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Efficient Translation of the RpoS Sigma Factor in *Salmonella typhimurium* Requires Host Factor I, an RNA-Binding Protein Encoded by the *hfq* Gene

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The RpoS transcription factor (also called σ^S or σ^{38}) is required for the expression of a number of stationary-phase and osmotically inducible genes in *Escherichia coli*. RpoS is also a virulence factor for several pathogenic bacteria, including *Salmonella typhimurium*. The activity of RpoS is regulated in response to several different signals, at the transcriptional and translational levels as well as by proteolysis. Here we report that host factor I (HF-I), the product of the *hfq* gene, is required for efficient expression of *rpoS* in *S. typhimurium*. HF-I is a small, heat-stable, site-specific RNA-binding protein originally characterized for its role in replication of the RNA bacteriophage Qβ of *E. coli*. Its role in the uninfected bacterial cell has previously been unknown. Assays of β-galactosidase in strains with *rpoS-lac* fusions, Western blot (immunoblot) analysis, and pulse-labeling and immunoprecipitation of both fusion proteins and native RpoS show that an *S. typhimurium hfq* mutant has a four- to sevenfold reduction in expression of *rpoS* that is attributable primarily to a defect in translation. These results add a new level of complexity to the regulation of RpoS activity.

Enteric bacteria such as *Salmonella typhimurium* and *Escherichia coli* live in vertebrate hosts as pathogens or commensals but also survive in nutrient-deficient soils and water (43). In both habitats they lead a "feast-and-famine existence" (34), mainly famine punctuated by rare periods of nutritional surplus. Recent work with *E. coli* has focused on the physiological changes that occur in batch cultures as growth slows and cells enter the stationary phase (35). The *rpoS* gene encodes a specificity factor for RNA polymerase (RpoS [also called σ^{38} or σ^S]) (46, 50, 62) that is required for the transcription of many genes expressed during the onset of stationary phase (40). RpoS-dependent adaptations include not only shifts in metabolic pathways but also resistance mechanisms developed as insurance against life-threatening stresses such as high osmolarity, heat shock, an elevated H_2O_2 level, and UV light (26, 40).

Experimentally, many inducing treatments have been shown to increase both the abundance of RpoS and expression of RpoS-dependent genes. These include the exhaustion of a single nutrient in minimal medium containing limiting sources of carbon and energy, nitrogen, or phosphorus (21, 66), as well as growth into stationary phase, where additional signals may include oxygen limitation, low pH, and high cell density (38, 47, 53). Furthermore, differences in stationary-phase induction are seen when a defined minimal medium and a rich medium such as Luria-Bertani (LB) medium are compared (38, 44), and there are reports of induction by specific metabolites, including acetate, benzoate, and homoserine lactone (28, 47, 53). Osmotic challenge or growth in a high-osmolarity medium also increases RpoS activity (4, 27). Not all RpoS-dependent genes are induced in every condition that increases the amount of RpoS protein; this probably reflects the involvement of additional factors specific for each stimulus and regulated gene (40).

Both transcriptional and posttranscriptional control of RpoS in response to various inducing treatments have been demonstrated (27, 38, 40, 42, 44, 53). Control of RpoS abundance at the translational level and by proteolysis (38, 66) is reminiscent of heat shock sigma factor (RpoH) regulation (67). It has also been demonstrated that RpoS abundance is positively regulated by ppGpp (21) during limitation of different nutrients, including glucose. Since glucose limitation is reported not to affect transcription of *rpoS* (38), this again suggests posttranscriptional control mechanisms. An *hns* mutant, lacking the abundant DNA-binding protein H-NS, has increased RpoS levels in exponential phase through effects on both translation and protein stability (4, 66); RpoS stability was recently shown to be controlled by the ClpXP protease (55). Additionally, several mutants that apparently alter RpoS levels by affecting UDP-glucose, a putative intracellular signal molecule (6), have been found.

The *E. coli hfq* gene product, host factor I (HF-I), was discovered through its role in the in vitro replication of Qβ, an RNA bacteriophage (18, 19, 31). The function of HF-I in uninfected cells has been unknown, but it is an RNA-binding protein associated with ribosomes (9, 32, 56), and *hfq* mutants are pleiotropic (64). In this report, we show that an *S. typhimurium* mutant defective in the *hfq* gene has substantially reduced expression of *rpoS*. The pleiotrophy of *hfq* mutants may be partly explained by a requirement for HF-I in efficient translation of *rpoS*.

MATERIALS AND METHODS

Bacterial strains and construction. The strains used in this study are listed in Table 1; the wild-type *S. typhimurium* strains LT2 and ATCC 14028s do not carry the *lac* operon. The high-frequency generalized transducing bacteriophage P22 mutant HT*105/1 int-201* (54) was used for transduction in *S. typhimurium* by standard methods (12). Strain ATCC 14028s shows a phage P22-resistant phenotype in cross-streak tests but is competent as a recipient in transduction. To use ATCC 14028s and derivatives as transductional donors, P22 lysates were grown by zygotic induction by the method described previously (13), except that the conjugational donor was an *S. typhimurium* strain.

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TABLE 1. Bacterial strains

Species and strain	Genotype or description[a]	Source (reference)
E. coli		
DH5α	F$^-$ *endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-1 recA1 gyrA96* (Nal^r) *relA1* Δ(*lacZYA-argF*)*U169* (ϕ80*lacZ*Δ*M15*)	P. Higgins (25)
MG1655	Wild type	D. Biek
UM122	*thi-1* HfrH *katF13*::Tn*10*	P. Loewen
TE6256	MG1655 *katF13*::Tn*10*	P1.UM122 × MG1655
S. typhimurium[b]		
LT2	Wild type	J. Roth
ATCC 14028s	Virulent, wild type	S. Libby (17)
SF1005	ATCC 14028s *rpoS*::pRR10 (Amp^r)	S. Libby (17)
DB7000	*leuA414*(Am)	P. Youderian
TE4521-4	*putPA1303*::Kan^r-*hemA-lac* [pr] *rpoS1071*::Tn*10d*-Cam	1
TE5314	*putPA1303*::Kan^r-*hemA-lac* [pr] *hfq-1*::Mud-Cam	1
TE6133	*rpoS1071*::Tn*10d*-Cam	P22.TE4521-4 × LT-2
TE6153	*putPA1303*::Kan^r-*katE-lac* [op]	This study
TE6154	ATCC 14028s *putPA1303*::Kan^r-*katE-lac* [op]	P22.TE6153 × ATCC 14028s
TE6167	*putPA1303*::Kan^r-*katE-lac* [op] *hfq-1*::Mud-Cam	P22.TE5314 × TE6153
TE6168	*putPA1303*::Kan^r-*katE-lac* [op] *rpoS1071*::Tn*10d*-Cam	P22.TE6133 × TE6153
TE6170	ATCC 14028s *putPA1303*::Kan^r-*katE-lac* [op] *hfq-1*::Mud-Cam	P22.TE5314 × TE6154
TE6171	ATCC 14028s *putPA1303*::Kan^r-*katE-lac* [op] *rpoS1071*::Tn*10d*-Cam	P22.TE6133 × TE6154
TE6193	DB7000 *putPA1303*::Kan^r-*katE-lac* [op]	P22.TE6153 × DB7000
TE6212	*putPA1303*::Kan^r-*katE-lac* [op] *zfd-6825*::Tn*10d*-Tet *rpoS*$^+$ (50% linked)	
TE6225	ATCC 14028s *putPA1303*::Kan^r-*katE-lac* [op] *zfd-6825*::Tn*10d*-Tet *rpoS* (ATCC)	P22.TE6212 × TE6154
TE6226	ATCC 14028s *putPA1303*::Kan^r-*katE-lac* [op] *zfd-6825*::Tn*10d*-Tet *rpoS* (LT2)	P22.TE6212 × TE6154
TE6227	*putPA1303*::Kan^r-*katE-lac* [op] *zfd-6825*::Tn*10d*-Tet *rpoS* (ATCC)	P22.TE6225 × TE6153
TE6228	*putPA1303*::Kan^r-*katE-lac* [op] *zfd-6825*::Tn*10d*-Tet *rpoS* (LT2)	P22.TE6225 × TE6153
TE6231	DB7000 *putPA1303*::Kan^r-*katE-lac* [op] *hfq-1*::Mud-Cam	P22.TE5314 × TE6193
TE6232	DB7000 *putPA1303*::Kan^r-*katE-lac* [op] *rpoS1071*::Tn*10d*-Cam	P22.TE6133 × TE6193
TE6241	*putPA1303*::Kan^r-*katE-lac* [op] *zfd-6825*::Tn*10d*-Tet *rpoS* (ATCC) *hfq-1*::Mud-Cam	P22.TE5314 × TE6227
TE6244	*putPA1303*::Kan^r-*katE-lac* [op]/pTE577 (P_{ara}-*hfq*)	
TE6245	*putPA1303*::Kan^r-*katE-lac* [op]/pTE570 (P_{ara})	
TE6246	*putPA1303*::Kan^r-*katE-lac* [op] *hfq-1*::Mud-Cam/pTE577 (P_{ara}-*hfq*)	
TE6247	*putPA1303*::Kan^r-*katE-lac* [op] *hfq-1*::Mud-Cam/pTE570 (P_{ara})	
TE6248	*putPA1303*::Kan^r-*katE-lac* [op] *zfd-6825*::Tn*10d*-Tet *rpoS1071*::Tn*10d*-Cam	P22.TE6133 × TE6227
TE6253	*putPA1303*::Kan^r-*rpoS-lac* [pr]	This study
TE6266	*putPA1303*::Kan^r-*rpoS-lac* [pr] *hfq-1*::Mud-Cam	P22.TE5314 × TE6253
TE6317	*putPA1303*::Kan^r-*rpoS-lac* [op]	This study
TE6318	*putPA1303*::Kan^r-*rpoS-lac* [op] *hfq-1*::Mud-Cam	P22.TE5314 × TE6317
TE6321	*putPA1303*::Kan^r-*rpoS-lac* [pr]/pTE570 (P_{ara})	
TE6322	*putPA1303*::Kan^r-*rpoS-lac* [pr]/pTE577 (P_{ara}-*hfq*)	
TE6323	*putPA1303*::Kan^r-*rpoS-lac* [pr] *hfq-1*::Mud-Cam/pTE570 (P_{ara})	
TE6324	*putPA1303*::Kan^r-*rpoS-lac* [pr] *hfq-1*::Mud-Cam/pTE577 (P_{ara}-*hfq*)	

