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How a Mutation in the Gene Encoding σ^{70} Suppresses the Defective Heat Shock Response Caused by a Mutation in the Gene Encoding σ^{32}

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In *Escherichia coli*, transcription of the heat shock genes is regulated by σ^{32}, the alternative sigma factor directing RNA polymerase to heat shock promoters. σ^{32}, encoded by *rpoH* (*htpR*), is normally present in limiting amounts in cells. Upon temperature upshift, the amount of σ^{32} transiently increases, resulting in the transient increase in transcription of the heat shock genes known as the heat shock response. Strains carrying the *rpoH165* nonsense mutation and *supC*(Ts), a temperature-sensitive suppressor tRNA, do not exhibit a heat shock response. This defect is suppressed by *rpoD800*, a mutation in the gene encoding σ^{70}. We have determined the mechanism of suppression. In contrast to wild-type strains, the level of σ^{32} and the level of transcription of heat shock genes remain relatively constant in an *rpoH165 rpoD800* strain after a temperature upshift. Instead, the heat shock response in this strain results from an approximately fivefold decrease in the cellular transcription carried out by the RNA polymerase holoenzyme containing mutant RpoD800σ^{70} coupled with an overall increase in the translational efficiency of all mRNA species.

The heat shock response, characterized by the transient induction of a set of proteins upon temperature upshift, occurs in all organisms. In *Escherichia coli*, about 20 heat shock proteins are transiently induced during the heat shock response. Other stresses, such as bacteriophage infection, ethanol, and DNA damage, also induce these proteins (21), suggesting that the heat shock proteins have a general protective role against stress.

In *E. coli*, transcription of the heat shock genes is under control of σ^{32}, an alternative σ factor encoded by *rpoH* (*htpR*, *hin*) (12, 21, 32). The first indication that this locus encodes the regulator of the heat shock response came from studies on a strain with an amber mutation in the *rpoH* gene (*rpoH165* [*htpR165*]) and a temperature-sensitive tRNA suppressor [*supC*(Ts)]. Such a strain is temperature sensitive for growth and fails to induce the synthesis of heat shock proteins upon temperature upshift (5, 21, 32). Several other alleles of *rpoH* also produce these phenotypes (13, 29). Additional studies demonstrated that *rpoH* encodes a 32-kDa σ factor, called σ^{32}, which directs core RNA polymerase to transcribe heat shock promoters in vitro (6, 12). Strains lacking σ^{32} because of a deletion or insertion in *rpoH* show no transcription from heat-inducible promoters in vivo, indicating that σ^{32} is responsible for transcription from heat shock promoters in vivo as well (34).

The regulation of the heat shock response has been studied extensively. The transient increase in expression of heat shock genes after temperature upshift results from increased transcription initiation at heat shock promoters (6, 28, 31, 32, 33), which is mediated by a transient 20-fold increase in the amount of σ^{32} per cell (17, 26, 27). Two distinct mechanisms govern the transient increase in σ^{32} after temperature upshift. First, under steady-state growth conditions, σ^{32} is a very unstable protein, with a half-time for degradation of only 1 min (27). Immediately after temperature upshift, σ^{32} is transiently stabilized, leading to its accumulation (27). Second, the synthesis of σ^{32} is repressed at the translational level (15, 20, 27). Immediately after temperature upshift, translational repression is transiently lifted, leading to an increase in the rate of synthesis of σ^{32} (15, 20, 27). Therefore, σ^{32} not only directs the core RNA polymerase to transcribe the heat shock genes but also regulates the heat shock response directly.

One way to gain further understanding of how the heat shock response is regulated is to analyze mutations that alter the response. The *rpoD800* mutation, a 14-amino-acid in-frame deletion in σ^{70} (14), encodes an unstable σ^{70} and leads to a temperature-sensitive cell growth phenotype (1, 10, 11, 18). Strains carrying this mutation exhibit a heat shock response of greater magnitude and duration than that of isogenic wild-type strains (9). To explore the mechanism which is responsible for this effect, we examined the influence of the *rpoD800* allele on the heat shock defect of an *rpoH165 supC*(Ts) strain. We found that *rpoD800* restores the heat shock response to this strain and describe studies which led us to conclude that the presence of the *rpoD800* allele allows an additional mechanism of heat shock induction, which differs significantly from the mechanism in wild-type strains.

MATERIALS AND METHODS

Bacteria and plasmids. The *E. coli* strains used in this work are all derivatives of *E. coli* K-12 and are listed in Table 1. The plasmids utilized in this work are listed in Table 2.

Total RNA and protein synthesis. Cells growing in M9-glucose medium containing all of the amino acids except leucine and lysine were pulse-labeled for 1 min by adding 100 μl of exponentially growing cells to 100 μl of identical medium containing either 0.4 μCi of [^{3}H]uridine and 0.2 μg of uridine (for RNA determinations) or 0.25 μCi of [^{3}H] lysine or leucine (for protein determinations). Incorporation

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TABLE 1. Bacterial strains

Strain	Relevant genotype	Origin or reference
SC122	*supC*(Ts) *lacZ*(Am) *trp*(Am) *pho*(Am) *mal*(Am) *rpsL*	5
CAG456	SC122 *rpoH165*	1
CAG481	SC122 *rpoD800 rpoH165*	1
CAG1831	SC122 *galK*	Our laboratory
CAG1832	CAG456 *galK*	Our laboratory
CAG1833	CAG481 *galK*	Our laboratory
CAG1834	CAG510 *galK*	Our laboratory
CAG1880	CAG1831(pKG1900)	Our laboratory
CAG1886	CAG1832(pKG1900)	Our laboratory
CAG1894	CAG1833(pKG1900)	Our laboratory
CAG1901	CAG1834(pKG1900)	Our laboratory
CAG9356	CAG1833(pDC441)	Our laboratory
CAG9357	CAG1834(pDC441)	Our laboratory
CAG9358	CAG1831(pDC441)	Our laboratory
CAG9359	CAG1832(pDC441)	Our laboratory

was terminated by adding 5% trichloroacetic acid. Samples were filtered onto a type GF/C disc and counted.

