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Concentrations of 4.5S RNA and Ffh Protein in *Escherichia coli*: the Stability of Ffh Protein Is Dependent on the Concentration of 4.5S RNA

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We measured the concentrations of both 4.5S RNA and Ffh protein under a variety of growth conditions and found that there were 400 molecules of 4.5S RNA per 10,000 ribosomes in wild-type cells and that the concentration of Ffh protein was one-fourth of that. This difference in concentration is 1 order of magnitude less than that previously reported but still significant. Pulse-chase labeling experiments indicated that Ffh protein is unstable in cells carrying *ffh* on high-copy-number plasmids and that simultaneous overproduction of 4.5S RNA stabilizes Ffh protein. Our analyses show that free Ffh protein is degraded with a half-life of approximately 20 min. We also tested whether three previously isolated suppressors of 4.5S RNA deficiency could reduce the requirement for Ffh protein. Since the two *sffE* suppressors do not suppress the Ffh requirement, we suggest that 4.5S RNA either acts in a sequential reaction with Ffh or has two functions.

4.5S RNA, encoded by the *ffs* gene of *Escherichia coli*, shows structural homology to the 7S RNA of the signal recognition particle (SRP) in eukaryotic cells (26). This ribonucleoprotein particle consists of 7S RNA and six proteins (34, 35), and one of these, SRP54, is homologous to the *E. coli* Ffh protein, the product of the *ffh* gene (1, 28). Recently, the FtsY protein, encoded by the *ftsY* gene, was suggested to be a member of a possible SRP secretion pathway in bacteria because of its homology to SRP-α protein from the eukaryotic SRP receptor (17, 28). Furthermore, since Ffh protein and 4.5S RNA constitute a ribonucleoprotein particle in *E. coli* (25, 27) and Ffh protein is reported to participate in secretion (24), 4.5S RNA may also be involved in this process.

In spite of the homology between 4.5S RNA and 7S RNA, it has not been demonstrated that all nondispensable functions of 4.5S RNA involve secretion. The 4.5S RNA molecule has been shown to be essential for cell growth (7). The function of 4.5S RNA is connected with translation because 4.5S RNA is bound to ribosomes and suppressors of the 4.5S RNA requirement reside in genes encoding components of the translation system, such as elongation factor G, 23S RNA, and tRNA-synthetases (4, 5). During depletion of 4.5S RNA, the rate of protein synthesis is reduced and the synthesis of heat shock proteins is induced (2, 7). A recent study of the maturation kinetics of several secreted proteins in a Δ*rpoH* strain unable to induce the synthesis of heat shock proteins indicated that the primary function of 4.5S RNA involves protein synthesis rather than protein secretion (15). Therefore, this study supports the conclusion from genetic studies that the primary defect in 4.5S RNA-deficient cells is in the translation, most likely the translocation process (6), of mRNAs in general and suggests that the defective processing of secreted proteins might be a secondary effect. The casual relationship between secretion defects and severe induction of heat shock response in *rpoH*$^+$ cells (15, 25) remains to be elucidated. In *E. coli*, a number of Sec mutants with defects in secretion have been isolated, but none of these were due to alterations in either the *ffs*, *ffh*, or *ftsY* genes (for a review, see reference 29). However, it cannot been excluded that the Sec pathway and an SRP-like secretion pathway are partially redundant, making it difficult to observe any specific effect from a deficient SRP-like pathway.

To further investigate if 4.5S RNA has any function separate from its function in the ribonucleoprotein particle with Ffh protein, we first determined the molar amounts of these two molecules, particularly in Δ*ffs* P_{tac}-*ffs* strains (7), in order to determine the minimal concentration of 4.5S RNA which sustains growth. Previously, it was determined that the concentration of 4.5S RNA was 2,000 molecules per cell or 1 per five ribosomes (13, 16), which is 30-fold higher than the estimated concentration of Ffh protein (36). This great difference between the concentrations of 4.5S RNA and Ffh made it less likely that the only role of 4.5S RNA is to complex with Ffh protein. However, we discovered that the previously determined concentrations of both 4.5S RNA and Ffh protein are erroneous and have redetermined them.

MATERIALS AND METHODS

Strains. The genotypes of the strains used in this study are shown in Table 1.

Plasmids. To construct plasmid pCGJ1, the low-copy-number plasmid pDPT487 (31) was digested with *Bgl*II and *Bam*HI and the resulting large fragment was ligated with the *Bam*HI fragment from pKK235b-15 containing the P_{tac}-*ffs* fusion (3). The orientation of the cloned P_{tac}-*ffs* fusion in pCGJ1 is such that the P_{tac} promoter is closest to the *Bam*HI-*Bam*HI junction. Plasmid pSB432 carrying *ffs* has been described previously (7). Plasmid pMAS49, constructed by M. Sørensen (30a), carries *recA*$^+$ on a plasmid with the replication origin from the low-copy-number plasmid pHSG575 (32). Plasmid pBY03, carrying the *ffh* gene, and plasmid pBY15, with *ffh* deleted from pBY03, were described by Byström and Björk (9, 10).

