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Genome-Wide Analysis of the General Stress Response Network in *Escherichia coli*: σ^S-Dependent Genes, Promoters, and Sigma Factor Selectivity†

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The σ^S (or RpoS) subunit of RNA polymerase is the master regulator of the general stress response in *Escherichia coli*. While nearly absent in rapidly growing cells, σ^S is strongly induced during entry into stationary phase and/or many other stress conditions and is essential for the expression of multiple stress resistances. Genome-wide expression profiling data presented here indicate that up to 10% of the *E. coli* genes are under direct or indirect control of σ^S and that σ^S should be considered a second vegetative sigma factor with a major impact not only on stress tolerance but on the entire cell physiology under nonoptimal growth conditions. This large data set allowed us to unequivocally identify a σ^S consensus promoter in silico. Moreover, our results suggest that σ^S-dependent genes represent a regulatory network with complex internal control (as exemplified by the acid resistance genes). This network also exhibits extensive regulatory overlaps with other global regulons (e.g., the cyclic AMP receptor protein regulon). In addition, the global regulatory protein Lrp was found to affect σ^S and/or σ^{70} selectivity of many promoters. These observations indicate that certain modules of the σ^S-dependent general stress response can be temporarily recruited by stress-specific regulons, which are controlled by other stress-responsive regulators that act together with σ^{70} RNA polymerase. Thus, not only the expression of genes within a regulatory network but also the architecture of the network itself can be subject to regulation.

The general stress sigma factor σ^S (or RpoS) is strongly induced when *Escherichia coli* cells are exposed to various stress conditions, which include starvation, hyperosmolarity, pH downshift, or nonoptimal high or low temperature (for a review of σ^S regulation, see reference 24). By standard genetic and molecular biology methods, more than 80 σ^S-controlled genes have been identified to date, indicating that σ^S is the master regulator of a rather large regulon which represents the genetic basis of the *E. coli* general stress response (for summaries, see references 23 and 41).

In their regulatory patterns, many σ^S-controlled genes just follow the cellular σ^S level; i.e., they are activated whenever σ^S and therefore σ^S-containing RNA polymerase ($E\sigma^S$) accumulate in the cell. Other σ^S-dependent genes, however, exhibit highly specific regulation, with a narrow window of expression only under some sort of stress condition. The best-studied example of this type of σ^S-controlled gene is the *csiD* gene, which is mainly induced by carbon starvation because the cyclic AMP (cAMP)-cAMP receptor protein (CRP) acts as an essential activator for σ^S-containing RNA polymerase at the *csiD* promoter (21, 46, 49). Also, the leucine-responsive regulatory protein (Lrp) is involved in the regulation of certain σ^S-dependent genes (9, 13, 33, 64). These findings indicate that the σ^S-containing RNA polymerase holoenzyme has the ability to cooperate with additional regulatory factors, just as the vegetative RNA polymerase containing σ^{70} does.

The identification of a clearly defined σ^S consensus promoter sequence and therefore the prediction of σ^S-controlled promoters in upstream regions of genes in the *E. coli* genome have been notoriously difficult. σ^S is highly related to σ^{70}, and genes that are dependent on $E\sigma^S$ in vivo can often be transcribed in vitro by $E\sigma^{70}$, and vice versa. The current view of this "sigma selectivity paradox" is that $E\sigma^S$ and $E\sigma^{70}$ in principle use very similar promoters but that minor differences, e.g., in the extended −10 region (6), can shift the preference towards one or the other holoenzyme. Also, transcription initiation by $E\sigma^S$ is less affected by various deviations from the classical promoter consensus sequence (e.g., by degeneration of the −35 sequence) (20), which gives $E\sigma^S$ an advantage at nonoptimal promoters (summarized in reference 25).

The present study was undertaken with a number of questions and goals in mind. How many and which genes in the *E. coli* genome are under σ^S control? Does a more or less complete set of these genes provide us with novel insights into the physiological function of the σ^S regulon? Can we use such a database of σ^S-dependent genes for unequivocal in silico identification of a σ^S consensus promoter sequence? Does expression of the majority of these genes just follow σ^S levels, or is differential regulation common among σ^S-dependent genes? In view of the similarity between σ^{70}- and σ^S-controlled promoters, can $E\sigma^S$ selectivity be conditional and can histone-like proteins globally affect $E\sigma^S$ and/or $E\sigma^{70}$ promoter selectivity (as suggested for Lrp in a few cases so far)? The present study provides answers to these questions from a genome-wide perspective.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used in this study are derivatives of *E. coli* K-12 strain MC4100 (11). Mutant alleles previously described are *rpoS359*::Tn*10* (35), *rpoS*::*kan* (7), and *lrp-201*::Tn*10* (17). The *gadX*::*cat* insertion was isolated by one-step inactivation (15) with the following primers: 5′-GCGTGCTACATTAATAAACAGTAATATGTTTATGTAATATTAAGTCAACT**GTGTAGGCTGGAGCTGCTTC**-3′ and 5′-ATGTCTGAGTAAAACTCTATAATCTTATTCCTTCCGCAGAACGGTCAGTG**CATATGAATATCCTCCTTAG**-3′ (nucleotides shown in boldface type deviate from the *gadX* sequence). These mutations were introduced into MC4100 by P1 transduction (50).

Standard batch cultures were grown at 37°C under aeration in a rotary shaker in Luria-Bertani (LB) medium. Ampicillin (100 μg/ml) was added for plasmid-carrying strains. For hyperosmotic shift experiments, cultures were grown in M9 (50) with glycerol (0.4%) for three or more generations before 0.3 M NaCl was added. For pH downshift experiments, cultures were grown in LB medium for four or more generations before 170 mM 4-morpholine-methanesulfonic acid (MES) was added (this procedure acidifies the medium to pH 5). Growth was monitored by measuring the optical density at 578 nm (OD_{578}).

Origin of *E. coli* DNA microarrays. Genomic *E. coli* K-12 DNA microarrays were made by robotically spotting PCR products. The PCR products were generated with an ORFmer primer set (Genosys Biotechnologies, Cambridge, United Kingdom) giving full-length open reading frames as double-stranded DNA. Details of the spotting procedure and quality control of the microarrays were described previously (39, 54, 71).

RNA preparation and cDNA labeling. For total RNA preparation, cultures were grown and harvested under three different conditions known to produce high σ^S levels and σ^S-dependent target gene expression in wild-type strains: (i) during transition into stationary phase in LB medium at an OD_{578} of 4.0, (ii) 20 min after the addition of 0.3 M NaCl (added at an OD_{578} of 0.3) in M9–0.4% glycerol, and (iii) 40 min after a shift to pH 5 in LB medium (MES was added at an OD_{578} of 0.4).

One volume of cell suspension was harvested on 0.5 volume of ice (−20°C) and centrifuged immediately for 2 min at 4,500 × *g* at 4°C. The pellet was resuspended in 700 μl of RLT buffer (RNeasy; QIAGEN, Hilden, Germany) and transfered to a vial with 1 g of 0.1-mm Zirconia/Silica beads (Roth, Karlsruhe, Germany). Cells were disrupted with a Mini-BeadBeater (Biospec Products, Inc.) at 5,000 rpm for three intervals of 30 s each. After centrifugation, the lysate was supplemented with 500 μl of ethanol and split in two portions, and total RNA was extracted with two RNeasy mini-columns according to the manufacturers instructions (QIAGEN). The isolated RNA was treated with 30 U of DNase I (RNase free; Roche, Mannheim, Germany) in DNase I buffer (1 M sodium acetate, 50 mM $MgSO_4$ [pH 5.0]) for 20 min at 37°C, incubated for 10 min at 70°C for inactivation of DNase I, and extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1), followed by ethanol precipitation (39, 54, 58). RNA concentration and quality were checked photometrically and on formaldehyde gels according to standard procedures (58). Equal amounts of total RNA (each, 20 to 25 μg) were used for random hexamer-primed synthesis of fluorescence-labeled cDNA with the fluorescent nucleotide analogues Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia), as described previously (39, 54, 71).

DNA microarray analysis. Hybridization of the fluorescence-labeled cDNA to the microarrays and the washing protocol were described previously (39, 54, 71). Fluorescence at 532 nm (Cy3-dUTP) and 635 nm (Cy5-dUTP) was determined at a 10-μm resolution with a GenePix 4000 (Axon, Inc.) laser scanner. TIFF images were analyzed with the software GenePix Pro 3.0 (Axon). The normalized Cy5/Cy3 ratio for the median was taken to reflect relative RNA level changes (39, 54).

Data analysis. Each microarray experiment was repeated independently at least three times (biological replicates). Genes were considered differentially expressed according to the following criteria. (i) Reliable detection was based on signal-to-noise ratios exceeding a factor of 3. (ii) Reliable detection was confirmed in at least two out of three repetitions. (iii) In a paired Student's *t* test, relative RNA levels were significantly different from the levels of the genomic DNA controls ($P < 0.05$) (39, 54). (iv) Average relative RNA level changes were at least twofold (in all three replicate experiments).

