The RNA-binding protein HF-I, known as a host factor for phage Qβ RNA replication, is essential for *rpoS* translation in *Escherichia coli*

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The *rpoS*-encoded σ^S subunit of RNA polymerase in *Escherichia coli* is a global regulatory factor involved in several stress responses. Mainly because of increased *rpoS* translation and stabilization of σ^S, which in nonstressed cells is a highly unstable protein, the cellular σ^S content increases during entry into stationary phase and in response to hyperosmolarity. Here, we identify the *hfq*-encoded RNA-binding protein HF-I, which has been known previously only as a host factor for the replication of phage Qβ RNA, as an essential factor for *rpoS* translation. An *hfq* null mutant exhibits strongly reduced σ^S levels under all conditions tested and is deficient for growth phase-related and osmotic induction of σ^S. Using a combination of gene fusion analysis and pulse–chase experiments, we demonstrate that the *hfq* mutant is specifically impaired in *rpoS* translation. We also present evidence that the H-NS protein, which has been shown to affect *rpoS* translation, acts in the same regulatory pathway as HF-I at a position upstream of HF-I or in conjunction with HF-I. In addition, we show that expression and heat induction of the heat shock σ factor σ^{32} (encoded by *rpoH*) is not dependent on HF-I, although *rpoH* and *rpoS* are both subject to translational regulation probably mediated by changes in mRNA secondary structure. HF-I is the first factor known to be specifically involved in *rpoS* translation, and this role is the first cellular function to be identified for this abundant ribosome-associated RNA-binding protein in *E. coli*.

[*Key Words*: σ^S; σ factor; stationary phase; osmotic regulation; mRNA secondary structure]

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The σ^S subunit of RNA polymerase in *Escherichia coli*, which is encoded by the *rpoS* gene, is a key regulator for the enhanced expression of many genes during entry into stationary phase or in response to increased medium osmolarity. At present, >40 genes are known to belong to the σ^S regulon. Many of the corresponding gene products play a role in long-term starvation and stress adaptation and survival (Hengge-Aronis 1993; Loewen and Hengge-Aronis 1994).

The cellular concentration of σ^S itself increases >10-fold during transition into stationary phase or upon osmotic upshift (Gentry et al. 1993; Tanaka et al. 1993; Lange and Hengge-Aronis 1994a; Muffler et al. 1996a,b). Several studies have shown that post-transcriptional mechanisms are of primary importance in the control of the cellular σ^S level (Loewen et al. 1993; McCann et al. 1993; Lange and Hengge-Aronis 1994a). *rpoS* translation is already stimulated during the late exponential phase, that is, under conditions where nutrients are still present and the growth rate is not yet reduced. This may be attributable to a cell density-dependent mechanism (Lange and Hengge-Aronis 1994a). Moreover, osmotic upshift also results in increased *rpoS* translation (Muffler et al. 1996b). In addition, σ^S is a highly unstable protein in exponentially growing nonstressed cells (with a half-life between 1.4 and 3 min). This rapid turnover is inhibited in response to starvation and high osmolarity (Lange and Hengge-Aronis 1994a; Takayanagi et al. 1994; Muffler et al. 1996b).

The molecular mechanisms underlying this complex regulation are not yet understood. Recently however, a few components involved in the post-transcriptional regulation of σ^S have been identified. One of these factors is the histone-like protein H-NS, which despite its DNA-binding properties is involved in the post-transcriptional regulation of σ^S. *hns* mutants exhibit increased *rpoS* translation and reduced turnover of σ^S during exponential phase and no longer show growth phase-associated or osmotic induction of σ^S. However, the mechanism by which H-NS inhibits σ^S expression has not been characterized further (Barth et al. 1995; Yamashino et al. 1995). Two factors have been found to be essential for σ^S turnover. One is the Clp protease, with the subunits ClpP

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and ClpX (Schweder et al. 1996), the other is a two-component-type response regulator, RssB, with a unique carboxy-terminal output domain of not yet defined molecular function (Muffler et al. 1996a).

In this study we identify a component that, in contrast to RssB and ClpXP, is essential for *rpoS* translation. This factor is the HF-I protein (encoded by the *hfq* gene), which has been known as a host factor for phage Qβ RNA replication, but whose function for the *E. coli* cell has remained elusive since it was first described in 1968 (Franze de Fernandez et al. 1968). HF-I is part of the Qβ replicase (Kamen 1970; Kondo et al. 1970) and is required for the synthesis of the minus strand from the original viral RNA (Franze de Fernandez et al. 1972; Barrera et al. 1993). A recently isolated *hfq* mutant exhibits a pleiotropic phenotype, indicating that HF-I plays an important role in the physiology of an *E. coli* cell, but none of the phenotypes observed provided a clear hint of its molecular function (Tsui et al. 1994). The role of HF-I in σ^S expression reported here is therefore the first known cellular function of HF-I, and in view of the role of σ^S as a key global regulator, provides a direct explanation for the pleiotropic phenotype of *hfq* mutants.

