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An N-Terminally Truncated RpoS (σ^S) Protein in *Escherichia coli* Is Active In Vivo and Exhibits Normal Environmental Regulation Even in the Absence of *rpoS* Transcriptional and Translational Control Signals

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RpoS (σ^S) in *Escherichia coli* is a stationary-phase-specific primary sigma factor of RNA polymerase which is 330 amino acids long and belongs to the eubacterial σ^{70} family of proteins. Conserved domain 1.1 at the N-terminal end of σ^{70} has been shown to be essential for RNA polymerase function, and its deletion has been shown to result in a dominant-lethal phenotype. We now report that a σ^S variant with a deletion of its N-terminal 50 amino acids ($\sigma^S\Delta1–50$), when expressed in vivo either from a chromosomal *rpoS*::IS*10* allele (in *rho* mutant strains) or from a plasmid-borne arabinose-inducible promoter, is as proficient as the wild type in directing transcription from the *proU* P1 promoter; at three other σ^S-dependent promoters that were tested (*osmY*, *katE*, and *csiD*), the truncated protein exhibited a three- to sevenfold reduced range of activities. Catabolite repression at the *csiD* promoter (which requires both σ^S and cyclic AMP [cAMP]-cAMP receptor protein for its activity) was also preserved in the strain expressing $\sigma^S\Delta1–50$. The intracellular content of $\sigma^S\Delta1–50$ was regulated by culture variables such as growth phase, osmolarity, and temperature in the same manner as that described earlier for σ^S, even when the truncated protein was expressed from a template that possessed neither the transcriptional nor the translational control elements of wild-type *rpoS*. Our results indicate that, unlike that in σ^{70}, the N-terminal domain in σ^S may not be essential for the protein to function as a sigma factor in vivo. Furthermore, our results suggest that the induction of σ^S-specific promoters in stationary phase and during growth under conditions of high osmolarity or low temperature is mediated primarily through the regulation of σ^S protein degradation.

The σ factor is a subunit of RNA polymerase in all eubacteria that confers on the enzyme the property of promoter specificity in the initiation of transcription. Based on amino acid sequence similarity as well as organization of the cognate promoters, two families of σ factors have been identified (19); the σ^{70} family is by far the larger one. The prototypic example of this family is *Escherichia coli* RpoD or σ^{70} (with 613 amino acid residues), the primary or housekeeping σ factor in this organism, which is essential for its viability.

Four conserved regions, numbered 1 to 4 beginning from the N-terminal end, have been identified in the σ^{70} family of proteins; some of these are further divided into distinct subregions (19). Regions 2 and 4 are the most highly conserved among different members of the σ^{70} family, and these regions are involved in recognition of and binding to promoter DNA by the enzyme. On the other hand, subregion 1.1 appears to be conserved only in the housekeeping or indispensable σ proteins of different bacteria, as well as in σ^S (see below). In σ^{70}, subregion 1.1 (comprising the segment from approximately residues 30 through 100) has been suggested to function as a mask in free σ protein for DNA-binding domains 2 and 4, so that the latter are exposed only when the σ factor is associated with the core enzyme, that is, the other subunits of the RNA polymerase holoenzyme (6). Other studies have suggested that subregion 1.1 is also required for (i) the initial binding of σ^{70} to the core enzyme (10, 26); (ii) masking of other core-binding regions in the free σ^{70} subunit that are later involved in a holoenzyme interface (10); (iii) influencing the efficiency of transcription initiation, perhaps by constraining the holoenzyme to assess the fitness of a promoter by its −10 and −35 sequences (7, 40, 42); and (iv) imparting stability to the protein in vivo (42). Consistent with these findings, mutant versions of σ^{70} with a deletion of subregion 1.1 confer a dominant-lethal phenotype in vivo (5, 34).

In addition to σ^{70}, *E. coli* cells possess a second primary sigma factor, RpoS or σ^S (with 330 amino acid residues), that is encoded by *rpoS* and that is important for stationary-phase gene expression and survival (reviewed in references 12 and 14). As a member of the σ^{70} family, σ^S also has conserved regions 1 through 4. The N-terminal stretch of 60 amino acid residues of σ^S has been reported to share moderate sequence similarity with domain 1.1 of σ^{70} (19, 25), and both are also rich in acidic amino acid residues; however, the function of this region in σ^S is not known. An alignment of these two segments of σ^S and σ^{70} is shown in Fig. 1.

The genes of the σ^S regulon are induced under different environmental conditions, such as the stationary phase of growth, low pH, high osmolarity, or low incubation temperature, consequent to an elevation of the cytoplasmic concentra-

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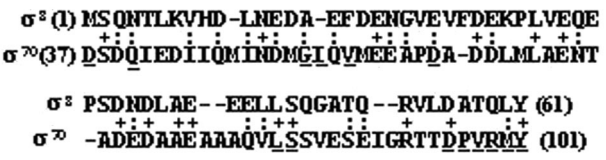

FIG. 1. Similarity between the N-terminal region of σ^S and domain 1.1 of σ^{70}. In the alignment shown, individual amino acids are represented in the one-letter code, and plus symbols and colons are used to indicate identity and conservative substitution (within one or another of the following groups: D, E, N, or Q; S or T; G or A; and I, V, L, or M), respectively, between the two proteins. When necessary, gaps (dashes) have been introduced in the sequences to maximize the similarity in alignment. Sequence numbers of the N- and C-terminal residues of each of the two polypeptide stretches are given in parentheses. Residues in σ^{70} that are highly conserved among the primary sigma factors of both gram-negative and gram-positive bacteria (42) are underlined.

tion of σ^S under each of these conditions (12, 14). Cellular σ^S content itself is determined by the interplay of a complex set of regulatory mechanisms that operate at the levels of *rpoS* transcription and translation as well as the stability of the protein.