Rates of synthesis of individual proteins. Aliquots of mid-log-phase cultures growing in M9-glucose medium containing all of the amino acids except methionine were pulse-labeled with 10 μCi of [^{35}S]methionine (800 to 1,000 Ci/mmol) for 1 min and then chased with a 200-fold excess of cold methionine for 1 min. The labeled cultures were precipitated on ice in 5% trichloroacetic acid for at least 30 min. Precipitates were collected by centrifugation at 0°C, washed with 80% acetone, dried, and utilized for further analysis. For qualitative investigations, samples were resuspended in sodium dodecyl sulfate (SDS) sample buffer, equal counts of each sample were subjected to SDS-polyacrylamide gel electrophoresis (16), and the distribution of radioactivity among cellular proteins was visualized following autoradiography of dried gels. To quantify protein synthesis rates, aliquots of experimental cells containing 1×10^6 cpm of [^{35}S]methionine (800 to 1,000 Ci/mmol) were mixed with control cell samples containing 1×10^6 to 2×10^6 cpm of [^{3}H]leucine and lysine, immunoprecipitated with an appropriate polyclonal antibody, resuspended in SDS sample buffer, and analyzed by SDS-polyacrylamide gel electrophoresis. The portion of the gel containing the immunoprecipitate was excised, solubilized, and counted by using a double-label program which corrects for chemiluminescence. The control cells served as a standard to correct for losses during the procedure. The relative rate of synthesis of each protein is expressed as follows (34): (ratio of ^{35}S counts to ^{3}H counts for the individual protein)/(ratio of ^{35}S counts to ^{3}H counts for the total trichloroacetic acid-precipitable protein).

σ^{32} level. The level of σ^{32} in the cells was measured by Western blotting as described by Straus et al. (27). Total protein extracts prepared from trichloroacetic acid-precipitated proteins were separated on SDS-polyacrylamide gels and transferred to nitrocellulose filters. The nitrocellulose filters were blocked by incubation with BLOTTO and were exposed to anti-σ^{32} antibody and then to goat anti-rabbit antibody conjugated with alkaline phosphatase. The blots were developed with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

RNA preparation and S1 nuclease mapping. RNA isolated by the method of Salser et al. (25) was used for S1 nuclease mapping experiments performed by the method of Berk and Sharp (3) as described by Erickson et al. (7). The amount of RNA used in each reaction mixture is indicated in the legend to Fig. 4. Control experiments established that the labeled DNA probe was present in excess. Samples were hybridized overnight at 48°C in deionized formamide, treated with 170 U of S1 nuclease at 37°C for 60 min and analyzed on a 6% polyacrylamide–50% (wt/vol) urea sequencing gel. To quantify various mRNAs, the gel slices which contained the protected transcripts were cut out and counted.

TABLE 2. Plasmids used in this study[a]

Plasmid	Relevant markers	Origin or reference
pKG1900	P*gal-galK*[b]	19
pDC441	P*groE-galK*[c]	6
pS2	Contains the *groES* and *groEL* genes and their promoters	C. Georgopoulos
pdnaK	Contains the *dnaK* and *dnaJ* genes and their promoters	2

[a] All plasmids were derivatives of plasmid pBR322 (4).

[b] The *gal* promoter is contained on an *Eco*RI-*Hin*dIII fragment extending from 500 bp upstream of the transcription start point (position +1) to position +421 in the *galE* structural gene and is inserted by replacing the *Eco*RI-*Hin*dIII fragment of pKO1.

[c] The *groE* promoter is contained on an *Hin*dIII-*Ssp*I fragment extending from 60 bp upstream of the transcription start point (position +1) to position +77 downstream and is inserted in pKO500 between the *Hin*dIII and *Xba*I sites.

RESULTS

***rpoD800* restores the heat shock response to *rpoH165* cells.** We monitored the heat shock response in mutant and wild-type cells by examining the proteins synthesized by cells that had been pulse-labeled at 30°C or at various times after temperature upshift. A strain carrying the *rpoH165* nonsense mutation and the temperature-sensitive suppressor tRNA, *supC*(Ts), has a relatively normal complement of heat shock proteins at 30°C but little or no induction of heat shock proteins after temperature upshift (5, 21, 32) (Fig. 1, lanes 1 through 3). Surprisingly, when the *rpoD800* mutation was introduced into the *rpoH165* strain, the double mutant exhibited a heat shock response (Fig. 1, lanes 4 through 6).

To further characterize the heat shock response in the *rpoD800 rpoH165* double mutant, we measured the rate of synthesis of GroEL, one of the major heat shock proteins in *E. coli*, by immunoprecipitating it from pulse-labeled cells (Fig. 2). The magnitude of GroEL induction in the double mutant was similar to the magnitude in the wild-type strain, with GroEL exhibiting an approximately 10-fold increase in synthesis at the peak of the heat shock response. However, the kinetics of the response in the double mutant were similar to those in the *rpoD800* single mutant, which exhibits a high rate of synthesis of the heat shock proteins for an extended period of time. Similar results were obtained for DnaK, another major heat shock protein (33a).

Level of σ^{32} shows little change in the *rpoD800 rpoH165* strain after temperature upshift. In wild-type cells, the transient increase in heat shock protein expression is mediated by a transient increase in the intracellular concentration of σ^{32} (17, 26, 27). We examined the level of σ^{32} by a Western blotting protocol to determine whether heat shock induction in the double mutant resulted from an increase in the σ^{32} level. Surprisingly, the double mutant, like the heat shock-defective *rpoH165* single mutant, showed little increase in σ^{32} level after the temperature upshift, while the *rpoD800* single mutant showed the dramatic increase in σ^{32} level