Functional grouping of genes was made according to the data from GenProtEC (http://genprotec.mbl.edu/) (60). MEME (http://meme.sdsc.edu/) (3) and BioProspector (http://robotics.stanford.edu/~xsliu/BioProspector/) (40) were used for sequence analysis of upstream regions of coregulated open reading frames. Both algorithms seek conserved motifs in sets of unaligned sequences. The sequence logo was created with WebLogo (http://weblogo.berkeley.edu/) (14).

RNA preparation and primer extension. For *gadE* mRNA detection by primer extension experiments, cells were grown under the same conditions as for the microarray experiments (see above). Total RNA was prepared and subject to reverse transcription as previously described (49). The primer used for reverse transcription was 5′-ATCTTTCAACTGCCAAAAGCCCTGT-3′.

Construction of *lacZ* reporter fusions and their transfer into the chromosome. Chromosomal *lacZ* fusions to the *gadA* and *gadB* genes (as well as to the regulatory gene *gadE*) were isolated with the fusion vectors pJL28 and pJL30, respectively (42), which are pMLB1034 (62) derivatives carrying the polylinkers of pNM480 and pNM482 (52). The inserts were generated by PCR with MC4100 chromosomal DNA as a template, digested with EcoRI and SalI, and cloned into the fusion vector, which was digested with the same enzymes. The following primers were used for PCR: (i) 5′-GTGGATGAATTCGTAGCTTTCCTGC-3′ (upstream of *gadA*), (ii) 5′-GTGAGAATTCAGGAGACACAGAATGC-3′ (upstream of *gadB*), (iii) 5′-GATAATCTGAAAGTCGACATCATCGC-3′ (used for *gadA* and *gadB*, since the coding regions for the isoenzymes GadA and GadB are nearly identical), (iv) 5′-TTGAATTCCGCATAAATATCCGTGTCTCCAGACG-3′ (upstream of *gadE*), and (v) 5′-ATCTATAAGCTTTATCTTTCAACTGCCAAAAGCCCTG-3′ (reverse primer complementary to the coding sequence of *gadE*). These constructs result in translational fusions inserted after nucleotide 138 of the coding regions of *gadA* and *gadB* and after nucleotide 61 of *gadE*. Translational fusions were converted to transcriptional fusions, as previously described (56) (with a HindIII-ClaI fragment to replace a corresponding fragment carrying the fusion joint and thereby introducing stop codons in all three reading frames, a Shine-Dalgarno sequence, and an initiation codon for *lacZ*). PCR-derived parts of the resulting plasmids carrying all fusions were sequenced. All constructs were crossed onto λRS45, followed by lysogenization into MC4100 according to the method described in reference 63. Single lysogeny was tested by a PCR method (55).

β-Galactosidase assay. β-Galactosidase activity was assayed with *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate and is reported as the number of micromoles of *o*-nitrophenol per minute per milligram of cellular protein (50).

RESULTS

Genome-wide identification of σ^S-dependent genes under three different growth and stress conditions. We used genome-wide transcription profiling of isogenic *rpoS*$^+$ and *rpoS*::Tn*10* strains to identify σ^S-dependent genes of *E. coli*. To find as many such genes as possible, we used not only one but three different culture growth conditions, which are known to result in high cellular σ^S levels. These were (i) growth in LB medium to an OD_{578} of 4.0 (this corresponds to transition into stationary phase), (ii) growth in minimal medium to which 0.3 M NaCl was added at an OD_{578} of 0.3 (cells were harvested 20 min after this hyperosmotic shift), and (iii) growth in LB medium which was acidified by the addition of MES at an OD_{578} of 0.4 (cells were harvested 40 min after this shift to pH 5). Total RNA and labeled cDNA were prepared and hybridized to genomic *E. coli* microarrays (for details, see Materials and Methods).

We identified a total of 481 genes, which exhibited >2-fold-higher expression in the *rpoS*$^+$ strain than in the *rpoS* mutant (Fig. 1). In addition, 95 genes showed >2-fold-higher expression in the *rpoS* mutant (at least under one condition tested); i.e., they appeared to be negatively controlled by σ^S (Fig. 1A). Most likely, these latter genes are expressed by σ^{70}-containing RNA polymerase ($E\sigma^{70}$) from promoters that are sensitive to the increase in the cellular concentration of $E\sigma^{70}$ that results from a lack of competition between σ^S and σ^{70} for core polymerase in the *rpoS* mutant. Alternatively, some of the negatively σ^S-controlled genes may be subject to repression by σ^S-dependent regulatory proteins.

Interestingly, only 140 of the positively σ^S-controlled genes

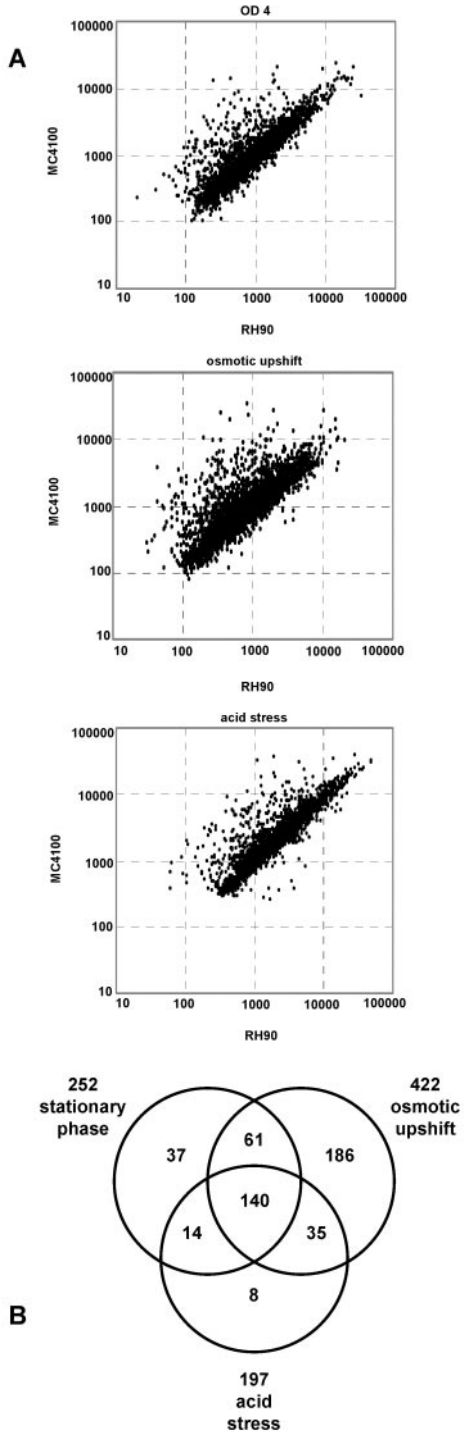

FIG. 1. (A) Comparison of genome-wide gene expression in *rpoS*$^+$ and *rpoS*::Tn*10* strains (MC4100 and RH90, respectively) under three different growth and stress conditions. RNA was prepared during entry into stationary phase in LB medium (OD_{578} = 4), 20 min after the addition of 0.3 M NaCl in minimal medium and 40 min after a shift to pH 5 in LB medium. Cy3- and Cy5-labeled cDNAs obtained from these RNA preparations were analyzed by whole-genome microarray analysis, and normalized intensities are visualized as scatter plots (MC4100 versus RH90). All data are the average of three independent identical experiments. (B) The numbers of σ^S-controlled genes (i.e., genes with an at least twofold difference in expression in *rpoS*$^+$ and *rpoS*::Tn*10* strains) identified under one, two, or all three conditions tested are shown as a Venn diagram.

were found under all three growth and stress conditions (Fig. 1B); expression ratios for *rpoS*$^+$ and *rpoS*-negative strains as well as assigned functions of these genes are listed in Table 1. The expression of these genes may just change in parallel with σ^S levels, and we henceforth refer to them as the core set of σ^S-controlled genes. The other 341 genes revealed their σ^S dependence under only one or two of the growth and stress conditions used (with genes in all possible combinatorial groups) (Fig. 1B). Especially noteworthy was a large set of 186 genes, which was observed as σ^S controlled only under conditions of osmotic upshift (Fig. 1B). Thus, the majority of σ^S-controlled genes require not only the presence of σ^S but also specific environmental conditions for expression. Alternatively, certain subsets of genes may switch from σ^S to σ^{70} dependence under certain conditions. Taken together, these data indicate that the σ^S regulon (i) is much larger (up to 10% of all *E. coli* K-12 genes) and (ii) displays a higher degree of internal differential regulation than previously suspected.

To our knowledge, previous publications have described 87 *E. coli* genes as being σ^S controlled. With our microarrays, we identified 54 of these genes as σ^S dependent (with 36 belonging to the core set of σ^S-controlled genes). Many of the other 33 genes exhibited ratios of expression of just below 2. Likely explanations are that these genes can be expressed from more than one promoter with not all promoters being σ^S dependent, as is known, for example, for *glgS* (26), *proP* (48), or *ftsQAZ* (19) or from promoters which can be activated by Eσ^S as well as by Eσ^{70}, as demonstrated for *osmE* (8) and *csiE* (45).