[a] [op], operon (transcriptional) fusion; [pr], protein (translational, gene) fusion.
[b] Except as noted, all *S. typhimurium* strains are derived from LT2.

Fusions of DNA fragments derived from the *E. coli katE* and *rpoS* genes to *lac* were constructed as described below; these *lac* fusions were transferred to the chromosome of an *E. coli recD* mutant by linear transformation as described previously (14). Phage P22 transducing lysates were grown in *E. coli* (13, 14) and used to transduce the fusions into the *S. typhimurium* chromosome. Each resulting strain carries a *lac* fusion in a single copy as an insertion of a Kan^r-promoter-*lac* fragment in the *put* operon.

Tn*10d*-Tet and Tn*10d*-Cam are derivatives of the transposon Tn*10* that confer resistance to tetracycline and chloramphenicol, respectively. These elements lack transposase but can move if transposase is also provided. Their construction and use have been described previously (16, 65). The transposition-defective Mu derivative Mud-Cam has also been described previously (15).

The level of *lac* expression from the *katE-lac* [op] fusion in a single copy in an LT2 background is sufficient to give a weak Lac$^+$ phenotype on MacConkey lactose indicator plates (bull's-eye colonies with red centers and a white periphery at 24 h), whereas the same fusion gives a strong Lac$^+$ phenotype in ATCC 14028s and DB7000. To exchange the *rpoS* region between LT2 and ATCC 14028s, we first isolated a Tn*10d*-Tet insertion 50% linked to *rpoS* in LT2 and then performed transductions as detailed in Table 1 and in Results. In these crosses, transductants showing the Lac phenotype of the donor were presumed to have inherited the donor's *rpoS* allele, while those with the Lac phenotype of the recipient have retained the recipient's *rpoS* allele.

Media and growth conditions. Bacteria were grown at 37°C in LB medium (58) or in minimal MOPS (morpholinepropanesulfonic acid) medium (49) as modified (5), with 0.2% glucose as the carbon and energy source. Plates were prepared by using nutrient agar (Difco) with 5 g of NaCl per liter. Antibiotics were added to final concentrations as follows: 100 μg of sodium ampicillin per ml, 20 μg of chloramphenicol per ml, 50 μg of kanamycin sulfate per ml, and 20 μg of tetracycline hydrochloride per ml. MacConkey lactose agar was prepared as described previously (45). X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) was used at 50 μg/ml.

Construction of *lac* fusions. The system we used has been described previously (14). Fusions were made in the pRS series of plasmids constructed by Simons et al. (59). The *katE-lac* fusion was originally obtained from P. C. Loewen as pRSkatE16 (47). This plasmid contains a 1.4-kb *Sma*I-*Eco*RV fragment of the *E. coli katE* gene inserted into the *Sma*I site of pRS415, creating an operon fusion of *katE* to *lac* at codon 181 of the *katE* gene. The pRSkatE16 insert was excised by digestion with *Eco*RI and *Bam*HI and cloned into pRS551. This step added a Kan^r gene upstream of the *katE-lac* fusion, which is necessary for the chromosomal substitution system.

The *rpoS-lac* protein fusion was constructed by digesting pMMkatF2 (obtained from P. C. Loewen) (46) with *Cla*I and *Eag*I, filling in the ends with the Klenow fragment of DNA polymerase I, and cloning the 1.6-kb fragment into pRS552 that had been digested with *Eco*RI and *Bam*HI and filled in.

We constructed a new *lac* operon fusion vector, pTE583, derived from pRS552 by substituting a 2.2-kb *Bam*HI-*Sac*I fragment from pTL61T (39). This construction places an RNase III site upstream of the *lacZ* gene in the pRS backbone. The RNase III site should help to insulate *lac* expression from indirect effects on

messenger half-life (39), but we do not known how efficient this insulating effect is. To make the *rpoS-lac* operon fusion, the 1.6-kb *Cla*I-*Eag*I fragment of pMM katF2 was filled in and inserted into the *Sma*I site of pRS415, subsequently excised by digestion at the flanking *Eco*RI and *Bam*HI sites, and inserted into pTE583. The *lac* operon and protein fusions to *rpoS* are made at the identical *Eag*I site at codon 73 of the *rpoS* gene and extend to the *Cla*I site upstream of *nlpD*.

For complementation studies of *hfq*, we modified the pBAD18 plasmid of Guzman et al. (24) (a gift of T. Silhavy). First, the *Nde*I site of pBAD18 was removed by filling in and religating to give pTE570. Next, an unrelated gene (*arcA*) with a strong ribosome binding site from pTE553 (10) was inserted as a *Dra*I-*Kpn*I fragment between the *Nhe*I (filled in) and *Kpn*I sites of the vector. This resulted in pTE571, a plasmid that when digested with *Nde*I and an enzyme cutting in the multiple cloning site of pBAD18 will accept any gene having an *Nde*I site overlapping its ATG codon. The inserted gene will be expressed as the native protein under the control of the arabinose promoter.

The *hfq* gene was PCR amplified from *E. coli* W3110 by using the primers 5′ GGGAA TTCCA TATGG CTAAG GGGCA ATCTT TACAA G 3′ and 5′ CCCGG ATCCT TATTC GGTTT CTTCG CTGTC CTG 3′. The resulting fragment was digested with *Eco*RI and *Bam*HI, cloned into pK184 (30), and sequenced. The *hfq* fragment was subsequently excised by digestion with *Nde*I and *Hin*dIII and cloned into pTE571 to give the *hfq* expression plasmid, pTE577.

