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Posttranscriptional Autoregulation of *Escherichia coli* Threonyl tRNA Synthetase Expression In Vivo

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Five mutations in *thrS*, the gene for threonyl-tRNA synthetase, have been characterized, and the sites of the mutations have been localized to different regions of the *thrS* gene by recombination with M13 phage carrying portions of the *thrS* gene. Quantitative immunoblotting shows that some of these mutations cause the overproduction of structurally altered threonyl-tRNA synthetase in vivo. The amounts of in vivo *thrS* mRNA as measured by quantitative hybridization are, however, the same as wild-type levels for each mutant. These results demonstrate that the expression of threonyl-tRNA synthetase is autoregulated at the posttranscriptional level in vivo.

Aminoacyl-tRNA synthetases are a particularly important and interesting class of enzymes with respect to both enzymology and control of gene expression. Since these enzymes aminoacylate tRNAs, they probably play a role in processes sensitive to tRNA aminoacylation (i.e., the stringent response and the attenuation of transcription). Little is known, however, about how the concentrations of aminoacyl-tRNA synthetases themselves are controlled. Neidhardt and his co-workers showed that aminoacyl-tRNA synthetase levels increase with increases in cell growth rate (18), and it is known that the levels of some aminoacyl-tRNA synthetases undergo transient, or in some cases permanent, derepression when starved for their cognate amino acids (16). More recently, alanyl-tRNA synthetase was shown in vitro to control its own expression at the level of transcription initiation (24). Phenylalanyl-tRNA synthetase expression is also controlled at the transcriptional level, but in this case by an attenuation mechanism mediated by the level of charged $tRNA^{Phe}$ (4, 27).

The gene (*thrS*) for threonyl-tRNA synthetase (ThrRS) is located at 38 min on the *Escherichia coli* chromosome (5). The stop codon of *thrS* is followed three nucleotides later by the initiation codon of the gene for translation initiation factor IF3 (*infC*) (12). A multicopy plasmid which carries *thrS* as well as *pheS* and *pheT* (the genes for the small and large subunit of phenylalanyl-tRNA synthetase, respectively) overproduces phenylalanyl-tRNA synthetase and ThrRS in a ratio of 10 to 1 in vivo (21), yet the amounts of mRNA for each synthetase are the same (22). In an in vitro transcription translation system the addition of ThrRS specifically inhibits ThrRS synthesis, but has no effect on the synthesis of *thrS* mRNA (11). These results suggest that the expression of *thrS* may be regulated posttranscriptionally. This hypothesis is supported by experiments which demonstrate that the expression of protein fusions, but not operon fusions, between *thrS* and *lacZ* is derepressed in two *thrS* mutants which overproduce defective ThrRS (25a).

The use of gene fusions is, however, only an indirect way of monitoring gene expression. Thus we decided to measure directly the quantities of ThrRS and *thrS* mRNA in vivo in five *thrS* mutants. The results of the measurements show that the overproduction of ThrRS in some of these mutants is not a result of derepression of transcription of *thrS*. Thus, it is likely that the expression of *thrS* is translationally autoregulated in vivo.

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MATERIALS AND METHODS

Bacterial strains and general methods. The *E. coli* strains used in this work are listed in Table 1. The structure of pB21 is described by Plumbridge and Springer (21). General genetic techniques are described elsewhere (27). Restriction enzymes were either from Boehringer Mannheim or New England Biolabs and were used according to the recommendations of the manufacturer. [5,6-^{3}H]uridine ([^{3}H]U) (43 Ci/mmol) was purchased from Amersham.

Isolation of *thrS* thermosensitive mutants. P1 *vir*, grown on strain YMC, was mutagenized with hydroxylamine as described by Murgola and Yanofsky (15) until the survival (in PFU) was about 10^{-4} and the transduction capacity of the lysate (as measured by the transduction of pps^- to pps^+) was reduced to about 1%. After mutagenesis the phage were dialyzed against P1 buffer (14) and stored at 4°C. Transduction with the mutagenized phage was performed (14) using IBPC4901 (λ) (a pps^- strain not able to grow on pyruvate or lactate) and minimal A plates (14) supplemented with 0.2% sodium pyruvate, 50 μg of arginine per ml, 50 μg of histidine per ml, and 200 μg of proline per ml (S plates). Transductions were performed at 30°C, and transductants were tested for growth on S plates at 30 and 42°C. Strains with impaired growth at high temperature were purified twice and screened for complementation with phage λ *B*1 (23), which carries *thrS* as well as *infC*, *rplT*, and *pheST*.