In principle, σ^S-dependent genes are scattered all over the *E. coli* chromosome. Nevertheless, there were a few clusters with a rather high local density of σ^S-controlled genes. One example was a region of approximately 91 kb around 79.3 min on the chromosome, which features 29 σ^S-controlled genes, including several regulatory and structural genes involved in acid resistance (further analyzed and discussed below) (Fig. 2). Another cluster of approximately 13 kb included the previously described *csiD-ygaF-gabDTP* operon (49), as well as the genes *ygaU*, *yqaE*, *yqaM*, and *nrdE* (around 60.2 min on the chromosome). Additional such regions included *dps* and *poxB* (among 27 σ^S-dependent genes clustered over approximately 89 kb, located at around 18.6 min of the chromosome) or *sodC*, *cfa*, *ihfA*, and *katE* (among 34 σ^S-dependent genes clustered over approximately 120 kb, located around 38.4 min of the chromosome). On the other hand, the only region which over a long distance (approximately 540 kb) was essentially free of σ^S-dependent genes (a single exception is the *ysgG* gene) included the replication initiation region *oriC* (data not shown). The biological significance of this absence of σ^S control in this large segment of the genome is currently unknown.

In silico identification of a σ^S consensus promoter sequence. To identify putative promoter sequences recognized by σ^S-containing RNA polymerase, the upstream noncoding regions (200 bp) of the 140 σ^S-dependent core genes were searched for common motifs with the programs MEME (3, 4) and BioProspector (40). Both algorithms identified the sequence 5′-TCTATACTTAA-3′ with high statistical significance. This sequence is shown in Fig. 3 as a sequence logo with base frequencies represented by the height of a stack of letters at each position; note that even at positions where the sequence logo does not appear impressive, preference for one or

TABLE 1. Expression ratios of σ^S-controlled genes identified under three different σ^S-inducing conditions

Function (reference) and gene	b no.	Gene product	Ratio		
			OD 4[a]	NaCl[b]	pH 5[c]
Metabolism (26)					
adhP	b1478	Alcohol dehydrogenase, propanol preferring	5.38	9.19	3.43
amyA	b1927	Cytoplasmic alpha-amylase	12.21	11.02	10.57
dkgV	b3012	2,5-Diketo-D-gluconate reductase A	4.37	6.33	2.37
fbaB	b2097	Fructose-bisphosphate aldolase class I	8.00	9.61	9.33
gabD	b2661	Succinate-semialdehyde dehydrogenase NADP dependent	4.81	4.08	2.89
hdhA	b1619	alpha-hydroxysteroid dehydrogenase, NAD dependent	2.71	6.97	2.43
hycF	b2720	Hydrogenase 3, putative quinone oxidoreductase, FeS related	10.67	24.06	9.11
IdcC	b0186	Lysine decarboxylase 2, constitutive	3.76	3.72	3.88
narU	b1469	Nitrate extrusion protein	2.75	3.62	2.07
narY	b1467	Nitrate reductase 2, beta-subunit	5.24	5.70	4.57
poxB	b0871	Pyruvate dehydrogenase/oxidase:FAD and thiamine PP_i-binding	17.67	45.89	29.60
qor	b4051	Quinone oxidoreductase, NADPH dependent	2.39	2.73	2.64
talA	b2464	Transaldolase A	6.79	13.08	13.83
tam	b1519	Trans-aconitate methyltransferase	2.76	8.68	3.53
tktB	b2465	Transketolase 2, thiamin binding, isozyme	14.08	35.87	10.76
ugp	b3449	Glycerophosphodiester phosphodiesterase, cytosolic	2.30	3.69	2.01
ybaS	b0485	Putative glutaminase/carboxypeptidase with beta-lactamase/D-Ala-carboxypeptidase domain	14.05	9.46	2.70
ybaY	b0453	Glycoprotein/polysaccharide metabolism	6.15	8.67	6.09
ycaC	b0897	Putative enzyme with cysteine hydrolase domain	13.34	5.92	5.88
yfcF	b2301	Putative glutathione *S*-transferase enzyme with thioredoxin-like domain	2.55	2.44	2.40
ygjG	b3073	Putrescine:2-oxoglutaric acid aminotransferase	8.93	16.90	7.45
yhfW	b3380	Putative phosphopentomutase, alkaline phosphatase-like domain	4.00	6.67	4.46
yjcS	b4083	Putative enzyme with 2 metallohydrolase/oxidoreductase domains	10.73	10.38	4.71
yjgB	b4269	Putative alcohol dehydrogenase with NAD(P)-binding and GroES domains	6.98	10.80	7.40
yncG	b1454	Putative glutathione *S*-transferase with thioredoxin-like and glutathione *S*-transferases, C-terminal domain	3.42	13.77	3.64
ysgA	b3830	Putative dienelactone hydrolase	2.69	2.27	3.68
Regulation (11)					
bolA	b0435	Transcriptional activator of morphogenic pathway (BolA family), important in general stress response	4.74	8.47	4.77
chaB	b1217	Cation transport regulator	3.36	5.54	3.38
csiR	b2664	Putative transcriptional repressor with DNA-binding winged helix domain (GntR family)	4.84	10.09	4.30
gadW	b3515	Transcriptional regulator for GadX (regulatory protein), glutamic acid decarboxylase (GadA, -B), and glutamate transport protein (GadC) (AraC-XylS family)	6.12	5.98	3.09
gadX	b3516	Transcriptional regulator for glutamic acid decarboylase and transporter (GadA, GadBC) (AraC/XylS family)	6.09	7.45	3.14
gem	b1285	RNase II modulator with PYP-like sensor domain	6.78	3.83	6.94
pdhR	b0113	Transcriptional repressor for pyruvate dehydrogenase complex (GntR family)	5.01	3.42	2.86
rssB	b1235	Response regulator involved in protein turnover, controls stability of RpoS	4.68	7.19	4.08
wrbA	b1004	Flavodoxin-like protein, trp repressor-binding protein	9.38	6.83	14.77
yiaG	b3555	Putative transcriptional regulator with DNA-binding domain	8.40	9.20	4.08
yjdC	b4135	Putative regulator with homeodomain-like DNA-binding domain (TetR/AcrR family)	2.49	2.34	2.46
Transport and membrane (20)					
artM	b0861	Arginine transport protein (ABC superfamily, membrane)	2.20	2.79	2.61
artP	b0864	Arginine transport protein (ABC superfamily, ATP-binding subunit)	3.50	4.56	4.71
blc	b4149	Outer membrane lipoprotein (lipocalin)	4.77	8.99	8.10
eutH	b2452	Putative transport protein, ethanolamine utilization	3.11	3.06	2.59
gabP	b2663	Gamma-aminobutyrate transport protein	4.06	4.70	2.83
mscL	b3291	Mechanosensitive channel	2.94	2.31	2.59
potF	b0854	Putrescine transport protein (ABC superfamily, periplasmic binding protein)	2.76	4.25	2.24
rssA	b1234	Putative transmembrane protein	2.50	3.19	2.26
ugpB	b3453	Sn-Glycerol 3-phosphate transport protein (ABC superfamily, periplasmic binding protein)	3.29	5.55	3.29
ugpC	b3450	Sn-Glycerol 3-phosphate transport protein (ABC superfamily, ATP-binding subunit)	2.39	2.50	2.44
ybiO	b0808	Putative transport protein, integral membrane location	8.49	4.51	2.02
ybiP	b0815	Putative transmembrane protein (N terminal); putative phosphatase (C terminal)	7.73	5.89	2.43
ydcS	b1440	Putative transport protein (ABC superfamily, periplasmic binding protein)	21.66	4.83	4.36
ydhJ	b1644	Putative multidrug resistance membrane protein	2.21	4.29	2.22
yeaY	b1806	Putative membrane protein	2.43	3.21	4.06
yebS	b1833	Putative membrane protein	2.08	2.50	2.48
yhhT	b3474	Putative permease (PerM family)	2.30	3.89	2.92
yhiD	b3522	Putative membrane protein	4.11	5.93	7.24
yphA	b2543	Putative transmembrane protein	2.35	3.38	2.53
yqaE	b2666	Putative transport protein (YqaE family)	2.42	4.78	3.16

