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

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The \textit{rpoS}-encoded σ^5 subunit of RNA polymerase in \textit{Escherichia coli} is a global regulatory factor involved in several stress responses. Mainly because of increased \textit{rpoS} translation and stabilization of σ^5, which in nonstressed cells is a highly unstable protein, the cellular σ^5 content increases during entry into stationary phase and in response to hyperosmolarity. Here, we identify the \textit{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 \textit{rpoS} translation. An \textit{hfq} null mutant exhibits strongly reduced σ^5 levels under all conditions tested and is deficient for growth phase-related and osmotic induction of σ^5. Using a combination of gene fusion analysis and pulse-chase experiments, we demonstrate that the \textit{hfq} mutant is specifically impaired in \textit{rpoS} translation. We also present evidence that the H-NS protein, which has been shown to affect \textit{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 \textit{rpoH}) is not dependent on HF-I, although \textit{rpoH} and \textit{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 \textit{rpoS} translation, and this role is the first cellular function to be identified for this abundant ribosome-associated RNA-binding protein in \textit{E. coli}.

[Key Words: σ^5; σ factor; stationary phase; osmotic regulation; mRNA secondary structure]

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The σ^5 subunit of RNA polymerase in \textit{Escherichia coli}, which is encoded by the \textit{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^5 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^5 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. 1996b]. Several studies have shown that post-transcriptional mechanisms are of primary importance in the control of the cellular σ^5 level [Loewen et al. 1993; McCann et al. 1993; Lange and Hengge-Aronis 1994a]. \textit{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 \textit{rpoS} translation [Muffler et al. 1996b]. In addition, σ^5 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^5 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^5. \textit{hns} mutants exhibit increased \textit{rpoS} translation and reduced turnover of σ^5 during exponential phase and no longer show growth phase-associated or osmotic induction of σ^5. However, the mechanism by which H-NS inhibits σ^5 expression has not been characterized further [Barth et al. 1995; Yamashino et al. 1995]. Two factors have been found to be essential for σ^5 turnover. One is the Clp protease, with the subunits ClpP...
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 hfg 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 $\sigma^5$ expression reported here is therefore the first known cellular function of HF-I, and in view of the role of $\sigma^5$ as a key global regulator, provides a direct explanation for the pleiotropic phenotype of hfg mutants.

**Results**

**Cellular $\sigma^5$ levels are reduced in the HF-I-deficient hfg1::Ω mutant**

Some of the phenotypes of an hfg 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 $\sigma^5$ 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 hfg of an Ω[Kan] cassette that carries transcriptional terminators on both ends [Tsui et al. 1994]. While the hfg1::Ω mutation is a disruption approximately in the middle of the hfg gene, the insertion of the same Ω cassette close to the 3’ end of hfg [hfg2::Ω] does not produce the pleiotropic phenotype even though 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 hfg accounts for the observed physiological and morphological alterations [Tsui et al. 1994].

$\sigma^5$ levels in hfg1::Ω and hfg2::Ω mutants were determined by immunoblot experiments. Figure 1 demonstrates that during exponential growth as well as during entry into stationary phase, $\sigma^5$ levels were barely detectable in the hfg1::Ω mutant, whereas in the otherwise isogenic hfg- strain, $\sigma^5$ levels increased more than fivefold during the growth cycle. In the hfg2::Ω mutant, however, expression of $\sigma^5$ was similar to that observed in the hfg- strain [data not shown]. Also, osmotic upshift during the exponential growth phase did not result in accumulation of $\sigma^5$ in the hfg1::Ω mutant, which was in pronounced contrast to the regulation of $\sigma^5$ in the hfg- and hfg2::Ω strains [Fig. 2]. We conclude that the HF-I protein, the gene product of hfg, is required for the expression of wild-type levels of $\sigma^5$ protein.

**The hfg1::Ω mutation interferes with rpoS translation**

To determine which level of $\sigma^5$ control was affected by a defect in hfg, 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 hfg1::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 hfg mutant background [Fig. 3C,D]. In addition, in quantitative primer extension experiments similar levels of rpoS mRNAs [originating at the main promoter, rpoSp1] have been observed [data not shown]. These results demonstrate that the HF-I protein is involved in the post-transcriptional control of $\sigma^5$.