We have found in this study that a mutant σ^S protein which is missing its N-terminal 50 amino acid residues retains in vivo activity at σ^S-regulated promoters. Our results obtained with strains expressing the mutant protein also suggest that environmental regulation of cellular σ^S content may occur primarily at the posttranslational level.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* K-12 strains that were used in this study are listed in Table 1. Five plasmids, pHYD275, pHYD373, pETF, pBAD24, and pHYD408, were used in this study. The first two are IncW-based single-copy-number plasmids encoding trimethoprim resistance and carrying the *lacZ* reporter gene (as an operon fusion) downstream of the *proU* P1 promoter of, respectively, *E. coli* and *Salmonella enterica* serovar Typhimurium (4, 28). The third is a ColE1 replicon encoding ampicillin resistance and carrying the *E. coli* *rpoS*$^+$ gene (39). Plasmid vector pBAD24, which is also ColE1 based and encodes ampicillin resistance, is designed for achieving L-arabinose (Ara)-induced expression of target genes cloned into its multiple-cloning-site region (11). Plasmid pHYD408 was contructed in this study by cloning into the appropriate sites in pBAD24 a *Sal*I-*Hin*dIII fragment obtained by PCR from the mutant *rpoS* locus of strain GJ875 (see below). This PCR was done with a pair of primers (5′-TATG *GTCGA CACATGGTTACGCTTTGG*-3′ and 5′-TCGT *AAGC TT*T CTGACAGATGCTTACTT-3′) whose sequences (to the 3′ side of the residue marked in bold in each case) correspond, respectively, to the sequence near one end of IS*10* and to the sequence immediately downstream of the termination

TABLE 1. *E. coli* K-12 strains used in this study

Strain	Genotype[a]	Reference or source
MC4100	Δ(*argF-lac*)*U169 rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25*	8
RH90	MC4100 *rpoS359*::Tn*10*	21
RH100[b]	MC4100 Δ(*nlpD-rpoS*)*360 zgc-3251*::Tn*10*	13
GJ146	MC4100 Δ*putPA101 proP222* Δ(*zfi-900*::Tn*10-proU*)*233* Δ(*pyr-76*::Tn*10*) *rpoS365*::IS*10*	9
GJ829	GJ146 *ilv-3164*::Tn*10*Kan	This study
GJ830	GJ146 *rho-4 ilv-3164*::Tn*10*Kan	This study
GJ862	Like MC4100	27
GJ863	MC4100 *rho-4*	27
GJ875	GJ862 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ876	GJ862 *zgc-912*::Tn*10*dCm	This study
GJ884	GJ862 *csiD*::*lac*(Kan)	27
GJ885	GJ863 *csiD*::*lac*(Kan)	27
GJ888	GJ862 *osmY*::*lac*(Kan)	27
GJ889	GJ863 *osmY*::*lac*(Kan)	27
GJ893	GJ884 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ894	GJ888 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ2731	GJ885 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ2732	GJ889 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ2755	GJ863 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ2756	GJ862 *ara*$^+$ *zac-3093*::Tn*10*Kan *rpoS359*::Tn*10*	This study
GJ2758	GJ875 *ara*$^+$ *zac-3093*::Tn*10*Kan	This study
GJ2765	GJ863 *zgc-912*::Tn*10*dCm	This study
GJ2770	GJ862 [λ *katE*::*lac*(Kan)]	This study
GJ2771	GJ863 [λ *katE*::*lac*(Kan)]	This study
GJ2778	RH100 *ara*$^+$	This study
GJ2780	GJ2778 *csiD*::*lac*(Kan)	This study
GJ2781	GJ2778 *osmY*::*lac*(Kan)	This study
GJ2782	GJ2778 [λ *katE*::*lac*(Kan)]	This study
GJ2785	GJ884 *ara*$^+$ *zac-3051*::Tn*10*	This study
GJ2787	GJ885 Δ(*nlpD-rpoS*)*360 zgc-3251*::Tn*10*	This study
GJ2788	GJ889 Δ(*nlpD-rpoS*)*360 zgc-3251*::Tn*10*	This study
GJ2789	GJ2770 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ2790	GJ2771 *rpoS365*::IS*10 zgc-912*::Tn*10*dCm	This study
GJ2791	GJ2771 Δ(*nlpD-rpoS*)*360 zgc-3251*::Tn*10*	This study

[a] Genotype designations are like those in the work of Berlyn (2). All strains are F$^-$. In the strains listed, the following mutations were transduced from strains previously described: *rho-4* from CGSC5072 (*E. coli* Genetic Stock Center); *ilv-3164*::Tn*10*Kan, *zac-3093*::Tn*10*Kan, and *zac-3051*::Tn*10* from CAG18599, CAG12131, and CAG12095, respectively (35); *csiD*::*lac*(Kan) from DW12 (21); *osmY*::*lac*(Kan) (previously called *csi-5*::*lac*) from R0151-a (13); and λ *katE*::*lac*(Kan) from KT1008EL (38).

[b] The Tn*10* allele in RH100 was originally designated *zfi-3251*::Tn*10*, based on the calibration in an earlier edition of the *E. coli* K-12 linkage map.

TABLE 2. Effect of *rpoS-rho* interaction on *proU* P1-*lac* expression[a]

Strain	Genotype	β-Galactosidase sp act with the following plasmid and conditions:					
		pHYD275 (*E. coli* P1-*lac*)			pHYD373 (*S. enterica* P1-*lac*)		
		No NaCl, 30°C	NaCl, 30°C	No NaCl, 10°C	No NaCl, 30°C	NaCl, 30°C	No NaCl, 10°C
GJ862	*rpoS*$^+$ *rho*$^+$	63	275	962	4	4	54
GJ863	*rpoS*$^+$ *rho*	126	322	885	51	69	463
GJ829	*rpoS*::IS*10 rho*$^+$	7	14	56	1	2	2
GJ830	*rpoS*::IS*10 rho*	157	467	1,360	26	104	849

[a] Cultures were grown to mid-exponential phase in trimethoprim-supplemented K-tryptone medium without or with 0.3 M NaCl at 30 or 10°C for β-galactosidase assays. Enzyme specific activities are reported in Miller units (22).

codon of *rpoS*; the *Sal*I and *Hin*dIII recognition sites, respectively, provided in the two primers are italicized.