Continued on following page

TABLE 1—*Continued*

Function (reference) and gene	b no.	Gene product	Ratio		
			OD 4[a]	NaCl[b]	pH 5[c]
Adaptation to stress (16)					
aidB	b4187	Putative acyl-CoA dehydrogenase (flavoprotein), adaptive response (transcription activated by Ada)	4.11	2.08	3.47
bfr	b3336	Bacterioferritin, an iron storage homoprotein	8.84	3.28	4.72
dps	b0812	Stress response DNA-binding protein with ferritin-like domain	8.92	24.19	4.32
gadA	b3517	Glutamate decarboxylase, isozyme A	32.38	5.80	2.33
gadB	b1493	Glutamate decarboxylase, isozyme B	16.44	3.96	2.22
katE	b1732	Catalase; hydroperoxidase HPII (III), RpoS dependent	11.14	9.00	7.37
osmB	b1283	Lipoprotein, osmotically inducible	3.34	2.36	2.34
osmC	b1482	Resistance protein, osmotically inducible	4.93	6.19	3.51
osmY	b4376	Hyperosmotically inducible periplasmic protein, RpoS-dependent stationary-phase gene	50.66	68.76	19.08
otsA	b1896	Trehalose-6-phosphate synthase	10.27	52.44	13.79
otsB	b1897	Trehalose-6-phosphate phosphatase, biosynthetic	5.56	38.22	9.09
psiF	b0384	Induced by phosphate starvation	9.48	10.53	7.42
treA	b1197	Trehalase, periplasmic	7.98	12.71	2.50
treF	b3519	Trehalase, cytoplasmic	2.07	9.25	5.20
uspB	b3494	Universal stress protein B	4.52	5.91	3.61
gadC	b1492	Putative glutamate:gamma-aminobutyric acid antiporter (APC family)	10.28	3.48	2.22
Protein processing (7)					
cbpA	b1000	Curved DNA-binding protein, cochaperone of DnaK (Hsp40 family)	4.97	4.16	2.05
hchA	b1967	Molecular chaperone independent of ATP/ADP cycle, with class I glutamine amidotransferase-like domain	6.02	15.64	4.89
hycI	b2717	Protease involved in processing C-terminal end of HycE	4.00	4.16	2.79
rpsV	b1480	30S ribosomal subunit protein S22	5.20	6.07	4.60
ycfF	b1103	Putative inhibitor of protein kinase C; contains a transferase domain	2.68	2.99	2.10
ylgH	b4248	Putative translation factor	6.04	4.70	3.06
yhbO	b3153	Putative intracellular proteinase with class I glutamine amidotransferase-like domain	4.94	23.00	12.10
Other or undefined function (60)					
csiD	b2659	Conserved protein with clavaminate synthase-like domain	6.90	7.82	3.68
elaB	b2266	Unknown CDS[d]	8.11	17.82	11.80
erfK	b1990	Conserved hypothetical protein with NAD(P)-binding Rossmann-fold domain	2.24	4.55	2.18
fic	b3361	Possible cell filamentation protein, induced in stationary phase	12.94	17.76	10.82
msyB	b1051	Acidic protein suppresses mutants lacking function of protein export	17.37	8.60	8.89
phnB	b4107	Conserved protein with glyoxalase and dihydroxybiphenyl dioxygenase domain	4.81	5.37	3.38
yaiA	b0389	Unknown CDS	3.92	4.72	2.41
yajO	b0419	Putative oxidoreductase, NAD(P) dependent	2.21	6.76	3.42
ybdK	b0581	Conserved hypothetical protein	8.78	14.70	12.71
ybeL	b0643	Conserved hypothetical protein	2.42	9.80	4.27
ybgA	b0707	Conserved protein	6.53	16.97	13.24
ybgS	b0753	Conserved protein	8.43	38.08	16.12
ybhE	b0767	Putative isomerase	2.66	5.57	2.38
yhjP	b0865	Conserved hypothetical protein	3.66	6.53	6.24
yccJ	b1003	Unknown CDS	10.42	17.09	16.33
yceK	b1050	Unknown CDS	14.93	14.50	10.14
ycgB	b1188	Conserved hypothetical protein	10.36	35.43	11.19
ycgZ	b1164	Unknown CDS	5.11	5.53	4.00
yciF	b1258	Conserved protein	2.98	20.43	2.04
yciG	b1259	Conserved hypothetical protein	18.16	84.92	19.19
ydaM	b1341	Conserved protein with PYP-like sensor domain	6.74	9.54	3.15
ydfN	b1547	Qin prophage; putative tail fiber protein	2.25	4.71	2.35
ydgA	b1614	Conserved protein	2.99	5.89	2.63
yeaG	b1783	Conserved protein, nucleotide triphosphate hydrolase domain	19.44	26.61	21.48
yeaH	b1784	Conserved hypothetical protein	11.89	14.66	14.73
yebF	b1847	Conserved hypothetical protein	4.04	2.79	2.17
yeeP	b1999	CP4-44 prophage; putative GTP binding factor	2.80	4.87	3.08
yegP	b2080	Conserved hypothetical protein	17.85	17.67	7.86
yegS	b2086	Conserved protein with PDZ-like domain	8.87	6.52	13.93
yfiL	b2602	Conserved hypothetical protein	3.22	3.63	3.75
ygaF	b2660	Putative enzyme	5.28	6.57	5.18
ygaM	b2672	Conserved hypothetical protein	9.39	14.13	13.02
ygaU	b2665	Conserved hypothetical protein with LysM domain	7.54	11.75	8.51

Continued on following page

TABLE 1—*Continued*

Function (reference) and gene	b no.	Gene product	Ratio		
			OD 4[a]	NaCl[b]	pH 5[c]
ygbA	b2732	Conserved hypothetical protein	2.67	2.80	2.08
ygfS	b2886	Putative 4Fe-4S ferredoxin-type protein	3.06	5.92	3.66
yggE	b2922	Conserved protein	3.35	2.57	2.52
yghA	b3003	Putative oxidoreductase; NAD(P)-binding domain	4.55	26.14	6.54
ygiW	b3024	Conserved hypothetical protein	2.86	2.67	2.16
yhfG	b3362	Conserved hypothetical protein	6.03	8.40	5.95
yhhA	b3448	Conserved protein	4.05	8.31	3.28
yhjG	b3524	Conserved protein	3.89	7.11	4.58
yjbJ	b4045	Unknown CDS with YmbJ domain	21.96	20.09	7.60
yjdI	b4126	Conserved hypothetical protein	8.41	9.78	7.15
yjdJ	b4127	Putative acyl-CoA *N*-acyltransferase	6.34	9.34	6.96
yjeB	b4178	Conserved protein, winged helix domain	2.22	2.49	2.08
yjgG	b4247	Unknown CDS	3.62	3.87	2.22
yjgR	b4263	Putative enzyme contains nucleoside *triP* hydrolase domain	2.70	2.94	2.74
yjhT	b4310	Putative enzyme contains galactose oxidase-like central domain	3.05	7.04	3.05
ymgA	b1165	Unknown CDS	4.98	5.87	4.63
yncB	b1449	Putative dehydrogenase, with NAD(P)-binding and GroES-like domains	2.08	9.30	2.70
ynhG	b1678	Putative ATP synthase subunit with LysM domain	5.46	8.64	6.71
ynjF	b1758	Putative transferase	2.95	2.87	2.69
yodC	b1957	Unknown CDS	5.46	3.02	2.15
yodD	b1953	Unknown CDS	8.49	18.20	15.16
yohF	b2137	Putative oxidoreductase with NAD(P)-binding domains	3.97	11.26	5.21
ygjC	b3097	Conserved protein	6.77	14.06	5.17
ygjD	b3098	Conserved hypothetical protein	4.88	4.75	4.59
ygjE	b3099	Conserved protein	5.26	5.36	4.65
ygjG	b3102	Putative glutathione *S*-transferase enzyme with thioredoxin-like domain	4.77	9.03	5.65
ygjK	b3100	Conserved hypothetical protein	3.43	5.60	3.80

[a] Average relative mRNA levels (rpoS$^+$/rpoS mutant ratio) determined at transition into stationary phase in LB medium. OD 4, OD_{578} value of 4.0.
[b] Hyperosmotic shift in M9 medium.
[c] pH downshift in LB medium.
[d] CDS, coding sequence.

two nucleotides is still highly significant, as can be seen in the accompanying table.

This sequence motif represents an extended version of the sequence previously discussed as a putative −10 region for σ^S-specific promoters (see discussion below and reference 25). In several cases where genes were already known to be σ^S dependent, this sequence coincided with experimentally demonstrated promoters (e.g., for *dps*, *poxB*, *osmC*, and *otsB*; for compilations of experimentally verified σ^S-dependent promoters, see references 6, 18, 37, and 41). The presence of such a common promoter motif suggests that these promoters are subject to the same regulatory mechanism, i.e., direct recognition by σ^S-containing RNA polymerase. This motif could be clearly identified from the core set of σ^S-dependent genes, but neither MEME nor BioProspector recognized this pattern upstream of noncore σ^S-controlled genes, i.e., genes which display σ^S dependence under only one or two of the three growth and stress conditions tested (Fig. 1). This indicates either that these noncore σ^S-controlled genes have more degenerate σ^S-dependent promoters, which require additional activation mechanisms, or that they are under the indirect control of σ^S.