The RpoS742::LacZ hybrid protein is subject to rapid and regulated turnover, just like $\sigma^5$ itself [Muffler et al. 1996b]. While this large fusion protein contains a se-
Role of HF-I in rpoS translation

Figure 3. The hflQ::fl 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 hflQ+ (A,C,E) or hflQ::fl (B,D,F) 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.

A consequence element that recently was shown to be involved in σ5 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 σ5 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 σ5 degradation. Figure 3, E and F, demonstrate that the expression of the shorter rpoS379::lacZ was also strongly reduced in the hflQ+::fl mutant and that its induction during the late exponential phase was abolished completely. From these data it is evident that the hflQ+::fl mutation interferes with rpoS translation.

Osmotic upshift results in increased translation of rpoS as well as an inhibition of σ5 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 hflQ+::fl mutant.

Changes in the rate of σ5 synthesis in response to os-
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Figure 4. The hfq1::Ω mutation interferes with osmotic induction of the translational rpoS742::lacZ fusion. Strains RO91 (hfq1::Ω), 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 nm of ~0.3, the cultures were divided and 0.3 M NaCl was added to one of the aliquots. Optical densities (O) and specific β-galactosidase activities (A) were determined in the absence (O) and presence (A) of salt.

motic upshift were also monitored directly in pulse-chase experiments (with short labeling and chase times to minimize the influence of σ5 degradation). Figure 5 demonstrates that upon the addition of 0.3 M NaCl, σ5 synthesis was more than sixfold stimulated in the hfq1::Ω strain, whereas no significant increase was observed in the otherwise isogenic hfq1::Ω strain. Because these conditions only affect the post-transcriptional regulation of σ5, 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 σ5, 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 σ5 bands (Figure 6). In the absence of NaCl, no labeled σ5 could be detected after a 6-min chase with nonradioactive methio-

nine, whereas after osmotic upshift, no degradation could be observed during a 3-min chase and at least 50% of the initially synthesized σ5 was still present after 6 min. This indicates that the low residual levels of σ5 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].

Relationship between HF-I and other components involved in the regulation of σ5: RssB and H-NS

We have reported previously that the response regulator RsS is essential for σ5 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 σ5 control, we performed a double mutant analysis. We found that in the hfq1::Ω mutant background, the introduction of an rssB::Tn10 mutation re-

Figure 5. The hfq1::Ω mutant exhibits reduced rates of σ5 synthesis before and after osmotic upshift. Strains MC4100 (hfq1::Ω, lanes 1, 2) and AM111 (hfq1::Ω, lanes 3, 4) were grown in M9 with 0.4% glycerol. Samples were taken at ODs87 of ~0.3 and labeled with [35S]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. σ5 bands on the autoradiograph (bottom arrowhead) were quantitated in relation to an internal standard (an unidentified nonosmotically regulated protein that weakly reacts with the σ5 antisera, marked by the top arrowhead). Relative density values for the σ5 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 σ5-deficient control (lane 5). For all experimental details, see Materials and methods.

![Figure 5](https://example.com/figure5)

Figure 6. Residual amounts of σ5 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::Tn10 mutant RH90 (lane 9) was used to identify the weak σ5 bands (arrowhead).
sulted in a severalfold increase in the expression of the translational \textit{rpoS742::lacZ} fusion, whereas this expression in the double mutant was still lower than that in the strain carrying \textit{rpsS::Tn10} alone and was not stimulated during the late exponential phase [data not shown]. We conclude that there is no epistasis relationship between \textit{hfq} and \textit{rpsS}, 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 \textit{\sigma^9} is the H-NS protein. \textit{hns} mutants exhibit high levels of \textit{\sigma^9} protein that are no longer subject to growth phase-dependent or osmotic regulation. Although H-NS is a DNA-binding protein, it affects \textit{rpoS} translation as well as \textit{\sigma^9} 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 \textit{rpoS} translation, we made a double mutant analysis using the translational \textit{rpoS379::lacZ} fusion. Because the \textit{RpoS379::LacZ} hybrid protein is not subject to normal RpoS turnover, only effects on \textit{rpoS} translation are monitored with this fusion. An \textit{hns} mutant exhibited an increased basal level of \textit{rpoS379::lacZ} expression during the exponential phase [Fig. 7A,B]. When the \textit{hfq1::\Omega} mutation was introduced into the \textit{hns} strain, a strong reduction in \textit{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 \textit{hfq} alone [cf. to Fig. 3F]. These data suggest that H-NS most likely affects \textit{rpoS} translation by influencing the expression or activity of HF-I.