Results

Cellular σ^S levels are reduced in the HF-I-deficient hfq1::Ω *mutant*

Some of the phenotypes of an *hfq* null mutant, such as an increased osmosensitivity and elongated cell shape during the exponential and stationary phases (Tsui et al. 1994), would be consistent with a reduced expression of the σ^S subunit of RNA polymerase because similar phenotypes have also been observed with *rpoS* mutants (Lange and Hengge-Aronis 1991a; McCann et al. 1991). We wanted to test whether HF-I is involved in the regulation of *rpoS*. For this study, we used previously described insertions in *hfq* of an Ω(Kan) cassette that carries transcriptional terminators on both ends (Tsui et al. 1994). While the *hfq1*::Ω mutation is a disruption approximately in the middle of the *hfq* gene, the insertion of the same Ω cassette close to the 3′ end of *hfq* (*hfq2*::Ω) does not produce the pleiotropic phenotype even though

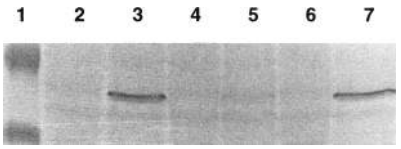

Figure 1. The *hfq1*::Ω mutant has strongly reduced σ^S levels during entry into stationary phase. Strains MC4100 (*hfq*$^+$; lanes *2–4*), AM111 (*hfq1*::Ω; lanes *5–7*), and RH90 (*rpoS*::Tn*10*; lane *8*) were grown in M9 medium with 0.1% glucose. Samples were taken at OD_{578} of 0.35 (lane *2*), 0.68 (lane *3*), 1.35 (i.e., 1 hr after the onset of starvation; lane *4*), 0.32 (lane *5*), 0.75 (lane *6*), 0.95 (1 hr after the onset of starvation; lane *7*) and 0.48 (lane *8*) and subject to immunoblot analysis with antibodies against σ^S. Size standard proteins (49 and 32 kD) are shown in lane *1*.

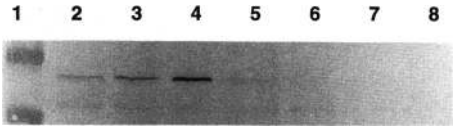

Figure 2. In the *hfq1*::Ω mutant the cellular σ^S content does not increase in response to osmotic upshift. Strains MC4100 (*hfq*$^+$; lanes *2,3*), AM111 (*hfq1*::Ω; lanes *4,5*), and AM112 (*hfq2*::Ω; lanes *6,7*) were grown in M9 with 0.4% glycerol. At an OD_{578} of 0.3, the cultures were divided and 0.3 M NaCl was added to one of the aliquots. Thirty minutes after the addition of NaCl, samples obtained from NaCl-free (lanes *2,4,6*) and NaCl-containing (lanes *3,5,7*) cultures were subject to immunoblot analysis with antibodies against σ^S. Size standard proteins (49 and 32 kD) are shown in lane *1*.

complete polarity on the downstream gene *hflX* was demonstrated. Therefore, a loss of HF-I itself and not polarity on the genes located downstream of *hfq* accounts for the observed physiological and morphological alterations (Tsui et al. 1994).

σ^S levels in *hfq1*::Ω and *hfq2*::Ω mutants were determined by immunoblot experiments. Figure 1 demonstrates that during exponential growth as well as during entry into stationary phase, σ^S levels were barely detectable in the *hfq1*::Ω mutant, whereas in the otherwise isogenic *hfq*$^+$ strain, σ^S levels increased more than fivefold during the growth cycle. In the *hfq2*::Ω mutant, however, expression of σ^S was similar to that observed in the *hfq*$^+$ strain (data not shown). Also, osmotic upshift during the exponential growth phase did not result in accumulation of σ^S in the *hfq1*::Ω mutant, which was in pronounced contrast to the regulation of σ^S in the *hfq*$^+$ and *hfq2*::Ω strains (Fig. 2). We conclude that the HF-I protein, the gene product of *hfq*, is required for the expression of wild-type levels of σ^S protein.

The hfq1::Ω *mutation interferes with* rpoS *translation*

To determine which level of σ^S control was affected by a defect in *hfq*, various transcriptional and translational *rpoS::lacZ* fusions were used. These fusions are located on λ phages integrated in single copy at the *att*(λ) site of the *E. coli* chromosome (for details, see Materials and methods). A transcriptional fusion inserted after nucleotide 742 within *rpoS* was not affected significantly by the *hfq1*::Ω mutation (Fig. 3A,B). However, the expression of the corresponding translational *rpoS742::lacZ* fusion, which exhibits a growth phase-related induction in wild-type strains of more than fivefold, was nearly abolished in the *hfq* mutant background (Fig. 3C,D). In addition, in quantitative primer extension experiments similar levels of *rpoS* mRNA (originating at the main promoter, *rpoS*p1) have been observed (data not shown). These results demonstrate that the HF-I protein is involved in the post-transcriptional control of σ^S.

The RpoS742::LacZ hybrid protein is subject to rapid and regulated turnover, just like σ^S itself (Muffler et al. 1996b). While this large fusion protein contains a se-