β-Galactosidase assays. Cells were centrifuged, resuspended in Z buffer (100 mM KPO_4 [pH 7.0], 10 mM KCl, 1 mM $MgSO_4$), and then permeabilized by treatment with sodium dodecyl sulfate (SDS) and chloroform (45). Assays were performed in Z buffer containing 50 mM β-mercaptoethanol by a kinetic method with a plate reader (Molecular Devices). Activities (change in optical density at 420 nm [ΔOD_{420}] per minute) are normalized to the actual cell density (OD_{650}) and were always compared with activities of appropriate controls assayed at the same time. For experiments employing cultures grown to different densities, the number of cells harvested was adjusted to provide approximately equal cell densities in the assay. The results shown are from a single experiment; each experiment was repeated several times with similar results.

DNA sequencing. Sequencing of double-stranded plasmid DNA was done as previously described (13) by using Sequenase version 2.0 (U.S. Biochemical Corp.) according to the manufacturer's instructions. Plasmid DNA for sequencing was isolated by column chromatography (Qiagen).

Two-milliliter overnight cultures grown in LB medium were used to prepare chromosomal DNA essentially as described previously (20) except that the DNA was recovered after ethanol precipitation by spooling on a microcapillary pipette and the RNase digestion was omitted. The *hfq*::Mud-Cam insertion was cloned from chromosomal DNA of strain TE5314 by digesting with *Hin*dIII and ligating to *Hin*dIII-digested pBR322. Transformants carrying the *hfq*::Mud-Cam insertion were selected as Cam^r colonies after electroporation of *E. coli* DH5α; the resulting plasmid is pTE512. Sequencing utilized the MuL and MuR primers described previously (15). The *hfq-1*::Mud-Cam insertion duplicates bp 935 to 939 of the *S. typhimurium* homolog of the sequence listed under accession no. U00005 (31); the product of this disrupted gene is predicted to be identical to HF-I for 68 amino acids and then to terminate after an additional 16 amino acids.

The *rpoS107I*::Tn*10d*-Cam insertion was cloned from chromosomal DNA of strain TE4521-4 that had been partially digested with *Sau*3A and ligated to *Bam*HI-digested pBR322. Sequencing utilized primers directed to the ends of the Cam^r determinant: 5′ GTTTC TATCA GCTGT CCCTC CTGTT C 3′ and 5′ GACGA TATGA TCATT TATTC TGCCT C 3′. The *rpoS107I*::Tn*10d*-Cam insertion duplicates by 509 to 517 of the sequence listed under accession no. U05011 (52); the product of this disrupted gene is predicted to be identical to RpoS for 53 amino acids and then to terminate immediately.

Immunological detection of proteins. For Western blots (immunoblots), cultures were grown overnight in LB medium, and the cells were centrifuged and resuspended in sample buffer (13). For a culture with an OD_{600} of 4.0, an equal volume of sample buffer was used; otherwise, volumes were adjusted to give the same cell density. Samples of 10 μl of total protein were separated by standard SDS-polyacrylamide gel electrophoresis (36) and transferred to a polyvinylidene difluoride membrane (Micron Separations) by semidry electroblotting (Bio-Rad) in buffer containing 25 mM Tris base, 192 mM glycine, 20% methanol, and 0.05% SDS (21, 63). Blots were blocked overnight at 4°C with 5% nonfat milk in phosphate-buffered saline (PBS) (10 mM $NaPO_4$ [pH 7.5], 140 mM NaCl, 0.1% NaN_3), rinsed with 0.1% Tween 20 in PBS, and incubated sequentially at room temperature with ascites fluid containing anti-RpoS, diluted 1:2,500 in 0.1% Tween 20 in PBS (monoclonal antibody 1RS1; a gift of L. H. Nguyen and R. R. Burgess [50]), with biotin-conjugated goat anti-mouse immunoglobulin G1 (diluted 1:2,500), and finally with streptavidin-conjugated horseradish peroxidase (diluted 1:5,000; both from Southern Biotechnology Associates). Detection was by enhanced chemiluminescence (Amersham).

Pulse-labeling and immunoprecipitation were carried out essentially as described previously (2). Labeling of LacZ and RpoS-LacZ used 25 μCi, and labeling of RpoS used 120 μCi, of [^{35}S]methionine-[^{35}S]cysteine (Tran^{35}S-label; ICN) (≈1,000 Ci/mmol) per 0.5 ml of culture grown in minimal MOPS medium with 0.2% glucose. LacZ and RpoS-LacZ were immunoprecipitated (29) by using 1 μg of a monoclonal antibody to β-galactosidase (Promega) and protein A–Sepharose CL-4B (Sigma) for precipitation of antigen-antibody complexes. RpoS was immunoprecipitated by the same method, using culture supernatants containing a specific monoclonal antibody. This antibody will be described elsewhere; however, no significant cross-reaction is observed by either Western blotting or immunoprecipitation with extracts of *rpoS* mutants. The amount of radioactivity in individual bands was determined with a PhosphorImager (Molecular Dynamics) with background correction by the histogram peak method.

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ATG GCT AAG GGG CAA TCT TTA CAA GAT CCG TTC CTG AAG CCA TTG CGT CGG GAA CGT GTT    60
 M   A   K   G   Q   S   L   Q   D   P   F   L   K   P   L   R   R   E   R   V    ST
                                             N   A                                EC

CCA GTT TCT ATT TAT TTG GTG AAT GGT ATT AAG CTG CAA GGT CAA ATC GAG TCC TTT GAT   120
 P   V   S   I   Y   L   V   N   G   I   K   L   Q   G   Q   I   E   S   F   D

CAG TTC GTG ATC CTG TTG AAG AAC ACG GTC AGC CAG ATG GTT TAT AAG CAC GCG ATT TCT   180
 Q   F   V   I   L   L   K   N   T   V   S   Q   M   V   Y   K   H   A   I   S
                     * *** *
ACT GTT GTC CCG TCT CGC CCG GTT TCC CAT CAC AGC AAC AAT GCC GGT GGC GGC GCC AGC   240
 T   V   V   P   S   R   P   V   S   H   H   S   N   N   A   G   G   G   A   S
                                                                         T
AAT AAC TAC CAT CAC GGT AGC AAC GCG CAG GGG TCT ACT GCG CAA CAG GAC AGC GAA GAG   300
 N   N   Y   H   H   G   S   N   A   Q   G   S   T   A   Q   Q   D   S   E   E
 S                           S           N   T   S

ACT GAA TAA                                                                     309
 T   E   *
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FIG. 1. DNA sequence of the *S. typhimurium hfq* gene. The sequence starts from the ATG initiation codon. Also shown are the deduced amino acid sequence of the product of the *hfq* gene, HF-I, and residues that differ in *E. coli* HF-I. The five nucleotides duplicated in the *hfq-1*::Mud-Cam insertion mutant are overlined with asterisks. The *S. typhimurium* (ST) and *E. coli* (EC) HF-I proteins are predicted to have 93% identity.

RESULTS

In previous work, we isolated a series of *S. typhimurium* insertion mutants defective in *rpoS* (the gene encoding the stationary-phase sigma factor) and one mutant defective in *hfq*, a gene encoding an RNA-binding protein of unknown function (1, 2). The *rpoS* and *hfq* mutants were identified by sequencing the sites of transposon insertions (as described in Materials and Methods); the DNA sequence of the *S. typhimurium hfq* gene and the deduced amino acid sequence of the *S. typhimurium* HF-I protein are shown in Fig. 1 and compared with the sequence of *E. coli* HF-I. In searching for common properties of these strains, we tested cultures that were grown to stationary phase in LB medium for the production of bubbles after challenge with H_2O_2. Both the *hfq* mutant (TE5314 *hfq-1*::Mud-Cam) and the *rpoS* mutants were defective by this test. This result suggested that the *hfq* mutant might have lower levels of catalase HPII (*katE*), whose induction during stationary phase requires RpoS (8, 41).

