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Processing of the leader mRNA plays a major role in the induction of *thrS* expression following threonine starvation in *Bacillus subtilis*

(mRNA processing/threonyl tRNA synthetase/mRNA stability/antitermination)

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ABSTRACT **The threonyl-tRNA synthetase gene, *thrS*, is a member of a family of Gram-positive genes that are induced following starvation for the corresponding amino acid by a transcriptional antitermination mechanism involving the cognate uncharged tRNA. Here we show that an additional level of complexity exists in the control of the *thrS* gene with the mapping of an mRNA processing site just upstream of the transcription terminator in the *thrS* leader region. The processed RNA is significantly more stable than the full-length transcript. Under nonstarvation conditions, or following starvation for an amino acid other than threonine, the full-length *thrS* mRNA is more abundant than the processed transcript. However, following starvation for threonine, the *thrS* mRNA exists primarily in its cleaved form. This can partly be attributed to an increased processing efficiency following threonine starvation, and partly to a further, nonspecific increase in the stability of the processed transcript under starvation conditions. The increased stability of the processed RNA contributes significantly to the levels of functional RNA observed under threonine starvation conditions, previously attributed solely to antitermination. Finally, we show that processing is likely to occur upstream of the terminator in the leader regions of at least four other genes of this family, suggesting a widespread conservation of this phenomenon in their control.**

Unlike in *Escherichia coli*, where the genes encoding the aminoacyl-tRNA synthetases and the amino acid biosynthetic operons are induced by a wide variety of mechanisms in response to starvation for their cognate amino acid (1, 2), many of these genes appear to be regulated by a common transcription antitermination mechanism in *Bacillus subtilis* and other Gram-positive bacteria (3–6). The genes of this family all have a highly structured leader region of about 300 nucleotides (nt), followed by a Rho-independent transcription terminator, just upstream of the translation initiation site. Upstream of the leader transcription terminator is a highly conserved sequence of about 14 nt, known as the T-box, and part of the T-box sequence can base pair with a complementary sequence in the 5′ strand of the terminator stem to form an alternative, but much weaker, antiterminator structure (2, 4). The antitermination conformation of the mRNA is thought to be stabilized in each case by the cognate uncharged tRNA, thus accounting for the induction of these genes by starvation for their corresponding amino acid (4, 7, 8).

The identity of the stabilizing tRNA is specified by a codon bulged out of a conserved secondary structure called the specifier domain, which occurs early in the leader regions of these genes (4, 8). The tRNA is thought to recognize the bulged triplet via a classical codon/anticodon interaction. Changing the specifier codon in the tyrosyl-tRNA synthetase gene, *tyrS*, from UAC to UUC, switches the specificity of regulation from tyrosine to phenylalanine (4). Similarly, changing the UUC codon of the phenylalanyl-tRNA synthetase gene to ACC permits induction of *pheS* by starvation for threonine (8). A second interaction occurs between the NCCA-3′ end of the acceptor arm of the uncharged tRNA and a complementary sequence (UGGN′), which is part of the T-box and is bulged out of the antiterminator structure (4, 5, 8). Although the formation of these two interactions is likely to be the critical element in the stabilization of the antitermination conformation of the RNA, it is likely that other tRNA-leader interactions and possibly some protein factors are required to make this a productive complex *in vivo* (4, 8).

We have been studying the mechanism of tRNA-mediated antitermination in the control of the *thrS* gene, one of two threonyl-tRNA synthetase genes in *Bacillus subtilis*. Here we show that the *thrS* leader mRNA is cleaved between the T-box and the leader terminator structure, and that the cleaved transcript is significantly more stable than the full-length RNA, particulary under starvation conditions. Thus, termination/antitermination is not the only determinant of the level of functional *thrS* mRNA in the cell. We show that while the interaction between uncharged tRNA-Thr and the *thrS* leader is not necessary for processing to occur, the cleavage efficiency is nonetheless stimulated by threonine starvation. Finally, we show that cleavage between the T-box and the terminator probably occurs in several other members of this family of genes, suggesting that this phenomenon may have a general importance in their regulation.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions. The *B. subtilis* strain used for these studies was 168, or derivatives thereof, containing *thrS–lacZ* transcriptional fusions integrated into the *amy* locus. Strains were in general grown in M9 minimal medium (9) at 37°C to an OD_{600} of about 1.0 before harvesting for RNA isolation. For starvation experiments, cells were grown to an OD_{600} of about 0.3 in M9 medium, divided, and then starved for 2 h in the presence of either 600 μg of threonine hydroxamate per ml (Sigma) or 3 μg of arginine hydroxamate per ml (Sigma), each of which caused about a two-fold decrease in growth rate.