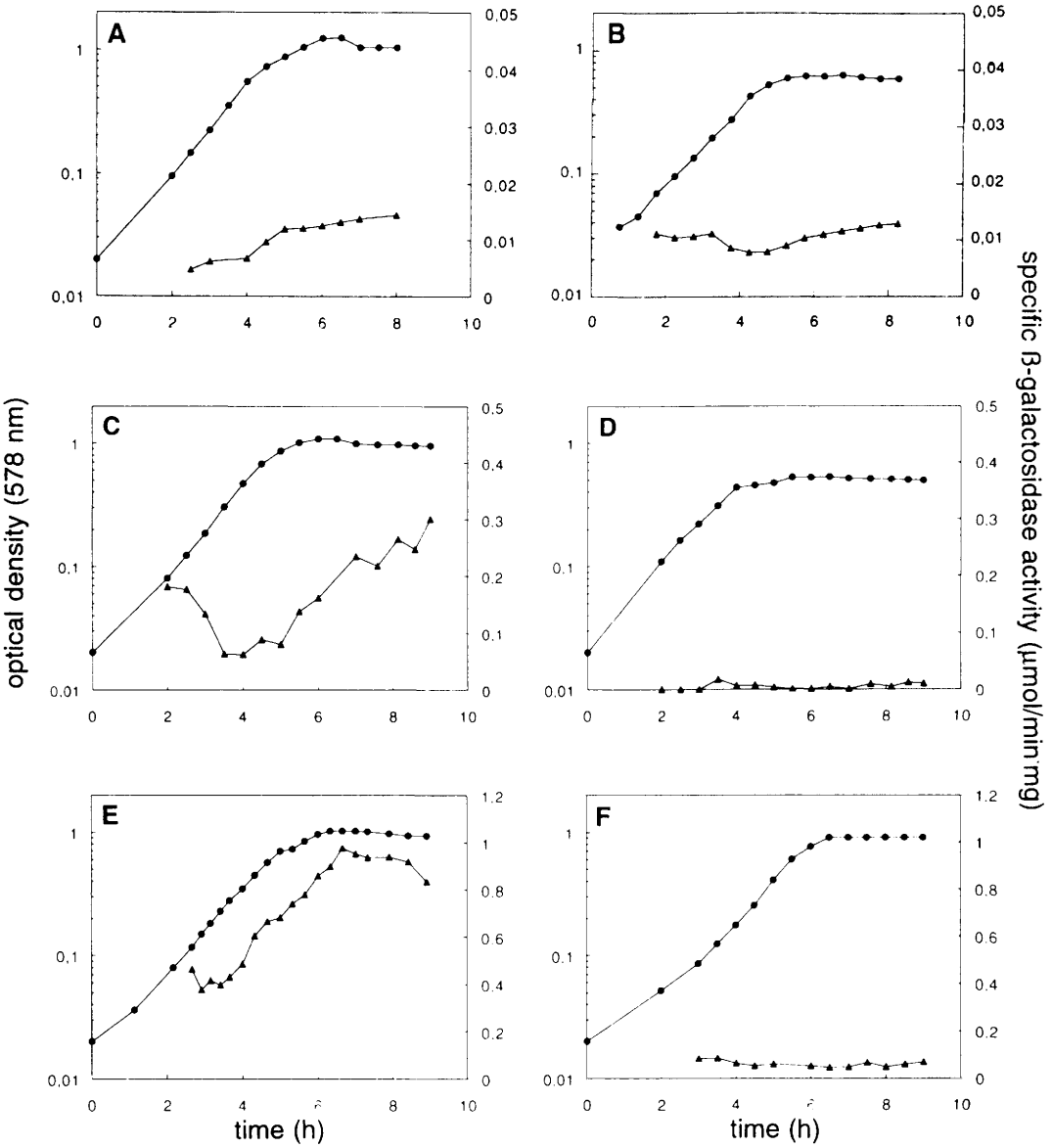

Figure 3. The *hfq1*::Ω mutation interferes differentially with the expression of various transcriptional and translational *rpoS::lacZ* fusions. Strains carrying the transcriptional *rpoS742::lacZ* fusion (*A,B*), or the translational *rpoS742::lacZ* (*C,D*) or *rpoS379::lacZ* (*E,F*) fusions in either *hfq*$^+$ (*A,C,E*) or *hfq1*::Ω backgrounds were grown in M9 medium with 0.1% glucose. Optical densities (●) and specific β-galactosidase activities (▲) were determined along the growth curve. Scales for β-galactosidase activities are different for transcriptional and translational fusions, as the former possess a relatively inefficient translational start site for *lacZ*.

quence element that recently was shown to be involved in σ^S degradation, this "turnover element" is not present in the shorter RpoS379::LacZ hybrid protein, which is completely stable (Muffler et al. 1996b; Schweder et al. 1996). β-Galactosidase activities expressed from *rpoS379::lacZ* are therefore relatively high and, in the absence of changes in transcript levels, reflect translational control only. In contrast, the activities from *rpoS742::lacZ* are severalfold lower in exponentially growing cells (Fig. 3, cf. C and E) and reflect the regulation of both *rpoS* translation and σ^S turnover (Muffler et al. 1996b). A comparison of the effects on these two translational fusions therefore allows one to distinguish whether a given mutation affects *rpoS* translation or σ^S degradation. Figure 3, E and F, demonstrate that the expression of the shorter *rpoS379::lacZ* was also strongly reduced in the *hfq1*::Ω mutant and that its induction during the late exponential phase was abolished completely. From these data it is evident that the *hfq1*::Ω mutation interferes with *rpoS* translation.

Osmotic upshift results in increased translation of *rpoS* as well as an inhibition of σ^S turnover. Accordingly, the *rpoS742::lacZ* fusion exhibits strong osmotic induction, for example, in response to the addition of NaCl or sucrose (Lange and Hengge-Aronis 1994a; Muffler et al. 1996b). Figure 4 shows that this osmotic induction is almost abolished completely in the *hfq1*::Ω mutant.

