Processing of a Multimeric tRNA Precursor from Bacillus subtilis by the RNA Component of RNase P*

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Processing of multimeric precursor tRNAs from Bacillus subtilis by the catalytic RNA component of RNase P was studied in vitro. Previous studies on processing by either Escherichia coli or B. subtilis RNase P-RNA utilized monomeric or dimeric substrates. In the experiments described here, a multimeric precursor tRNA containing six complete tRNA sequences and the partial sequence of a seventh were used. One species did not encode the 3′-terminal CCA sequence and the partial tRNA lacked 3′ nucleotides and could form only a 3-base pair instead of a 7-base paired aminoacyl stem. Two species had the potential for forming extended base-paired aminoacyl stems.

Processing was studied under varied ionic conditions. Chemical sequencing of the products showed that the RNase P-RNA cleavage produced the proper mature 5′ termini for all of the six complete tRNA species, but no 5′-cleavage of the partial species was observed. At suboptimal ionic concentrations, the two species capable of forming extended base-paired aminoacyl stems were not observed. Thus, encoding of the 3′-CCA in a tRNA species is not critical for processing, but the formation of an aminoacyl stem with more than 3 base pairs is necessary.

Particularly noteworthy was the observation that all species of the multimeric precursor could be processed at significantly lower ionic conditions than monomeric precursors used previously by ourselves and others. However, a single precursor species produced from the multimeric precursor could also be processed at the same lower ionic conditions as the multimeric precursor. This demonstrates that precursor tRNA species can differ widely in their ionic requirements for processing and that, to a large extent, the optimal conditions of MgCl₂ or NH₄Cl are a function of the substrate which is used.

EXPERIMENTAL PROCEDURES

Transfer RNA precursors from eubacteria are transcribed with extra sequences on the 5′ and 3′ termini. RNase P cleavage forms the mature 5′ terminus. RNase P from eubacteria is a ribonucleoprotein consisting of a protein component of approximately 14,000 daltons and an RNA component approximately 400 nucleotides in length (Pace, 1984). The RNA is the catalytic component (Guerrier-Takada et al., 1983) and the protein may facilitate bringing the charged catalytic RNA and RNA substrate into proximity at low ionic strengths. High ionic conditions can substitute for the presence of the protein in vitro and enable the catalytic RNA to function by itself. It has been reported, however, that the protein may still be required for some aspects of specificity of the reaction, such as whether a precursor tRNA species lacking the 3′-CCA can be used as a substrate by catalytic RNA alone (Guerrier-Takada et al., 1984).

Many tRNA genes from Escherichia coli and other organisms have been utilized as substrates for in vitro processing by eubacterial catalytic RNAs; however, these have all been monomeric or dimeric substrates. Bacillus subtilis tRNA gene organization differs from that of E. coli in that B. subtilis contains highly clustered tRNA gene regions (Vold, 1985; Fournier and Ozeki, 1985), which might make a multimeric precursor tRNA a more natural substrate for study of processing reactions by B. subtilis RNase P RNA. Investigations of processing of this multimeric precursor offer the advantage of comparing RNase P RNA activity of various precursor tRNAs, one of which does not encode the 3′-terminal CCA sequence and one of which does not have a complete 3′ terminus.

EXPERIMENTAL PROCEDURES

Plasmids—A 2.5-kb Bell-EcoRI fragment from the tRNA gene region from B. subtilis described by Green and Vold (1983) was cloned in pSP64, designated pSP64BE2STG, containing the bacteriophage SP6 RNA polymerase promoter for use in in vitro transcription reactions (Melton et al., 1984, Promega Biotec). This DNA region contained 21 tRNA genes preceded on the 5′-end by the last 79 bases of the 23 S rRNA gene and a 5 S rRNA gene (Vold and Green, 1985).

The plasmid containing a single B. subtilis tRNA* gene cloned into pSP64 was used in transcribing a monomeric tRNA precursor (Green and Vold, 1988). This plasmid was linearized with EcoRI and was used to produce a precursor with 26 extra residues on the 5′ side and 15 residues 3′ to the CCA sequence.

The plasmid containing the cloned B. subtilis P-RNA was a gift of Claudia Reich and Norman Pace, University of Indiana (Reich et al., 1986). The plasmid containing the cloned E. coli M1-RNA was a gift of LaNita Nichols and Francis Schmidt, University of Missouri.