Plasmid Construction. The wild-type *thrS–lacZ* fusion plasmid (pHMS69) was constructed by cloning a 0.44-kb *Eco*RI–*Bam*HI fragment from pHMS25 (8) into the *lacZ* fusion vector, pHM2 (10), and cut with *Eco*RI and *Bam*HI.

RNA Isolation. Frozen pellets of cells (from 20 ml of culture) were resuspended in 4 ml ice-cold TE buffer (pH 8.0). RNA

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Abbreviation: RT, reverse transcriptase.

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was isolated by vortex mixing the cells in an ice-cold mixture of glass beads, phenol, chloroform, and SDS, as described (3), followed by two further cold phenol extractions and three phenol/chloroform extractions. It was finally precipitated in ethanol and resuspended in an appropriate volume of diethylpyrocarbonate (DEPC)-treated water.

Reverse Transcriptase (RT) Assays. RT assays were carried out as described (10) by using 15 μg of total RNA and about 1 pmol of 5′-end labeled oligonucleotide. RNA and oligonucleotide were heated together at 65°C for 5 min, and then frozen in a mixture of dry ice and ethanol and allowed to thaw on ice. Reactions contained 2 units of avian myeloblastosis virus RT (Eurogentec, Brussels) and were allowed to run for 30 min at 48°C. Oligonucleotides were synthesized by Oligo-Express (Montreuil, France), and the sequences are available on request.

S1 Nuclease Assays. S1 probes were amplified by PCR from plasmids containing either part of the *thrS* gene or *thrS–lacZ* fusions. Probes complementary to the *thrS* leader and part of the structural gene, which are specific for the native *thrS* gene transcripts, were amplified from pHMS2, a pBluescript-derived plasmid described previously (see figure 3 in ref. 11). Thirty picomol of a 5′-end labeled oligonucleotide (28-mer) starting at position +503 of the *thrS* transcript and 30 pmol of the Universal primer were used in the PCR. Probes specific for the wild-type and mutant *thrS–lacZ* fusions were amplified from their respective plasmids pHMS69 (wild type; this paper), pHMS34 (stop codon mutant), and pHMS29 (deletion of specifier domain) described previously (8), and pHMS21 (truncated leader fusion) also described previously (3). Thirty picomol of a labeled oligonucleotide starting at codon 42 of the *lacZ* gene and 30 pmol of an oligonucleotide specific for the *cat* gene, also contained on the plasmid, were used for the PCR. The PCR products were purified from agarose gels (Jetsorb; Bioprobe, Montreuil, France) and counted in a scintillation counter. Fifty micrograms of RNA and ≈100,000 Cerenkov counts of probe were precipitated together in ethanol and resuspended in 40 μl deionized formamide and 10 μl of 5× hybridization buffer (0.2 M Pipes, pH 6.4/2 M NaCl/5 mM EDTA). Samples were denatured at 75°C for 10 min, hybridized overnight at 48°C, and then diluted in 450 μl ice cold S1 reaction buffer [0.25 M NaCl/30 mM NaOAc, pH 4.6/1 mM ZnSO4/5% glycerol/20 μg of denatured herring sperm DNA per ml/120 units S1 nuclease (Boehringer Mannheim)]. Samples were incubated for 30 min at 37°C, ethanol precipitated, and run on polyacrylamide gels. Quantification of both RT and S1 products was done by using a PhosphorImager apparatus (Molecular Dynamics).

Half-Life Measurements. For half-life measurements, RNAs were prepared as described above from cells harvested at 0, 2, 4, and 6 min after addition of rifampicin (Sigma) at 150 μg/ml.

RESULTS

The *thrS* Leader Region Is Cleaved Just Upstream of the Transcription Terminator. Primer extension analysis of total *B. subtilis* RNA, using a labeled oligonucleotide complementary to a sequence about 200 nt downstream of the *thrS* leader transcription terminator, produced three major bands on polyacrylamide gels (Fig. 1*B*, lane 1). The basic structure of the *thrS* gene and RT strategy are shown in Fig. 1*A*. The band of the lowest molecular weight corresponds to a RT stop at the terminator itself, presumably caused by a difficulty in traversing the rather stable secondary structure, and the largest band corresponds to the full-length messenger RNA generated by the *thrS* promoter. We mapped the third RT stop to a point between the T-box and the terminator, 9 nt upstream of the terminator stem (Fig. 1*C*). To determine whether this RT stop corresponded to a mature 5′ end *in vivo*, we also performed S1 nuclease mapping experiments on the same RNA preparation. The S1 probe was made by PCR amplification of a fragment containing the promoter and about 850 nt of the *thrS* gene cloned in pBluescript, using the same labeled oligonucleotide as for the RT reactions and the Universal primer as the upstream oligonucleotide (see *Materials and Methods*). The band corresponding to the RT stop between the T-box and the terminator was also a product of the S1 nuclease reaction (Fig. 1*B*, lane 2), confirming that this 5′ end exists *in vivo*. Control reactions with total *E. coli* tRNA or the probe alone (lanes 3 and 4) did not show a band in this position. Since no such 5′ end is observed in *in vitro* transcription experiments, and the 160 bp upstream of this site show no promoter activity *in vivo* when fused to *lacZ* (data not shown), the possibility of transcription initiation from a second gene promoter is eliminated. We thus concluded that the shorter *thrS* transcript is a cleavage product of the full-length mRNA.