The *hfq* insertion mutant is defective in *katE* expression. To test whether the *hfq* mutant is defective in *katE* expression, we obtained a *lac* fusion to the *E. coli katE* gene (47) and transferred it to the chromosome of *S. typhimurium* as described in Materials and Methods. Assays of β-galactosidase in LB overnight cultures showed that an *hfq* mutant derivative of the wild-type strain LT2 expressed *katE-lac* at about 25% of the level seen in the parent (Fig. 2, bars a and b). Because the activity of the RpoS sigma factor is variable even among wild-type isolates of *E. coli* K-12 (40) and *S. typhimurium* LT2 (60), we tested *katE-lac* expression in two additional *S. typhimurium* strains: the mouse-virulent strain ATCC 14028s and strain DB7000 (genotype given in Table 1). In comparing these strains with LT2, expression of *katE-lac* varied over a threefold range, but in each strain background it was substantially dependent on *hfq* and nearly completely dependent on *rpoS* (Fig. 2). In each background, the fraction of activity remaining in the *hfq* mutant was about 25% of that in the parent strain. These results indicate that *hfq* function is required for a high level of *katE* gene expression. Residual expression of *katE-lac* was also similar in each background when an *rpoS* insertion mutation was present (about 3% of the parental level). This might indicate that the variation in *katE* expression among different strains is superimposed on *rpoS* regulation and independent of

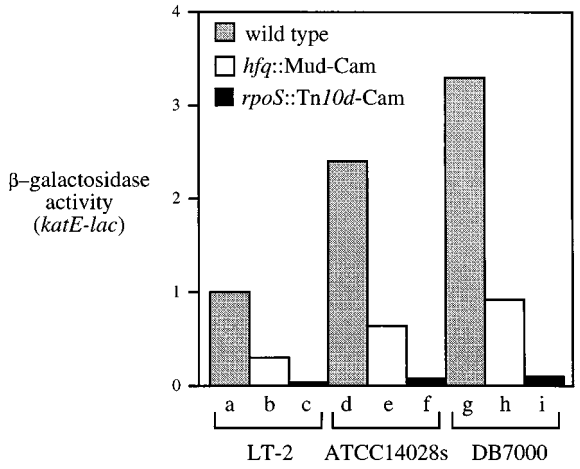

FIG. 2. Dependence of *katE-lac* expression on *hfq* and *rpoS*. A *katE-lac* operon fusion was placed in three different backgrounds (indicated at the bottom), and then an *hfq* or *rpoS* insertion mutation was introduced. β-Galactosidase activity was assayed in cultures grown overnight to stationary phase in LB medium, and results are presented in arbitrary units, normalized to the activity in a wild-type LT2 background. Strains: a, TE6153; b, TE6167; c, TE6168; d, TE6154; e, TE6170; f, TE6171; g, TE6193; h, TE6231; i, TE6232. See Table 1 for complete genotypes.

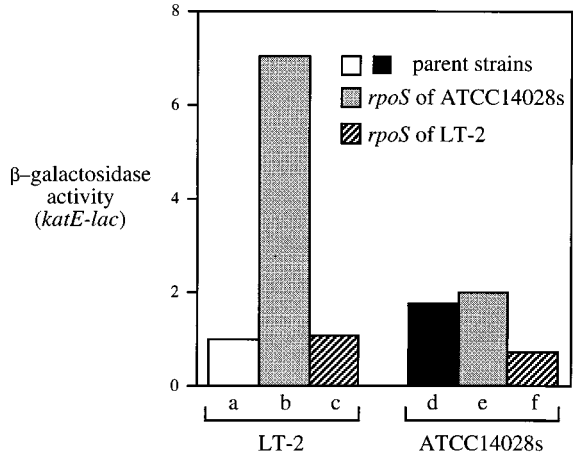

FIG. 3. Exchange of the *rpoS* region between LT2 and ATCC 14028s and its effect on *katE-lac* expression. A Tn*10d*-Tet insertion linked to *rpoS* was used to construct strains with either the LT2 or ATCC 14028s background (indicated at the bottom) in which the *rpoS* region was derived from either LT2 or ATCC 14028s. The parent strains do not carry the linked Tn*10d*-Tet insertion. All strains also carried a *katE-lac* fusion, and β-galactosidase activity was assayed as described for Fig. 2. Strains: a, TE6153; b, TE6227; c, TE6228; d, TE6154; e, TE6225; f, TE6226.

it. However, other results, presented below, show that expression of *katE-lac* in LT2 and ATCC 14028s is at least partly determined by differences in the *rpoS* region.

An *S. typhimurium* strain with a high level of RpoS activity. We investigated the strain dependence of RpoS activity, as measured with the *katE-lac* reporter, by using a transposon insertion linked to *rpoS* to exchange this region between LT2 and the virulent strain ATCC 14028s in phage P22-mediated transductional crosses (see Materials and Methods for details). Analysis of the resulting recombinant strains shows that there is a genetic difference in the *rpoS* region, likely reflecting differences in *rpoS* gene structure or regulatory elements, that determines the different levels of RpoS activity and *katE-lac* expression in LT2 and ATCC 14028s. Thus, LT2 with the *rpoS* allele derived from ATCC 14028s exhibits a higher level of *katE-lac* expression, while ATCC 14028s carrying the *rpoS* allele of LT2 shows reduced activity, compared with the activities of the respective parental strains (Fig. 3). However, expression with the ATCC 14028s *rpoS* allele is much higher in LT2 than in the ATCC 14028s background (Fig. 3, bar b). The recombinant strain TE6227 has about sevenfold higher RpoS activity, and this was confirmed by Western blots (see below). Multiple mechanisms that regulate RpoS expression and stability are already known from studies with *E. coli* (see the introduction) and *S. typhimurium* (60); we have not yet determined the genetic factors other than *rpoS* that are responsible for the differences between LT2 and ATCC 14028s. Significantly, we found that the *hfq* mutation reduced *katE-lac* expression in TE6227 by the same factor as for the strains shown in Fig. 2 (7); it was also found to reduce synthesis of RpoS (see below).

The defect of the *hfq* mutant is not due to polarity. The region directly downstream of *hfq* contains the genes of the *hfl* locus, previously described for *E. coli* (reviewed in reference 23). The *hflK* and *hflC* genes encode a protease active on the cII protein of phage λ, and hence, *hflK* and *hflC* mutants of *E. coli* show an increased frequency of lysogeny upon infection by phage λ. The *hflX* gene lies between *hfq* and *hflKC*, and *hflX* is cotranscribed with *hfq* in *E. coli* (64); *hflX* encodes a putative GTP-binding protein of unknown function (51). It was possible that the *hfq-1*::Mud-Cam insertion might affect *katE-lac* expression indirectly through polarity on *hflX*. We tested this by constructing a plasmid, pTE577, that expresses the *E. coli hfq* gene under the control of the *araBAD* promoter. In this plasmid (a derivative of pBAD18 [24]), *hfq* expression is inducible by arabinose. Cultures of an *S. typhimurium hfq* mutant with a *katE-lac* fusion and carrying either pTE577 or the parent vector were grown into stationary phase in the presence of different amounts of arabinose (Fig. 4A). Inducer-dependent complementation was observed, demonstrating that the defect in *katE-lac* expression of the *hfq* insertion mutant is corrected by a functional copy of *hfq* and is therefore not due to polarity on *hflX*. Even at high concentrations of inducer, the level of expression was comparable to that observed with hfq^+ in a single copy on the chromosome.

Subsequent experiments demonstrated that the effect of *hfq* on *katE* expression is a consequence of reduced levels of RpoS (see below). We used an *rpoS-lac* fusion to perform a similar complementation test by expressing *E. coli hfq* in an *rpoS-lac* [pr] *hfq*::Mud-Cam strain (Fig. 4B). Again, the effect of the *hfq* mutant was corrected by expression of *E. coli hfq* alone and is therefore not due to polarity.

