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σ^S-Dependent Gene Expression at the Onset of Stationary Phase in *Escherichia coli*: Function of σ^S-Dependent Genes and Identification of Their Promoter Sequences†

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The σ^S subunit of RNA polymerase, the product of the *rpoS* gene, controls the expression of genes responding to starvation and cellular stresses. Using gene array technology, we investigated *rpoS*-dependent expression at the onset of stationary phase in *Escherichia coli* grown in rich medium. Forty-one genes were expressed at significantly lower levels in an *rpoS* mutant derived from the MG1655 strain; for 10 of these, we also confirmed *rpoS* and stationary-phase dependence by reverse transcription-PCR. Only seven genes (*dps*, *osmE*, *osmY*, *sodC*, *rpsV*, *wrbA*, and *yahO*) had previously been recognized as *rpoS* dependent. Several newly identified *rpoS*-dependent genes are involved in the uptake and metabolism of amino acids, sugars, and iron. Indeed, the *rpoS* mutant strain shows severely impaired growth on some sugars such as fructose and *N*-acetylglucosamine. The *rpoS* gene controls the production of indole, which acts as a signal molecule in stationary-phase cells, via regulation of the *tnaA*-encoded tryptophanase enzyme. Genes involved in protein biosynthesis, encoding the ribosome-associated protein RpsV (*sra*) and the initiation factor IF-1 (*infA*), were also induced in an *rpoS*-dependent fashion. Using primer extension, we determined the promoter sequences of a selection of *rpoS*-regulated genes representative of different functional classes. Significant fractions of these promoters carry sequence features specific for Eσ^S recognition of the −10 region, such as cytosines at positions −13 (70%) and −12 (30%) as well as a TG motif located upstream of the −10 region (50%), thus supporting the TGN$_{0-2}$C(C/T)ATA(C/A)T consensus sequence recently proposed for σ^S.

Bacterial cells undergo a variety of morphological and physiological changes as they enter stationary phase. Several global regulators, involved in a complex regulatory network, induce the expression of stationary-phase-responsive genes (reviewed in reference 34). Among these, the *rpoS*-encoded alternative sigma factor σ^S plays a major role as a regulator of genes involved in stress responses (32). The σ^S protein accumulates in stationary phase as well as in response to stress conditions and directs transcription from about 100 genes (41, 47, 85). The high conservation (61) and the tight control of RpoS accumulation in response to a wide range of environmental stimuli are consistent with its crucial role in bacterial physiology (36). *rpoS*-regulated genes encode a variety of proteins with unrelated physiological functions, and mutations in *rpoS* have pleiotropic effects. Strains carrying nonfunctional *rpoS* alleles fail to express acid phosphatase (*appA*) (91), as well as oxidative stress genes such as superoxide dismutase (*sodC*) and catalase (*katE* and *katG*). *rpoS*-dependent regulation of oxidative stress genes is often mediated by additional regulatory proteins such as FNR, OxyR, or Fur (69). The *rpoS* gene also regulates the expression of DNA repair enzymes such as the exonuclease *xthA* (83), the methyl transferase *ada* (90), and the nonspecific DNA binding protein *dps* (3). The σ^S protein is also required for expression of the acid resistance *gadA* and *gadB* genes (21), for the response to either osmotic or temperature shifts (32), and in the expression of genes determining cell morphology, such as *bolA* (51). Finally, it regulates the expression of several virulence factors in pathogenic *Escherichia coli* strains e.g., *csgBA* genes encoding curli (6).

Sigma factors associate with core RNA polymerase (RNAP) to determine specific promoter recognition. The σ^{70} protein, encoded by the *rpoD* gene, is the main σ factor in *Escherichia coli* and is responsible for the transcription of most genes. Alternative σ factors compete with σ^{70} for core RNA polymerase to form the corresponding holoenzymes (e.g., σ^S-associated RNAP, or Eσ^S) and direct transcription of genes belonging to specific functional classes (41, 58). Unlike other alternative σ factors, σ^S recognizes promoters with sequences very similar to σ^{70}-dependent promoters, raising the problem of specific promoter recognition in vivo. Indeed, promoter alignment of *rpoS*-dependent genes suggests a −10 consensus sequence for Eσ^S [CTATA(A/C)T] basically identical to the Eσ^{70} consensus (TATAAT) (23, 55). This was confirmed by in vitro systematic evolution of ligands by exponential enrichment (SELEX), which also suggested recognition of an identical −35 sequence (TTGACA) (24). These data are consistent with the strong similarity in the DNA binding domains (2.4 and 4.2) between the two σ factors (57) and with the observation that Eσ^S can initiate transcription at many σ^{70}-dependent promoters in vitro (88). On the other hand, Eσ^{70} can transcribe some σ^S-dependent genes in the presence of additional factors or in the absence of specific repressors such as H-NS (6, 22, 87; reviewed in reference 56). Thus, at least for a subclass of

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promoters, promoter selectivity between $E\sigma^{70}$ and $E\sigma^{S}$ in vivo might be determined by factors other than DNA sequence, such as increased intracellular salt concentrations, degree of DNA supercoiling, modulation by additional regulators (37, 41; reviewed in reference 34), or signal molecules such as the ppGpp alarmone (43, 46). However, specific deviations from the common consensus sequence might favor either form of RNAP; for instance, the presence of a C immediately upstream of the −10 element (−13C [9]), flexibility in the location of the TG motif, and the presence of a C as the first nucleotide of the −10 sequence might favor promoter recognition by $E\sigma^{S}$ (47).

In this work, we have studied the expression of *rpoS*-dependent genes at the onset of stationary phase in cell grown in rich medium. We observed *rpoS*-dependent induction of amino acid and carbohydrate metabolism genes, consistent with the role of RpoS in response to starvation. We show that *rpoS* controls the production of indole, which plays a role as a signal molecule in stationary phase. Finally, our results show that *rpoS* also regulates the expression of genes involved in protein synthesis and that of a large number of genes with unknown function, underlining the complexity of the RpoS regulon. We characterized the promoter sequences in a selection of these genes by using primer extension. Our data strongly support the importance of the proposed σ^{S}-specific features in the −10 region (−12C, −13C, and TG) as well as the lack of conservation of the −35 promoter element.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and determination of indole production. The *rpoS* derivative (EB1.3 [78]) of *E. coli* strain MG1655 that was used in the present study was obtained by phage P1 transduction from strain MV2792 (93). For RNA extraction, bacterial cultures were grown overnight in Luria broth (LB) medium at 37°C with vigorous aeration, diluted 1:100 in LB medium, and then grown to an optical density at 600 nm (OD_{600}) of 0.2 and rediluted 1:100 in 15 ml of prewarmed LB in 100-ml flasks to avoid carryover of proteins accumulated in late-stationary phase. Samples for RNA isolation, either for gene array and primer extension or for real-time PCR experiments, were collected either at an OD_{600} of 2.5 to 3, a cell density that in our growth curve corresponds to the onset of stationary phase, or at an OD_{600} of 0.6 to 0.8 (mid-exponential phase of growth). For growth on limiting sugar concentrations, 1 ml of a bacterial suspension from an overnight culture in LB medium was centrifuged, washed, and resuspended in sterile phosphate-buffered saline. Twenty microliters of bacterial suspensions was used to inoculate 2 ml of M9 medium supplemented with different sugars as the sole carbon source, either at 10 mM (control cultures) or at 0.1 mM (carbon-limiting conditions), and bacteria were grown at 37°C with full aeration for 24 h. Aliquots of the overnight cultures prior to and after centrifugation were plated on L agar plates; plate counts showed very similar bacterial concentrations for strains MG1655 and EB1.3 (ca. 5×10^{9} CFU/ml).

For determination of indole production, we followed the method of indole conversion into indigo described in reference 71, with minor modifications. Both strains MG1655 and EB1.3 were transformed with the pStyABB plasmid, which constitutively expresses the styrene monooxygenase *styAB* genes from *Pseudomonas* strain S12 (71). In addition to styrene oxidation, the StyAB protein also catalyzes the transformation of indole into indigo, whose blue color can be determined spectrophotometrically at OD_{620} after lysis of the cells with dimethyl sulfoxide (DMSO) (71). Aliquots (1 ml) of either MG1655/pStyABB or EB1.3/pStyABB cultures grown in LB medium were taken at different times; the bacterial cells were centrifuged and lysed in DMSO, and the presence of indigo was determined spectrophotometrically. In an alternative method, 1-ml supernatants from overnight cultures of either MG1655 or EB1.3 were used to resuspend an equal volume of MG1655/pStyABB culture from the early-exponential phase of growth (OD_{600}, 0.25 to 0.3). The cells were incubated with the spent medium for 30 min at 37°C before DMSO lysis and spectrophotometric determination of indigo production.

RNA isolation, cDNA labeling, and hybridization. Cells were harvested by centrifugation, and RNA was isolated with the RNeasy mini kit (QIAGEN). Total-RNA purification was performed by on-column DNase I digestion according to the manufacturer's instructions. RNA samples were quantified by using a spectrophotometer (260 nm), checked by gel electrophoresis, and stored at −80°C until further use. For microarray hybridization, 25 μg of RNA samples was used as a template for reverse transcriptase to produce cDNA labeled with either Cy3- or Cy5-dCTP (Cyscribe first-strand cDNA kit; Amersham Biosciences). Four different array hybridizations were performed using RNA samples extracted from two independent cultures under analogous conditions. To correct for possible differences in Cy3 and Cy5 dye incorporation, the cDNAs were labeled with Cy3 dye in two hybridizations and with Cy5 dye in the other two (dye swapping). For each of the four experiments, the Cy3- and Cy5-labeled cDNAs, products of the reverse transcriptase reactions, were pooled, purified by using QIAGEN Qiaquick spin columns, and concentrated with a Microcon-30 concentrator (Millipore) prior to addition of the hybridization buffer. For our investigation we used the *E. coli* K-12 V2 array (MWG), which contains 4,286 genes (http://www.mwg-biotech.com). Hybridizations were run overnight at 42°C in the buffer supplied, and subsequent washing steps with SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) were performed at room temperature (22°C).

