Rho-independent transcription terminators inhibit RNase P processing of the secG leuU and metT tRNA polycistronic transcripts in Escherichia coli

Bijoy K. Mohanty and Sidney R. Kushner*

Department of Genetics, University of Georgia, Athens, GA 30602, USA

Received August 6, 2007; Revised October 17, 2007; Accepted October 22, 2007

ABSTRACT
The widely accepted model for the processing of tRNAs in Escherichia coli involves essential initial cleavages by RNase E within polycistronic transcripts to generate pre-tRNAs that subsequently become substrates for RNase P. However, recently we identified two polycistronic tRNA transcripts whose endonucleolytic processing was solely dependent on RNase P. Here we show that the processing of the secG leuU and metT leuW glnU glnW metU glnV glnX polycistronic transcripts takes place through a different type of maturation pathway. Specifically, RNase P separates the tRNA units within each operon following the endonucleolytic removal of the distal Rho-independent transcription terminator, primarily by RNase E. Failure to remove the Rho-independent transcription terminator inhibits RNase P processing of both transcripts leading to a decrease in mature tRNA levels and dramatically increased levels of full-length transcripts in an RNase E deletion strain. Furthermore, we show for the first time that RNase G also removes the Rho-independent transcription terminator associated with the secG leuU operon. Our data also demonstrate that the Rne-1 protein retains significant activity on tRNA substrates at the non-permissive temperature. Taken together it is clear that there are multiple pathways involved in the maturation of tRNAs in E. coli.

INTRODUCTION
The processing of tRNA genes in Escherichia coli has been studied extensively over the past 15 years (1–9). Based on these experiments, a general model for the maturation of tRNAs, particularly those contained within polycistronic operons, has emerged in which the primary transcripts are presumed to be cleaved by RNase E to yield pre-tRNAs that are subsequently matured by RNase P at their 5’ ends and by a combination of RNase T, RNase PH, RNase BN, RNase II and RNase D at their 3’ ends (5,10).

However, the observed differential efficiency of RNase E cleavages among a large number of tRNA precursors as well as the absence of consensus RNase E cleavage sites in some of them (6,7,10) raised the possibility that alternative processing pathways existed. In fact, the recent analysis of the valV valW and leuQ leuP leuV operons has demonstrated a new processing pathway in which RNase P is the only endonuclease involved in the separation of tRNAs within these polycistronic transcripts (10).

In the experiments described here, we sought to determine if additional endoribonucleases, other than RNase E and RNase P, are involved in tRNA maturation. These include RNase G (encoded by rng), which is homologous to the catalytic domain of RNase E (11) and has been shown to be involved in the maturation of the 5’ end of the 16S rRNA (12,13) and to serve as a less efficient alternative to RNase E for initiating the decay of some mRNAs in wild-type cells (14). RNase Z (encoded by rnz) is known to be essential for the maturation of tRNA precursors that do not contain a chromosomally encoded CCA determinant in eukaryotes (15,16), archaea (17) and certain prokaryotes (18). However, since all of the tRNA genes in E. coli contain chromosomally encoded CCA sequences (19), it seemed likely that its RNase Z ortholog functioned in a different pathway. In fact, it has now been implicated in the decay of mRNAs, suggesting that it also functions as an alternative enzyme for RNase E in certain aspects of RNA metabolism (20). Similarly, RNase LS (encoded by rnlA), which is involved in the degradation of T4 late-gene mRNAs, has been suggested to play a role in E. coli RNA metabolism (21).

We now show that the processing of secG leuU and metT (metT leuW glnU glnW metU glnV glnX) polycistronic transcripts is accomplished through a distinct tRNA processing pathway involving multiple endonucleases. In this pathway, RNase E and/or RNase G-dependent removal of the Rho-independent transcription terminator significantly improves the efficiency of

*To whom correspondence should be addressed. Tel: +1 706 542 8000; Fax: +1 706 542 3910; Email: skushner@uga.edu

© 2007 The Author(s)
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.0/uk/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
RNase P-mediated processing of the polycistronic transcripts. However, unlike with *leuT* and *leuZ* (5), the RNase E cleavages are not absolutely essential for processing to take place, in part because of the ability of RNase G to remove the transcription terminator and/or processing to take place, in part because of the ability of RNase E cleavages are not absolutely essential for transcripts. However, unlike with *leuT* and *leuZ* (5), the RNase E cleavages are not absolutely essential for processing to take place, in part because of the ability of RNase G to remove the transcription terminator and/or processing to take place, in part because of the ability of RNase E cleavages are not absolutely essential for

**MATERIALS AND METHODS**

**Bacterial strains and plasmids**

The *E. coli* strains used in this study were all derived from MG1693 (*thyA715 rph-1*), which was provided by the *E. coli* Genetic Stock Center (Yale University), and are listed in Table 1. The *rne-1* and *rneA49* alleles encode temperature-sensitive RNase E and RNase P proteins, respectively, that are unable to support cell viability at 44°C (22–24). SK3564 (*rneA1018::bla thyA715 rph-1 recA56 rulD::Tn10/pDHK30 (rng219 Smr/Spr)/pWSK129 (Km')) is an RNase E deletion strain in which cell viability is supported by a mutant RNase G (*rne-1* rntiA::kan thyA715 rph-1 (Kmr)) protein synthesized from a single copy plasmid. The details of the construction of this strain will be published separately (D. Chung, Z. Min, B.-C. Wang and S.R. Kushner, manuscript in preparation).

**Growth of bacterial strains and isolation of total RNA**

The growth of the bacterial strains used in this work as well as the isolation, quantification and normalization of RNA samples have been described previously (10). Northern analyses were carried out as outlined by Mohanty and Kusher (10).

**Primer extension experiments**

Primer extension analysis of the *secG leuU* transcript was carried out essentially as described previously (10) with the following modifications. The nucleotide sequences were obtained from a PCR DNA product (amplified from wild-type genomic DNA using primers upstream and downstream of the *secG leuU* operon) using the primer SECG-227 (primer a, Figure 1A), which was also used for the reverse transcription reaction.