Changes in the rate of σ^S synthesis in response to os-

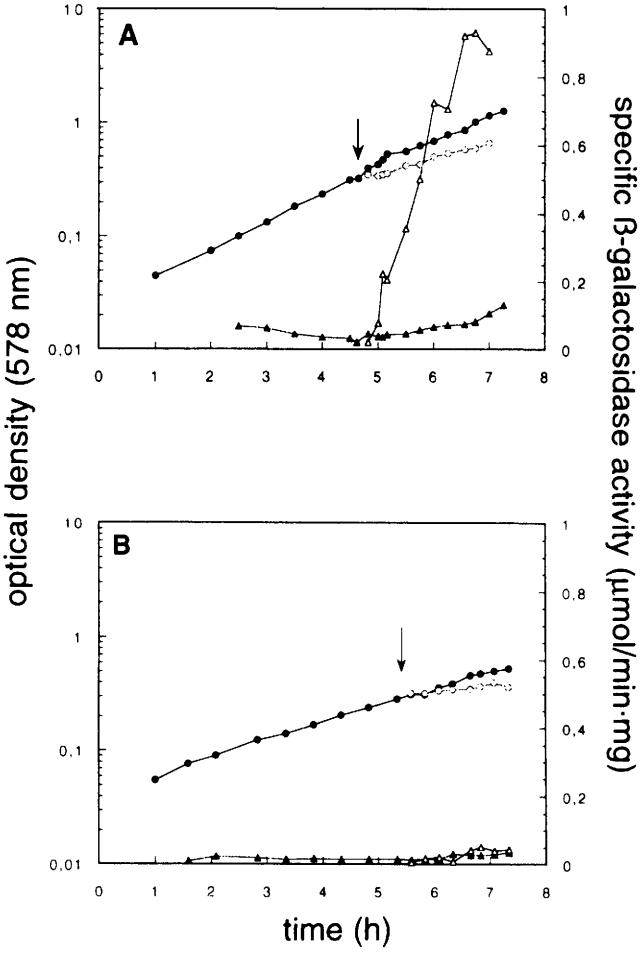

Figure 4. The *hfq1*::Ω mutation interferes with osmotic induction of the translational *rpoS742::lacZ* fusion. Strains RO91 (*hfq*$^+$; *A*), which carry the translational *rpoS742::lacZ* fusion and its *hfq1*::Ω derivative AM117 (*B*), were grown in M9 with 0.4% glycerol. At an OD_{578} of ~0.3, the cultures were divided and 0.3 M NaCl was added to one of the aliquots. Optical densities (●,○) and specific β-galactosidase activities (▲,△) were determined in the absence (●,▲) and presence (○,△) of salt.

motic upshift were also monitored directly in pulse–chase experiments (with short labeling and chase times to minimize the influence of σ^S degradation). Figure 5 demonstrates that upon the addition of 0.3 M NaCl, σ^S synthesis was more than sixfold stimulated in the *hfq*$^+$ strain, whereas no significant increase was observed in the otherwise isogenic *hfq1*::Ω strain. Because these conditions only affect the post-transcriptional regulation of σ^S, we conclude that the HF-I protein plays a crucial role in determining the rate of *rpoS* translation.

Finally, we have tried to assay whether or not low levels of σ^S, still potentially synthesized in the *hfq1*::Ω mutant, exhibit normal turnover. The *hfq1*::Ω mutant was pulse-labeled with increasing chase times before and after osmotic upshift, and extended exposure of autoradiographs allowed the detection of weak σ^S bands (Figure 6). In the absence of NaCl, no labeled σ^S could be detected after a 6-min chase with nonradioactive methionine, whereas after osmotic upshift, no degradation could be observed during a 3-min chase and at least 50% of the initially synthesized σ^S was still present after 6 min. This indicates that the low residual levels of σ^S in the *hfq1*::Ω mutant are subject to normal turnover control. This is corroborated by an approximately fivefold higher basal level of expression of *rpoS379::lacZ* than that of the *rpoS742::lacZ* fusion in the *hfq1*::Ω background (Fig. 3, cf. D and F).

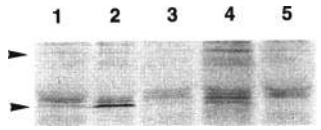

Figure 5. The *hfq1*::Ω mutant exhibits reduced rates of σ^S synthesis before and after osmotic upshift. Strains MC4100 (*hfq*$^+$; lanes *1,2*) and AM111 (*hfq1*::Ω; lanes *3,4*) were grown in M9 with 0.4% glycerol. Samples were taken at OD_{578} of 0.32 and labeled with [^{35}S]methionine (60-sec pulse, followed by a 30-sec chase with nonradioactive methionine; lanes *1,3*). Immediately thereafter, 0.3 M NaCl was added to the cultures, followed by a similar labeling 10 min later (lanes *2,4*). Labeled samples were subject to immunoprecipitation and SDS-PAGE. σ^S bands on the autoradiograph (*bottom* arrowhead) were quantitated in relation to an internal standard (an unidentified nonosmotically regulated protein that weakly reacts with the σ^S antiserum, marked by the *top* arrowhead). Relative density values for the σ^S band (after background subtraction and in relation to the internal standard) were 1.0 (lane *1*), 6.15 (lane *2*), 0.55 (lane *3*), and 0.51 (lane *4*). The *rpoS* mutant RH90 was used as a σ^S-deficient control (lane *5*). For all experimental details, see Materials and methods.

Relationship between HF-I and other components involved in the regulation of σ^S: RssB and H-NS

We have reported previously that the response regulator RssB is essential for σ^S turnover. Translational control of *rpoS*, however, is normal in *rssB* mutants (Muffler et al. 1996a). In contrast, data presented above indicate that the HF-I protein is involved in *rpoS* translation. To test more directly whether RssB and HF-I act in separate pathways of σ^S control, we performed a double mutant analysis. We found that in the *hfq1*::Ω mutant background, the introduction of an *rssB*::Tn*10* mutation re-

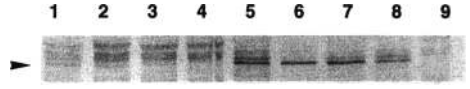

Figure 6. Residual amounts of σ^S in the *hfq1*::Ω mutant exhibit normal regulation of turnover. Strain AM111 (*hfq1*::Ω; lanes *1–8*) was grown in M9 with 0.4% glycerol. Samples were pulse-labeled before (lanes *1–4*) and after treatment with 0.3 M NaCl (lanes *5–8*) as described in the legend to Fig. 5. Chase times with nonradioactive methionine, however, varied between 25 sec (lanes *1,5*), 85 sec (lanes *2,6*), 3 min (lanes *3,7*), and 6 min (lanes *4,8*). Autoradiography exposure was for 8 days. The *rpoS*::Tn*10* mutant RH90 (lane *9*) was used to identify the weak σ^S bands (arrowhead).

sulted in a severalfold increase in the expression of the translational *rpoS742::lacZ* fusion, whereas this expression in the double mutant was still lower than that in the strain carrying *rssB*::Tn*10* alone and was not stimulated during the late exponential phase (data not shown). We conclude that there is no epistasis relationship between *hfq* and *rssB*, and therefore the corresponding gene products, HF-I and RssB, act independently from each other in different pathways.