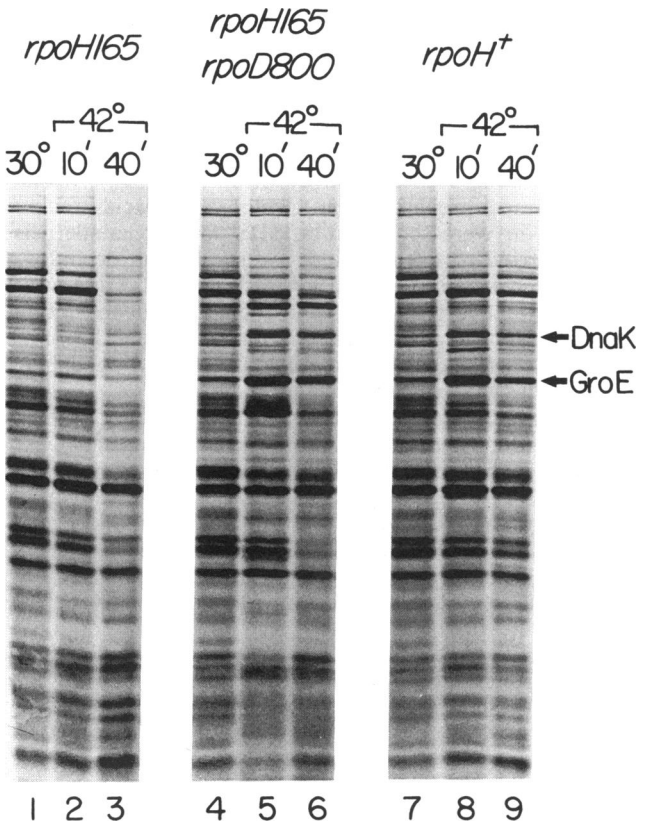

FIG. 1. Heat shock response in various strains. Exponentially growing cells were pulse-labeled with [^{35}S]methionine at 30°C or at the indicated times at 42°C and then analyzed on a 10% SDS–polyacrylamide gel as described in Materials and Methods. The gel was dried and subjected to autoradiography. The arrows indicate the positions of the DnaK and GroE heat shock proteins.

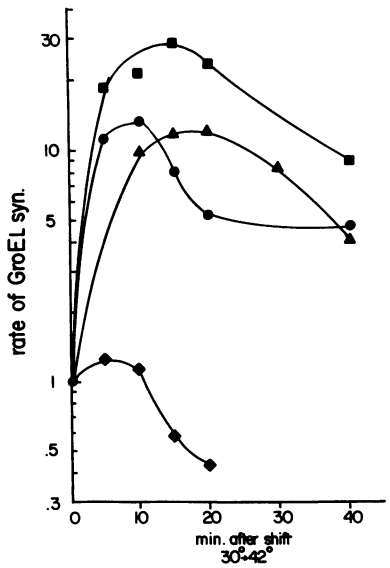

FIG. 2. Rate of synthesis of GroEL protein after temperature upshift. Cells of *rpoD*$^+$ *rpoH*$^+$ (●), *rpoD800 rpoH*$^+$ (■), *rpoD*$^+$ *rpoH165* (◆), and *rpoD800 rpoH165* (▲) strains were pulse-labeled with [^{35}S]methionine at 30°C or at various times after a shift to 42°C, immunoprecipitated with anti-GroEL antibody as described in Materials and Methods, and separated by SDS-polyacrylamide gel electrophoresis. The gels were dried and subjected to autoradiography. The protein bands were excised, solubilized, and counted as described in Materials and Methods. The values for the rates of synthesis (syn.) of GroEL after heat shock are shown relative to the rates of synthesis at 30°C.

characteristic of wild-type cells (Fig. 3A). Quantitative measurements indicated that the *rpoD800 rpoH165* strain exhibited less than a twofold increase in the σ^{32} level after temperature upshift (Fig. 3B). This extent of accumulation is virtually identical to that exhibited by the heat shock-defective *rpoH165* single mutant after temperature upshift (Fig. 3B). Clearly, the heat shock response in the double mutant is not mediated by an increase in the amount of σ^{32}.

Transcription of heat shock genes in *rpoD800 rpoH165* strain is not induced after temperature upshift. Although the amount of σ^{32} does not increase in the double mutant after temperature upshift, the increased rate of synthesis of the heat shock proteins could still reflect increased transcription initiation. Either an increase in the activity of Eσ^{32} (the holoenzyme containing σ^{32}) or transcription by another form of holoenzyme could result in increased transcription of heat shock genes in the double mutant. To investigate this, we used S1 nuclease mapping to examine the nature and amounts of the *groE* and *dnaK* mRNA transcripts both at a low temperature and after temperature upshift. The results of these experiments indicated that the transcripts are characteristic of transcripts that originate from heat shock promoters and that their amounts increase less than twofold after temperature upshift (Fig. 4A and B). Similar results were obtained in S1 nuclease mapping experiments with *htpG*, another heat shock mRNA (data not shown). Thus, the extent of induction in the *rpoH165 rpoD800* double mutant is similar to the extent of induction exhibited by the *rpoH165* single mutant that lacks the heat shock response (Fig. 1 and 2). The small increase in the heat shock transcripts is consistent with the small increase in the level of σ^{32} after temperature upshift in these strains. These findings establish that the mechanism of induction in the double mutant differs from the mechanism in wild-type cells, where induction results from the increased transcription of heat shock genes.

Altered macromolecular synthesis in *rpoD800* strains can explain restoration of the heat shock response in *rpoD800 rpoH165* strains. The effects of temperature upshift on macromolecular synthesis in *rpoD800* strains have been investigated previously (9). These studies indicated that the rate of RNA synthesis, as measured by [^{3}H]uridine uptake, drops immediately upon temperature upshift, while the rate of protein synthesis, as measured by [^{14}C]leucine uptake, remains relatively constant for some time after upshift. The simplest interpretation of these results is that RpoD800σ^{70} is defective in transcription at high temperatures, leading to an overall decrease in Eσ^{70}-mediated transcription and that the cells compensate for the decreased synthesis of mRNA by increasing the translational efficiency of mRNA.

This straightforward interpretation of the *rpoD800* phenotype could provide a simple explanation for the heat shock response of the *rpoH165 rpoD800* double mutant. We have shown that the amount of heat shock transcription stays the same or slightly increases in the double mutant (Fig. 4). If the level of Eσ^{70} transcripts decreased fivefold in the double mutant as a consequence of the *rpoD800* mutation, then the fraction of total RNA as heat shock mRNA would have increased at least fivefold. This, coupled with an increase in