Transcription Reaction for the Multimeric Precursor—Since analysis of the products of the precursor containing 21 tRNAs was somewhat cumbersome, a system was designed for the production of a precursor containing six complete precursor tRNAs and one partial tRNA sequence (Vold and Green, 1985). This was accomplished by cleaving the DNA template with AucI before transcription and allowing the transcript to terminate by run-off. The AucI cut template generates a tRNA precursor containing all of the first six tRNAs and ends 8 bases before the 3′-end of the seventh, tRNA*5. The sequence of the precursor RNA generated in this way is given in Fig. 1.

The transcription reaction was carried out essentially as described by Green et al. (1983), Zinn et al. (1983), and the instructions to the Riboprobe system (Promega Biotec). Materials were autoclaved or prepared in diethyl pyrocarbonate (DEP)-treated water (0.1% DEP overnight then autoclaved). Transcription reactions using [α-32P]}

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** The abbreviations used are: kb, kilobase; DEP, diethyl pyrocarb-
CTP contained 40 mM Tris (pH 7.5), 6 mM MgCl₂, 2 mM spermidine, 100 mM dithiothreitol, 50 units of RNasin (Promega Biotech), 400 µM of all four ribonucleotide triphosphates, 50 µCi (0.065 nmol) [α-32P]CTP, 2 µg of template DNA, and 28 units of SP6 RNA polymerase (Du Pont-New England Nuclear or Promega Biotech) in 20 µl, incubated at 37 °C for 1 h. Reactions were then precipitated with ethanol, collected by centrifugation, dissolved in urea loading buffer, heated at 65 °C for 2 min, and electrophoresed on a 5% polyacrylamide-7 M urea gel. Gels were run and in buffer containing 89 mM Tris base, 89 mM borax acid, and 2.8 mM disodium EDTA, pH 8.3. The gel piece containing the purified transcript was then cut, crushed, and extracted in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. The purified transcript was precipitated with ethanol, collected by centrifugation, dried, and dissolved in DEP-treated water.

Transcription and Processing Reactions for Generating the Precursor Valine tRNA—Two precursors containing valine tRNA were produced. The first precursor contained the same upstream sequences as the multimeric precursor and was transcribed from the same DNA template except that the template was cleaved before transcription with HpaII instead of AuaI (see Fig. 1). This contains the 5' end of tRNA and 3' terminal sequences. The fragment containing the purified transcript was then cut, crushed, and extracted in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. The purified transcript was precipitated with ethanol, collected by centrifugation, dried, and dissolved in DEP-treated water.

Sequence Analysis of Processed Products—Products from the P-RNA processing reaction were separated by electrophoresis on a 5 or 8% polyacrylamide-7 M urea gel, eluted from the gel, and labeled at the 3' terminus with [5'-32P]cytidine 3',5'-bis(phosphate) as described by Bruce and Uhlenbeck (1976). Sequence determination was done by the chemical sequencing technique of Peattie (1979).

RESULTS

Processing of Six-tRNA Transcript in P-RNA Buffer—The reactions optimized for a monomeric precursor tRNA for the B. subtilis catalytic RNA, P-RNA, contained 1.2 M NH₄Cl and 250 mM MgCl₂ (Gardiner et al., 1985). Products of processing for the six-tRNA precursor under these conditions are shown in Fig. 2, lane 2. The fragment indicated as Rib contained the 3' terminus of the 23 S RNA, a spacer sequence, the 5 S RNA, and the 3' flanking sequence. The 5 S RNA is not processed because RNase M5, responsible for 5 S RNA cleavage (Meyhack et al., 1978), was not present in these processing reactions. The Rib fragment is generated by the 5' processing of the first tRNA precursor in the transcript, prRNA. The tRNAs labeled as ptRNA represent the 5'-processed tRNAs still containing the 3'-flanking sequences. The fragment labeled ptRNA represents the 5'-processed tRNA, a spacer sequence, and part of the tRNA sequence. The partial tRNA lacks 8 nucleotides from the 3'-end of the mature tRNA sequence. The Rib fragment and ptRNA are released by one processing event. The production of the other ptRNAs requires the 5'-cleavage of a particular tRNA and the 5' cleavage of the tRNA species on its 3' side.