Procedure for microarray data analysis. Sixteen-bit tagged-image format file (TIFF) images produced by the scan of the microarray slides were analyzed with Affymetrix 428 Scanalyse and Jaguar software (version 2; Affymetrix), and data were managed by using Microsoft Excel. No ribosomal DNA spots are available on the MWG array slides for normalization. Thus, normalization was computed by using Jaguar software (average method): the mean values of an array are corrected by a factor which equalizes the global intensity of the signals resulting from each channel. An average background was determined for each individual hybridization experiment from the background values given by Jaguar for each spot with a specific dye. The normalized intensities of the spots were sorted according to the signals obtained for the wild-type MG1655 control samples (Cy3- or Cy5-labeled cDNA), and values lower than 1.3 times the average background were eliminated. This subtraction was performed in order to eliminate spots with nonsignificant expression levels in the wild-type strain, which were therefore unsuitable for addressing down-regulation due to the *rpoS* mutation. Several known *rpoS*-dependent genes (e.g., *bolA*, *katE*) fell into this category and were not further considered in our analysis, despite a high wild-type/*rpoS* ratio. Data from the four array experiments were flagged, pooled, and sorted to identify reproducible spots. Genes corresponding to spots with a wild type/*rpoS* signal ratio (R) of >2.5 in at least three out of four hybridizations, which never showed an R of <1 and whose final average ratio was ≥2.5, were considered down-regulated in the *rpoS* strain and are listed in Table 2.

RT PCR. For real-time PCR (RT-PCR), reverse transcription from RNA samples (extracted as for the microarray experiments) was carried out using 62.5 U of MultiScribe (Applied Biosystems) reverse transcriptase per μg of total RNA, in the presence of 1.25 μM random hexamers. Amplification reactions were carried out in an Applied Biosystems ABI Prism 7000 sequence detection system using the SYBR Green PCR master mix according to the manufacturer's standard protocol. cDNA produced from 20 ng of total RNA was used for each reaction. The internal-control 16S rRNA gene, used to normalize the values obtained for the genes analyzed, was amplified by primers *rrsB*fw (5′-GAATG CCACGGTGAATACGTT) and *rrsB*rev (5′-ACCCACTCCCATGGTGTGA). The PCR products of the genes of interest (shown in Fig. 1) were generated by using the primers listed in Table 1. All reactions were performed in triplicate, in addition to samples using DNase I-digested RNA as templates, to verify the lack of residual DNA. Real-time PCR data were analyzed by using ABI PRISM 7000 SDS software (version 1.0) and according to *Relative Quantitation of Gene Expression* (P/N 4303859), issued by the manufacturer.

Primer extension. Samples were collected, and RNA was extracted, in the same way as for microarray hybridization (see above). Twelve micrograms of RNA was used by the primer extension assay with ImpromII reverse transcriptase (Promega) according to the manufacturer's instructions. RNA and labeled primers were annealed at 70°C (for 5 min), and the reaction proceeded at 42°C for 1.5 h. The labeled extended fragments were separated from mRNA by heating at 95°C and were placed on ice prior to addition of formamide dye (MWG); the samples were then loaded onto a 7% polyacrylamide–urea denaturing sequencing gel, and transcription start sites were determined by comparison with a known DNA sequence used as a molecular weight marker. Reaction products were quantified with ImageQuant (Molecular Dynamics) after background subtraction from the TIFF images generated by the laser scanner (Li-Cor 4200 long reader; MWG). Primers used in this assay are shown in Table 1.

Other genetic methods. The *tnaA* gene was inactivated by the λ *red gam* method as described in reference 20, producing strain PL614. Insertion was verified by direct amplification of the *tnaA* gene. To measure *tnaA* gene expres-

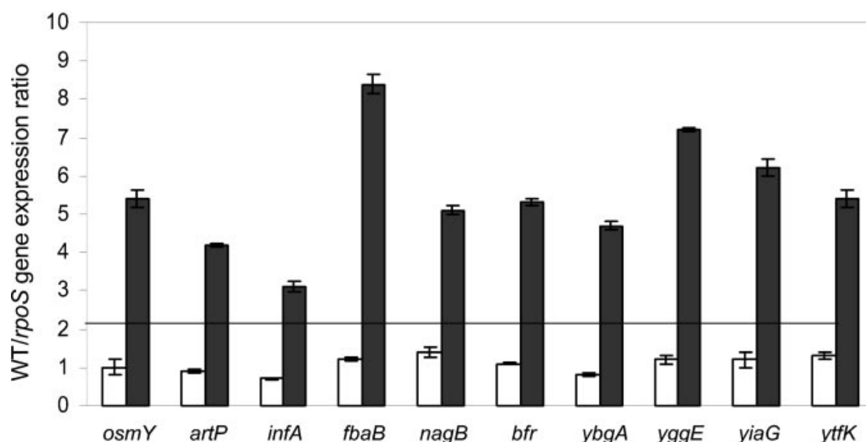

FIG. 1. Real-time PCR experiments. Values are shown as induction factors for expression in the wild-type (WT) strain MG1655 versus the *rpoS* mutant strain EB1.3 in the exponential (white bars) and stationary (black bars) phases of growth. The genes tested are indicated in the figure. Values were normalized to those for the 16S rRNA gene, and results shown are averages of three experiments. The horizontal line shows the 2.5-fold threshold used to select *rpoS*-dependent genes in the microarray experiments.

sion, its promoter region, including the short leader peptide for the TnaA protein encoded by the *tnaL* gene, was amplified by PCR using primers tnaA1 (CTGAAGCTTGATTGTGATTCGATTC, annealing at positions −392 to −376 relative to the *tnaA* start codon) and tnaA2 (CGGAATTCCTCTTCACGATAAGC, annealing at positions +76 to +90 relative to the *tnaA* start codon). The primers introduced a HindIII and an EcoRI site (underlined in the primer sequences) into the amplified fragment, which was then subcloned into the multicopy plasmid pRS1274 (49) by using the corresponding restriction sites. β-Galactosidase activity was determined as described in reference 66.

TABLE 1. Primers used in this study

Name	Sequence
For RT-PCR	
osmYfw	5′-GTCGATAGCTCTATG-3′
osmYrev	5′-GAAGGCGGCCCTGGG-3′
artPfw	5′-GAGTATTCAATTAAACGGC-3′
artPrev	5′-CCCTGTGGGCAATCCAGCGTG-3′
infAfw	5′-CGGTCACGTGGTTACTGCAC-3′
infArev	5′-GTCGCCCGTCAGGATGCG-3′
fbaBfw	5′-CGGGAGCATAGTAATGAC-3′
fbaBrev	5′-ACGGTGCTGTAAAAGGTTGTCG-3′
nagBfw	5′-CTCAACGGCAACGCCCCGG-3′
nagBrev	5′-GGCTCGTTACCTACACCGCCC-3′
bfrfw	5′-CTGGGGTCTCAAACGTCTC-3′
bfrrev	5′-CGGCGTGTTTCATCTCATC-3′
ybgAfw	5′-GATTTGATGACAGCACCCTAATC-3′
ybgArev	5′-AGCGGCTGTGATAATCGAGAAGC-3′
yggEfw	5′-GTGAAGTTCAAAGTTATCGCC-3′
yggErev	5′-TGACAATATGCGGTCCATCCG-3′
yiaGfw	5′-GATCCAATGCATGAGCTGTTGAG-3′
yiaGrev	5′-AGGACGTTGTTCTGTGCGTCAGG-3′
ytfkfw	5′-GGTACAACCCACTTCAGGTG-3′
ytfKrev	5′-TCCTTGATGTATAACCGTCC-3′
For determination of transcription start sites	
ansP	5′-AATGGCGATCATCTGCACCTGG-3′
artP	5′-CCCTGTGGGCAATCCAGCGTG-3′
dps	5′-AACTGGATAACCTGGCGATTCAG-3′
fbaB	5′-ACGGTGCTGTAAAAGGTTGTCG-3′
mysB	5′-TTTGGGCATTGAACTGTTGCAC-3′
pfkB	5′-AACACCGGTGCGGTACAGCG-3′
rpsV	5′-TGCCGGTTGGGTTATTTACTACG-3′
talA	5′-TGAGTAACAGCGAAGGATTGGTG-3′
ybjP	5′-TTCAACGCAAGGACCACTGCG-3′
ybgA	5′-AGCGGCTGTGATAATCGAGAAGC-3′
yggE	5′-TGACAATATGCGGTCCATCCG-3′
yiaG	5′-AGGACGTTGTTCTGTGCGTCAGG-3′
ytfK	5′-TCCTTGATGTATAACCGTCC-3′

Sequence analysis and gene function. The pattern search tools of Colibri (http://genolist.pasteur.fr/Colibri/genome.cgi) (65) were used to search for putative −10 promoter elements upstream of the primer extension products. The Colibri, PromEC (38) (http://bioinfo.md.huji.ac.il/marg/promec/prom.seq.final.html), and RegulonDB (84) (http://www.cifn.unam.mx/Computational_Genomics/regulondb) databases, and J. E. Mitchell's collection of σ^{70}-dependent promoters described in reference 67 (http://www.biosciences.bham.ac.uk/labs/minchin/mitchell2003), were used for analysis of promoter and operon structures. Finally, the Swiss-Prot (http://www.expasy.org/) and ProDom (http://prodes.toulouse.inra.fr/prodom/current/html/home.php) databases were used to gather more information about genes encoding hypothetical proteins.