**RT-PCR cloning and sequencing of 5′–3′ ligated transcripts**

The 5′ and 3′ ends of various transcripts described in the text were identified by cloning and sequencing the

---

### Table 1. Strains used in this study

| Strains   | Genotype                                      | Reference/source  |
|-----------|-----------------------------------------------|-------------------|
| MG1693    | *thyA715 rph-1*                               | B. Bachmann       |
| SK2525    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | (7)               |
| SK2534    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | (7)               |
| SK2538    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | (14)              |
| SK2541    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | (14)              |
| SK3166    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | Perwez and Kushner, unpublished data |
| SK3170    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | Perwez and Kushner, unpublished data |
| SK3564    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | Perwez and Kushner, unpublished data |
| SK5665    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | (24)              |
| SK9795    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | (20)              |
| SK9797    | *rne-1* *rneA49* *thyA715 rph-1* *rneB2::Tn10* | (20)              |

---

**Figure 1.** Schematic representation of *secG leuU* operon (not drawn to scale). Relative positions of the oligonucleotide probes (a: SECG-227, b: SECG3, c: LEU and d: LEUU-TER) used in the northern analysis are shown below the diagram. (B) Northern analysis of *secG leuU* transcript. Total RNA (12 µg/lane) was separated on 6% PAGE, transferred to a nylon membrane and probed multiple times as described in the Materials and Methods. Only the blot probed with c is shown. The genotypes of the strains used are noted above each lane. The deduced structures and the names for the processing intermediates of *secG leuU* transcript are shown to the right. The RQ of the mature tRNA*leu* (M) in the various genetic backgrounds was calculated by setting the wild-type level at 1.0. PF denotes the fraction of the mature tRNA relative to the total amount of both processed and unprocessed species in each specific genetic background. RQ and PF were obtained from the average of at least three independent experiments. The RNA size standards (nucleotide) (Invitrogen) are shown to the left.
RT-PCR products obtained from 5′ to 3′ end-ligated circular RNAs (25–28). Total steady-state RNA (3 μg) was self-ligated in the presence of T4 RNA ligase (NEB). For analysis of the secG leuU and secG transcripts, total RNA was initially treated with tobacco acid pyrophosphatase (TAP) according to the manufacturer’s instructions (Epitect Technologies, Madison, WI, USA) to convert the 5′-triphosphate termini to phosphomonoesters prior to self-ligation. The circular transcripts were reverse transcribed using a gene-specific primer close to the 5′–3′ junction in the presence of Superscript III reverse transcriptase (Invitrogen) according to the manufacturer’s specifications. The 5′–3′ junctions of the resulting cDNAs were amplified using either a pair of gene-specific primers (secG, leuU, hisR and cysT) or one upstream and one downstream gene-specific primers (secG leuU) in the presence of JumpStart REDTaqTM DNA polymerase (Sigma). All the primers used in the cDNA amplifications were engineered to contain a suitable restriction site for directed cloning into pWSK29 (29). DNA sequencing was carried out using an automated sequencer (Applied Biosystems 3730 x1 DNA analyzer).

Oligonucleotide probes and primers

The sequences of the primers used in the experiments reported here are available on request.

RESULTS

Processing of the secG leuU transcript is RNase P dependent

The secG leuU operon is unique in E. coli in that it contains both a protein encoding gene (secG) and a tRNA gene (leuU) (Figure 1A). The product of the leuU gene (tRNA Leu2) recognizes the CUC and CUU leucine codons and is essential for cell viability (30). The secG leuU transcript is terminated with a Rho-independent transcription terminator and the two genes are separated by a 14 nt spacer region that has been predicted to contain a putative terminator (31). Initially, we examined the processing of this transcript in wild-type, rne-1, rnpA49 and rne-1 rnpA49 strains by northern analysis using a series of oligonucleotides (Figure 1A-a, b, c and d). For the sake of simplicity, only the results with the leuU-specific probe (Figure 1A-c) are shown (Figure 1B).

An expected band of 87 nt, corresponding to the mature tRNA Leu2 (M) was observed in all the strains (Figure 1B, lanes 1 and 4), with its level primarily a function of RNase P activity. Thus the loss of RNase P activity led to reductions of ~50% in the relative quantity (RQ) and ~70% in the processed fraction (PF) of mature tRNA compared to the wild-type strain (Figure 1B, lanes 1 and 3). The reduction in the level of mature tRNA Leu2 in the rnpA49 mutant was consistent with the accumulation of significant amounts of a high-molecular weight processing intermediate (GU2) and a mixture of pre-tRNA Leu2 species (pre-leuU) that were 6–11 nt longer than the mature tRNA Leu2 (Figure 1B, lane 3).

In contrast, inactivation of RNase E had only a minor effect on either the RQ or PF of tRNA Leu2 compared to the wild-type strain (Figure 1B, lanes 1 and 2). However, a different high-molecular weight intermediate (GU1) that was ~40 nt longer than GU2 appeared in the rne-1 strain, but was only present in very low levels (Figure 1B, lane 2). When both RNase E and RNase P were inactivated there was a further reduction of the RQ and PF of the mature tRNA to ~0.35 and ~0.19, respectively, compared to the wild-type control along with a concomitant increase in both the GU1 and GU2 species (Figure 1B, lane 4). In addition, the pre-leuU species that were present in the rnpA49 single mutant (Figure 1B, lane 3) disappeared, suggesting that they were generated by RNase E cleavages in the intergenic region between secG and leuU. In the wild-type control, both the GU1 and GU2 species were only visible after a much longer exposure of the membrane (data not shown).

A secG-specific probe (Figure 1A-a) hybridized only to bands GU1 and GU2 (data not shown) indicating that these transcripts contained both secG and leuU-coding sequences. Surprisingly, a band corresponding to the full-length secG mRNA (~420 nt) was only visible after a much longer exposure (data not shown).

The two larger secG leuU transcripts (GU1 and GU2) have identical 5′ termini

Although the data presented in Figure 1 clearly indicated that the two larger transcripts contained both secG and leuU-coding sequences, it was not clear what accounted for their difference in electrophoretic mobility. We hypothesized that the smaller transcript (GU2) was derived from larger GU1 species by endonucleolytic cleavage(s) either at the 5′ or 3′ end. Accordingly, we analyzed the 5′ ends of the secG leuU transcripts employing primer extension analysis (Figure 2A). A strong primer extension product (I) terminating at 81 nt (G) and a second weaker primer extension product (II) (~10% of I) terminating at 84 nt (C) upstream of the putative secG translation start codon were detected in the wild-type strain. Since no other longer primer extension products were observed, I and II represented the major and minor transcription initiation sites, respectively, for the secG leuU transcript. The sequence upstream of these transcription start sites (I and II) contained consensus (4/6) −10 and −35 sequences of a σ70 promoter (Figure 2B).

