Annu. Rev. Microbiol. 1994. 48:53–80

THE ROLE OF THE SIGMA FACTOR σ^S (KatF) IN BACTERIAL GLOBAL REGULATION

Peter C. Loewen
Department of Microbiology, University of Manitoba, Winnipeg, MB R3T N2 Canada

Regine Hengge-Aronis
Department of Biology, Universität Konstanz, Postfach 5560, D-78434 Konstanz 1, Germany

KEY WORDS: starvation, stationary phase, survival, osmoprotection, virulence

CONTENTS

0066-4227/94/1001-0053$05.00

ABSTRACT

The protein encoded by *katF* (also known as *nur, appR, csi-2, abrD,* and *rpoS* in various alleles) has been biochemically confirmed to be an alternate sigma transcription factor and renamed σ^S. Its synthesis is controlled transcriptionally and posttranscriptionally by as yet undefined mechanisms that are active well into stationary phase. σ^S controls a regulon of 30 or more genes expressed in response to starvation and during the transition to stationary phase. Proteins in the regulon, many of which have not been characterized, enhance long-term survival in nutrient-deficient medium and have a diverse group of functions including protection against DNA damage, the determination of morphological changes, the mediation of virulence, osmoprotection, and thermotolerance. Differential expression of subfamilies of genes within the regulon is effected by supplementary regulatory factors, working both individually and in combination to modulate activity of different σ^S-dependent promoters.

INTRODUCTION

In their natural environment, bacteria frequently experience nutrient limitation and, as a result, suffer prolonged periods of negligible growth or apparent dormancy. Only the occasional nutrient-rich condition can support the rapid growth rates normally seen in the laboratory. To allow bacteria to survive the long periods of starvation and still be capable of periods of rapid growth, specialized metabolic states have evolved. For example, in some gram-positive organisms such as *Bacillus subtilis,* the survival strategy involves differentiation to spores, whereas other organisms such as *Escherichia coli* enter what is normally referred to as stationary phase. In this apparently nondifferentiated state, significant physiological changes have taken place that allow the cells, like spores, to survive a wide variety of environmental stresses including starvation, near-UV radiation, hydrogen peroxide, heat, and high salt. Entry into stationary phase is accompanied by changes in the synthesis patterns of glycogen and trehalose, which are important for long term survival of the cell under a variety of conditions, and by changes in gross cell morphology from filamentous to spherical and compact. The phenomena associated with starvation and stationary phase have been the subjects of recent reviews (54, 57).

At one time stationary phase was considered a period of metabolic inactivity, during which the cell simply waited for the next period of nutrient surplus. However, bacterial adaptation to nutrient limitation during the entry into and the maintenance of stationary phase is characterized by an intricate series of metabolic changes that continue over a very long time. All adaptive responses, whether to changing nutrients or to various stresses, involve a series of genetic switches that control the metabolic changes taking place. A common regulatory mechanism involves the modification of sigma factors whose primary role is to bind to the core RNA polymerase conferring promoter specificity. The main, or housekeeping, sigma factor (σ^{70} in *E. coli* and σ^{43} in *B. subtilis*) is responsible for transcription from a majority of the promoters either independently or in conjunction with other regulatory transcription factors. Alternate sigma factors have different promoter specificities, directing expression of specialty regulons involved in the heat-shock response, the chemotactic response, phage expression, and sporulation, in which a cascade of sigma factors mediate progression through the different stages of the sporulation process (30, 40, 70, 75, 106). The similarities in physiological properties between sporulation and stationary phase, and the intricate involvement of sigma factors in *B. subtilis* sporulation, suggested that one or more alternate sigma factors might be found mediating the stationary-phase adaptive process in *E. coli*. To date one such sigma factor has been identified and is named σ^S for starvation sigma factor.

σ^S is encoded by the gene most commonly referred to as *katF*, the first allele of which was identified 15 years ago. This gene has been known as *nur*, *katF*, *appR*, *csi-2*, and *abrD* a result of independent studies of different phenotypes, and finally *rpoS*, following its characterization as a sigma factor. Once investigators recognized commonality of the various alleles, they realized that mutations in *rpoS* (*katF*) resulted in a pleiotropic phenotype with a rapid loss of viability under various conditions, including exposure to near-UV radiation, elevated temperature, high salt, hydrogen peroxide, and prolonged starvation. σ^S or KatF has emerged as a key factor in the control of an overlapping group of stress-response proteins induced during the transition to stationary phase. This review focuses on its identification and characterization as a sigma factor, the regulation of its synthesis, its role in the regulation of expression of other genes, and the identity and function of its target genes (for previous reviews see 42, 43).

IDENTIFICATION AND CHARACTERIZATION OF *rpoS* (*katF*)

Genetic Characterization

Several alleles of *rpoS* (*katF*) imparting different phenotypes were isolated and studied over a 10-year period before the gene product was identified. The

first report came from Tuveson & Jonas in 1979 (115), who found it in the guise of a gene, *nur,* that enhanced resistance to near-UV radiation. They had observed that near-UV sensitivity did not correlate with far-UV sensitivity in strains carrying two different *recA* alleles and resolved the contradiction by assuming and then demonstrating the presence of a second mutation at *nur,* a new locus that mapped near *recA* at around 58 min (115). *nur* controlled near-UV sensitivity while *recA* was responsible for far-UV sensitivity. Significantly, the resistance to near-UV was growth-phase dependent; *nur*-dependent resistance began to increase as cells entered stationary phase and continued to increase into late stationary phase (92, 115).

The gene *katF* was first reported in 1984 to map at 59.0 min and to be required for the synthesis of the catalase hydroperoxidase II (HPII) but not the induction of catalase-peroxidase HPI (67). Shortly thereafter, *nur* was confirmed as an allele of *katF* (98). The gene *appR,* required for the synthesis of an acid phosphatase from *appA* and imparting pleiotropic properties to mutants, was mapped at 59 min (111) and was also subsequently shown to be an allele of *katF* (113). Confirmation of a role for the *katF* gene product in the expression of *xthA,* which encodes exonuclease III and is required for resistance to near-UV radiation, provided a mechanism for the involvement of *katF* (*nur*) in the mediation of resistance to near-UV radiation (97).

A search for carbon starvation–inducible genes using *lacZ* fusions yielded an *E. coli* containing a *csi-2::lacZ* (*csi* = carbon starvation inducible) fusion. This strain responded to starvation by synthesizing β-galactosidase (from the fusion) but not by inducing H_2O_2 resistance, thermotolerance, or *appA* expression. Furthermore, as many as 16 proteins that normally appeared in stationary-phase cells were not synthesized in the *csi-2*-containing mutant (62). A comparison of restriction patterns and map location confirmed that *csi-2* was an allele of *katF.* Finally, a study of *aidB,* part of the adaptive response to methylation, identified *abrD* (for *aidB* regulatory gene) as a suppressor of mutations that made *aidB* hyperinducible and subsequently showed that *abrD* is an allele of *rpoS* (M Volkert, unpublished information). Thus, several groups had identified the single locus known variously as *nur, katF, appR, csi-2,* and *abrD* and found that it affected a diverse group of cellular functions, suggesting that it encoded a regulatory protein controlling a significant number of genes involved in the stationary-phase response. Physical characterization of the locus to identify the gene product and its regulatory role then became an important next step.

Physical Characterization

The *katF* gene was cloned at the same time as *katE* (80), and sequence analysis of the *katF* gene revealed an open reading frame of 1086 bp, thus predicting the sequence of a 362–amino acid, 41.5-kDa protein (79). This predicted size was

consistent with the size of the predominant protein, approximately 44 kDa, found in maxicell extracts of cells harboring the *katF* plasmid. Subsequent work revealed a single-base deletion in the *katF* sequence that shortened the open reading frame to 1026 bp, which indicated a 342–amino acid, 38-kDa protein (48, 110). Of the two potential start codons separated by 13 codons at the 5′ end of the open reading frame (79), the second, or internal, ATG was confirmed as the initiation codon (68). The predicted sequence of the KatF protein revealed that it was closely related to the σ^{70} family of transcription factors. This observation suggested a possible role for the protein (79) and led to the suggestion that *katF* and its alleles be renamed *rpoS* and that the KatF protein be named σ^S (62). Strengthening this hypothesis was a detailed comparison of 31 σ factor sequences from various bacteria that included σ^S in the second of three evolutionarily related groupings of sigma factors in the σ^{70} family (69). Group 1 contained the primary sigma factors; group 2 contained the alternate sigma factors, including σ^S, that had diverged moderately from σ^{70}; and group 3 contained alternate sigma factors that had diverged more extensively. In particular, the DNA-binding regions of σ^S and σ^{70} were very similar, suggesting that similar promoters might be recognized by the two factors.

Biochemical Characterization

A direct demonstration that KatF binds to the core RNA polymerase (E) and enhances transcription of *katF*-dependent genes was required to confirm the sigma-factor role predicted by the sequence. Surprisingly, the first verification of $E\sigma^S$-directed in vitro transcription came from the typical σ^{70} promoters *lac*UV5p, *trp*p, and *dnaQ*p2, confirming the predicted overlap (69) in promoter sequences recognized by the two sigma factors. Efforts to demonstrate σ^S-dependent transcription from *katE*p, known to be *katF* dependent, were unsuccessful (110; PC Loewen, unpublished data), suggesting that yet another factor may be involved in activating *katE* transcription. In a parallel study, both $E\sigma^{70}$ and $E\sigma^S$ recognized the promoters of two *katF*-dependent genes, *bolA* and *xthA* (84), further confirming the sigma-factor property of KatF and the overlap in holoenzyme recognition sequences. The identification of a σ^S-specific promoter, transcribed by $E\sigma^S$ but not by $E\sigma^{70}$, upstream of the *fic-pabA* operon led to the proposal of three classes of housekeeping promoters: $E\sigma^S$ specific, $E\sigma^{70}$ specific, and those recognized by either $E\sigma^S$ or $E\sigma^{70}$ (110).