RESULTS AND DISCUSSION

***rpoS*-dependent genes expressed at the onset of stationary phase.** Whole-genome expression in either *E. coli* MG1655 or its *rpoS* mutant derivative was determined at the onset of stationary phase (i.e., cells were harvested as soon as an increase in cell turbidity was no longer detectable) for cells grown in LB (rich) medium. At this stage of bacterial growth, we expected that the subset of *rpoS*-regulated genes mostly involved in the establishment of stationary-phase physiology would be maximally expressed. Through microarray experiments we detected 41 genes whose expression was significantly reduced in the *rpoS* strain (Table 2); only 7 of these had already been described as *rpoS* dependent (*dps*, *osmE*, *osmY*, *rpsV*, *sodC*, *wrbA*, and *yahO*). Known *rpoS*-dependent genes not detected in our experiment might be expressed only at later stages of stationary phase or in different growth media and conditions; however, lack of detection of some *rpoS*-dependent genes might be due to shortcomings of the microarray technique and to the criteria chosen for microarray analysis (see Materials and Methods). The known *rpoS*-dependent genes found in our experiments mainly encode stress response proteins with either regulatory or detoxification functions (35, 41; reviewed in reference 33). Among the newly identified σ^S-regulated genes, almost 50% have unknown functions. The others belong to four main functional classes: genes involved in amino acid transport and metabolism, iron uptake and storage,

TABLE 2. Genes induced at the onset of stationary phase with σ^s -dependent expression as identified by microarray analyses

Gene	Gene product	Functional class[a]	Mean WT value[b]	Ratio (WT/*rpoS*) SD[c]	Regulation[d]
Starvation and stress response genes					
dps^S	DNA protection protein, bacterial ferritin	SSR	1,597	2.5 ± 0.4	IHF^S, OxyR^S, *rssB*^{TC}, MMC, PQ, NaSal, Lrp, BG, DHCP, OS
osmE^S	Stress-inducible outer membrane lipoprotein	SSR	1,728	9.6 ± 2.1	Fis^S, NaSal, Lrp, OS
osmY^S	Hyperosmotically inducible periplasmic protein	SSR	424	4.4 ± 1.3	CRP^S, Lrp^S, IHF^S, H-NS^S, *arcB*^{TC}, MMC, Lrp, Rcs, BG, DHCP
psiF	Induced by phosphate starvation		656	4.8 ± 0.4	*ompR/envZ*^{TC}, CD
sodC^S	Superoxide dismulase precursor (Cu-Zn)	SSR	459	2.7 ± 0.5	*uvrY*^{TC}, *arcB*^{TC}
Protein synthesis					
rpsV^S	30S ribosomal subunit protein S22	PS	3,466	5.2 ± 2.8	FIS^S, IHF^S, ppGpp^S
infA	Protein chain initiation factor IF-1	PS	1,011	2.7 ± 0.8	
Amino acid transport and metabolism					
artP	Arginine transport, ATP-binding component	AATM	714	2.6 ± 0.7	PQ, NaSal, Lrp
artI	Arginine transport, periplasmic binding protein	AATM	346	3.8 ± 1.9	PQ, Lrp
ansP	L-Asparagine permease	AATM	572	2.5 ± 0.4	DHCP
ilvD	Dihydroxyacid dehydratase	AATM	364	4.8 ± 2.1	*ompR/envZ*^{TC}
tnaA	Tryptophanase	AATM	7,808	13.7 ± 4.3	*phoQ*^{TC}, *ompR/envZ*^{TC}, CRP, *uvrY*^{TC}, *arcA*^{TC}, *arcB*^{TC}, *rcsB*^{TC}, *citAB*^{TC}, MMC, PQ (*ilvG*: Lrp)
wrbA^S	Flavodoxin-like protein; binds the *trp* repressor	SSR	364	5.0 ± 2.0	NaSal, Lrp, BG, DHCP, OS
Iron uptake and storage					
bfr	Bacterioferrin, iron storage homoprotein	IUS	905	5.1 ± 2.1	DHCP, *ryhB* RNA
entD	Enterochelin synthetase, component D	IUS	508	4.2 ± 0.5	*acrB*^{TC}
Carbohydrate metabolism					
crr	Phosphotransferase system, glucose-specific IIA component	CHM	443	2.7 ± 0.6	CD
fbaB	Fructose 1,6-bisphosphate aldolase (b2097)	CHM	1,264	4.6 ± 1.4	MMC, Lrp
gip	Glyoxylate-induced protein		384	3.6 ± 1.0	*kdpABCDE*^{TC}
glcG	Putative oxidase, glycolate utilization (b2977)	CHM	462	3.0 ± 1.7	*uvrY*^{TC}, *arcA*^{TC}, *arcB*^{TC}
nagB	Glucosamine-6-phosphate deaminase	CHM	860	2.6 ± 0.6	*ompR/envZ*^{TC}, *arcB*^{TC}
pfkB	Phosphofructokinase-2	CHM	512	2.5 ± 2	MMC, CD, DHCP
talA	Transaldolase A	CHM	1,097	2.9 ± 1.8	*rssB*^{TC}, MMC, DHCP, CreBC
Miscellaneous function or unknown function					
deoD	Purine nucleoside phosphorylase	NM	349	4.8 ± 3.0	
moeB	Molybdopterin biosynthesis	CB	1,100	3.4 ± 1.2	
msyB	Membrane protein, protein export (b1051)	MF	516	6.1 ± 4.0	CD, Lrp, DHCP
yafN	Hypothetical (adherence) protein (b0232)	(MF)	354	2.6 ± 0.1	MMC, GadX
yahO^S	Hypothetical protein (b0329)		933	4.5 ± 2.2	Lrp
ybgA	Hypothetical (inner membrane) protein (b0707)		2,038	4.5 ± 1.7	
ybjP	Putative enzyme (b0865)		1,317	4.2 ± 2.5	*rssB*^{TC}, *baeSR*^{TC}, *atoSC*^{TC}, Lrp
yddX	Hypothetical protein, biofilm dependent (b1481)	(EF)	376	4.2 ± 1.4	
ydiZ	Hypothetical protein (b1724)		707	3.1 ± 1.7	*rssB*^{TC}, OS
yfeT	Hypothetical protein (b2427)		341	3.5 ± 0.9	*ompR/envZ*^{TC}
ygaF	Putative dehydrogenase protein (b2660)		453	2.8 ± 0.5	*rssB*^{TC}, NarXL^{TC}
yggE	Putative immunogenic protein (b2922)	(EF)	813	4.8 ± 1.7	*rssB*^{TC}, MMC; Rcs, *phoQP*
ygiS	Putative transport periplasmic protein (b3020)	(MF)	436	2.7 ± 0.8	
ygiW	Hypothetical (outer membrane) protein (b3024)	(MF)	892	4.0 ± 1.9	*rssB*^{TC}, *citAB*^{TC}, *arcA*^{TC}, NaSal
yiaG	Hypothetical (regulatory) protein (b3555)		1,227	2.7 ± 0.5	*rssB*^{TC}, *ntrBC*^{TC}, NaSal
yjbJ	Hypothetical protein (b4045)		601	4.5 ± 1.2	
ynfA	Hypothetical (membrane) protein (b1582)	(MF)	3,334	3.5 ± 1.1	
yqjC	Hypothetical (periplasmic) protein (b3097)		1,121	5.9 ± 1.3	
ytfK	Hypothetical protein (b4217)		556	3.9 ± 3.0	PQ, Fur, BG

[a] SSR, starvation and stress response; PS, protein synthesis; AATM, amino acid transport and metabolism; IUS, iron uptake and storage; CHM, carbohydrate metabolism; NM, nucleoside metabolism; CB, cofactor biosynthesis; MF, membrane function; EF, extracellular function. Parentheses indicate a putative function.

[b] Mean relative expression level in the wild-type (WT) strain; average values of the signal intensities obtained in the wild-type strain in four experiments without correction (i.e., without dye swapping).

[c] Averages and standard deviations from four experiments are presented. The genes listed in this table presented a ratio of expression in the wild-type versus the *rpoS* strain (R) greater than 1.9 in at least three out of four experiments and never presented an R of <1. Genes with an average R lower than 2.5 were excluded from this table, except for *ytfK* and *pfkB,* which were confirmed by primer extension, and *artI,* located in a cluster with *ybjP* and *artP,* both of which are down-regulated in the *rpoS* strain.

[d] Genes known to be regulated by RpoS are marked with a superscript capital S. Relevant references for these genes are given in parentheses *dps* (3), *osmE* (11, 17), *osmY* (8, 50, 101), *sodC* (27), *rpsV* (*sra* [42]), *wrbA* (100), and *yahO* (40). Growth conditions or regulatory pathways known (by either genomic or genetic analysis) to affect expression of the genes listed include the following: (i) disruption of known two- component (TC) regulatory systems (74); (ii) knockout of the specific regulatory gene(s) *fur* (10), *rcs* or *phoQP* (30), *lrp* (39, 89), *gadX* (64), *creBC* (7), or *ryhB* (63); (iii) specific growth conditions, including exposure to mitomycin C (MMC) (44), oxidative stress induced by either paraquat (PQ) or sodium salicylate (NaSal) (76), biofilm growth (BG) (86), 4,5-dihydroxy-2-cycloenten-1-one (DHCP) treatment (antimicrobial agent [75]), high-cell-density (CD) culture (102), osmotic stress (OS) response (supercoiling- dependent genes [16]), and minimal versus LB medium (MD) (97).