More importantly, the major primer extension products (I and II) in both the rne-1 and rnpA49 mutants were at positions identical to those observed in the wild-type strain (Figure 2A), suggesting that transcription initiation started at the same locations in all genetic backgrounds. Consistent with the northern analysis (Figure 1B), the amount of primer extension products increased significantly in both the rne-1 and rnpA49 mutants compared to the wild-type strain. The additional minor primer extension products visible in the rnpA49 strain (arrows and asterisk in Figure 2A) presumably arise from endonucleolytic cleavages downstream of the two transcription initiation sites.
Table 2. RQ of secG leuU full-length transcripts and PF of mature tRNA\[^{120}\] in various strains

| Strain      | Genotype       | RQ[^a] | PF[^b]  |
|-------------|----------------|--------|---------|
| MG1693      | Wild type      | 1      | 0.95 ± 0.05 |
| SK5665      | rne-1          | 16 ± 2 | 0.95 ± 0.05 |
| SK5665      | rneA1018/rng-219 | 98 ± 10 | 0.5 ± 0.05 |
| SK2525      | rnpA49         | 1.5 ± 0.2 | 0.3 ± 0.05 |
| SK2525      | rne-1 rnpA49   | 32 ± 2  | 0.3 ± 0.05 |
| SK2538      | rnp::cat       | 1 ± 0.1 | 0.95 ± 0.05 |
| SK2541      | rne-1 rnp::cat | 29 ± 2  | 0.7 ± 0.05 |
| SK9795      | Δrne::kan      | 1 ± 0.1 | 0.95 ± 0.05 |
| SK9797      | rne-1 Δrne::kan | 20 ± 2 | 0.9 ± 0.05 |
| SK3140      | Δrne::kan      | 1 ± 0.1 | 0.95 ± 0.05 |
| SK3166      | rne-1 Δrne::kan | 12 ± 2 | 0.95 ± 0.05 |

[^a]: The RQ was calculated as described in Figure 1.
[^b]: The PF was calculated as described in Figure 1.

Data represents the average of at least two independent experiments.

The full-length secG leuU transcript that includes the Rho-independent transcription terminator only accumulates in the absence of RNase E

Since both larger processing intermediates (GU1 and GU2) still retained the 5′ transcription start site (Figure 2), we surmised that the difference in their electrophoretic mobility arose from a processing event at the 3′ end. As shown in Figure 1A, the secG leuU operon contains a strong Rho-independent transcription terminator predicted to be 36 nt in length. Since the nucleotides immediately downstream of the leuU CCA determinant were A/U-rich, we suspected that RNase E might be responsible for removal of the terminator region. To test this hypothesis directly, we probed northern blots with an oligonucleotide (Figure 1A-d) specific to the terminator region of the secG leuU transcript. As predicted, only the larger of the two species (GU1) was detected in the strains tested, indicating that it represented the full-length secG leuU transcript (data not shown). While the full-length transcript was very weakly visible in the wild-type and rnpA49 strains after long exposure, there was an ~16-fold increase in the rne-1 strain and ~32-fold in the rne-1 rnpA49 double mutant compared to the wild-type control (Table 2), indicating a significant role of RNase E in the generation of the secG leuU species that lacked the terminator (GU2), which accumulated in the rnpA49 mutant (Figure 1A, lane 3).

However, the importance of the RNase E cleavage for removal of the terminator from the secG leuU transcript was not completely clear from the above experiment because the PF of mature tRNA\[^{120}\] did not change significantly in the rne-1 strain compared to the wild-type control (Figure 1B, lanes 1 and 2, Table 2). If the removal of the terminator by RNase E were a prerequisite for RNase P processing of the secG leuU transcript, we expected to see comparable reductions in the PF of the mature tRNA\[^{120}\] in both the rne-1 and rnpA49 strains (Figure 1B, lanes 2 and 3, Table 2).

We, therefore, hypothesized that either there was residual RNase E activity in the rne-1 mutant at the non-permissive temperature or that additional endoribonucleases were involved in the removal of the terminator, particularly because there was also ~10-fold difference between the level of the full-length transcript (GU1) in the rne-1 strain compared to the terminator-less species (GU2) in the rnpA49 mutant (Figure 1B). To address the possibility of residual RNase E activity in the rne-1 strain, we tested an rne deletion strain [rneA1018, (32)] in which cell viability was restored by a mutant RNase G protein (encoded by rng-219, D. Chung, Z. Min, B.-C. Wang and S. R. Kushner, manuscript in preparation). In this case, the RQ of GU1 increased ~98-fold compared to the wild-type strain (Table 2). More importantly,
the PF of the mature tRNA\textsubscript{Leu} decreased to \(\sim50\%\) of the wild-type level (Table 2). In addition, a small amount of a processing intermediate containing the \textit{leuU} tRNA with the terminator still attached was now present in this genetic background (data not shown), indicating that either RNase P or the mutant RNase G protein could inefficiently process the full-length transcript at or upstream of the 5' end of \textit{leuU}.

To investigate the potential involvement of other endoribonucleases, we measured the level of the full-length transcript (GU1) in a series of mutants defective in RNase E, RNase G (14), RNase LS (21) and RNase Z (20). While the level of the full-length sec\textit{G} \textit{leuU} transcripts (GU1) in the \textit{rne}, \textit{mnlA} and \textit{nrz} single mutants was identical to the wild-type strain within experimental error (Table 2), it increased significantly in the \textit{rne-1} \textit{nrz} double mutant compared to the \textit{rne-1} single mutant (Table 2).

**The stability of the sec\textit{G} \textit{leuU} transcript is dependent on RNase \textit{P} processing**

The significant difference in the steady-state levels of the sec\textit{G} \textit{leuU} transcript with terminator (GU1) in the \textit{rne-1} strain and without terminator (GU2) in the \textit{rnpA49} mutant (Figure 1B, lanes 2 and 3) suggested that processing of the sec\textit{G} \textit{leuU} transcript was highly dependent on an RNase \textit{P} cleavage event. Accordingly, we determined the half-lives of both sec\textit{G} \textit{leuU} transcripts by using either \textit{leuU}-specific or sec\textit{G}-specific probes (Figure 1A-a and c). Since both probes yielded identical results, only the results obtained with probe c (\textit{leuU}-specific) are shown in Figure 3.