Growth conditions. Cultures were grown exponentially at 37°C in A+B minimal medium (11) supplemented with either 0.2% glucose, 0.2% arabinose, 0.4% glycerol, or 0.4% acetate as indicated and at least 20 μg of any required amino acid per

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TABLE 1. Strains used in this study

Strain	Genotype (construction)	Source or reference
CGJ42	S1192 *lacY*::Tn*10* mini-tet [S1192 × P1(S1773)]	This work
CGJ117	MAS142 *ffs*::*kan591*/F′ *proAB*$^+$ *lacI*^{q1} Δ*lacZY* pCGJ1 P_{tac}-*ffs*	This work
CGJ142	NF1830 carrying pBY03 (*ffh*$^+$)	This work
CGJ143	NF1830 carrying pBY15 (Δ*ffh*)	This work
CGJ145	WAM113 *sffE47 zjg*::Tn*10* mini-tet [WAM113 × P1(S1582)]	This work
CGJ147	WAM113 *sffE53 zjg*::Tn*10* mini-tet [WAM113 × P1(S1587)]	This work
CGJ173	CGJ142(pBY03)(pSB432)	This work
CGJ175	CGJ143(pBY15)(pSB432)	This work
CGJ195	WAM113 *rpsL*$^+$ *sffA35* [WAM113 × P1(S1235 × P1(S1243))]	This work
CGJ196	S971 Tn*10* mini-tet [S971 × P1(S1243)]	This work
CGJ200	WAM113 *rpsL*$^+$ Tn*10* mini-tet [WAM113 × P1(CGJ196)]	This work
NF1830	*galUK* Δ*lacX74 rpsL thi recA1 araD139* Δ(*araABOIC-leu*)*7679*/F′ *proAB*$^+$ *lacI*^{q1} *lacZ*::Tn*5* *lacY*$^+$	N. Fiil
MAS142	*recA1 thi* Δ(*pro-lac*) *rel*$^+$ carrying pMAS49	M. Sørensen
S971	HfrH *relA1 spoT1 lacI*^{q}	4
S1192	HfrH *relA1 spoT1 lacI*^{q} *ffs*::*kan591* [λ*imm*^{434} *nin5 XhoI*::Φ(P_{tac}-*ffs*)]	4
S1235	S1192 *sffA35*	4
S1243	S1192 *rpsL zhd*::Tn*10* mini-tet	S. Brown
S1582	F$^-$ Δ*lacU169 araD139 rpsL thi sffE47 zjg*::Tn*10* mini-tet	5
S1587	F$^-$ Δ*lacU169 araD139 rpsL thi sffE53 zjg*::Tn*10* mini-tet	5
S1773	F$^-$ *lacI*^{q} *rpsL rpoH30*::*kan ffs*::*kan591 lacY*::Tn*10* mini-tet [λ*imm*^{434} *nin5 Xho*I::Φ(P_{tac}-*ffs*)] pKV3	S. Brown
WAM113	F$^-$ Δ*lacU169 araD139 rpsL thi ffh1*::*kan* λ(*ara-ffh*$^+$ Amp^r) Ara$^+$	24

ml (Table 1). All cultures had been growing exponentially for at least five generations before being used in experiments.

Identification of the positions of proteins on two-dimensional gels. The positions of elongation factors EFG, EFTu, and EFTs had previously been determined (21), and to locate the position of the Ffh protein, we calculated its approximate position in the two-dimensional gel system from its sequence. This position was verified for the nonequilibrium gel system by analyzing labeled extracts from pBY03- and pBY15-carrying cells.

Examination of synthesis of individual proteins. At a cell density (A_{436}) of approximately 0.6, 1 ml of culture was labeled for 2 min with 20 to 40 μCi of carrier-free [^{35}S]methionine. Protein synthesis was stopped by the addition of chloramphenicol to a final concentration of 2.5 mg/ml, and total cell extracts were separated by equilibrium or nonequilibrium (14 h at 170 V) electrophoresis (20). By using autoradiograms of two-dimensional gels, the complete spots of elongation factors EFTs, EFTu, and EFG and of Ffh protein were cut out. For each gel, a background spotless area of the same size was also cut out, and the radioactivity in each spot was determined (22).

To determine the stability of Ffh protein, 325 μCi of [^{3}H]lysine was incorporated in a 13-ml culture of CGJ173 for 10 min. A constant amount of ^{3}H-labeled cells was added to 1 ml of [^{35}S]methionine-labeled samples. ^{35}S-labeled cells were pulse-labeled with [^{35}S]methionine as described above and chased with methionine (100 μg/ml) for the indicated period prior to being mixed. Extracts of such mixtures of ^{3}H- and ^{35}S-labeled cells were separated by two-dimensional electrophoresis, and ^{3}H-to-^{35}S isotope ratios in radioactive spots were determined.

Labeling of RNA. Cells were grown at 37°C in MOPS (morpholinepropanesulfonic acid) medium (19), with either glucose or acetate as the carbon source, supplemented with required amino acids and containing 0.33 mM phosphate and 30 μCi of [^{32}P]phosphate per ml. This is the lowest concentration of phosphate that does not interfere with the growth rate (19). The 4.5S RNA concentration in cells grown in YT medium was also determined (18) by labeling with 0.33 mCi of [^{32}P]phosphate per ml. All cultures were labeled for approximately three generations in medium supplemented with isopropyl-β-D-thiogalactoside (IPTG) as indicated. RNA was extracted by following the procedure described by von Gabain et al. (33) and separated either on polyacrylamide gels as described by Brosius and Holy (3) or under denaturing conditions on gels containing 6 M urea. The resulting abundances of 4.5S and 5S RNAs did not vary with the type of gel used. After autoradiography, the radioactivities in 5S and 4.5S RNA bands and a similar-sized background band were determined.