To establish that the tRNA precursors were being cleaved at the appropriate sites, unlabeled P-RNA-processed transcripts from the AuaI cut template were 3'-end-labeled and chemically sequenced. Fig. 1 shows the sequence of the region containing tRNA precursors that was transcribed from this template. Cleavage sites are indicated by a P; no other sites of cleavage were observed. The figure also shows the AuaI site used to terminate the six-tRNA transcript and a HaeIII site, which was used to generate a five-tRNA transcript (Vold and Green, 1985). The specificity of the processing reaction with

\[^3\text{ptRNA}\] is used as an abbreviation for a complete or partially processed precursor containing tRNA sequences.
Lack of a Complete 3'–Aminoacyl Stem Sequence Prevents 5' Processing—The AuaI cut template produces a transcript with a complete sequence of ptRNA<sub>Leu</sub> and missing the last 8 nucleotides of the 3' mature sequence. This results in a potential 3-base pair aminoacyl stem instead of the normal 7-base pair aminoacyl stem for tRNA<sup>Leu</sup>. Although the ptRNA<sub>Leu</sub> precursor does not contain the CCA 3'-terminal sequence, which (as discussed below) has been suggested to be important for P-RNA processing, P-RNA makes the appropriate cut in this tRNA precursor to produce a mature 5'-end of ptRNA<sub>Leu</sub>. However, the incomplete precursor tRNA<sup>Leu</sup> is not processed by P-RNA, resulting in the formation of a dimeric tRNA precursor fragment. This phenomenon is not peculiar to the ptRNA<sub>Leu</sub>-tRNA<sup>Leu</sup> arrangement. When the template is cut with a HaeIII site, which produces a five-tRNA precursor ending in a partial ptRNA<sub>Leu</sub> also lacking an aminoacyl stem, then a tRNA<sup>15</sup>ptRNA<sup>15</sup> dimer appears (Vold and Green, 1985). The partial ptRNA<sub>Leu</sub> generated by the HaeIII cut cannot form any base pairs in the aminoacyl stem. Thus, when part of the aminoacyl stem of the tRNA on the 3'-end of the precursor for either tRNA<sup>15</sup> or ptRNA<sub>Leu</sub> was deleted but the 5'-end was intact, the incomplete ptRNA could not be processed. Although results discussed in the next section will indicate that the CCA sequence itself is not necessary for processing, formation of a base-paired structure longer than 3 base pairs in the aminoacyl stem region seems to be important.

3'-CCA Sequence Is Not Critical for Processing—In the E. coli system, it has been suggested that the presence of the 3'-terminal CCA sequence is necessary for proper processing by M1-RNA. The in vivo RNase P processing of phage T4 tRNA precursors in E. coli has been shown to be dependent on the creation of a mature CCA end by tRNA nucleotidyl transferase (McClain, 1977; Fukada and Abelson, 1980). Transfer RNA precursors that lack the CCA sequence have also been shown to be poor substrates for the purified M1-RNA in vitro (Guerrier-Takada et al., 1984). In the B. subtilis system described here, the lack of the CCA end does not seem to interfere with proper processing. For instance, a mature 5'-end of the precursor for either tRNA<sup>15</sup> or ptRNA<sub>Leu</sub> was made even though the CCA terminus of this tRNA was not encoded in the gene (Fig. 1). The 5'-cleavage of ptRNA<sub>Leu</sub> occurs even under suboptimal ionic conditions, as discussed later in this paper.

Two recent studies also support the idea that the lack of the 3'-terminal sequence does not necessarily prevent cleavage by catalytic RNA. In studies with E. coli M1-RNA, Nichols and Schmidt (1988) have shown that both tRNAs from a dimeric precursor, can be processed even though one tRNA species lacks the 3'-CCA; however, the cleavage of the tRNA lacking the CCA was more dependent on a higher Mg<sup>2+</sup> concentration. Studies using synthetic B. subtilis tRNA<sup>15</sup> also demonstrated that catalytic RNA from E. coli or B. subtilis RNase P cleaved the substrate if the CCA was replaced with other nucleotides, however the K<sub>m</sub> was increased (Green and Vold, 1988).