As expected, neither of the sec\textit{G} \textit{leuU} transcripts was detected in the wild-type strain (Figure 3, lanes 1–7), indicating a half-life of \(<30\) s (10). The full-length sec\textit{G} \textit{leuU} transcript (GU1) had a half-life of \(1.5\pm0.4\) min in the \textit{rne-1} strain (Figure 3, lanes 8–14). In contrast, in the \textit{rnpA49} mutant the terminator-less sec\textit{G} \textit{leuU} species (GU2) was significantly stabilized with a half-life of \(17\pm2\) min (Figure 3, lanes 15–21). When both the RNase \textit{E} and RNase \textit{P} were inactivated (\textit{rne-1} \textit{rnpA49}), the half-lives of both the GU1 and GU2 transcripts increased further to \(3\pm0.5\) and \(>32\) min, respectively (Figure 3, lanes 22–28). Furthermore, in the \textit{rne-1} \textit{rnpA49} double mutant, the amount of the GU2 species increased slowly during the course of the experiment (Figure 3, lanes 22–28). Also of interest was that the level of pre-tRNA (pre-\textit{leuU}) species in the \textit{rnpA49} mutant increased \(\sim2\)-fold during the course of the experiment (Figure 3, lanes 15–21), while their complete absence in the \textit{rne-1} \textit{rnpA49} double mutant (lanes 22–28) was consistent with them being derived from inefficient RNase \textit{E} cleavages upstream of \textit{leuU}.

**Separation of the sec\textit{G} mRNA from \textit{leuU} by RNase \textit{P} leads to its rapid degradation**

While the data described in Figures 1 and 3 clearly showed that processing of the sec\textit{G} \textit{leuU} transcript was primarily dependent on the action of RNase \textit{P}, surprisingly no significant amount of sec\textit{G} transcript was detected using either sec\textit{G}-specific oligonucleotides (probes a and b, Figure 1A) or a full-length sec\textit{G} DNA probe in all the strains tested (wild-type, \textit{rne-1}, \textit{rneA1018/rng-219}, \textit{rnpA49} and \textit{rne-1} \textit{rnpA49}, \textit{rne-1} \textit{rng::cat}, \textit{rne-1} \textit{nrz::kan} and \textit{rne-1} \textit{mnlA::kan} (data not shown). These observations indicated that the sec\textit{G} mRNA was rapidly degraded with a half-life of \(<30\) s, after cleavage of the sec\textit{G} \textit{leuU} transcript by RNase \textit{P}.

**RNase \textit{E} cleaves in the A/U-rich region downstream of \textit{leuU} CCA mature terminus**

Although the primer extension and northern blot analysis revealed that the full-length sec\textit{G} \textit{leuU} transcript (GU1) was processed in part by RNase \textit{E} to GU2, we were
interested in determining the actual cleavage site(s). We employed an RT-PCR technique using 5′–3′ end-ligated transcripts (25) to clone and sequence both the 5′ and 3′ termini of the transcripts simultaneously. Since both the secG leuU transcripts appeared to retain the 5′ end of the primary transcript (Figure 2), the triphosphate was converted to monophosphate employing TAP treatment to facilitate the self-ligation of 5′ and 3′ ends of transcripts in presence of T4 RNA ligase.

Consistent with the primer extension experiments (Figure 2), the majority of the 5′ ends of the transcripts were located at either 81 nt (11/15 in the wild-type strain, 8/14 in the rne-1 and 7/16 in the rnpA49 mutant) or in a limited number of cases at 82–84 nt upstream of the putative translation start codon (Figure 4A–D). Three of the clones (1/14 in the rne-1 and 2/16 in the rnpA49 mutants) had 5′ ends at 66 and 59 nt upstream of the translation start (Figure 4C and D), which corresponded to primer extension products seen in Figure 2 (marked with asterisk). These particular termini were also identified in clones generated from self-ligated transcripts independent of prior TAP treatment, suggesting that these ends most likely arose from endonucleolytic cleavages (data not shown). In contrast, none of the 5′ ends at −81 to −84 nt were detected in self-ligated transcripts in the absence of treatment with TAP (data not shown), indicating that the majority of these ends had retained a 5′ triphosphate. In fact, the presence of only a very limited number of primary transcripts with 5′-phosphomonoesters were indicated, since there was a >100-fold reduction in the level of PCR amplification products from the self-ligated transcripts in the absence of TAP treatment (data not shown).

Sixty percent (9/15) of the 3′ ends of the secG leuU transcripts in the wild-type strain occurred after the Rho-independent transcription terminator (Figure 4B). However, transcripts from 6/15 (40%) wild-type clones ended at or after 1–2 residues downstream of the mature CCA terminus within the AAUU sequence (Figure 4B, 430–432 nt). When RNase E was inactivated, the number of the 3′ ends of the secG leuU transcripts terminating after the Rho-independent transcription terminators increased to 93% (13/14) (Figure 4C). The lone transcript in the rne-1 mutant that had a terminus 2 nt downstream from the CCA (Figure 4C) may have arisen from a cleavage by RNase G, as suggested from the data presented in Table 2.

Furthermore, the secG leuU transcripts from all of the 16 clones derived from the rnpA49 mutant were missing the terminator and ended at 430–432 nt (Figure 4D), indicating that the RNase E and/or RNase G cleavages occurred within the AAUU sequence (Figure 4A). Finally, it should be noted that the number of secG leuU transcripts, with and without a transcription terminator, obtained in this experiment reflect the distribution of particular species in each genetic background, in agreement with the northern analysis shown in Figure 1. Interestingly, ~24% of all the clones contained post-transcriptionally added A residues of up to 5 nt (Figure 4B and C, red arrows).

A single RNase P cleavage of the secG leuU transcript releases tRNALeu2 with a mature 5′ terminus

The data described in Figures 1 and 3 suggested that RNase P, not RNase E, was essential for the removal of tRNALeu2 from the secG leuU poly-cistronic transcript. To provide further support for this conclusion, we mapped the 5′ and 3′ ends of tRNALeu2 in various genetic backgrounds by cloning and sequencing of 5′–3′ end-ligated junctions. As controls, we also identified the 5′ and 3′ ends of tRNA His (hisR) and tRNA Cys (cysT) using identical techniques, since both of these RNAs are present in single copy, are part of poly-cistronic transcripts, and have been shown to be dependent on RNase E for their initial processing (5,7).

In the case of tRNALeu2, we expected that all of the clones would have mature 5′ ends in a wild-type strain because the RNase P cleavage would generate them directly. In contrast, with tRNA His and tRNA Cys, we hypothesized that some of them would have 5′ extensions resulting from initial upstream cleavages by RNase E. As predicted, all the tRNALeu2 clones (23/23) in the wild-type strain had the mature 5′ end (Figure 5C). Three of these
clones (3/23, 13%) had immature 3' ends occurring 1–2 nt downstream of the CCA terminus in the AAUU sequence (Figure 5C). Similarly, 3' termini were also obtained with the secG leuU-specific transcripts (Figure 4, 431–432 nt).

