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RNase E plays an essential role in the maturation of *Escherichia coli* tRNA precursors

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ABSTRACT

Conversion of tRNA precursors to their mature forms requires the action of both endo- and exoribonucleases. Although studies over many years identified the endoribonuclease, RNase P, and several exoribonucleases as the enzymes responsible for generating the mature 5′ and 3′ termini, respectively, of *Escherichia coli* tRNAs, relatively little is known about how tRNAs are separated from long multimeric or multifunction transcripts, or from long leader and trailer sequences. To examine this question, the tRNA products that accumulate in mutant strains devoid of multiple exoribonucleases plus one or several endoribonucleases were analyzed by northern analysis. We find that the multifunction *tyrT* transcript, which contains two $tRNA_1^{Tyr}$ sequences separated by a 209-nt spacer region plus a downstream mRNA, is cleaved at three sites in the spacer region by the endoribonuclease, RNase E. When both RNase E and RNase P are absent, a product containing both tRNAs accumulates. Two multimeric tRNA transcripts, those for tRNA Arg-His-Leu-Pro and tRNA Gly-Cys-Leu also require RNase E for maturation. For the former transcript, products with long 3′ extensions on $tRNA^{Arg}$, $tRNA^{His}$, and $tRNA^{Pro}$, as well as the primary transcript, accumulate in the absence of RNase E. For the latter transcript, RNase E cleaves downstream of each tRNA. Little processing of either multimeric transcript occurs in the absence of both RNase E and RNase P. These data indicate that RNase E is a major contributor to the initial processing of *E. coli* tRNA transcripts, providing substrates for final maturation by RNase P and the 3′ exoribonucleases. Based on this new information, a detailed model for tRNA maturation is proposed.

Keywords: ribonuclease; RNA processing

INTRODUCTION

During their biosynthesis, tRNA precursors undergo a series of processing events that convert a primary transcript to a mature, functional molecule (Deutscher, 1995). However, despite work over many years, we still lack information about some major steps of the maturation process. In *Escherichia coli,* where our knowledge is most advanced, tRNA maturation requires removal of precursor-specific residues from both the 5′ and 3′ termini of the RNA chain (Deutscher, 1995). At the 5′ terminus, this is accomplished by RNase P, which generates the mature 5′ end of the tRNA by a single endonucleolytic cleavage (Altman et al., 1995). In contrast, maturation of the 3′ terminus appears to be a multistep process that utilizes a number of different enzymes (Deutscher, 1990).

Early studies suggested that 3′ processing of bacterial tRNA precursors would involve an initial endonucleolytic cleavage followed by one or more exonucleolytic trimming reactions (Bikoff & Gefter, 1975; Sekiya et al., 1979). Endonucleolytic cleavage is needed because tRNA precursors often contain long, highly structured 3′ trailer sequences that would be difficult to remove by exoribonuclease action. Secondly, multimeric tRNA precursors require endonucleolytic cleavages to separate individual tRNAs from each other. Thirdly, because RNase P is inhibited by the presence of long 3′ precursor sequences (Altman et al., 1987), an initial endonucleolytic cleavage at the 3′ end would be necessary for 5′ maturation to proceed efficiently. Numerous attempts to identify the relevant endoribonuclease(s) were undertaken and potential activities were observed in crude extracts or in partially purified preparations (Deutscher, 1995). These activities were given various names by different investigators such as RNase P2, P4, O, F, and PC, but their genetic identities

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were unknown, and their structural and catalytic properties were not well characterized.

Other reports suggested that the known endoribonucleases, RNase III and RNase P, might participate in 3′ maturation of tRNA precursors; however, their actions were observed in only a few cases and were not studied further (Deutscher, 1995). A role for the endoribonuclease, RNase E, was also suggested (Ray & Apirion, 1981a, 1981b). For example, in RNase E^-, RNase P^- double mutant cells, little 4S (tRNA-sized) RNA was made after inactivation of both enzymes, but a specific role for RNase E was not determined. Secondly, a precursor of $tRNA^{Ser}$ containing extra 3′ sequences was found to accumulate in an RNase E^- strain. However, this observation was not extended to other tRNA species, and it was unclear whether RNase E played a general role in the processing of tRNA precursors or how it might act.

One problem in defining a role for an endoribonuclease is that the downstream cleavage product is often rapidly degraded. Without identification of this product, it is difficult to confirm that an endonucleolytic cleavage has occurred, and it is not possible to determine the cleavage site. This problem can be overcome partially by the use of mutant *E. coli* strains in which multiple exoribonucleases are eliminated, especially the active enzymes, RNase II and polynucleotide phosphorylase (PNPase; Li & Deutscher, 1994, 1996). Thus, in an earlier study using strains with a low level of exoribonuclease activity, we showed that a long 3′ trailer sequence that was cleaved from a tRNA precursor could accumulate, directly demonstrating the involvement of an endoribonuclease in the 3′ processing of this precursor (Li & Deutscher, 1996).

In this article, we extend these observations to identification of the endoribonuclease responsible for maturation of many tRNA precursors and of the specific cleavage sites generated by the enzyme. To do this, we carried out extensive northern and primer extension analyses of strains lacking the endoribonucleases, RNase P, E, or III, and also deficient in multiple exoribonucleases. Our data show that RNase E is a major endoribonuclease involved in the maturation of tRNA.

RESULTS

Analysis of endonucleolytic processing of complex tRNA transcripts in an exoribonuclease-deficient background

As noted, a major difficulty in the analysis of endonucleolytic processing is that the non-tRNA cleavage products often are too unstable to detect due to trimming or degradation by exoribonucleases. Knowledge of the amount and sequence of such products are important to confirm an endonucleolytic cleavage and to identify cleavage sites. For example, in our initial studies of the processing of the *tyrT* transcript in wild-type backgrounds, we were unable to compare cleavage products or to analyze cleavage sites because of product destruction (data not shown). In an attempt to circumvent such problems, we constructed a mutant strain, $II^{ts}T^-\ D^-\ BN^-\ PNP^-$, which is deficient in the exoribonucleases, RNase T, D, BN, and PNPase, and also contains a temperature-sensitive RNase II (Li & Deutscher, 1996). At high temperature, this strain lacks five activities, including the two most active exoribonucleases, RNase II and PNPase. Temperature-sensitive mutations in RNases E and P or a null mutation in RNase III were then introduced into this strain, either alone or in combination, and the tRNA products that accumulated for 30 min after the temperature shift were examined to define the roles of these endoribonucleases in tRNA maturation.

In this work, we have examined three representative tRNA transcripts. The first is the *tyrT* transcript (Fig. 1A). This is a mixed-function transcript that contains in its 5′ half a tandem repeat of $tRNA_1^{Tyr}$ separated by a long spacer. The 3′ half consists of rtT mRNA separated from the downstream $tRNA_1^{Tyr}$ by a short spacer. Including the 5′ leader and 3′ trailer sequences, the length of the entire transcript is 648 nt. The second is the *argX-hisR-leuT-proM* multimeric transcript, which contains $tRNA_3^{Arg}$, $tRNA^{His}$, $tRNA_1^{Leu}$, and $tRNA_3^{Pro}$ separated by spacer sequences. Its total length is 480 nt (Fig. 4A). The third is the *glyW-cysT-leuZ* multimeric transcript. It contains $tRNA_3^{Gly}$, $tRNA^{Cys}$, and $tRNA_5^{Leu}$ separated by spacers. Its total length is 436 nt (Fig. 5A). All three transcripts are initiated from their own promoter and are derived from chromosomal genes. Whereas the *tyrT* transcript terminates primarily at a rho-dependent terminator (Rossi et al., 1981), the other two transcripts terminate at rho-independent terminators (Hsu et al., 1984; Komine et al., 1990).

Maturation of these tRNA transcripts would be expected to require endonucleolytic cleavages to remove leader and trailer sequences, to separate tRNAs or to separate tRNA from mRNA, all in defined steps. We would also expect that processing intermediates containing these extra sequences would accumulate when the required endoribonuclease was eliminated by mutation. We detect the products that accumulate in such mutant strains by northern blotting using probes complementary to specific regions of the transcripts. In many cases, the 5′ termini of the products also were determined by primer extension. For the *tyrT* transcript, an in vitro experiment was also carried out to examine the capabilities of various endoribonucleases for RNA processing.