The Processed *thrS* Messenger RNA Is More Stable Than the Full-Length Transcript, Particularly Under Starvation Conditions. The processed transcript is likely to have a terminator structure at both its 5′ and its 3′ end. Since stable secondary structures at the ends of messenger RNAs have been associated with increased stability in *E. coli* (12–15), we examined the stability of the *thrS* full-length and processed mRNAs under normal and starvation conditions. RNA was isolated from *B. subtilis* strains at 2-min intervals following the addition of rifampicin, which prevents further transcription initiation, and subjected to S1 nuclease analysis as above (Fig. 2*A*). The same results were obtained with the RT assay or by Northern blot analysis (data not shown). On minimal medium, the processed transcript had a half-life of about 2.0 min (Fig. 2*B*; Table 1), about three times that of the full-length mRNA (0.7 min). Upon starvation for threonine, the total amount of *thrS* mRNA increased dramatically as expected, and the stability of the processed transcript increased significantly to a half-life of about 3.5 min, while the stability of the full-length transcript remained unchanged. To see whether the increase in stability under starvation conditions was specific for threonine, we repeated the experiment, this time starving for arginine. Under arginine starvation conditions, the amount of *thrS* readthrough mRNA decreased by half, consistent with previous observations using *thrS–lacZ* fusions (3), and the half-life of the processed *thrS* transcript increased to 3.1 min, whereas the full-length transcript remained relatively unstable (0.7 min; Table 1). Thus, at least part of the increased stability of the processed transcript appears to be a nonspecific effect of amino acid starvation.

By comparing the zero-time points (0′) for each of the three conditions examined (Fig. 2*A*), we noticed that the relative amount of the processed transcript was significantly higher following threonine starvation than under either nonstarvation or arginine starvation conditions. Starvation for threonine caused a 5-fold increase in the amount of full-length transcript; however, the intensity of the band corresponding to the cleaved RNA was increased 35-fold in the same RNA preparation [compare M9 (0′) and threonine hydroxamate (THX; 0′) in Fig. 2*A* and Table 1]. This results in there being 6-fold more of the more stable, cleaved form of the *thrS* mRNA than the full-length transcript under inducing conditions, and the processed transcript represents 86% of the total *thrS* mRNA. Under arginine starvation conditions, the intensity of both the full-length and the processed RNAs was decreased [compare lanes M9 (0′) and arginine hydroxamate (AHX; 0′) in Fig. 2*A* and Table 1], and ratio of processed to full-length mRNAs was 10-fold lower (0.6) than that observed following starvation for threonine and unchanged from that measured on minimal medium. Under noninducing conditions, therefore, the processed transcript only accounts for 38% of *thrS* mRNA.

Cleavage of the *thrS* Leader mRNA Can Also Occur Independently of the Specifier Codon and Specifier Domain. The