Media and growth conditions. For routine experiments, Luria-Bertani (LB) medium (22) and glucose (Glu)-minimal A medium (22) were used as the nutrient and defined media, respectively, and the incubation temperature for growth was 37°C. MacConkey lactose agar was obtained from Difco. Cultures for β-galactosidase assays were incubated with shaking (i) until mid-exponential phase (A_{600} of 0.4 to 0.8) or until 1 h after entry into stationary phase; (ii) at 30 or 10°C; and (iii) in LB medium or low-osmolarity K-tryptone medium (27), the latter supplemented when necessary with NaCl to 0.3 M. Unless otherwise indicated, Ara induction experiments (11) were performed by supplementation of the growth medium with 0.2% Ara (or 0.2% Glu, as a control). Concentrations of antibiotics used were as described earlier (20).

Immunoblot analysis. The procedures used for electrophoresis of cell extracts on 10% polyacrylamide gels with sodium dodecyl sulfate, electroblotting to a polyvinylidene difluoride membrane, staining with Ponceau S, and sequential treatments with blocking reagent, primary antibody, alkaline phosphatase-conjugated secondary antibody, and chromogenic substrates were essentially as previously described (32). As part of the protocol, 2.5 μl of rabbit anti-σ^S antiserum (kindly provided by Regine Hengge-Aronis) was preadsorbed at 4°C overnight with 1 ml of a sonicated cell extract (containing approximately 2 mg of protein) of either the *rpoS*::Tn*10* strain RH90 or the Δ*rpoS* strain RH100 in 50 mM Tris-Cl buffer (pH 8) containing 1 mM each EDTA and phenylmethylsulfonyl fluoride; following centrifugation at 12,000 × *g* for 20 min at 4°C, the supernatant was recovered and used in a total volume of 10 ml as the primary antibody preparation.

Other experimental techniques. The procedures used for phage P1 transduction (8), transposon tagging with Tn*10*dCm and genetic mapping (22), and experiments involving DNA manipulations (32) were as described previously. Three primers, 5′-GAACCAGTTCAACACGCT-3′, 5′-ACCGAGGTAATGC GCTCGT-3′, and 5′-CCGATGGGCATCGAC-3′, which were specific, respectively, for the 5′ end, the middle, and the 3′ end of *rpoS*, were used for PCR amplification and DNA sequence determination of the *rpoS* locus. β-Galactosidase assays were performed by the method of Miller (22); enzyme specific activity values are reported in Miller units.

RESULTS

An *rpoS*::IS*10* insertion mutant with *rho*-modulated σ^S activity in vivo. It was shown earlier that the P1 promoter of the *E. coli proU* operon is σ^S regulated both in vivo and in vitro (20, 27–29); its in vivo activity (for example, in a wild-type strain such as GJ862) (Table 2) can be assayed by using plasmid pHYD275, which is a very-low-copy-number replicon that carries P1 upstream of the *lacZ* reporter gene (4). The starting point for this study were the unexpected observations (Table 2) that *lac* expression from pHYD275 was abolished in one of our laboratory strains, GJ829, but that it was restored in an isogenic derivative, GJ830, that was defective in the *rho* gene (encoding the transcription termination factor Rho; for a review, see reference 31). The *rho* mutation had little effect in a wild-type strain (Table 2, compare values for GJ862 and GJ863; see also reference [27]). An *rpoS*::Tn*10* derivative of GJ830 was also negative for pHYD275-driven *lac* expression (data not shown), indicating that the *rho* mutation acted specifically and in a σ^S-dependent manner to restore *proU* P1-*lac* expression in GJ829.

We used classical genetic techniques (22) first to obtain a new transposon Tn*10*dCm insertion allele (designated *zgc-912*::Tn*10*dCm) 85% cotransducible with the locus in GJ829 that confers *rho*-modulated P1 -*lac* expression and then to map the chloramphenicol resistance (Cm^r) marker to the 62-min region of the chromosome (data not shown). The Cm^r marker exhibited 85% linkage to *rpoS*, raising the possibility that the mutation in GJ829 that affects P1-*lac* expression is itself an *rpoS* allele.

The linked Cm^r marker also allowed us to construct, from wild-type strain GJ862, an isogenic pair of derivatives, GJ875 and GJ876, that carried, respectively, the mutant locus of GJ829 and its wild-type allele. PCR amplification of the *rpoS* gene from the two strains by using a pair of primers specific to the 5′ and 3′ ends of the gene (1.2 kb apart) indicated that the GJ829 mutation is associated with an insertion of 1.3 kb of DNA in the *rpoS* locus (data not shown). DNA sequence analysis revealed it to be an IS*10* insertion disrupting the *rpoS* open reading frame after codon 53 (Fig. 2), and the mutation was designated *rpoS365*::IS*10*. The stock of strain GJ146 that had served as the immediate ancestor for GJ829 and GJ830 was also shown to carry the *rpoS*::IS*10* mutation (data not shown); since GJ146 had been derived during several steps of Tet^s selection following successive transductions with Tn*10* insertions (9), we presume that the IS*10* transposition must have occurred subsequently, during routine strain maintenance, spontaneously, and unselected from one of the original Tn*10*-bearing loci in the chromosome. Incidentally, the site of *rpoS365*::IS*10* insertion was identical to that for each of two different *rpoS*::Tn*10* insertions oriented opposite one another and sequenced earlier (15) in strains RH90 and UM22 (Fig. 2), indicating that it is a hot-spot site for transpositions involving IS*10* and its derivative composite transposons. Furthermore, data from an earlier study (27) indicated that the *rho* mutation does not restore *proU* P1-*lac* expression in strains carrying the *rpoS359*::Tn*10* mutation of RH90.

A plasmid (pETF) carrying the *rpoS*$^+$ gene (39) was able to complement the *rpoS*::IS*10* mutation for pHYD275-directed *lac* expression in the *rho*$^+$ strain GJ829 (data not shown).