RESULTS

Concentration of 4.5S RNA. We first determined the molar amounts of 4.5S RNA in wild-type cells and Δ*ffs* P_{tac}-*ffs* cells used for previous genetic studies (4, 5). The latter strain has a normal growth rate even when growing with a low concentration of IPTG. We measured the concentration of 4.5S RNA relative to that of 5S ribosomal RNA by labeling exponentially growing cultures for three generations with [^{32}P]phosphate both in minimal medium and YT medium, in which all genetic experiments were performed. The concentrations of 4.5S RNA per 5S RNA, i.e., per ribosome, were calculated after corrections for the number of nucleotides in the two RNAs were made (8, 13) and are shown in Table 2. These results show that Δ*ffs* P_{tac}-*ffs* cells grow at a normal rate in the presence of only 1/10 of the normal concentration of 4.5S RNA, as expected from the low concentration of IPTG. Strain CGJ117, with P_{tac}-*ffs* on a low-copy-number plasmid, requires IPTG for growth but grows at a normal rate in the presence of 5 μM IPTG even when two-dimensional gel analysis shows that the cells in this condition are in permanent heat shock (data not shown). From the data for CGJ117 in Table 2, we see that basal transcription from the repressed P_{tac} promoter on the low-copy-number plasmid supplies approximately 29 molecules of 4.5S RNA per 10,000 ribosomes. This is not enough to sustain growth. However, a marginal increase in the synthesis rate of 4.5S RNA, to approximately 37 molecules per 10,000 ribosomes (Table 2), results in a normal growth rate. The concentration of 4.5S RNA in wild-type cells is fivefold lower than that found previously (13, 16), but this is still sevenfold above the previously reported concentration of Ffh protein (36).

TABLE 2. Cellular concentrations of 4.5S RNA

Strain (relevant genotype)	Medium	Doubling time (min)	IPTG concn (μM)	4.5S RNA/5S RNA ratio (no. of expts)[a]
S971 (*ffs*$^+$)	Acetate	234	0	0.0518 ± 0.0032 (6)
S971 (*ffs*$^+$)	Glucose	56	0	0.0365 ± 0.0017 (10)
S971 (*ffs*$^+$)	YT	33	0	0.0409 ± 0.0016 (2)
S1192 (P_{tac}-*ffs*)	Glucose	60	100	0.0715 ± 0.0052 (3)
S1192 (P_{tac}-*ffs*)	Glucose	56	10	0.0063 ± 0.0010 (9)
S1192 (P_{tac}-*ffs*)	Glucose	55	0[b]	0.0011 ± 0.0001 (2)
CGJ117 (P_{tac}-*ffs*)	Glucose	56	5	0.0037 ± 0.0011 (3)
CGJ117 (P_{tac}-*ffs*)	Glucose	55	0[b]	0.0029 ± 0.0004 (3)
MAS142 (*ffs*$^+$)	Glucose	48	0	0.0406 ± 0.0022 (4)
WAM113 (*ffs*$^+$)	Arabinose	68	0	0.0371 ± 0.0017 (5)
WAM113 (*ffs*$^+$)	Glucose + arabinose	51	0	0.0302 ± 0.0026 (6)

[a] Calculated from the radioactivity in radioactively labeled bands as described in Materials and Methods, with the standard error of the mean and the number of determinations indicated.

[b] Labeled after the removal of IPTG, and RNA was extracted before the growth rate was affected by a lack of 4.5S RNA.

Concentration of Ffh protein. The position of Ffh protein on an equilibrium two-dimensional gel has previously been reported by Wikström and Björk (36). However, examining the sequence of the Ffh protein (1), we realized that this protein is basic (pI ≈ 10 to 11) and should not focus at equilibrium on these two-dimensional gels. The protein previously identified as Ffh protein was located at an acidic position (pI ≈ 4 to 5) (36). The estimate of the concentration of this acidic protein does not therefore reflect the concentration of Ffh protein. Figure 1 shows the position of Ffh protein as determined by the two-dimensional nonequilibrium gel system described in Materials and Methods, and we determined the amounts of Ffh, EFTu, EFG, and EFTs proteins in cells carrying either plasmid pBY03 (*ffh*$^+$) or pBY15 (Δ*ffh*) (36). The synthesis rates of Ffh are given in Table 3 and were calculated by normalizing to the amounts of the three elongation factors after corrections for the number of methionines in each protein were made. The amounts of these three elongation factors have been determined under two-dimensional gel electrophoresis conditions similar to ours to be one per ribosome for EFTs and EFG and 6.5 for EFTu (21). Table 3 gives the results of one experiment. Taking all six determinations into consideration, we find 110 ± 16 molecules of Ffh protein per 10,000 ribosomes in the strain with only the chromosomal copy of *ffh* and this protein is stable (see below). In addition, we confirmed that the level of the acidic protein previously identified as Ffh protein (36) is elevated significantly by the presence of *ffh* on a high-copy-number plasmid (Fig. 1). The regulation of the synthesis of this protein is therefore affected by the concentration of Ffh. This acidic protein has a gel position that is the same as that calculated from the sequence of the FtsY protein (12), i.e., pI ≈ 4 to 5 and a molecular weight of 54,000, but since the reported apparent molecular weight of FtsY on SDS gels is 92,000 (12), identification of this protein awaits further investigation.