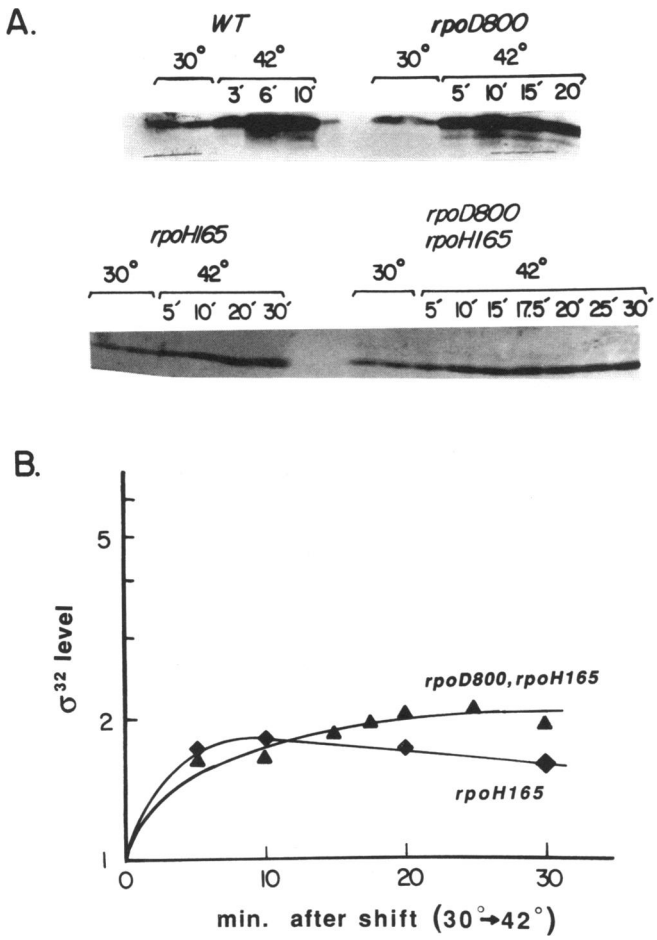

FIG. 3. Measurement of σ^{32} levels in various strains. (A) Equal volumes of cells growing at 30°C or cells at various times after a shift to 42°C were analyzed by Western blotting, using anti-σ^{32} antiserum to visualize the amount of σ^{32}. WT, wild type. (B) σ^{32} levels in *rpoD*$^+$ *rpoH165* (◆) and *rpoD800 rpoH165* (▲) strains before and after temperature upshift were measured by scanning the Western blots shown in panel A as described in Materials and Methods. The levels of σ^{32} after the shift to 42°C were normalized to the level at 30°C.

translational efficiency of all mRNAs, would give an absolute increase in the rate of synthesis of heat shock proteins, leading to an apparent heat shock response in the double mutant. We performed two experiments to determine whether such a mechanism could explain the heat shock response of the double mutant. First, we quantified the overall rates of protein synthesis and RNA synthesis after temperature upshift to determine whether the double mutant shows a protein and RNA synthesis profile similar to that exhibited by the *rpoD800* strain. Second, we analyzed the mRNA level and the rate at which protein was synthesized from an Eσ^{70} transcript and an Eσ^{32} transcript to directly determine whether translatability of mRNAs changes in *rpoD800* strains after temperature upshift. The latter set of experiments is important because it is independent of the uptake of radioactive label and thus controls for a major possible flaw in these experiments. It is known that a decrease in the rate of stable RNA synthesis without a decrease in the rate of synthesis of mRNAs is sufficient to inhibit uptake of ^{3}H-labeled nucleotides (30). If the *rpoD800* strain exhibited selective inhibition of stable RNA synthesis upon temperature upshift, the observed decrease in [^{3}H]uridine uptake would not indicate a corresponding decrease in mRNA synthesis.

The overall rates of RNA synthesis (as measured by uptake of [^{3}H]uridine) and protein synthesis (as measured by uptake of [^{3}H]lysine) at 30°C and after shift to 42°C are shown in Fig. 5. As expected, the *rpoH*$^+$ strain and the *rpoH165* strain with an *rpoD*$^+$ allele both showed slight increases in both the rate of RNA synthesis (Fig. 5A) and the rate of protein synthesis (Fig. 5B) after temperature upshift. In contrast, the *rpoH*$^+$ strain and the *rpoH165* strain with the *rpoD800* mutation both exhibited immediate and severe decreases in the rate of RNA synthesis (Fig. 5A) and either no decrease (*rpoD800 rpoH*$^+$) or less than a 50% decrease (*rpoD800 rpoH165*) in the rate of protein synthesis after temperature upshift (Fig. 5B). If [^{3}H]uridine incorporation is a valid measure of the rate of mRNA synthesis, then the results of these experiments indicate that mRNA synthesis is more severely inhibited than protein synthesis in the *rpoD800 rpoH165* double mutant, as well as in the *rpoD800* single mutant, after temperature upshift. Assuming that this is true, a comparison of the ratio of protein synthesis to RNA synthesis suggests that the general translation efficiency of overall mRNA increases about fourfold after temperature upshift in *rpoD800* strains but remains unchanged in *rpoD*$^+$ strains (Fig. 5C). We found that overall RNA synthesis is more inhibited in the *rpoH165 rpoD800* strain than in the *rpoD800* single mutant. Immediately after the temperature upshift, a significant amount of the total mRNA synthesis in wild-type strains is from heat shock promoters. The fact that the *rpoH165* strain does not increase transcription of heat shock mRNAs after temperature upshift could account for the increased inhibition of total RNA synthesis in the *rpoH165 rpoD800* double mutant.

To provide an independent measure of the mRNA translation efficiency in *rpoD800* strains, we determined the amounts of mRNA and the rates of protein synthesis with an Eσ^{70} (non-heat shock) transcript and an Eσ^{32} (heat shock) transcript before and after temperature upshift. The P*gal* promoter directing transcription of the galactokinase (*galK*) structural gene served as the Eσ^{70} model transcript, while a similar P*groE-galK* fusion construction served as the Eσ^{32} model transcript. The amount of each mRNA was quantified by S1 nuclease mapping, and the rate of translation of this mRNA was quantified by immunoprecipitating galactokinase from pulse-labeled cells.

The results of measurements on the Eσ^{70} P*gal-galK* transcript are in general agreement with the idea that the amount of protein synthesized from a given amount of mRNA increases after temperature upshift in *rpoD800* strains but not in *rpoD*$^+$ strains (Table 3). After temperature upshift, the amount of the P*gal-galK* transcript decreases three- to fourfold in both the *rpoD800* and *rpoD800 rpoH165* mutant strains, while the rate of galactokinase synthesis remains relatively constant. Thus, in *rpoD800* strains, the amount of galactokinase synthesized per minute from a given amount of the Eσ^{70} P*gal-galK* transcript increases three- to fourfold after temperature upshift. Our measurements indicate that the single and double mutants are not identical in this regard. Increased translation of the P*gal-galK* transcript is maximal within 10 min after upshift in the *rpoD800 rpoH165* strain but increases more slowly in the *rpoD800* strain. It is conceivable that the more severe inhibition of RNA synthesis in the double mutant after temperature upshift accounts for this difference.