Does HF-I play a role in \textit{\sigma^{32}} regulation?

\textit{rpoH}, the gene encoding the heat shock \textit{\sigma} factor \textit{\sigma^{32}}, is another prominent example of translational control of gene expression in \textit{E. coli}. Evidence has been presented that secondary structure formation of the \textit{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 \textit{rpoS} [see below], we wanted to know whether HF-I, as an RNA-binding protein, is also involved in the translation of \textit{rpoH}.

Using immunoblot analysis, we determined cellular \textit{\sigma^{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 \textit{hfq} and \textit{hfq1::\Omega} strains contained very similar \textit{\sigma^{32}} levels (Fig. 8A). Moreover, the basal level of \textit{\sigma^{32}} before heat shock was shown to be slightly higher in the \textit{hfq} mutant. In addition, the rapid increase in \textit{\sigma^{32}} synthesis in response to heat shock, which is attributable to increased \textit{rpoH} translation, was not affected by the \textit{hfq1::\Omega} mutation, as demonstrated in a pulse-chase experiment [Fig. 8B]. These findings are in pronounced contrast to the results obtained for \textit{\sigma^9} 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 \textit{\sigma^{32}}.

**Figure 7.** Effects of single and double mutations in \textit{hfq} and \textit{hns} on the expression of the translational \textit{rpoS379::lacZ} fusion. Strains RO90 (carrying the translational \textit{rpoS379::lacZ} fusion, \textit{Al} and its \textit{hns::Tn10} and \textit{hns::Tn10 hfq1::\Omega} derivatives AM123 [\textit{B}] and AM124 [\textit{C}], respectively, were grown in M9 with 0.1% glucose. Optical densities (●) and specific \textit{\beta}-galactosidase activities (▲) were determined.

**Discussion**

The RNA-binding protein HF-I, which is encoded by the \textit{hfq} gene [Kajitani and Ishihama 1991], has long been known as a host factor essential for the replication of QB-RNA phage [Franze de Fernandez et al. 1968, 1972]. While the pleiotropic phenotype of \textit{hfq} mutants indicated that HF-I is clearly important for \textit{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 \textit{\sigma^9} subunit of RNA
polymerase that is a global regulatory factor for the ex-
by immunoblot analysis (A); 42.5~ For immunoblot analysis (A), additional samples were
rpoS
Figure
an ODs78 of 0.3, samples were taken (lanes
lanes
shock induction of 0.32. Total cellular levels of 0.32 were assayed
into stationary phase or in response to hyperosmolarity.

4.5,42 pulse-chase experiments as well as the analysis of
ors synthesis and degradation of ors cannot be separated com-

HF-I induces a conformational change near the 3' end of the ors gene [hfq1::Ω] that produces a trun-
cated HF-I protein with at least partial activity (Tsui et al. 1994). Because both mutations are completely polar,
but ors levels were reduced only in the hfq1::Ω mutant, we can conclude that the observed effects on ors are a
result of the absence of HF-I protein itself.

The cellular ors content is controlled at the levels of
rpoS transcription and translation as well as of ors 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 rpo-
S::lacZ fusions. Whereas the former, in conjunction with
immunoprecipitation, represents a direct assay for ors,
synthesis and degradation of ors cannot be separated com-
pletely, because ors 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
ors turnover, because the hybrid protein encoded by
rpoS379::lacZ does not contain the ors internal turnover
element required for degradation (Muffler et al. 1996b).

Several lines of evidence indicate that HF-I is in-
volved 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]; and, finally, (4) the very low levels of ors still detect-
able in the hfq1::Ω mutant exhibit normally regulated
turnover (Fig. 6). Our data indicate that HF-I is a posi-
tively 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
ors in the hfq1::Ω mutant even after a short chase time
(25 sec; Fig. 6) does not seem to argue against this hy-
pothesis, as an apparent twofold induction can be ex-
plained by inhibition of degradation of ors if the very
short half-life of ors 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 ors.