Genetic mapping of *thrS* mutations. We wanted to map *thrS* mutations by recombination with the male-specific phage M13 carrying parts of the *thrS* gene. Thus it was necessary to introduce an F factor into each mutant. Overnight cultures were crossed with strain TT628 (2) as described by Miller (14), except that the mating was allowed to occur for 3 h at 30°C. Tetracycline-resistant recombinants were selected on glucose minimal plates supplemented with arginine, histidine, and proline, each at 50 μg/ml, tetracycline at 20 μg/ml, and, for strain IBPC4771, threonine at 50 μg/ml.

Each F^+ derivative was infected with a different M13 phage at a multiplicity of infection of 100. After incubation at 30°C for 10 min, the mixtures were diluted with 1 ml of LB broth supplemented with tetracycline (10 μg/ml) and streptomycin (25 μg/ml) and grown with shaking at 30°C for 48 h. The mixtures were then diluted and plated on LB plates at

TABLE 1. Bacterial strains

Strain	Relevant genotype	Source (reference)
IBPC4901(λ)	F⁻ *thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 pps xyl-5 tsx-29 supE44 rpsL*, λ⁺ lysogen, λˢ	This work
MHB4	F⁻ *thi-1 argE3 his-4 proA2 lacY1 galK2 m[illegible] l xyl-5 tsx-29 supE44 rpsL thrS204*, λ⁺ lysogen, λˢ	This work
MHB19	F⁻ *thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44 rpsL thrS219*, λ⁺ lysogen, λˢ	This work
MHB35	F⁻ *thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44 rpsL thrS235*, λ⁺ lysogen, λˢ	This work
MHC37	F⁻ *thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44 rpsL thrS337*, λ⁺ lysogen, λˢ	This work
IBPC4771	F⁻ *thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44 rpsL thrS1029*, λ⁺ lysogen, λˢ	By P1 transduction from strain GT302 (8)
IBPC1365	F⁻ *thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx29 supE44* λ⁻	(22)
TT628	*pyrC rpsL*/F′ ts114 *lac*⁺ *zzF-21*::Tn*10*	(2)
JM101	Δ(*lac-pro*) *supE thi*/F⁻ *traD36 proAB lacI*ᵠ *lacZΔM15*	(13)
YMC	*supF lacY*	(3)

44°C (strains MHB19, MHC37, and MHB35) or on Glu Arg His Pro plates at 42°C (strain MHB4) and at 37°C (strain IBPC4771). Mixtures were also plated on the same media at 30°C or, in the case of strain IBPC4771, on the same medium plus threonine at 37°C. The number of colonies under these conditions is considered as the number of viable cells.

Determination of cellular levels of ThrRS and IF3. Mutant and wild-type strains were grown in LB broth at 30°C to approximately A_{650} = 0.5. The cells were lysed by the addition of sample buffer containing sodium dodecyl sulfate (SDS) (9), and the proteins were separated by electrophoresis on 15 or 12.5% polyacrylamide-SDS gels. The proteins were then electrophoretically transferred to nitrocellulose paper (6). The nitrocellulose paper was treated with antibodies to ThrRS or IF3, followed by treatment with ^{125}I-labeled *Staphylococcus aureus* protein A. The position of ThrRS and IF3 on the paper was determined by autoradiography, the appropriate portions were cut out, and the amount of bound ^{125}I was determined in a gamma radiation counter. In each case, doubling the amount of protein loaded on the gel resulted in a doubling of the ^{125}I-protein A bound to the nitrocellulose paper.