Another component involved in the post-transcriptional control of σ^S is the H-NS protein. *hns* mutants exhibit high levels of σ^S protein that are no longer subject to growth phase-dependent or osmotic regulation. Although H-NS is a DNA-binding protein, it affects *rpoS* translation as well as σ^S turnover by unknown mechanisms (Barth et al. 1995; Yamashino et al. 1995). For addressing the question of whether H-NS and HF-I act in the same pathway controlling *rpoS* translation, we made a double mutant analysis using the translational *rpoS379::lacZ* fusion. Because the RpoS379::LacZ hybrid protein is not subject to normal RpoS turnover, only effects on *rpoS* translation are monitored with this fusion. An *hns* mutant exhibited an increased basal level of *rpoS379::lacZ* expression during the exponential phase (Fig. 7A,B). When the *hfq1*::Ω mutation was introduced into the *hns* strain, a strong reduction in *rpoS379::lacZ* expression was observed (Fig. 7C) and the basal level of expression was very similar to that observed in the strain that was deficient for *hfq* alone (cf. to Fig. 3F). These data suggest that H-NS most likely affects *rpoS* translation by influencing the expression or activity of HF-I.

Does HF-I play a role in σ^{32} regulation?

rpoH, the gene encoding the heat shock σ factor σ^{32}, is another prominent example of translational control of gene expression in *E. coli*. Evidence has been presented that secondary structure formation of the *rpoH* mRNA interferes with translational initiation, and the current model proposes that temperature upshift may cause a change in mRNA secondary structure by a mechanism not yet identified (Nagai et al. 1991; Yura et al. 1993; Yuzawa et al. 1993). Because mRNA secondary structure may also play a role in the translational control of *rpoS* (see below), we wanted to know whether HF-I, as an RNA-binding protein, is also involved in the translation of *rpoH*.

Using immunoblot analysis, we determined cellular σ^{32} levels before and during the first 12 min after a temperature shift from 28°C to 42.5°C. Heat-shocked cells of otherwise isogenic *hfq*$^+$ and *hfq1*::Ω strains contained very similar σ^{32} levels (Fig. 8A). Moreover, the basal level of σ^{32} before heat shock seemed to be even slightly higher in the *hfq* mutant. In addition, the rapid increase in σ^{32} synthesis in response to heat shock, which is attributable to increased *rpoH* translation, was not affected by the *hfq1*::Ω mutation, as demonstrated in a pulse–chase experiment (Fig. 8B). These findings are in pronounced contrast to the results obtained for σ^S reported above (Figs. 1, 2, and 5). We conclude that HF-I does not play a crucial role in the expression and heat shock regulation of σ^{32}.

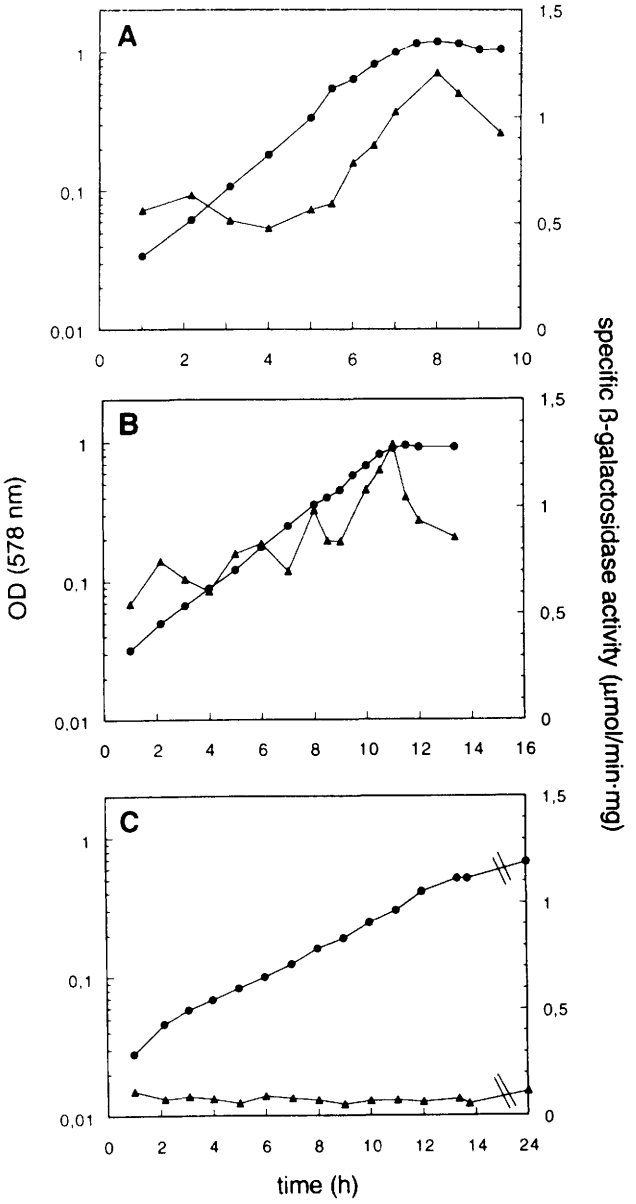

Figure 7. Effects of single and double mutations in *hfq* and *hns* on the expression of the translational *rpoS379::lacZ* fusion. Strains RO90 (carrying the translational *rpoS379::lacZ* fusion; *A*) and its *hns*::Tn*10* and *hns*:.Tn*10 hfq1*::Ω derivatives AM123 (*B*) and AM124 (*C*), respectively, were grown in M9 with 0.1% glucose. Optical densities (●) and specific β-galactosidase activities (▲) were determined.