Maturation of the *tyrT* transcript in vivo

The *tyrT* transcript is one of the more complex tRNA transcripts generated in *E. coli.* Nevertheless, its mat-

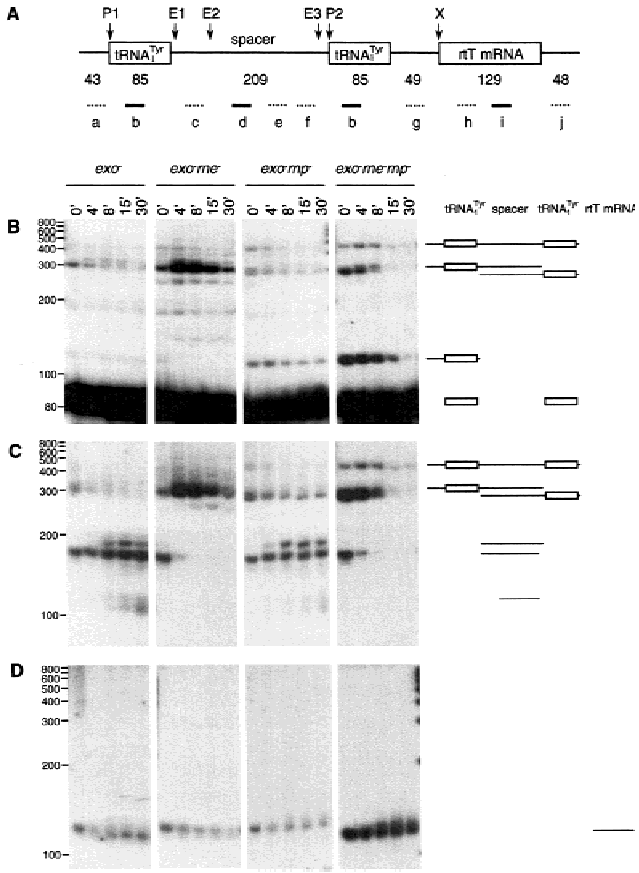

FIGURE 1. Northern analysis of the *tyrT* transcript. The structure of the 648-nt RNA is shown in **A**. The tRNA and mRNA regions are shown as boxes and the extra sequences are shown as lines. The length in nucleotides of each part of the transcript is shown below. The probes used for the analysis are also positioned below the transcript. Solid bars represent probes for which the data are shown in this figure, and dashed bars are for probes for which data is not shown. Based on the results obtained, the derived endonucleolytic cleavage sites are marked on the top with arrows. The northern analysis was carried out as described in Materials and Methods using 15 μg of RNA in each lane. Four panels are presented for each section of the figure detailing the results of northern blotting with RNA from each of the four mutant strains indicated on the top of **B**. The *exo*$^-$ strain, CA244 II^{ts}T$^-$D$^-$BN$^-$PNP$^-$, and its derivatives, are described in the text. Cells were grown at 30 °C, and RNA was prepared after shifting to 44 °C for the indicated period of time. DNA size markers, labeled with ^{32}P at their 5′ end, were used to determine the lengths of the RNA product. RNA markers of lengths 218 to 700 nt were also used in some experiments. Inasmuch as migration of the DNA and RNA markers was very similar in this system, only the DNA lengths are shown at the left. Based on the lengths of the products, and on patterns of hybridization with other probes, the deduced structures for the major bands are diagramed on the right. **B**, **C**, and **D** represent the results using probes, b, d, and i, respectively. Note that the mature tRNA^{Tyr} seen in **B** is due to molecules synthesized before the temperature shift that inactivates the RNases.

uration to tRNA$_1^{Tyr}$ has received extensive study. It was suggested that two rho-dependent terminators act on this transcript, with the more downstream one being responsible for about 80% of the termination events (Rossi et al., 1981). In another study, it was reported that the major transcript also contains a messenger RNA (rtT), 171 nt long, that encodes a small, basic protein (Bosl & Kersten, 1991). This mRNA is thought to begin at the eighth nucleotide downstream of the second tRNA, and that the maturation process initiates with an endonucleolytic cleavage after the seventh nucleotide downstream of the second tRNA. We would expect that the resulting extra seven-nucleotide sequence on the tRNA is removed by exoribonucleases, and that the 5′ leader sequence would be removed by RNase P. However, the 3′ endonucleolytic cleavage that removes the mRNA and the cleavage in the spacer region that separates the two tRNAs are not well understood.

To identify enzymes responsible for these reactions, we have undertaken a detailed examination of the maturation of the *tyrT* transcript in vivo. This was carried out with a variety of mutant cells using the endogenous chromosomal transcript to avoid possible artifacts due to overexpression. Total RNA was prepared from late log phase cells before and after inactivation of temperature-sensitive RNases by elevation of the temperature to 44 °C, and the products derived from the *tyrT* transcript were identified by northern blotting using multiple probes specific to different regions of the transcript, as outlined in Figure 1A. Although experiments were carried out with each of the probes shown, data from only a few of them (probes b, d, and i) are described in detail here to simplify the presentation. Nevertheless, data from all the probes, together with size determination and primer extension analysis, were used to verify the identities of the observed *tyrT* products and to relate them to the absence of specific RNases.

Northern analysis using probe b, specific for the tRNA part of the transcript, is shown in Figure 1B. Not shown is the analysis of RNA from a wild-type strain, which revealed that precursors do not accumulate when all the RNases are present. However, when RNA from the exoribonuclease-deficient strain was examined, a small amount of tRNA precursor was seen. This material rapidly disappeared, presumably due to its conversion to mature tRNA (Fig. 1B, left panel). Upon additional inactivation of RNase E (Fig. 1B, second panel), precursors accumulated in large amount. Based on size and hybridization with various probes, the major product contains the upstream tRNA attached to both the long 3′ spacer and the 5′ leader sequences, as shown on the right side of Figure 1B. The presence of the 5′ leader sequence is not unexpected because RNase P is known to work poorly removing the 5′ extra sequence on tRNA precursors containing a long 3′ trailer (Altman et al., 1987). The data suggest that RNase E is required to remove the spacer sequence from the 3′ end of the upstream tRNA.

Upon inactivation of RNase P (Fig. 1B, third panel), four tRNA-containing products are observed, although they are present in relatively small amounts. The shortest product is the upstream tRNA with its 5′ leader sequence. Its identity was confirmed by the fact that it

could be detected by probe a as well as probe b (data not shown). The second product is the downstream tRNA with a long 5′ sequence derived from the spacer region. This product was not observed in the RNase E single mutant because RNase P would have cleaved it. Its presence in the *rnp* mutant confirms that RNase E can cleave close to the 3′ end of the upstream tRNA, as concluded above from the product accumulated in the *rne* mutant. The third product is the same as that present in the *rne* mutant. The longest product in the *rnp* mutant contains both copies of $tRNA^{Tyr}$, as well as the leader and spacer regions. The presence of the two latter products suggests that some RNase E cleavages may be relatively slow. It should be noted that all the products seen in the *rnp* mutant are already present before the temperature shift because the mutant RNase P is probably partially inactivated even at low temperature. The various products do not increase after the temperature shift, indicating that other enzymes present in the *rnp* strain can process them to mature tRNA or degrade them.

In the *rne, rnp* double mutant (Fig. 1B, right panel), the same products as in the *rnp* mutant are present, but in this strain, the two largest products actually accumulate upon elevation of the temperature. These data indicate that maturation of the 5′ half of the *tyrT* transcript essentially stops in the absence of RNase E and P. The amounts of the two smaller products in the double mutant are much higher than in the *rnp* single mutant, even at zero time, suggesting that RNase E is responsible for their conversion or degradation and that it may be partially inactivated before the temperature shift. As with the single mutant, all the products that are present in the double mutant eventually disappear, presumably due to degradation by other unidentified activities.

An RNase III mutation was also introduced into the exoribonuclease-deficient (exo^-) background, alone or in combination with RNase E or P mutations. However, no additional effect was observed upon inactivation of this enzyme in any of the strains (data not shown). Based on this information, it appears that RNase III does not participate in maturation of the *tyrT* transcript.

Many of the same products that were seen when northern analysis was performed with probe b also are observed using probe d, which is specific for the spacer region (Fig. 1C). This observation confirms that these products contain spacer sequences. In addition, several new, spacer-specific products can be detected in the exo^- strain (Fig. 1C, left panel). These products, which must contain only spacer sequences, accumulate to higher levels upon elevation of the temperature and subsequent inactivation of RNase II, indicating that exoribonucleases normally are responsible for their degradation. They disappear after inactivation of RNase E, simultaneous with the increase of even longer precursors (Fig. 1C, second panel), indicating that RNase E participates in their release from the longer precursor by cutting near the 3′ end of the upstream tRNA (designated at E1 in Fig. 1A). RNase E must also cut near the 3′ end of the spacer (designated as E3) because the spacer-specific products are still produced even when RNase P is inactivated (Fig. 1C, third panel). However, the E3 cleavage may not be very efficient, as a product with the spacer attached to the downstream tRNA is present in the *rnp* mutant in which RNase E remains active. A third RNase E cleavage (designated E2 in Fig. 1A) is also thought to occur and is responsible for formation of the shortest spacer product. This product is not detected by probe c, but is seen with probes e and f in addition to d (data not shown). However, it could be formed after the spacer is released, and may actually be a degradation intermediate.