Construction of M13 probes. Plasmid pB1 (21), which carries all of *thrS* and *infC*, was digested with *Hpa*I and added to M13mp8 cut by *Hin*dII. The DNAs were ligated and used to transform strain JM101. White or light-blue plaques were picked and purified three times, and the identity and orientation of the insert were determined by restriction endonuclease digestion. All of the probes shown in Fig. 1, except plasmid M13mp8H$_{54}$, were found in this way and were the generous gift of G. Fayat and M. Panvert. Attempts to clone the *Eco*RI-*Hin*dIII fragment of plasmid M13mp8H$_{45}$ into the *Eco*RI-*Hin*dIII site of M13mp9 to give the insert in the opposite orientation relative to M13mp8H$_{45}$ were unsuccessful. Instead, we cloned the *Bam*HI-*Hin*dIII fragment of M13mp8H$_{45}$ into the same sites in pBR322. The only clone (pSB1) found to contain the correct fragment also contains an approximately 250-base-pair *Hin*dIII fragment of unknown origin in the *Hin*dIII site. We tried to clone the *Bam*HI-*Hin*dIII fragment of pSB1 in the same sites in M13mp9, but we found no clones with the insert in the desired orientation. Instead, we cloned the *Eco*RI-*Bam*HI fragment of pSB1 into the same sites of M13mp8 to give M13mp8H$_{54}$. This recombinant M13mp8 carries the 250-base-pair fragment of unknown origin. We wanted to be sure that no RNA hybridizes to this *Hin*dIII fragment, so we deleted the portion of M13mp8H$_{54}$ corresponding to *Hpa*I$_4$-*Hpa*I$_5$ (see Fig. 1) by digesting the plasmid with *Hin*dII and religating it back together. The resulting recombinant, M13mp8H$_{54}$Δ1, carries only the 250-base-pair *Hin*dIII fragment of unknown origin. In the hybridization assay described below we found no hybridization of RNA to single-stranded DNA probes derived from plasmid M13mp8H$_{54}$Δ1.

The M13mp8 probe M13mp8*lac*14 was constructed by inserting the 1.8-kilobase *Hpa*I-*Eco*RI fragment of pMC871 (1) into the *Eco*RI-*Hin*dII site of mp8. In m13mp8 *lac-14* there is no complementarity between the portion of *lacZ* carried in M13mp8 and the 1.8-kilobase *Hpa*I-*Eco*RI fragment of pMC871.

Isolation of pulse-labeled, [^{3}H]U-labeled RNA. Mutant or wild-type strains were grown from A_{650} = 0.05 to A_{650} = 0.4 in the MOPS glucose medium of Neidhardt et al. (17) supplemented with arginine, histidine, and proline (50 μg/ml) for all strains except IBPC4771, for which threonine (50 μg/ml) was also added. At A_{650} = 0.4, 0.50 ml of culture was labeled with 500 μCi of [^{3}H]U (43 Ci/mmol). Cells were then lysed at the times indicated by the addition of 0.5 ml of boiling SDS solution, and the [^{3}H]U-RNA was extracted twice with phenol saturated with 0.2 M sodium acetate (pH 5.2), followed by three extractions with diethyl ether. The

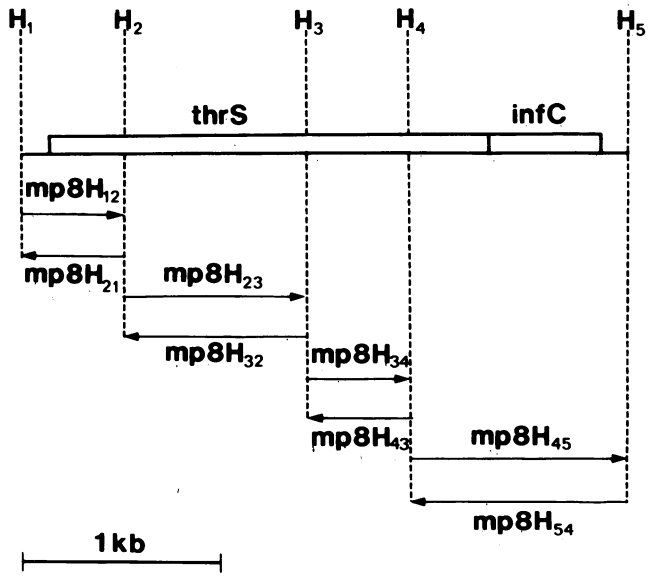

FIG. 1. Physical map of the region of the *E. coli* chromosome carrying the genes for ThrRS (*thrS*) and initiation factor IF3 (*infC*). Arrows represent the size of DNA inserts in phage M13mp8. The direction of the arrows represents the orientation of the insert in the *Hin*dII site of the M13mp8 linker with left being towards the *Eco*RI site and right being towards the *Hin*dIII site. H, *Hpa*I.