In contrast, 6/24 (25%) of the tRNAHis and 8/36 (22%) of the tRNACys clones had immature 3' ends in the wild-type strain (Figure 5A and B). These immature termini were 8 and 4 nt upstream of the tRNAHis and tRNACys mature 5' termini, respectively, in what appeared to be RNase E consensus cleavage sites (A/U-rich regions). Furthermore, ~63% (15/24) of the tRNAHis and ~67% (24/36) of the tRNACys clones had immature 3' ends that were up to 2 nt (U or UU) for tRNAHis and C or CU for tRNACys downstream of the mature CCA terminus. The sites of the 5' and 3' immature termini of tRNAHis and tRNACys were consistent with the previous reports of RNase E-processing sites to release these pre-tRNAs from their respective polycistronic transcripts (5). The presence of a higher percentage of unprocessed 3' ends of tRNAHis (~63%) and tRNACys (~67%) compared to tRNALen2 (~13%) in the wild-type strain suggests the possibility of differential rates of 3' end maturation by RNase T, RNase D and RNase BN in this genetic background (MG1693 contains the rph-1 allele, which inactivates RNase PH). Interestingly, ~9% of the tRNALeu clones and ~20–25% of both the tRNAHis and tRNACys clones contained 1–3 nt of untemplated A residues downstream of the immature terminus (Figure 5A–C).

Since we observed some pre-leuU species in the rnpA49 single mutant, but not in the rne-1 rnpA49 double mutant (Figure 1B, lanes 3 and 4), we also mapped the 5' and 3' ends of the tRNALen2 in an rnpA49 single mutant. Under these circumstances, 18/24 (75%) of the tRNALen2 clones contained immature 5' ends with 6–11 unprocessed nucleotides (Figure 5C). There were four different termini within a 6-nt region (GUAGUA) of the 14 nt secG leuU intercistronic spacer (Figure 5C), indicative of inefficient, non-specific cleavages by RNase E upstream of tRNAHis. Generally, almost all of the tRNALen2 clones (23/24) had mature 3' ends even if their 5' ends were not processed by RNase P. Furthermore, the mature tRNALeu3 was reduced only marginally in the rnpA49 mutant (Figure 5C).

To obtain further evidence that RNase P cleavage of the secG leuU transcript directly generated the mature 5' terminus of the tRNALen2, we examined the 3' termini of secG transcripts. We hypothesized that a single RNase P cleavage of secG leuU transcript to release the mature 5' end of the tRNALen2 would yield secG transcripts containing the intact 14 nt spacer present between secG and leuU (Figure 5C). In fact, when we mapped the 3' termini of secG mRNAs, ~20% (8/40) of the clones contained the entire 14 nt spacer in both the wild-type and rne-1 strains (data not shown). The rest of the clones contained secG transcripts with 3' ends distributed throughout the coding sequence (data not shown).

### Processing of the metT operon

We subsequently turned our attention to leuW (tRNALeu3), which is part of the metT operon (metT leuW glnU glnW metU glnV glnX) (Figure 6A). In addition to the presence of a leucine tRNA, this operon was of interest because Li et al. (33) observed recently that the steady-state level of all the methionine (met) tRNA transcripts increased significantly in an rnpA49 mutant. Accordingly, we analyzed the processing of this complex operon, employing the nine distinct oligonucleotide probes (a–i) shown in Figure 6A.

As the leuW-coding sequence is unique to this operon and differs significantly from the other seven leucine tRNAs, we initially probed the northern blot with the probe c (Figure 6A). As expected a band corresponding to the mature tRNALeu3 (M1) was observed in all the strains (Figure 6B, lanes 1–5). While both the RQ and PF of the mature tRNALeu3 were reduced only marginally in the rne-1 mutant compared to the wild-type control, the decreases were much more significant in both the rnpA49 and rne-1 rnpA49 mutants. Besides the mature tRNA band (M1), five other processing intermediates (I, II, IV, V...
and VII, Figure 6B, lanes 2–4) were observed in various genetic backgrounds.

In order to determine the composition of these intermediates, we hybridized the blot with two additional oligonucleotides (Figure 6A-a and b) that were complementary to the 5′ non-coding and tRNA Met (metT and metU)-coding regions, respectively. Both of these probes hybridized to bands I, II, IV and V but not to band VII (Figure 6B, lanes 8–9 and 13–14), which was thus identified as leuW with an unprocessed 5′ end that occurred in the absence of RNase P (Figure 6B, lane 3). However, both the probes a and b hybridized to a new processing intermediate (VI) of ~125 nt that was present in rnpA49 mutants (lanes 8–9 and 13–14) and was absent in the c-probed blot. Thus species VI represented metT containing an unprocessed 5′ end.

Furthermore, probe b hybridized to an additional high-molecular weight processing intermediate (III) in the rne-1 mutant (lane 12), and, as expected, also hybridized to the mature tRNAMet in all genetic backgrounds (M2, Figure 6B, lanes 11–15). Similar to what was observed with tRNALeu3 (M1), inactivation of RNase E led to only a small reduction in the relative level of the mature tRNA Met (lane 12). In contrast, the loss of RNase P activity had a much greater effect on the amount of mature tRNAMet (Figure 6B, lanes 13 and 14).

Probing the blot with oligonucleotide i (Figure 6A), which was complementary to the terminator sequences of the operon, identified bands I and III (Figure 6B) in all strains defective in RNase E (data not shown). Since these bands were missing in the rne-1 rnpA49 double mutant (Figure 6B, lanes 2, 4, 7, 9, 12 and 14), suggesting roles for both RNase E and
RNase P in initiating the processing of this transcript. As was seen with the secG leuU transcript (Table 2), the level of the full-length transcript that retained the downstream Rho-independent transcription terminator sequences of the metT operon increased ~5-fold along with a concomitant reduction in the PF of mature tRNA^{Leu3} and tRNA^{Met} in an rne deletion mutant compared to the temperature-sensitive rne-1 mutant (Figure 6B, lanes 2, 5, 7, 10, 12 and 15, data not shown).

The composition of all the processing intermediates shown in Figure 6B was further confirmed using the additional oligonucleotide probes d, e, f, g and h (Figure 6A). For example, oligonucleotides g and h (Figure 6A), which were specific for the intergenic regions between metU, glnV and glnX, hybridized to bands I and III in the rne-1 single mutant but only to band I in rne-1 rnpA49 double mutant (Figure 6B, lanes 17 and 19, data not shown). In the rnpA49 mutant, the only intermediate observed was glnX containing extra nucleotides at its 5' terminus with probe h (Figure 6B, lane 18). Similarly, while probe d hybridized to bands I, II and IV, probe e hybridized to bands I and II only (data not shown). Furthermore, probe f (Figure 6A) did not hybridize to band III containing metU (Figure 6B, data not shown) suggesting that it was missing the 5' upstream sequences.

