulators, there is a clear requirement for RpoS for the acid resistance of stationary-phase cells (11, 78). Required for this acid resistance are RpoS-dependent genes (155), including *gadC*, a gene of the glutamate-dependent low-pH-resistance network of genes, *hdeAB*, encoding pH-regulated periplasmic chaperones, and *cfa*, encoding a gene for cyclopropane fatty acid synthesis. *hdeAB* was termed an H-NS-dependent operon; it is strongly repressed by H-NS (161). HdeA binds proteins at low pH, helping to protect misfolded or damaged proteins from aggregating and then releasing them to refold when the pH returns to neutral (140). The product of the *cfa* gene leads to modification of phospholipids that render the cell able to tolerate low pH (27). In addition to these genes, *gadY*, encoding an sRNA that positively regulates the synthesis of two other regulators of the *gad* genes, GadX and GadW, is also RpoS dependent (103, 142).

The pathway of induction of RpoS by low pH is not fully understood. One component of induction is RpoS stabilization (11), now known to be mediated by the two-component PhoPQ system, the activator of the antiadaptor IraM (15). The importance of coupling PhoPQ to RpoS is demonstrated by the observation that in *Salmonella* another antiadaptor, IraP, is expressed not only from the (p)ppGpp-dependent promoter found in *E. coli* but also from a second

PhoPQ-dependent promoter (149). PhoPQ is activated at low Mg and in response to antimicrobial peptides, both encountered in eukaryotic hosts. Another two-component system, EvgA and EvgS, participates in activation of the low-pH response, at least in part by activating PhoPQ and thus increasing synthesis of the IraM antiadaptor (37). PhoP directly and indirectly regulates some of the pH-resistance genes and *phoP* mutants are sensitive to acid stress (170). Some of these effects seem likely to involve the PhoPQ-dependent stabilization of RpoS.

RprA, the Rcs phosphorelay, and biofilm maturation. An important developmental pathway for *E. coli* and most other bacteria is the development of biofilms. Cells in biofilms are relatively quiescent, and *rpoS* mutants are defective in formation of mature biofilms. Some but not all genes expressed in biofilms are RpoS dependent (1, 32, 60). Thus, it is not surprising that some of the signals leading to RpoS accumulation are also tied to biofilm development.

One regulator of RpoS that may be important in biofilms is the sRNA RprA. RprA is activated by the Rcs phosphorelay (84). The Rcs phosphorelay, important for biofilm formation, is necessary for expression of the genes needed for colanic capsule synthesis in *E. coli* K12, activates a large number of genes, and directly represses others, including genes for flagellar synthesis (reviewed in References 29 and 83). It is not clear which ligand or physiological condition(s) activates the Rcs phosphorelay. However, the genes in the Rcs regulon and the observation that the system is induced by solid surfaces suggest that the Rcs phosphorelay may be important during the development of biofilms, when motility needs to be shut down and capsule accumulates. RpoS is needed for biofilm maturation, and too much or too little RpoS interferes with biofilm development (40). It is attractive to suggest that RprA activation of *rpoS* translation helps to ensure properly timed expression of RpoS during biofilm maturation. Increased osmolarity also induces the Rcs system (43, 129), and in the absence of DsrA, RprA activates *rpoS* translation under osmotic-shock conditions (81).

The interaction of the Rcs phosphorelay and RpoS extends well beyond RprA. There are in fact a number of other genes for which the Rcs phosphorelay may provide regulation, both directly, via RcsB, and indirectly, via stimulation of RpoS. *ftsZ*, encoding the primary septation protein, is synthesized from multiple promoters, among them the RcsB-dependent pZ1 promoter and the RpoS-dependent pQ1 promoter (8, 41). *osmB*, an osmotically inducible gene encoding a periplasmic protein of unknown function, is transcribed from two promoters, one activated by RcsB and one dependent on RpoS (18). As mentioned below, LrhA represses RpoS, probably via an unidentified sRNA. RcsB also represses *lrhA* expression; thus, in this case, RcsB acts via the LrhA-dependent pathway to stimulate RpoS expression (106).

Another regulatory protein, CsgD, reinforces the connection between RpoS and biofilm development. CsgD, a transcriptional factor important for biofilm development, promotes stabilization of RpoS by increasing synthesis of the IraP antiadaptor (50) (**Figure 1**). Thus, at the level of both RpoS translation and stabilization, induction of biofilm formation leads to increased RpoS accumulation. CsgD is under complex transcriptional and post-transcriptional control, including a promoter stimulated by RpoS, providing a positive-feedback loop for promoting RpoS accumulation under specific conditions (58, 102).

Too much and too little oxygen: OxyR and ArcB/ArcA negatively regulate RpoS. Two sRNAs, OxyS and ArcZ, play opposite roles in regulating RpoS levels as a function of the oxygen/energy status of the cell. Stationary-phase cells are resistant to oxidative damage such as hydrogen peroxide, and one of the first RpoS-dependent genes found was *katE*, encoding a catalase (123). A parallel pathway for resistance to hydrogen peroxide is regulated by OxyR, which in turn activates another catalase gene, *katG* (reviewed in Reference 136). OxyS,

an sRNA transcribed in response to OxyR activation, negatively regulates *rpoS* translation (165). Thus, expression of the OxyR response to oxidative damage or the RpoS response may be mutually exclusive, although they share some effector genes, including *dps*. Possibly, the specific OxyR response is preferable when the cell encounters oxidative stress during exponential growth, and the RpoS response is used only when RpoS is induced in response to stationary-phase transition and/or another stress.