Variability in katF

Because of the diversity of genes controlled by *katF*, the diversity of phenotypes among the *katF* alleles is not surprising. However, phenotypes also varied among alleles. For example, the *appR190* allele, which was identified on the basis of its reduced acid phosphatase levels, did not seem to affect *katE* expression, whereas a *katF::*Tn*10* mutation reduced both acid phosphatase and

HPII production (113). This is probably a reflection of a subtle change in promoter specificity in the *appR190* allele compared to a complete absence of σ^S in the transposon mutant. The apparent prevalence of *katF* mutations in laboratory strains (113) suggests that the gene is easily mutated but that the mutations may not manifest themselves in changes to all expected phenotypes because of the diversity of promoters recognized by σ^S and overlap with σ^{70} promoters. The σ^S genes of several laboratory strains have been sequenced, revealing the locations of a number of mutations and transposon insertions, but no single region with a high frequency of mutation was identified (48). These authors noted differences from the original sequence, but the fact that the same changes were found in each strain probably reflects errors in the original sequence rather than new alleles.

What may be the most interesting mutation and its associated phenotypic change occur spontaneously in stationary-phase cells (129). Prolonged incubation of a culture for up to 10 days in stationary phase resulted in a phenotype of selective growth advantage over cells that had recently, within one day, entered stationary phase. Additionally, these mutants were partially deficient in the production of HPII. This phenotype was traced to a mutation in *rpoS* (labeled *rpoS819*) comprising a 46-bp duplication near the end of the gene that caused a frame shift replacing the final four amino acids with 39 additional residues (129). Whereas cells normally had to experience stationary-phase conditions for up to 10 days to obtain the growth advantage, cells containing the 46-bp duplication exhibited the growth advantage after just one day in stationary phase. Not all of the *rpoS* alleles with the selective-growth phenotype contained this duplication, leading to the conjecture that other types of mutations in *rpoS* may elicit a similar phenotype. At least one *rpoS819*-dependent gene involved in the selective growth phenomenon has been identified but not characterized (129). A mutation identical to the *rpoS819* allele was identified independently (48) in a laboratory strain exhibiting small quantitative differences in several *rpoS*-related phenotypes, including reduced levels of HPII and greater sensitivity to acid and heat. Evidently, changes in gene expression take place even late in stationary phase, and extension of the σ^S sequence that might perturb the adjacent helix-turn-helix region 4.2, a feature common to all sigma factors and believed to interact with the –35 region of promoters, is probably one mechanism for the mediation of these changes. The mechanism responsible for duplicating this small portion of the *rpoS* gene, and possibly for removing the duplication during the return to an actively growing state, has not been characterized.

rpoS (katF) Homologues in Other Bacterial Species

The principal sigma factor in *E. coli* encoded by *rpoD* has homologues in most bacterial species studied. In addition, the presence of multiple sigma factors

controlling different regulons in response to different environmental demands seems to be the rule rather than the exception, suggesting that *rpoS* homologues should be found in other bacterial species exhibiting similar starvation responses. Among the enteric bacteria, the sequence and genome similarity is well known, and *rpoS* homologues have been identified in *Salmonella typhimurium* (32), *Klebsiella pneumoniae* (R Hengge-Aronis, unpublished information), and *Shigella flexneri* (105). In less closely related bacterial species, only *Pseudomonas aeruginosa* has been identified as containing a *rpoS*-like gene (K Tanaka & H Takahashi, in preparation). Thus, at least in gram-negative bacteria exhibiting similar responses to starvation, σ^S homologues may prove to be a common regulatory component.

CONTROL OF THE CELLULAR LEVELS OF σ^S (KatF)

Transcriptional Control

Considerable evidence accumulated prior to 1990 suggested that *katF* was expressed primarily in stationary phase. As already noted, *nur*-mediated protection against near-UV radiation depends on cells having attained stationary phase (92, 115), while the synthesis of both HPII (66) and acid phosphatase (112) is turned on only as cells enter stationary phase. However, researchers had no direct assay for RpoS expression until fusions of its promoter to *lacZ* were constructed (81) or isolated from λp*lac*Mu insertions (62, 101). As expected, *rpoS* (*katF*) transcriptional expression in cells growing in rich medium was low in early exponential phase and increased gradually two- to threefold during exponential phase. The most substantial increase to 20-fold above basal levels occurred during and after the transition to stationary phase (62, 81). In minimal medium, unexplained strain-specific differences have arisen. In one report, transferring cells from rich to minimal medium resulted in a 10-fold increase in expression during the lag period prior to the commencement of growth, and *rpoS* expression was elevated throughout exponential phase, increasing only slightly upon entry to stationary phase (81). A second report indicated that *rpoS* expression did not turn on in minimal medium except in a Δ*cya* background in which *rpoS* expression also significantly increased early in exponential phase. This result was attributed to conditions in which limiting but sufficient nutrients allowed the expression of *rpoS*, but did not allow for rapid growth (62). Increasing the growth rate of the Δ*cya* strain by adding cAMP resulted in a decrease in *rpoS* expression. Starvation could also elicit an increase in *rpoS* expression depending on the missing component. For example, starvation for carbon resulted in limited expression, whereas starvation for nitrogen (81) or phosphate (62) resulted in maximal

expression of *rpoS.* Anaerobiosis reduced the growth rate and stimulated *rpoS* expression during exponential growth in Luria-Bertani (LB) medium (81).

Like the expression of *katE* (100), expression of *rpoS* is induced by a dialyzable, heat-stable factor in spent LB medium (81). Attempts to identify the component by adding fermentation by-products to fresh LB medium were unsuccessful, but aromatic acids such as benzoic acid induced expression (81). Subsequent experiments showed that other weak acids such as acetate and propionate could induce expression in minimal medium, leading to the suggestion that *rpoS* expression is modulated by the internal pH of the cell (101)—a factor in the expression of several uncharacterized genes in *E. coli* (89, 104). Although accumulated fermentation by-products and a lower medium pH in stationary phase may cause a lower internal pH and result in induction of *rpoS,* elevated expression in anaerobic LB or during slow growth in minimal medium cannot be explained in this way, but can instead be explained in terms of subtle changes in ΔpH or $\Delta\psi$ (65, 103). Whether two or more independent mechanisms control *rpoS* transcription, or if the correlation between lower pH and increased expression is simply that, a correlation, and not a direct cause-and-effect relationship, remains undetermined.

Further complicating the picture are the contradictory reports about the role of cAMP in *rpoS* expression. One phenotype of the *appR* mutations allows a *crp*-containing mutant to grow on succinate (on which it normally cannot grow) (111). Subsequent studies showed that a Δ*cya* mutation increased *rpoS* expression in minimal medium and that the addition of cAMP could reverse this action (62). Higher levels of *rpoS* expression were observed in exponential-phase Δ*cya* and Δ*crp* cells in LB medium, in stationary phase Δ*cya* cells in LB medium (PC Loewen, unpublished information), and in wild-type cells growing in minimal succinate as compared with minimal glucose medium (R Hengge-Aronis, unpublished information). Thus cAMP-CRP may negatively control *rpoS* transcription (R Lange & R Hengge-Aronis, in preparation).

The level of induction of *rpoS* during growth, however, was no different on either glucose or succinate minimal medium, although *katE* expression was reduced in glucose medium (81). Also, a Δ*cya* mutation caused reduced *rpoS* expression during exponential-phase growth and no induction during the onset of glucose starvation (73). One explanation for these divergent results involving *cya* and *crp* mutations is that cAMP and CRP may act indirectly, and their influence may depend on the genetic background in the strains used or on the composition of the growth medium.

Autoregulation of *rpoS* transcription during entry into stationary phase in LB medium is not an important factor (81), although a twofold increase in expression in the absence of σ^S was noted in minimal medium (101). However,

σ^S may indirectly affect translational expression of *rpoS* during starvation in minimal medium (73; R Lange & R Hengge-Aronis, in preparation).

The signaling role of guanosine tetraphosphate (ppGpp) as both a negative and positive effector of transcription in the bacterial response to starvation for amino acids and carbon has been well documented (15). Recently, several starvation-inducible genes in *S. typhimurium* were found to be positively regulated by ppGpp during both carbon and nitrogen, but not phosphate, starvation, suggesting a link between ppGpp control and the more general starvation phenomenon involving *rpoS* (108). These results implicated two independent pathways for ppGpp action, but the possible involvement of σ^S was not studied. A link between σ^S and ppGpp was suggested by the observation that *relA spoT* strains of *E. coli,* lacking any detectable ppGpp, have a pleiotropic phenotype similar to *rpoS* strains (35). Further study revealed that σ^S levels determined using Western immunoblot analysis responded normally to starvation but at a much reduced level in the *relA spoT* mutant. Increasing ppGpp levels, either by inducing a plasmid-encoded *relA* gene or by impairing decay, caused a corresponding increase in σ^S with similar kinetics of synthesis. Thus, ppGpp may be a positive regulator of σ^S synthesis at the level of *rpoS* transcription but is not involved in the specific starvation-induction system. This hypothesis suggests a simple model for *rpoS* transcriptional regulation in which starvation lowers the amino acid pools, resulting in ppGpp synthesis and, as a result, σ^S synthesis. Clearly this is not the whole picture because it does not explain posttranscriptional factors affecting σ^S synthesis (see below), nor do we know how ppGpp modulates RNA polymerase activity at the *rpoS* promoter.

Structure of the rpoS (katF) Promoter Region

The initial sequence of *rpoS* extended upstream only 300 bp from the first possible initiation codon. Within that region, several possible promoter motifs for σ^{70}- and σ^F-containing holoenzymes were evident (79), and segments of this region contained active promoters in operon and protein fusions to *lacZ* used to assess transcriptional and translational control factors (68, 73, 81).

More recently, the sequence was extended upstream 1400 bp (Y Takayanagi, K Tanaka & H Takahashi, in preparation), and four promoters have been identified, one of which corresponded to the promoter included in the fusions. All four promoters were induced during the transition to stationary phase, suggesting that they were all subject to the same regulatory mechanism. The principal promoter responsible for more than 75% of transcription in fusion systems was promoter P2, which is located within an adjacent open reading frame approximately 550 bp upstream from the open reading frame of *rpoS*. It is preceded by a σ^{70} promoter sequence motif, although both σ^{70}- and

σ^S-containing holoenzymes recognized the promoter in vitro. In addition, two potential weak CRP-binding domains were identified in the P2 region, thus providing a possible explanation for the observed effects of *cya* and *crp* on *rpoS* transcription. The identification of four promoters, the putative CRP-binding sites, and recognition by at least two sigma factors provide the necessary components for a complicated multilayered regulatory picture, not inconsistent with much of what has already been observed and not properly explained.