Taken together, we conclude that the band I (Figure 6B) represents the full-length transcript containing all seven tRNAs. Bands II, IV, V and VI are processing intermediates that retain the 5' non-coding region along with one or more tRNA species. Thus band II has the first five tRNAs (metT leuW glnU glnW metU), band IV has the first three tRNAs (metT leuW glnU), band V had two tRNAs (metT leuW) and band VI has only metT tRNA. On the other hand, band III is derived from the 3' end of the transcript by an RNase P cleavage at 5' end of metU and contains metU glnV glnX as well as the intact Rho-independent transcription terminator.

**DISCUSSION**

Previous studies have identified two distinct tRNA processing pathways in *E. coli* involving either RNase E or RNase P as the primary endoribonuclease responsible for generating pre-tRNAs from polycistronic transcripts (5,10). In the RNase E-dependent pathway, polycistronic tRNA transcripts are separated into pre-tRNAs solely by RNase E with the pre-tRNAs being subsequently matured at their 5' termini by RNase P (5). In contrast, in the RNase P-dependent pathway, polycistronic tRNA transcripts are separated into pre-tRNAs by RNase P, generating the mature 5' ends in a single step independent of RNase E (10). In this report, we describe for the first time an alternative tRNA processing pathway for the secG leuU and metT (metT leuW glnU glnW metU glnV glnX) polycistronic transcripts (Figure 7A and B) that involves certain aspects of both the RNase E- and RNase P-dependent pathways. What makes this pathway distinct in wild-type *E. coli* is that the removal of the Rho-independent transcription terminator associated with each polycistronic transcript, primarily by RNase E, significantly stimulates their rapid processing by RNase P into pre-tRNAs containing mature 5' termini. However, RNase E is not absolutely required for this step, in part because RNase G can also remove the terminator.
sequences and RNase P can inefficiently process a full-length transcript that retains the terminator. The role of RNase G in the removal of the terminator is partially masked by the fact that there is ~10 times more RNase E in the cell than RNase G (34).

Evidence for RNase P being the primary ribonuclease involved in the separation of tRNA\textsubscript{Leu\textsuperscript{2}} from secG (Figure 7A) was derived from the fact that secG leuU transcripts lacking the Rho-independent transcription terminator (GU2) accumulated in the rnpA49 single mutant but not in either the rne-1 (Figure 1B) or rneA1018 deletion strains (data not shown). Furthermore, the identification of secG mRNA fragments with the intact 14 nt secG leuU intergenic sequence along with no unprocessed 5’ nucleotides in the pre-tRNA\textsubscript{Leu\textsuperscript{2}} species (Figure 5, data not shown) confirmed that a single-RNase P cleavage generated the mature 5’ terminus of tRNA\textsubscript{Leu\textsuperscript{2}} and separated it from the upstream secG mRNA.

Similarly, inactivation of RNase P led to the dramatic accumulation of several large precursor species of the metT operon (bands II and IV) that contained either the first five (metT leuV glnU glnW metU) or first three (metT leuW glnU) tRNAs, respectively. (Figure 6), suggesting that these tRNAs were also endonucleolytically processed primarily by RNase P (Figure 7B). The significant drop in the RQ and PF of the mature tRNA\textsubscript{Leu\textsuperscript{2}}, tRNA\textsubscript{Leu\textsuperscript{3}} and tRNA\textsubscript{Met} species in rnpA49 mutants (Figures 1B and 6B, data not shown) was consistent with this model (Figure 7).

Interestingly, the presence of a Rho-independent transcription terminator appears to significantly inhibit the ability of RNase P to process both transcripts. This was evidenced by the accumulation of full-length transcripts with intact terminators only in RNase E-deficient strains as opposed to either no accumulation in the wild-type strain or only accumulation of transcripts with the terminator removed in the RNase P-deficient strain (Figures 1 and 6, Table 2). Thus, the role of RNase E and/or RNase G for the processing of the secG leuU and metT polycistrionic transcripts is primarily to remove the Rho-independent transcription terminator, thereby facilitating the RNase P-dependent cleavages that generate pre-tRNAs with mature 5’ termini. This is in marked contrast to the requirement for initial RNase E cleavages to generate pre-tRNAs with immature 5’ ends in the processing of the glyW and argX polycistrionic transcripts (5,7).

Although it is not clear at this time why the presence of the Rho-independent transcription terminator inhibits RNase P activity, it appears from the data presented in Table 2 that under normal physiological conditions, the terminator sequences are endonucleolytically removed by RNase E and/or RNase G prior to RNase P action to generate the pre-tRNAs. In addition, the last three tRNAs in the complex metT operon are also separated by RNase E as suggested by the accumulation of band III (metU glnV glnX) in the rne-1 strain (Figure 6B). This species was generated by an RNase P cleavage at the mature 5’ terminus of metU (Figure 7B, data not shown). The disappearance of this band in the rnpA49 single mutant and rnpA49 rne-1 double mutants supports this interpretation.

An unexpected observation was the involvement of RNase G in the removal of the Rho-independent transcription terminator associated with the secG leuU transcript (Figure 4, Table 2). This is the first demonstration that RNase G plays any role in the processing of E. coli transcripts containing tRNAs. While the removal of the Rho-independent transcription terminator associated with the metT polycistrionic transcript appeared independent of RNase G activity (data not shown), it seems likely that other polycistrionic tRNA transcripts terminated in a Rho-independent fashion may also be partially processed by RNase G.

Even though the presence of the Rho-independent transcription terminator inhibited processing by RNase P, the relatively low steady-state level of full-length transcripts of both operons in the rne-1 strain indicates that RNase P can process the full-length transcript, although more slowly than in the presence of functional RNase E. This was consistent with a significant increase in the half-life of full-length secG leuU transcript (Figure 3) and the level of both the secG leuU and metT operon full-length transcripts (Figures 1 and 6) in the rne-1 rnpA49 double mutant compared to either of the single mutants. These results indicate that either RNase E or RNase P can independently initiate the processing of these transcripts, although removal of the Rho-independent transcription terminator is normally the preferred first step in a wild-type strain.