Discussion

The RNA-binding protein HF-I, which is encoded by the *hfq* gene (Kajitani and Ishihama 1991), has long been known as a host factor essential for the replication of Qβ-RNA phage (Franze de Fernandez et al. 1968, 1972). While the pleiotropic phenotype of *hfq* mutants indicated that HF-I is clearly important for *E. coli* physiology (Tsui et al. 1994), this report is the first to identify a cellular process in which HF-I is involved. HF-I is required for normal expression of the σ^S subunit of RNA

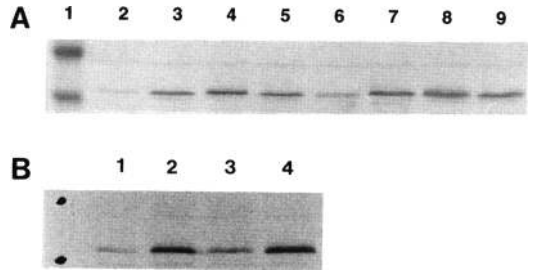

Figure 8. The *hfq1*::Ω mutation does not interfere with heat shock induction of σ^{32}. Total cellular levels of σ^{32} were assayed by immunoblot analysis (*A*); σ^{32} synthesis was monitored by pulse–chase experiments (*B*). Strains MC4100 (lanes *2–5* in *A*, lanes *1,2* in *B*) and its *hfq1*::Ω derivative AM111 (lanes *6–9* in *A*, lanes *3,4* in *B*) were grown in M9 with 0.1% glucose at 28°C. At an OD_{578} of 0.3, samples were taken (lanes *2,6* in *A*, lanes *1,3* in *B*), and immediately thereafter the cultures were shifted to 42.5°C. For immunoblot analysis (*A*), additional samples were withdrawn 4 min (lanes *3,7*), 8 min (lanes *4,8*), and 12 min (lanes *5,9*) after temperature upshift. For pulse–chase experiments (*B*), samples were taken 3 min after heat shock (lanes *2,4*). The samples were processed as described in Materials and methods. Size standard proteins (49 and 32 kD) are shown in lane *1* in (*A*).

polymerase that is a global regulatory factor for the expression of many genes that are induced during entry into stationary phase or in response to hyperosmolarity. Much of the pleiotropy of *hfq* mutants is therefore probably explained by the pleiotropic regulatory function of σ^S. In this study we have used an *hfq* null mutant (*hfq1*::Ω) as well as an insertion mutation located near the 3′ end of the *hfq* gene (*hfq2*::Ω) that produces a truncated HF-I protein with at least partial activity (Tsui et al. 1994). Because both mutations are completely polar, but σ^S levels were reduced only in the *hfq1*::Ω mutant, we can conclude that the observed effects on σ^S are a result of the absence of HF-I protein itself.

The cellular σ^S content is controlled at the levels of *rpoS* transcription and translation as well as of σ^S protein turnover (Lange and Hengge-Aronis 1994a). Here we demonstrate that HF-I is crucial for *rpoS* translation. Our conclusion is based on a complementary approach using pulse–chase experiments as well as the analysis of *rpoS::lacZ* fusions. Whereas the former, in conjunction with immunoprecipitation, represents a direct assay for σ^S, synthesis and degradation of σ^S cannot be separated completely, because σ^S half-life is ~1.5 min under conditions of normal growth. On the other hand, the analysis of fusions is a more indirect approach but allows for the assaying of *rpoS* translation in a way that is unaffected by σ^S turnover, because the hybrid protein encoded by *rpoS379::lacZ* does not contain the σ^S internal turnover element required for degradation (Muffler et al. 1996b).

Several lines of evidence indicate that HF-I is involved in *rpoS* translation: (1) Whereas the expression of transcriptional *rpoS::lacZ* fusions was not affected by the *hfq1*::Ω mutation, β-galactosidase levels from corresponding translational fusions were reduced strongly (Fig. 3); (2) the expression of the translational *rpoS379::lacZ* fusion was affected strongly (Fig. 3), that is, of the fusion that does not reflect σ^S turnover; (3) the osmotic stimulation of σ^S synthesis, which we have shown to be attributable to increased translation (Muffler et al. 1996b), was reduced in the *hfq1*::Ω mutant (Fig. 5); and, finally, (4) the very low levels of σ^S still detectable in the *hfq1*:Ω mutant exhibit normally regulated turnover (Fig. 6). Our data indicate that HF-I is a positively acting factor crucial for establishing normal rates of *rpoS* translation. Whether HF-I is also involved in translational up-regulation in response to environmental signals is not yet clear, although the hypothesis that HF-I activity may be controlled by other components of the respective signal transduction pathway seems attractive. The residual osmotic induction of the very low levels of σ^S in the *hfq1*::Ω mutant even after a short chase time (25 sec; Fig. 6) does not seem to argue against this hypothesis, as an apparent twofold induction can be explained by inhibition of degradation of σ^S if the very short half-life of σ^S is taken into account and if it is assumed that even nascent polypeptide chains become accessible for proteolysis as soon as they contain the turnover element that is located approximately in the middle of σ^S.