Processing of the Multimeric Transcript under Conditions of Low Ionic Strength—in the original paper, demonstrating that the RNA components of RNase P from both E. coli and B. subtilis were the catalytically active moieties of the enzyme, it was shown that the E. coli holoenzyme could cleave E. coli ptRNA<sup>15</sup> or E. coli ptRNA<sup>3</sup> in buffers containing 5–10 mM Mg<sup>2+</sup>, but the RNA component by itself required higher ionic concentrations of 60 mM Mg<sup>2+</sup> using 100 mM NH₄Cl (Guerrier-Takada et al., 1983). Optimal conditions for the RNA component of B. subtilis RNase P with the semisynthetic substrate, E. coli ptRNA<sup>3</sup>, were subsequently determined to

FIG. 2. Processing of the six-tRNA precursor with diluted P-RNA buffer and M1 buffer conditions. Processing products were electrophoresed on a 5% acrylamide–7 M urea gel. After electrophoresis, the gel was cut for drying, the figure shows the reassembled photograph. All reactions contained 17 fmol of 32P-labeled precursor. Lane 1 represents the precursor incubated for 30 min without P-RNA. Reactions represented in lanes 2–7 contained 50 ng of P-RNA and, lane 2, undiluted P-RNA buffer; lane 3, P-RNA buffer at 1/2 strength; lane 4, P-RNA buffer at 1/4 strength; lane 5, P-RNA buffer at 1/8 strength; lane 6 the same amount of precursor and P-RNA in M1 buffer. Lane 7 represents the same reaction as in lane 6, incubated in M1 buffer for 30 min, followed by addition of the NH₄Cl and MgCl₂ to concentrations of straight P-RNA buffer and the reaction for 15 min. Lane 8 contained the precursor and self-processing conditions of Cech (Cech et al., 1981). Lane 9 contained the precursor and self-processing conditions of Apirion (Watson et al., 1984).
be considerably higher with Mg\(^{2+}\) concentrations decreasing from about 400 to 250 mM as the NH\(_4\)Cl concentration was increased from 0.6 to 2.0 M (Gardiner et al., 1985; Marsh et al., 1985). Furthermore, these higher ionic conditions are probably preferable for processing monomeric transcripts with M1-RNA from E. coli (Green and Vold, 1988). The monovalent and divalent cations were to some extent able to complement each other, suggesting that part of the effect of the high cation concentration is to fulfill a requirement for ionic strength (Gardiner et al., 1985).

To approximate what ionic conditions might be required for P-RNA with the six-tRNA precursor, the processing reaction was carried out in P-RNA buffer (1.2 M NH\(_4\)Cl and 250 mM MgCl\(_2\) or \(\frac{1}{2}\) strength P-RNA buffer (0.6 M NH\(_4\)Cl, 125 mM MgCl\(_2\)). Processing was still evident at \(\frac{1}{4}\)th strength P-RNA buffer (240 mM NH\(_4\)Cl, 50 mM MgCl\(_2\)), but there was a noticeable reduction in the amount of ptRNA\(^{39}\) and, to a lesser extent, ptRNA\(^{Thr}\). No processing took place in \(\frac{1}{10}\)th diluted P-RNA buffer (120 mM NH\(_4\)Cl, 25 mM MgCl\(_2\)) with an incubation time of 30 min. Also, in M1 buffer, processing occurred but, again, the ptRNA\(^{39}\) was almost completely missing and the amount of ptRNA\(^{Thr}\) was noticeably reduced. Raising the ionic strength of the M1 buffer to that of P-RNA buffer and continuing the incubation for 15 min resulted in the reappearance of the ptRNA\(^{39}\) and increase of ptRNA\(^{Thr}\) to normal levels (Fig. 2, lane 7). Thus, at suboptimal ionic strength, ionic conditions affected the amount of processing of the various ptRNAs to different extents.

The potential for self-processing of the six-tRNA precursor was also examined. Lanes 8 and 9 of Fig. 2 show processing under the self-processing conditions used by Cech (Cech et al., 1981) and Apirion (Watson et al., 1984), respectively. No self-processing of the precursor was observed.