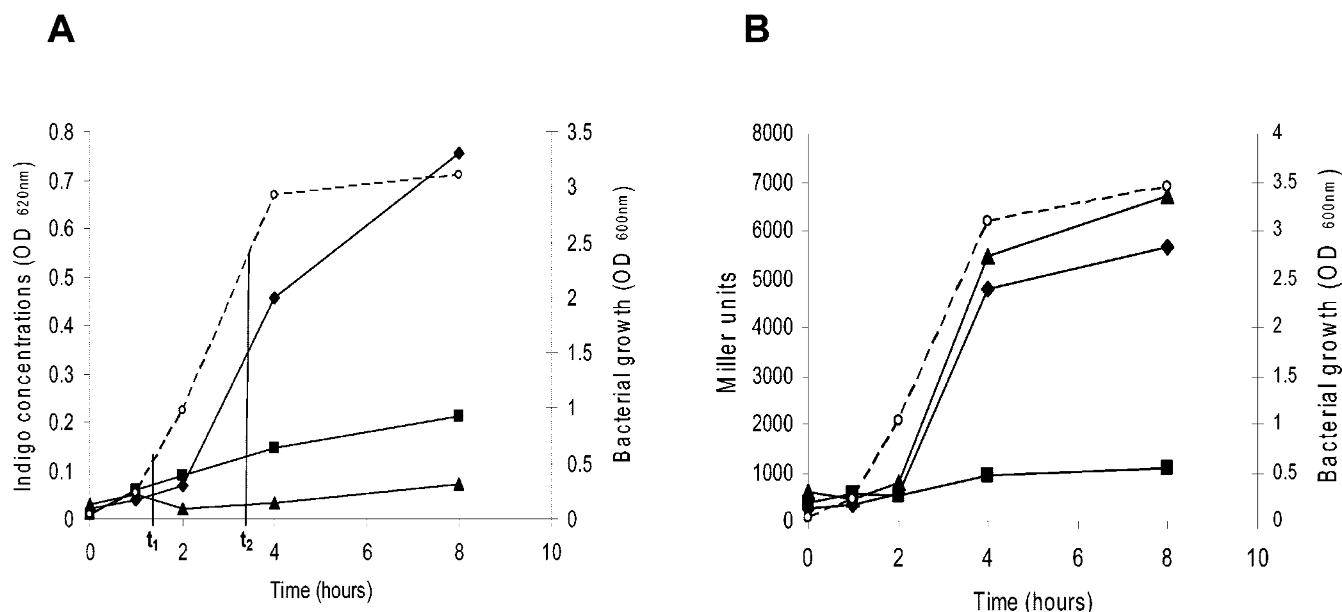

FIG. 2. (A) Indole production in strains MG1655 (wild type) (black diamonds), EB1.3 (*rpoS*) (black squares), and PL614 (*tnaA*) (black triangles), all carrying the pStyABB plasmid. The indole concentration was determined through its conversion into indigo by the StyAB monooxygenase as described in Materials and Methods. The growth curve in LB for MG1655 is shown by the dashed line and open circles. The growth rate of either the EB1.3 or the PL614 strain was not affected by the mutation. Samples for RT-PCR (Fig. 1) from exponential- and stationary-phase cells were collected at time points t_1 (OD_{600}, 0.6 to 0.8) and t_2 (OD_{600}, 2.5 to 3), respectively. (B) *tnaA* promoter activity from a multicopy plasmid in strains MG1655, EB1.3, and PL614. Symbols are as explained for panel A.

protein synthesis, and carbohydrate and nucleoside metabolism. It is noteworthy that the newly identified *rpoS*-dependent genes do not appear to be essential genes as defined by Gerdes et al. (26), except for *infA* (18), a finding consistent with the lack of major effects of the *rpoS* mutation on cell viability.

To confirm the results of the whole-genome expression analysis, we performed RT-PCR experiments on a selection of differentially expressed genes belonging to each functional category (Fig. 1). RT-PCR experiments confirmed the increased expression of all the genes tested in strain MG1655 compared to the *rpoS* mutant strain EB1.3. Increased gene expression in MG1655 was detectable only at the onset of stationary phase; samples taken in mid-exponential phase (OD_{600}, 0.6 to 0.8) showed no significant differences in gene expression between the two strains (Fig. 1).

Genes involved in cell metabolism. We found several genes involved in amino acid transport (*artI*, *artP*, and *ansP*) or metabolism (*tnaA* and *ilvD*) to be induced in a σ^S-dependent fashion at the onset of stationary phase. In particular, *rpoS* appears to promote tryptophan degradation by inducing the tryptophanase gene *tnaA*, which results in the production of indole, and by stimulating expression of the WrbA protein, which might strengthen negative regulation of the *trp* biosynthetic operon via binding to the TrpR repressor protein (100). Expression of the *wrbA* gene has already been shown to be *rpoS* dependent (41, 100). Interestingly, indole has been proposed to act as an extracellular signal in stationary cells of *E. coli* (95), and *tnaA*-mediated indole biosynthesis appears to stimulate biofilm formation (62). We investigated the possibility that indole production is negatively affected by *rpoS* mutation. Indole is converted into indigo (which is not further degraded in *E. coli*) by several monooxygenases, thus providing an easy method for its determination (71). We transformed both strains MG1655 and EB1.3 with pStyABB, a plasmid expressing styrene monooxygenase, and monitored indole production through its conversion into indigo (Fig. 2A). The production of indole closely follows the growth curve in MG1655, as revealed by indigo accumulation; in contrast, indole production is totally abolished in a *tnaA*-null mutant and severely affected by inactivation of the *rpoS* gene (Fig. 2A). To rule out the possibility that indigo production might be affected by different expression of styrene monooxygenase in the *rpoS* mutant strain, we exposed MG1655/pStyABB cells in early-exponential phase to filtered supernatants of either MG1655 or EB1.3 cultures. MG1655/pStyABB produced indigo only when treated with MG1655 conditioned medium, confirming that the *rpoS* mutant strain EB1.3 is deficient in indole production (data not shown). The results of the microarray experiments strongly suggest that lack of indole production in the *rpoS* mutant strain depends on reduced *tnaA* expression, which was further confirmed by *tnaA* gene expression measurement using the *lacZ* reporter gene (Fig. 2B).

While tryptophan is degraded in stationary phase, accumulation of the amino acids arginine and asparagine appears to be stimulated by *rpoS* through activation of the *artP* and *artI* genes, whose products are components of the arginine transport system and of the asparagine transporter *ansP*. Thus, the *rpoS* gene seems to play an important role in determining intracellular amino acid concentrations in stationary phase. This function might be related to the synthesis of amino acid- or peptide-derived signal molecules, as for indole, or to the need to redirect general amino acid metabolism in stationary phase. It is noteworthy that both the *artP* and the *wrbA* promoter are controlled by the global regulator Lrp (leucine-

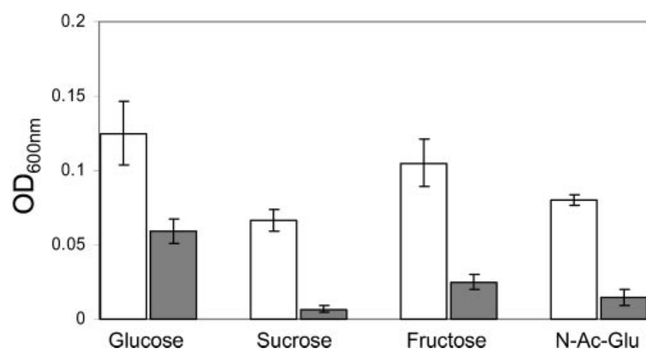

FIG. 3. Growth of MG1655 (wild type) (white bars) or EB1.3 (*rpoS*) (grey bars) on limiting concentrations (0.1 mM) of either glucose, sucrose, fructose, or *N*-acetylglucosamine (N-Ac-Glu). The growth experiments were performed as described in Materials and Methods. Values represent the final OD_{600} reached at the end of the 24-h incubation and are averages from three experiments.

responsive protein) (Table 2), the main regulator of amino acid biosynthesis and metabolism. The unknown *ybjP* gene, located immediately upstream of the *art* operon but controlled by its own promoter (see Fig. 4), is also regulated by *lrp* (39) as well as by *rpoS*. Concerted regulation by RpoS and Lrp has already been reported for a number of genes (*osmC*, *osmY*, *aidB*, and *csiD* [13, 48, 50, 59]), suggesting tight integration of Lrp- and RpoS-dependent regulation.

We also detected *rpoS*-dependent activation of genes encoding carbohydrate metabolism proteins such as the carbohydrate phosphotransferase systems (*crr*) or glycolysis enzymatic proteins (*fbaB* and *pfkB*). Interestingly, both the *fbaB* and *talA* genes encode isoenzymes of *fbaA* and *talB*, genes that do not appear to be regulated by *rpoS*, suggesting that σ^S could upregulate the expression of isoenzymes that are possibly more functional during the stationary phase of growth. The *rpoS* gene also appears to be involved in alternative metabolic pathways for carbohydrate utilization such as the pentose phosphate or the glyoxylate shunt to the carboxylic acid pathway (*glcG* or *gip*). In addition, *rpoS* activates the catabolism of *N*-acetylglucosamine through induction of *nagB*. We directly tested the possible role of *rpoS* in the expression of carbohydrate metabolism genes by growing strains MG1655 and EB1.3 on limiting amounts of different sugars as sole carbon sources. Both the parental and *rpoS* strains were able to grow to an OD_{600} of >1.0 on 10 mM concentrations of glucose, fructose, *N*-acetylglucosamine, or sucrose, with similar growth rates (data not shown). However, in the presence of a growth-limiting sugar concentration (0.1 mM), the *rpoS* mutant showed significantly impaired ability to grow, ranging from a twofold reduction for growth on glucose to an eightfold reduction for growth on sucrose (Fig. 3), despite the fact that the inocula contained similar numbers of viable cells (data not shown) (see Materials and Methods). Although *rpoS* dependence of sugar uptake or catabolism genes would be consistent with their induction in response to starvation, it is in contrast with previous observations suggesting that *rpoS* might negatively regulate the *nagB* and *crr* genes in continuous cultures grown under sugar-limited conditions (92). It is possible that *rpoS* might be involved in feedback regulation of carbohydrate metabolism genes. *rpoS* could activate the expression of sugar uptake and metabolism genes at the onset of stationary phase, to scavenge for the presence of low concentrations of sugars. However, in the absence of the specific inducers, expression would be shut down by *rpoS* itself through indirect regulation at a later stage of the stationary phase. An example of feedback loop regulation by *rpoS* has already been described for the curli-encoding *csg* operon (6, 78).

Iron-dependent genes. Iron is an essential cofactor of many enzymes, and bacteria have evolved a range of strategies to acquire and store iron, which is often not bioavailable in the environment. *E. coli* possesses two main iron storage proteins: bacterioferritin and ferritin (4). Bacterioferritin, whose precise function is still unclear (1), is induced during slow growth or at the transition to stationary phase, consistent with its regulation by *rpoS*. The *entD* gene encodes enterochelin, a synthase of a catechol siderophore from chorismic acid; the EntD protein is located in the inner membrane, but its function is not fully understood (5, 29). The gene is located downstream of the *fepA* gene, apart from the rest of the *ent* cluster, suggesting possible differential regulation. Regulation of ferritins and iron-dependent genes by *rpoS* is not entirely surprising, since *rpoS* is already known as the main regulator of *dps* expression. Although the Dps protein was originally described as a nonspecific DNA binding protein involved in resistance to oxidative stress (60), it is actually a bacterial ferritin (28), whose main function could be to chelate intracellular iron and thus to prevent DNA damage through Fenton reaction-catalyzed oxyradical formation (31). Many iron-dependent genes are regulated by Fur (ferric uptake regulator), which, in the presence of iron, represses the transcription of iron-responsive genes (25, 63, 80). A recent report proposes that Fur accumulation would be modulated by *rpoS* in *Vibrio vulnificus* (52), pointing to a possible overlap of *fur*- and *rpoS*-dependent gene regulation in enterobacteria.