Our data also clearly show that the temperature-sensitive Rne-1 protein, which is the most widely used allele to characterize which substrates are degraded in an RNase E-dependent fashion, clearly retains significant activity on tRNA substrates at the non-permissive temperature. Thus, there was a 5–6-fold increase in the transcript level in the rneA1018 strain compared to the rne-1 strain (2 h after shift to 44°C) for both the secG leuU and metT operons as well as concomitant reductions in the PF levels of tRNA\textsubscript{Leu\textsuperscript{2}}, tRNA\textsubscript{Leu\textsuperscript{3}} and tRNA\textsubscript{Met} (Figure 6B, Table 2). An independent study from this laboratory has shown identical results for the argX (argX hisR leuT proM) and glyW (glyW cysT leuZ) operons when comparing the rne-1 and rneA1018 strains (D. Chung, Z. Min, B.-C. Wang and S. R. Kushner, manuscript in preparation).

Previous microarray analysis of the E. coli transcriptome in an rnpA49 mutant led to the hypothesis that inactivation of RNase P specifically destabilized transcripts downstream of its cleavage sites (31). However, the dramatic destabilization of the secG mRNA after RNase P processing of the secG leuU transcript (Figures 1 and 3) clearly demonstrates that this idea is not entirely correct. In addition, the failure to stabilize the secG mRNA in any of the endonuclease mutants tested suggests that once the RNase P cleavage occurs, the upstream secG mRNA is rapidly degraded exonucleolytically.

Interestingly, a very low level of RNase E cleavages within the intergenic regions of both operons was observed in the absence of RNase P. Thus, the pre-leuU species were the result of RNase E cleavages upstream of leuU in the secG leuU transcript (Figures 1B, lane 3 and 7A). These cleavages were inefficient (Figure 1B,
### Table 3. Analysis of RNase E cleavage sites in tRNA precursors

| tRNA gene | 3' terminal region | Efficiency of RNase E cleavage | Source/reference |
|-----------|--------------------|-------------------------------|-----------------|
| lysY      | CCAG[UU]UUUA       | High                          | (7)             |
| tyrT      | CCAC[A]AAUUC       | High                          | (5)             |
| hisR      | CCAC[U]AAUUA       | High                          | (5), This study |
| cysT      | CCAC[C][UU]UCU     | High                          | (5), This study |
| metU      | CCAA[AA]UCU        | This study                    |                 |
| aspT      | CCACCU[A]A         | High                          | Mohanty and Kushner, unpublished data |
| trpT      | CCAGA[AA]U         | High                          | Mohanty and Kushner, unpublished data |
| proK      | CCACA[CG]A         | High                          | Mohanty and Kushner, unpublished data |
| lysW      | CCAG[UU]UUUA       | High                          | Mohanty and Kushner, unpublished data |
| leuU      | CCAC[A]A[UU]C      | High                          | This study      |
| ghvV      | CCACUUAUU          | High                          | This study      |
| ghxX      | CCAAUUAA           | High                          | This study      |
| mctT      | CCACUCUU           | Medium                        | This study      |
| glhU      | CCACUCUCU          | Medium                        | This study      |
| leuQ      | CCACAACC           | Low                           | (10)            |
| leuP      | CCACCAGAG          | Low                           | (10)            |
| ghW       | CCACGAAAG          | Very Low                      | This study      |
| leuW      | CCACUCACC          | Very Low                      | This study      |
| valV      | CCACUCCUGC         | None                          | (10)            |

Downward arrows indicate RNase E cleavage sites that have been mapped.

Based on the experiments reported above and our recent observations with the valV valW and leuQ leuP leuV operons (10), it is now clear that a significant number of tRNA precursors are dependent on RNase P for their initial separation from polycistronic transcripts rather than RNase E. In fact, a preliminary analysis indicates that the metZ operon (metZ metW metV) is also highly dependent on RNase P for processing (data not shown) as predicted from the microarray data of Li et al. (33). These observations provide further support for our hypothesis that the essential function of RNase P may be related to its requirement for the processing of polycistronic tRNA transcripts rather than the processing of the 5' ends of pre-tRNAs (10). Finally, it would not be surprising if there were, in fact still yet, unidentified additional pathways for the maturation of specific tRNAs. Thus the mechanisms of tRNA maturation in E. coli are far more diverse than has previously been envisioned.

**ACKNOWLEDGMENTS**

We are indebted to V. Maples, T. Perwez and D. Chung for invaluable technical assistance in the construction of various multiple mutants used in this work. This work was supported in part by a grant from the National Institutes of General Medical Sciences (GM57220) to S.R.K. Funding to pay the Open Access publication charges for this article was provided by GM57220.

**Conflict of interest statement.** None declared.

**REFERENCES**

1. Kelly, K.O. and Deutscher, M.P. (1992) The presence of only one of five exoribonucleases is sufficient to support the growth of *Escherichia coli*. J. Bacteriol., 174, 6682–6684.
2. Reuven, N.B. and Deutscher, M.P. (1993) Multiple exoribonucleases are required for the 3' processing of *Escherichia coli* tRNA precursors *in vivo*. *FASEB J.*, 7, 143–148.
3. Altman, S., Kirsebom, L. and Talbot, S. (1995) Recent studies of *E. coli* RNase P. In SoI.L. and RajBhandary. (eds), *tRNA: Structure and Function*, American Society for Microbiology Press, Washington, DC, pp. 67–78.
4. Li, Z. and Deutscher, M.P. (1996) Maturation pathways for *E. coli* tRNA precursors: a random multienzyme process *in vivo*. *Cell*, 86, 503–512.
5. Li, Z. and Deutscher, M.P. (2002) RNase E plays an essential role in the maturation of *Escherichia coli* tRNA precursors. *RNA*, 8, 97–109.
6. Li, Z., Gong, X., Joshi, V.H. and Li, M. (2005) Co-evolution of tRNA 3' trailer sequences with 3' processing enzymes in bacteria. *RNA*, 11, 567–577.
7. Ow, M.C. and Kushner, S.R. (2002) Initiation of tRNA maturation by RNase E is essential for cell viability in *Escherichia coli*. *Genes Dev.*, 16, 1102–1115.
8. Zuo, Y. and Deutscher, M.P. (2002) The physiological role of RNase T can be explained by its unusual substrate specificity. *J. Biol. Chem.*, 277, 29654–29661.
9. Soderblom, F., Svard, S.G. and Kirsebom, L.A. (2005) RNase E cleavage in the 5' leader of a tRNA precursor. *J. Mol. Biol.*, 352, 22–27.
10. Mohanty, B.K. and Kushner, S.R. (2007) Ribonuclease P processes polycistronic tRNA transcripts in *Escherichia coli* independent of ribonuclease E. *Nucleic Acids Res.*, doi:10.1093/nar/gkm971.
11. McDowell, K.J., Hernandez, R.G., Lin-Chao, S. and Cohen, S.N. (1992) The ans-l and rne-3071 temperature-sensitive mutations in the ans gene are in close proximity to each other and cause
substitutions within a domain that resembles a product of the *Escherichia coli* rne locus. *J. Bacteriol.*, **175**, 4245–4249.