HF-I is the first *trans*-acting factor known to be involved specifically in the control of *rpoS* translation. Recently, two components involved in the regulation of σ^S turnover, the response regulator RssB (Muffler et al. 1996a) and the ClpXP protease (Schweder et al. 1996), have also been identified. Our double mutant analysis with mutations in *hfq* and *rssB* reported here confirmed that HF-I and RssB act in separate pathways of σ^S control. Figure 9 summarizes our present knowledge of environmental and physiological conditions, *trans*-acting regulatory factors, and *cis*-acting regulatory regions involved at the different levels of control of the cellular σ^S content.

mRNA secondary structures in the translational initiation region (TIR) of *rpoS* may interfere with the binding of the ribosome and thus play a role in translational regulation. Depending on the length of RNA sequence used for secondary structure prediction, various such structures seem possible. Recent results with a *lacZ* fusion containing only 70 nucleotides of *rpoS* (Muffler et al. 1996b) and with a point mutation shortly upstream of the *rpoS* TIR (S. Bouché and R. Hengge-Aronis, unpubl.) indicate that the mRNA secondary structure that is likely to be relevant for *rpoS* translational control involves base-pairing between the TIR and a region located directly upstream of the TIR. It has been proposed that in association with Qβ–replicase, the RNA-binding protein HF-I induces a conformational change near the 3′ end of the stably folded Qβ–RNA that is required for the replicase to initiate the synthesis of the minus strand (Franze de Fernandez et al. 1972; Senear and Steitz 1976; Barrera et al. 1993). In view of these properties of the protein HF-I, we speculate that it may bind to *rpoS* mRNA and perhaps affect its secondary structure in the TIR in such a way that translational initiation is stimulated. HF-I therefore appears to be a rare example of an RNA-binding

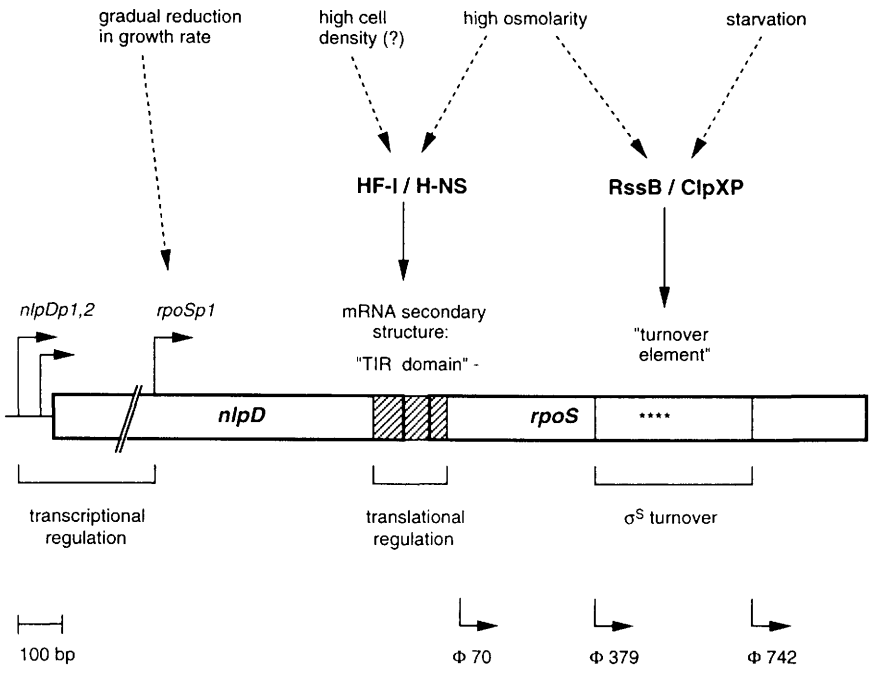

Figure 9. Regulation of the cellular σ^S content. Summarized is the present knowledge of environmental and physiological conditions that affect σ^S levels (Lange and Hengge-Aronis 1994a; Muffler et al. 1996b), as well as of *trans*-acting regulatory components and *cis*-acting regulatory regions that play a role in *rpoS* transcription and translation and σ^S turnover. The involvement of H-NS (Barth et al. 1995; Yamashino et al. 1995), RssB (Muffler et al. 1996a), and the ClpXP protease (Schweder et al. 1996) has been reported previously. Although RssB and ClpXP are each essential for σ^S turnover, the relationship between these two factors is unclear. The localization of the promoters that contribute to *rpoS* expression (Lange and Hengge-Aronis 1994b; Takayanagi et al. 1994; Lange et al. 1995) and the approximate location of a turnover element located within σ^S (Muffler et al. 1996a,b; Schweder et al. 1996) have been published. Asterisks indicate the position of a sequence that is important for ClpXP-mediated σ^S degradation (Schweder et al. 1996). A region able to fold into a stable mRNA secondary structure includes the translational initiation region (TIR domain) and may interfere with translational initiation (D. Traulsen and R. Hengge-Aronis, unpubl.). In addition, the positions of *rpoS::lacZ* fusions (Φ70,379,742) used or mentioned in this study are indicated.

protein that affects translation positively. In eukaryotic cells, nearly all RNA-binding proteins known to be involved in translational regulation act as translational repressors (Standart and Jackson 1994).

We have shown previously that *hns* mutants exhibit constitutively high expression of *rpoS* that is not affected by osmotic upshift or entry into stationary phase, which may indicate that the H-NS protein is involved in this control by environmental signals (Barth et al. 1995). While *hns* mutants are affected in *rpoS* translation as well as in σ^S turnover, the use of the translational *rpoS379::lacZ* fusion allowed us to show that an *hns* mutation does not affect *rpoS* translation when present in an *hfq* mutant background (Fig. 7), suggesting that H-NS acts upstream of or at the same level as HF-I in a hypothetical signal transduction pathway. As a histone-like DNA-binding protein, H-NS may influence the expression of HF-I. On the other hand, it has been reported that in vitro H-NS binds tightly to HF-I (Kajitani and Ishihama 1991), which raises the possibility that in vivo H-NS may interfere with the activity of HF-I by direct protein–protein interaction. The environmental signals that control *rpoS* translation may then affect the interaction between H-NS and HF-I. This would be an entirely novel function for H-NS, which so far has been known as an abundant DNA-binding protein that specifically regulates the expression of some genes, such as the *proU* operon, by directly binding to a *cis*-regulatory region (Lucht and Bremer 1994), and that is involved in the determination of chromosomal superstructure (Spassky et al. 1984; Owen-Hughes et al. 1992; Tupper et al. 1994).