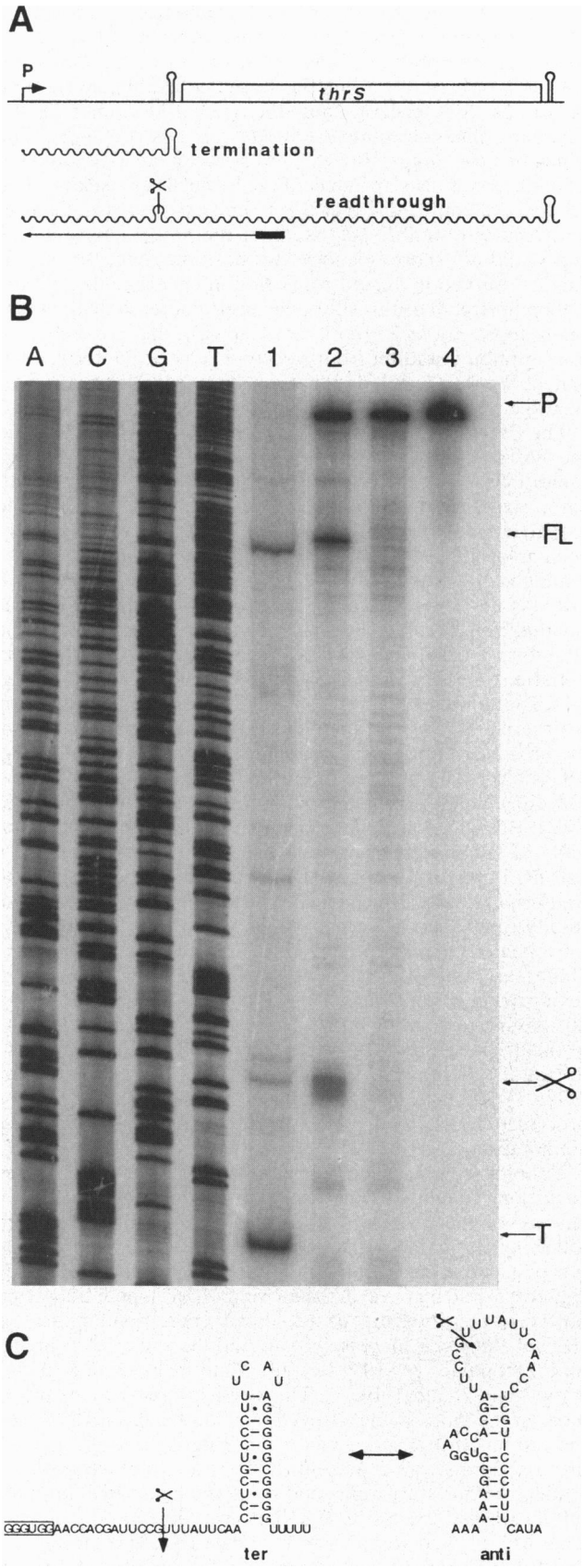

FIG. 1. (*A*) Schematic of the *thrS* gene, showing terminated and readthrough transcripts and RT strategy. The gene promoter is marked as P; the scissors symbol represents the proposed endonucleolytic cleavage. The oligonucleotide for the RT assay (and for the synthesis of the S1 probe) is indicated as a short thick line, and the reverse transcript is shown as a thinner line ending in an arrowhead.

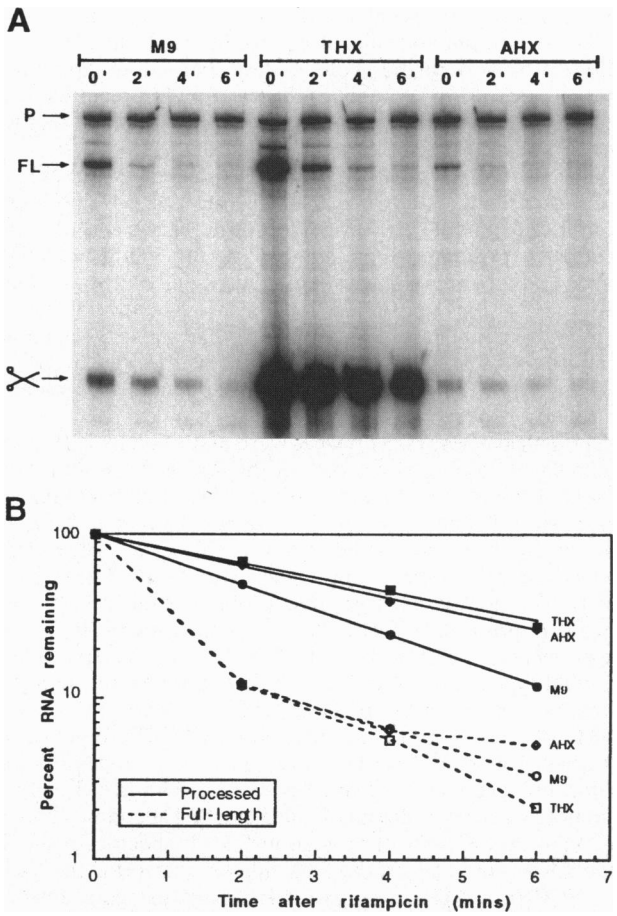

FIG. 2. (*A*) S1 analysis showing differential stabilities of the full-length (FL) and processed (scissors) transcripts. RNA (50 μg) were used in each lane. The S1 probe is marked as P. Lanes marked 0′, 2′, 4′, and 6′ correspond to the number of minutes after addition of rifampicin that RNA samples were prepared. Samples harvested on M9 minimal medium (M9), and in the presence of threonine hydroxamate (THX), and arginine hydroxamate (AHX) are as indicated. (*B*) Graph of data from the autoradiograph in *A*, showing the rate of degradation of the individual transcripts.

high levels of induction of *thrS* expression typically seen under conditions of threonine starvation are thought to be caused by a stabilization of the antiterminator structure by uncharged tRNA-Thr (4, 8). Because the amount of processed RNA was significantly increased under inducing conditions, we wished to see whether the interaction between uncharged tRNA-Thr and the *thrS* leader were necessary for cleavage to occur. We have previously shown that changing the ACC specifier codon in the *thrS* leader to a stop codon, UAA, abolishes induction of *thrS–lacZ* transcriptional fusions (8). These fusions contain the leader, terminator, and about 80 nt of *thrS* coding sequence. A fusion containing a 58-nt deletion that removes the 5′ stem of the specifier domain, thus preventing its proper formation, is