Free Ffh protein is unstable. In the strain carrying *ffh* on pBY03, we initially found 17 times more Ffh protein than in the pBY15-carrying strain. However, this amount was found to decrease with increasing labeling periods. Therefore, we performed a pulse-chase experiment with several chase periods and also tested whether a simultaneously increased concentration of 4.5S RNA could stabilize Ffh protein. The results are shown in Fig. 2. Clearly, the overexpression of Ffh protein causes a large fraction of the protein to be unstable. Figure 2 also shows that the amount of Ffh made in an *ffh* haploid is stable and that the presence of a wild-type *ffs* gene on a high-copy-number plasmid stabilizes a large fraction of the Ffh protein synthesized from pBY03. The labeled amounts of EFTu, indicating the stability of EFTu, are not presented here but vary less than 10% for all time points. We therefore suggest

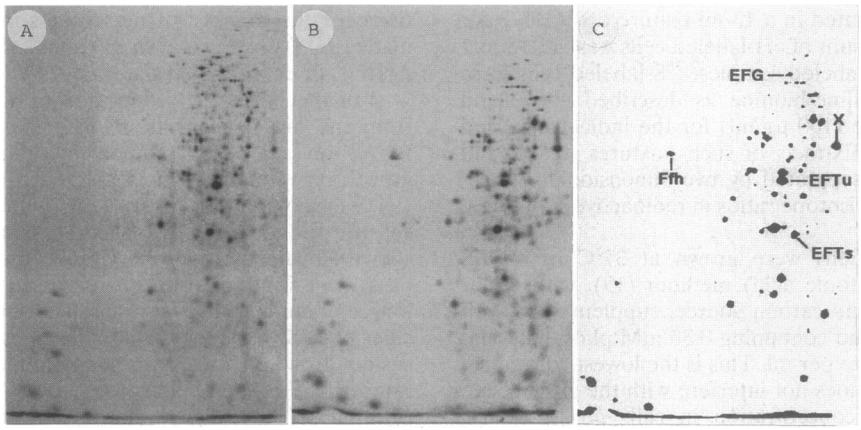

FIG. 1. Localization of Ffh protein on the two-dimensional gel spot pattern. Analysis of proteins labeled for 2 min with carrier-free [^{35}S]methionine for strains CGJ143 (Δ*ffh*) (A) and CGJ142 (*ffh*$^+$) (B). (C) Outline of panel B, with Ffh protein and elongation factors EFG, EFTu, and EFTs indicated. X, the acidic protein overexpressed in cells carrying pBY03.

TABLE 3. Synthesis rates of Ffh protein[a]

Strain (plasmid)	Synthesis rate (cpm)				Ffh/ribosome ratio
	Ffh	EFTu	EFG	EFTs	
CGJ142(pBY03)	3,646	21,768	6,942	3,178	0.3901
CGJ143(pBY15)	59	15,068	4,387	3,102	0.0086
CGJ173(pBY03)(pSB432)	5,002	28,565	ND[b]	5,178	0.3585
CGJ175(pBY15)(pSB432)	86	13,319	4,511	3,300	0.013

[a] The indicated cultures were pulse-labeled for 2 min and chased for 2 min. Proteins were cut out in their entirety, radioactivity was determined, and the amount of Ffh was calculated after corrections for the number of methionine residues (28, 10, 22, and 10 for Ffh, EFTu, EFG, and EFTs, respectively) and the number of EFTu molecules per ribosome (21) were made.

[b] ND, not determined.

that the Ffh protein is stable in the 4.5S RNA-Ffh particle, but that excess is rapidly degraded. The data in Fig. 2 give a first-order decay of free Ffh protein, with a half-life of approximately 20 min if 75 and 5% of the Ffh protein in strains CGJ173 and CGJ142, respectively, are stable.

One or two essential functions of 4.5S RNA? Does 4.5S RNA have only one function, i.e., to complex with Ffh protein, or is there an additional function for free 4.5S RNA? To address this question, we first determined, by labeling with [^{32}P]phosphate as described above, the synthesis rates of 4.5S RNA at various IPTG concentrations in the parental strain used previously for suppressor analysis. The strains able to grow at the lowest 4.5S RNA concentration are the *sff* strains which require only 4.5S RNA resulting from transcription from the repressed P_{tac} promoter (4, 5). Table 2 indicates that this amount is approximately 11 molecules of 4.5S RNA per 10,000 ribosomes.