Posttranscriptional Control

Differences between *katE* and *rpoS* transcription patterns prompted the proposal of control elements supplementary to *rpoS* transcriptional controls (81). An hour or two prior to a cessation of growth brought on by glucose starvation in minimal medium, both transcriptional and translational expression of *rpoS* were induced, but translation increased by eightfold while transcription increased by only twofold, leading to the conclusion that posttranscriptional control was the primary determinant in the appearance of σ^S (73). This observation was confirmed in rich medium (68), where transcriptional expression increased 20-fold throughout the exponential-growth phase into stationary phase while translational expression was induced 100-fold only during the transition to stationary phase. Even in the presence of benzoic acid, which fully induced *rpoS* transcription, σ^S translation was only partially induced (68). These results, which were based on β-galactosidase assays in transcriptional and translational fusions, were confirmed with Western analysis (35).

The mechanism of posttranscriptional control is, as yet, undetermined, but investigators have noted at least two possibilities for the control of translation and protein stability. A segment of RNA immediately downstream of the initiation AUG in *rpoS* is required for efficient translation of σ^S (68), and RNA further downstream has been implicated in controlling the translation of σ^{32} (51, 82) and σ^S (R Lange & R Hengge-Aronis, in preparation). An alternative explanation—that the stability of σ^S may change during growth—comes from Western analyses and pulse chase experiments that have revealed that σ^S is degraded much more rapidly in exponential phase (half-life 1.5 to 2.5 min) as compared with stationary phase (half-life 25 min) (Y Takayanagi, K Tanaka & H Takahashi, in preparation; R Lange & R Hengge-Aronis, in preparation). How this increase in stability, in the face of higher protease levels in stationary-phase cells, is mediated remains to be determined. Furthermore, an increase in protease resistance cannot explain the patterns of β-galactosidase synthesis from fusion studies, further implicating the concept of a multilayered control system. Indeed, induction of transcription, translation, and σ^S stabilization may occur sequentially during the transition to stationary phase (R Lange & R Hengge-Aronis, in preparation).

GENE REGULATION BY σ^S (KatF)

Consensus Sequence for σ^S-Recognized Promoters

In general, alternative sigma subunits of RNA polymerase are synthesized or activated under certain environmental conditions and can temporarily or partially replace the main vegetative sigma factor as the promoter-recognizing subunit. Consequently, other sets of promoters characterized by sequences that deviate from the consensus for the vegetative sigma factor are transcribed. This selection may be mediated, in part, by residues in regions 2.3, 2.4, and 4.2 of the sigma factor (40). For example, vegetative sigma factors such as σ^{70} in *E. coli* and σ^{43} in *B. subtilis* recognize similar or identical promoter sequences and exhibit a high degree of homology in these regions, whereas alternative sigma factors recognizing different sequence determinants diverge from σ^{70} in these regions (40).

σ^S is exceptionally homologous to σ^{70} in regions 2.3, 2.4, and 4.2, suggesting that it may be a second vegetative sigma factor rather than an alternative sigma factor, and that σ^S promoters may be quite similar to σ^{70} promoters. Indeed σ^S-dependent transcription was first observed in vitro for templates carrying the typical σ^{70}-dependent promoters, *lac*UV5p, *dnaQ*p2, *trp*p (110), and *tac*p (K Tanaka, personal communication). These promoters are recognized in vitro by both $E\sigma^{70}$ and by $E\sigma^S$, but others are exclusively transcribed by either $E\sigma^{70}$ (mostly stringently controlled promoters), or $E\sigma^S$ (*fic*p) (110).

Most σ^S-controlled genes have been identified by comparing their expression (often assayed as *lacZ* or *phoA* gene fusions) in wild-type and σ^S-deficient strains. Presently, more than 20 genes or operons are known to be under σ^S control (see below), but even though there is mounting evidence for a multilayered, cascade-regulation mechanism within the *rpoS* regulon (see below), all the respective promoters are probably not directly recognized by σ^S. Therefore, derivation of a consensus sequence for σ^S-dependent promoters will be difficult because of the similarity to σ^{70} promoters and because of sequence variations arising from the involvement of additional regulatory factors. The unambiguous identification of a consensus for σ^S recognition will require mapping and sequencing of many more σ^S-controlled promoters and confirmation that they are indeed directly σ^S dependent. Whether a gene is predominantly transcribed by $E\sigma^S$ or $E\sigma^{70}$ in vivo is probably not only determined by the promoter-recognition specificities of the two sigma factors, but also by the relative amounts of the two holoenzymes, by the type of core enzyme involved, and by the activity of *cis*-acting regulatory factors that differentially influence binding and open complex formation at a given promoter by the two holoenzymes. In vitro systems containing only purified

RNA polymerase and a single sigma factor do not reflect such complexity and therefore do not produce the degree of specificity observed in vivo.

The promoter for *osmY* illustrates this complexity. Osmotic induction does not require σ^{70} (127), but in the absence of σ^S, expression and induction still occur at a low level, indicating that σ^{70} can take over to some extent (60). A comparison of *osmY*p to several other σ^S-dependent promoters (*glgS*p2, *treA*p, *osmB*p2, and *cyxA*p) and in vitro Eσ^S-transcribed promoters (*fic*p, *dnaQ*p2, *lac*UV5p, *rnaI*p, and *trp*p) suggests a −10 consensus (TATACT) very similar to the σ^{70} consensus (43). However, a −35 consensus is not apparent, suggesting that a requirement for additional activating factors may be as common for σ^S-controlled genes as for σ^{70}-dependent genes.

A small group of σ^S-regulated promoters including *xthA*p, *bolA*p1 (43), and apparently *otsB*p (109) have sequences that seem to have diverged from a σ^{70}-like consensus. An indirect regulation by σ^S of these genes seems to be ruled out by the finding that *bolA*p1 and *xthA*p can be transcribed by purified Eσ^S in vitro (84). However, only a putative −35 consensus (GTTAAGC) can be derived from these promoters, with the −10 regions being more heterogeneous (43). One might speculate that the sequence GTTAAGC is the −35 region recognized by σ^S while the −10 consensus is obscured in these cases by overlap with binding sites for regulatory proteins. Indeed, *bolA*p1 contains the gearbox motif (CGGCAGTA) (1, 2) in its −10 region. Despite being σ^S-dependent in vivo (97), both *bolA*p1 and *xthA*p were transcribed by both Eσ^S and Eσ^{70} in vitro, albeit with slight differences in specificity and transcript size (84).

The gearbox sequence may be a potential recognition site for σ^S (120), but its presence does not correlate with σ^S control. For example, the gearbox-containing *mcbA* promoter is σ^{70} dependent (11, 61), and most σ^S-dependent genes do not have gearboxes in their promoter regions (43). In order to avoid confusion, the term gearbox promoter should be restricted to promoters that possess the gearbox motif and exhibit inversely growth rate–regulated expression (e.g. *bolA*p1, *mcbA*p, and *ftsQ*p1). Because this pattern of expression is exactly the opposite of positive growth-rate control as observed for ribosomal genes (37), a tempting speculation is that the regulatory mechanisms involved are related. For instance, a yet to be identified gearbox-binding protein could be a repressor protein that is itself under positive growth-rate regulation and that can act on σ^S-dependent (*bolA*p1) as well as on σ^{70}-dependent (*mcbA*p) promoters.

A recently published study reported that DNA fragments carrying the promoters of *xthA*, *bolA*, and *katE* exhibited temperature-dependent gel mobilities indicative of intrinsic DNA curvature (31). No evidence for DNA bending was found for *mcbA*p, which is very similar to *bolA*p1 but is σ^{70} dependent. In conclusion, the authors proposed that DNA curvature upstream of *rpoS*-dependent promoters may compensate for the absence of a clearly defined consensus for recognition by σ^S (31). However, since bent DNA regions are often

found upstream of strong σ^{70}-dependent promoters (93), and the artificial insertion of bending sequences at such a position can increase the rate of in vitro transcription initiation (34), DNA curvature upstream of σ^S-dependent promoters probably contributes to promoter strength rather than to σ^S-specific recognition.

Cascade Regulation Within the rpoS Regulon

Several σ^S-dependent genes identified to date (*bolA, appY, dps*) encode regulatory proteins. Both BolA (1) and AppY (5) contain helix-turn-helix motifs typical of DNA-binding proteins, and Dps forms highly ordered complexes with DNA (4). Genes controlled by these secondary regulators are therefore under indirect regulation by σ^S, putting σ^S at the top of a branched regulatory cascade. For example, the penicillin-binding protein PBP6 is controlled by σ^S through BolA (1, 14), and expression of two adjacent operons, *hyaABCDEF* (T Atlung, personal communication) and *cyxABappA* (5, 23), is controlled by σ^S through AppY. Furthermore, AppY and the operons under its control represent a subclass of σ^S-dependent genes that are also anaerobically induced (23) by a mechanism involving ArcA and Fnr (T Atlung, personal communication). The *appY* subregulon thus illustrates one of the advantages of cascade regulation, namely that the secondary regulatory proteins can be the point of integration of additional signals that influence the expression of only certain subsets of genes within the larger regulon.

Dps is a regulatory protein still synthesized in late (3-day-old) stationary-phase cells that controls the synthesis of as many as 23 strongly starvation-induced proteins (4), none of which have yet been identified. Dps does not exhibit a typical DNA-binding motif but forms highly structured complexes with DNA in vitro. Therefore, like H-NS (90) and possibly Lrp (22), Dps may be one of several abundant DNA binding proteins that have chromosome-organizing as well as regulatory functions.