Evidence for the synthesis of an σ^S polypeptide with a deletion of its N-terminal 50 amino acids (σ^SΔ1–50) in

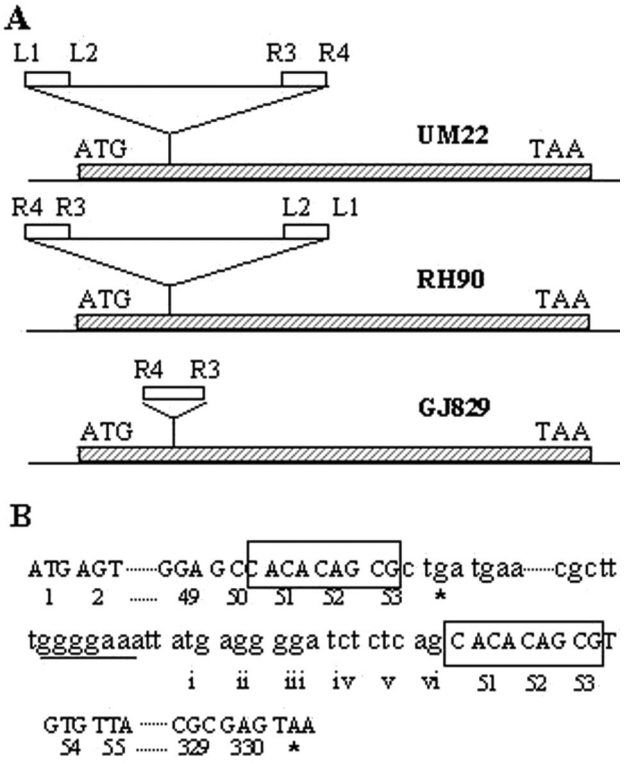

FIG. 2. Molecular description of the *rpoS365*:: IS*10* insertion. (A) The positions (and orientations) of *rpoS*::Tn*10* insertions previously characterized (15) in strains UM22 and RH90 are compared with those of the *rpoS*:: IS*10* insertion in strain GJ829 (this study). The coding region of *rpoS* is shown as a hatched bar, and IS*10* sequences (including those found in Tn*10*) are shown as open bars. Representations of Tn*10* and IS*10* are not to scale. To facilitate comparison, the four distinct ends of IS*10* present in Tn*10* (3) are marked as L1 and L2 (from IS*10*-L) and R3 and R4 (from IS*10*-R). (B) DNA sequence of the *rpoS*:: IS*10* insertion, with particular reference to the sequence in the vicinity of the ends of IS*10*. Codons of *rpoS* are indicated by arabic numerals (and stop codons are indicated by asterisks); the 9-bp duplications flanking the ends of IS*10* (whose sequence is shown in lowercase letters) are boxed. Codons from IS*10* that are predicted to encode an N-terminal extension of the $\sigma^S\Delta1$–50 peptide are indicated by small roman numerals, and the polypurine stretch (putative ribosome-binding site motif) is underlined.

***rpoS*::IS*10 rho* mutants.** There were two conceivable explanations, not mutually exclusive, for the above results. Transcription initiating from *proU* P1 of *S. enterica* is subject to premature termination (that is, attenuation), and this phenomenon is known to be Rho dependent (27, 28). It was therefore possible that the *rpoS*::IS*10* insertion mutation somehow accentuated the effect of Rho factor on transcription initiating from *E. coli proU* P1 as well. For example, even in an $rpoS^+$ background, *lacZ* reporter gene expression from *S. enterica proU* P1 (on plasmid pHYD373) is absent in a rho^+ strain (GJ862) and is stimulated in a *rho* mutant (GJ863) (27) (Table 2); in the *rpoS*::IS*10* rho^+ and *rho* derivatives (GJ829 and GJ830, respectively), *lac* expression from pHYD373 was virtually indistinguishable from that in the corresponding $rpoS^+$ strains (Table 2).

The second explanation (31) is that the *rho* mutation acted only to relieve the polarity effect of the IS*10* insertion in *rpoS*. The location of the *rpoS*::IS*10* insertion is such that (i) translation initiating from the start codon of *rpoS* will be terminated immediately within the insertion element after codon 53, and (ii) a potential start site for translation exists (with a polypurine stretch situated upstream of the putative initiation codon stretch to serve as a ribosome-binding site) proceeding outward from the other end of IS*10* to produce an in-frame fusion of six codons of IS*10* with codons 51 to 330 of *rpoS* (Fig. 2). (The latter feature is absent from either of the Tn*10* insertions at this same site in strains RH90 and UM22.) It was therefore possible that the *rho* mutation permitted transcription initiating from the *rpoS* promoter (and upstream promoters) to proceed through IS*10* into the 3′ end of the *rpoS* gene and that the N-terminally truncated mutant σ^S polypeptide (hereafter referred to as $\sigma^S\Delta1$–50) synthesized under these conditions contributed, either alone or in combination with the σ^S N-terminal fragment from positions 1 to 53 [$\sigma^S(1–53)$] to *proU* P1 expression in vivo.

As a test of this second model, an immunoblot experiment was performed with anti-σ^S antiserum against cell extracts prepared from rho^+ and *rho* derivatives of the $rpoS^+$ and *rpoS*::IS*10* strains (Fig. 3A). An immunoreactive protein corresponding to σ^S was present in the $rpoS^+$ strains and was more prominent in the *rho* mutant (Fig. 3A, lane 4) than in the rho^+ strain (lane 1), perhaps reflecting increased readthrough in the former from the promoter(s) for gene *nlpD* situated upstream of *rpoS* (17); the σ^S band was absent in the *rpoS*::IS*10* strains (lanes 2 and 3) as well as in the *rpoS*::Tn*10* mutant control (lane 5). On the other hand, a new protein cross-reacting with the anti-σ^S antiserum and whose faster migration was consistent with that for the postulated $\sigma^S\Delta1$–50 polypeptide was detected only in the cell extract from the *rpoS*::IS*10 rho* derivative (Fig. 3A, lane 3). These observations lent support to the suggestion that, in a *rho* background, an N-terminally truncated σ^S protein was expressed from the chromosomal *rpoS*::IS*10* allele.