Inasmuch as the 5′ leader sequence is still present on the longer products, these data demonstrate that the RNase E cleavages occur prior to 5′ processing of the upstream tRNA by RNase P (cleavage P1). Thus, RNase E plays a role in the early steps of *tyrT* RNA processing. Again, the presence of RNase III does not have an effect on the products from the spacer region (data not shown).

Using probe i to examine the downstream portion of the *tyrT* transcript, a product corresponding in size to the rtT mRNA is present in all the strains (Fig. 1D). This product is not detected by either probe g or probe j, confirming that it encompasses just rtT mRNA. There is no change in the size of the product when RNase E and/or P is inactivated. Likewise, removal of RNase III also does not have any effect. In addition, long precursors containing both tRNA and rtT mRNA were not observed to be major products in any experiments, nor did they accumulate upon inactivation of RNases. These observations suggest that an activity other than RNase E, P, or III may be responsible for cleavage between the tRNA and the mRNA. RNase G, an endoribonuclease sharing sequence and functional homology with RNase E (Li et al., 1999a; Wachi et al., 1999) is a good candidate for such an activity. Alternatively, the possibility also must be considered that rtT is synthesized separately from *tyrT* when chromosomal rather than cloned genes are transcribed, as previous work was carried out with cloned genes (Rossi et al., 1981; Bosl & Kersten, 1991).

Experiments were performed with all the probes shown in Figure 1A. Analysis of the patterns of the products accumulated in each of the mutant strains leads to the conclusion that RNase E is a major processing enzyme for *tyrT* RNA. It appears to cleave at three positions within the spacer region. Two of these cleavage sites could be defined by primer extension experiments using oligonucleotide e as the primer (Fig. 2A). Based on this analysis, the E1 cleavage is at a position 3 nt downstream of the 3′ terminus of the upstream tRNA. The E2 cleavage results in several

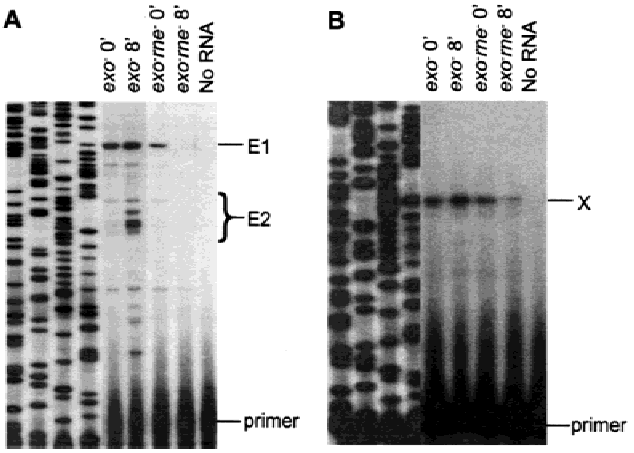

FIGURE 2. Primer extension analysis of the spacer and mRNA regions of *tyrT* RNA. Probes e and i were used for the spacer and rtT mRNA, respectively, and the results are presented in **A** and **B**. The experiment was carried out as described in Materials and Methods using the same RNA samples as for the northern analysis. A DNA sequencing ladder was run together with the products for size determination. The identified extension products are indicated on the right using the cleavage designations shown in Figure 1A. Zero and 8 min refer to the times after the temperature shift.

primer extension bands, the major one being 49 nt downstream of the tRNA. In keeping with the northern analysis, both the E1 and E2 cleavage products are more abundant when RNase II is inactivated (compare 8 min to 0 min after the temperature shift). In addition, these products are missing in the absence of RNase E after 8 min supporting their formation by RNase E and their degradation by RNase II and other exoribonucleases. The third RNase E cleavage site, E3, is proposed to be 10 to 20 nt upstream of the 5′ end of the downstream tRNA based on length measurements, for example, Figure 1C. However, we have been unable to get a primer extension product corresponding to that position.

Primer extension analysis using oligonucleotide i as the primer generated a single band corresponding to a cleavage 49 nt downstream of the second $tRNA^{Tyr}$ (Fig. 2B). The enzyme responsible for this cleavage has not been determined, and is designated X in Figures 1A and 2B.

Processing of the $tRNA^{Tyr}su_3^+$ transcript in vitro

To examine further the actions of various endoribonucleases on the precursor of $tRNA^{Tyr}$, we carried out experiments to determine whether the enzymes can act on the precursor in vitro. This was done by generating a simpler transcript from a construct that contains the amber suppressor form of $tRNA^{Tyr}$, $tRNA^{Tyr}su_3^+$. The in vitro-synthesized transcript, uniformly labeled with [α-^{32}P] CTP, contains the leader sequence, one copy of $tRNA^{Tyr}su_3^+$, followed by the downstream rtT mRNA and the 3′ UTR (Fig. 3A). Its total length is 404 nt. The in vitro-synthesized RNA was treated with cell extracts prepared from mutant strains deficient in exo- and endoribonucleases, or with purified RNase E.

As shown in Figure 3B, the full-length transcript disappears quickly after mixing with an extract from the exo$^-$ strain (temperature-sensitive RNase II had been inactivated prior to the reaction by incubation at 45 °C for 30 min). The major product already formed after 10 min, was the size of mature tRNA. Another stable product ~120 nt in length was also produced. This product probably derives from the rtT mRNA region, as it was also produced in vivo, as shown in Figure 1D. Removal of RNase III had no effect on processing of $tRNA^{Tyr}$ transcript; however, inactivation of RNase E largely blocked processing. Essentially no tRNA or 120-nt products were produced, suggesting that RNase E is largely responsible for their production. As might be expected, removal of RNase P also has an effect. Two products, both a few nucleotides longer than mature $tRNA^{Tyr}$, were generated in its absence. The longer one is produced first and is gradually converted to the shorter one over time. These products probably are generated by endonucleolytic cleavages upstream of the mature 5′ end in the absence of RNase P.

The in vitro transcript was also treated with purified RNase E. As shown in Figure 3C, two major products close to tRNA in size were produced, in agreement with the results seen with the *rnp* cell extract (Fig. 3B), and indicating that RNase E is able to cleave near both termini of the tRNA. These results strongly suggest that RNase E is responsible for generating the products seen in the extract lacking RNase P. Another product of ~120 nt also was generated by purified RNase E (Fig. 3C), suggesting that the enzyme is able to release this product derived from the mRNA region of the transcript. These data suggest that RNase E has the capability to make specific cleavages in the 3′ half of the *tyrT* transcript, although this was not seen in vivo where alternative activities may also function.

Maturation of the tRNA Arg-His-Leu-Pro transcript

The second transcript examined is the multimeric molecule that contains $tRNA_3^{Arg}$, $tRNA^{His}$, $tRNA_1^{Leu}$, and $tRNA_3^{Pro}$ (reading from the 5′ to the 3′ end; Fig. 4A). $tRNA_3^{Arg}$ is preceded by a 13-nt leader sequence and it is separated from $tRNA^{His}$ by a 57-nt spacer region. Northern and primer extension analyses were carried out as with the *tyrT* transcript. Only data with probes directed against $tRNA_3^{Arg}$ and $tRNA^{His}$ are presented, as they clearly demonstrate the role of RNase E in maturation of this transcript.

Northern analysis with a probe complementary to $tRNA_3^{Arg}$ is presented in Figure 4B. Using RNA from the