(*B*) Processing in the leader of the *thrS* gene. Lanes: 1, RT products from total *B. subtilis* RNA using a primer specific for the coding region of *thrS*; 2, S1 products from the same RNA; 3, control lane using the same quantity of *E. coli* tRNA; 4, a 1:100 dilution of the S1 probe alone. Lanes A, C, G, and T represent the dideoxy sequencing reactions using the same oligonucleotide as primer as was used for both the RT and the S1 nuclease reactions. The bands corresponding to the probe (P), full-length (FL), processed (scissors symbol) transcripts, and RT stop at the terminator structure (T) are marked on the right-hand side of the gel. (*C*) Location of the endonucleolytic cleavage (scissors) in the leader region of *thrS* in the termination (ter) and antitermination (anti) conformations. The core sequence of the T-box is framed.

Table 1. Summary of data from Fig. 2

	M9	THX	AHX
Half-life of *thrS* transcripts, min			
Full-length	0.7	0.7	0.7
Processed	2.0	3.5	3.1
Fold-induction of *thrS* transcripts, times			
Full-length	1.0	5	0.4
Processed	1.0	35	0.7
Distribution of *thrS* RNAs, %			
Full-length	62	14	62
Processed	38	86	38
Ratio (P/FL)	0.6	6.1	0.6

M9, M9 minimal medium; THX, threonine hydroxamate; AHX, arginine hydroxamate; P/FL, processed/full-length.

also uninducible by threonine starvation (8). Thus, neither of these mutant leaders is likely to interact with uncharged tRNA-Thr in a productive manner. We used these two mutant fusions to test the necessity of the interaction between the tRNA and the *thrS* leader for processing. S1 analysis of RNA isolated from cells growing in minimal medium showed that both of these fusions are cleaved with a similar efficiency to a wild-type fusion (Fig. 3, lanes 1–3). Thus, processing can occur in the absence of the tRNA-leader mRNA interaction. Because the same amount of RNA (50 μg) was used in each lane, and the probes had the same specific activity, the different intensities of the bands in each lane accurately reflect the amount of readthrough transcript for each of these fusions and correspond quite well to their relative β-galactosidase activities (see ref. 8). Following threonine starvation, only the wild-type fusion showed an accumulation of readthrough RNA, confirming that neither the specifier codon mutant nor the specifier domain deletion are inducible by uncharged tRNA-Thr (Fig. 3, lanes 5–7). The processing efficiency of the wild-type fusion differs somewhat from the native *thrS* gene under both noninducing and inducing conditions for reasons we do not yet understand. Thus, we could not use the mutant fusions to accurately quantify the role played by uncharged tRNA.

Leader Processing Is Eliminated in a Truncated *thrS* Leader–*lacZ* Fusion. We also tested processing efficiency in a *thrS–lacZ* fusion where the leader region was truncated at an *Eco*NI site in the loop of the terminator. Thus, this fusion lacks the 3′ half of the terminator stem and about 100 nt of downstream *thrS* sequence, but contains all of the sequences necessary for the formation of the antiterminator structure. Since cleavage seemed likely to occur in the antitermination conformation of the leader transcript (see *Discussion*), we expected the processing efficiency to increase significantly in this mutant. However, no trace of the leader processing was observed under either non-starvation or threonine starvation conditions (Fig. 3, lane 4 and 8). All of the radioactivity from the wild-type induced fusion accumulated in the full-length transcript in this deletion mutant (Fig. 3, compare lanes 5 and 8), suggesting that cleavage of the transcript is abolished, rather than normal cleavage and decreased stability of the processed mRNA. Thus, the leader terminator structure, or sequences 3′ to it, are necessary for cleavage of the *thrS* mRNA.

Cleavage of the Leader mRNA in the Antiterminator Structure Appears to be Widely Conserved. The second threonyl-tRNA synthetase gene in *B. subtilis*, *thrZ*, is normally not expressed except under threonine starvation conditions or when *thrS* expression is reduced (3, 11). By using RNA isolated under these conditions, we had previously seen evidence that *thrZ* was also processed between the T-box and the terminator, 7 nt upstream of the terminator stem. This suggested that leader processing might play a general role in the regulation of the aminoacyl-tRNA synthetase genes and amino acid biosynthetic operons in *B. subtilis*. By using oligonucleotides complementary to the published sequences of several of these genes, we performed primer extension experiments on total *B. subtilis* RNA to see whether there were RT stops which would correspond to processing between the T-box and the terminator structure of their leader regions. Fig. 4*A* shows the band corresponding to the RT stop in this position in *thrZ*, and that a similar band occurs in three other systems we tested: *tyrS*, *serS*, and *trpS*. A similar analysis of the *pheS*, *leuS*, and *valS* genes failed to produce conclusive results (data not shown). However, we noticed a band corresponding to an equivalent position in the *ilv–leu* operon in a similar experiment by Grandoni *et al.* (6). Thus, it would appear that cleavage of the mRNA in the antiterminator structure is widely conserved within this family of genes. There are often one or two minor bands associated with the RT stop corresponding to the processed RNA, suggesting that the cleavage reaction is not very precise. The proposed major cleavage site of each gene is estimated in Fig. 4*B* and was deduced either by RNA sequenc-