The *hfq* mutant has reduced amounts of RpoS protein. To investigate whether reduced expression of the *katE-lac* reporter in the *hfq* mutant can be ascribed to lower levels of the RpoS sigma factor, we used a monoclonal antibody (1RS1 [50]), reactive with *E. coli* RpoS, to probe Western blots of total cell proteins (Fig. 5). A protein reactive with the monoclonal antibody against RpoS was present in $rpoS^+$ strains but not in otherwise isogenic *rpoS* mutant derivatives (for *E. coli*, compare lanes a and b of Fig. 5; for *S. typhimurium*, compare lanes c and e as well as lanes f and h). The abundance of RpoS in *S. typhimurium* correlated with RpoS activity as determined in the same strains by using the *katE-lac* reporter (compare lanes c and f of Fig. 5 with bars b and a of Fig. 3). RpoS is less abundant in the LT2 *hfq* mutant (compare lanes f and g of Fig. 5). This overexposed blot does not clearly show the decreased abundance of RpoS in the *hfq* mutant derivative of TE6227, although it can be discerned in the original film. However, other data, including *katE-lac* expression (Fig. 3) and pulse-

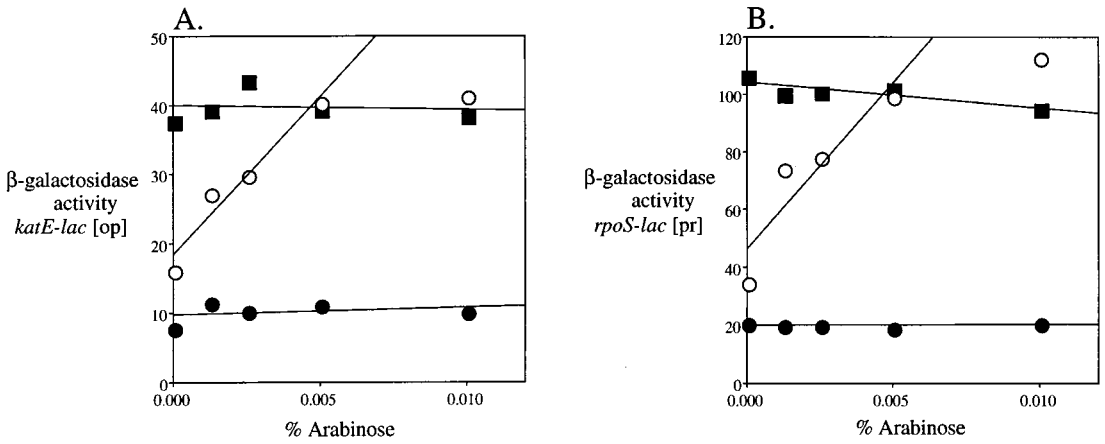

FIG. 4. Complementation test of the effect of *hfq* on *katE-lac* and *rpoS-lac* expression. Strains carried either a *katE-lac* operon fusion (A) or an *rpoS-lac* protein fusion (B). In each experiment, three strains were analyzed: an *hfq*::Mud-Cam insertion mutant carrying the P_{ara} vector (filled circles), an hfq^+ strain carrying the P_{ara} vector (filled squares), and an *hfq*::Mud-Cam insertion mutant carrying the P_{ara}-*hfq* expression plasmid (open circles). Cultures were grown overnight to stationary phase in LB medium containing ampicillin and the indicated amount of arabinose and then assayed for β-galactosidase. The slope was plotted by using only the first four datum points for the strain carrying the expression plasmid (open circles).

labeling of RpoS (see below), also show a defect for the *hfq* mutant in this strain background. We have found a reduction of RpoS synthesis in *hfq* mutant derivatives of all *S. typhimurium* strains examined.

Effect of the *hfq* mutant on expression of *rpoS-lac* fusions. We constructed *lac* protein and operon fusions to the *E. coli* *rpoS* gene and placed them in single copy in the *S. typhimurium* chromosome as described in Materials and Methods. The fusions contain a DNA segment starting at the *Cla*I site upstream of the *nlpD* gene and extending to the *Eag*I site at codon 73 of *rpoS*. The inserted fragment includes both the basal and the stationary-phase inducible promoter for *rpoS* (37, 61). Strains carrying an *rpoS-lac* protein fusion were assayed for β-galactosidase during growth into stationary phase in LB medium (Fig. 6). Expression of *rpoS-lac* was highly inducible in the wild type, but the level was substantially reduced in the *hfq* mutant, reaching only 30% of that in the hfq^+ parent at 24 h. In contrast, when a similar experiment was performed with strains carrying an *rpoS-lac* operon fusion, the *hfq* mutant had β-galactosidase levels almost as high as those in the hfq^+ strain: expression at 24 h was 85% of that in the parent. These results indicate that the lack of *hfq* function primarily affects translation and not transcription of *rpoS*. The small effect on transcription could be a secondary consequence of reduced translation (e.g., see reference 11).

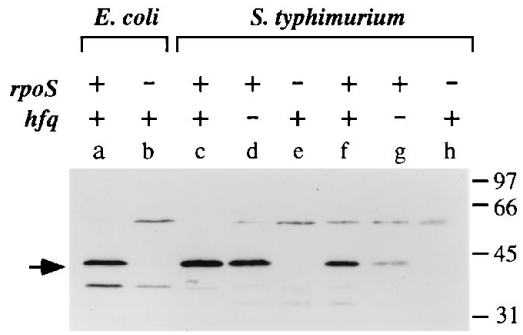

FIG. 5. Western blot analysis of RpoS levels. The arrow indicates RpoS. Cultures were grown overnight to stationary phase in LB medium. Strains: a, MG1655 (*E. coli* wild type); b, TE6256 (*E. coli* MG1655 *rpoS*::Tn*10*); c, TE6227 (*S. typhimurium* LT2 with *rpoS* of ATCC 14028s); d, TE6241 (as TE6227 but *hfq*::Mud-Cam); e, TE6248 (as TE6227 but *rpoS*::Tn*10d*-Cam); f, TE6153 (*S. typhimurium* LT2); g, TE6167 (as TE6153 but *hfq*::Mud-Cam); h, TE6168 (as TE6153 but *rpoS*::Tn*10d*-Cam). The positions of molecular mass markers (in kilodaltons) are indicated on the right.

Pulse-labeling and immunoprecipitation. We carried out pulse-labeling experiments to demonstrate directly that the effect of the *hfq* mutation is primarily on translation of *rpoS*. To do this, cells were labeled during exponential growth in minimal glucose medium at a low density. Under these conditions, assay of β-galactosidase shows a six- to sevenfold reduction in expression of an *rpoS-lac* [pr] fusion in the *hfq* mutant compared with that in the wild type (7).

In the first experiment, a monoclonal antibody to β-galactosidase was used to immunoprecipitate labeled proteins (2-min pulse) from the *rpoS-lac* protein and operon fusion strains described above (Fig. 7). The ratio of RpoS-LacZ expression from the protein fusion construct in *hfq* mutant and hfq^+ strains was computed and was compared with a similar ratio of LacZ expression from the operon fusion in mutant and wild-type strains (Table 2). A comparison of the relative effects on protein and operon fusions eliminated potential confounding effects of the pleiotropic *hfq* mutation, such as the slower growth rate of *hfq* mutants (80-min doubling time compared with 49 min for the wild type), and possible effects of *hfq* on transcriptional control. The results clearly show a differential effect on the protein fusion and agree reasonably well with measurements of β-galactosidase activity. These pulse-labeling results reflect only rates of synthesis rather than stability of RpoS-LacZ: a pulse-chase protocol demonstrated that the hybrid protein was stable in both *hfq* mutant and wild-type cells (>90% remaining in a 12-min chase) (7).

Although we did not succeed in using the 1RS1 monoclonal antibody to immunoprecipitate RpoS, we have recently isolated other monoclonal antibodies that are active both on Western blots and in immunoprecipitation by our standard protocol (12a, 29). Figure 8 shows the results of pulse-labeling (1 min) and immunoprecipitation with one antibody (R12) in the hfq^+ strain TE6227 (duplicate cultures analyzed in lanes a and b) and its *hfq* mutant derivative (lanes c and d). Quantitation of the radioactivity in these bands, corrected for the total incorporation into protein, reveals that the rate of synthesis of RpoS in the *hfq* mutant is about 20% of that in the wild type.