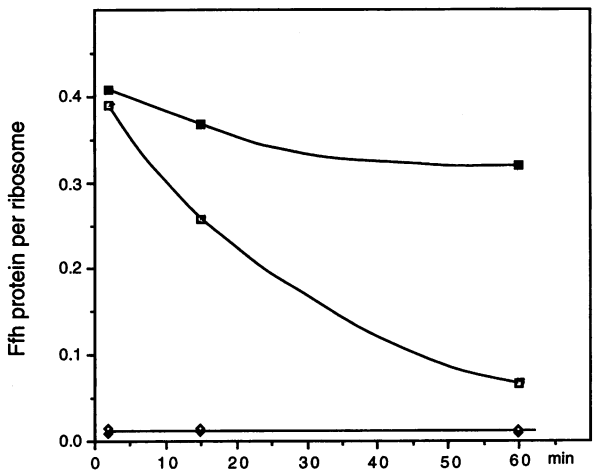

FIG. 2. Stability of Ffh protein. Cultures were pulse-labeled with [^{35}S]methionine for 2 min, and samples were taken as indicated. Before two-dimensional gel electrophoresis, cells labeled with [^{3}H] lysine were mixed into samples to enable the normalization of recovery. The isotope ratios of Ffh and EFTu were determined, and the ratio of Ffh was normalized to the ratio of EFTu. Therefore, each calculated ratio represents the amount of Ffh remaining per ribosome synthesized during the pulse. To calculate the points shown, these relative amounts were multiplied by the synthesis rates of Ffh per ribosome in Table 3. ◆, CGJ175, carrying Δ*ffh* and *ffs*$^+$ plasmids; ◇, CGJ143, carrying the Δ*ffh* plasmid; □, CGJ142, carrying the *ffh*$^+$ plasmid; ■, CGJ173, carrying *ffh*$^+$ and *ffs*$^+$ plasmids.

If the only active form of 4.5S RNA is as a complex with Ffh protein, one might expect suppressors that reduce the requirement for 4.5S RNA also to reduce the requirement for Ffh protein. By transduction with phage P1, we transferred two *sffE* suppressor mutations to strain WAM113, constructed by Phillips and Silhavy (24), in which the synthesis of Ffh protein can be modulated by the concentration of arabinose. In these strains, the requirement for Ffh protein can be measured in two ways. By one method, the requirement for Ffh protein can be estimated by the time it takes from the removal of arabinose (i.e., the end of Ffh protein synthesis) until the growth rate of cells is reduced because of a lack of Ffh protein. The time lags in WAM113 and the two *sffE* strains were found to be very similar (Fig. 3). By another method, the requirement for Ffh protein can be estimated by measuring the efficiency of plating (EOP) on media with and without arabinose. The results are shown in Table 4; the EOPs of *sffE* strains are reduced 5 orders of magnitude by the absence of arabinose, as was the case for the *sff* wild-type strain. The effects of these *sffE* alleles on the EOPs of Δ*ffs* P_{tac}-*ffs* strains in the absence of IPTG were not reported by Brown (5) and are given in Table 4 for comparison. Therefore, we conclude that the *sffE* suppressors of the 4.5S requirement cannot suppress the requirement for Ffh protein.

We also tested one of the EFG suppressors of the 4.5S RNA requirement (4), *sffA35*, for its ability to suppress the Ffh requirement by measuring the EOP on plates with and without arabinose. Approximately 10 times more colonies appear with the *sffA35* strain than with the parent. If the *sffA35* suppressor could suppress this requirement and the basal level of transcription from the *ara* promoter was high enough, an EOP close to 1 would be expected. If the *sffA35* suppressor could suppress this requirement but the basal level of transcription was too low, we would expect to see a significant increase in the number of revertants appearing on plates without arabinose. The arabinose-independent revertants of the *sffA35* strain appear to be of two classes. Twenty percent of the revertants from the *sffA35* strain form large colonies similar to the arabinose-independent colonies from the *sff*$^+$ strain. However, unlike the revertants from the *sff*$^+$ strain, 80% of the revertants from the *sffA35* strain formed slowly growing colonies.

DISCUSSION

The synthesis of 4.5S RNA, like that of rRNA, has previously been shown to be under stringent control (14). Thus, we may expect the synthesis of 4.5S RNA to depend on growth rate, as does that of rRNA. Table 2 shows that this expectation is fulfilled and that the molar amount of 4.5S RNA in wild-type

TABLE 4. 4.5S RNA and Ffh protein requirements of *sff* strains

Strain (relevant genotype)	EOP (no. of expts)[a]
WAM113 (*sff*$^+$)	$(6.3 \pm 1.2) \times 10^{-6}$ (6)
WAM113 (*sffE47*)	$(7.8 \pm 0.9) \times 10^{-6}$ (3)
WAM113 (*sffE53*)	$(8.2 \pm 1.0) \times 10^{-6}$ (6)
WAM113 (*sff*$^+$ *rpsL*$^+$)	$(4.6 \pm 0.9) \times 10^{-6}$ (3)
WAM113 (*ssfA35 rpsL*$^+$)	$(41.6 \pm 5.0) \times 10^{-6}$ (6)
CGJ42 (*sff*$^+$)	3.6×10^{-5} (1)
S1547 (*sffE47*)	0.92 (1)
S1553 (*sffE53*)	0.97 (1)

[a] Relative EOP on broth plates with or without 0.4% arabinose and 0.1 mM IPTG. EOPs are calculated from the number of colonies counted after 2 days of incubation at 30°C.