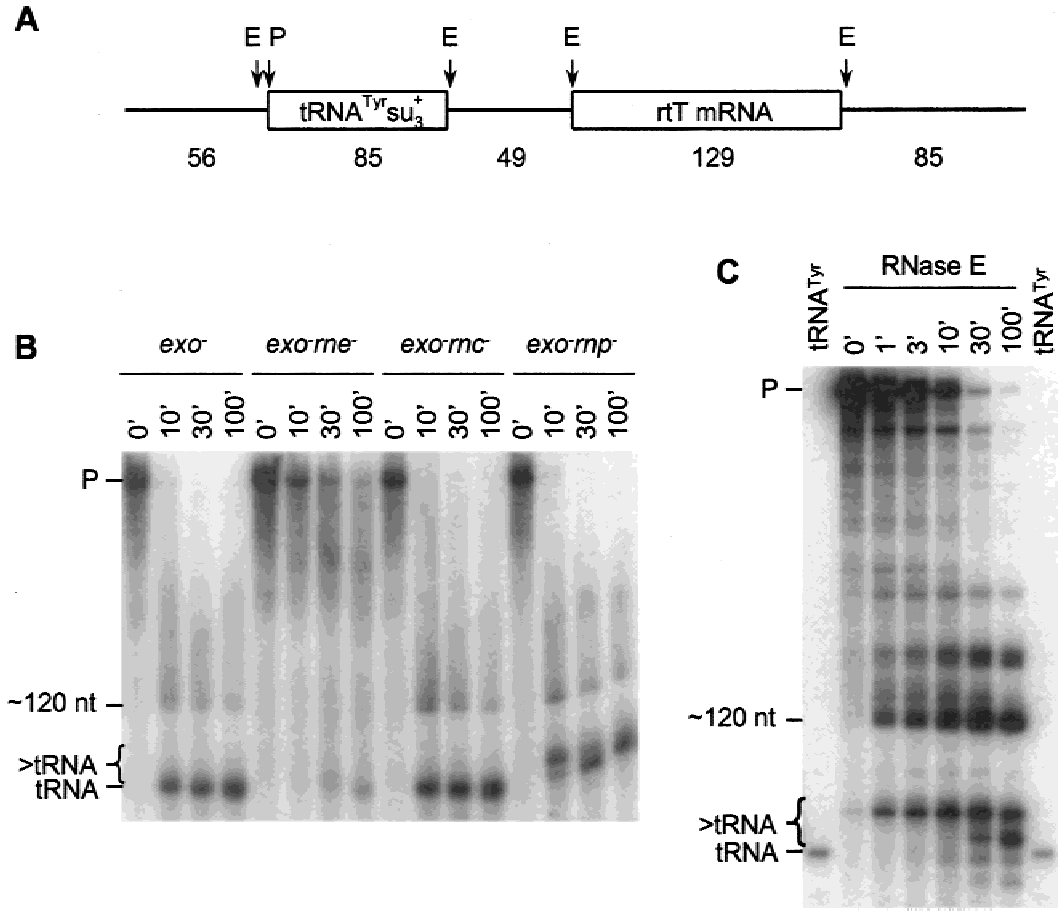

FIGURE 3. In vitro analysis of processing of the $tRNA^{Tyr}su_3^+$ transcript. The structure of the 404 nt in vitro run-off transcript, synthesized as described in Materials and Methods, is shown in **A** using the same format as in Figure 1A. The transcript was treated either with cell extracts (**B**) or purified RNase E (**C**) for the indicated times. Cell extracts were preincubated at 45 °C for 30 min to inactivate the temperature-sensitive RNases II, E, and P. $tRNA^{Tyr}$ and 5S rRNA (120 nt) were used as size markers. The products are marked on the left. "P" in **B** and **C** denotes precursor.

exo$^-$ parent strain (Fig. 4B, left panel), multiple bands are evident. Interestingly, some full-length transcript can be seen at all the time points, suggesting that the reactions that separate individual tRNAs must be relatively slow. The other major products are larger than mature $tRNA_3^{Arg}$, and all of these molecules shift to longer forms upon elevation of the temperature that inactivates the temperature-sensitive RNase II. The longest of these products corresponds in size to a molecule containing the 5′ leader, $tRNA_3^{Arg}$ and most of the spacer sequence, as shown in the diagram on the right side of Figure 4B. The shorter products are similar except that the spacer sequences have undergone some trimming. It is not surprising that the 5′ leader is present on all these product because, as noted earlier, the long 3′ sequence inhibits RNase P action. In fact, the identical products are present in the *rnp* mutant (Fig. 4B, third panel), as would be expected. These data show that removal of the long spacer sequence is dependent on the exoribonucleases absent from the exo$^-$ strain, although some 3′ terminal trimming by residual RNases can still occur.

These intermediate-sized precursors of $tRNA_3^{Arg}$ disappear upon removal of RNase E (Fig. 4B, second panel) indicating that it is responsible for their production by cleaving at E1 (Fig. 4A). Instead, what accumulates are large amounts of the full-length transcript, demonstrating that RNase E is required for the initial steps of maturation of this multimeric transcript. This product also accumulates in the *rne, rnp* double mutant (Fig. 4B, right panel). Also present in the double mutant, and in the *rnp* strain (Fig. 4B, third panel) is $tRNA_3^{Arg}$ with its 5′ leader that results from the absence of RNase P. As noted earlier with the *tyrT* transcript, such products are seen even before the temperature shift, because the mutant RNase P activity is low even at the permissive temperature. Taken together, these data show that $tRNA_3^{Arg}$ is separated from the multimeric transcript by an RNase E cleavage upstream, but near, the 5′ end of $tRNA^{His}$. Primer extension from a primer in $tRNA^{His}$ revealed three positions for this RNase E cleavage; the two major ones are 7 and 8 nt upstream from $tRNA^{His}$ and a minor one is 16 nt upstream (data not shown).

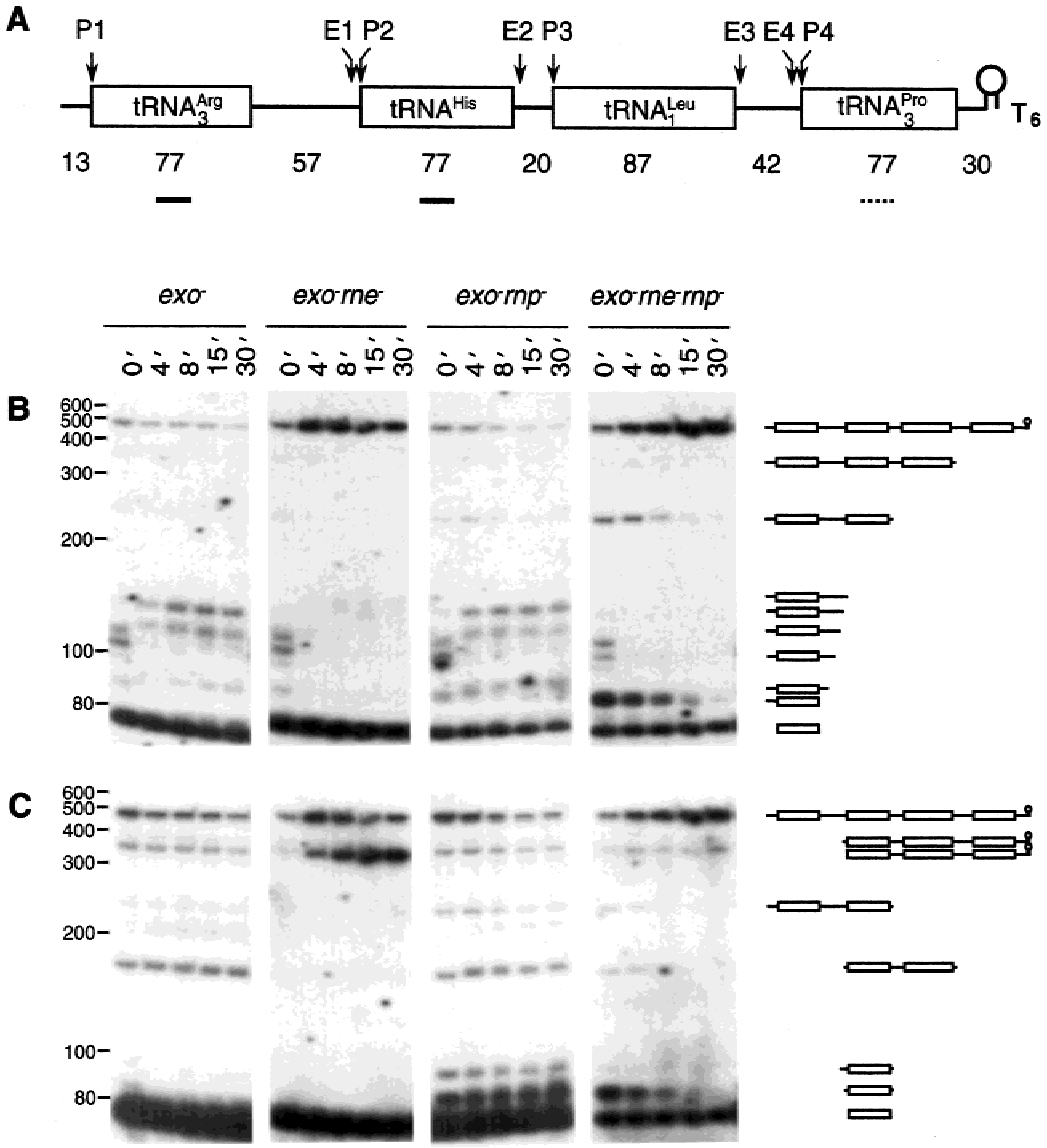

FIGURE 4. Northern analysis of the *argX-hisR-leuT-proM* transcript. **A** details the structure of this 480-nt RNA using the same format as in Figure 1A. **B** and **C** present the results of northern blotting using probes for $tRNA_3^{Arg}$ and $tRNA^{His}$, respectively, carried out as in Figure 1. Sizes of the bands from DNA size markers are shown on the left, and the structure of each product is shown on the right.