ArcZ, the third sRNA activator of RpoS, is negatively regulated by the two-component system ArcB/ArcA (86). ArcZ is processed to a 56-nt-long RNA from the 3′ end of the initial 120-nt transcript; the 56-nt form of ArcZ can pair with the same region of the *rpoS* hairpin as DsrA and RprA and activate translation similarly (86). Under aerobic conditions, ArcZ is well expressed, but under anaerobic conditions, ArcA represses ArcZ and thus, indirectly, downregulates *rpoS* translation (86). As with RprA and DsrA, ArcZ sRNA serves as a connector between the general stress response and a more specific response mediated by the ArcB/ArcA two-component system. ArcA and ArcB have previously been implicated in negative regulation of RpoS at the level of transcription (ArcA represses the *rpoS* promoter) and degradation (ArcB-P stimulates RpoS degradation, by increased phosphorylation of RssB) (92). Although the relative contributions of each of these pathways is not yet clear, this concerted downregulation of RpoS by Arc suggests that the cell does not perceive anaerobiosis as a stress or that the specific Arc response is preferable to the RpoS-dependent response.

CONCLUSIONS

The discussion above touches on some of what we now know about the RpoS system, how it is induced, and what genes regulated by RpoS may do for the cell. The combination of years of work on *E. coli* as an experimental system and the advent of genome-wide approaches to monitoring gene expression have left us with a long list of stresses that induce the RpoS system and genes that get induced. However, we are still far from having a complete picture of the network for this response. We study RpoS in the laboratory, under the conditions easiest for us to manipulate. We hope that a full understanding of the functions of downstream genes will allow a better understanding of the stresses that the bacteria has evolved to respond to outside the laboratory.

In the paper that first named RpoS, Lange & Hengge-Aronis (75) noted that many of the resistance phenotypes of the RpoS system were also dealt with by very specific stress systems independent of RpoS. In fact, we now see that, whereas some specific stress systems collaborate and overlap with RpoS (for instance, the Rcs phosphorelay, the PhoPQ two component system, and the (p)ppGpp-dependent stringent response), others act as negative regulators of RpoS or RpoS-dependent genes. Included in the latter are the OxyR-dependent oxidative stress response and the ArcA/ArcB system. Others are likely to be found. For instance, stationary-phase cells show resistance to DNA damage, yet this response is induced independent of the classic LexA-dependent SOS response (90). PhoBR, the two-component system for P starvation, does not contribute significantly to the induction of RpoS after P starvation via IraP (17). What the advantage or disadvantage of keeping these systems distinct (for instance, not simultaneously inducing the specific and the general response for oxidative stress resistance) is not yet clear, but it may reflect only the need to minimize sigma factor competition, allowing either the RpoD-dependent systems or the RpoS-dependent systems to induce fully.

Why does acid stress or P starvation lead to a general stress response, giving overall resistance to many other stresses? Are these conditions usually encountered by the cell either during an infection and development of a biofilm or when cells reach stationary phase? The general stress response may in fact have evolved as a

response to stationary phase in which multiple nutrients are limiting and toxic compounds are accumulating. If these stresses are always associated as the end of growth nears, it would make sense for signals of one sort to be interpreted by the cell as evidence for the coming disaster. Inducing a specific stress response may not be feasible when a cell is truly starving. One possible solution for invoking a general rather than a specific response would be if induction of the general stress response is organized such that multiple stresses must coincide for a robust response.

Recent progress has uncovered many of the mechanisms for regulating RpoS rapidly in response to changes in growth conditions. However, new inducing signals continue to be found, and many are not fully understood (**Table 1**). Unexplored are the physiological roles of most of the RpoS-dependent genes; the advent of genome-wide studies of phenotypes may begin to uncover their functions.

SUMMARY POINTS

1. The RpoS regulon is induced when RpoS levels rise, primarily by changes in translation of *rpoS*, mediated by sRNAs, and by changes in RpoS degradation, mediated by antiadaptors, each induced under different conditions.
2. There is major overlap between the (p)ppGpp-induced stringent response and the RpoS response.
3. Many RpoS-dependent genes can also be read by RpoD under specific circumstances; H-NS plays a significant role in repressing the basal expression of these genes by RpoD and also negatively affects RpoS. The combined effect is to limit expression of the RpoS regulon to conditions of stress.
4. Overlap between genes necessary for resistance to high osmolarity as well as low and high temperature suggests common intracellular stresses under these conditions.
5. RpoS and some of the downstream genes have key roles in biofilm formation; the inducing environment in this case may be contact with a surface.
6. The Rcs phosphorelay and the PhoPQ two-component system contribute to RpoS induction through downstream connectors (sRNAs and antiadaptors), whereas the Arc and OxyR systems, among others, negatively regulate RpoS.

FUTURE ISSUES

1. What are the "core" RpoS regulon genes, dependent only on RpoS? What stress do these genes deal with, and does this define the broadest role for RpoS?
2. What RpoS-dependent genes are necessary for surviving starvation for C, P, and N, and how do they contribute to survival?
3. A variety of experiments and isolation of *E. coli* from the environment suggest that there are many conditions under which the cell accumulates mutations in *rpoS*. Why then is RpoS retained, when is it critical, and when is it bad for the cell?
4. What other stresses feed into the RpoS system? Have all inducing signals been defined?

5. When the RpoS system is induced, the cell is resistant to a wide range of stress and starvation treatments. During exponential growth, when RpoS is not induced, the cell has specific responses to specific stresses. To what extent do the effector genes for specific and global stress responses overlap, and if they do, how is specificity achieved? If they do not, why is it preferable to have different responses?
6. To what extent do the lessons learned from the *E. coli* general stress response extrapolate to other bacteria, and what can be learned from other bacteria to help in understanding the *E. coli* response?

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Contents