The organization of the σ^S regulon in functional cascades may also be the basis for a temporal order of σ^S-dependent events occurring after the onset of starvation. When expression is assayed in rich LB medium (for which most data are available), the products of *otsBA* (45) and *bolA* (1, 11, 61) are expressed early in the transition phase, whereas those of *osmY* (123), *treA* (45), and *glgS* (44) are induced somewhat later. The expression of *osmB* (45, 49) and the *S. typhimurium spv* genes cloned into *E. coli* (32, 85) is stimulated even later, at the beginning of the stationary phase, and acid phosphatase (AppA) activity, indirectly σ^S controlled via AppY, starts to increase only after at least two hours in stationary phase (38). Finally, the cellular concentration of Dps continuously increases over several days after the onset of starvation (4). Depending on the relative affinities of Dps for binding sites in the chromosome, the increasing level of Dps could result in differential and temporal

effects on chromosomal domain organization and, therefore, gene expression in stationary phase.

σ^S *Is a Central Component in a Larger Regulatory Network*

Evidence for the differential regulation of subfamilies of genes within the *rpoS* regulon in response to environmental signals besides starvation indicates that other regulatory components are involved in the control of σ^S-dependent genes. We have noted the influence of ArcA and Fnr on AppY synthesis, the effect of hyperosmolarity on *osmY,* and the temporal order in the expression of σ^S-controlled genes. Moreover, regulatory patterns of certain σ^S-dependent genes including *osmY* (60) and *mcc* (76) are retained in *rpoS* null mutants, although the absolute levels of expression are greatly reduced. Several additional factors that operate independently from σ^S on the same genes have been identified.

INVOLVEMENT OF cAMP-CRP cAMP-CRP has been implicated as an activator for many stationary phase–induced genes. Approximately two-thirds of the carbon starvation–induced proteins originally identified by two-dimensional gel electrophoresis are not expressed or at least are not starvation induced in *cya* or *crp* mutants (39). Some of these genes have been characterized, including several *cst* genes (10, 102), *glgS* (44), *csiD,* and *csiE* (123), as well as *mcc* (76). The transcriptional start sites of *cstA* (102) and *glgS* (44) have been mapped, and CRP boxes have been found upstream of the promoters at locations consistent with activator function. However, when assayed in *cya* mutant backgrounds, the external addition of cAMP during exponential phase did not immediately stimulate the expression of some of these genes, and a positive effect can be seen only after the onset of starvation (10, 123). Thus, another factor present only in starved cells is required for induction; σ^S (or a certain minimum cellular concentration of it) may be the stationary phase–specific factor for *glgS, csiD,* and *csiE.*

On the other hand, some *pex* genes (72) and several σ^S-dependent genes (123) are under negative control by cAMP-CRP. This factor repressed expression of a chromosomal *osmY::lacZ* transcriptional fusion during all growth phases in minimal medium but only early in the transition to stationary phase in rich medium. Therefore, other factors are responsible for keeping *osmY* expression low in cells growing rapidly in rich medium, and repression by cAMP-CRP is relieved during entry into stationary phase by some unknown mechanism. cAMP-CRP acts independently from σ^S, because repression by exogenous cAMP could still be observed in a *cya rpoS* double mutant. A potential CRP binding site with very good homology to the consensus (as well as a second site with somewhat less homology on the opposite strand) overlaps with the −10 region and transcriptional start site of *osmY,* indicating that

cAMP-CRP most likely acts as a direct repressor (60). The correlation of global expression patterns with gene location on the Kohara phages (55) has revealed that repression by CRP under conditions of glucose starvation is very common (17) , and *osmY* may be a prototype of a rather large regulatory class of genes.

INVOLVEMENT OF Lrp Lrp (leucine-responsive regulatory protein) has been characterized either as a negative regulator of genes required for rapid growth in rich medium or as an activator of biosynthetic genes required for slow growth in minimal medium (83). A recent study showed that Lrp represses *osmY* expression in minimal medium, thereby implicating both σ^S induction and relief of Lrp repression as overlapping control mechanisms (60). In rich medium, Lrp acts as a negative transition-state regulator of *osmY* in a way that seems similar to the role of CRP (60). In addition, Lrp activates *csiD* expression during starvation (C Marschall & R Hengge-Aronis, unpublished results). While few data exist concerning the regulation of *lrp,* Lrp is synthesized at low levels in rich medium and the expression increases in minimal medium despite negative autoregulation (64).

INVOLVEMENT OF IHF Integration host factor (IHF) is a sequence-specific histone-like protein with a wide range of functions. Originally identified as a factor involved in site-specific recombination during lysogenization of phage λ, it also participates in transposition, in plasmid replication, partitioning, and transfer, as well as in gene expression as either a positive or negative effector of transcription initiation. In all these processes, IHF is part of higher-order nucleoprotein structures, and its DNA-bending activity could be crucial for proper arrangement of the other components (28, 33).

Investigations recently demonstrated that IHF negatively controls *osmY* expression and, like Lrp and CRP, acts as a transition-state repressing regulator. Sequences similar to the IHF binding–site consensus overlap the −10 region and the transcriptional start site of *osmY* (60). A role for IHF in growth phase–dependent gene regulation is, however, not restricted to σ^S-controlled genes. For instance, stationary-phase induction of *mcbA* [the first gene in the σ^{70}-controlled (11) operon for microcin B17 production and immunity] is abolished in IHF-deficient mutants (76). The finding that the intracellular level of IHF increases during entry into stationary phase (H Gilladi, personal communication) suggests that IHF may be generally involved in the expression of growth phase–regulated genes.

INVOLVEMENT OF H-NS The expression of the abundant histone-like protein H-NS (or H1) is further stimulated during transition into stationary phase (25, 116), but this induction does not require σ^S (25). H-NS has been implicated as a repressor of *mcc* (plasmid-encoded genes for microcin C7 production and

immunity) (76) and *csgA* (which encodes the structural protein of curli surface fibers) (87). Expression of both genes is reduced in *rpoS* mutants and is restored in *rpoS hns* double mutants, indicating that σ^S, or a σ^S-controlled protein, interferes with H-NS-mediated repression. *hns* mutations were recently found to increase the expression of *osmY* and several other stationary phase–induced proteins, implicating H-NS as an exponential phase repressor of these genes (M Barth & R Hengge-Aronis, unpublished results).

COMBINATIONS OF FACTORS Not only are many factors besides σ^S involved in the regulation of stationary phase–induced genes, but different genes are under the control of different combinations of regulators. For example, *osmY* is under positive control by σ^S but is negatively controlled by cAMP-CRP, Lrp, IHF, and H-NS (60); *mcc* requires σ^S and cAMP-CRP as positive factors, but is repressed by H-NS (76); and *csiD* is positively controlled by σ^S, cAMP-CRP, and Lrp (C Marschall & R Hengge-Aronis, unpublished results). As a result, σ^S-dependent genes can have very different patterns of expression. For example, *osmY* exhibits a strong stationary-phase induction in LB medium but a weak increase of expression upon carbon starvation in minimal medium, whereas *csiD* is equally well induced under both conditions. The advantage of such a modular system is that many regulatory patterns can be produced by using a relatively small number of regulators, especially if these regulators can act either positively or negatively.

How the architecture of the control regions of the target genes reflects this complex control by σ^S and various regulator proteins is slowly becoming evident. The few data available suggest that the organization of these regulatory regions will be complex and diverse. *osmY*, for instance, has a single σ^S-recognized promoter and several potential binding sites for additional regulatory factors upstream or overlapping the promoter (60, 127). *glgS*, on the other hand, is transcribed from four transcriptional start sites, only one of which is σ^S dependent, whereas the other three transcripts require cAMP-CRP, with a CRP-box present upstream (44). Several other σ^S-controlled genes, including *bolA* (1), *appA* (23), and *osmB* (49), also have more than one promoter. The presence of multiple promoters in the control regions of *rpoS*-regulated genes could indicate a high degree of overlap in the control mechanisms of the σ^S regulon and other regulons or stimulons.

Interestingly, most of the additional regulatory factors mentioned above are not specific activators or repressors, but occur at copy numbers of at least several thousand per cell. Binding sites for these proteins are found at high frequencies in the chromosome, suggesting some of them could also be involved in chromosome organization. These possibilities include H-NS and IHF, which have long been classified as histone-like proteins (28), and Lrp (22). Chromosomal organization could change considerably during entry into sta-

tionary phase because of the activities of these DNA-binding proteins. Examples of such phenomena include alterations in the negative superhelical density of reporter plasmids after several hours in stationary phase (7) and the condensation of the nucleoid of *Vibrio* sp. in starved cells (6, 78). These changes could be intimately connected to alterations in gene expression and, therefore, to modulation of the expression of σ^S-dependent genes.

Influence of Core Modification

Another level of complexity has been introduced into attempts to explain changes in promoter specificity by the identification of three new chromatographically separable forms of RNA polymerase in stationary-phase cells (91). The nature of the changes to the core components have not been identified, but they seem to involve charge rather than size modifications. The significance of these changes in the core is that they elicit changes in promoter recognition independent of the sigma factor bound thus complicating the definition of $E\sigma^S$-recognized promoters.

σ^S *in Exponential-Phase Gene Expression*

The primary role of σ^S seems to lie in mediating the transition to stationary-phase metabolism in response to starvation, but this regulator also mediates exponential-phase expression of some genes. The expression of *xthA* was initially considered to be unique among σ^S-dependent genes in that it increased by 50% during exponential phase and dropped slightly as the cells entered stationary phase (97). However, the uninduced levels of *katG* expression (not the H_2O_2-induced levels) that essentially parallel the pattern of *xthA* expression (66) are also controlled by *rpoS* (A Eisenstark, personal communication; H Schellhorn, personal communication), and *aidB* expression in exponential phase cells is *rpoS* dependent (M Volkert, personal communication). σ^S must therefore be present and active in exponential-phase cells despite low levels of transcription (62, 81) and translation (68, 73). Western analysis has confirmed this supposition (35, 110).

If *xth*Ap and *katG*p are recognized by $E\sigma^S$, why doesn't an increase in expression occur in stationary phase when the levels of $E\sigma^S$ are higher? The simplest explanation lies in competition among promoters for $E\sigma^S$ whereby secondary transcription factors synthesized in stationary phase increase the affinity of $E\sigma^S$ for other promoters relative to *katG*p and *xth*Ap. Alternatively, an exponential phase–specific positive effector, a stationary phase–specific negative effector, or modifications to core enzymes in stationary phase may affect promoter selection.