Protein synthesis. We found two genes involved in protein synthesis to be activated in an *rpoS*-dependent fashion under the conditions tested. Interestingly, in addition to the already known *rpsV* gene, we could show that transcription of the *infA* gene is also stimulated by *rpoS* (Table 2; Fig. 1). The *infA* gene encodes the initiation factor IF-1, whose precise role has not yet been assigned (77). Similarly, no precise function has yet been assigned to the product of *rpsV*, a small, stationary-phase-induced, ribosome-associated protein (SRA) which appears to be specific to enterobacteria (42). The RpsV protein might play a role similar to that of the ribosome-modulating factor (RMF), i.e., to stabilize ribosomes in stationary phase. The RMF protein is also produced in stationary phase, although not in an *rpoS*-dependent fashion (94), and *E. coli* mutants with defects in *rmf* cannot survive long periods of starvation (99). Recent findings point to a close relationship between protein synthesis and entrance into stationary phase: aberrant proteins result from increased mistranslation in nonproliferating bacteria and are likely to accumulate in stationary phase (70). We propose that σ^S-dependent induction of IF-1 and SRA might either prevent translation errors or modulate protein synthesis by blocking ribosomes in an inactive form.

Genes of unknown function. The *rpoS* mutation affects the expression of 16 genes encoding hypothetical proteins of unknown function, several of which might have functions associated with membrane trafficking (*ybgA*, *ygiS*, *ygiW*, *ynfA*, and

yqjC) or might be located at the cell surface (*yafN*, *yddX*, and *yggE*). Some of these proteins are common to a wide number of enterobacteria or, in the case of *ynfA* and *yiaG*, even to a larger group including gram-positive bacteria. Interestingly, the *yahO* gene has previously been reported to be regulated by RpoS in *Salmonella enterica* (40); the YahO protein shares 66% identity and 79% similarity with its *E. coli* homologue. The *yggE* gene, conserved in other enterobacteria such as *Shigella flexneri* and *Edwardsiella ictaluri* at an identical locus (68, 96), encodes a putative extracellular protein, considered a potential antigenic protein for development of a vaccine against *E. ictaluri* in catfish (68). Two *rpoS*-dependent genes encode either a potential adhesion factor (*yafN*) or a factor involved in biofilm formation (*yddX* [79]). Another gene, *ytfK*, is up-regulated in *E. coli* cells growing as a biofilm (86). These observations would support a general positive role of *rpoS* in biofilm formation (2), possibly mediated by indole production (Fig. 2) (62), consistent with the dependence on *rpoS* of known adhesion and biofilm formation determinants such as the *csg* genes in *Salmonella* and in pathogenic *E. coli* strains (73).

Promoter sequences of σ^S-regulated genes. Primer extension assays were performed to determine the promoter sequences of the genes identified and to confirm the results of the gene array experiment. We selected 11 genes belonging to different functional classes and showing variable expression levels and wild-type/*rpoS* mutant ratios in the gene array experiment. In addition, we performed primer extension assays on the *dps* and *rpsV* genes, whose σ^S-dependent promoters have already been characterized, as a control for our experimental conditions. The promoter sequences found for both *dps* and *rpsV* correspond to those previously described (3, 42); this was also the case for the known *pfkB* promoter, which, however, had not yet been identified as a σ^S-dependent promoter (19). Primer extension was performed from total RNAs of both wild-type and *rpoS* strains, and transcription start sites were determined by comparison with a DNA sequencing reaction ladder (see Fig. S1 in the supplemental material). Quantification of primer extension products consistently showed lower levels of expression in the *rpoS* mutant, confirming the results of the gene array experiments (Fig. 4). For several *rpoS*-dependent genes, multiple signals were detected in the primer extension experiments (*ansP*, *artP*, *talA*, *pfkB*, *ybgA*, and *yggE*), which might depend either on secondary RNA structures resulting in pausing of reverse transcriptase, or on the presence of multiple promoters. Indeed, well-conserved promoter-like sequences could be detected upstream of most reverse transcriptase extension products, suggesting that the presence of multiple promoters might be a rather common feature for *rpoS*-dependent genes.

Our results do not allow us to ascertain that all *rpoS*-dependent genes identified are directly transcribed by $E\sigma^S$. However, we expect that most promoters induced at the onset of stationary phase should belong to the class of promoters recognized with high affinity by the σ^S subunit of RNA polymerase and thus should display promoter features favorable for $E\sigma^S$. Indeed, the sequences of the −10 regions of the promoters tested are in good agreement with previous compilations of σ^S-dependent promoters (23, 47, 54) and reflect the recently proposed motif $TGN_{0-2}CYATAMT$ (47). The −10 sequence of around 70% (12 of 18) of the newly characterized σ^S-dependent promoters is immediately preceded by a C at the position conventionally indicated as −13; −13C is a widely conserved feature for promoter recognition by $E\sigma^S$ (9, 15). Six (30%) promoters (*ansPp*$_1$, *ansPp*$_2$, *fbaB*, *talAp*$_2$, *pfkBp*$_1$, and *yggEp*$_2$) display a C as the first nucleotide of the −10 promoter element (conventionally −12C), a feature that might prevent their transcription by $E\sigma^{70}$ (47). A TG motif is present upstream of the −10 element in 10 (50%) of the newly determined promoters, at positions from −17 to −14. In a previous study (47), we proposed that σ^S can recognize the TG motif at different positions, in contrast to σ^{70}, which can interact only with a TG placed at −15/−14 (14, 67). In the novel σ^S-dependent promoters identified in this report, the distribution of the TG motif is not as wide as that which we had found at the 56 previously characterized σ^S-dependent promoters (47), as it is more restricted to the −15/−14 position. Interestingly, the *artPp*$_3$ promoter, one of the promoters with the lowest σ^S/σ^{70} activity ratio, harbors a double TG, which is supposed to further enhance promoter recognition and transcription initiation by both forms of polymerase (24, 67).

The conservation of the amino acids of region 4.2 of both σ factors and the results obtained by the SELEX approach (24) suggest that $E\sigma^S$ might be able to recognize a −35 element and would have the same optimal sequence as σ^{70} (TTGACA). At some *rpoS*-dependent promoters, such as *osmE* (12), disruption of the −35 sequence (TTGAAA) has a negative effect on σ^S-mediated transcription. However, data on the importance of a −35 sequence for $E\sigma^S$ are controversial, and very few known σ^S-dependent promoters possess a −35 sequence similar to that recognized by $E\sigma^{70}$ (12, 72, 87, 98). Among the newly identified promoters, only three (*pfkB*, *ytfK*, and *yggE*) have a discernible −35 element with four of six matches to the σ^{70} consensus sequence, possibly suggesting that $E\sigma^S$-specific −35 sequences might strongly diverge from the $E\sigma^{70}$ consensus (Fig. 4). It has been proposed that DNA bending upstream of the promoter region as well as motifs rich in C/G in the −35 region would favor σ^S selectivity (45, 98). Although our results do not allow us to confirm this hypothesis, we did find fairly high occurrence of GC or CG dinucleotides in the −35 area (Fig. 4). Indeed, the *artPp*$_1$, *artPp*$_2$, and *yiaG* promoters carry a CCG sequence, proposed to be a σ^S-specific −35 promoter sequence by Wise et al. (98), while *dps* and *ytfK* display the GCGG motif recently proposed as an osmotic shock-responding module in a subset of σ^S-dependent promoters (53).

Finally, five promoters (*ansPp*$_1$, *fbaBp*$_1$, *pfkB*, *talA*, and *ytfK*) possess UP-like elements, i.e., putative binding sites for the α subunit of the RNAP (82). The role of the UP element in promoter recognition by $E\sigma^S$ has not yet been addressed in detail; at the *aidB* promoter, an UP-like element functional for $E\sigma^{70}$ also contributes to $E\sigma^S$-dependent transcription in vitro, suggesting that UP elements might indeed play a role in $E\sigma^S$-promoter interactions (S. Lacour and P. Landini, unpublished data).

Conclusions. In this report, we compared the expression profiles of strain MG1655 and its *rpoS* derivative in LB (rich) medium. Since we tested *rpoS*-dependent expression at only one time point (the onset of stationary phase) and in one growth medium, our results give only an incomplete picture of the *rpoS* regulon; however, they provide information on genetic and physiological adaptation to stationary phase under