Modules within the σ^S network: the case of acid resistance genes. Our finding that many regulatory genes are under σ^S control (Table 1; Fig. 6) suggested that the σ^S regulon constitutes a large regulatory network with a hierarchical (cascade-like), modular internal architecture. Thus, secondary regulators may impose special regulatory patterns upon subsets of σ^S-dependent genes (modules). This includes a high potential for additional signal input into specific modules. From our data, it is directly seen that a group of known acid resistance genes constitutes such a module with an interesting regulatory pattern. These include *gadA* and the *gadBC* operon (encoding two glutamate decarboxylases and a glutamate-GABA exchange carrier involved in cytoplasmic proton scavenging) (57), the *hde* genes (which have also been implicated in acid resistance) (68), and the regulatory genes *gadX* (*yhiX*), *gadW* (*yhiW*), and *gadE* (*yhiE*). With the exception of the *gadBC* operon, all these genes are located in one of the chromosomal

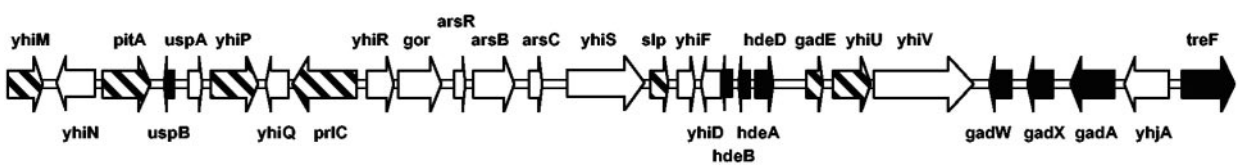

FIG. 2. Cluster of σ^S-controlled genes at around 79 min on the *E. coli* chromosome that includes several acid resistance genes (*gad* and *hde* genes). Genes identified as σ^S controlled under all three growth and stress conditions (core genes) are shown by black arrows, and σ^S-controlled genes identified only under one or two conditions are indicated by hatched arrows.

Blk1	A	C	G	T	Con	Deg
1	0.17	0.08	**0.34**	**0.41**	T	K
2	0.00	**1.00**	0.00	0.00	C	C
3	0.00	0.05	0.00	**0.95**	T	T
4	**1.00**	0.00	0.00	0.00	A	A
5	0.09	**0.44**	0.00	**0.47**	T	Y
6	**0.59**	0.06	**0.34**	0.00	A	R
7	0.06	**0.73**	0.17	0.03	C	C
8	0.00	0.00	0.00	**1.00**	T	T
9	0.09	0.06	0.03	**0.81**	T	T
10	**0.64**	0.00	0.17	0.19	A	A
11	**0.50**	0.20	0.09	0.20	A	A

FIG. 3. A common sequence pattern in the 200-bp regions upstream of σ^S-controlled core genes strongly resembles an extended −10 region previously discussed for σ^S-dependent promoters. Relative frequencies of nucleotides as identified by BioProspector (40) are shown in the table and correspond to positions −14 to −4 in putative σ^S-dependent promoters. A consensus sequence (Con) is given as well as a degenerate consensus (Deg), which also takes into account the second-most-frequent nucleotide if it occurs in more than 30% of the sequences identified (K stands for T or G, Y stands for T or C, and R stands for A or G). The consensus sequence is also shown as a sequence logo (14).

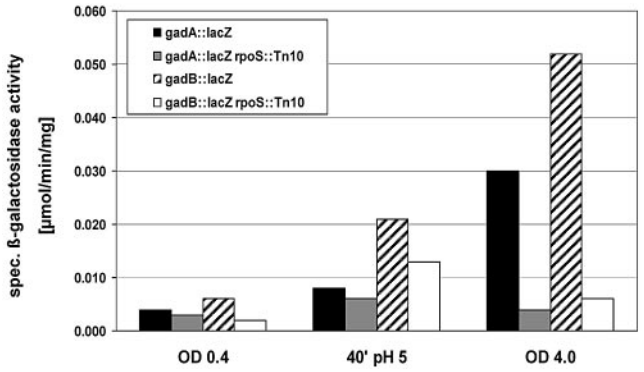

FIG. 4. Expression and σ^S dependence of *gadA*::*lacZ* and *gadB*::*lacZ* fusions under different growth and stress conditions. Strains JK86 and JK87, which carry transcriptional single-copy *lacZ* fusions in *gadA* and *gadB* (black and hatched bars, respectively), as well as their *rpoS*::Tn*10* derivatives (grey and white bars, respectively) were grown in LB medium. During log-phase growth, an aliquot of the culture was shifted to pH 5 (see Materials and Methods for details). Samples were taken during log phase (OD_{578} = 0.4; pH 7), 40 min after shift to pH 5, and during entry into stationary phase (OD_{578} = 4; pH 7). Specific β-galactosidase activities were measured (the data given represent the average of three independent experiments each).

clusters of σ^S-dependent genes (Fig. 2), which has also been referred to as a "fitness island for acid adaptation" in *E. coli* (27). At least under certain conditions, the regulators GadX and GadW seem to play opposite roles in the expression of *gadA*, *gadBC*, and the *hde* genes, whereas GadE seems to be an essential activator for these genes (27, 43, 44, 47, 66). Our microarray data (Table 2) not only demonstrated that all these genes are under σ^S control (previously demonstrated for some genes) (43, 44, 65) but also revealed an interesting pattern of regulation: while these genes were strongly σ^S dependent during entry into stationary phase, their σ^S dependence was reduced or even abolished under acid stress conditions (Table 2).

To confirm this apparent change in sigma factor dependence and to assay induction ratios in response to acid shift or entry into stationary phase, we constructed *lacZ* reporter fusions in the two target genes *gadA* and *gadB* and in the regulatory gene *gadE*. Expression of the single-copy transcriptional *lacZ* fusions in *gadA* and *gadB* was tested in wild-type and *rpoS* mutant backgrounds under the same conditions used for array analysis. The results (Fig. 4) demonstrated that expression of *gadA* and

TABLE 2. Expression ratios of σ^S-controlled acid resistance genes[a]

Gene	b no.	Gene product	Ratio			
			OD 4	NaCl	pH 5	OD 4 (Irp mutant)[b]
gadA	b3517	Glutamate decarboxylase A, isozyme, PLP dependent	32.38	5.80	2.33	39.58
gadB	b1493	Glutamate decarboxylase, PLP dependent, isozyme beta	16.44	3.96	2.22	21.23
gadC	b1492	Putative glutamate gamma-aminobutyric acid antiporter (APC family)	10.28	3.48	2.22	29.72
hdeA	b3510	Conserved protein with protein HNS-dependent expression	11.26	9.16	1.65	9.17
hdeB	b3509	Conserved hypothetical protein	6.61	4.46	1.28	5.87
hdeD	b3511	Putative membrane protein	2.52	2.07	1.27	3.64
slp	b3506	Outer membrane protein, induced after carbon starvation	3.68	2.79	1.08	5.19
yhiU	b3513	Multidrug resistance protein (lipoprotein)	3.48	1.38	0.99	3.23
gadE	b3512	Transcriptional regulator for *gadABC* operon, activates glutamate decarhboxylase-dependent acid resistance	13.06	8.68	1.14	19.38
gadW	b3515	Transcriptional regulator for GadX (regulatory protein), glutamic acid decarboxylase (GadA, -B), and glutamate transport protein (GadC)	6.12	5.98	3.09	12.39
gadX	b3516	Transcriptional regulator for glutamic acid decarboylase and transporter (*gadA, gadBC*)	6.09	7.45	3.14	9.39

[a] For explanations of data, see the footnotes to Table 1.
[b] Average relative mRNA levels (ratio of the *Irp rpoS*$^+$ mutant to the *Irp rpoS* mutant) determined at transition into stationary phase in LB medium.