σ^S *in the Control of Osmotically Regulated Genes*

The genes *otsBA*, *treA*, and *osmB* were initially identified as osmotically regulated genes (12, 36, 41) before their stationary phase induction (45, 49)

and σ^S-dependence (45) were recognized. The expression of *bolA* (46) and *osmY* (*csi-5*) (46, 128) is also stimulated by an increase in medium osmolarity with little change in growth rate. The hyperosmotic induction of these, and 18 additional proteins identified on two-dimensional gels as having similar regulation, was abolished or at least strongly reduced in *rpoS* mutants (46). However, because a number of σ^S-controlled genes are not hyperosmotically induced, including *csiD* and *csiE* (C Marschall & R Hengge-Aronis, unpublished information), as well as *csgA*, which requires low osmolarity for full expression (87), the osmotically regulated genes represent a subfamily within the larger σ^S regulon.

Transcription and translation initiation of *rpoS* remained essentially unchanged after osmotic upshift (46), but osmotic control of translational elongation was suggested by the induction of a late translational fusion, encoding a hybrid protein with the first 247 amino acids of σ^S after osmotic upshift (R Lange & R Hengge-Aronis, in preparation). The osmotic signal and regulatory mechanism have not been identified. Increased σ^S levels in osmotically stressed cells is not the only factor involved in osmotic regulation, however, because *osmY* is still induced 10-fold in a *rpoS* mutant albeit at a lower level and with slower kinetics (46, 60). An inverted repeat upstream of the −35 region was implicated in osmotic control of *osmB*p (49) but has not been found in *osmY*p (60, 127), suggesting a multiplicity of mechanisms for osmotic control. Lrp, CRP, and IHF are not involved (60) and other factors including supercoiling and intracellular K^+ concentration that control other osmotically induced genes (47, 94) will have to be investigated.

σ^S-REGULATED GENES AND THEIR FUNCTIONS

The preceding discussion clearly indicates that σ^S controls a large group of genes. Analysis of two-dimensional gels has revealed that 18 (62) to 32 (74) proteins are missing or are present in smaller amounts, as well as the presence of some new proteins, in *rpoS* mutants. In addition, other genes may be transcribed by both $E\sigma^S$ and $E\sigma^{70}$ that cannot be identified on gels. Several of the identified σ^S-controlled proteins encompass an extremely diverse group of functions, as outlined below, illustrating the global importance of σ^S in cellular metabolism.

Prevention and Repair of DNA Damage: katE, katG, xthA, dps, aidB

Of the many protective and repair mechanisms that have evolved in *E. coli*, several are σ^S dependent. Protection against H_2O_2 involves *katE* and *katG*, encoding the catalases HPII and HPI respectively, which destroy H_2O_2 before it can cause damage. Dps forms nuclease-resistant complexes with DNA that

presumably also protect the cells from killing by H_2O_2 (4). Alternatively, several enzymes, including *xthA*-encoded exonuclease III, can repair damage to DNA caused by H_2O_2 and near-UV radiation (24, 99) while a pathway involving *aidB* repairs methylation damage to DNA. These five genes all require σ^S for some aspect of their expression, but each exhibits a different pattern of control, illustrating the complexity possible with overlapping $E\sigma^S$ and $E\sigma^{70}$ promoters.

HPII synthesis is σ^S dependent (66, 81, 100) and may involve an as yet undefined factor (81, 110). Induced as cells enter stationary phase (66), production of this protein enhances survival (74, 81). The induction of HPI synthesis that occurs in response to H_2O_2 is σ^S independent (16, 66, 77), but the twofold increase in HPI in late exponential phase (66) appears to be controlled by $E\sigma^S$ (A Eisenstark, personal communication; H Schellhorn, personal communication). The *rpoS*-dependent pattern of exonuclease III synthesis from *xthA* (97) essentially parallels uninduced HPI synthesis by increasing 50% in exponential phase and dropping slightly in stationary phase, with no significant increase equivalent to the increase in HPII levels. Whereas *xthA*p is recognized by both $E\sigma^S$ and $E\sigma^{70}$ in vitro (84), it is σ^S dependent in vivo, indicating a higher promoter specificity in vivo. In addition to the σ^S-independent induction pathway for *aidB* involving *ada* (121, 122), basal levels of *aidB* expression in exponential-phase cells and *aidB* induction during anaerobiosis or treatment with acetate at low pH are σ^S dependent (M Volkert, unpublished information). Expression of *dps* is unique in that it continues for several days in stationary phase but has not been characterized further (4).

Cell Morphology: bolA, ficA

Two σ^S-dependent genes that influence cell morphology, *bolA* and *fic*, have been identified. Overexpression of *bolA* caused cells to be osmotically stable, compact, and spherical, typical of stationary-phase morphology (3). The expected absence of morphological change during the transition to stationary phase has not been reported for a *bolA* mutant but has been observed in *rpoS* mutants (61). *bolA* encodes a 13.5-kDa protein required for expression of the penicillin-binding protein PBP6, which plays a role in cell-wall synthesis at the septum (8) possibly through stabilization of the peptidoglycan (118). In vivo, promoter *bolA*p1 is growth-phase regulated (1) and σ^S dependent (61), whereas in vitro it is recognized preferentially, but not exclusively, by $E\sigma^S$ (84), providing another example of enhanced promoter selectivity in vivo. The as yet undefined role of BolA in cell division requires *ftsZ*, a cell-division gene from the *ftsAQZ* operon, the expression of which is growth-phase controlled (2, 26) but apparently not σ^S dependent.

A second σ^S-dependent (110) morphological determinant, *fic*, is situated immediately upstream of *pabA* (114) and encodes a protein whose function is

unknown but that shares sequence similarity with other cell-division proteins (53). *fic*-containing mutants are short rods compared to the longer wild-type strains, and presence of the wild-type gene promotes filamentation in conjunction with cAMP (52).

Modulation of Virulence Genes in Salmonella, Shigella, and E. coli

Expression of the *Salmonella* plasmid virulence genes (*spv*), including *spvR* and the operon *spvABCD* required by a number of *Salmonella* species to confer lethal disease (9, 59), increases as cells enter stationary phase (58). Transcription from *spvA*p is σ^S-dependent both in *S. typhimurium* (32) and *E. coli* (85) and is modulated by the positive effector SpvR, a member of the LysR family of regulatory proteins. *rpoS*-containing mutants are 1000-fold less virulent than a wild-type, plasmid-containing strain (32) and 100-fold less virulent than the plasmid-cured parent (32). Whether this is a result of reduced survival or of changes in expression of other unidentified virulence genes was not determined. In *Shigella flexneri,* a *rpoS* mutation makes the cells much more sensitive to acid and much less infective, presumably because the cells can no longer survive passage through the gut to the intestines (105). This observation implies that *rpoS* controls the synthesis of proteins that protect the cell from low pH. σ^S may also play a role in the interaction of *E. coli* with the host intestinal tissue through its control of *csgA*. Certain *E. coli* strains produce surface fibers called curli, the subunit of which is encoded by *csgA*. These fibers play a role in the binding of fibronectin and laminin and thus in the adhesion of eukaryotic tissue (88). Consistent with σ^S control of *csgA* expression, *rpoS* mutants do not bind fibronectin.

Osmoprotection and Thermotolerance: otsBA, treA, csiD, htrE

The σ^S-dependent *otsBA* operon encodes trehalose-6-phosphate synthase (OtsA) and trehalose-6-phosphate phosphatase (OtsB), which together produce large amounts of trehalose in osmotically stressed cells (36). Trehalose acts as an osmoprotectant (21), and consequently, *otsBA* (36) and *rpoS* (45) mutants exhibit an osmosensitive growth phenotype. Trehalase, which degrades trehalose either excreted by *E. coli* during growth or taken up as a carbon source in high osmolarity medium (13, 109), is encoded by *treA* (12), the expression of which is also σ^S dependent (45). Trehalose also acts as a thermoprotectant in a wide variety of species (119), presumably through its membrane and protein-protecting properties (19, 20, 63, 124). Not surprisingly mutations in *otsBA* impart a heat-sensitive phenotype in stationary phase following growth in minimal medium (43, 45).

A mutation in the σ^S-dependent *csiD* gene (123) causes a similar heat-sensitive phenotype in addition to causing pleiotropic changes in protein patterns

on two-dimensional gels, which suggests a regulatory function (R Hengge-Aronis, unpublished information). Mutations in yet another σ^S-regulated gene, *htrE,* which produces proteins of unknown function but of similar sequence to the pilin porin PapC, impart a heat-sensitive phenotype and sensitivity to high osmolarity (95).

Because thermosensitivity of the mutants described above is always significantly less than that of a *rpoS* mutant, σ^S-dependent stationary-phase thermotolerance is mediated by the products of several genes. Furthermore, it is distinct from adaptive thermotolerance, mediated by σ^{32}- and σ^E-controlled heat-shock genes (29, 117, 125), which is normal in *rpoS* and *otsBA* mutants (45).

Glycogen Synthesis: glgS

Even though *rpoS*-containing mutants are deficient in glycogen synthesis (62) and the *glgCAP* operon is induced in stationary phase (86), *glgCAP* is not controlled by σ^S (44). Other σ^S-regulated genes must therefore be involved in glycogen synthesis, and one such gene, *glgS,* was isolated on a plasmid that strongly stimulated glycogen production. Although *glgS* is regulated by both σ^S and cAMP-CRP from separate promoters, *glgS* expression depends entirely on σ^S under conditions of maximal glycogen synthesis, excess glucose, and no nitrogen (44). GlgS is a small 7.8-kDa protein that is glycosylated in vivo (D Fischer & R Hengge-Aronis, unpublished data), which suggests it may play a role as a primer for glycogen synthesis, similar to that of glycogenin in animal cells (107).