Additional information about maturation of the multimeric transcript came from northern analysis using a probe complementary to $tRNA^{His}$ (Fig. 4C). In the exo$^-$ parent strain, besides the full-length transcript, products containing two or three tRNAs are observed that were generated by RNase E cleavage at position E1. These products do not change with time, indicating that they are always present at a certain level due to the relatively slow maturation of this transcript. Upon elimination of RNase E (Fig. 4C, second panel), the product with three tRNAs, as well as the full-length product, accumulate with time. Thus, as concluded from the data in Figure 4B, RNase E is needed to initiate the maturation process that leads to the smaller multimers seen the exo$^-$ parent. In its absence, RNase P can cleave at the 5 end of $tRNA^{His}$, albeit quite slowly, to separate the three downstream tRNAs from $tRNA_3^{Arg}$. Thus, the multimer of three tRNAs does not accumulate in the *rnp* strain (Fig. 4C, third panel), and the larger products seen are the same as those in the exo$^-$ parent. However, two smaller products are present; they contain $tRNA^{His}$ plus some of the upstream spacer and were generated by the RNase E cleavages in the spacer region. The 5′ termini of these products were confirmed by primer extension (data not shown), and they correspond to the expected RNase E cleavages already discussed.

In the *rne, rnp* double mutant, only the full-length transcript accumulates after the temperature shift. Comparing the products in the double-mutant to those in the

rnp single-mutant strain, it becomes clear that the products with two or three tRNAs must arise from RNase E cleavages at multiple positions, such as downstream of $tRNA^{His}$ and $tRNA_1^{Leu}$ (designated as E2 and E3). The latter cleavage is also seen by northern blotting using a probe directed against $tRNA_3^{Pro}$. A product containing $tRNA_3^{Pro}$ with a long 5′ leader was observed (data not shown). This piece of data suggests that E3 cleavage is close to the 3′ end of $tRNA_1^{Leu}$. Primer extension analysis, using primers in $tRNA_3^{Pro}$, and the 5′ region of $tRNA_1^{Leu}$, located the RNase E cleavages 2 nt downstream of $tRNA^{His}$ (E2), and 3 or 4 nt upstream of $tRNA_3^{Pro}$ (designated as E4, data not shown). Based on these analyses, RNase E is responsible for separating each of the tRNAs in this multimeric transcript.

Maturation of the tRNA Gly-Cys-Leu transcript

The third transcript examined to ascertain the importance of RNase E for tRNA maturation is the 436-nt multimer that contains $tRNA_3^{Gly}$, $tRNA^{Cys}$, and $tRNA_5^{Leu}$. Its structure is shown in Figure 5A. Northern and primer extension analyses were carried out in the same manner as for the previous transcripts studied. Interestingly, when RNA from the exo$^-$ parent was probed with an oligonucleotide complementary to $tRNA^{Cys}$, only the mature tRNA is seen (Fig. 5B, left panel). This contrasts with what was observed for other tRNAs, and indicates that $tRNA^{Cys}$ can be processed rapidly in vivo even in the absence of multiple exoribonucleases.

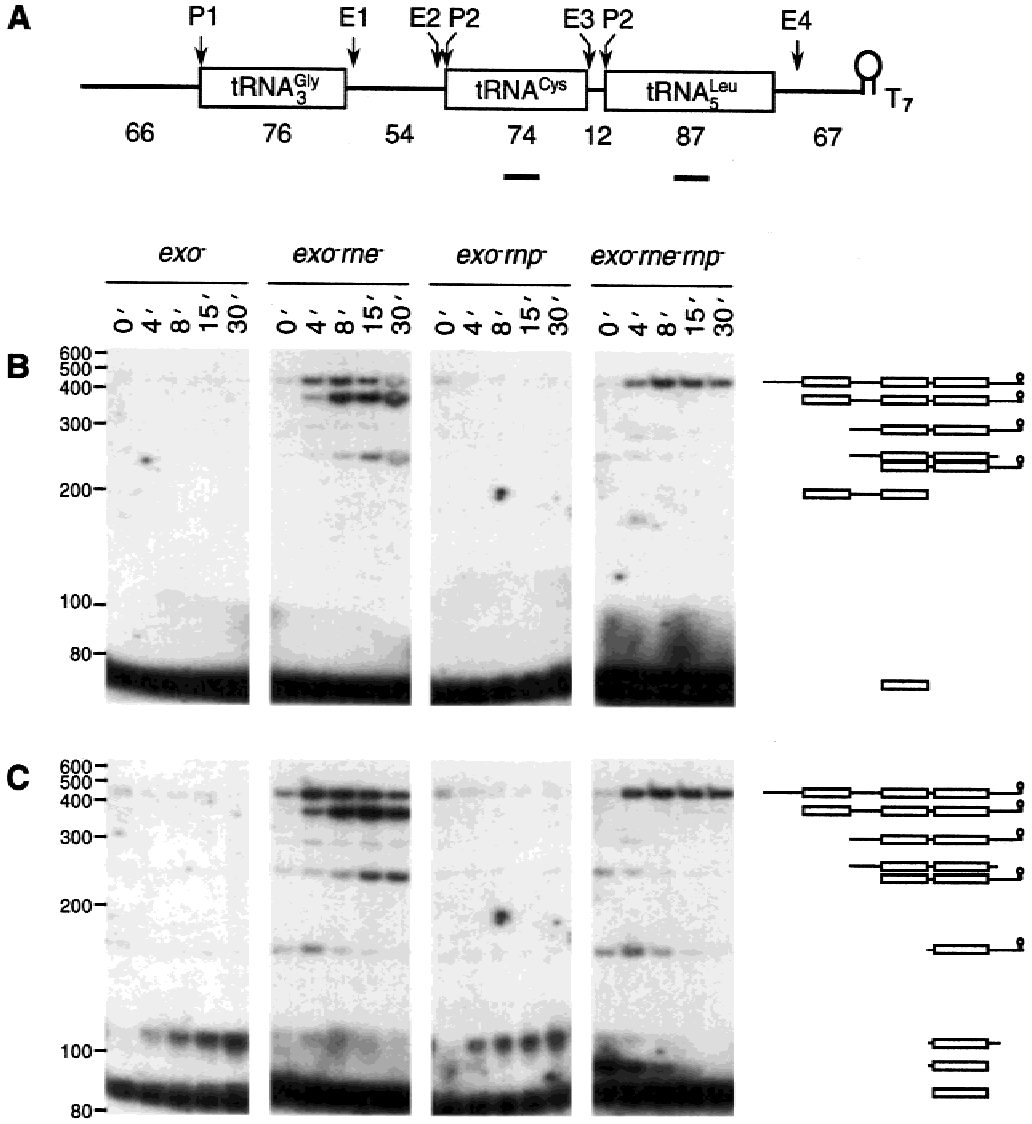

FIGURE 5. Northern analysis of the *glyW-cysT-leuZ* transcript. The structure of the 436-nt transcript is shown in **A**. The results of northern blotting, carried out as in Figure 1, using probes for $tRNA^{Cys}$ and $tRNA_5^{Leu}$ are shown in **B** and **C**, respectively. DNA size markers are shown on the left and the structure of each product is shown on the right.

Upon additional inactivation of RNase E, a number of products accumulate (Fig. 5B, second panel). These include the full-length transcript, the full-length transcript lacking its 5′ leader sequence, and a product containing the two downstream tRNAs plus the 3′ trailer. The latter two products accumulate more slowly than the full-length transcript, and must be due to slow RNase P cleavage of the full-length molecule at the P1 and P2 positions (Fig. 5A) because they are absent in the *rne, rnp* strain in which RNase P is also eliminated (Fig. 5B, right panel). Only the full-length transcript accumulates to any degree in the double mutant, although a few other minor products also can be seen and probably are found before RNase E and RNase P are fully inactivated. Several of these products, which are diagramed on the right side of the figure, suggest that one RNase E cleavage site (designated E1 in Fig. 5A) is present close to the 3′ end of $tRNA_3^{Gly}$.

Surprisingly, no processing intermediates of $tRNA^{Cys}$ are present when RNase P is inactivated (Fig. 5B, third panel). This contrasts to what is seen when RNase E is missing (Fig. 5B, second panel), and indicates that the initial steps of maturation can proceed when RNase P is absent. In addition, because no product containing $tRNA^{Cys}$ plus the 5′ spacer is seen in the *rnp* mutant, it implies either that RNase E can cut near the 5′ end of the tRNA or that this intermediate is unstable and is degraded.

Use of a probe directed against $tRNA_5^{Leu}$ further clarified the maturation process of this multimeric transcript (Fig. 5C). With this probe, one major product is seen in the exo$^-$ parent. This product corresponds to $tRNA_5^{Leu}$ with a long 3′ trailer sequence, and it accumulates after the temperature shift that inactivates the temperature-sensitive RNase II. Thus, exoribonucleolytic trimming of the long trailer is required to mature the 3′ end of $tRNA_5^{Leu}$. It is likely that this product also contains 5′ extra residues from the spacer region between $tRNA^{Cys}$ and $tRNA_5^{Leu}$, and the product with just the 5′ extra residues can be seen in the *rnp* strains (Fig. 5C, two right panels). Confirmation of this point comes from primer extension analysis (see below). Accumulation of this product suggests that there is an RNase E cleavage at the E3 position.