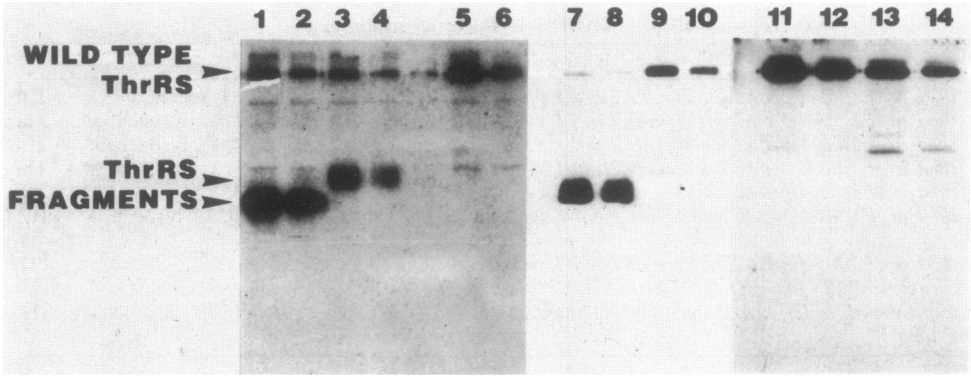

FIG. 2. Autoradiogram of immunoblots of protein extracts from wild-type and *thrS* mutant strains. Samples of 5 to 8 μg (even-numbered lanes) or 10 to 16 μg (odd-numbered lanes) of protein were separated by electrophoresis on 12.5% polyacrylamide-SDS gels. The proteins were transferred to nitrocellulose paper and treated as described in the text. Lanes 1 and 2, MHB19 (*thrS219*); lanes 3 and 4, MHB4 (*thrS204*); lanes 5 and 6, IBPC4901(λ) (wild type); lanes 7 and 8, MHC37 (*thrS337*); lanes 9 and 10, IBPC4901(λ) (wild type); lanes 11 and 12, IBPC4771 (*thrS1029*); lanes 13 and 14, IBPC4901(λ) (wild type).

[^{3}H]U-RNA was precipitated with ethanol and stored in sterile water at −20°C. The doubling times and labeling times are, respectively, for each strain: IBPC4901(λ), 70 min and 105 s; MHB4, 90 min and 135 s; MHB19, 75 min and 110 s; MHB35, 60 min and 90s; MHC37, 115 min and 170 s; and IBPC4771, 90 min and 135 s.

Hybridization of [^{3}H]U-RNA to strand-specific M13 probes. Single-stranded phage M13mp8 DNAs were purified (13) and stored at −20°C in sterile water. The M13 single-stranded probes were fixed to nitrocellulose filters (25-mm diameter) by filtration (2 ml/min) of 10 μg of each probe in 50 ml of 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Each filter was cut into four smaller filters (6-mm diameter), and the filters were dried under vacuum at 80°C for 3 h.

Hybridizations were carried out in sterile 5-ml glass vials with 167 μl of hybridization buffer (0.1 M Tris hydrochloride, pH 8, 0.6 M NaCl, 0.02 M disodium EDTA, 0.1% SDS, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 50% deionized formamide) and 2 × 10^6 dpm of [^{3}H]U-RNA per filter at 41°C for 16 h with gentle shaking. In general, six filters were incubated in each vial. Three of these filters carried a probe with the insert in one orientation, and the other three carried a probe with the same insert in the opposite orientation. The fourth 6-mm filter cut for each probe was incubated with [^{3}H]U-RNA extracted from strain IBPC1365 carrying the plasmid pB21 (21). This plasmid produces approximately 10 times more *thrS* and *infC* mRNA than the chromosome (see Fig. 2). The large amount of [^{3}H]U-RNA hybridized to the fourth filter establishes that the other three filters carry enough DNA to saturate all of the specific [^{3}H]U-RNA available for hybridization and that there are no significant differences between batches of filters.

After incubation, the filters were washed four times in 50 ml of 2× SSC for 10 min at 30°C, treated with 0.75 ml of RNase A (20 μg/ml; initially preincubated at 100°C for 2 min) per filter for 1 h at 30°C, and washed twice with 50 ml of 2× SSC for 10 min at 30°C. The filters were then washed in 100 ml of 90% ethanol and dried before scintillation counting (twice, 10 min each, for each filter).

RESULTS

Genetic mapping of *thrS* mutations. We decided to map the mutations of a number of *thrS* thermosensitive mutants isolated by P1 mutagenesis and of one additional mutation in the hope that this might give us more insight into the regulation of expression of *thrS*. First, we screened each *thrS* thermosensitive mutant plus the one *thrS* auxotrophic mutant strain IBPC4771, using λ *B*1 (23). We then chose for further study only those mutants complemented by λ phage carrying a complete *thrS* gene.