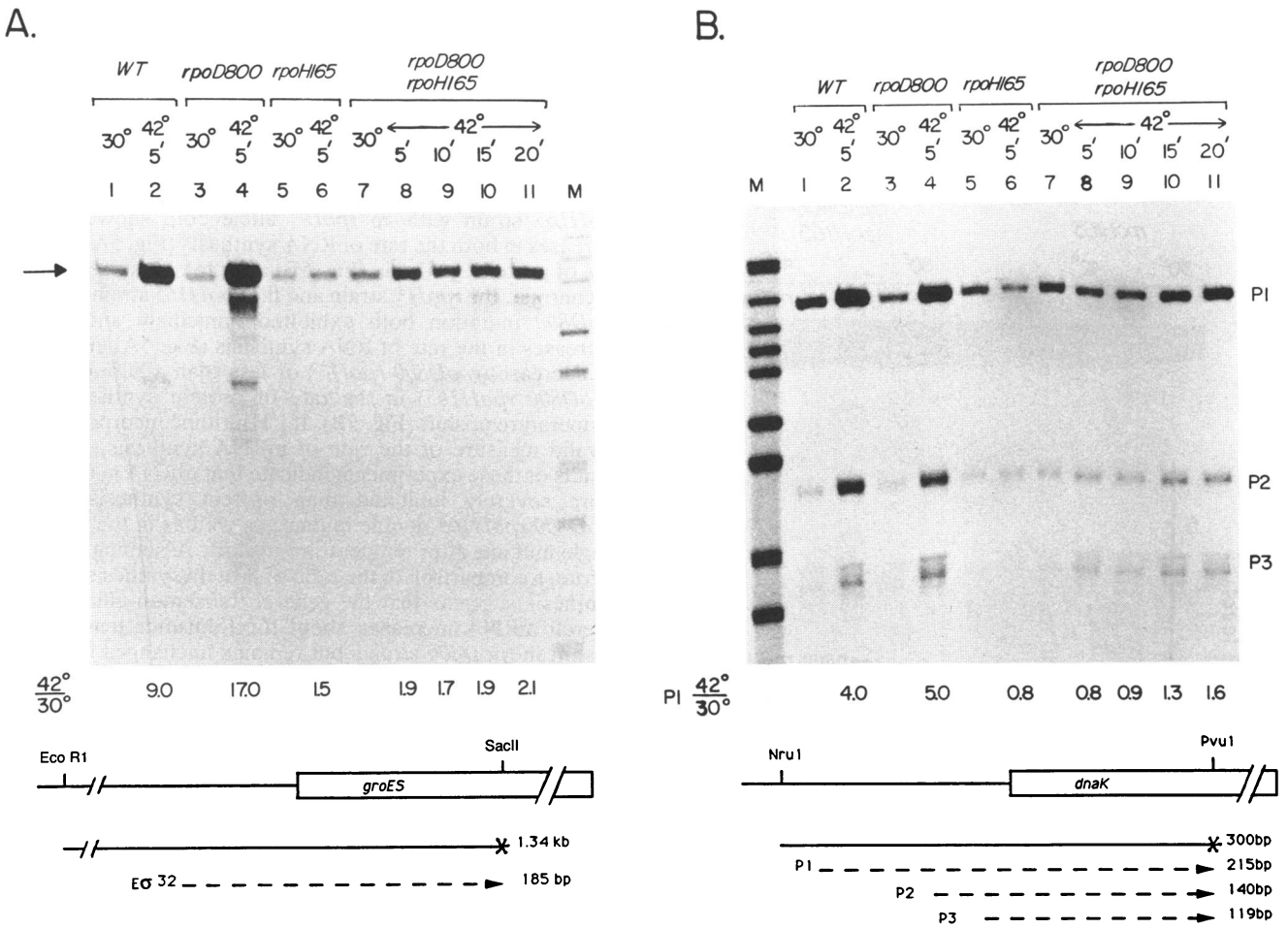

FIG. 4. S1 nuclease mapping of the 5′ ends of heat shock gene transcripts from different strains. The schematic diagrams at the bottom illustrate the relevant genes and restriction sites. The solid lines below the restriction maps indicate the probes used for S1 nuclease mapping. The dashed lines indicate the transcripts. Portions (40 μg) of RNAs isolated from cells at 30°C or from cells at various times after a shift to 42°C were hybridized to an excess of DNA probe and subjected to S1 nuclease mapping as described in Materials and Methods. (A) *groES* mRNA. A 1.34-kb *groE*-specific probe that was 5′ end labeled at the *Sac*II site was used. The arrow indicates the position of the *groE* transcript. Below each lane 42° is a number indicating the fold increase in mRNA level compared with the 30°C level. The RNA bands were cut from the gel and counted in a scintillation counter. The fold increase is expressed as follows: (disintegrations per minute for the 42°C band)/(disintegrations per minute for the 30°C band). (B) *dnaK* mRNA. A 300-bp probe that was 5′ end labeled at the *Pvu*I site was used. The positions of the three RNA transcripts of the *dnaK* promoter are indicated. The quantitative data presented below lanes 42° were obtained as described above. Only data for *dnaK* P1 are shown. For abbreviations see the legend to Fig. 3.

The results of measurements on the $E\sigma^{32}$ P*groE-galK* transcript suggest that the increase in the efficiency of mRNA translation observed for an $E\sigma^{70}$ transcript in *rpoD800* strains after temperature upshift extends to an $E\sigma^{32}$ transcript as well since strains exhibited an increase in the rate of galactokinase synthesis without a concomitant increase in the level of *galK* mRNA (Table 4). However, the situation is somewhat complex. In the *rpoD800* strain background, the $E\sigma^{32}$ transcript exhibited a two- to threefold increase in translation efficiency, which is somewhat less than the three- to fivefold increase exhibited by the $E\sigma^{70}$ transcript. Conversely, in the *rpoD800 rpoH165* strain background, the $E\sigma^{32}$ transcript exhibited a 10- to 12-fold increase in translation efficiency, which is somewhat greater than the 5-fold increase exhibited by the $E\sigma^{70}$ transcript. These differences do not result from random experimental error, as the values which we obtained were quite reproducible from experiment to experiment.