Inactivation of RNase E leads to visualization of the same products seen with the probe against $tRNA^{Cys}$. In addition, two other products are also evident. One appears transiently at 4 min and contains $tRNA_5^{Leu}$ and the complete 3′ trailer, and presumably is produced by RNase E acting at E3 before it inactivates. The other has the shortened 3′ trailer already seen in the exo$^-$ strain. This latter product, however, does not accumulate in the *rne* strain, indicating that its production depends on RNase E cleavage in the 3′ trailer. We estimate, based on size, that this cleavage at E4 occurs ~15 nt downstream of the 3′ end of $tRNA_5^{Leu}$.

As noted, in both the *rnp* mutant and the *rnp, rne* double mutant, a product with just the 5′ extra sequence is observed, but it is highest at zero time, and does not accumulate after the temperature shift in either strain. The existence of these products strongly suggested another RNase E cleavage near the 3′ end of $tRNA^{Cys}$, and this was confirmed by primer extension from a primer in $tRNA_5^{Leu}$. A stop was found 11 nt upstream of the 5′ end of $tRNA_5^{Leu}$, which is 1 nt downstream from the 3′ end of $tRNA^{Cys}$ (data not shown), which is consistent with the limited need for exoribonucleases for $tRNA^{Cys}$ maturation (Fig. 5B, left panel). These data show that RNase E makes multiple cleavages in the tRNA Gly-Cys-Leu transcript, and that in its absence the multimeric transcript accumulates.

DISCUSSION

By elimination of specific RNases in mutant strains, and analysis of the products that accumulate under such conditions, we conclude that the endoribonuclease, RNase E, plays an essential role in the maturation of many tRNAs in *E. coli.* RNase E has already been implicated in the maturation of ribosomal RNAs (Ghora & Apirion, 1978; Li et al., 1999a; Wachi et al., 1999), in the decay of a wide range of mRNAs (Regnier & Arraiano, 2000; Steege, 2000), and in the degradation of antisense RNA I (Lin-Chao & Cohen, 1991). In the present study, we have shown that the maturation of three complex tRNA transcripts essentially ceases upon inactivation of RNase E. In its absence, long processing intermediates or full-length transcripts accumulate to high levels. Thus, the data indicate that RNase E acts early in the maturation pathways, and that it is the primary enzyme responsible for separating individual tRNAs within complex transcripts. RNase E cleavage sites were identified downstream of the 3′ termini of most tRNAs examined. Inasmuch as RNase P works inefficiently when long 3′ sequences remain downstream of a tRNA (Altman et al., 1987), the absence of RNase E effectively eliminates RNase P action as well, and little processing occurs. These results agree with the previous findings of Apirion's group (Ray & Apirion, 1981a, 1981b) that little tRNA-size material is produced after the inactivation of both RNase P and RNase E. The data presented here clarifies the specific role of each enzyme in the maturation process.

Maturation of the *tyrT* mixed-function transcript

RNase E was found to cleave at least three times in the *tyrT* transcript in the spacer region between the two tRNAs. One more cleavage, 49 nt downstream of the second tRNA, was also suggested, based on the 5′ end of rtT mRNA, but this could not be ascribed to RNase E in vivo. Two additional cleavages, near the 3′

terminus of the downstream tRNA and of rtT mRNA also would be expected because products with 3′ ends at those positions were detected. Interestingly, purified RNase E can cleave at all three of these positions in vitro, suggesting that it may also work at these sites in vivo. However, this could not be shown by northern analysis, perhaps because of the presence of alternative activities, for example, RNase G, in vivo.

The longest product that we could detect from *tyrT* was about 600 nt, and this was present in very small amounts. Thus, even in the absence of RNase P and RNase E, the full-length *tyrT* transcript apparently is rapidly converted to shorter products. Again, this may be due to other endoribonucleases that can cleave the full-length molecule. However, it is also possible that the full-length transcript is rarely made in vivo. The earlier work by Landy's group (Rossi et al., 1981) with this transcript were all done with overexpressing systems that may have overwhelmed termination at the terminator shown to be present between the tRNAs and the mRNA. The downstream rtT mRNA, whose function is unclear, may be needed in only small amounts or under special environmental conditions, such that only a shorter transcript was produced in our experiments.

Based on our data, the rtT mRNA is 129 nt long. This contrasts with the report of Bosl and Kersten (1991) that the mRNA is 171 nt long. The shorter product does contain a Shine–Dalgarno sequence and the protein-coding region. We have no explanation for the difference in results, although the earlier size measurement was based on a weak primer extension signal and used a primer that covered the 5′ end of the 129-nt product. Nevertheless, the existence of the ~120 nt product in our experiments confirms a requirement for endonucleolytic processing in this region of the *tyrT* transcript.

Maturation of the multimeric tRNA transcripts

A stronger effect was observed for maturation of the two multimeric transcripts. In both cases, elimination of RNase E resulted in the accumulation of large amounts of the full-length transcript, and the almost complete elimination of processing when both RNase P and RNase E are absent. Because inactivation of only RNase P, by itself, did not block the initial processing events, these data show that RNase E is responsible for cleaving the primary transcripts into shorter intermediates. Four RNase cleavages were identified in each transcript, which served to separate the tRNAs from each other or from the long 3′ trailers.

Most of the RNase E cleavages occur close to the 3′ end of the tRNA, requiring only short trimming, primarily by RNase T and/or RNase PH, to generate the mature 3′ terminus (Li & Deutscher, 1994, 1996). However, the cleavage downstream of $tRNA_3^{Arg}$ leaves a long 3′ trailer, and the products that accumulated in the exo$^-$ strain indicated that this trailer was removed by exoribonucleases. We had suggested earlier, based on in vitro studies (Li & Deutscher, 1994), that RNase II and PNPase would be needed to remove long 3′ trailers. The shift to longer lengths of the $tRNA_3^{Arg}$ precursor upon inactivation of the temperature-sensitive RNase II supports a role for this enzyme in the 3′ shortening in vivo. However, because PNPase is already absent from this strain, we have no direct evidence for its role in vivo. RNase II is also responsible for shortening of the ~15 nt long 3′ trailer downstream of $tRNA_5^{Leu}$ in the trimeric transcript. As RNase II cannot remove residues close to the secondary structure of the aminoacyl stem, we suspect that the last few residues of each of the long trailers are removed by RNase T or RNase PH.

RNase E cleavage sites

Based on the present examples, it appears that RNase E cleaves at least once between tRNAs and between tRNAs and mRNAs or terminator sequences. These cleavages are very efficient, preceding other processing reactions and producing shorter precursors to individual tRNAs that are subsequently matured by RNase P and exoribonucleases. In some cases, two tRNAs in a transcript may be separated by only a few nucleotides, and in these cases RNase E may not be needed because the action of RNase P at the 5′ terminus of the downstream tRNA would be sufficient to separate the tRNAs. In other cases, in which a long spacer is present, RNase E may cleave at multiple positions, as seen here in *tyrT,* to aid in the removal of the spacer.

Analysis of the sequences around all of the RNase E cleavage sites identified here reveals that all are in long AU-rich stretches. Previous examination of RNase E cleavage sites in mRNA, rRNA, and RNAI also indicated a propensity for cleavage in AU-rich regions, although specific recognition sequences for RNase E are not known (McDowall et al., 1994, 1995; Li et al., 1999a). To extend these observations, the 3′ trailer sequences of all *E. coli* tRNAs were examined for AU-rich regions that could serve as potential RNase E cleavage sites. AU-rich sequences of at least 5 nt were found in every tRNA that had a 3′ trailer 6 nt or longer (Z. Li, unpubl. observation). Further work will be necessary to determine whether these sequences actually serve as RNase E cleavage sites.

tRNA maturation: A refined model

Based on the new information presented here, together with earlier work, we propose a more detailed model for tRNA maturation in *E. coli* (Fig. 6). RNase E (and perhaps other endoribonucleases in a few cases) cleaves tRNA-containing primary transcripts in the 3′