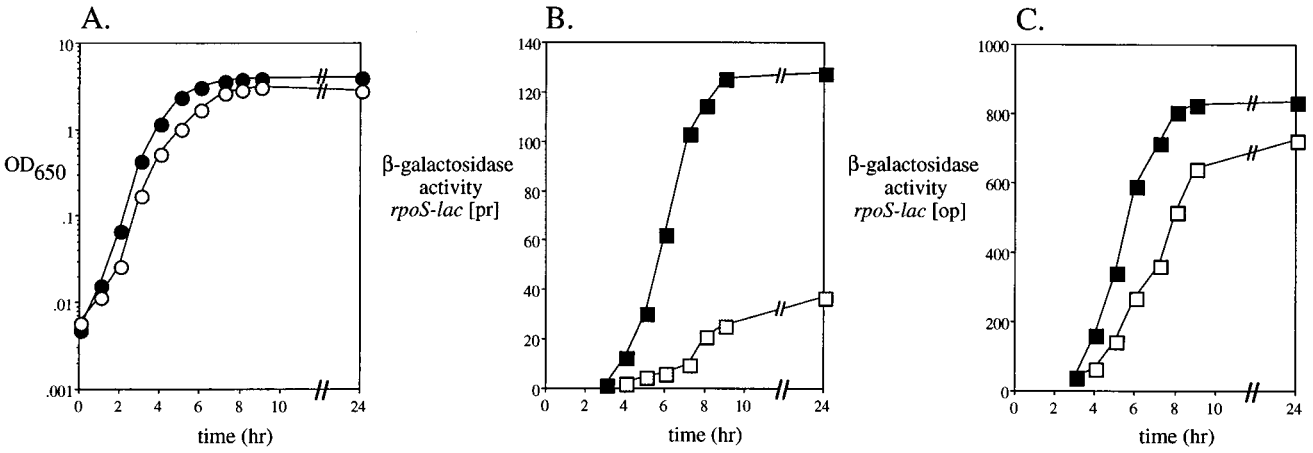

FIG. 6. Effect of the *hfq*::Mud-Cam insertion on *rpoS-lac* expression. (A) Growth curves (OD_{650}). Closed circles, TE6253 *hfq*$^+$; open circles, TE6266 *hfq-1*::Mud-Cam. Cultures were grown in LB medium, and samples were assayed for β-galactosidase activity. (B) *rpoS-lac* protein fusion. Closed squares, TE6253 *hfq*$^+$; open squares, TE6266 *hfq-1*::Mud-Cam. (C) *rpoS-lac* operon fusion. Closed squares, TE6317 *hfq*$^+$; open squares, TE6318 *hfq-1*::Mud-Cam.

DISCUSSION

The experiments reported here demonstrate that a functional *hfq* gene is required for efficient translation of the stationary-phase sigma factor RpoS in *S. typhimurium*. Residual expression in an *hfq* mutant varies depending on the medium and stage of growth but is four- to sevenfold lower than in a wild-type strain. Two previous reports have documented a role for *hfq* homologs in enhancing gene expression: for the nitrogen-regulatory protein *nifA* in *Azorhizobium caulinodans* (33) and for *yst*, encoding the heat-stable enterotoxin in *Yersinia enterocolitica* (48). Neither in this work nor in the previous studies has it been determined whether the effect of *hfq* is direct; however, it would not be surprising to find that an *rpoS* homolog mediates the *hfq* effect on either *nifA* or *yst* expression.

The *E. coli hfq* gene product, HF-I, was discovered as a protein required for in vitro replication of the RNA bacteriophage Qβ (18, 19, 31). Like ribosomal protein S1, HF-I is specifically required for copying a Qβ plus-strand template, whereas neither S1 nor HF-I is required with a Qβ minus-strand template (19, 32a). Unlike S1, HF-I does not copurify with the Qβ replicase. The HF-I protein is a hexamer (19) or pentamer (32) of 11-kDa subunits, and it is capable of binding specifically to a small number of sites on Qβ and R17 phage RNAs (3, 56). Three HF-I binding sites on Qβ RNA have been characterized as being rich in A residues, and importantly, HF-I binds tightly to isolated oligonucleotides containing these sites and lacking significant secondary structure (56). HF-I also binds to poly(A) (9). No significant homology was found between the sequenced HF-I binding sites in Qβ RNA and the first 70 codons of *rpoS* (7), a region suggested to contain important regulatory sequences (38). It has been suggested that HF-I might serve during the initiation of replication to bring the 3′ end of the template RNA to a replicase complex bound at two internal sites (3). Replicase binding to internal sites could help to ensure that replication and translation do not occur on the same RNA molecule simultaneously. The gene for HF-I was described only recently, and it is not known whether *hfq* function is required for phage Qβ infection in vivo. It is possible that there is more than one protein of this type, and if so, the requirement for HF-I-like proteins may actually be more strict than is apparent from the defect observed in the *hfq* mutant. The insertion mutant we used contains a Mud transposon at codon 68 (of a total of 102); it is also possible that this mutant retains partial function.

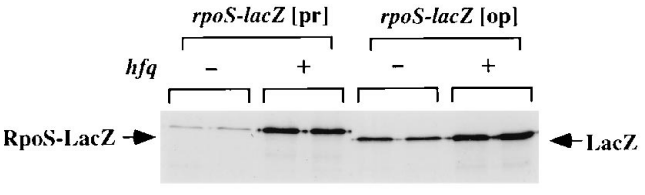

FIG. 7. Pulse-labeling and immunoprecipitation of β-galactosidase from strains carrying *rpoS-lac* fusions. Each strain was pulse-labeled in duplicate, and the samples were analyzed in parallel. Cultures were grown in minimal MOPS medium with 0.2% glucose to an OD_{600} of 0.4, pulse-labeled for 2 min, and immunoprecipitated with anti-β-galactosidase antibody. Strains were TE6266 (LT2 *rpoS-lac* [pr] *hfq-1*::Mud-Cam), TE6253 (LT2 *rpoS-lac* [pr]), TE6318 (LT2 *rpoS-lac* [op] *hfq-1*::Mud-Cam), and TE6317 (LT2 *rpoS-lac* [op]). The protein fusions substitute 73 amino acids from *rpoS*, plus the linker, for 9 amino acids of *lacZ*. One-tenth of the amount of immunoprecipitated protein was analyzed from the operon fusion strains.

The function of HF-I in uninfected cells has not been defined, but it is associated with ribosomes (9, 32), and *hfq* mutants are pleiotropic (64). Several facts have suggested that HF-I and the DNA-binding protein H-NS might have related functions. HF-I and H-NS were observed to copurify through several steps; however, purified H-NS cannot substitute for HF-I in Qβ replication (31). Phenotypes reported for an *E. coli hfq* mutant included osmosensitivity, SOS-independent cell elongation, and altered plasmid supercoiling; similar defects are also seen with mutants that lack abundant DNA-binding proteins, including H-NS (64). A mutation in *hfq* suppressed (reduced) activation of the cryptic *bgl* operon in an *hns* mutant (64); both effects could be explained if *bgl* activation requires RpoS. Finally, multicopy plasmids bearing *hfq* or the *hns*-like gene *stpA* complement some defects of *hns* mutants (57).

We favor a model in which the translational efficiency of *rpoS* mRNA depends on HF-I; an alternative possibility would be a direct effect of HF-I on message stability. Low translational efficiency could be due, in part, to the extensive secondary structure postulated to occur in this RNA (38). Since HF-I is reported to bind tightly to unstructured Qβ RNA (56), it may not act simply to unzip duplex RNA and allow access to the ribosome. Alternatively, HF-I may bind only to particular duplex RNA structures like one found in *rpoS*; S1 protein exhibits this type of dual specificity for structured and unstruc-

TABLE 2. Quantitation of *hfq* effect on *rpoS-lac* protein and operon fusions

Genotype of labeled strain	Radioactivity in LacZ or RpoS-LacZ[a] (avg)	Ratio of radioactivity (*hfq*/*hfq*$^+$)
rpoS-lac [pr] *hfq*::Mud-Cam	127, 147 (137)	0.13
rpoS-lac [pr] *hfq*$^+$	1,070, 1,012 (1,041)	
rpoS-lac [op] *hfq*::Mud-Cam	5,560, 4,710 (5,140)	0.4
rpoS-lac [op] *hfq*$^+$	11,730, 13,960 (12,850)	

[a] Arbitrary units determined by use of the PhosphorImager (Molecular Dynamics). Values for operon fusion strains have been adjusted to reflect the fact that only 1/10 of the sample was analyzed for Fig. 7.

tured RNA targets (52a). We can also speculate on indirect models. Perhaps HF-I facilitates the synthesis or activity of an RNA helicase which then works on *rpoS* RNA, for example, one of the family of DEAD proteins found in *E. coli* and, presumably, in *S. typhimurium* (53a). The partners of HF-I contributed by *E. coli* for its only characterized function, in Qβ replication, are ET-Tu, EF-Ts, and S1. Perhaps HF-I simply facilitates the unwinding activity of S1, or HF-I might counteract a negative influence of a protein that binds to *rpoS* mRNA (e.g., EF-Tu or H-NS).