DISCUSSION

We have studied the mechanism by which *rpoD800*, a small in-frame deletion in the gene encoding σ^{70}, suppresses the heat shock defect of a strain carrying the *rpoH165* nonsense mutation in the gene encoding σ^{32} and a temperature-sensitive suppressor tRNA, *supC*(Ts). In wild-type cells the heat shock response results from a transient increase in the rate of transcription of the heat shock genes, which is mediated by a transient accumulation of σ^{32}. In contrast, neither increased transcription of heat shock genes nor accumulation of σ^{32} is characteristic of the heat shock response in the *rpoD800 rpoH165* strain. Instead, the heat shock response appears to result from a combination of two changes in macromolecular synthesis caused by the *rpoD800* allele. First, the levels of cellular transcripts made by $E\sigma^{70}$ decrease after temperature upshift in strains containing the *rpoD800* mutation. As a consequence, in the *rpoD800*

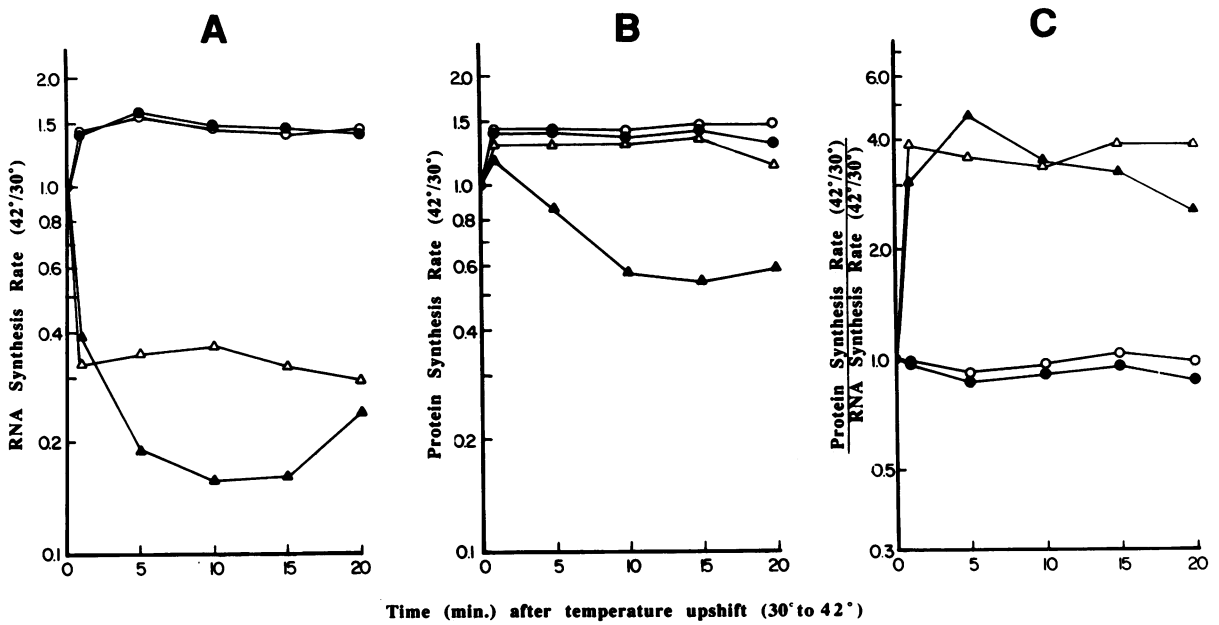

FIG. 5. Kinetics of RNA synthesis and protein synthesis after temperature upshift. Exponentially growing cells of strains CAG440 (*rpoD*$^+$ *rpoH*$^+$) (○), CAG510 (*rpoD800 rpoH*$^+$) (△), CAG456 (*rpoD*$^+$ *rpoH165*) (●), and CAG481 (*rpoD800 rpoH165*) (▲) were pulse-labeled with [^{3}H]uridine for RNA synthesis and with [^{3}H]lysine for protein synthesis at 30°C or at various times after a shift to 42°C as described in Materials and Methods. The rate of synthesis after the shift to 42°C was normalized to the rate of synthesis at 30°C. (A) Rate of total RNA synthesis. (B) Rate of total protein synthesis. (C) Translation efficiency expressed as the ratio of rate of total protein synthesis to rate of total RNA synthesis.

rpoH165 strain, Eσ^{32} transcripts, whose levels remain constant or increase slightly after temperature upshift, become a larger fraction of the total mRNA pool. Second, the overall translation efficiency of mRNA increases after upshift in *rpoD800* strains. As a consequence, in the *rpoD800 rpoH165* strain, the absolute amount of heat shock proteins increases after temperature upshift, resulting in an apparent heat shock response.

Two different kinds of evidence led us to the view that the translation efficiency of most mRNA species increases in *rpoD800* strains after temperature upshift. First, direct measurements of the rates of RNA synthesis and protein synthesis in *rpoD800* strains, obtained from the rates of incorporation of radioactive precursors, indicated that the overall rate of RNA synthesis declined but the overall rate of protein synthesis did not. After temperature upshift, three- to fourfold more protein appeared to be synthesized per given amount of mRNA than before the upshift (Fig. 6A). To verify that the decrease in incorporation of radioactive RNA precursors really reflected a decrease in the rate of mRNA synthesis, we measured the amount of a representative Eσ^{70} mRNA before and after temperature upshift by using an S1 nuclease mapping protocol. Consistent with the expectations from measurements of the overall rate of RNA synthesis, the

TABLE 3. *galK* mRNA levels and rates of galactokinase protein synthesis from P*gal-galK* after temperature upshift

Strain (genotype)	Time (min) at 42°C	Ratio of *galK* mRNA level at 42°C to *galK* mRNA level at 30°C[a]	Ratio of rate of GalK synthesis at 42°C to rate of GalK synthesis at 30°C[b]	Translatability[c]
CAG510	10	0.24 ± 0.04	0.41 ± 0.09	1.71
(*rpoD800 rpoH*$^+$)	20	0.27 ± 0.04	1.16 ± 0.02	4.30
	40	0.37	2.10	5.68
CAG481	10	0.24 ± 0.02	1.13 ± 0.07	4.71
(*rpoD800 rpoH165*)	20	0.27 ± 0.03	1.15 ± 0.15	4.26
	40	0.34	1.60	4.71
CAG456	10	0.34 ± 0.03	0.77 ± 0.03	2.26
(*rpoD*$^+$ *rpoH165*)	20	0.35 ± 0.04	0.57 ± 0.06	1.63
	40	0.57	0.83	1.46
SC122	10	0.68 ± 0.04	0.74 ± 0.04	1.09
(*rpoD*$^+$ *rpoH*$^+$)	20	0.80 ± 0.01	0.94 ± 0.04	1.18
	40	0.89	0.85	0.96