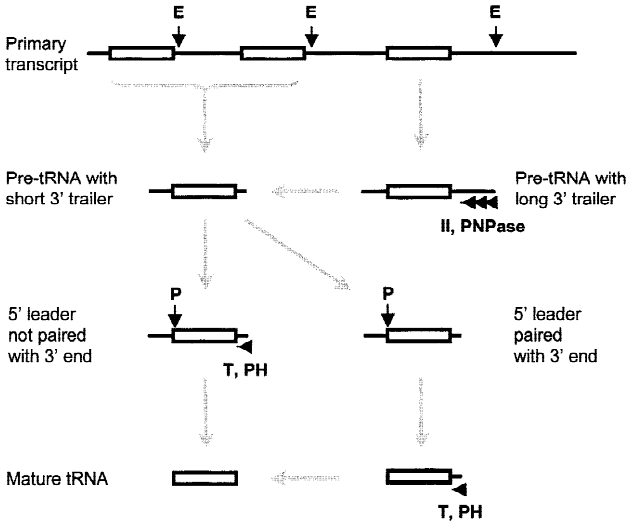

FIGURE 6. A refined processing model for *E. coli* tRNA. tRNA is shown as a box and extra sequences as lines. The 5′ ends of RNAs are on the left and 3′ ends are on the right. The vertical arrows indicate endonucleolytic cleavages by the RNase shown above, and horizontal arrows indicate exonucleolytic trimming reactions by the RNases shown below. Conversion from the primary transcript to the final product proceeds through the intermediates shown, which are connected by arrows. The primary transcript is first cleaved by RNase E. If the monomeric tRNA precursor contains a long 3′ trailer, it is shortened by RNase II and/or PNPase. Pre-tRNAs with short 3′ trailers are matured either by the stochastic action of RNase P (5′ end) and RNases T and PH (3′ end) when the 5′ extra sequence is not paired with the 3′ end, or by RNase P first, followed by RNases T and PH when the ends are paired.

trailer regions of the tRNAs. These cleavages serve to separate individual tRNA precursors or to separate tRNAs from mRNAs or from terminator sequences. Depending on the length of the resulting extra 3′ sequence, one of several possible reactions may take place next. If the sequence is short, RNase P will efficiently remove the 5′ leader to generate the mature 5′ end, and exoribonucleases, primarily RNase T and PH, will simultaneously trim the extra 3′ residues. If the extra 5′ residues base pair with the 3′ end, such as in the case of $tRNA_1^{Tyr}$ in which 2 bp are present, RNase P action will precede 3′ maturation because 3′ trimming is inhibited by the extended aminoacyl stem. This scenario is based on the observation that upon inactivation of RNase P, tRNAs with such base pairing accumulate precursors with extra 3′ residues (Schedl et al., 1976; Vogeli et al., 1977; Sakano & Shimura, 1978), whereas those without base pairing mature the 3′ end (Vogeli et al., 1977). This phenomenon agrees with our findings on the maturation of other stable RNAs (Li et al., 1998, 1999b).

If the extra 3′ sequence generated by the RNase E cleavage is long (~15 nt may be sufficient), RNase P action is inhibited, and 3′ shortening will then occur first. RNase II and PNPase efficiently and processively shorten the 3′ trailer (Li & Deutscher, 1994), followed by RNase P action and 3′ terminal trimming, as above, to generate the mature termini. The order of processing reactions is not obligatory, but is determined by the structure of the particular tRNA precursor. As we have postulated earlier (Li & Deutscher, 1996), RNA processing is stochastic, and reactions will occur whenever an RNase can act on a substrate. If the maturation pathway of a particular RNA precursor happens to follow a specific order of events, it is simply because the structure of a processing intermediate allows one enzyme to act more rapidly than another. Based on such a model, different tRNA precursors may follow different maturation pathways, and even a single precursor may proceed through a different sequence of processing steps on the way to the mature product.

MATERIALS AND METHODS

Bacterial strains

E. coli K12 strain CA244 (*lacZ*, *trp*, *relA*, *spoT*; Reuven & Deutscher, 1993) was considered wild type for these studies. The exo$^-$ derivative of CA244 (RNase II^{ts}T$^-$ D$^-$ BN$^-$ PNP$^-$) was reported previously (Li & Deutscher, 1994, 1996). The mutations in RNase T, RNase D, and PNPase are interruption mutations and lead to loss of the relevant activity. The mutations in RNase BN and RNase II have not been defined, but they lead to >98% loss of enzyme activity. Each of the endoribonuclease mutations was introduced into this strain by transduction. The *rnc::tet* mutation (RNase III gene interrupted by a tetracycline cassette, obtained from the Yale *E. coli* Genetic Stock Center) was introduced into a tetracycline-sensitive derivative of the exo$^-$ strain. Likewise, the *rne1* mutation, encoding the temperature-sensitive RNase E from strain SK5695 (Babitzke & Kushner, 1991) and the temperature-sensitive RNase P gene, *rnpA49*, were each introduced into the exo$^-$ strain by cotransduction with nearby Tn10 transposons obtained from the *E. coli* Genetic Stock Center. All the strains used in this study were stable, although some multiple RNase-deficient cells grew poorly. Previous work (Kelly & Deutscher, 1992) showed that inactivation of one or more exoribonucleases does not lead to overexpression of the remaining enzymes.

Materials

[γ-^{32}P]-ATP was purchased from DuPont-New England Nuclear. Phage T4 polynucleotide kinase and M-MLV reverse transcriptase were from Gibco BRL. RNasin was obtained from Promega. Sequagel for DNA sequencing was purchased from National Diagnostics. The oligonucleotides used for northern and primer extension analysis were prepared as in Table 1. All other chemicals were reagent grade.

RNA preparation

Cells were grown in YT medium to an $A_{550} \approx 1$. Total cellular RNA was isolated by phenol extraction as described (Deutscher & Hilderman, 1974). The RNA was used for analysis without further fractionation.

TABLE 1. Oligonucleotides used for northern and primer extension analysis of tRNA transcripts.

Name	Specific for transcript	Sequence	Complementary to residues
TyrT-a	$tRNA_1^{Tyr}$-5′ leader	CGGGGTAATGCTTTTTAC	25–42
TyrT-b	$tRNA_1^{Tyr}$-tRNA	TCGAAGTCGATGACGGCA	85–102, 379–396
TyrT-c	$tRNA_1^{Tyr}$-spacer	ATGAGTGCAAACTTTCACAATCT	162–184
TyrT-d	$tRNA_1^{Tyr}$-spacer	CTCTCGCTTTCGCTCGAATCGA	214–235
TyrT-e	$tRNA_1^{Tyr}$-spacer	ATTCGTCGCTTCGCTCCTCACC	263–284
TyrT-f	$tRNA_1^{Tyr}$-spacer	TTTGAGGTAATGCTTGAGATGG	301–322
TyrT-g	$tRNA_1^{Tyr}$-5′ to rtT	CGGATTCGTTGGGAAGTTCAGG	437–458
TyrT-h	$tRNA_1^{Tyr}$-rtT mRNA	CTCTCTCGCTGGCGCTCGAGTC	513–534
TyrT-i	$tRNA_1^{Tyr}$-rtT mRNA	ACTCAGCGCTGCGCGCTTCGCC	560–581
TyrT-j	$tRNA_1^{Tyr}$-3′ tail	CGGATTCGCTTGAGAGTTCAGG	616–637
CysT	$tRNA^{Cys}$	GGAGTCGAACCGGACTAGACGG	235–256
LeuZ	$tRNA_5^{Leu}$	CTACCGATTCCACCATCC	287–304
ArgX	$tRNA_3^{Arg}$	TGAGACCTCTGCCTCCGGAGGG	44–65
HisR	$tRNA^{His}$	GGATTCGAACCCACGACAACTG	189–210
LeuT	5′ to $tRNA_1^{Leu}$	CCTTCGCATTGTCACAAC	234–251
ProM	$tRNA_3^{Pro}$	GACCCACTGGTCCCAAAC	405–422

Northern analysis

Northern analysis was carried out according to the procedure described previously (Li & Deutscher, 1995, 1996) with some modifications. One volume of the total RNA sample at a concentration of 5 mg/mL was mixed with two volumes of gel loading buffer containing 96% formamide, 20 mM EDTA, pH 8.0, 0.1% xylene cyanol, and 0.1% bromphenol blue. The loading mixtures were boiled for 3 min and cooled on ice, and were loaded on a 6% polyacrylamide-urea (8.3 M) slab gel. The gel was run at 250 V until the xylene cyanol dye had migrated 7 cm. The RNA was transferred to a GeneScreen Plus membrane (DuPont) by electroblotting, and was hybridized separately with 5′ end ^{32}P-labeled synthetic oligonucleotides specific for different regions of the tRNA transcripts. The membrane was prehybridized for 30 min in buffer containing 4× SSC, 0.5% SDS, 1× Denhardt solution, 0.1 mg/mL denatured salmon sperm DNA. Hybridization was carried out overnight in the same buffer containing 0.1% SDS and the ^{32}P-labeled probe. The membrane was washed three times for 15 min each with 4× SSC–0.1% SDS prior to autoradiography. The same temperature, usually 45–50 °C, was used for prehybridization, hybridization, and washing for each specific probe. Synthetic oligonucleotides were used as probes for northern blotting. The sequences of the oligonucleotides are listed in Table 1.

Primer extension

The same oligonucleotides as for northern analysis were used for primer extension experiments. The protocol for primer extension was the same as previously described (Li & Deutscher, 1995) except that 20 μg of RNA and 2 pmol of ^{32}P-labeled primer were used in each reaction. The extension products were separated by electrophoresis on a 6% sequencing gel, and detected by autoradiography.