12. Wachi,M., Umitsu,G., Shimizu,M., Takada,A. and 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. *Biochim. Biophys. Res. Commun.*, **259**, 483–488.

13. Li,Z., Pandit,S. and Deutscher,M.P. (2002) Assigning a function to a conserved group of proteins: the tRNA 3'processing enzymes. *EMBO J.*, **18**, 2878–2885.

14. Ow,M.C., Perwez,T. and Kushner,S.R. (2003) tRNA 3' processing of *Escherichia coli* exhibits only limited functional overlap with its essential homologue, RNase E. *Mol. Microbiol.*, **49**, 607–622.

15. Schiffer,S., Rosch,S. and Marchfelder,A. (2002) Assigning a function to a conserved group of proteins: the tRNA 3' processing enzymes. *EMBO J.*, **21**, 2769–2777.

16. Dubrovskaia,V.A., Levinger,L., Schiffer,S. and Marchfelder,A. (2004) *Drosophila* RNase Z processes mitochondrial and nuclear pre-tRNA 3' ends in vivo. *Nucleic Acids Res.*, **32**, 255–262.

17. Schierling,K., Rosch,S., Rupprecht,R., Schiffer,S. and Marchfelder,A. (2002) tRNA 3' end maturation in archaea has eukaryotic features: the RNase Z from *Haloflexa volcanii*. *J. Mol. Biol.*, **316**, 895–902.

18. Pellegrini,O., Nezzar,J., Marchfelder,A., Putzer,H. and Condon,C. (2003) Endonucleolytic processing of CCA-less tRNA precursors by RNase E in *Bacillus subtilis*. *EMBO J.*, **22**, 4534–4543.

19. Blattner,F.R., Plunkett,G.III, Bloch,C.A., Perna,N.T., Burland,V., Riley,M., Collado-Vides,J., Glasner,J.D. et al. (1997) *Escherichia coli* K-12, 3166,573, 10432–10435.

20. Perwez,T. and Kushner,S.R. (2006) RNase Z in *Escherichia coli* plays a significant role in mRNA decay. *Mol. Microbiol.*, **60**, 723–737.

21. Otsuka,Y. and Yonesaki,T. (2005) A novel endoribonuclease, RNase LS, in *Escherichia coli*. *Genetics*, **169**, 13–20.

22. Schedl,P. and Primakoff,P. (1973) Mutants of *Escherichia coli* thermosensitive for the synthesis of transfer RNA. *Proc. Natl Acad. Sci. USA*, **70**, 2091–2095.

23. Ono,M. and Kawan,M. (1979) A conditional lethal mutation in an *Escherichia coli* strain with a longer chemical lifetime of mRNA. *J. Mol. Biol.*, **129**, 343–357.

24. Arraiato,C.M., Yancey,S.D. and Kushner,S.R. (1988) Stabilization of discrete mRNA breakdown products in *ans pop rnh* multiple mutants of *Escherichia coli* K-12. *J. Bacteriol.*, **170**, 4625–4633.

25. Yokobori,S.-I. and Paabo,S. (1995) Transfer RNA editing in land snail mitochondria. *Proc. Natl Acad. Sci. USA*, **92**, 10432–10435.

26. Aiso,T., Yoshida,H., Wada,A. and Ohki,R. (2005) Modulation of mRNA stability participates in stationary-phase-specific expression of ribosome modulation factor. *J. Bacteriol.*, **187**, 1951–1958.

27. Bensing,B.A., Meyer,B.J. and Dunn,G.M. (1996) Sensitive detection of bacterial transcription initiation sites and differentiation from RNA processing sites in the pheromone-induce plasmid transfer system of *Enterococcus faecalis*. *Proc. Natl Acad. Sci. USA*, **93**, 7794–7799.

28. Bensing,B.A. and Sullam,P.M. (2002) An accessory see locus of *Streptococcus gordoni* is required for export of the surface protein GspB and for normal levels of binding to human platelets. *Mol. Microbiol.*, **44**, 1081–1094.

29. Wang,R.F. and Kushner,S.R. (1991) Construction of versatile low-copy-number vectors for cloning, sequencing and expression in *Escherichia coli*. *Gene*, **100**, 195–199.

30. Nishiyama,K. and Tokuda,H. (2005) Genes coding for SecG and Leu2-tRNA form an operon to give an unusual RNA comprising mRNA and a tRNA precursor. *Biochim. Biophys. Acta*, **1729**, 166–173.

31. Li,Y. and Altman,S. (2003) A specific endoribonuclease, RNase P, affects gene expression of polycistronic operon mRNAs. *Proc. Natl Acad. Sci. USA*, **100**, 13213–13218.

32. Ow,M.C., Liu,Q. and Kushner,S.R. (2000) Analysis of mRNA decay and tRNA processing in *Escherichia coli* in the absence of RNase E-based degradosome assembly. *Mol. Microbiol.*, **38**, 854–866.

33. Li,Y., Cole,K. and Altman,S. (2003) The effect of a single, temperature-sensitive mutation on global gene expression in *Escherichia coli*. *RNA*, **9**, 518–532.

34. Briant,D.J., Hankins,J.S., Cook,M.A. and Mackie,G.A. (2003) The quartenary structure of RNase G from *Escherichia coli*. *Mol. Microbiol.*, **50**, 1381–1390.

35. Lin-Chao,S., Wong,T.-T., McDowall,K.J. and Cohen,S.N. (1994) A + U content rather than a particular nucleotide order determines the specificity of RNase E cleavage. *J. Biol. Chem.*, **269**, 10797–10803.

36. McDowall,K.J., Lin-Chao,S. and Cohen,S.N. (1994) A + U content rather than a particular nucleotide order determines the specificity of RNase E cleavage. *J. Biol. Chem.*, **269**, 10790–10796.

37. Kaberdin,V.R. (2003) Probing the substrate specificity of *Escherichia coli* RNase E using a novel oligonucleotide-based assay. *Nucleic Acids Res.*, **31**, 4710–4716.

38. Deutscher,M.P. (2006) Degradation of RNA in bacteria: comparison of mRNA and stable RNA. *Nucleic Acids Res.*, **34**, 659–666.