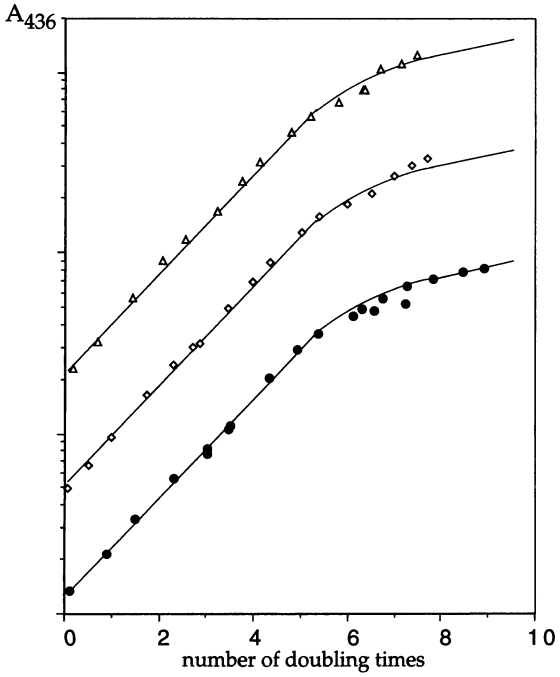

FIG. 3. Concentration of Ffh protein required to sustain growth. Strains were grown at 30°C in broth medium containing arabinose, filtered, and resuspended in broth medium without arabinose at time zero. A_{436} values of cultures are shown on a log scale, displaced by strain for clarity. Symbols: ●, WAM113 (*sffE*$^+$); ◇, CGJ145 (*sffE47*); △, CGJ147 (*sffE53*).

cells is 4% of that of rRNA at the growth rates investigated, that is, between 0.4 and 2 doublings per h. This concentration of 4.5S RNA is fivefold lower than that previously reported by Lee et al. (16) and Hsu et al. (13). Our lower estimate, we believe, might be caused by the fact that the cells in our experiment were growing exponentially and were not phosphate starved. Lee et al. grew cells in 0.2 mM phosphate and to such high cell densities that the phosphate concentration at the time of harvest can be estimated to have been reduced to approximately one-half of that. Such a low phosphate concentration gives rise to a lower growth rate (19) and the degradation of 16S and 23S rRNAs (16) as well as presumably the 5S RNA used by Lee et al. to normalize the number of 4.5S RNA molecules to the number of ribosomes. We are more uncertain as to why our results differ from those of Hsu et al. because we have used essentially the same method. As shown in Table 2, we have many independent determinations of the molar ratio of 4.5S RNA to 5S RNA and the standard error of the mean makes the difference from the results of Hsu et al. significant. We can only suggest that the difference results from the methods used to correct for background radioactivity, i.e., we counted the radioactivity and corrected for the background on the gel while Hsu et al. scanned their autoradiograms. From our determinations of the concentration of 4.5S RNA per ribosome, the amount of 4.5S RNA per ribosome is so small that it seems unlikely that 4.5S RNA is involved in every translation cycle.

The concentration of Ffh protein was previously reported to be approximately 40 molecules per gene (36). However, as described above, the protein in question was not Ffh but another protein, possibly FtsY, located at an acidic position but induced when the *ffh* gene is present on a high-copy-number plasmid. We have now determined the amount of Ffh to be 110 molecules per 10,000 ribosomes, i.e., approximately one-fourth of the molar concentration of 4.5S RNA.

From the time it takes to dilute the concentration of Ffh from the level produced by the fully induced P_{araBAD} promoter to the critical concentration starting to affect growth (Fig. 3), one can estimate the minimal requirement of Ffh protein. The minimal requirement of Ffh protein must be approximately 110 divided by 2^4 to 2^5 (from dilution by cell growth for four to five doublings) plus the contribution from basal P_{ara} transcription, which is almost nonexistent (30), i.e., approximately the same number as the minimal requirement of 4.5S RNA in suppressor strains (Table 2). Previous determinations of EOPs with different IPTG concentrations indicate that levels below 10 μM IPTG are not sufficient for normal growth in the wild-type parent strain from which the *sff* suppressors were isolated (5). The minimal requirement of 4.5S RNA in a wild-type strain is therefore fourfold higher than the minimal requirement of Ffh protein, i.e., approximately 50 molecules per 10,000 ribosomes (37 to 65 molecules per 10,000 ribosomes [Table 2]).

Figure 2 shows that Ffh protein is unstable in a molar concentration exceeding that of 4.5S RNA. Proteins from ribonucleoprotein particles, e.g., ribosomal proteins, are often unstable when synthesized in molar excess (23). The reason for the failure of pSB432 to stabilize all Ffh protein synthesized from pBY03 is most likely that pSB432 is not able to direct the synthesis of sufficient quantities of 4.5S RNA. The observation that this unstable Ffh protein is stabilized by increased synthesis of 4.5S RNA is additional evidence that 4.5S RNA and Ffh protein bind each other, as has been suggested by the genetic data of Brown (6) and the biochemical evidence of Poritz et al. (25) and Ribes et al. (27). Previous measurements indicated that 4.5S RNA is in 30-fold molar excess to Ffh protein. This large difference in concentration suggests that 4.5S RNA has another function in addition to that with Ffh protein. However, our measurements indicate that 4.5S RNA is in only fourfold molar excess to Ffh protein, consistent with the observation of Poritz et al. (25) that approximately one-fourth of 4.5S RNA can be precipitated by anti-Ffh.