Another important question is the role of HF-I in regulation of RpoS. In this work, we measured the rate of synthesis of RpoS during exponential growth in minimal glucose medium, a condition permitting only very low-level expression of *rpoS*, and found that synthesis was reduced in the *hfq* mutant by four- to fivefold. Assays of β-galactosidase expression from a *katE-lac* fusion suggested a deficit of about sixfold in RpoS activity caused by the *hfq* mutation in the same strain background and under the same growth conditions. These reductions are similar to, and even slightly more pronounced than, the decrease in RpoS activity reported by *katE-lac* at stationary phase in LB medium. These results suggested that HF-I might function not as a regulatory element but as a factor that is continuously required for stimulation of translation. However, preliminary experiments also show that *hfq* mutants are severely defective in induction of *rpoS-lac* expression upon osmotic challenge. Thus, HF-I may both stimulate basal expression and mediate osmotic regulation of translation. There is, in addition, a dramatic stabilization of the RpoS polypeptide after osmotic challenge (45a) which is unlikely to require HF-I. Further work should clarify the role of HF-I in RpoS regulation.

Finally, we constructed an *S. typhimurium* strain (TE6227) with high-level expression of RpoS due to a favorable combination of background (LT2) and *rpoS* allele (from ATCC 14028s). This strain was particularly useful for pulse-labeling studies. We noticed that the high-level expression of *rpoS* in this strain causes unusual phenotypes, including a striking deficit of porins (22) and more than a fourfold reduction in both aerobic oxidases as assayed by *cyo-lac* and *cyd-lac* expression (7). For both the porin- and cytochrome-related phenotypes, a mutation in *hfq* was observed to cause a partial reversal of the phenotype to one of a strain expressing moderate levels of RpoS. We postulate that particularly high levels of RpoS lead to competition between RpoS and σ^{70} for binding to RNA polymerase core, resulting in defective expression of some σ^{70}-dependent genes. This might also explain why we were unable to transfer the *rpoS* allele of ATCC 14028s into the DB7000 background (7), since this combination would be predicted to yield still higher levels of RpoS.

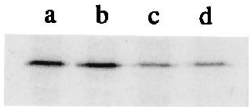

FIG. 8. Pulse-labeling and immunoprecipitation of RpoS. Each strain was pulse-labeled in duplicate, and the samples were analyzed in parallel. Cultures were grown in minimal MOPS medium with 0.2% glucose to an OD_{600} of 0.3, pulse-labeled for 1 min, and immunoprecipitated with a monoclonal anti-RpoS antibody. Strains were TE6227 *hfq*$^+$ (lanes a and b) and TE6241 *hfq-1*::Mud-Cam (lanes c and d).

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REFERENCES

1. **Archer, C. D., J. Jin, and T. Elliott.** 1996. Stabilization of a HemA-LacZ hybrid protein against proteolysis during carbon starvation in *atp* mutants of *Salmonella typhimurium*. J. Bacteriol. **178:**2462–2464.
2. **Archer, C. D., X. Wang, and T. Elliott.** 1993. Mutants defective in the energy-conserving NADH dehydrogenase of *Salmonella typhimurium* identified by a decrease in energy-dependent proteolysis after carbon starvation. Proc. Natl. Acad. Sci. USA **90:**9877–9881.
3. **Barrera, I., D. Schuppli, J. M. Sogo, and H. Weber.** 1993. Different mechanisms of recognition of bacteriophage Qβ plus and minus strand RNAs by Qβ replicase. J. Mol. Biol. **232:**512–521.
4. **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.
5. **Bochner, B. R., and B. N. Ames.** 1982. Complete analysis of cellular nucleotides by two-dimensional thin layer chromatography. J. Biol. Chem. **257:**9759–9769.
6. **Bohringer, J., D. Fischer, G. Mosler, and R. Hengge-Aronis.** 1995. UDP-glucose is a potential intracellular signal molecule in the control of expression of σ^S and σ^S-dependent genes in *Escherichia coli*. J. Bacteriol. **177:**413–422.
7. **Brown, L., and T. Elliott.** Unpublished data.
8. **Buchmeier, N. A., S. J. Libby, Y. Xu, P. C. Loewen, J. Switala, D. G. Guiney, and F. C. Fang.** 1995. DNA repair is more important than catalase for *Salmonella* virulence in mice. J. Clin. Invest. **95:**1047–1053.
9. **Carmichael, G. G., K. Weber, A. Niveleau, and A. J. Wahba.** 1975. The host factor required for RNA phage Qβ replication *in vitro*. Intracellular location, quantitation, and purification by polyadenylate-cellulose chromatography. J. Biol. Chem. **250:**3607–3612.
10. **Choi, P., L. Wang, C. D. Archer, and T. Elliott.** 1996. Transcription of the glutamyl-tRNA reductase (*hemA*) gene in *Salmonella typhimurium* and *Escherichia coli*: role of the *hemA* P1 promoter and the *arcA* gene product. J. Bacteriol. **178:**638–646.
11. **Cole, J. R., and M. Nomura.** 1986. Changes in the half-life of ribosomal protein messenger RNA caused by translational repression. J. Mol. Biol. **188:**383–392.
12. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

12a. **Elliott, M., and T. Elliott.** Unpublished data.

13. **Elliott, T.** 1989. Cloning, genetic characterization, and nucleotide sequence of the *hemA-prfA* operon of *Salmonella typhimurium*. J. Bacteriol. **171:**3948–3960.
14. **Elliott, T.** 1992. A method for constructing single-copy *lac* fusions in *Salmonella typhimurium* and its application to the *hemA-prfA* operon. J. Bacteriol. **174:**245–253.
15. **Elliott, T.** 1993. Transport of 5-aminolevulinic acid by the dipeptide permease in *Salmonella typhimurium*. J. Bacteriol. **175:**325–331.
16. **Elliott, T., and J. R. Roth.** 1988. Characterization of Tn*10d*-Cam: a transposition-defective Tn*10* specifying chloramphenicol resistance. Mol. Gen. Genet. **213:**332–338.
17. **Fang, F. C., S. J. Libby, N. A. Buchmeier, P. C. Loewen, J. Switala, J. Harwood, and D. G. Guiney.** 1992. The alternative σ factor KatF (RpoS) regulates *Salmonella* virulence. Proc. Natl. Acad. Sci. USA **89:**11978–11982.
18. **Franze de Fernandez, M. T., L. Eoyang, and J. T. August.** 1968. Factor fraction required for the synthesis of bacteriophage Qβ RNA. Nature (Lon-