$\sigma^S\Delta1$–50 expression from a heterologous promoter. As a further test of the model invoking a function for $\sigma^S\Delta1$–50, we designed experiments to address the question of whether the putative IS*10*-encoded translation initiation signals are indeed proficient for the expression of the truncated protein from a heterologous inducible promoter in rho^+ strains and, if so, whether the protein exhibits sigma activity in vivo. For this purpose, we used the thermostable high-fidelity *Pwo* DNA polymerase enzyme to amplify by PCR, from the chromosome of the *rpoS*::IS*10* strain GJ875, an 0.85-kb DNA segment comprising the coding region of the putative $\sigma^S\Delta1$–50 peptide along with the adjacent 43 bp from the end of IS*10*. The DNA segment was then cloned downstream of the Ara-inducible promoter in plasmid vector pBAD24 (11), and the resulting plasmid was designated pHYD408.

An immunoblot experiment with anti-σ^S antiserum was undertaken to demonstrate Ara-induced synthesis from the pHYD408 template of the N-terminally truncated $\sigma^S\Delta1$–50 polypeptide (Fig. 3B). As in the previous experiment, an immunoreactive protein corresponding to wild-type σ^S was detected only in cells of the $rpoS^+$ strain (Fig. 3B, lane 7) and was absent from those of the Δ*rpoS* derivatives (lanes 6, 8, and 9). Instead, a cross-reacting protein whose electrophoretic mobility was consistent with that expected for $\sigma^S\Delta1$–50 was present

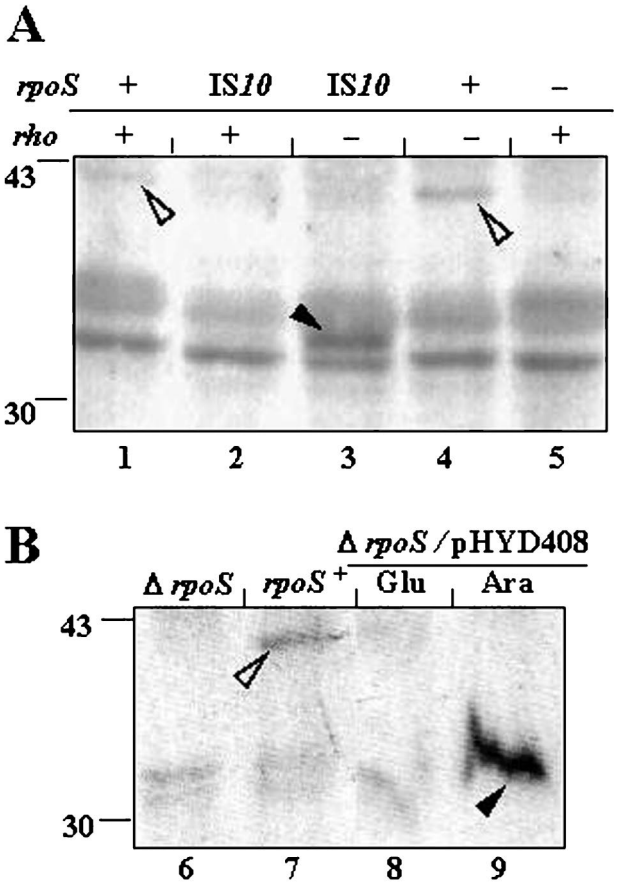

FIG. 3. Identification by immunoblotting of σ^SΔ1–50 expressed from the chromosome of an *rpoS*::IS*10* strain (A) and from plasmid pHYD408 by Ara induction in a Δ*rpoS* strain (B). Anti-σ^S antiserum was used for immunoblot analysis of cell extracts (of cultures grown at 30°C in LB medium until 1 h after entry into stationary phase) from strains with different combinations of chromosomal *rpoS* and *rho* alleles (A) or from *rho*$^+$ strains without or with plasmid pHYD408 (B). Cultures of the pHYD408 derivative were supplemented with either Glu or Ara and ampicillin. Strains were as follows: lane 1, GJ876; lane 2, GJ875; lane 3, GJ2755; lane 4, GJ2765; lane 5, RH90; lane 6, RH100; lane 7, GJ862; and lanes 8 and 9, GJ2782/pHYD408. To the left of each panel are shown the positions of migration of two marker proteins with sizes in kilodaltons. Open and closed arrowheads identify, respectively, bands corresponding to wild-type σ^S and σ^SΔ1–50.

in abundance in Ara-grown (Fig. 3B, lane 9) but not Glu-grown (lane 8) cells of the Δ*rpoS*/pHYD408 derivative.

In experiments aimed at testing in vivo σ activity, we were able to show that plasmid pHYD408 could also direct substantial *lac* expression, specifically in an Ara-inducible manner, from the *E. coli proU* P1 promoter on pHYD275 in *rho*$^+$ strains that carried the *rpoS*::IS*10*, the *rpoS359*::Tn*10*, or the Δ*rpoS* mutation on the chromosome (Table 3). These results provide additional support for the model invoking σ activity in vivo for the σ^SΔ1–50 protein and furthermore suggest that σ^S(1–53) encoded by the chromosomal *rpoS*::IS*10* allele may not be essential for this purpose (since considerable *proU* P1-*lac* expression was elicited in the Δ*rpoS* background as well).

Recognition of other σ^S-dependent promoters by σ^SΔ1–50.

TABLE 3. Ara-induced expression of *proU* P1 from pHYD275 in pHYD408 derivatives of *rpoS rho*$^+$ strains[a]

Strain	Chromosomal *rpoS* genotype	β-Galactosidase sp act with:	
		Glu	Ara
GJ2758	*rpoS*::IS*10*	17	783
GJ2756	*rpoS*::Tn*10*	11	447
GJ2778	Δ*rpoS*	10	263

[a] Cultures were incubated in LB medium supplemented with trimethoprim, ampicillin, and either Glu or Ara at 30°C until 1 h after entry into stationary phase for β-galactosidase assays. Enzyme specific activities are reported in Miller units (22).