As a loosely ribosome-associated RNA-binding protein (DuBow et al. 1977), HF-I may influence the translation of other mRNAs besides that of *rpoS*. The cellular level of the heat shock σ factor σ^{32} (encoded by the *rpoH* gene) is controlled by mechanisms that resemble, at least superficially, those found for σ^S. Also for σ^{32}, transcriptional regulation is of minor importance, and heat shock induction is caused by increased translation of *rpoH* mRNA (which is also folded into a complex secondary structure with the TIR region being base-paired) as well as an inhibition of σ^{32} turnover (summarized in Yura et al. 1993). However, HF-I is clearly dispensible for normal heat shock regulation of σ^{32} (Fig. 8). In *Azorhizobium caulinodans*, an HF-I homolog (encoded by the *nrfA* gene) was shown to be required for the expression of NifA, but its mechanism of action has not been studied in detail (Kaminski et al. 1994). In *E. coli*, *hfq* mutants exhibit somewhat reduced growth rates and growth yields (Tsui et al. 1994). These phenotypes have not been observed for *rpoS* mutants. Moreover, altered expression of various non-σ^S-dependent proteins in the *hfq1*::Ω mu-

Table 1. *Bacterial strains*

Strain	Relevant genotype	Reference
MC4100	F⁻ Δ(*arg–lac*)*U169 araD139 rpsL150 ptsF25 flbB5301 rbsR deoC relA1*	(Silhavy et al. 1984)
RH90	MC4100 *rpoS359*::Tn*10*	(Lange and Hengge-Aronis 1991b)
RO90	MC4100 [λRZ5: *rpoS379*::*lacZ*(hybr)]	(Lange and Hengge-Aronis 1994a)
RO91	MC4100 [λRZ5: *rpoS742*::*lacZ*(hybr)]	(Lange and Hengge-Aronis 1994a)
RO200	MC4100 (λRZ5: *rpoS742*::*lacZ*)	(Lange and Hengge-Aronis 1994a)
TX2808	*hfq1*::Ω (Km^r; *Bcl*I)	(Tsui et al. 1994)
TX2758	*hfq2*::Ω (Km^r; *Kpn*I)	(Tsui et al. 1994)
AM111	MC4100 *hfq1*::Ω	this study
AM112	MC4100 *hfq2*::Ω	this study
AM119	RO200 *hfq1*::Ω	this study
AM117	RO91 *hfq1*::Ω	this study
AM121	RO90 *hfq1*::Ω	this study
AM109	RO91 *rssB*::Tn*10*	(Muffler et al. 1996a)
AM122	RO91 *rssB*::Tn*10 hfq1*::Ω	this study
GM230	MC4100 Φ(*proU*::*lacZ*)hyb2 *hns205*::Tn*10*	(Higgins et al. 1988)
AM123	RO90 *hns205*::Tn*10*	this study
AM124	RO90 *hns205*::Tn*10 hfq1*::Ω	this study

tant was recently found (D. Traulsen and R. Hengge-Aronis, unpubl.) indicating that there are other targets for regulation by HF-I besides *rpoS*.

Materials and methods

Bacterial strains and growth conditions

The bacterial strains used in this work are listed in Table 1. *hfq*, *rssB*, and *hns* mutant alleles were introduced into various strain backgrounds by P1 transduction as described (Miller 1972). *hns* mutants were freshly constructed by P1 transduction for every experiment to avoid the occurrence of second-site suppressor mutations (Barth et al. 1995). Cultures were grown at 37°C or 30°C under aeration in Luria–Bertani (LB) medium or minimal medium M9 (Miller 1972) supplemented with 0.1% or 0.2% glucose or 0.4% glycerol as carbon sources. Antibiotics were added as recommended (Miller 1972). Growth was monitored by determining the optical density at 578 nm (OD_{578}). For osmotic or temperature-shift experiments, the cultures were grown exponentially for at least three generations before 0.3 M NaCl was added or the temperature was increased from 28°C to 42.5°C.

SDS-PAGE and immunoblot analysis

Sample preparation for SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblot analysis were performed as described previously (Lange and Hengge-Aronis 1994a). Fifteen micrograms of total cellular protein was applied per lane. Visualization of σ^S or σ^{32} bands was performed using polyclonal antisera against σ^S (Lange and Hengge-Aronis 1994a) or σ^{32} (kindly provided by B. Bukau, Zentrum für Molekular Biologie Heidelberg, Universität Heidelberg, Germany), a goat anti-rabbit IgG alkaline-phosphatase conjugate (Sigma), and a chromogenic alkaline phosphatase substrate (BCIP/NBT; Boehringer Mannheim).

Pulse-labeling of cells and immunoprecipitation

The procedure used for the pulse-labeling of cells with L-[^{35}S]methionine (Amersham; >1000 Ci/mmole) and immunoprecipitation of σ^S was performed as described (Lange and Hengge-Aronis 1994a). The OD_{578} of culture samples to be labeled was adjusted to 0.3 by dilution with supernatant from the same culture obtained by centrifugation immediately before taking the samples for pulse-labeling. For the determination of the rate of σ^S expression, pulse and chase times were 60 and 30 sec, respectively. As a σ^S-deficient control, exponential phase samples of strain RH90 were harvested at an OD_{578} of ~0.5 and labeled as described above. Bands on autoradiographs were analyzed using the NIH Image software for Macintosh.

β-Galactosidase assay

β-Galactosidase activity was assayed by use of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate and is reported as micromoles of *o*-nitrophenol per minute per milligram of cellular protein (Miller 1972).

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