[a] mRNA levels were measured by S1 nuclease mapping, as described in Materials and Methods.
[b] The rate of galactokinase (GalK) synthesis was determined by immunoprecipitation, as described in Materials and Methods.
[c] Translatability was determined as follows: (ratio of rate of galactokinase synthesis at 42°C to rate of galactokinase synthesis at 30°C)/(ratio of *galK* mRNA level at 42°C to *galK* mRNA level at 30°C).

TABLE 4. *galK* mRNA levels and rates of galactokinase protein synthesis from P*groE-galK* after temperature upshift

Strain (genotype)	Time (min) at 42°C	Ratio of *galK* mRNA level at 42°C to *galK* mRNA level at 30°C[a]	Ratio of rate of GalK synthesis at 42°C to rate of GalK synthesis at 30°C[b]	Translatability[c]
CAG510	10	13.2 ± 1.76	19.3 ± 4.20	1.46
(*rpoD800 rpoH*$^+$)	20	9.10 ± 0.90	19.0 ± 5.20	2.09
	40	6.20 ± 0.53	18.0 ± 2.90	2.90
CAG481	10	2.50 ± 0.57	14.2 ± 0.90	5.68
(*rpoD800 rpoH165*)	20	1.90 ± 0.27	17.4 ± 1.30	9.16
	40	1.20 ± 0.35	12.9 ± 1.70	10.8
CAG456	10	2.30 ± 0.20	4.00 ± 0.55	1.74
(*rpoD*$^+$ *rpoH165*)	20	1.70 ± 0.10	3.30 ± 0.60	1.94
	40	1.10 ± 0.13	2.00 ± 0.50	1.82
SC122	10	12.6 ± 1.22	13.5 ± 4.55	1.07
(*rpoD*$^+$ *rpoH*$^+$)	20	12.6 ± 1.50	9.70 ± 2.90	0.77
	40	4.40 ± 0.76	5.70 ± 1.36	1.30

[a] mRNA levels were measured by S1 nuclease mapping, as described in Materials and Methods.
[b] The rate of galactokinase (GalK) synthesis was determined by immunoprecipitation, as described in Materials and Methods.
[c] Translatability was determined as follows: (ratio of rate of galactokinase synthesis at 42°C to rate of galactokinase synthesis at 30°C)/(ratio of *galK* mRNA level at 42°C to *galK* mRNA level at 30°C).

steady-state amount of P*gal-galK* transcript decreased two- to threefold after temperature upshift. This result validates our assumption that the decreased rate of uptake of uridine in *rpoD800* strains reflects a decrease in the rate of synthesis of all RNA species rather than a preferential decrease in the rate of stable RNA synthesis. Moreover, since the rate of galactokinase synthesis per unit of mRNA increases at high temperatures, these measurements confirm our hypothesis that translational efficiency increases in *rpoD800* strains (Fig. 6B and Table 3). Our results indicate that there is a two- to fourfold increase in translation efficiency for the P*gal-galK* transcript after temperature upshift, in remarkable agreement with the increase in translation efficiency which we calculated from the overall incorporation data. The exact increase in translatability for the P*gal-galK* transcript depended on the time after upshift and whether the single mutant or the double mutant was assayed. Taken together, these measurements suggest that mRNA synthesis by Eσ^{70} decreases in *rpoD800* strains after temperature upshift, while translation efficiency increases concomitantly.

Our model for the mechanism of the heat shock response requires that Eσ^{32} transcripts also show an increase in translation efficiency in *rpoD800* strains. Therefore, we determined the amount of the Eσ^{32} P*groE-galK* transcript by S1 nuclease mapping and the rate of galactokinase synthesis of the transcript to determine the translation efficiency of this transcript. Our results indicated that the translation efficiency of Eσ^{32} transcripts also increases (Fig. 6C and Table 4C). Moreover, the increase in translation efficiency which we calculated from our data on the Eσ^{32} transcript is in quantitative agreement with the effects of the *rpoD800* allele on the expression of heat shock proteins in both the *rpoD800* strain and the *rpoD800 rpoH165* strain. The gradual two- to threefold increase in translational efficiency in the *rpoD800* strain after temperature upshift suggested by our measurements on P*groE-galK* accounts for both the change in magnitude and the kinetics of the heat shock response in this strain. Likewise, the 10- to 12-fold increase in translational efficiency observed for P*groE-galK* in the *rpoD800 rpoH165* strain is sufficient to account for the heat shock response observed in that strain background. This strong agreement between the observed heat shock response and the heat shock response predicted from the changes in translational efficiency which we calculated provides strong support for the idea that our model is correct.

However, a careful comparison of the translatability of P*gal-galK* with the translatability of P*groE-galK* in the *rpoH*$^+$ *rpoD800* and *rpoH165 rpoD800* strain backgrounds suggests that additional factors modulate the translatability of heat shock mRNAs. Except for the more rapid onset of increased translational potential in the *rpoH165 rpoD800* strain, the P*gal-galK* construction is translated equivalently in both strain backgrounds (Table 3 and Fig. 6B). In contrast, the P*groE-galK* construction is translated only half as well as the P*gal-galK* construction in the *rpoH*$^+$ *rpoD800* strain and twice as well as the P*gal-galK* construction in the *rpoH165 rpoD800* strain, leading to an approximately fourfold difference in translatability of P*groE-galK* in the two strain backgrounds (Table 4 and Fig. 6C). One possible origin for these effects could be differences in heat shock protein synthesis in the two strain backgrounds. Strains containing the *rpoD800* mutation have 1.5- to 2-fold more heat shock proteins and a more pronounced heat shock response than the wild-type strain (9) (Table 4). This oversupply of heat shock proteins could inhibit expression of heat shock mRNAs, while an undersupply of these proteins could specifically enhance expression of heat shock mRNAs. Alternatively, some other metabolic difference between these two strains could be responsible for the effect. However, such a model requires heat shock and non-heat shock mRNAs to be distinguished in some manner.