With the aid of the respective promoter-*lac* operon fusions, we examined the in vivo activity of σ^SΔ1–50 (expressed either from the chromosomal *rpoS*::IS*10* allele in a *rho* background or by Ara induction of Δ*rpoS rho*$^+$ strains carrying plasmid pHYD408) on the promoters of three other σ^S-dependent genes, *katE*, *osmY*, and *csiD*. Although each of the two methods used to obtain the expression of σ^SΔ1–50 protein had their individual shortcomings—namely, a nonspecific reduction, associated with the pleiotropic *rho* mutation, of *lac* expression from promoters other than *proU* P1 even in control *rpoS*$^+$ strains (Table 4) (see also reference 27) and considerable overproduction of the mutant protein in the Ara induction experiment—we reasoned that their potential confounding effects might not overlap.

The results from the experiments involving the *rpoS*::IS*10 rho* strains indicated that, unlike the situation with *proU* P1, the other three promoters were transcribed to the extent of only about 15 to 30% in the presence of σ^SΔ1–50 synthesized from the chromosomal allele, compared to their activities in the isogenic *rho rpoS*$^+$ strains (Table 4). Nevertheless, in vivo *lac* expression with σ^SΔ1–50 was higher than that in the corresponding *rho* Δ*rpoS* strains. The Lac phenotypes of the different promoter-*lac* fusion strains (*rho*$^+$ and *rho*, in combination with *rpoS*$^+$, *rpoS*::IS*10*, and Δ*rpoS*) on MacConkey lactose agar plates correlated well with the β-galactosidase specific activities reported in Table 4 (data not shown).

On the other hand, when σ^SΔ1–50 was overproduced in the Δ*rpoS rho*$^+$ strains by Ara induction from pHYD408, the level of expression of each of the three *lac* fusions was much higher and, at least for *katE*, comparable to that in the corresponding haploid *rpoS*$^+$ *rho*$^+$ derivatives (Table 4). A substantial level of expression was also obtained for each of the *lac* fusions with Ara induction of pHYD408 transformants of *rpoS*::IS*10 rho*$^+$ derivatives (data not shown).

In the course of these studies, we observed that whereas the activities of other promoters, such as *katE*, in the pHYD408 derivatives (Δ*rpoS*) were unaffected when the concentration of Ara as an inducer was varied between 0.2% (routinely used) and 0.02% (data not shown), the expression of *csiD-lac* was maximal at 0.02% Ara and then progressively declined with increasing sugar concentrations (Fig. 4). Even in a control *rpoS*$^+$ strain, GJ2785/pBAD24 (in which, as expected, the synthesis of σ^S could occur without Ara) (Fig. 4), *csiD-lac* expression was progressively repressed by Ara at concentrations above 0.02% (Fig. 4). It is known that the *csiD* promoter requires both σ^S and cyclic AMP (cAMP)-cAMP receptor pro-

TABLE 4. Activity of $\sigma^S\Delta1–50$ on σ^S-specific promoters[a]

Promoter-*lac* fusion	β-Galactosidase sp act in the following strain:							
	rpoS$^+$		Δ*rpoS*		*rpoS*::IS*10*		Δ*rpoS rho*$^+$/pHYD408 with:	
	rho$^+$	*rho*	*rho*$^+$	*rho*	*rho*$^+$	*rho*	Glu	Ara
katE	772	533	16	33	38	141	23	586
osmY	415	348	14	14	10	46	13	141
csiD	948	612	4	21	48	214	3	324[b]

[a] Cultures were incubated in LB medium (supplemented, for pHYD408 derivatives, with ampicillin and either Glu or Ara) at 30°C until 1 h after entry into stationary phase for β-galactosidase assays. Enzyme specific activities are reported in Miller units (22). Strains used for each promoter-*lac* fusion study were as follows (in the order *rpoS*$^+$ *rho*$^+$, *rpoS*$^+$ *rho*, *rpoS*::IS*10 rho*$^+$, *rpoS*::IS*10 rho*, Δ*rpoS rho*$^+$, and Δ*rpoS rho*): *katE*, GJ2770, GJ2771, GJ2789, GJ2790, GJ2782, and GJ2791; *osmY*, GJ888, GJ889, GJ894, GJ2732, GJ2781, and GJ2788; and *csiD*, GJ884, GJ885, GJ893, GJ2731, GJ2780, and GJ2787.

[b] Value obtained with 0.02% Ara (see Fig. 4).

tein for its activity (21), and the present results therefore suggest (i) that the promoter is catabolite repressed in the *rpoS*$^+$ strain at Ara concentrations above 0.02% and (ii) that a holoenzyme with the $\sigma^S\Delta1–50$ subunit is also proficient for catabolite repression at this promoter.

Environmental regulation of $\sigma^S\Delta1–50$. As mentioned above, it is believed that the regulation of cellular σ^S levels by environmental variables, such as growth phase, temperature, and osmolarity, is achieved by a combination of transcriptional, translational, and posttranslational control mechanisms (12, 14). On the other hand, Zgurskaya et al. (43) suggested that the increase in σ^S content in stationary-phase cells may be accounted for solely by the increased stability of σ^S, and Becker et al. (1) showed that the increase under these conditions occurs even in the absence of *rpoS* transcriptional or translational regulation.

In the present study, when we used the chromosomal *rpoS*::IS*10 rho* strain GJ830, we observed that *lac* reporter gene expression from *proU* P1 of *E. coli* or *S. enterica* was induced both by elevated osmolarity and by growth at 10°C and that the magnitude of such induction (4- and 10-fold, respectively) was comparable to that in the *rpoS*$^+$ strains (Table 2). Given that the *cis* regulatory sequences for native *rpoS* expression have been separated from the coding region of $\sigma^S\Delta1–50$ by the IS*10* insertion in GJ830, these results raised the possibility that environmental regulation of $\sigma^S\Delta1–50$ synthesis could occur even in the absence of such sequences, but alternative explanations were not excluded by the data.