don) **219:**588–590.
19. **Franze de Fernandez, M. T., W. S. Hayward, and J. T. August.** 1972. Bacterial proteins required for replication of phage Qβ ribonucleic acid. Purification and properties of Host Factor I, a ribonucleic acid-binding protein. J. Biol. Chem. **247:**824–831.
20. **Gay, N. J.** 1984. Construction and characterization of an *Escherichia coli* strain with a *uncI* mutation. J. Bacteriol. **158:**820–825.
21. **Gentry, D. R., V. J. Hernandez, L. H. Nguyen, D. B. Jensen, and M. Cashel.** 1993. Synthesis of the stationary-phase sigma factor σ^S is positively regulated by ppGpp. J. Bacteriol. **175:**7982–7989.
22. **Gibson, M. M., E. M. Ellis, K. A. Graeme-Cook, and C. F. Higgins.** 1987. OmpR and EnvZ are pleiotropic regulatory proteins: positive regulation of the tripeptide permease (*tppB*) of *Salmonella typhimurium*. Mol. Gen. Genet. **207:**120–129.
23. **Gottesman, S.** 1989. Genetics of proteolysis in *Escherichia coli*. Annu. Rev. Genet. **23:**163–189.
24. **Guzman, L.-M., D. Belin, M. J. Carson, and J. Beckwith.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J. Bacteriol. **177:**4121–4130.
25. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
26. **Hengge-Aronis, R.** 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*. Cell **72:**165–168.
27. **Hengge-Aronis, R., R. Lange, N. Henneberg, and D. Fischer.** 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. J. Bacteriol. **175:**259–265.
28. **Huisman, G. W., and R. Kolter.** 1994. Sensing starvation: a homoserine lactone-dependent signaling pathway in *Escherichia coli*. Science **265:**537–539.
29. **Ito, K. P. J. Bassford, and J. Beckwith.** 1981. Protein localization in *E. coli*: is there a common step in the secretion of periplasmic and outer membrane proteins? Cell **24:**707–717.
30. **Jobling, M. G., and R. K. Holmes.** 1990. Construction of vectors with the p15a replicon, kanamycin resistance, inducible *lacZ*α and pUC18 or pUC19 multiple cloning sites. Nucleic Acids Res. **18:**5315–5316.
31. **Kajitani, M., and A. Ishihama.** 1991. Identification and sequence determination of the host factor gene for bacteriophage Qβ. Nucleic Acids Res. **19:**1063–1066.
32. **Kajitani, M., A. Kato, A. Wada, Y. Inokuchi, and A. Ishihama.** 1994. Regulation of the *Escherichia coli hfq* gene encoding the host factor for phage Qβ. J. Bacteriol. **176:**531–534.
32a. **Kamen, R., M. Kondo, W. Romer, and C. Weissmann.** 1972. Reconstitution of Qβ replicase lacking subunit α with protein-synthesis interference factor i. Eur. J. Biochem. **31:**44–51.
33. **Kaminski, P. A., N. Desnouses, and C. Elmerich.** 1994. The expression of *nifA* in *Azorhizobium caulinodans* requires a gene product homologous to *Escherichia coli* HF-1, an RNA-binding protein involved in the replication of phage Qβ RNA. Proc. Natl. Acad. Sci. USA **91:**4663–4667.
34. **Koch, A. L.** 1971. The adaptive responses of *Escherichia coli* to a feast and famine existence. Adv. Microb. Physiol. **6:**147–217.
35. **Kolter, R., D. A. Siegele, and A. Tormo.** 1993. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47:**855–874.
36. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
37. **Lange, R., D. Fischer, and R. Hengge-Aronis.** 1995. Identification of the transcriptional start sites and the role of ppGpp in the expression of *rpoS*, the structural gene for the σ^S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **177:**4676–4680.
38. **Lange, R., and R. Hengge-Aronis.** 1994. The cellular concentration of the σ^S subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation and protein stability. Genes Dev. **8:**1600–1612.
39. **Linn, T., and R. St. Pierre.** 1990. Improved vector system for constructing transcriptional fusions that ensures independent translation of *lacZ*. J. Bacteriol. **172:**1077–1084.
40. **Loewen, P. C., and R. Hengge-Aronis.** 1994. The role of the sigma factor σ^S (KatF) in bacterial global regulation. Annu. Rev. Microbiol. **48:**53–80.
41. **Loewen, P. C., J. Switala, and B. L. Triggs-Raine.** 1985. Catalases HPI and HPII are induced independently. Arch. Biochem. Biophys. **243:**144–149.
42. **Loewen, P. C., I. von Ossowski, J. Switala, and M. R. Mulvey.** 1993. KatF (σ^S) synthesis in *Escherichia coli* is subject to posttranscriptional regulation. J. Bacteriol. **175:**2150–2153.
43. **Matin, A.** 1991. The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*. Mol. Microbiol. **5:**3–10.
44. **McCann, M. P., C. D. Fraley, and A. Matin.** 1993. The putative σ factor KatF is regulated posttranscriptionally during carbon starvation. J. Bacteriol. **175:**2143–2149.
45. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45a. **Muffler, A., D. D. Traulsen, R. Lange, and R. Hengge-Aronis.** 1996. Posttranscriptional osmotic regulation of the σ^S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **178:**1607–1613.
46. **Mulvey, M. R., and P. C. Loewen.** 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ transcription factor. Nucleic Acids Res. **17:**9979–9991.
47. **Mulvey, M. R., J. Switala, A. Borys, and P. C. Loewen.** 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. **172:**6713–6720.
48. **Nakao, H., H. Watanabe, S.-I. Nakayama, and T. Takeda.** 1995. *yst* gene expression in *Yersinia enterocolitica* is positively regulated by a chromosomal region that is highly homologous to *Escherichia coli* host factor 1 gene (*hfq*). Mol. Microbiol. **18:**859–865.
49. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
50. **Nguyen, L. H., D. B. Jensen, N. E. Thompson, D. R. Gentry, and R. R. Burgess.** 1993. *In vitro* functional characterization of overproduced *Escherichia coli katF/rpoS* gene product. Biochemistry **32:**11112–11117.
51. **Noble, J. A., M. A. Innis, E. V. Koonin, K. E. Rudd, F. Banuett, and I. Herskowitz.** 1993. The *Escherichia coli hflA* locus encodes a putative GTP-binding protein and two membrane proteins, one of which contains a protease-like domain. Proc. Natl. Acad. Sci. USA **90:**10866–10870.
52. **Prince, R. W., Y. Xu, S. J. Libby, and F. C. Fang.** 1994. Cloning and sequencing of the gene encoding the RpoS (KatF) sigma factor from Salmonella typhimurium 14028s. Biochim. Biophys. Acta **1219:**198–200.
52a. **Ringquist, S., T. Jones, E. E. Snyder, T. Gibson, I. Boni, and L. Gold.** 1995. High-affinity RNA ligands to *Escherichia coli* ribosomes and ribosomal protein S1: comparison of natural and unnatural binding sites. Biochemistry **34:**3640–3648.
53. **Schellhorn, H. E., and V. L. Stones.** 1992. Regulation of *katF* and *katE* in *Escherichia coli* K-12 by weak acids. J. Bacteriol. **174:**4769–4776.
53a. **Schmid, S. R., and P. Linder.** 1992. D-E-A-D protein family of putative RNA helicases. Mol. Microbiol. **6:**283–292.
54. **Schmieger, H.** 1972. Phage P22 mutants with increased or decreased transductional abilities. Mol. Gen. Genet. **119:**75–88.
55. **Schweder, T., K.-H. Lee, O. Lomovskaya, and A. Matin.** 1996. Regulation of *Escherichia coli* starvation sigma factor (σ^S) by ClpXP protease. J. Bacteriol. **178:**470–476.
56. **Senear, A. W., and J. A. Steitz.** 1976. Site-specific interaction of Qβ host factor and ribosomal protein S1 with Qβ and R17 bacteriophage RNAs. J. Biol. Chem. **251:**1902–1912.
57. **Shi, X., and G. N. Bennett.** 1994. Plasmids bearing *hfq* and the *hns*-like gene *stpA* complement *hns* mutants in modulating arginine decarboxylase gene expression in *Escherichia coli*. J. Bacteriol. **176:**6769–6775.
58. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
59. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:**85–96.
60. **Swords, E., and W. Benjamin.** Personal communication.
61. **Takayanagi, Y., K. Tanaka, and H. Takahashi.** 1994. Structure of the 5′ upstream region and the regulation of the *rpoS* gene of *Escherichia coli*. Mol. Gen. Genet. **243:**525–531.
62. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal σ factor in *Escherichia coli*: the *rpoS* gene product, σ^{38}, is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**3511–3515.
63. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
64. **Tsui, H.-C. T., H.-C. E. Leung, and M. E. Winkler.** 1994. Characterization of broadly pleiotropic phenotypes caused by an *hfq* insertion mutation in *Escherichia coli* K-12. Mol. Microbiol. **13:**35–49.
65. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.
66. **Yamashino, T., C. Ueguchi, and T. Mizuno.** 1995. Quantitative control of the stationary phase-specific sigma factor, σ^S, in *Escherichia coli*: involvement of the nucleoid protein H-NS. EMBO J. **14:**594–602.
67. **Yura, T., H. Nagai, and H. Mori.** 1993. Regulation of the heat-shock response in bacteria. Annu. Rev. Microbiol. **47:**321–350.