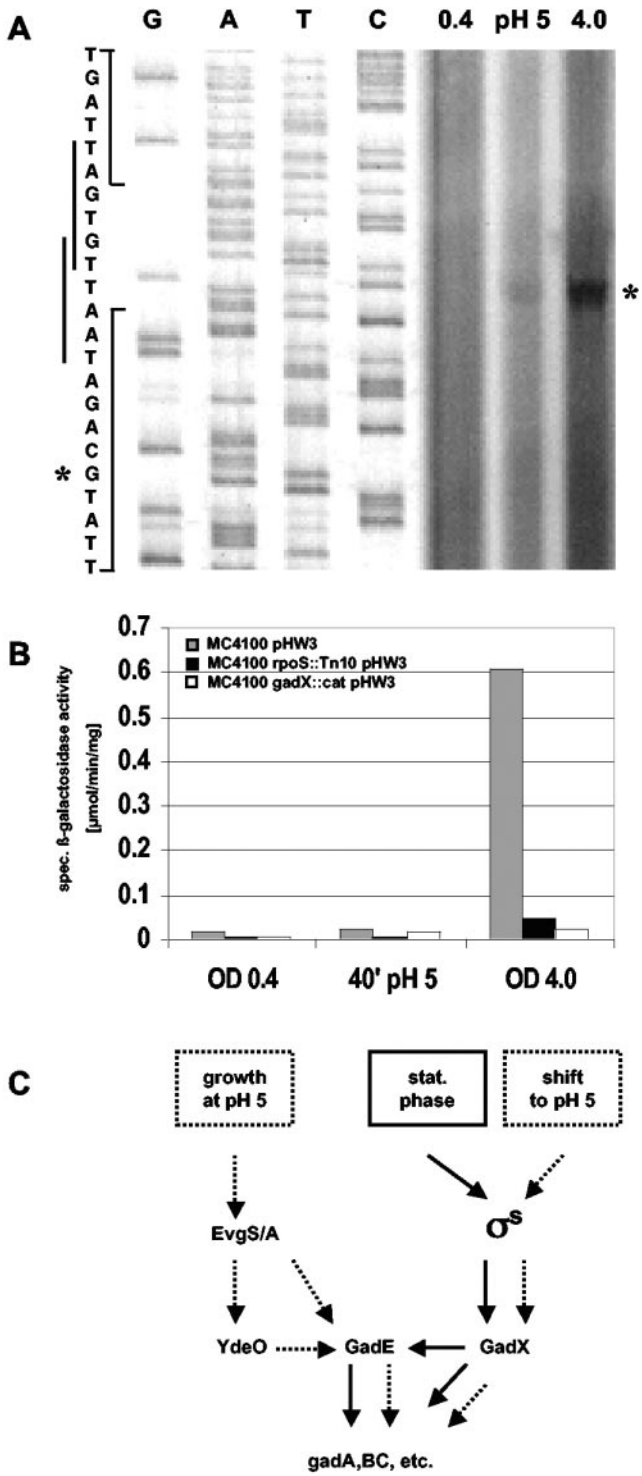

FIG. 5. The role of σ^S, GadX, and GadE in the expression of acid resistance genes under different stress conditions. (A) The *gadE* transcriptional start site was determined by primer extension experiments with RNA prepared from strain MC4100 carrying the translational *gadE*::*lacZ* fusion plasmid (see Materials and Methods for details). During log-phase growth in LB, an aliquot of the culture was shifted to pH 5. Samples for RNA preparation were taken during log phase (OD_{578} = 0.4; pH 7), 40 min after shift to pH 5, and during entry into stationary phase (OD_{578} = 4; pH 7). The reverse transcript and the transcriptional start site in the sequence are indicated by asterisks, and two putative −10 regions are indicated along the sequence. (B) Expression of *gadE* was assayed by a transcriptional *gadE*::*lacZ* fusion present on a plasmid, because in single-copy constructs, measurable activities were extremely low. Translational single-copy fusions, which exhibit higher activities, yielded results similar to those obtained with the multicopy transcriptional fusions. Strain MC4100 and its *rpoS* and *gadX* mutant derivatives carrying these fusions were grown and sampled as described in the legend to Fig. 4, and specific β-galactosidase activities were measured. (C) Summarizing model. Solid arrows indicate regulatory influences relevant during entry into stationary phase, and dotted arrows indicate regulatory influences upon shift to or growth at acidic pH.

gadB was activated under stress conditions and that this expression required σ^S during entry into stationary phase but was nearly σ^S independent after acid shift. By contrast, transcription of *gadE* was not stimulated by shift to pH 5 but was strongly activated during entry into stationary phase, as demonstrated by primer extension experiments (Fig. 5A) and transcriptional *gadE*::*lacZ* fusion analysis (Fig. 5B). The transcriptional start site shown in Fig. 5A was the same as for a previously described *gadE* transcript (a second transcript, which was observed in an *hns* mutant, could not be detected in any of our experiments and probably indicates the presence of a second H-NS-silenced promoter) (27). Also, stationary-phase induction of *gadE* was strongly dependent on σ^S and on GadX (Fig. 5B). Thus, σ^S dependence of *gadE* during entry into stationary phase was probably indirect via GadX, consistent with the putative −10 region of the stationary-phase-inducible *gadE* promoter (Fig. 5A) not showing similarity to the $E\sigma^S$ consensus promoter (Fig. 3). Taken together, these data suggest that this module of acid resistance genes can switch σ^S and/or σ^{70} dependence, depending on specific environmental or stress conditions, since σ^S is part of only one of the pathways that allow activation of these genes (Fig. 5C).

Relationship between σ^S and Lrp in global regulation. A factor that appears to directly affect σ^S and/or σ^{70} selectivity at certain promoters is the global regulatory protein Lrp, as was previously reported for the σ^S-controlled genes *osmY* (13), *osmC* (9), and *aidB* (32). To find out whether Lrp affects sigma factor selectivity of promoters in a more global way, we again determined ratios of expression in *rpoS*$^+$ and *rpoS* mutant strains, i.e., σ^S dependence on genomic microarrays but now for strains that were defective in the *lrp* gene. For these experiments, we chose one of the conditions previously tested, i.e., entry into stationary phase in LB medium (OD_{578}), since many putative stationary-phase-induced σ^S-controlled genes are repressed by Lrp (64). However, a difference in the σ^S dependence in *lrp*$^+$ and *lrp* mutant strains (in contrast to a mere difference in expression levels) would only be expected if Lrp differentially affected promoter access and/or activation by either σ^S-containing or σ^{70}-containing RNA polymerase, in other words, affected sigma factor selectivity.

As is demonstrated in Fig. 6, where σ^S dependence (i.e., ratios of expression in *rpoS*$^+$ and *rpoS* strains) is shown for *lrp*$^+$ and *lrp* mutant backgrounds, there are indeed σ^S-controlled genes with such changes in sigma factor selectivity. All genes for which such changes were more than twofold (Fig. 6) are listed in Table S3 in the supplemental material. An example of reduction or nearly complete loss of σ^S dependence is the *csiD-ygaF-gabDTP* operon (49). These microarray data are

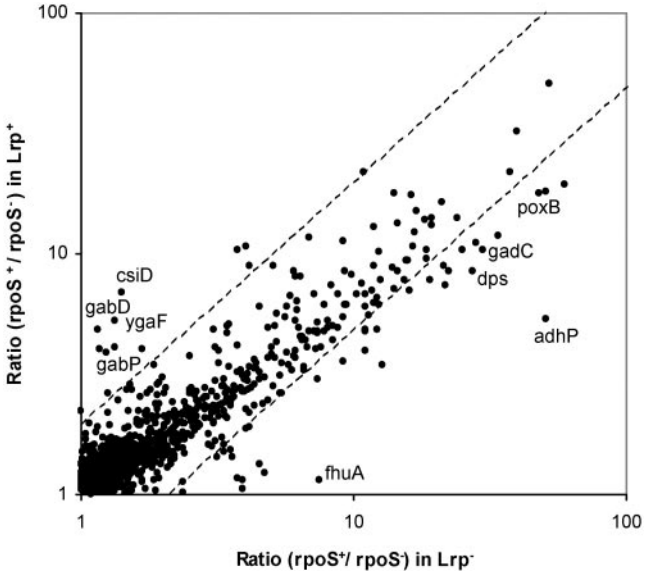

FIG. 6. σ^S dependence of many genes is altered in the absence of the global regulator Lrp. Ratios of expression in *rpoS*$^+$ and *rpoS* mutant strains were determined by microarray analysis of *lrp*$^+$ and *lrp*::Tn*10* backgrounds and are shown in a scatter plot. Genes with a >2-fold difference in this ratio (i.e., in σ^S dependence) in *lrp*$^+$ and *lrp* mutant strains fall outside of the diagonal field marked by hatched lines.

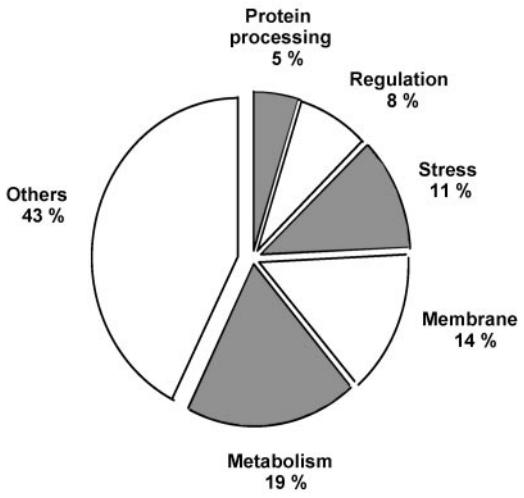

FIG. 7. Functional annotations of σ^S-controlled genes. Numbers shown were obtained for core genes. For gene names and further functional details, see Table 1 and the text.

consistent with a previous report of a modulatory role of Lrp in the control of the *csiD* promoter, observed with *lacZ* fusions (21). On the other hand, a number of genes exhibited a clear increase in σ^S dependence in the *lrp* mutant background (Fig. 6; Table S3 in the supplemental material). We identified 28 genes for which the ratio of σ^S dependence increased more than twofold in the *lrp* mutant background. Thirteen of these genes (*adhP*, *cfa*, *dps*, *gadC*, *gadW*, *mlrA*, *poxB*, *otsB*, *otsA*, *yciG*, *yeaG*, *yjgB*, and *yohF*) were also described as Lrp repressed by Tani et al. (64). Also, the acid resistance genes *gadA*, *gadB*, and *gadC*, as well as their regulatory genes *gadE*, *gadW* and *gadX*, exhibited increased σ^S dependence in the *lrp* mutant (although the increase in general was less than twofold) (Table 2).