Our concentration measurements thus weaken the hypothesis that 4.5S RNA has two functions. However, two observations imply that 4.5S RNA mediates an essential function in addition to the role in complex with Ffh. One is the observation by Brown (6) that increased intracellular concentrations of Ffh increase the required concentration of 4.5S RNA. If 4.5S RNA had only one function, this epistasis by *ffh* on the high-copy-number plasmid pBY03 would not have been observed unless excess Ffh was toxic, for example, by overwhelming a proteolysis pathway. The other is our determination of the 4.5S RNA and Ffh protein requirements of *sff* strains by measuring EOPs on media with and without IPTG and arabinose, respectively. If the only function of 4.5S RNA was in a stable complex with Ffh protein, suppressors of the 4.5S RNA requirement would be expected to be able to suppress the Ffh requirement also. We observe (Table 4) that the *sffE* suppressors fail to suppress the Ffh requirement, whereas the result with the *sffA35* suppressor suggests an alternative interpretation. If the *sffA35* suppressor reduced the requirement for Ffh relative to that of the *sff*$^+$ strain, we would expect more growth to be supported following the withdrawal of arabinose. If the Ffh requirement in the *sffA35* strain was low enough to be maintained by basal transcription from the arabinose promoter, we would see an EOP approaching 1. If the Ffh requirement was even higher than the basal level, we would

expect the EOP to be elevated relative to that of the *sff*$^+$ strain but still low. However, if the elevated EOP was due only to more generations occurring prior to lethal depletion of Ffh, we would expect the *sffA35* strain to generate the same classes of arabinose-independent mutants as the *sff*$^+$ strain. We do observe an increased frequency of the *sff*$^+$ class of revertants, suggesting that *sffA35* reduces the Ffh requirement, but the increase is small and barely significant. What we observe instead with the *sffA35* strain is that a majority of the arabinose-independent mutants are of a different class than those from the *sff*$^+$ strain. This suggests that a second mutation is necessary to allow *sffA35* to reduce the Ffh requirement.

We suggest two possibilities for 4.5S RNA function. 4.5S RNA might have two functions, one complexed to Ffh protein and one independent of Ffh protein. Alternatively, 4.5S RNA and Ffh protein participate in a sequential reaction at the ribosome, where at some point they are complexed to one another. If the *sffA35* suppressor suppressed both of the 4.5S RNA and Ffh requirements, then the sequential reaction would be one in which both 4.5S RNA and Ffh are at the ribosome at the same time as translation factor EFG, whereas only 4.5S RNA is at the ribosome as uncharged tRNAs interact with the ribosome. In the two-function model, the *sffE* suppressor suppresses only one of the functions, whereas the *sffA35* suppressor suppresses both functions. If there is in fact no suppression by the *sffA35* suppressor, then we are unable to distinguish between a two-function model and a sequential reaction. However, it is important to note that since the *sffE* suppressors do not suppress both requirements, it can be excluded that 4.5S RNA has only a function in a stable complex with Ffh.