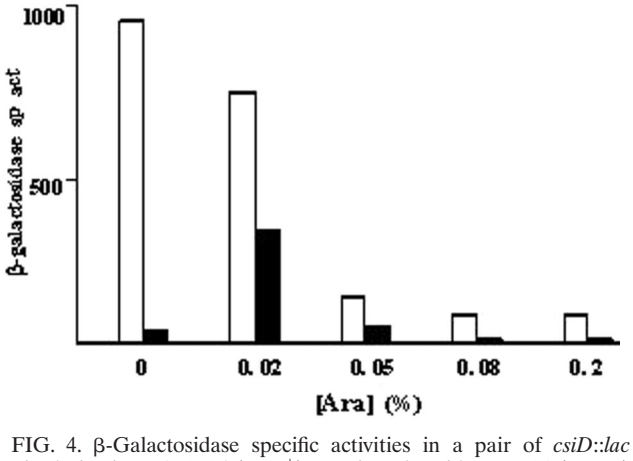

FIG. 4. β-Galactosidase specific activities in a pair of *csiD*::*lac* strain derivatives: GJ2785 (*rpoS*$^+$) carrying plasmid pBAD24 (vector) and GJ2780 (Δ*rpoS*) carrying plasmid pHYD408 (with the gene for $\sigma^S\Delta1–50$). Cultures were grown at 30°C in LB medium supplemented with ampicillin and the indicated concentrations of Ara until 1 h after entry into stationary phase. Enzyme specific activities are reported in Miller units (22). White bars, GJ2785/pBAD24; black bars, GJ2780/pHYD408.

We therefore examined whether the cellular content of $\sigma^S\Delta1–50$ could be regulated by the same environmental cues as those reported earlier for wild-type σ^S (12, 14), even when the former is expressed by Ara induction from pHYD408, that is, from a template devoid of the transcriptional and translational controls of native *rpoS*. The intracellular concentrations of $\sigma^S\Delta1–50$ were assayed both directly by immunoblotting with anti-σ^S antiserum (Fig. 5) and indirectly by determination of *lac* reporter gene expression from the *proU* P1 and *katE* promoters (Table 5).

The data from the immunoblotting experiment indicated that the levels of $\sigma^S\Delta1–50$ expressed following Ara induction in a Δ*rpoS* strain increase progressively from the early exponential to the stationary growth phase (Fig. 5, lanes 2 through 4) and that the levels are also increased by growth under conditions of elevated osmolarity (Fig. 5, compare lanes 5 and 6).

In the indirect assay (Table 5), *lacZ* reporter gene expression from the *proU* P1 and *katE* promoters was significantly higher in the stationary growth phase than in the exponential growth phase. The comparisons were done with cultures that had been subjected to Ara induction for both equal lengths of time and the same numbers of generations of growth. Similarly, the osmotic inducibility of both promoters was observed in derivatives expressing $\sigma^S\Delta1–50$ from pHYD408 to the same extent as in the *rpoS*$^+$ strain. Finally, both *proU* P1-*lac* transcription and *katE-lac* transcription directed by $\sigma^S\Delta1–50$ were substantially higher in cultures grown at 10°C than in those grown at 30°C, and the magnitude of low-temperature induction was equivalent to that seen with wild-type σ^S. The significance of these findings for the understanding of σ^S synthesis and turnover mechanisms is discussed below.

DISCUSSION

Properties of $\sigma^S\Delta1–50$ as a σ factor. Previous studies on σ^{70} showed that its N-terminal domain 1.1 is essential for the protein to function as a σ factor of RNA polymerase in bacterial cells (5, 6, 34). The present study demonstrates that a

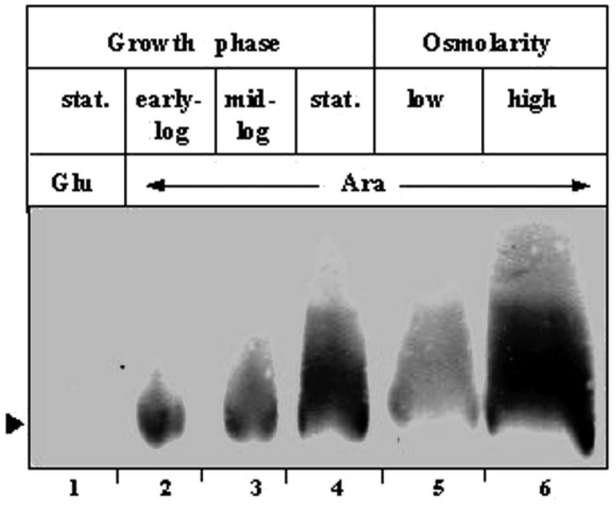

FIG. 5. Environmental regulation of cellular $\sigma^S\Delta1–50$ content. Anti-σ^S antiserum was used for immunoblot analysis of cell extracts of Δ*rpoS* strain GJ2782 carrying plasmid pHYD408 and grown with ampicillin and Ara supplementation in LB medium for the growth phase regulation studies to an A_{600} of 0.38 (lane 2), 0.7 (lane 3), or 1.2 (lane 4) or in K-tryptone medium without (lane 5) or with (lane 6) added 0.3 M NaCl for the osmotic regulation studies to an A_{600} of about 0.4. A control culture was grown in LB medium with Glu supplementation to an A_{600} of 1.2 (lane 1). stat., stationary phase. The arrowhead identifies the band corresponding to $\sigma^S\Delta1–50$.

mutant version of σ^S ($\sigma^S\Delta1–50$) in which the N-terminal 50 amino acids have been deleted is still capable of directing transcription in vivo from a variety of σ^S-dependent promoters.