Effects of Variations in Magnesium or Ammonium Chloride Concentrations—Fig. 3 shows the results obtained from the processing reactions when the NH\(_4\)Cl concentration was held at 100 mM and the magnesium chloride concentration was varied. No processing occurred under these conditions in the absence of magnesium (Fig. 3, lane 2). Some processing did occur at 25 mM Mg\(^{2+}\) (Fig. 3, lane 3); the first products were the Rib segment and the dimer, which represent processing of the 5' terminal ptRNA, ptRNA\(^{Val}\), and the ptRNA\(^{59}\) [tRNA\(^{AA}\) dimer]. The ptRNA\(^{59}\) is one ptRNA in the six-tRNA precursor that lacks the 3'-CCA sequence and yet it was processed at very low ionic strengths. At 50 mM, all of the final products could be seen except ptRNA\(^{Lys}\) although ptRNA\(^{Thr}\) was faint. In additional experiments, the magnesium concentration was increased, holding the NH\(_4\)Cl at 100 mM. Each of the final products was cut from the dried gel and the amount of radioactivity was measured in a scintillation counter. The results indicated a broad range of magnesium concentrations for processing, with a maximum at approximately 200–225 mM Mg\(^{2+}\) when the reactions included 100 mM NH\(_4\)Cl.

Fig. 4 shows results with increasing NH\(_4\)Cl at a suboptimal concentration of Mg\(^{2+}\) (50 mM). The question asked was whether processing to release ptRNA\(^{59}\) could be stimulated by raising either Mg\(^{2+}\) or NH\(_4\)\(^+\). The results show that when the concentration of NH\(_4\)Cl was raised to 500 mM (with 50 mM MgCl\(_2\) (Fig. 4, lane 7)), NH\(_4\)\(^+\) stimulated the release of ptRNA\(^{59}\) equivalent to that released in 100 mM MgCl\(_2\) and

\[ \text{Fig. 3. Magnesium ion dependence of processing reaction in } 100 \text{ mM NH}_{4}\text{Cl. Processing products were electrophoresed on a 5\% acrylamide-7 \text{ m urea gel. All reactions contained 122 fmol of } ^{32}\text{P}-\text{labeled precursor in a 10-\mu l reaction volume. Lane 1 represents the precursor incubated without P-RNA. Reactions represented in lanes 2–7 contained 50 ng of P-RNA, 100 mM NH}_{4}\text{Cl, 50 mM Tris-HCl (pH 7.5). MgCl}_{2} \text{ concentrations were 0 mM (lane 2), 25 mM (lane 3), 50 mM (lane 4), 75 mM (lane 5), 100 mM (lane 6), and 150 mM (lane 7). Reactions were incubated for 30 min.} \]
Processing of a Six-tRNA Precursor by Catalytic RNA

12345678
- Rib

**Fig. 4.** Ammonium ion dependence of processing reaction with suboptimal magnesium ion concentration. Processing reactions were done using the conditions described in the legend to Fig. 3 except that all reactions contained 50 mM MgCl₂. Lane 1 was incubated without P-RNA. Lanes 2–7 indicate NH₄Cl concentrations varied as follows: 0 mM (lane 2), 50 mM (lane 3), 100 mM (lane 4), 150 mM (lane 5), 200 mM (lane 6), and 500 mM (lane 7). Lane 8 is a control with 100 mM MgCl₂ and 100 mM NH₄Cl.

derived from the Multimeric Precursor, and Monomeric Precursor Not Included in the Multimeric Precursor—Magnesium was particularly critical for processing since the transcripts could not be processed without magnesium but could be processed without ammonium chloride (provided magnesium was present). Thus, processing as a function of magnesium concentration was performed with four different substrates: the multimeric precursor with 5 S rRNA and six-tRNAs, a shortened precursor with the 5 S rRNA and first tRNA, a monomer with only the first tRNA (a valine tRNA), and a monomer of a tRNA̅¹² not represented in the six-tRNA transcript. The precursor histidine tRNA was produced in vitro from a synthetic gene (Green and Vold, 1988). The precursor containing the 5 S rRNA and only the tRNAVal were transcribed from the same transcript as the multimeric precursor except that transcription was terminated by run-off at a HpaII instead of an AvaI restriction site. The monomeric ptRNAVal was prepared from the HpaII terminated transcript by isolation of the appropriate band from polyacrylamide gel electrophoresis after the transcript was treated with RNase M5 to cleave the 5 S rRNA.