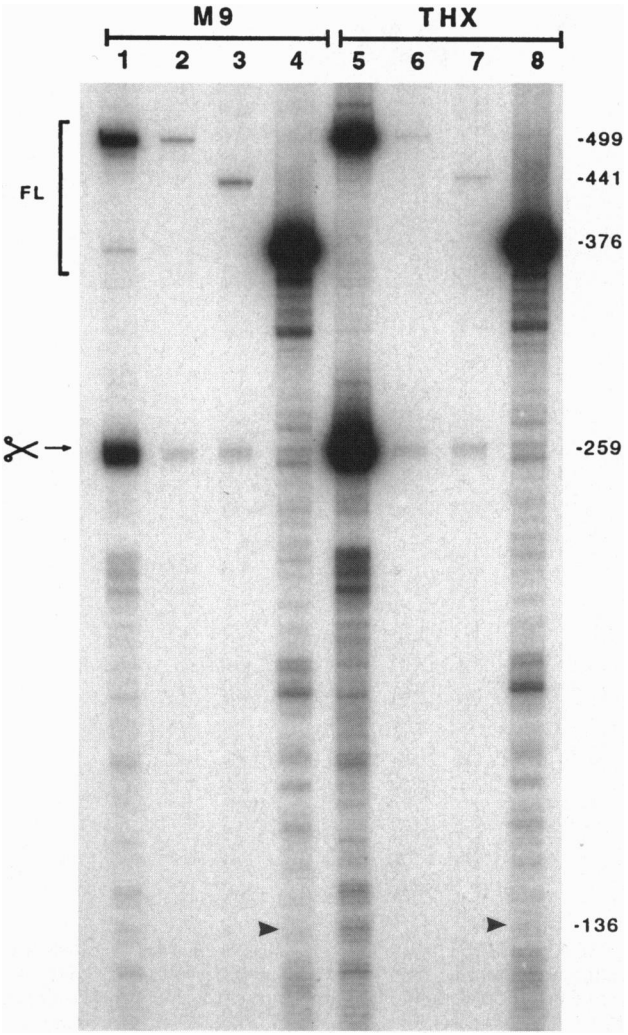

FIG. 3. Processing in wild-type and various mutant *thrS–lacZ* fusions. Fifty micrograms of RNA was used in each lane. S1 analysis of RNA from the wild-type fusion is shown in lanes 1 and 5; the stop codon mutant in lanes 2 and 6; the deletion of the specifier domain in lanes 3 and 7; and the fusion truncated in the loop of the leader terminator in lanes 4 and 8. Sizes are as indicated on the right-hand side of the gel. Samples harvested on M9 minimal medium (M9) and in the presence of threonine hydroxamate (THX) are as indicated. Full-length (FL) and processed (scissors) transcripts are as marked. The arrowheads within the gel depict where the processed RNA in the truncated fusion should migrate.