Gene	Promoter sequence (−35, −10, +1)	P_{+1}	WT / *rpoS*	R_{PE}	R_{MA}
ansP P1	TAGTTTTATACCCATAAAGAATAATGGTGATAACTATCATCGCCAGGATGAATAAACATTGTTCA**TGGCAACTT**ATAT ***g***	-31		2	2,5
ansP P2	ATGCTGCGCCGATAGTTTTATACCCATAAAGAATAATGGTGATAACTATCATCGCCAGGATGAATAAA**CATTGT**TCATGGC ***a***	-40		2	2,5
artP P1	ACATTTATGCTCGCCGACCACCGCCCCCGTTATTTTGTGCTATGTTTATTGAATAATGCGCTT**TG**CTT**TTAACT**TTTAA ***ag***	-81		1,2	2,6
artP P2	CGGCATTTCGTGAGGAATGCCGACATTTATGCTCGCCGACCACCGCCCCCGTTATTTTGTGCTA**TG**TT**TATTGA**ATAATGC ***g***	-99		1,5	2,6
artP P3	ATTGTCCGGCATTTCGTGAGGAATGCCGACATTTATGCTCGCCGACCACCGCCCCCGTTATTT**TGTGCTATGTT**TATTG ***aa***	-108		2	2,6
dps*	TTTTTCACGCTTGTTACCACTATTAGTGTGATAGGAACAGCCAGAATAGCGGAACACATAGCCGG**TGCTATACT**TAATCTC ***g***	-39		6	2,5
fbaB	TGCGGTGAAAACTTGACCGCGCTAACATTTTTTCTGATGAATCGAGCCAACAGAAAACGCTGAAAAAA**CATCCA**AAAG ***a***	-102		8	4,6
msyB	CGTCGTCGAATACCCCAGGTATCGAACTGATTTTTCGCCTTTCATACTTGCAAAAGCGGAGAATCAG**CTATCCT**TTTCCC ***tg***	-30		11	6,1
pfkB P1*	TGCAAAATTTTAAATAAAGCTCCAATAAATCATATTGTTAATTTC**TTCACT**TTCCGCTGATTCGG**TGCCAGACT**GAAAT ***c***	-19		1,2	2,5
pfkB P2	AAAACAGATTTTTATTTATATATATTTATCTGCAAAATTTTAAATAAAGCTCCAATAAATCATAT**TG**T**TAATTT**CTTCACT ***t***	-47		2	2,5
rpsV*	GTCAGTGATATCTACCAGCAAACGATCAATTATGTGGTCAGTGGCCAGCACCCTACGCTTTAAGG**TGCTATGCT**TGAT ***cg***	-86		5,5	5,2
talA P1	AATGTTTTGGTAATAATCCTATAACACTGATGTTACCTGCTTAATCCAGCAATACCATGCCTGTC**TGCTATGCT**TTTT ***t***	-77		2,5	2,9
talA P2	TCCAAACTCTCACCATTAATATAATGTTTTGGTAATAATCCTATAACACTGATGTTACCTGCTTAAT**CCAGCAA**TACCATGC***c***	-97		1,5	2,9
ybgA P1	GGGAGCAGGTTTCGTAAATTTGTCCTGCTACAACAGGATTAACTTCACAAATATCATTTCTCAACGT**CTACACT**TACTCCT ***g***	-21		3	4,5
ybgA P2	TCACGTTTGAATAGAAAGGGCAGCTATGGTTAGAAACTACCTGACGTCAGTCCTTGCGGGGAGCAGG**CTTTCGT**AAATTT ***g***	-82		3	4,5
ybjP	AAAATTGCATGATCGATCTCCTTGTTTGCTAAGCCTTCGATCTCAAAAGCATTATCAGACTGATACG**CTATTAT**TGAAA ***g***	-56		2	4,2
yggE P1	ACGGTATTGCTCATGCACAAGCCTTGTTCAGTTAGGCGCTATCTGA**TGGAAA**AATAAAACAGAGGCG**CTAAGCT**TGCCTCC ***a***	-46		9	4,8
yggE P2	TTATCAATCTGGTTGTGGGATGTGTTATGTGGTTTATTGCCTTGCAGCTGGCGAGAGACGGTAT**TG**CT**CATGCA**CAAGC ***c***	-105		25	4,8
yiaG	GACAGGCTGAATCGAATCATAGCCAGAGCATGCCCTGACTTCACCCCGCTGTGTCTGCTTTTCCCGA**CTATTCT**TAATGA ***g***	-47		6	2,7
ytfK	TAATTCTGAATAATTGTAACCTTTAGGTAAAAAAAGTTATACGCGG**TGGAAA**CATTGCCCGGATAGT**CTATAGT**CACTAA ***g***	-76		16	3,9
	Consensus **TG(N)$_{0-2}$CYATNCT**				

FIG. 4. Promoter sequences of σ^S-regulated genes induced at the onset of stationary phase, characterized by primer extension. The −10 sequence of each promoter is boldfaced, as is the conserved −35 element when present. Nucleotides that match the proposed consensus for the −10 element [(TGN$_{0-2}$)CYANNMT, where Y stands for C or T, M stands for A or C, and N stands for any nucleotide (47)] or the −35 element (TTGACA) at a functional location (15 to 17 nucleotides away from the −10 sequence) are underlined (for the −10 sequence) or double underlined (for the −35 sequence). The transcription start sites are lowercased, boldfaced, and italicized. Recurrent CG dinucleotides in the −35 region and the CCG motifs proposed by Wise et al. (98) are also underlined. Promoters marked with an asterisk are known promoters (3, 19, 42). P_{+1} indicates the position of the transcription start site relative to the start codon. WT and *rpoS* designate the products of primer extension obtained with total mRNA from the wild-type strain MG1655 and its *rpoS* derivative, respectively; the sizes of the primer extension products were determined by use of a DNA sequencing ladder of a known sequence run on the same gel. R_{PE} indicates the WT/*rpoS* expression ratio as determined from the primer extension experiments. R_{MA} indicates the WT/*rpoS* expression ratio as determined from the microarray experiments (Table 2).

the conditions tested, as well as on the structure of *rpoS*-dependent promoters. Indeed, in addition to known stress response genes, we found genes involved in a variety of metabolic functions to be induced in an *rpoS*-dependent fashion. The *rpoS* gene appears to be directly involved in the biosynthesis of signal molecules such as indole, which plays a role in biofilm formation in some *E. coli* strains (62) and might regulate gene expression in coordination with other cell-signaling systems directly related to quorum sensing and cell density (81). Our results also point to a direct role of σ^S in response to nutrient starvation, suggesting that the σ^S regulon overlaps with other nutrient-specific regulatory pathways such as cAMP/CRP, NtrB/NrtC/σ^{54}, and PhoB/PhoR. We could confirm with primer extension experiments that, for a large number of the newly identified σ^S-dependent promoters, the −10 promoter element matches the proposed consensus TGN$_{0-2}$CYATAMT. In contrast to strong conservation in the −10 region, little similarity to the −35 sequence for Eσ^{70} and no significant presence of UP element sequences could be detected.

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REFERENCES

1. **Abdul-Tehrani, H., A. J. Hudson, Y. S. Chang, A. R. Timms, C. Hawkins, J. M. Williams, P. M. Harrison, J. R. Guest, and S. C. Andrews.** 1999. Ferritin mutants of *Escherichia coli* are iron deficient and growth impaired, and *fur* mutants are iron deficient. J. Bacteriol. **181:**1415–1428.
2. **Adams, J. L., and R. J. McLean.** 1999. Impact of *rpoS* deletion on *Escherichia coli* biofilms. Appl. Environ. Microbiol. **65:**4285–4287.
3. **Altuvia, S., M. Almiron, G. Huisman, R. Kolter, and G. Storz.** 1994. The *dps* promoter is activated by OxyR during growth and by IHF and σ^S in stationary phase. Mol. Microbiol. **13:**265–272.
4. **Andrews, S. C., J. M. Smith, C. Hawkins, J. M. Williams, P. M. Harrison, and J. R. Guest.** 1993. Overproduction, purification and characterization of the bacterioferritin of *Escherichia coli* and a C-terminally extended variant. Eur. J. Biochem. **213:**329–338.
5. **Armstrong, S. K., G. S. Pettis, L. J. Forrester, and M. A. McIntosh.** 1989. The *Escherichia coli* enterobactin biosynthesis gene, *entD*: nucleotide sequence and membrane localization of its protein product. Mol. Microbiol. **3:**757–766.