Anaerobically Induced Genes: appY, appCBA, hyaABCDEF

This family of genes is σ^S dependent; is moderately induced in stationary phase, particularly by phosphate starvation; and is strongly induced by a shift to anaerobic conditions. The synthesis of AppY, a member of the AraC family of regulatory proteins and a positive regulator of *appCBA* and *hyaABCDEF* (5), is controlled by both σ^S and ArcA (T Atlung, unpublished information). *appCB* (also called *cyxAB*) and *hyaABCDEF* direct the synthesis of a third cytochrome oxidase and hydrogenase 1, respectively. *appA* encodes an acid phosphatase (112) that was recently shown to be a phytase that degrades phytic acid [myo-inositol(1, 2, 3, 4, 5, 6)hexakisphosphate] (38), a major phosphate-storage form in seeds and grains as well as a chelator of cations (96).

Membrane and Cell Envelope Functions: osmB, osmY, cfa

OsmB is a small lipoprotein localized in the outer membrane (50) that seems to play a role in cell surface alterations taking place in stationary phase (49). OsmY is a major constituent of the periplasmic space in high osmolarity medium (128) or in stationary phase (123) and may be involved in capsule

formation (127). None of the known *rpoS* phenotypes are exhibited by an *osmY* mutant (N Henneberg & R Hengge-Aronis, unpublished information). Cyclopropane fatty acid (CFA) synthase, encoded by *cfa,* produces CFAs that are nonessential under laboratory conditions and that have an unknown physiological function in phospholipid bilayers. One of the promoters upstream of *cfa* is stationary-phase induced and σ^S dependent (71).

The TrpR Repressor-Binding Protein WrbA

WrbA, which purification has shown as tightly bound to TrpR, enhances the binding of TrpR to its operator in vitro (126). Expression of *wrbA* increases during the transition to stationary phase and is controlled by two promoters, one of which is σ^S dependent (R Somerville, personal communication). WrbA may therefore enhance repression of the *trp* operon under starvation conditions.

Synthesis and Excretion of Microcin C7: The mcc Genes

The σ^S-dependent synthesis of microcin C7, a peptide antibiotic that inhibits protein synthesis, is induced from plasmid-encoded genes during entry into stationary phase (27, 56). Stationary phase–induced synthesis of microcins seems to be common and may confer a competitive advantage to the producing strain in a starvation situation. The synthesis of microcin B17 is also induced in stationary phase (18) but is not σ^S dependent (11, 61).

Genes of Unknown Function: csiE, csiF, pex

The expression of *csiE* and *csiF,* which were identified in a search for chromosomal stationary phase–induced *lacZ* fusions, is partially reduced in *rpoS*-containing mutants. The mutations did not affect long-term carbon starvation survival or confer any other apparent phenotypes (123). The Pex proteins were identified by two-dimensional electrophoresis as being induced by starvation but unaffected by cAMP-CRP (72). A subsequent study showed that expression of several Pex proteins is σ^S dependent (74), but to date the only *pex* genes identified are *pexA,* which is identical to *otsB* (109), and *pexB,* which is allelic with *dps* (R Kolter, personal communication).

SUMMARY AND PERSPECTIVES

The identification of σ^S and characterization of its role in cellular metabolism has been a major step forward in understanding stationary-phase gene expression. σ^S controls expression of many stationary-phase and, to a lesser extent, exponential-phase proteins in conjunction with various combinations of supplementary regulatory factors. The genes for these proteins exhibit a variety of expression patterns and a corresponding diversity of phenotypes. Additional layers of complexity have been superimposed on this already complicated

picture by four additional observations: sequence determinants in σ^S and σ^{70} promoters are very similar, such that a single promoter can be recognized by both holoenzymes; multiple forms of the core RNA polymerase exhibit different promoter specificities despite a common sigma factor; σ^S expression and accumulation change throughout the cell cycle; and changes in the sequence of *rpoS* occur late in stationary phase, resulting in a modified σ^S.

Evidently, diversity rather than uniformity will be the rule for describing regulatory patterns involving σ^S. The complexity of the control patterns so far described has raised almost as many questions as have been answered. Future work to address these questions will proceed in several directions. Many of the genes controlled by σ^S have yet to be identified and, if work to date is any indication, each new gene or operon identified may be controlled by a unique pattern of regulatory factors. The factors controlling σ^S expression and accumulation have not been fully defined. The mechanism of sequence duplication within *rpoS* giving rise to protein sequence changes remains unknown, as does the effect of these changes on gene expression. The challenge will be for workers to distinguish among the various overlapping levels of control to clearly define individual control elements and their impact.

Literature Cited

1. Aldea M, Garrido T, Hernandez-Chico C, Vicente M, Kushner SR. 1989. Induction of a growth-phase-dependent promoter triggers transcription of *bolA*, an *Escherichia coli* morphogene. *EMBO J.* 8:3923–31
2. Aldea M, Garrido T, Pla J, Vicente M. 1990. Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters. *EMBO J.* 9:3787–94
3. Aldea M, Hernandez-Chico C, de la Campa AG, Kushner SR, Vicente M. 1988. Identification, cloning, and expression of *bolA*, and *ftsZ*-dependent morphogenes of *Escherichia coli*. *J. Bacteriol.* 170:5169–70
4. Almiron M, Link A, Furlong D, Kolter R. 1992. A novel DNA binding protein with regulatory and protective roles in starved *Escherichia coli*. *Genes Dev.* 6:2646–54
5. Atlung T, Nielsen A, Hansen FG. 1989. Isolation, characterization, and nucleotide sequence of *appY*, a regulatory gene for growth phase-dependent gene expression in *Escherichia coli*. *J. Bacteriol.* 171:1683–91
6. Baker RM, Singleton FL, Hood MA. 1983. Effects of nutrient deprivation on *Vibrio cholerae*. *Appl. Environ. Microbiol.* 46:930–40
7. Balke VL, Gralla JD. 1987. Changes in the linking number of supercoiled DNA accompany growth transitions in *Escherichia coli*. *J. Bacteriol.* 169:4499–4506
8. Begg KJ, Takasuga A, Edwards DH, Dewar SJ, Spratt BG, et al. 1990. The balance between different peptidoglycan precursors determines whether *Escherichia coli* cells will elongate or divide. *J. Bacteriol.* 172:6697–6703
9. Beninger PR, Chikami G, Tanabe K, Roudier C, Fierer J, Guiney DG. 1988. Physical and genetic mapping of *Salmonella dublin* virulence plasmid pSDL2. Relationship to plasmids from other *Salmonella* strains. *J. Clin. Invest.* 81:1341–47

10. Blum PH, Jovanovich SB, McCann MP, Schultz JE, Lesley SA, et al. 1990. Cloning and in vivo and in vitro regulation of cyclic AMP-dependent carbon starvation genes from *Escherichia coli*. *J. Bacteriol.* 172:3813–20
11. Bohannon DE, Connell N, Tormo A, Espinosa-Urgel M, Zambrano MM, Kolter R. 1991. Stationary-phase-inducible "gearbox" promoters: differential effects of *katF* mutations and role of σ^{70}. *J. Bacteriol.* 173:4482–92
12. Boos W, Ehmann U, Bremer E, Middendorf A, Postma P. 1987. Trehalase of *Escherichia coli*. *J. Biol. Chem.* 262: 13212–18
13. Boos W, Ehmann U, Forkl H, Klein W, Rimmele M, Postma P. 1990. Trehalose transport and metabolism in *Escherichia coli*. *J. Bacteriol.* 172:3450–61
14. Buchanan CE, Sowell MO. 1982. Synthesis of penicillin-binding protein 6 by stationary-phase *Escherichia coli*. *J. Bacteriol.* 151:491–94
15. Cashel M, Rudd KE. 1987. The stringent response. In Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology,* ed. FC Neidhardt, JL Ingraham, KB Low, B Magasanik, M Schaechter, HE Umbarger, pp. 1410–38. Washington DC: Am. Soc. Microbiol.
16. Christman MF, Morgan RW, Jacobson FS, Ames BN. 1985. Positive control of a regulon for defenses against oxidative stress and some heat shock proteins in Salmonella typhimurium. *Cell* 41:753–62
17. Chuang SE, Daniels DL, Blattner FR. 1993. Global regulation of gene expression in *Escherichia coli*. *J. Bacteriol.* 175:2026–36
18. Connell N, Han Z, Moreno F, Kolter R. 1987. An *E. coli* promoter induced by the cessation of growth. *Mol. Microbiol.* 1:195–201
19. Crowe JH, Crowe LM, Carpenter JF, Rudolph AS, Aurell-Wistrom C, et al. 1988. Interactions of sugars with membranes. *Biochim. Biophys. Acta Biomembr. Rev.* 947:367–84
20. Crowe, JH, Crowe LM, Chapman D. 1984. Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science* 223:701–3
21. Csonka LN. 1989. Physiological and genetic responses of bacteria to osmotic stress. *Microbiol. Rev.* 53:121–47
22. D'Ari R, Lin RT, Newman EB. 1993. The leucine-responsive regulatory protein: more than a regulator? *Trends Biochem. Sci.* 18:260–63
23. Dassa J, Fsihi H, Marck C, Dion M, Kieffer-Bontemps M, Boquet PL. 1992. A new oxygen-regulated operon in *Escherichia coli* comprises the genes for a putative third cytochrome oxidase and for pH 2.5 acid phosphatase (*appA*). *Mol. Gen. Genet.* 229:342–52
24. Demple B, Halbrook J, Linn S. 1983. *Escherichia coli xth* mutants are hypersensitive to hydrogen peroxide. *J. Bacteriol.* 153:1079–82
25. Dersch P, Schmidt K, Bremer E. 1993. Synthesis of the *Escherichia coli* K12-nucleoid-associated DNA-binding protein H-NS is subjected to growth phase control and autoregulation. *Mol. Microbiol.* 8:875–89
26. Dewar SJ, Kagan-Zur V, Begg KJ, Donachi WD. 1989. Transcriptional regulation of cell division genes in *Escherichia coli*. *Mol. Microbiol.* 3:1371–77
27. Diaz-Guerra L, Moreno F, SanMillan JL. 1989. *appR* gene product activates transcription of microcin C7 plasmid genes. *J. Bacteriol.* 171:2906–8
28. Drlica K, Rouviere-Yaniv J. 1987. Histone-like proteins of bacteria. *Microbiol. Rev.* 51:301–19
29. Erickson JW, Gross CA. 1989. Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: a second alternate σ factor involved in high-temperature gene expression. *Genes Dev.* 3: 1462–71
30. Errington, J. 1993. *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. *Microbiol. Rev.* 57:1–33
31. Espinosa-Urgel M, Tormo A. 1993. σ^S-dependent promoters in *Escherichia coli* are located in DNA regions with intrinsic curvature. *Nucleic Acids Res.* 21: 3667–70
32. Fang FC, Libby SJ, Buchmeier NA, Loewen PC, Switala J, et al. 1992. The alternative σ factor KatF (RpoS) regulates *Salmonella* virulence. *Proc. Natl. Acad. Sci. USA* 89:11978–82
33. Friedman DI. 1988. Integration host factor: a protein for all reasons. *Cell* 55: 545–54
34. Gartenberg MR, Crothers DM. 1991. Synthetic DNA bending sequences increase the rate of in vitro transcription initiation at the *Escherichia coli lac* promoter. *J. Mol. Biol.* 219:217–30
35. Gentry DA, Hernandez VJ, Nguyen LH, Jensen DB, Cashel M. 1993. Synthesis of the stationary-phase sigma factor, σ^S, is positively regulated by ppGpp. *J. Bacteriol.* 175:7982–89
36. Giaever HM, Styrvold OB, Kaasen I, Storm AR. 1988. Biochemical and genetic characterization of osmoregulatory