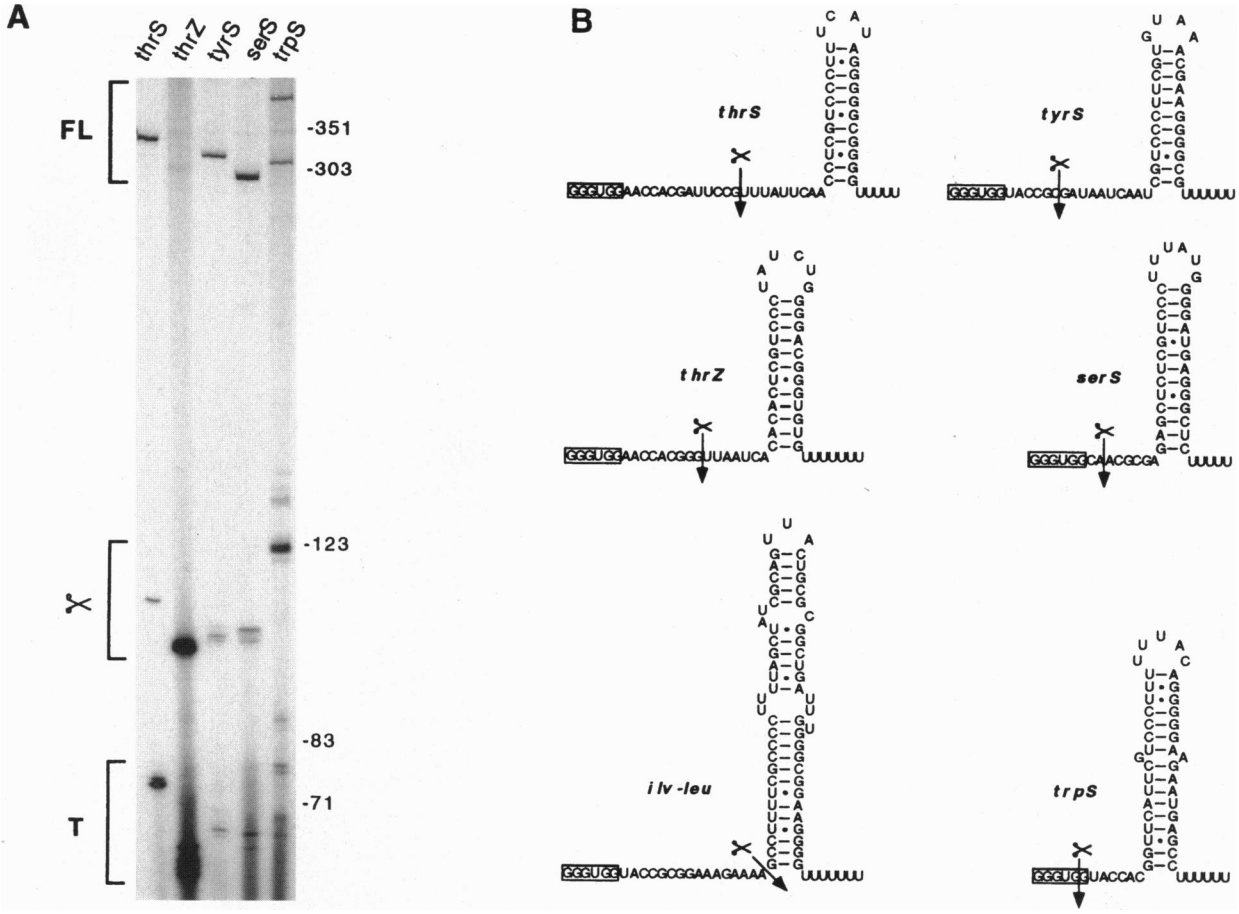

FIG. 4. (*A*) Processing in the genes for various aminoacyl-tRNA synthetases. RT products are shown using oligonucleotide primers specific for the threonyl- (*thrS* and *thrZ*), tyrosyl- (*tyrS*), seryl- (*serS*), and tryptophanyl- (*trpS*) tRNA synthetase genes. Sizes are as indicated on the right-hand side of the gel. The full-length transcript (FL), the proposed processing sites (scissors), and the nonspecific RT stop at the terminator structure (T) of each of these genes is marked. The RT enzyme is not processive enough to see the full-length *thrZ* transcript at >800 nt from the oligonucleotide. (*B*) Proposed processing sites (scissors) in each of the synthetase genes in *A* and the *ilv–leu* biosynthetic operon from ref. 6. The core sequence of the T-box is framed.

ing or by sequencing of DNA fragments containing the gene in question using the oligonucleotide used for the RT reaction as primer (data not shown).

DISCUSSION

We have shown that the *thrS* leader region is processed at a site between the T-box and the Rho-independent transcription terminator, 9 nt upstream from the terminator stem. This would place the cleavage in the loop of the antiterminator structure if cleavage were to occur in the antitermination conformation (see Fig. 1*C*). Cleavage of the transcript at this position and subsequent refolding of the terminator structure would leave the mature *thrS* mRNA possessing a "terminator" at both its 5′ and its 3′ end. In *E. coli*, stable secondary structures at either end of the transcript have been associated with increased mRNA stability (12–15). However, 5′ secondary structures no longer have a stabilizing effect if there are more than four unpaired nucleotides at the beginning of the message. Although the analogy with *E. coli* prompted us to do the initial mRNA stability studies, subsequent experiments (see below) suggest the terminator may play a more fundamental role in the processing event itself.

The processed *thrS* transcript is about three times as stable as the full-length mRNA under normal growth conditions on minimal medium, and becomes as much as 5-fold more stable than the full-length transcript following starvation for threonine. Thus, processing and the subsequent stabilization of the transcript contribute significantly to both basal and induced levels of *thrS* expression, since the yield of protein per mRNA is increased. The stability of the processed *thrS* transcript also increased in response to arginine starvation, although not quite to the same extent as that provoked by a lack of threonine. This suggests that the increased mRNA half-life seen under starvation conditions may be at least partially due to a non-specific effect of translation inhibition. Arginine starvation does not lead to an increase in *thrS* expression because so little of the mRNA is processed, and the increase in stability of the cleaved transcript is essentially canceled out by a decrease in total readthrough.