6. **Arnqvist, A., A. Olsen, and S. Normark.** 1994. σ^S-dependent growth-phase induction of the *csgBA* promoter in *Escherichia coli* can be achieved *in vivo* by σ^{70} in the absence of the nucleoid-associated protein H-NS. Mol. Microbiol. **13:**1021–1032.
7. **Avison, M. B., R. E. Horton, T. R. Walsh, and P. M. Bennett.** 2001. *Escherichia coli* CreBC is a global regulator of gene expression that responds to growth in minimal media. J. Biol. Chem. **276:**26955–26961.
8. **Barth, M., C. Marschall, A. Muffler, D. Fischer, and R. Hengge-Aronis.** 1995. Role for the histone-like protein H-NS in growth phase-dependent and osmotic regulation of σ^S and many σ^S-dependent genes in *Escherichia coli.* J. Bacteriol. **177:**3455–3464.
9. **Becker, G., and R. Hengge-Aronis.** 2001. What makes an *Escherichia coli* promoter σ^S-dependent? Role of the $-13/-14$ nucleotide promoter positions and region 2.5 of σ^S. Mol. Microbiol. **39:**1153–1165.
10. **Bjarnason, J., C. M. Southward, and M. G. Surette.** 2003. Genomic profiling of iron-responsive genes in *Salmonella enterica* serovar Typhimurium by high-throughput screening of a random promoter library. J. Bacteriol. **185:**4973–4982.
11. **Bordes, P., J. Bouvier, A. Conter, A. Kolb, and C. Gutierrez.** 2002. Transient repressor effect of Fis on the growth phase-regulated *osmE* promoter of *Escherichia coli* K12. Mol. Genet. Genomics **268:**206–213.
12. **Bordes, P., F. Repoila, A. Kolb, and C. Gutierrez.** 2000. Involvement of differential efficiency of transcription by $E\sigma^S$ and $E\sigma^{70}$ RNA polymerase holoenzymes in growth phase regulation of the *Escherichia coli osmE* promoter. Mol. Microbiol. **35:**845–853.
13. **Bouvier, J., S. Gordia, G. Kampmann, R. Lange, R. Hengge-Aronis, and C. Gutierrez.** 1998. Interplay between global regulators of *Escherichia coli*: effect of RpoS, Lrp and H-NS on transcription of the gene *osmC.* Mol. Microbiol. **28:**971–980.
14. **Burr, T., J. Mitchell, A. Kolb, S. Minchin, and S. Busby.** 2000. DNA sequence elements located immediately upstream of the -10 hexamer in *Escherichia coli* promoters: a systematic study. Nucleic Acids Res. **28:**1864–1870.
15. **Checroun, C., P. Bordes, O. Leroy, A. Kolb, and C. Gutierrez.** 2004. Interactions between the 2.4 and 4.2 regions of σ^S, the stress-specific sigma factor of *Escherichia coli*, and the -10 and -35 promoter elements. Nucleic Acids Res. **32:**45–53.
16. **Cheung, K. J., V. Badarinarayana, D. W. Selinger, D. Janse, and G. M. Church.** 2003. A microarray-based antibiotic screen identifies a regulatory role for supercoiling in the osmotic stress response of *Escherichia coli.* Genome Res. **13:**206–215.
17. **Conter, A., C. Menchon, and C. Gutierrez.** 1997. Role of DNA supercoiling and RpoS sigma factor in the osmotic and growth phase-dependent induction of the gene *osmE* of *Escherichia coli* K12. J. Mol. Biol. **273:**75–83.
18. **Cummings, H. S., and J. W. Hershey.** 1994. Translation initiation factor IF1 is essential for cell viability in *Escherichia coli.* J. Bacteriol. **176:**198–205.
19. **Daldal, F.** 1983. Molecular cloning of the gene for phosphofructokinase-2 of *Escherichia coli* and the nature of a mutation, *pfkB1*, causing a high level of the enzyme. J. Mol. Biol. **168:**285–305.
20. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
21. **De Biase, D., A. Tramonti, F. Bossa, and P. Visca.** 1999. The response to stationary-phase stress conditions in *Escherichia coli*: role and regulation of the glutamic acid decarboxylase system. Mol. Microbiol. **32:**1198–1211.
22. **Ding, Q., S. Kusano, M. Villarejo, and A. Ishihama.** 1995. Promoter selectivity control of *Escherichia coli* RNA polymerase by ionic strength: differential recognition of osmoregulated promoters by $E\sigma^D$ and $E\sigma^S$ holoenzymes. Mol. Microbiol. **16:**649–656.
23. **Espinosa-Urgel, M., C. Chamizo, and A. Tormo.** 1996. A consensus structure for σ^S-dependent promoters. Mol. Microbiol. **21:**657–659. (Letter.)
24. **Gaal, T., W. Ross, S. T. Estrem, L. H. Nguyen, R. R. Burgess, and R. L. Gourse.** 2001. Promoter recognition and discrimination by $E\sigma^S$ RNA polymerase. Mol. Microbiol. **42:**939–954.
25. **Garg, R. P., C. J. Vargo, X. Cui, and D. M. Kurtz, Jr.** 1996. A [2Fe-2S] protein encoded by an open reading frame upstream of the *Escherichia coli* bacterioferritin gene. Biochemistry **35:**6297–6301.
26. **Gerdes, S. Y., M. D. Scholle, J. W. Campbell, G. Balazsi, E. Ravasz, M. D. Daugherty, A. L. Somera, N. C. Kyrpides, I. Anderson, M. S. Gelfand, A. Bhattacharya, V. Kapatral, M. D'Souza, M. V. Baev, Y. Grechkin, F. Mseeh, M. Y. Fonstein, R. Overbeek, A. L. Barabasi, Z. N. Oltvai, and A. L. Osterman.** 2003. Experimental determination and system level analysis of essential genes in *Escherichia coli* MG1655. J. Bacteriol. **185:**5673–5684.
27. **Gort, A. S., D. M. Ferber, and J. A. Imlay.** 1999. The regulation and role of the periplasmic copper, zinc superoxide dismutase of *Escherichia coli.* Mol. Microbiol. **32:**179–191.
28. **Grant, R. A., D. J. Filman, S. E. Finkel, R. Kolter, and J. M. Hogle.** 1998. The crystal structure of Dps, a ferritin homolog that binds and protects DNA. Nat. Struct. Biol. **5:**294–303.
29. **Grossman, T. H., M. Tuckman, S. Ellestad, and M. S. Osburne.** 1993. Isolation and characterization of *Bacillus subtilis* genes involved in siderophore biosynthesis: relationship between *B. subtilis sfpo* and *Escherichia coli entD* genes. J. Bacteriol. **175:**6203–6211.
30. **Hagiwara, D., M. Sugiura, T. Oshima, H. Mori, H. Aiba, T. Yamashino, and T. Mizuno.** 2003. Genome-wide analyses revealing a signaling network of the RcsC-YojN-RcsB phosphorelay system in *Escherichia coli.* J. Bacteriol. **185:**5735–5746.
31. **Halsey, T. A., A. Vazquez-Torres, D. J. Gravdahl, F. C. Fang, and S. J. Libby.** 2004. The ferritin-like Dps protein is required for *Salmonella enterica* serovar Typhimurium oxidative stress resistance and virulence. Infect. Immun. **72:**1155–1158.
32. **Hengge-Aronis, R.** 1996. Back to log phase: σ^S as a global regulator in the osmotic control of gene expression in *Escherichia coli.* Mol. Microbiol. **21:**887–893.
33. **Hengge-Aronis, R.** 2000. The general stress response in *E. coli*, p. 161–178. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, D.C.
34. **Hengge-Aronis, R.** 1999. Interplay of global regulators and cell physiology in the general stress response of *Escherichia coli.* Curr. Opin. Microbiol. **2:**148–152.
35. **Hengge-Aronis, R.** 2002. Recent insights into the general stress response regulatory network in *Escherichia coli.* J. Mol. Microbiol. Biotechnol. **4:**341–346.
36. **Hengge-Aronis, R.** 2002. Signal transduction and regulatory mechanisms involved in control of the σ^S (RpoS) subunit of RNA polymerase. Microbiol. Mol. Biol. Rev. **66:**373–395, table of contents.
37. **Hengge-Aronis, R.** 2002. Stationary phase gene regulation: what makes an *Escherichia coli* promoter σ^S-selective? Curr. Opin. Microbiol. **5:**591–595.
38. **Hershberg, R., G. Bejerano, A. Santos-Zavaleta, and H. Margalit.** 2001. PromEC: an updated database of *Escherichia coli* mRNA promoters with experimentally identified transcriptional start sites. Nucleic Acids Res. **29:**277.
39. **Hung, S. P., P. Baldi, and G. W. Hatfield.** 2002. Global gene expression profiling in *Escherichia coli* K12. The effects of leucine-responsive regulatory protein. J. Biol. Chem. **277:**40309–40323.
40. **Ibanez-Ruiz, M., V. Robbe-Saule, D. Hermant, S. Labrude, and F. Norel.** 2000. Identification of RpoS (σ^S)-regulated genes in *Salmonella enterica* serovar Typhimurium. J. Bacteriol. **182:**5749–5756.
41. **Ishihama, A.** 2000. Functional modulation of *Escherichia coli* RNA polymerase. Annu. Rev. Microbiol. **54:**499–518.
42. **Izutsu, K., C. Wada, Y. Komine, T. Sako, C. Ueguchi, S. Nakura, and A. Wada.** 2001. *Escherichia coli* ribosome-associated protein SRA, whose copy number increases during stationary phase. J. Bacteriol. **183:**2765–2773.
43. **Jishage, M., K. Kvint, V. Shingler, and T. Nystrom.** 2002. Regulation of sigma factor competition by the alarmone ppGpp. Genes Dev. **16:**1260–1270.
44. **Khil, P. P., and R. D. Camerini-Otero.** 2002. Over 1000 genes are involved in the DNA damage response of *Escherichia coli.* Mol. Microbiol. **44:**89–105.
45. **Kusano, S., Q. Ding, N. Fujita, and A. Ishihama.** 1996. Promoter selectivity of *Escherichia coli* RNA polymerase $E\sigma^{70}$ and $E\sigma^{38}$ holoenzymes. Effect of DNA supercoiling. J. Biol. Chem. **271:**1998–2004.
46. **Kvint, K., A. Farewell, and T. Nystrom.** 2000. RpoS-dependent promoters require guanosine tetraphosphate for induction even in the presence of high levels of σ^S. J. Biol. Chem. **275:**14795–14798.
47. **Lacour, S., A. Kolb, and P. Landini.** 2003. Nucleotides from -16 to -12 determine specific promoter recognition by bacterial σ^S-RNA polymerase. J. Biol. Chem. **278:**37160–37168.
48. **Landini, P., L. I. Hajec, L. H. Nguyen, R. R. Burgess, and M. R. Volkert.** 1996. The leucine-responsive regulatory protein (Lrp) acts as a specific repressor for σ^S-dependent transcription of the *Escherichia coli aidB* gene. Mol. Microbiol. **20:**947–955.
49. **Landini, P., L. I. Hajec, and M. R. Volkert.** 1994. Structure and transcriptional regulation of the *Escherichia coli* adaptive response gene *aidB.* J. Bacteriol. **176:**6583–6589.
50. **Lange, R., M. Barth, and R. Hengge-Aronis.** 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. **175:**7910–7917.
51. **Lange, R., and R. Hengge-Aronis.** 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:**4474–4481.
52. **Lee, H. J., K. J. Park, A. Y. Lee, S. G. Park, B. C. Park, K. H. Lee, and S. J. Park.** 2003. Regulation of *fur* expression by RpoS and Fur in *Vibrio vulnificus.* J. Bacteriol. **185:**5891–5896.
53. **Lee, S. J., and J. D. Gralla.** 2004. Osmo-regulation of bacterial transcription via poised RNA polymerase. Mol. Cell **14:**153–162.
54. **Lee, S. J., and J. D. Gralla.** 2001. σ^{38} (RpoS)-RNA polymerase promoter engagement via -10 region nucleotides. J. Biol. Chem. **276:**30064–30071.
55. **Lisser, S., and H. Margalit.** 1993. Compilation of *E. coli* mRNA promoter sequences. Nucleic Acids Res. **21:**1507–1516.