Below, we consider the origin of the effects of the *rpoD800* allele on gene expression. Although RpoD800σ^{70} is unstable at high temperatures, the immediate decreases in RNA synthesis observed in *rpoD800* and *rpoD800 rpoH165* strains probably do not result from decreased amounts of σ^{70}. In *rpoD800*, increased synthesis of σ^{70} as a consequence of the heat shock response compensates for the instability of σ^{70} (9), while in the *rpoD800 rpoH165* strain the presence of the *rpoH165* mutation stabilizes the mutant σ^{70} (1). Instead, some defect in RpoD800σ^{70}, exhibited preferentially after temperature upshift, is likely to be the cause of the decreased Eσ^{70} transcription. Elsewhere (33b), we have spec-

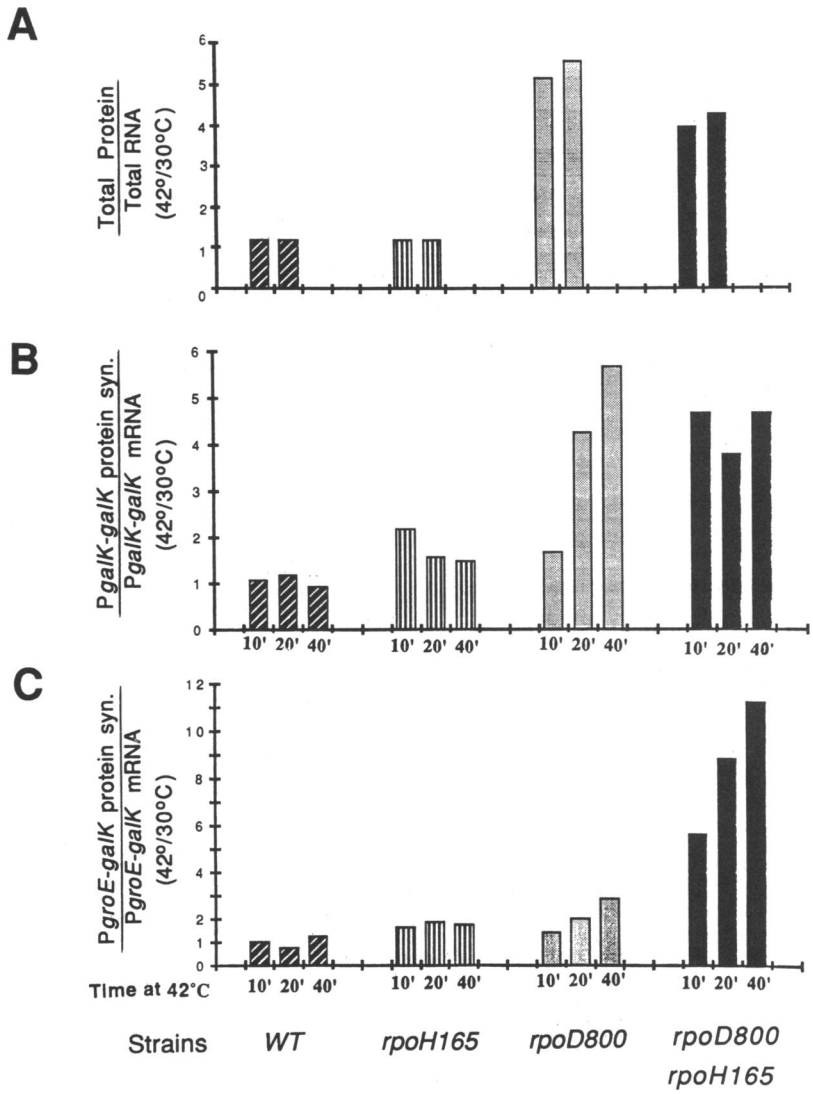

FIG. 6. Comparison of the translation efficiencies of different strains after temperature upshift. Translation efficiency is expressed as the rate of protein synthesis per mRNA molecule at 42°C normalized to that at 30°C. (A) Translation efficiency of total RNA. Data replotted from Fig. 5C. (B) Translational efficiency of *galK* transcripts from P*gal-galK*. Data replotted from Table 3. (C) Translational efficiency of heat shock transcripts from P*groE-galK*. Data replotted from Table 4. WT, wild type; syn., synthesis.

ulated that the *rpoD800* mutation may create a σ^{70} with decreased binding affinity for core RNA polymerase. Such a defect in the mutant sigma could account for the decreased transcription in these strains immediately after temperature upshift.

The increase in translation efficiency of all mRNA species in *rpoD800* strains may simply be a consequence of the abrupt decreases in the mRNA contents of the cells. There is precedent for this suggestion. When the cellular concentration of RNA polymerase is decreased by a factor of two, total mRNA synthesis declines proportionately while protein synthesis remains unchanged (22). Likewise, when the mRNA pool is decreased by adding rifampin, a similar, but less dramatic increase in translation efficiency is observed (24). However, the reason why a decrease in the amount of mRNA results in an increase in translation efficiency is not clear. In these situations, the number of ribosomes per mRNA molecule increases rapidly until it is severalfold higher than the preshift ratio. There have been many papers which suggest that, throughout the normal growth range, ribosomes are used at a constant efficiency in translation (23). By decreasing the amount of mRNA per cell, the *rpoD800* mutation effectively increases the number of ribosomes per mRNA molecule. If ribosomes are limiting for translation, the expected consequence would be to increase the translatability of each remaining mRNA molecule. This is in fact what we observe. However, the results of other experiments argue against this interpretation. Increasing the number of ribosomes per mRNA molecule by a factor of two does not increase translation (8), suggesting that ribosomes themselves may not limit translation. It has been suggested that initiation is the rate-limiting step in translation (8, 22); perhaps some initiation factors are limited from reaching maximum translation efficiency in balanced growing cells. It may be this reaction which increases when the level of mRNA decreases.

In summary, we have determined the mechanism of heat shock in an *rpoD800 rpoH165* mutant. Our results suggest that heat shock results from a selective decrease in $E\sigma^{70}$ transcripts coupled with increased translation of the reduced amounts of mRNA in the cells. These results suggest that under balanced growth conditions, most mRNA molecules are not translated at maximal efficiency. It remains to be determined why this is the case.

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