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REFERENCES

1. **Bernstein, H. D., M. A. Poritz, K. Strub, P. J. Hoben, S. Brenner, and P. Walter.** 1989. Model for signal sequence recognition from amino acid sequence of 54K subunit of signal recognition particle. Nature (London) **340**:482–486.
2. **Bourgaize, D. B., T. A. Phillips, R. A. VanBogelen, P. G. Jones, F. C. Neidhardt, and M. J. Fournier.** 1990. Loss of 4.5S RNA induces the heat shock response and lambda prophage in *Escherichia coli*. J. Bacteriol. **172**:1151–1154.
3. **Brosius, J., and A. Holy.** 1984. Regulation of ribosomal RNA promoters with a synthetic *lac* operator. Proc. Natl. Acad. Sci. USA **81**:6929–6933.
4. **Brown, S.** 1987. Mutations in the gene for EF-G reduce the requirement for 4.5S RNA in the growth of *E. coli*. Cell **49**:825–833.
5. **Brown, S.** 1989. Time of action of 4.5S RNA in *Escherichia coli* translation. J. Mol. Biol. **209**:79–90.
6. **Brown, S.** 1991. 4.5S RNA: does form predict function? New Biol. **3**:430–438.
7. **Brown, S., and M. J. Fournier.** 1984. The 4.5S RNA gene of *E. coli* is essential for cell growth. J. Mol. Biol. **178**:533–550.
8. **Brownlee, G. G., F. Sanger, and B. G. Barrell.** 1967. Nucleotide sequence of 5S ribosomal RNA from Escherichia coli. Nature (London) **215**:735–736.
9. **Byström, A. S., and G. Björk.** 1982. Chromosomal location and cloning of the gene (*trmD*) responsible for the synthesis of tRNA (m^1G)-methyltransferase in *Escherichia coli* K12. Mol. Gen. Genet. **188**:440–446.
10. **Byström, A. S., and G. R. Björk.** 1982. The structural gene (*trmD*) for the tRNA (m^1G)methyltransferase is part of a four polypeptide operon in *Escherichia coli* K12. Mol. Gen. Genet. **188**:447–454.
11. **Clark, B., and O. Maaløe.** 1967. DNA and the division cycle in *Escherichia coli*. J. Mol. Biol. **23**:99–112.
12. **Gill, D. R., G. F. Hatfull, and G. P. C. Salmond.** 1986. A new cell division operon in *Escherichia coli*. Mol. Gen. Genet. **205**:134–145.
13. **Hsu, L. M., J. Zagorski, and M. J. Fournier.** 1984. Cloning and sequence analysis of the *Escherichia coli* 4.5S RNA gene. J. Mol. Biol. **178**:509–531.
14. **Ikemura, T., and J. E. Dahlberg.** 1973. Small ribonucleic acids of *Escherichia coli*. J. Biol. Chem. **248**:5033–5041.
15. **Jensen, C. G., S. Brown, and S. Pedersen.** 1994. Effect of 4.5S RNA depletion on *Escherichia coli* protein synthesis and secretion. J. Bacteriol. **176**:2502–2506.
16. **Lee, S. Y., S. C. Bailey, and D. Apirion.** 1978. Small stable RNAs from *Escherichia coli*: evidence for the existence of new molecules and for a new ribonucleoprotein particle containing 6S RNA. J. Bacteriol. **133**:1015–1023.
17. **Miller, J. D., H. D. Bernstein, and P. Walter.** 1994. Interaction of *E. coli* Ffh/4.5S ribonucleoprotein and FtsY mimics that of mammalian signal recognition particle and its receptor. Nature (London) **367**:657–659.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.
20. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
21. **Pedersen, S., P. L. Bloch, S. Reeh, and F. C. Neidhardt.** 1978. Patterns of protein synthesis in *E. coli*: a catalog of the amount of 140 individual proteins at different growth rates. Cell **14**:179–190.
22. **Pedersen, S., S. Reeh, J. Parker, R. J. Watson, J. D. Friesen, and N. P. Fiil.** 1976. Analysis of the proteins synthesized in ultraviolet light-irradiated *Escherichia coli* following infection with the bacteriophages λ*drif*^{ts}18 and λd*fus*-3. Mol. Gen. Genet. **144**:339–343.
23. **Petersen, C.** 1990. *Escherichia coli* ribosomal protein L10 is rapidly degraded when synthesized in excess of ribosomal protein L7/L12. J. Bacteriol. **172**:431–436.
24. **Phillips, G. J., and T. J. Silhavy.** 1992. The *E. coli ffh* gene is necessary for viability and efficient protein export. Nature (London) **359**:744–746.
25. **Poritz, M. A., H. D. Bernstein, K. Strub, D. Zopf, H. Wilhelm, and P. Walter.** 1990. An *E. coli* ribonucleoprotein containing 4.5S RNA resembles mammalian signal recognition particle. Science **250**:1111–1117.
26. **Poritz, M. A., K. Strub, and P. Walter.** 1988. Human SRP RNA and *E. coli* 4.5S RNA contain a highly homologous structural domain. Cell **55**:4–6.
27. **Ribes, V., K. Römisch, A. Giner, B. Dobberstein, and D. Tollevey.** 1990. *E. coli* 4.5S RNA is part of a ribonucleoprotein particle that has properties related to signal recognition particle. Cell **63**:591–600.
28. **Romisch, K., J. Webb, J. Herz, S. Prehn, R. Frank, M. Vingron, and B. Dobberstein.** 1989. Homology of 54K protein of signal-cognition particle, docking protein and two *E. coli* proteins with putative GTP-binding domains. Nature (London) **340**:478–482.
29. **Schatz, P. J., and J. Beckwith.** 1990. Genetic analysis of protein export in *Escherichia coli*. Annu. Rev. Genet. **24**:215–248.
30. **Schleif, R.** 1987. The L-arabinose operon, p. 1473–1481. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaecter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington D.C.

30a. **Sørensen, M.** 1989. Ph.D. thesis. University of Copenhagen, Copenhagen, Denmark.

31. **Steen, R., D. K. Jemiolo, R. H. Skinner, J. J. Dunn, and A. E. Dahlberg.** 1986. Expression of plasmid-coded mutant ribosomal RNA in *E. coli*: choice of plasmid vectors and gene expression systems. Prog. Nucleic Acid Res. Mol. Biol. **33**:1–18.
32. **Takeshita, S., M. Sato, M. Toba, W. Masahashi, and T. Hashimoto-Gotoh.** 1987. High-copy-number and low-copy-number plasmid vectors for *lacZ* α complementation and chloramphenicol- or

kanamycin-resistance selection. Gene **61**:63–74.
33. **von Gabain, A., J. G. Belasco, J. L. Schottel, A. C. Y. Chang, and S. N. Cohen.** 1983. Decay of mRNA in *Escherichia coli*: investigation of the fate of specific segments of transcripts. Proc. Natl. Acad. Sci. USA **80**:653–657.
34. **Walter, P., and G. Blobel.** 1980. Purification of a membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **77**:7112–7116.
35. **Walter, P., and G. Blobel.** 1983. Disassembly and reconstitution of signal recognition particle. Cell **34**:525–533.
36. **Wikström, P. M., and G. R. Björk.** 1988. Noncoordinate translation-level regulation of ribosomal and nonribosomal protein genes in the *Escherichia coli trmD* operon. J. Bacteriol. **170**:3025–3031.