The second method of genetic mapping that we employed was recombination of F^+ derivatives (see Materials and Methods) of each mutant by M13 phage carrying *Hpa*I fragments of *thrS* inserted into the *Hin*dII site of the M13mp8 DNA (Fig. 1). After infection, cultures were grown for 48 h to allow recombination to occur and then plated at high temperature, or at low temperature in the absence of threonine in the case of strain IBPC4771. Table 2 lists the number of thermoresistant (or Thr^+) colonies per 10^6 viable cells. For strains MHB19 F′ and IBPC4771 F′ the background level of transduction was relatively high, probably as a result of the relatively high reversion frequency of these strains. The values in Table 2 show that the *thrS* mutations fall into two categories. First, there are three mutants (strains MHB4, MHB19, and MHC37) whose mutations are in the internal portion of *thrS* between the $HpaI_2$ and $HpaI_3$ sites and thus are structural mutations in *thrS*. Second, there are two mutants (strains MHB35 and IBPC4771) whose mutations are in the 5′-terminal region of *thrS* between the $HpaI_1$ and $HpaI_2$ sites and so may be either structural or regulatory mutations. Most importantly, these mapping experiments demonstrate that the mutations in strains MHB4, MHB19, and MHC37 are in the *thrS* gene and thus all phenotypic irregularities in these strains must be a result of mutations in *thrS*.

Measurement of in vivo levels of ThrRS. It was possible that some of these *thrS* mutations might result in variations of ThrRS levels if the synthetase is involved in its own

TABLE 2. Recombination mapping of *thrS* mutations

Strain	No. of transductants per 10^6 cells with M13 phage:			
	$mp8H_{12}$	$mp8H_{23}$	$mp8H_{34}$	$mp8H_{45}$
MHB4 F′ (*thrS204*)	73	4.0 × 10^4	25	47
MHB19 F′ (*thrS219*)	200	1.3 × 10^5	167	666
MHC37 F′ (*thrS337*)	<50	1.5 × 10^4	<50	<50
MHB35 F′ (*thrS235*)	2 × 10^3	11	33	12
IBPC4771 F′ (*thrS1029*)	7 × 10^4	<10	250	300

TABLE 3. Relative concentrations of ThrRS and initiation factor IF3 in *thrS* mutants

Strain	Relative concn[a] (%)	
	ThrRS	IF3
IBPC4901λ (wild type)	100	100
MHB4[b] (*thrS204*)	62 ± 27	134 ± 27
MHB19[b] (*thrS219*)	351 ± 48	162 ± 27
MHC37[b] (*thrS337*)	334 ± 28	106 ± 29
MHB35 (*thrS235*)	147 ± 23	108 ± 26
IBPC4771 (*thrS1029*)	323 ± 32	104 ± 2

[a] Average ± SD of three separate experiments in which two different concentrations of protein were tested.
[b] In three strains ThrRS is recognized as degraded fragments.

regulation. To test this possibility, we determined ThrRS and IF3 protein levels in exponentially growing cells by quantitative immunoblotting (Fig. 2). We determined IF3 levels in addition to ThrRS levels because there are only three nucleotides between the stop condon of *thrS* and the initiation codon of *infC* (12). Since there are no transcription termination signals between the two genes, they must be coexpressed. Table 3 lists the in vivo levels of ThrRS and IF3 for each mutant relative to the wild type. The level of IF3 for each mutant is about the same as in the wild-type strain. However, there is significant overproduction of ThrRS or fragments of ThrRS for a number of the mutants (strains MHB19, MHC37, and IBPC4771). In the case of strains MHB4, MHB19, and MHC37, ThrRS antibodies recognize lower-molecular-weight forms of ThrRS. Thus, it is likely that these mutant forms of ThrRS are susceptible to proteolysis. This fact, with the possibility that the affinity of ThrRS antibodies could be lower for these defective forms, suggests that the levels of ThrRS determined for the mutants by this method must represent the minimum amounts of ThrRS in vivo. The fact that IF3 is not overproduced in mutants which overproduce ThrRS suggests that IF3 expression can be controlled independently of ThrRS.