56. **Loewen, P. C., B. Hu, J. Strutinsky, and R. Sparling.** 1998. Regulation in the *rpoS* regulon of *Escherichia coli.* Can. J. Microbiol. **44:**707–717.
57. **Lonetto, M., M. Gribskov, and C. A. Gross.** 1992. The σ^{70} family: sequence conservation and evolutionary relationships. J. Bacteriol. **174:**3843–3849.
58. **Maeda, H., N. Fujita, and A. Ishihama.** 2000. Competition among seven *Escherichia coli* sigma subunits: relative binding affinities to the core RNA polymerase. Nucleic Acids Res. **28:**3497–3503.
59. **Marschall, C., V. Labrousse, M. Kreimer, D. Weichart, A. Kolb, and R. Hengge-Aronis.** 1998. Molecular analysis of the regulation of *csiD*, a carbon starvation-inducible gene in *Escherichia coli* that is exclusively dependent on σ^S and requires activation by cAMP-CRP. J. Mol. Biol. **276:**339–353.
60. **Martinez, A., and R. Kolter.** 1997. Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. J. Bacteriol. **179:**5188–5194.
61. **Martinez-Garcia, E., A. Tormo, and J. M. Navarro-Llorens.** 2001. Further studies on RpoS in enterobacteria: identification of *rpoS* in *Enterobacter cloacae* and *Kluyvera cryocrescens.* Arch. Microbiol. **175:**395–404.
62. **Martino, P. D., R. Fursy, L. Bret, B. Sundararaju, and R. S. Phillips.** 2003. Indole can act as an extracellular signal to regulate biofilm formation of *Escherichia coli* and other indole-producing bacteria. Can. J. Microbiol. **49:**443–449.
63. **Masse, E., F. E. Escorcia, and S. Gottesman.** 2003. Coupled degradation of a small regulatory RNA and its mRNA targets in *Escherichia coli.* Genes Dev. **17:**2374–2383.
64. **Masuda, N., and G. M. Church.** 2003. Regulatory network of acid resistance genes in *Escherichia coli.* Mol. Microbiol. **48:**699–712.
65. **Medigue, C., A. Viari, A. Henaut, and A. Danchin.** 1993. Colibri: a functional database for the *Escherichia coli* genome. Microbiol. Rev. **57:**623–654.
66. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
67. **Mitchell, J. E., D. Zheng, S. J. Busby, and S. D. Minchin.** 2003. Identification and analysis of 'extended −10' promoters in *Escherichia coli*. Nucleic Acids Res. **31:**4689–4695.
68. **Moore, M. M., D. L. Fernandez, and R. L. Thune.** 2002. Cloning and characterization of *Edwardsiella ictaluri* proteins expressed and recognized by the channel catfish *Ictalurus punctatus* immune response during infection. Dis. Aquat. Organ. **52:**93–107.
69. **Mulvey, M. R., and P. C. Loewen.** 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel sigma transcription factor. Nucleic Acids Res. **17:**9979–9991.
70. **Nystrom, T.** 2003. Conditional senescence in bacteria: death of the immortals. Mol. Microbiol. **48:**17–23.
71. **O'Connor, K. E., A. D. Dobson, and S. Hartmans.** 1997. Indigo formation by microorganisms expressing styrene monooxygenase activity. Appl. Environ. Microbiol. **63:**4287–4291.
72. **Ojangu, E. L., A. Tover, R. Teras, and M. Kivisaar.** 2000. Effects of combination of different −10 hexamers and downstream sequences on stationary-phase-specific sigma factor σ^S-dependent transcription in *Pseudomonas putida.* J. Bacteriol. **182:**6707–6713.
73. **Olsen, A., A. Arnqvist, M. Hammar, S. Sukupolvi, and S. Normark.** 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:**523–536.
74. **Oshima, T., H. Aiba, Y. Masuda, S. Kanaya, M. Sugiura, B. L. Wanner, H. Mori, and T. Mizuno.** 2002. Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12. Mol. Microbiol. **46:**281–291.
75. **Phadtare, S., I. Kato, and M. Inouye.** 2002. DNA microarray analysis of the expression profile of *Escherichia coli* in response to treatment with 4,5-dihydroxy-2-cyclopenten-1-one. J. Bacteriol. **184:**6725–6729.
76. **Pomposiello, P. J., M. H. Bennik, and B. Demple.** 2001. Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. J. Bacteriol. **183:**3890–3902.
77. **Pon, C. L., B. Wittmann-Liebold, and C. Gualerzi.** 1979. Structure-function relationships in *Escherichia coli* initiation factors. II. Elucidation of the primary structure of initiation factor IF-1. FEBS Lett. **101:**157–160.
78. **Prigent-Combaret, C., E. Brombacher, O. Vidal, A. Ambert, P. Lejeune, P. Landini, and C. Dorel.** 2001. Complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli* via regulation of the *csgD* gene. J. Bacteriol. **183:**7213–7223.
79. **Prigent-Combaret, C., O. Vidal, C. Dorel, and P. Lejeune.** 1999. Abiotic surface sensing and biofilm-dependent regulation of gene expression in *Escherichia coli.* J. Bacteriol. **181:**5993–6002.
80. **Quail, M. A., P. Jordan, J. M. Grogan, J. N. Butt, M. Lutz, A. J. Thomson, S. C. Andrews, and J. R. Guest.** 1996. Spectroscopic and voltametric characterisation of the bacterioferritin-associated ferredoxin of *Escherichia coli.* Biochem. Biophys. Res. Commun. **229:**635–642.
81. **Ren, D., L. A. Bedzyk, R. W. Ye, S. M. Thomas, and T. K. Wood.** 2004. Stationary-phase quorum-sensing signals affect autoinducer-2 and gene expression in *Escherichia coli.* Appl. Environ. Microbiol. **70:**2038–2043.
82. **Ross, W., K. K. Gosink, J. Salomon, K. Igarashi, C. Zou, A. Ishihama, K. Severinov, and R. L. Gourse.** 1993. A third recognition element in bacterial promoters: DNA binding by the alpha subunit of RNA polymerase. Science **262:**1407–1413.
83. **Sak, B. D., A. Eisenstark, and D. Touati.** 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:**3271–3275.
84. **Salgado, H., A. Santos-Zavaleta, S. Gama-Castro, D. Millan-Zarate, E. Diaz-Peredo, F. Sanchez-Solano, E. Perez-Rueda, C. Bonavides-Martinez, and J. Collado-Vides.** 2001. RegulonDB (version 3.2): transcriptional regulation and operon organization in *Escherichia coli* K-12. Nucleic Acids Res. **29:**72–74.
85. **Schellhorn, H. E., J. P. Audia, L. I. Wei, and L. Chang.** 1998. Identification of conserved, RpoS-dependent stationary-phase genes of *Escherichia coli.* J. Bacteriol. **180:**6283–6291.
86. **Schembri, M. A., K. Kjaergaard, and P. Klemm.** 2003. Global gene expression in *Escherichia coli* biofilms. Mol. Microbiol. **48:**253–267.
87. **Tanaka, K., S. Kusano, N. Fujita, A. Ishihama, and H. Takahashi.** 1995. Promoter determinants for *Escherichia coli* RNA polymerase holoenzyme containing σ^{38} (the *rpoS* gene product). Nucleic Acids Res. **23:**827–834.
88. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal sigma factor in *Escherichia coli*: the *rpoS* gene product, σ^{38}, is a second principal sigma factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**3511–3515. (Erratum, **90:**8303.)
89. **Tani, T. H., A. Khodursky, R. M. Blumenthal, P. O. Brown, and R. G. Matthews.** 2002. Adaptation to famine: a family of stationary-phase genes revealed by microarray analysis. Proc. Natl. Acad. Sci. USA **99:**13471–13476.
90. **Taverna, P., and B. Sedgwick.** 1996. Generation of an endogenous DNA-methylating agent by nitrosation in *Escherichia coli.* J. Bacteriol. **178:**5105–5111.
91. **Touati, E., E. Dassa, and P. L. Boquet.** 1986. Pleiotropic mutations in *appR* reduce pH 2.5 acid phosphatase expression and restore succinate utilisation in CRP-deficient strains of *Escherichia coli.* Mol. Gen. Genet. **202:**257–264.
92. **Ueguchi, C., N. Misonou, and T. Mizuno.** 2001. Negative control of *rpoS* expression by phosphoenolpyruvate: carbohydrate phosphotransferase system in *Escherichia coli.* J. Bacteriol. **183:**520–527.
93. **Volkert, M. R., L. I. Hajec, Z. Matijasevic, F. C. Fang, and R. Prince.** 1994. Induction of the *Escherichia coli aidB* gene under oxygen-limiting conditions requires a functional *rpoS* (*katF*) gene. J. Bacteriol. **176:**7638–7645.
94. **Wada, A.** 1998. Growth phase coupled modulation of *Escherichia coli* ribosomes. Genes Cells **3:**203–208.
95. **Wang, D., X. Ding, and P. N. Rather.** 2001. Indole can act as an extracellular signal in *Escherichia coli.* J. Bacteriol. **183:**4210–4216.
96. **Wei, J., M. B. Goldberg, V. Burland, M. M. Venkatesan, W. Deng, G. Fournier, G. F. Mayhew, G. Plunkett III, D. J. Rose, A. Darling, B. Mau, N. T. Perna, S. M. Payne, L. J. Runyen-Janecky, S. Zhou, D. C. Schwartz, and F. R. Blattner.** 2003. Complete genome sequence and comparative genomics of *Shigella flexneri* serotype 2a strain 2457T. Infect. Immun. **71:**2775–2786.
97. **Wei, Y., J. M. Lee, C. Richmond, F. R. Blattner, J. A. Rafalski, and R. A. LaRossa.** 2001. High-density microarray-mediated gene expression profiling of *Escherichia coli.* J. Bacteriol. **183:**545–556.
98. **Wise, A., R. Brems, V. Ramakrishnan, and M. Villarejo.** 1996. Sequences in the −35 region of *Escherichia coli rpoS*-dependent genes promote transcription by $E\sigma^S$. J. Bacteriol. **178:**2785–2793.
99. **Yamagishi, M., H. Matsushima, A. Wada, M. Sakagami, N. Fujita, and A. Ishihama.** 1993. Regulation of the *Escherichia coli rmf* gene encoding the ribosome modulation factor: growth phase- and growth rate-dependent control. EMBO J. **12:**625–630.
100. **Yang, W., L. Ni, and R. L. Somerville.** 1993. A stationary-phase protein of *Escherichia coli* that affects the mode of association between the Trp repressor protein and operator-bearing DNA. Proc. Natl. Acad. Sci. USA **90:**5796–5800.
101. **Yim, H. H., R. L. Brems, and M. Villarejo.** 1994. Molecular characterization of the promoter of *osmY*, an *rpoS*-dependent gene. J. Bacteriol. **176:**100–107.
102. **Yoon, S. H., M. J. Han, S. Y. Lee, K. J. Jeong, and J. S. Yoo.** 2003. Combined transcriptome and proteome analysis of *Escherichia coli* during high cell density culture. Biotechnol. Bioeng. **81:**753–767.