In vitro treatment of $tRNA_1^{Tyr}$ precursor

Cell extracts were prepared as described previously (Li & Deutscher, 1994). Late log-phase cells, grown in YT medium at 31 °C, were collected and resuspended in a buffer containing 10 mM Tris-Cl, pH 7.5, 500 mM NH_4C1, 300 mM KC1, 0.5 mM Na_2EDTA, 0.5% (v/v) Triton X-100, 6 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride and 5% (v/v) glycerol. Cells were opened by two passages through a French press. Portions of the 100,000 × *g* supernatant fraction (S100) were stored at −80 °C until use. To inactivate the temperature-sensitive RNases, extracts were first incubated for 30 min at 45 °C.

The gene for full-length monomeric $tRNA^{Tyr}su_3^+$ was cloned as follows: A *Hin*dIII fragment of 178 bp carrying part of the downstream rtT gene sequence was inserted into pBSCCA31 (Li & Deutscher, 1994) to generate plasmid pBSCCA31H. The correct orientation was confirmed by restriction analysis. To prepare ^{32}P-labeled pre-$tRNA^{Tyr}su_3^+$ substrate, the plasmid was opened by the enzyme *Mnl*I. The linearized DNA was then used as a template for in vitro transcription (Li & Deutscher, 1994). The resulting transcript is 404 nt in size, and contains the leader sequence, $tRNA^{Tyr}su_3^+$, the downstream rtT mRNA, the terminator sequence, plus additional residues at each end.

^{32}P-labeled substrates were treated with cell extracts in a mixture containing 30 mM Tris-Cl, pH 7.5, 10 mM $MgCl_2$, 100 mM NH_4Cl, 60 mM KCl, 0.1 mM Na_2EDTA, 6 mM β-mercaptoethanol, 0.1% Triton X-100, and 6% glycerol. The mixture was incubated at 30 °C and the reaction was terminated and RNA precipitated by addition of 2.5 vol of cold ethanol. The products were then analyzed by gel electrophoresis as described previously (Li & Deutscher, 1994). ^{32}P-substrates were also treated with partially purified RNase E (a gift from Dr. George Mackie, University of British Columbia) in a mixture containing 25 mM HEPES-KOH, pH 7.5,

6 mM $MgC1_2$, 100 mM NH_4Cl, 60 mM KCl, 0.1 mM Na_2EDTA, 0.6 mM β-mercaptoethanol, 0.1 mM dithiothreitol, 8% polyethylene glycol, 0.07% Triton X-100, and 10% glycerol. The reaction was incubated at 30 °C, and the RNA was analyzed as above.

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REFERENCES

Altman S, Baer M, Gold H, Guerrier-Takada C, Lawrence N, Lumelsky N, Vioque A. 1987. Cleavage of RNA by RNase P. In: Inouye M, Dudock BS, eds. *Molecular biology of RNA*. Orlando, Florida: Academic Press. pp 3–15.

Altman S, Kirsebom L, Talbot S. 1995. Recent studies of RNase P. In: Söll D, RajBhandary, UL, eds. *tRNA structure, biosynthesis and function.* Washington, DC: ASM Press. pp 67–78.

Babitzke P, Kushner SR. 1991. The Ams (altered mRNA stability) protein and ribonuclease E are encoded by the same structural gene of *Escherichia coli. Proc Natl Acad Sci USA 88*:1–5.

Bikoff EK, Gefter ML. 1975. In vitro synthesis of transfer RNA. *J Biol Chem 250*:6240–6247.

Bosl M, Kersten H. 1991. A novel RNA product of the tyrT operon of *Escherichia coli. Nucleic Acids Res 19*:5863–5870.

Deutscher MP. 1990. Ribonucleases, tRNA nucleotidyltransferase and the 3′ processing of tRNA. *Prog Nucleic Acid Res Mol Biol 39*:209–240.

Deutscher MP. 1995. tRNA processing nucleases. In: Söll D, Rajhandary UL, eds. *tRNA structure, biosynthesis and function.* Washington, DC: ASM Press. pp 51–65.

Deutscher MP, Hilderman RN. 1974. Isolation and partial characterization of *Escherichia coli* mutants with low levels of tRNA nucleotidyltransferase. *J Bacteriol 118*:621–627.

Ghora BK, Apirion D. 1978. Structural analysis and in vitro processing to p5 rRNA of a 9S RNA molecule isolated from an rne mutant of *E. coli. Cell 15*:1055–1066.

Hsu LM, Klee HJ, Zagorski J, Fournier MJ. 1984. Structure of an *Escherichia coli* tRNA operon containing linked genes for arginine, histidine, leucine, and proline tRNAs. *J Bacteriol 158*:934–942.

Kelly KO, Deutscher MP. 1992. The presence of only one of five exoribonucleases is sufficient to support the growth of *Escherichia coli. J Bacteriol 174*:6682–6684.

Komine Y, Adachi T, Inokuchi H, Ozeki H. 1990. Genomic organization and physical mapping of the transfer RNA genes in *Escherichia coli* K12. *J Mol Biol 212*:579–598.

Li Z, Deutscher MP. 1994. The role of individual exoribonucleases in processing at the 3′ end of *Escherichia coli* tRNA precursors. *J Biol Chem 269*:6064–6071.

Li Z, Deutscher MP. 1995. The tRNA processing enzyme RNase T is essential for maturation of 5S RNA. *Proc Natl Acad Sci USA 92*:6883–6886.

Li Z, Deutscher MP. 1996. Maturation pathways for *E. coli* tRNA precursors: A random multienzyme process in vivo. *Cell 86*:503–512.

Li Z, Pandit S, Deutscher MP. 1998. 3′ exoribonucleolytic trimming is a common feature of the maturation of small, stable RNAs in *Escherichia coli. Proc Natl Acad Sci USA 95*:2856–2861.

Li Z, Pandit S, Deutscher MP. 1999a. RNase G (CafA protein) and RNase E are both required for the 5′ maturation of 16S ribosomal RNA. *EMBO J 18*:2878–2885.

Li Z, Pandit S, Deutscher MP. 1999b. Maturation of 23S ribosomal RNA requires the exoribonuclease RNase T. *RNA 5*:139–146.

Lin-Chao S, Cohen SN. 1991. The rate of processing and degradation of antisense RNAI regulates the replication of ColE1-type plasmids in vivo. *Cell 65*:1233–1242.

McDowall KJ, Kaberdin VR, Wu SW, Cohen SN, Lin-Chao S. 1995. Site-specific RNase E cleavage of oligonucleotides and inhibition by stem-loops. *Nature 374*:287–290.

McDowall KJ, Lin-Chao S, Cohen SN. 1994. A+U content rather than a particular nucleotide order determines the specificity of RNase E cleavage. *J Biol Chem 269*:10790–10796.

Ray BK, Apirion D. 1981a. RNAase P is dependent on RNase E action in processing monomeric RNA precursors that accumulate in an RNase E^- mutant of *Escherichia coli. J Mol Biol 149*:599–617.

Ray BK, Apirion D. 1981b. Transfer RNA precursors are accumulated in *Escherichia coli* in the absence of RNase E. *Eur J Biochem 114*:517–524.

Regnier P, Arraiano CM. 2000. Degradation of mRNA in bacteria: Emergence of ubiquitous features. *BioEssays 22*:235–244.

Reuven NB, Deutscher MP. 1993. Multiple exoribonucleases are required for the 3′ processing of *Escherichia coli* tRNA precursors in vivo. *FASEB J 7*:143–148.

Rossi J, Egan J, Hudson L, Landy A. 1981. The tyrT locus: Termination and processing of a complex transcript. *Cell 26*:305–314.

Sakano H, Shimura Y. 1978. Characterization and in vitro processing of transfer RNA precursors accumulated in a temperature-sensitive mutant of *Escherichia coli. J Mol Biol 123*:287–326.

Schedl P, Roberts J, Primakaff P. 1976. In vitro processing of *E. coli* tRNA precursors. *Cell 8*:581–594.

Sekiya T, Contreras R, Takeya T, Khorana HG. 1979. Total synthesis of a tyrosine suppressor transfer RNA gene. XVIII. Transcription in vitro of the synthetic gene and processing of the primary transcript to transfer RNA. *J Biol Chem 254*:5802–5816.

Steege DA. 2000. Emerging features of mRNA decay in bacteria. *RNA 6*:1079–1090.

Vogeli G, Stewart TS, McCutchan T, Söll D. 1977. Isolation of *Escherichia coli* precursor tRNAs containing modified nucleoside Q. *J Biol Chem 252*:2311–2318.

Wachi M, Umitsuki G, Shimizu M, Takada A, Nagai K. 1999. *Escherichia coli* cafA gene encodes a novel RNase, designated as RNase G, involved in processing of the 5′ end of 16S rRNA. *Biochem Biophys Res Commun 259*:483–488.