The data presented in Table 2 suggest that chromosomally expressed $\sigma^S\Delta1–50$ may be as proficient as wild-type σ^S in its ability to initiate transcription from the *E. coli* and *S. enterica* *proU* P1 promoters. On the other hand, the in vivo activities of three other promoters, *katE*, *csiD*, and *osmY*, in the presence of $\sigma^S\Delta1–50$ were only fractions (15 to 30%) of the values obtained with σ^S (Table 4).

Two alternative explanations, not mutually exclusive, are possible for the latter finding. The first is that it represents a defect in σ^S activity associated with the N-terminal truncation (that can be overcome at least partially by increased expression of the mutant protein from plasmid pHYD408). With a K173E mutant of σ^S, Becker et al. (1) had earlier analogously demonstrated normal activity at one promoter (*csiD*) and three- to fivefold reduced activities at several others (*osmY*, *bolA*, and *otsB*). The second explanation is that of a confounding effect of the *rho* mutation on some σ^S-dependent promoters. As reported earlier (27) and as is also clear from the data in Tables 2 and 4, even in an *rpoS*$^+$ background the *rho* mutation has opposite effects on expression from *proU* P1 (increase) and expression from the *csiD*, *osmY*, or *katE* promoter (decrease).

In vitro experiments to assess the binding affinity of $\sigma^S\Delta1–50$ for core RNA polymerase and to determine the transcription activity of the reconstituted enzyme at the various promoters will help address these questions. Catabolite repression at *csiD* was preserved under conditions where the promoter was active in vivo with $\sigma^S\Delta1–50$, suggesting that an interaction of RNA polymerase bearing the mutant subunit with cAMP-cAMP receptor protein is normal (21).

$\sigma^S\Delta1–50$ and environmental regulation of σ^S-specific promoters. As mentioned above, the intracellular σ^S concentration varies according to the growth conditions and is regulated by different mechanisms, including those that operate at the steps of *rpoS* transcription, *rpoS* translation, and σ^S proteolysis (12, 14). There also exists substantial evidence for the notion that regulation of the intracellular σ^S content explains the regulation of expression of genes of the σ^S regulon by environmental variables, such as osmolarity (13, 16), growth phase (16, 41), and temperature (36), or by factors such as Hfq (23, 24) and ClpXP (33).

In the present study, therefore, the first suggestion that $\sigma^S\Delta1–50$ may exhibit normal environmental regulation even in the absence of the *rpoS* transcriptional and translational control signals came from the data in Table 2, which showed that osmotic and cold induction of *proU* P1 was unaffected in the chromosomal *rho rpoS*::IS*10* strain (in which the *rpoS* expression control sequences, although still retained in *cis*, are separated from the coding region for $\sigma^S\Delta1–50$ by IS*10*).

This possibility was then validated in experiments in which $\sigma^S\Delta1–50$ was designed to be expressed from a plasmid construct (pHYD408) that completely lacked the *rpoS* transcriptional and translational control sequences. Although Ara-induced synthesis of $\sigma^S\Delta1–50$ occurred at higher-than-physiological levels in these experiments, environmental regulation by growth phase, low temperature, and high osmolar-

TABLE 5. Environmental regulation with $\sigma^S\Delta1–50$[a]

Variable(s)	Culture condition	β-Galactosidase sp act for *lac* fusion with the following promoter in the indicated strain:			
		proU P1		*katE*	
		rpoS$^+$	Δ*rpoS*/pHYD408	*rpoS*$^+$	Δ*rpoS*/pHYD408
Temp and growth phase	30°C, log	63	27	110	50
	30°C, stationary	536	263	772	586
	10°C, log	279	263	1,686	645
Osmolarity	No NaCl	63	21	297	65
	0.3 M NaCl	275	193	576	333

[a] Cultures for β-galactosidase assays were grown in LB medium for experiments involving growth phase (log or mid-exponential phase and 1 h after entry into stationary phase) and temperature as variables and in K-tryptone medium (to mid-exponential phase at 30°C) for those involving osmolarity as the variable. For strain derivatives with the *proU* P1-*lac* fusion (on plasmid pHYD275), the medium was supplemented with trimethoprim; for derivatives with plasmid pHYD408, it was supplemented with ampicillin and Ara. Enzyme specific activities are reported in Miller units (22). Strains used were as follows (in the order *rpoS*$^+$ and Δ*rpoS*): for *proU* P1-*lac*, GJ862 and GJ2778 (each with pHYD275); and for *katE-lac*, GJ2770 and GJ2782.

ity was demonstrated by direct immunoblotting and indirect reporter gene expression assays. The nature and extent of regulation so identified for $\sigma^S\Delta1–50$ are similar to those described earlier for wild-type σ^S (12, 14).

Since normal environmental regulation of $\sigma^S\Delta1–50$ could be demonstrated even in the absence of the transcriptional and translational control elements of wild-type *rpoS*, our data suggest that alternative mechanisms, such as those affecting proteolytic degradation of σ^S, may be the primary determinants for regulation under these conditions. The $\sigma^S\Delta1–50$ protein retains the σ^S turnover element, which has been implicated in the regulation by RssB of ClpXP-catalyzed degradation of the protein (1).

Although regulation at the level of σ^S proteolysis was suggested earlier as one of several mechanisms involved in osmotic and stationary-phase induction of the cognate σ^S-specific promoters (12), its relative importance in either of these phenomena had so far not been defined. Nor had such a mechanism been postulated to participate in the phenomenon of low-temperature induction, since previous studies had described roles for only DsrA RNA and Hfq protein in the stimulation of *rpoS* translation during low-temperature growth (18, 30, 36, 37).

In summary, therefore, three major conclusions that have emerged from the present investigation are that (i) the N-terminal region of σ^S is not essential for in vivo sigma activity on at least one promoter (*proU* P1), although its removal leads to an apparent partial reduction of activity at three other promoters; (ii) environmental regulation of the σ^S regulon is largely unaffected even in the absence of *cis* elements needed for the transcriptional and translational control of *rpoS*; and (iii) the N-terminal region is also not necessary for the post-translational regulation of σ^S.

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