Measurement of in vivo levels of *thrS* mRNA. If the overproduction of ThrRS is the result of derepression of transcription of *thrS*, then proportionate increases in *thrS* mRNA should be detected by hybridization of in vivo-labeled mRNA to single-stranded DNA probes. We measured the amount of in vivo-labeled [^{3}H]U-RNA transcribed from the *thrS* region by hybridization to single-stranded phage M13 DNA carrying different *Hpa*I fragments from in and around *thrS* (Fig. 1). The data in Fig. 3 demonstrate that the hybridization system was sensitive to small changes in [^{3}H]U-RNA levels. For each probe, increasing the amounts of specific [^{3}H]U-RNAs resulted in proportionate increases in the amounts of specifc [^{3}H]U-RNAs hybridized to each probe. The amount of [^{3}H]U-RNA hybridizing per kilobase of probe along the *thrS* transcription unit was not constant, probably as a result of small differences in hybridization efficiencies between each probe. More importantly, the linear response between the total amount of [^{3}H]U-RNA added and the amounts of [^{3}H]U-RNA found as hybrids guarantees that increases in in vivo levels of *thrS* or *infC* mRNA will be detected by hybridization to these probes.

The in vivo levels of *thrS* and *infC* mRNA determined by hybridization (Table 4) indicate two important features of *thrS* and *infC* transcription. First, there was a three- to fivefold greater amount of hybridization to the M13mp8H$_{54}$ probe than to the preceding three probes (also compare Fig. 3A and B). This large increase in hybridization could reflect differences in hybridization efficiencies, but is most likely due to a real increase in transcription near to the *Hpa*I$_4$ site. Significantly, this increase in transcription agrees quantitatively with measurements showing that there is about fivefold more IF3 than ThrS in vivo (7, 16). It thus seems likely that *infC* can be transcribed independently of *thrS* and that this transcription is initiated near to or after the *Hpa*I$_4$ site. This is consistent with in vivo experiments which demonstrate that *infC* can be expressed independently of the promoter for *thrS* (26). There also seems to be a slight increase in hybridization to probe M13mp8H$_{43}$ for strains MHB4 and MHB19. This is reflected by a slight elevation of IF3 levels (Table 2) for each of these two strains. It is possible that the sensitivity of the hybridization system allows one to detect relatively minor changes in *infC* transcription initiated near the *Hpa*I$_3$ site.

The second and most important feature of the hybridization results is that for each *thrS* mutant the in vivo level of *thrS* mRNA was not significantly different from that of the wild-type strain. Thus, the nearly fourfold overproduction of

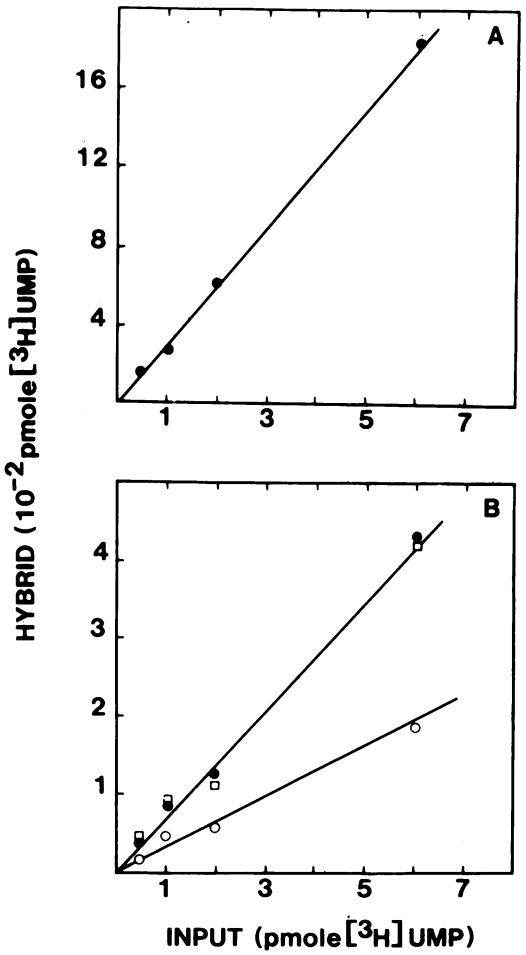

FIG. 3. Relative amounts of [^{3}H]U-RNA hybridizing to M13mp8 probes. Increasing amounts of [^{3}H]U-RNA from strain IBPC1365 carrying plasmid pB21 were hybridized to a fixed amount of each M13mp8 probe as described in the text. The amount of [^{3}H]U-RNA hybridizing to probes containing inserts in the opposite orientation has been subtracted. Symbols: (A) ●, M13mp8H$_{54}$; (B) ●, M13mp8H$_{21}$; ○, M13mp8H$_{32}$; □, M13mp8H$_{43}$.