Extensive overlap between σ^S and cAMP-CRP in global regulation. CRP plays a complex role in the regulation of the expression of *rpoS* itself (34; F. Scheller and R. Hengge, unpublished results). In addition, several previously identified σ^S-dependent genes (e.g., *csiD* and *osmY*) are also under direct positive or negative control by cAMP-CRP (13, 46). To find out whether coregulation by σ^S-containing RNA polymerase and cAMP-CRP is a more general phenomenon, the upstream regions (200 bp) of all 481 positively σ^S-controlled genes identified here were screened for putative cAMP-CRP boxes, defined as TGTGA(N6)TCACA, with a maximum of three mismatches allowed.

We identified 263 σ^S-controlled genes (i.e., 55%) featuring putative cAMP-CRP binding sites (with one, two, or three mismatches from the consensus) upstream of their coding regions. Some of these genes have two or more cAMP-CRP boxes. Even though not all of these putative binding sites may actually play physiologically relevant regulatory roles, this is a striking number that indicates a strong overlap between the σ^S and cAMP-CRP regulons.

For 55 of 140 σ^S-dependent core genes, putative extended −10 promoter regions (Fig. 3) could be unequivocally identified or were known before (this corresponds to a total of 64 such regions, as 9 genes displayed 2 of these putative promoter regions). More than half of these genes (30 of 55) also contained putative cAMP-CRP-binding sites in their promoter regions. Nineteen genes exhibited one cAMP-CRP box, 9 had two, and 2 (*osmC* and *talA*) had three such sites. The maximum number of cAMP-CRP boxes, i.e., five, was found in the *pdhR* promoter region, where the first such site overlapped with the putative promoter and the additional ones followed further downstream. Among these 48 putative cAMP-CRP-binding sites, 5 sites were located at typical activator positions (class I or II) (10), 14 sites were found at typical repressor positions (i.e., overlapping with the promoter and/or the transcriptional start site), 3 sites were situated between typical activator and repressor sites (around −50), 12 sites were situated too far upstream to exert a direct activating effect (but may act indirectly, e.g., by bending DNA), and 14 sites were located downstream of the transcriptional start site (the latter groups could in principle also serve other non-σ^S-dependent promoters that may contribute to the expression of the respective genes).

Physiological functions of σ^S-dependent genes. For approximately 57% of all σ^S-dependent genes identified here, functional annotations exist. In Table 1, the core set of σ^S-controlled genes is ordered in functional categories (according to a simplified version of the system used by Riley and coworkers) (60, 61), and the relative occurrence of genes belonging to each category is shown in Fig. 7. Besides genes with known functions in stress management (11%), nearly all σ^S-controlled core genes with known or probable functions fall into three groups. They encode either metabolic enzymes (19%), transport proteins and/or intrinsic membrane proteins of unclear function (which are likely to be transporters as well) (14%), or regulatory proteins (8%).

Upon closer inspection of the metabolic genes, an interest-

ing pattern became apparent. A number of genes involved in central energy metabolism (glycolysis, fermentation, anaerobic respiration, and the pentose phosphate shunt) exhibited positive σ^S control at least under one condition tested (Table S4 in the supplemental material). In addition, interesting regulatory antagonisms were observed. Thus, not only pyruvate oxidase (*poxB*) was strongly σ^S activated, but also the repressor (encoded by *pdhR*) for the housekeeping pyruvate-oxidizing enzyme, i.e., pyruvate dehydrogenase, was under σ^S control. While fumarate reductase (*frdA*) was positively σ^S controlled, succinate dehydrogenase (*sdhCDAB*) was negatively affected by σ^S. In the pentose phosphate shunt, there was opposite σ^S control of the two genes for transketolase (*tktA* and *tktB*), indicating that in stationary phase or under other stress conditions, the *tktA*-encoded major enzyme may be replaced by TktB, for which only very low expression levels were previously reported (29). Overall, these data indicate that induction of σ^S in starving or otherwise stressed cells may contribute to decreasing aerobic respiration in favor of a more fermentative and/or anaerobic respiration-based energy metabolism.

DISCUSSION

Genome-wide identification of σ^S-dependent genes and in silico identification of a σ^S consensus promoter sequence. Among the 481 positively σ^S-controlled genes identified here, there is a core group of 140 genes which were found to be σ^S controlled under all three growth and stress conditions used here, which known to result in high cellular σ^S levels (Fig. 1). Thus, the expression of these genes may just follow the cellular σ^S concentration. Expression of the remaining 341 genes seems to require some special conditions and therefore specific regulation in addition to high σ^S levels (see also below).

The large number of σ^S-dependent genes identified here provided an ideal database for an in silico search for common regulatory motifs upstream of the coding sequences. From the core group of σ^S-dependent genes, two pattern-searching programs, MEME and Bioprospector, identified a common motif with high statistical significance. The motif consensus sequence is TCTATACTTAA (or KCTAYRCTTAA, which takes into account the second-most-frequent nucleotides when present in more than 30% of the sequences analyzed; K stands for T or G, Y stands for T or C, and R stands for A or G) (Fig. 3). This sequence represents an extended version (nucleotide −14 to −4) of a −10 promoter region previously proposed to be recognized by $E\sigma^S$ (6, 18, 25, 38). By contrast, it was recently suggested that a C instead of a T at the −12 position strongly contributes to $E\sigma^S$ selectivity of a promoter (30). However, our compilation of putative −10 regions found upstream of σ^S-dependent core genes (as well as the previously published shorter lists of σ^S-controlled promoters mentioned above) indicates that a C (−12) is possible but actually quite rare (5%) (Fig. 3, with A and G being completely absent). Thus, a C (−12) is obviously not part of an $E\sigma^S$-selective consensus promoter but may be an occasionally occurring deviation from the consensus that is better tolerated by $E\sigma^S$ than by $E\sigma^{70}$ and thereby contributes to $E\sigma^{70}$ selectivity.

The extended −10 consensus sequence identified here features all the nucleotides, which have been found experimentally to be important in promoter binding and activation by $E\sigma^S$. T (−14) and above all C (−13) were shown to directly interact with a specific amino acid (K173) in region 3.0 (2.5) of σ^S (6). Strong conservation of T (−12), A (−11), and T (−7) reflects the special importance of these nucleotides in $E\sigma^S$-mediated promoter melting (36–38). Finally, in the context of the σ^S-controlled *rssAB* promoter, the TAA motif (−6 to −4) has also been found to stimulate σ^S-dependent activation in stationary phase (56). Interestingly, both pattern identification algorithms used here could not identify a motif corresponding to a −35 region, which is consistent with suggestions that the −35 regions of naturally evolved σ^S-dependent promoters may be more degenerate (20, 36, 37). Taking the data together, we would like to suggest that the motif identified here, KCTAYR CTTAA, represents an extended −10 region of a directly $E\sigma^S$-recognized and -activated promoter. The length of this sequence motif should allow the identification of putative σ^S-controlled promoters in silico with high probability.

For many of the 140 core σ^S-dependent genes, this putative extended −10 region can easily be recognized with only one or two mismatches. In those cases where such a sequence is less apparent, several explanations are possible. Either the promoter is further upstream than the 200 upstream nucleotides screened in our study (e.g., the *osmY* promoter) (33), especially if genes are part of an operon. On the other hand, such a gene may be under the control of a σ^S-dependent activator (whose gene would belong to the core set of σ^S-controlled genes), which may then activate promoters in conjunction with $E\sigma^{70}$.

σ^S-controlled genes represent a large and complex network with differentially controlled modules and connections to other global regulons. Two major observations reported here indicate that σ^S controls not only a regulon but rather a regulatory network with an intrinsic hierarchical and modular structure. (i) The majority (71%) of the positively σ^S-dependent genes were found only under one or two conditions characterized by high cellular σ^S levels, and even the core genes exhibited very different degrees of σ^S dependence under different conditions (Table 1). (ii) Quite a large number of σ^S-dependent genes identified here encode regulatory proteins (Table 1 and Fig. 6), which can be expected not only to affect the expression of subsets of σ^S-dependent genes but also to serve as additional signal integrators. If the target genes of these regulators are also directly dependent on σ^S-containing RNA polymerase, this would establish feed-forward regulatory circuits (51), which may fine-tune or boost the expression of subsets of σ^S-controlled genes (i.e., modules) under specific conditions. Knockout mutants of such secondary regulatory genes are currently isolated to identify their spectrum of target genes.

The internal architecture of the σ^S network is also dynamic, i.e., subject to environmental regulation. σ^S-controlled target genes may be flexibly allocated to additional global regulons, as suggested by the apparently strong overlap with cAMP-CRP control. More than half of all σ^S-controlled genes exhibit putative cAMP-CRP binding sites in their 200-bp upstream regions. The locations of these sites in cases where the promoters were either known or can be identified with high probability with our −10 region consensus (Fig. 3) indicate that cooperation between σ^S and cAMP-CRP can be positive, negative, or more complex (e.g., involving several cAMP-CRP boxes or additional promoters). These connections between the σ^S and

the cAMP-CRP networks also extend to the level of the master regulators, as cAMP-CRP controls σ^S itself in a complex and as-yet-unclarified manner (reference 34; Scheller and Hengge, unpublished). In other words, σ^S and cAMP-CRP seem to tightly cooperate in integrating the responses to multiple (general) stresses and specific C starvation, respectively. Thus, cAMP-CRP may have a global regulatory role that goes beyond mediating catabolite control of gene expression. In a recent transcriptome study of CRP-dependent catabolite control, the strong overlap with the σ^S network did not become apparent, although many stress genes encoding chaperones and proteases, for example, exhibited glucose-CRP-dependent regulation (22). An obvious explanation is that this study used cells that grew rapidly in LB medium under conditions in which σ^S is hardly present in the cell. To further analyze the cooperation between σ^S and cAMP-CRP, future experiments will have to assay the effects of *crp* mutations under σ^S-inducing stress conditions (such as those used in the present study).