trehalose synthesis in *Escherichia coli*. *J. Bacteriol.* 170:2841–49
37. Gourse RL, deBoer HA, Nomura M. 1986. DNA determinant of rRNA synthesis in *E. coli:* growth rate dependent regulation, feedback inhibition, upstream activation, antitermination. *Cell* 44:197–200
38. Greiner R, Konietzny U, Jany KD. 1993. Purification and characterization of two phytases from *Escherichia coli*. *Arch. Biochem. Biophys.* 303:107–13
39. Groat RG, Schultz JE, Zychlinski E, Bockman AT, Matin A. 1986. Starvation proteins in *Escherichia coli:* kinetics of synthesis and role in starvation survival. *J. Bacteriol.* 168:486–93
40. Gross CA, Lonetto M, Losick R. 1992. Bacterial sigma factors. In *Transcription Regulation,* ed. K Yamamoto, S McKnight, pp. 129–76. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory
41. Gutierrez C, Barondess J, Manoil C, Beckwith J. 1987. The use of transposon Tn*phoA* to detect genes for cell envelope proteins subject to a common regulatory stimulus. *J. Mol. Biol.* 195:289–97
42. Hengge-Aronis R. 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in E. coli. *Cell* 71:165–68
43. Hengge-Aronis R. 1993. The role of *rpoS* in early stationary phase gene regulation in *Escherichia coli* K12. See Ref. 54, pp. 171–200
44. Hengge-Aronis R, Fischer D. 1992. Identification and molecular analysis of *glgS,* a novel growth phase–regulated and *rpoS*-dependent gene involved in glycogen synthesis in *Escherichia coli*. *Mol. Microbiol.* 6:1877–86
45. Hengge-Aronis R, Klein W, Lange R, Rimmele M, Boos W. 1991. Trehalose synthesis genes are controlled by the putative sigma factor encoded by *rpoS* and are involved in stationary phase thermotolerance in *Escherichia coli*. *J. Bacteriol.* 173:7918–24
46. Hengge-Aronis R, Lange R, Henneberg N, Fischer D. 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. *J. Bacteriol.* 175:259–65
47. Higgins CF, Dorman CJ, Stirling DA, Waddell L, Booth IR, et al. 1988. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in S. typhimurium and E. coli. *Cell* 52:569–84
48. Ivanova A, Renshaw M, Guntaka V, Eisenstark A. 1992. DNA base sequence variability in *katF* (putative sigma factor) gene of *Escherichia coli*. *Nucleic Acids Res.* 20:5479–80
49. Jung JU, Gutierrez C, Martin F, Ardourel M, Villarejo M. 1990. Transcription of *osmB,* a gene encoding an *Escherichia coli* lipoprotein, is regulated by dual signals. *J. Biol. Chem.* 265: 10574–81
50. Jung JU, Gutierrez C, Villarejo MR. 1989. Sequence of an osmotically inducible lipoprotein gene. *J. Bacteriol.* 171:511–20
51. Kamath-Loeb A, Gross CA. 1991. Translational regulation of σ^{32} synthesis: requirement for an internal control element. *J. Bacteriol.* 173:3904–6
52. Kawamukai M, Matsuda H, Fujii W, Nishida T, Izumoto Y, et al. 1988. Cloning of the *fic-1* gene involved in cell filamentation induced by cyclic AMP and construction of a Δ*fic Escherichia coli* strain. *J. Bacteriol.* 170:3864–69
53. Kawamukai M, Matsuda H, Fujii W, Utsumi R, Komano T. 1989. Nucleotide sequences of *fic* and *fic-1* genes involved in cell filamentation induced by cyclic AMP in *Escherichia coli*. *J. Bacteriol.* 171:4525–29
54. Kjelleberg S, ed. 1993. *Starvation in Bacteria.* New York: Plenum
55. Kohara Y, Akiyama K, Isono K. 1987. The physical map of the whole Escherichia coli chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. *Cell* 50:495–508
56. Kolter R, Moreno F. 1992. Genetics of ribosomally synthesized peptide antibiotics. *Annu. Rev. Microbiol.* 46:141–63
57. Kolter R, Siegele DA, Tormo A. 1993. The stationary phase of the bacterial life cycle. *Annu. Rev. Microbiol.* 47:855–74
58. Krause M, Fang FC, Guiney DG. 1992. Regulation of plasmid virulence gene expression in *Salmonella dublin* involves an unusual operon structure. *J. Bacteriol.* 174:4482–89
59. Krause M, Roudier C, Fierer J, Harwood J, Guiney DG. 1991. Molecular analysis of the virulence locus of *Salmonella dublin* plasmid pSLD2. *Mol. Microbiol.* 5:307–16
60. Lange R, Barth M, Hengge-Aronis R. 1993. Complex transcriptional control of the σ^S-dependent stationary phase-induced and osmotically regulated *osmY* (*csi-5*) gene suggests novel roles for Lrp, cyclic AMP (cAMP), receptor protein–cAMP complex, and integration host factor in the stationary phase response of *Escherichia coli*. *J. Bacteriol.* 1757910–17
61. Lange R, Hengge-Aronis R. 1991.

Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^S. *J. Bacteriol.* 173:4474–81
62. Lange R, Hengge-Aronis R. 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. *Mol. Microbiol.* 5:49–59
63. Lee CWB, Waugh JS, Griffin RG. 1986. Solid-state NMR study of trehalose/1,2-dipalmitoyl-*sn*-phosphatydylcholine interactions. *Biochemistry* 25:3737–42
64. Lin R, D'Ari R, Newman EB. 1992. λ*plac*Mu insertions in genes of the leucine regulon: extension of the regulon to genes not regulated by leucine. *J. Bacteriol.* 174:1948–55
65. Loewen PC. 1992. Regulation of bacterial catalase synthesis. In *Molecular Biology of Free Radical Scavenging Systems,* ed J Scandalios, pp. 97–115. Cold Spring Harbor NY: Cold Spring Harbor Laboratory
66. Loewen PC, Switala J, Triggs-Raine BL. 1985. Catalases HPI and HPII in *Escherichia coli* are induced independently. *Arch. Biochem. Biophys.* 243:144–49
67. Loewen PC, Triggs BL. 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. *J. Bacteriol.* 160:668–75
68. Loewen PC, von Ossowski I, Switala J, Mulvey MR. 1993. KatF (σ^S) synthesis in *Escherichia coli* is subject to post-transcriptional regulation. *J. Bacteriol.* 175:2150–53
69. Lonetto M, Gribskov M, Gross CA. 1992. The σ^{70} family: sequence conservation and evolutionary relationships. *J. Bacteriol.* 174:3843–49
70. Losick R, Stragier P. 1992. Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. *Nature* 355:601–4
71. Magnuson K, Jackowski S, Rock CO, Cronan JE. 1993. Regulation of fatty acid biosynthesis in *Escherichia coli*. *Microbiol. Rev.* 57:522–42
72. Matin A. 1991. The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*. *Mol. Microbiol.* 5:3–10
73. McCann MP, Fraley CD, Matin A. 1993. The putative σ factor KatF is regulated posttranscriptionally during carbon starvation. *J. Bacteriol.* 175: 2143–49
74. McCann MP, Kidwell JP, Matin A. 1991. The putative σ factor KatF has a central role in development of starvation-mediated general resistance in *Escherichia coli*. *J. Bacteriol.* 173: 4188–94
75. Moran CP. 1989. Sigma factors and the regulation of transcription. See Ref. 106a, pp.167–84
76. Moreno F, San Millan JL, del Castillo I, Gomez JM, Rodriguez-Sainz MC, et al. 1992. *Escherichia coli* genes regulating the production of microcins MccB17 and MccC7. In *Bacteriocins, Microcins and Antibiotics,* ed. R James, C Lazdunski, F Pattus, pp. 3–13. Berlin Heidelberg: Springer-Verlag
77. Morgan, RW, Christman MF, Jacobson FS, Storz G, Ames BN. 1986. Hydrogen peroxide-inducible proteins in *Salmonella typhimurium* overlap with heat shock and other stress proteins. *Proc. Natl. Acad. Sci USA* 83:8059–63
78. Moyer CL, Morita RY. 1989. Effect of growth rate and starvation-survival on the viability and stability of a psychrophilic marine bacterium. *Appl. Environ. Microbiol.* 55:1122–27
79. Mulvey MR, Loewen PC. 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests that KatF protein is a novel σ transcription factor. *Nucleic Acids Res.* 17:9979–91
80. Mulvey MR, Sorby PA, Triggs-Raine BL, Loewen PC. 1988. Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*. *Gene* 73:337–45
81. Mulvey MR, Switala J, Borys A, Loewen PC. 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. *J. Bacteriol.* 172: 6713–20
82. Nagai H, Yuzawa H, Yura T. 1991. Interplay of two cis-acting regions in translation control of σ^{32} synthesis during heat shock response of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 88: 10515–19
83. Newman EB, D'Ari R, Lin RT. 1992. The leucine-Lrp regulon in *E. coli:* a global response in search of a raison d'etre. *Cell* 68:617–19
84. Nguyen LH, Jensen DB, Thompson NE, Gentry DR, Burgess RR. 1993. In vitro functional characterization of overproduced *Escherichia coli katF/rpoS* gene product. *Biochemistry* 32: 11112–17
85. Norel F, Robbe-Saule V, Popoff MY, Coynault C. 1992. The putative sigma factor KatF (RpoS) is required for the transcription of the *Salmonella typhimurium* virulence gene *spvB* in *Es-*