The intensity of band corresponding to the processed RNA increases 35-fold (compared with 5-fold for the full-length transcript), and accounts for 86% of the *thrS* readthrough RNA under threonine starvation conditions. Under nonstarvation conditions, or following starvation for arginine, the situation is the reverse, and the majority of the *thrS* mRNA exists in its more labile, full-length form. Thus, there is a substantial amplification of the amount of cleaved relative to the amount of full-length RNA under inducing conditions. While the increase in stability of the processed transcript (1.6-fold) accounts for part of its amplification under threonine starvation conditions, the greatest proportion is due to an increased processing efficiency of the full-length RNA. The increased efficiency of *thrS* mRNA cleavage following threonine starvation would suggest that cleavage occurs in the antitermination conformation of the leader mRNA and could

be explained as follows. Under nonstarvation conditions, there is very little transcriptional readthrough and the majority of transcripts that escape termination might be predicted to quickly refold into the terminator conformation in the absence of uncharged tRNA. These full-length transcripts are consequently unlikely to be cleaved and are labile. In contrast, under inducing conditions, the vast majority of the full-length transcripts would be predicted to be held in the antitermination conformation by the uncharged tRNA and subject to cleavage and stabilization. The increased cleavage efficiency and stabilization of the processed transcript contribute greatly to the level of functional *thrS* mRNA in the cell under inducing conditions. Previously, the increase in expression of genes of this family following starvation for the cognate amino acid has been solely attributed to antitermination.

Since uncharged tRNA-Thr is thought to stabilize the antiterminator structure, and given the proximity of the cleavage site to the proposed site of tRNA-antiterminator interaction, we decided to test mutations in the *thrS* leader that would allow us to determine whether interaction with the tRNA were necessary for processing to occur. We did this experiment in the context of *thrS–lacZ* fusions we had previously constructed in the laboratory. Two mutant *thrS* fusions, the first where the ACC specifier codon was changed to the UAA stop codon, and the second where the 5′ half of the specifier domain was deleted, both of which would be predicted to prevent proper interaction with tRNA-Thr, were still processed efficiently under nonstarvation conditions *in vivo*. Thus, processing of the *thrS* leader can occur in the absence of the specifier codon or specifier domain, and probably, therefore, does not require the presence of the uncharged tRNA. However, that cleavage of *thrS* mRNA is increased specifically under threonine starvation conditions would suggest that, nevertheless, stabilization of the antiterminator structure by uncharged tRNA stimulates processing.

We have previously shown that antitermination can occur independently of uncharged tRNA-Thr, and that the tRNA-independent antitermination mechanism is used to control expression of *thrS* by growth rate (8). Since processing can occur in the absence of uncharged tRNA-Thr, but appears to be stimulated by its presence, the data presented in this paper suggest that processing of the *thrS* leader is important for the stabilization of readthrough transcripts resulting from both the tRNA-independent and tRNA-dependent antitermination mechanisms.

Processing was abolished in the *thrS–lacZ* fusion truncated in the loop of the leader terminator, suggesting that the terminator itself, or sequences 3′ to it, are necessary for cleavage *in vivo*. An alternative possibility was that processing occurred as normal in this mutant fusion, but that because the processed RNA lacked the terminator structure at its 5′ end, it became unstable to the point of nondetection. However, the accumulation of the readthrough mRNA in the full-length form in the mutant, compared with the wild-type fusion under inducing conditions, leads us to favor the former interpretation. While a requirement for an intact terminator for processing may seem at odds with the idea that cleavage occurs in the antitermination conformation, the apparent paradox could be explained if a pause of RNA polymerase in the terminator region were required for proper folding of the mRNA into the antitermination conformation for processing.

Our primer extension analysis of the genes for other aminoacyl-tRNA synthetases suggests that processing of the leader regions of these genes may be widely conserved. In five of the eight *B. subtilis* synthetase genes we examined, there was a clear RT stop that could be mapped to just upstream of the terminator structure in each case. There also appears to be an RT stop in an equivalent position in the *ilv–leu* operon. It obviously remains to be proven that this RT stop corresponds to a mature 5′ end in each case; however, its conservation of position is highly suggestive that these mRNAs are also cleaved *in vivo*. There is no obvious sequence or positional homology between the proposed cleavage sites of the various genes. The processing site is not consistently the same distance from either the T-box or the terminator stem, the most obvious points of reference given their conservation. If the antitermination conformation of these mRNAs were preferred, as we suspect is the case for *thrS*, cleavage would occur in single stranded regions in the antiterminator structure of *thrS, ilv-leu,* and *serS* but would occur in a region predicted to be double stranded in *thrZ*, *tyrS*, and *trpS*. The specificity of the endonuclease activity will be the focus of future studies.

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