Depending on specific stress conditions, acid resistance genes belong to the σ^S network or not. By our microarrays and reporter gene fusion analysis, we identified a subset of σ^S-controlled genes, which were clearly stationary-phase-induced in a σ^S-dependent manner but exhibited only minor or no σ^S dependence upon acidic shift (Table 2; Fig. 4). The expression of these genes probably becomes σ^{70} dependent, as no other *E. coli* sigma factor is known to be induced or activated under acidic conditions. These genes are crucial for acid resistance and include *gadA*, *gadBC*, and the *hde* genes (as well as other less-well-characterized genes), together with their regulatory genes *gadE* (*yhiE*), *gadX* (*yhiX*), and *gadW* (*yhiW*) (Table 2). Thus, whether these genes belong to the σ^S network or not depends on environmental conditions.

The GadE regulator was reported to be essential for the expression of *gadA*, *gadBC*, and the *hde* genes, whereas GadX and GadW play a complex and conditional modulatory role, which becomes dispensable if GadE is overproduced (27, 43, 47). Reduced σ^S dependence and even a lack of induction upon acid shift is especially pronounced for the regulator GadE (Fig. 5; Table S3 in the supplemental material), and the corresponding regulatory pattern of the downstream target genes may be a reflection of this change in *gadE* control. Moreover, we show here that strong *gadE* expression during entry into stationary phase also requires the GadX regulator. This generates a feed-forward loop in which the target genes *gadA* and *gadB* and probably others are under both direct and indirect control of GadX (the latter via GadE) (Fig. 5C).

These and the previously published data on the complex control of acid resistance genes can be summarized in a model in which the essential module activator GadE is under the control of two pathways (Fig. 5C): (i) the stationary-phase induction pathway using σ^S, which is required for GadX expression (this control includes the small σ^S-dependent *gadY* RNA which affects the stability of *gadX* mRNA) (53), which in turn activates *gadE*, and (ii) a regulatory cascade involved in *gadE* expression in cells growing at low pH, which comprises the EvgS-EvgA two-component system and YdeO (47). Thus, the switch in sigma factor dependence of the acid resistance genes would be due to σ^S being part of only one of the activating pathways for *gadE*. This model explains earlier reports of acid tolerance being σ^S dependent only in stationary phase (12) and is also reflected in the absence of σ^S in the regulatory network of these acid resistance genes as presented by Masuda and Church (47). The previously reported strong derepression of acid resistance genes in *hns* mutants involves *gadX* (28) and most likely reflects the strong negative regulation of σ^S by H-NS (5, 69). Interestingly, σ^S itself is strongly induced by a shift to pH 5, so why is σ^S not sufficient and apparently not even relevant for activation of these acid resistance genes at low pH? We have recently observed that acid induction of σ^S is transient, i.e., σ^S levels do not remain high in cells growing continuously at pH 5 (M. Metzner and R. Hengge, unpublished results) and this transient σ^S induction upon sudden acid shift does not result in the activation of *gadE* (Fig. 5A and B). However, continuous expression of *gadA*, *gadBC*, and the *hde* genes is likely necessary to cope with permanent acid stress; this requires continuously high expression of GadE, probably mediated by the EvgSA/YdeO pathway.

Eσ^S-Eσ^{70} promoter selectivity can be conditional and influenced by the abundant nucleoid protein Lrp. As outlined above, a switch between σ^S and σ^{70} dependence can be a consequence of using different activating pathways that converge on a module regulator (such as GadE). On the other hand, the similarity of Eσ^S- and Eσ^{70}-recognized promoters allows a switch between σ^S and σ^{70} dependence, even at the same promoter, i.e., the sigma factor selectivity of a promoter can be conditional. An example of such regulation is provided by the *dps* gene, which is strongly activated by Eσ^S in stationary phase or under other stress conditions (*dps* is a core gene) (Table 1) but which can also be activated by Eσ^{70} cooperating with the H_2O_2-activated regulator OxyR (1). In fact, of the 22 genes that belong to the OxyR regulon (70), 10 genes were observed here to be under σ^S control, including the core genes *dps* and *yaiA*, the noncore genes *dsbG* and *hemH*, and the *sufABCDSE* operon (data not shown). The general pattern that seems to emerge here is that certain modules of genes within the σ^S network can be recruited as subsets of other regulons under special growth or stress conditions. This also indicates that the internal architecture of stress-responsive regulatory networks is not static but is itself subject to regulation.

Another module within the σ^S network comprises genes that are also under the control of the global regulator Lrp. Approximately half of the 140 core σ^S-dependent genes identified here are also under repression by Lrp (64). Previous studies with the *osmY*, *osmC*, and *aidB* promoters suggested that Lrp not only acts as an ordinary repressor but can also affect sigma factor selectivity (9, 13, 32). Our data presented here indicate that this is a more general phenomenon. We identified 28 genes for which σ^S dependence was >2-fold higher in the *lrp* mutant background (Fig. 6; Table S3 in the supplemental material). For almost half of these genes, this correlates with repression by Lrp (64). On the other hand, 13 genes showed a reduction or even loss of σ^S dependence in the *lrp* mutant background. The most striking example is the *csiD-ygaF-gabDTP* operon (Fig. 5), which lost σ^S dependence in the *lrp* mutant background and for which a role of Lrp as a positive modulator at the *csiD* promoter was suggested previously (21).

How can Lrp affect the preference for either σ^S- or σ^{70}-containing RNA polymerase holoenzyme? Lrp is known as an abundant regulatory and chromosome-organizing protein that is further induced during entry into stationary phase (2, 31).

Lrp can bend DNA and often assembles at multiple adjacent sites along one side of the DNA helix (67). This means that Lrp can induce complex DNA superstructures, which in general would have an inhibitory effect on gene expression but at some promoters may less affect $E\sigma^S$ than $E\sigma^{70}$. At other promoters, Lrp may also somewhat optimize the positioning of the −35 and −10 regions and perhaps also of operator sites required for expression by $E\sigma^{70}$. In all these cases, the absence of Lrp would shift relative $E\sigma^S$-$E\sigma^{70}$ dependence, as $E\sigma^S$ is less demanding with respect to using a nonoptimal −35 region (20, 25) or to nonoptimal spacing of the −35 and −10 regions (for a summary, see reference 25; A. Typas and R. Hengge, unpublished data). Taken together, our data suggest that the abundant Lrp protein not only acts as a global repressor (or activator in some cases) but also affects $E\sigma^S$-$E\sigma^{70}$ selectivity at many stationary-phase-induced promoters, probably by modulating local DNA topology.

Physiological functions of the σ^S network. Besides genes directly involved in coping with the detrimental effects of stress (e.g., *dps*, *katE*, the *otsBA* operon, or the *gad* genes), the other annotated σ^S-dependent genes (core and noncore genes) in principle encode three major functional groups of proteins (Fig. 7): (i) regulatory factors (about 8%) (implications are discussed above), (ii) known transport systems or other intrinsic membrane proteins (14%), and (iii) metabolic enzymes, many of which belong to central energy metabolism (19%) (Table S4 in the supplemental material). This suggests that overall membrane traffic is significantly altered in stressed or stationary-phase cells. Thus, σ^S control may contribute to scavenging of various nutrients under nutrient-limiting conditions, as well as to increased resistance against various toxic compounds, by inducing putative efflux pumps. Moreover, σ^S seems to have a more pronounced influence on energy metabolism than previously suspected and may be crucial in the transition from growth to maintenance metabolism in stressed or stationary-phase cells. Important genes involved in glycolysis, fermentation, anaerobic respiration, electron transport, and the pentose phosphate shunt turned out to be under positive σ^S control (Table S4 in the supplemental material). These data suggest that induction of σ^S may prepare the cells for a shift away from oxidative respiration towards a more fermentative or anaerobic respiratory energy metabolism. This may serve to counteract the increased production of reactive oxygen species in aerobic respiration during entry into a starvation situation (16), but detailed implications will have to be studied in the future.

Finally, it will certainly be interesting to compare the σ^S networks of various bacterial species. So far, genome-wide profiling of σ^S-controlled genes has only been reported for *Pseudomonas aeruginosa* (59). There, the σ^S network is at least as large as in *E. coli* (14% of the genes in the genome are affected). A large group of σ^S-dependent genes specifically found in *Pseudomonas* is involved in quorum sensing. As *E. coli* does not possess the corresponding quorum-sensing systems, this demonstrates that even in relatively closely related species, the same global regulators and their regulatory networks have been recruited to serve different functions in ways that may reflect differences in natural environments and lifestyles.

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