ThrRS in strains MHB19, MHC37, and IBPC4771 is not due to derepression of transcription of *thrS* but is most likely due to increased translation of *thrS* mRNA.

For the determination of *thrS* and *infC* mRNA levels, we chose to pulse-label cells for time periods proportionate to the growth rates of the cells. We did this because both ThrRS and IF3 levels are known to increase in proportion to the growth rate of the cell (7, 18). If this metabolic control is exerted at the transcriptional level, then pulse-labeling all strains for the same amount of time might result in an underestimate of the contribution of factors other than the growth rate to the relative synthesis rates of *thrS* and *infC* mRNAs in cells with relatively long doubling times. On the other hand, it is possible that the use of different pulse-labeling times might result in the underestimate of relative mRNA synthesis rates in slowly growing cells if the degradation rate of the mRNA has a significant effect on the incorporation of label into mRNA during the pulse. The experimental results shown in Fig. 4 demonstrate that the incorporation of [^{3}H]U into *thrS* mRNA in the wild-type strain IBPC4901(λ) and the ThrRS-overproducing mutant MHB19 is linear and identical for the two strains for pulse-labeling times up to at least 150 s. Thus, we do not believe that the half-life of *thrS* mRNA has a significant effect on the *thrS* mRNA levels listed in Table 4, because all of the strains were labeled for less than 150 s (except strain MHC37, which was labeled for 170 s).

To compare the strength of transcription of *thrS* and *infC* with a well-characterized gene, we used a *lacZ* mRNA-specific single-stranded M13 DNA probe, mp8*lac*14, to measure the amount of *lacZ* mRNA in strain IBPC4901(λ) grown in the presence of glucose and isopropyl-thio-β-D-galactoside (5×10^{-4} M) and pulse-labeled as described for strain IBPC4901(λ) in Materials and Methods. The amount of *lacZ*-specific mRNA hybridized to an excess of M13mp8*lac*14 was 1.2×10^{-2} pmol of [^{3}H]U-RNA per kilobase of *lacZ*-specific probe, corresponding to 3,000 Miller units (14) of β-galactosidase. Thus, transcription of *thrS* and *infC* in strain IBPC4901(λ) (see Table 4) is respectively, about one-half and three times as strong as *lacZ* transcription under these conditions (isopropyl-thio-β-D-galactoside does not affect *thrS* or *infC* transcription).

TABLE 4. ThrRS and initiation factor IF3 mRNA levels in *thrS* mutants

Strain	[^{3}H]UMP hybridized per kilobase of probe insert[a] (pmol × 10^{-2}):			
	mp8H$_{21}$	mp8H$_{32}$	mp8H$_{43}$	mp8H$_{54}$
IBPC4901(λ) (wild type)	0.84 ± 0.13 (100)	0.54 ± 0.07 (100)	0.60 ± 0.06 (100)	3.24 ± 0.20 (100)
MHB4 (*thrS204*)	0.98 ± 0.02 (117)	0.50 ± 0.13 (93)	1.01 ± 0.12 (167)	3.30 ± 0.59 (102)
MHB19 (*thrS219*)	0.76 ± 0.22 (90)	0.58 ± 0.12 (107)	0.97 ± 0.16 (162)	3.59 ± 0.92 (110)
MHC37 (*thrS337*)	1.18 ± 0.16 (140)	0.64 ± 0.01 (89)	0.64 ± 0.06 (106)	2.66 ± 0.07 (92)
MHB35 (*thrS235*)	0.84 ± 0.12 (100)	0.35 ± 0.07 (66)	0.47 ± 0.09 (78)	3.11 ± 0.66 (96)
IBPC4771 (*thrS1029*)	1.02 ± 0.14 (121)	0.72 ± 0.07 (133)	0.64 ± 0.10 (107)	2.57 ± 0.99 (79)

[a] Values are averages ± SD of at least four measurements of at least two independent RNA preparations and represent the quantity of RNA hybridized to a single filter. Values in parentheses are percentages of wild type.