HF-I is the first trans-acting factor known to be in-
volved specifically in the control of rpoS translation. Re-
cently, two components involved in the regulation of ors
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 ors con-
trol. Figure 9 summarizes our present knowledge of en-
vironmental and physiological conditions, trans-acting
regulatory factors, and cis-acting regulatory regions in-
volved at the different levels of control of the cellular ors
content.

mRNA secondary structures in the translational initi-
ation region [TIR] of rpoS may interfere with the binding of
the ribosome and thus play a role in translational reg-
ulation. Depending on the length of RNA sequence used
for secondary structure prediction, various such struc-
tures 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. Bouc6 and R. Hengge-Aronis, unpubl.)
indicate that the mRNA secondary structure that is
likely to be relevant for rpoS translational control in-
volves base-pairing between the TIR and a region located
directly upstream of the TIR. It has been proposed that
in association with QB–replicase, the RNA-binding protein
HF-I induces a conformational change near the 3' end
of the stably folded QB–RNA that is required for the repli-
case to initiate the synthesis of the minus strand [Franze
de Fernandez et al. 1972; Sencar 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
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 $\sigma^5$ 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 $\sigma$ factor $\sigma^5$ (encoded by the rpoH gene) is controlled by mechanisms that resemble, at least superficially, those found for $\sigma^7$. Also for $\sigma^5$, transcriptional regulation is of minor importance, and heat shock induction is caused by increased translation of rpoH mRNA (which is also folded 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 (Fig. 7, 379, 742) used or mentioned in this study are indicated.
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Table 1. Bacterial strains

| Strain       | Relevant genotype | Reference |
|--------------|-------------------|-----------|
| MC4100       | F- Δarg-lacU169 araD139 rpsL150 ptsF25 flbB5301 rbsR deoC relA1 | [Silhavy et al. 1984] |
| RH90         | MC4100 rpsS359::Tn10 | [Lange and Hengge-Aronis 1991b] |
| RO90         | MC4100 [rZ5: rpoS579::lacZ] [hybR] | [Lange and Hengge-Aronis 1994a] |
| RO91         | MC4100 [rZ5: rpoS5742::lacZ] [hybR] | [Lange and Hengge-Aronis 1994a] |
| RO200        | MC4100 [rZ5: rpoS5742::lacZ] | [Lange and Hengge-Aronis 1994a] |
| TX2780       | hft1::Ω (Km<sup>+</sup>, BclI) | (Tsuji et al. 1994) |
| TX2785       | hft2::Ω (Km<sup>+</sup>, KpmI) | (Tsuji et al. 1994) |
| AM111        | MC4100 hft1::Ω | this study |
| AM112        | MC4100 hft2::Ω | this study |
| AM119        | RO200 hft1::Ω | this study |
| AM117        | RO91 hft1::Ω | this study |
| AM121        | RO90 hft1::Ω | this study |
| AM109        | RO91 rssB::Tn10 | [Muffler et al. 1996a] |
| AM122        | RO91 rssB::Tn10 hft1::Ω | this study |
| GM230        | MC4100 Φ[pOPE::lacZ] [hyb2 hns205::Tn10] | [Higgins et al. 1988] |
| AM123        | RO90 hns205::Tn10 | this study |
| AM124        | RO90 hns205::Tn10 hft1::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. hftq, 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<sub>578</sub>). 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 α<sup>32</sup> or α<sup>35</sup> bands was performed using polyclonal antiserum against α<sup>32</sup> [Lange and Hengge-Aronis 1994a] or α<sup>35</sup> [kindly provided by B. Bukau, Zentrum für Molekular Biologie Heidelberg, University 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-[<sup>35</sup>S]methionine [Amersham, >1000 Ci/mmol] and immunoprecipitation of α<sup>32</sup> was performed as described [Lange and Hengge-Aronis 1994a]. The OD<sub>578</sub> 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 α<sup>32</sup> expression, pulse and chase times were 60 and 30 sec, respectively. As a α<sup>32</sup>-deficient control, exponential phase samples of strain RH90 were harvested at an OD<sub>578</sub> 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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