cherichia coli. FEMS Microbiol. Lett. 99:271–76
86. Okita TW, Rodriguez RL, Preiss J. 1981. Biosynthesis of bacterial glycogen: cloning of the glycogen enzyme structural genes in *Escherichia coli. J. Biol. Chem.* 256:6944–52
87. Olsen A, Arnqvist A, Sukupolvi S, Normark S. 1993. The RpoS sigma factor relieves H-NS-mediated transcriptional repression of *csgA*, the subunit gene of fibronectin binding curli in *Escherichia coli. Mol. Microbiol.* 7:523–36
88. Olsen A, Jonsson A, Normark S. 1989. Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli. Nature* 338:652–55
89. Olson ER. 1993. Influence of pH on bacterial gene expression. *Mol. Microbiol.* 8:5–14
90. Owen-Hughes TA, Pavitt GD, Santos DS, Sidebotham JM, Hulton CS, et al. 1992. The chromatin-associated protein H-NS interacts with curved DNA to influence DNA topology and gene expression. *Cell* 71:255–65
91. Ozaki M, Wada A, Fujita N, Ishihama A. 1991. Growth phase-dependent modification of RNA polymerase in *Escherichia coli. Mol. Gen. Genet.* 230: 17–23
92. Peak MJ. 1970. Some observations on the lethal effects of near-ultraviolet light on *Escherichia coli,* compared with the lethal effects of far-ultraviolet light. *Photochem. Photobiol.* 12:1–8
93. Plaskon RR, Wartell RM. 1987. Sequence distributions associated with DNA curvature are found upstream of strong *E. coli* promoters. *Nucleic Acids Res.* 15:785–96
94. Prince WS, Villarejo MR. 1990. Osmotic control of *proU* transcription is mediated through direct action of potassium glutamate on the transcription complex. *J. Biol. Chem.* 265:17673–79
95. Raina S, Missiakas D, Baird L, Kumar S, Georgopoulos C. 1993. Identification and transcriptional analysis of *Escherichia coli htrE* operon which is homologous to *pap* and related pilin operons. *J. Bacteriol.* 175:5009–21
96. Reddy NR, Sathe SK, Salunkhe DK. 1982. Phytates in legumes and cereals. *Adv. Food Res.* 28:1–92
97. Sak BD, Eisenstark A, Touati D. 1989. Exonuclease III and the catalase hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* gene product. *Proc. Natl. Acad. Sci. USA* 86:3271–75
98. Sammartano LJ, Tuveson RW, Davenport R. 1986. Control of sensitivity to inactivation by H_2O_2 and broad-spectrum near-UV radiation by the *Escherichia coli katF* locus. *J. Bacteriol.* 168:13–21
99. Saporito SM, Smith-White BJ, Cunningham RP. 1988. Nucleotide sequence of the *xthA* gene of *Escherichia coli* K12. *J. Bacteriol.* 170:4542–47
100. Schellhorn HE, Hassan HM. 1988. Transcriptional regulation of *katE* in *Escherichia coli* K12. *J. Bacteriol.* 170: 4286–92
101. Schellhorn HE, Stones VL. 1992. Regulation of *katF* and *katE* in *Escherichia coli* K-12 by weak acids. *J. Bacteriol.* 174:4769–76
102. Schultz JE, Matin A. 1991. Molecular and functional characterization of a carbon starvation gene of *Escherichia coli. J. Mol. Biol.* 218:129–40
103. Siegle DA, Kolter R. 1992. Life after log. *J. Bacteriol.* 174:345–48
104. Slonczewski JL, Gonzalez TN, Bartholomew FM, Holt NJ. 1987. Mu d-directed *lacZ* fusions regulated by low pH in *Escherichia coli. J. Bacteriol.* 169: 3001–6
105. Small PLC, Falkow S. 1992. A genetic analysis of acid resistance in *Shigella felxneri:* the requirement for a *katF* homologue. *Abstr. 92nd Annu. Meet. Am. Soc. Microbiol.* 92:38 (Abstr.)
106. Smith I. 1989. Initiation of sporulation. See Ref. 106a, pp.185–210
106a. Smith I, Slepecky RA, Setlow P, eds. 1989. *Regulation of Procaryotic Development.* Washington, DC: Am. Soc. Microbiol.
107. Smythe C, Cohen P. 1991. The discovery of glycogenin and the priming mechanism for glycogen biogenesis. *Eur. J. Biochem.* 200:625–31
108. Spector MP, Cubitt CL. 1992. Starvation-inducible loci of *Salmonella typhimurium:* regulation and roles in starvation-survival. *Mol. Microbiol.* 6: 1467–76
109. Strom AR, Kaasen I. 1993. Trehalose metabolism in *Escherichia coli:* stress protection and stress regulation of gene expression. *Mol. Microbiol.* 8: 205–10
110. Tanaka K, Takayanagi Y, Fujita N, Ishihama A, Takahashi H. 1993. Heterogeneity of the principal σ factor in *Escherichia coli:* the *rpoS* gene product σ^{38} is a secondary principal σ factor of RNA polymerase in stationary-phase *Escherichia coli. Proc. Natl. Acad. Sci. USA* 90:3511–15
111. Touati E, Dassa E, Boquet PL. 1986. Pleiotropic mutations in *appR* reduce pH 2.5 acid phosphatase expression and restore succinate utilisation in CRP-de-

ficient strains of *Escherichia coli*. *Mol. Gen. Genet.* 202:257–64
112. Touati E, Dassa E, Dassa J, Boquet P. 1987. Acid phosphatase (pH2.5) of *Escherichia coli:* regulatory characteristics. In *Phosphate Metabolism and Cellular Regulation in Microorganisms*, ed. A Torriani-Gorini, FG Rothman, S Silver, A Wright, E Yagil, pp. 31–40. Washington DC: Am. Soc. Microbiol.
113. Touati E, Dassa E, Dassa J, Boquet PL, Touati D. 1991. Are *appR* and *katF* the same *Escherichia coli* gene encoding a new sigma transcription initiation factor? *Res. Microbiol.* 142:29–36
114. Tran PV, Bannor TA, Doktor SZ, Nichols BP. 1990. Chromosomal organization and expression of *Escherichia coli pabA*. *J. Bacteriol.* 172: 397–410
115. Tuveson RW, Jonas RB. 1979. Genetic control of near-UV (300–400 nm) sensitivity independent of the *recA* gene in strains of *Escherichia coli* K12. *Photochem. Photobiol.* 30:667–76
116. Ueguchi C, Kakeda M, Mizuno T. 1993. Autoregulatory expression of the *Escherichia coli hns* gene encoding a nucleoid protein: H-NS functions as a repressor of its own transcription. *Mol. Gen. Genet.* 236:171–78
117. Van Bogelen RA, Acton MA, Neidhardt FC. 1987. Induction of the heat shock regulon does not produce thermotolerance in *Escherichia coli*. *Genes Dev.* 1:525–31
118. Van der Linden MPG, De Haan L, Hoyer MA, Keck W. 1992. Possible role of *Escherichia coli* penicillin-binding protein 6 in stabilization of stationary-phase peptidoglycan. *J. Bacteriol.* 174:7572–78
119. Van Laere A. 1989. Trehalose, reserve and/or stress metabolite? *FEMS Microbiol. Rev.* 63:201–10
120. Vicente M, Kushner SR, Garrido T, Aldea M. 1991. The role of the "gearbox" in the transcription of essential genes. *Mol. Microbiol.* 5:2085–91
121. Volkert MR, Hajec LI, Nguyen DC. 1989. Induction of the alkylation-inducible *aidB* gene of *Escherichia coli* by anaerobiosis. *J. Bacteriol.* 171: 1196–98
122. Volkert MR, Nguyen DC. 1984. Induction of specific *Escherichia coli* genes by sublethal treatments with alkylating agents. *Proc. Natl. Acad. Sci. USA* 81: 4110–14
123. Weichart D, Lange R, Henneberg N, Hengge-Aronis R. 1993. Identification and characterization of stationary phase-inducible genes in *Escherichia coli*. *Mol. Microbiol.* 10:407–20
124. Womersley C, Uster PS, Rudolph AS, Crowe JH. 1986. Inhibition of dehydration-induced fusion between liposomal membranes by carbohydrates as measured by fluorescence energy transfer. *Cryobiology* 23:245–55
125. Yamamori T, Yura T. 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in *Escherichia coli* K-12. *Proc. Natl. Acad. Sci. USA* 79:860–64
126. Yang W, Ni L, Somerville RL. 1993. A stationary phase protein of *Escherichia coli* that affects the mode of association between the *trp* reressor protein and operator-bearing DNA. *Proc. Natl. Acad. Sci. USA* 90:5796–5800
127. Yim HH, Brems RL, Villarejo M. 1993. Molecular characterization of the promoter of *osmY*, an *rpoS*-dependent gene. *J. Bacteriol.* 176:100–7
128. Yim HH, Villarejo M. 1992. *osmY*, a new hyperosmotically inducible gene, encodes a periplasmic protein in *Escherichia coli*. *J. Bacteriol.* 174:3637–44
129. Zambrano MM, Siegele DA, Almiron M, Tormo A, Kolter R. 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. *Science* 259:1757–60

Annual Review of Microbiology
Volume 48, 1994

CONTENTS

vi *(continued)*