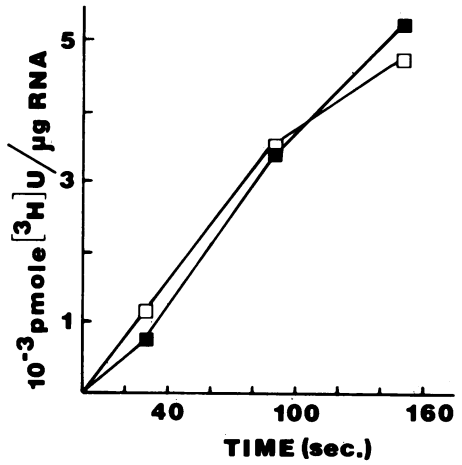

FIG. 4. Relative synthesis rates of *thrS* mRNA in strains IBPC4901(λ) and MHB19. Strains were grown and labeled as described in the text. At the times indicated, 1.5-ml samples were withdrawn and lysed, the [^{3}H]U-RNA was prepared, and the amount of [^{3}H]U in *thrS* mRNA was determined as described in the text by hybridization of 10 to 16 μg of [^{3}H]U-RNA to single-stranded probes M13mp8H$_{23}$ and M13mp8H$_{32}$. The data points are the average of three measurements with a standard deviation of less than 10%. Symbols: ■, IBPC4901(λ); □, MHB19.

DISCUSSION

This paper reports evidence that the expression of ThrRS is autoregulated at the level of its own translation. Two of the mutants studied here (strains MHB19 and MHC37) overproduce (at least threefold) structurally altered forms of ThrRS. The *thrS* allele (*thrS1029*) in one of these mutants (strain IBPC4771) produces ThrRS with a K_m for threonine 200-fold higher than that for wild type (8) and overproduces ThrRS by a factor of at least three (Table 3). The overproduction of a modified form of ThrRS in *thrS* mutants having mutations in the structural gene suggests strongly that the synthetase controls its own gene expression and is consistent with in vitro experiments that show that ThrRS represses translation of *thrS* mRNA in vitro (11). An alternative explanation would be that each of these mutants carries a second mutation which causes overproduction of ThrRS. This explanation is not likely for strains MHB4, MHB19, and IBPC4771 because recent experiments demonstrate that the mutant *thrS* alleles in these strains cause a derepression in *trans* of β-galactosidase synthesis from a *thrS-lacZ* protein fusion, whereas the wild-type allele has no effect. (Springer et al., in press). More significantly, when a wild-type *thrS* allele is placed in *trans* to the *thrS1029* allele (strain IBPC4771), the expression of *thrS* returns to normal (Springer et al., in press). This suggests that the *trans*-acting factor affecting *thrS* expression is ThrRS itself. The fact that the mutation carried by strain MHB4 causes derepression of a *thrS-lacZ* protein fusion suggests that this strain also overproduces an apparently unstable form of ThrRS (Fig. 2) and is consistent with the idea that ThrRS regulates the expression of *thrS*.

Autoregulation of gene expression has been shown for two other aminoacyl-tRNA synthetases. Alanyl-tRNA synthetase regulates its own expression at the level of transcriptional initiation in vitro by acting as a repressor (24). Phenylalanyl-tRNA synthetase expression is controlled by a transcription attenuation system which is sensitive to levels of charged tRNA^{Phe} and thus to the concentration of the

enzyme itself (4, 27). The experiments reported in this paper show that although in vivo levels of ThrRS are at least threefold greater in some mutants than in the wild type, the levels of specific in vivo *thrS* mRNAs are not significantly different between the wild type and these mutants. This suggests that ThrRS expression is regulated at the translational level, and the data as a whole indicate that ThrRS expression is autoregulated at the translational level in vivo.

There are already a number of proteins which are known to regulate their own expression at the level of translation. These include some of the ribosomal proteins (19) and the gene 32 protein (10, 25) of phage T4. ThrRS shares with the ribosomal proteins the feature that one of its substrates is an RNA. It is now fairly well accepted that in the case of the ribosomal proteins, regulation is effected by the protein binding to its own mRNA at a site which shares some structural features with the protein-binding site of the substrate rRNA. In the case of ThrRS, the nucleotide sequence of the DNA in the region of its ribosome binding site shows a number of sequences capable of forming secondary structures in a *thrS* mRNA (11). The actual existence of such structures and their potential as a site for ThrRS translational autoregulation are now being studied in our laboratory.

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