The results are shown in Fig. 5. Using 1.2 M NH₄Cl, the multimeric substrate was optimally processed at approximately 20 mM MgCl₂. This graph represents the summation of all 5’ processed fragments or intermediates; however, each of the six-tRNA species showed almost identical curves when the 5’ processed fragments were measured independently. The precursor containing the first tRNA, tRNAVal, or the 5 S rRNA and first tRNA also could be processed at approximately 20 mM MgCl₂. The monomeric precursor histidine tRNA, however, which is not represented in any of the six-tRNA species in the multimer, required approximately 250 mM MgCl₂.

**Fig. 5.** Optimal MgCl₂ concentration for cleavage of monomeric and a multimeric precursor tRNA in 1.2 M NH₄Cl. The top graph shows the concentration of MgCl₂ required for processing a monomeric precursor histidine tRNA (left side) or monomeric precursor valine tRNA (right side). The lower graph shows the concentration of MgCl₂ required for processing the precursor containing six-tRNAs (left side) or a precursor containing 5 S rRNA and valine tRNA. Processing reactions for each experiment contained 50 fmol of precursor, 44 ng of P-RNA, 1.2 M NH₄Cl, 50 mM Tris-HCl, pH 8.0, in a 20-μl volume. Reactions were incubated at 37 °C for 1 h. Products were separated on an acrylamide-7 M urea gel. Fragments were located by autoradiography of the dried gel, cut, and their radioactivity measured by liquid scintillation counting: ○, unprocessed transcript; ●, 5’ processed products; ■, processing intermediates.

**DISCUSSION**

These experiments shed light on structural and ionic requirements for processing of B. subtilis precursor RNAs by B. subtilis RNase P-RNA. In our discussion of precursor structure, we assume that tRNA precursors have conformational domains resembling those of mature tRNAs. This is supported by studies on the analysis of solution conformations of tRNA precursors by chemical and enzymatic probes (Swerdlow and Guthrie, 1984). In addition, studies on conformational changes in tRNAs suggest that folding into the mature tRNA structure is possible during biosynthesis even before the tRNA
sequence is complete (Stein and Crothers, 1976). Thus, it seems reasonable to assume that the multimeric precursor tRNA made in in vitro transcription reactions will exist with the tRNA coding sequences folded into typical tRNA domains.

Our experiments demonstrate one of the structural features important for correct processing is the aminoacyl stem region. The need to form an aminoacyl stem of more than 3 base pairs is critical. Evidence for this is that the 5' processing of the last, partial tRNA never was observed. Since this tRNA lacking nucleotides at the 3'-end, it could only form a 3 base-paired aminoacyl stem. This phenomenon is not unique to the structure of the tRNA since using a shorter precursor which terminates in the preceding tRNA, partial tRNA, which cannot form any of the aminoacyl stem, results in complete lack of processing of the partial tRNA. The presence of the 3'-CCA sequence in the aminoacyl stem region, however, does not seem to be critical for processing in this multimeric precursor. The tRNA lacking the CCA 3' terminus was processed efficiently even under conditions of low ionic strength.

Formation of an extended aminoacyl stem may slightly inhibit processing. As indicated in Fig. 6, tRNA and tRNA could form extended aminoacyl stems, a stem loop structure could form between the 5 S tRNA and tRNA, and tRNA could have 1 extra base pair in the aminoacyl stem. It is interesting to note that under conditions of suboptimal ionic concentrations, cleavage of the two tRNAs capable of forming extended base-paired aminoacyl stems is considerably reduced compared to other tRNA species. Extending the base-paired stem, however, did not affect the cleavage site. Precursor tRNAs with an extended aminoacyl stem structure have also been shown to be processed at a slower rate in eukaryotic cells (Castagnoli et al., 1982). On the other hand, tRNA has a potential for a 1-base-paired extension of the aminoacyl stem and a 7-base-paired stem-loop structure two nucleotides before the 5'-cleavage site, and this precursor is efficiently processed even at suboptimal ionic strengths (resulting in the release of the ribosomal fragment).

Another important parameter is the ionic requirements for processing of the multimeric and monomeric substrates. Substrates previously used to examine the in vitro specificity of catalytic RNAs have been monomorphic. Perhaps the most unique aspect demonstrated by these studies is that the transcript containing six complete precursor tRNAs or a monomeric precursor derived from the multimeric sequence are able to be processed by P-RNA at much lower ionic concentrations than previously reported for other monomeric precursors. Optimal ionic conditions were determined to be 200-225 mM MgCl2 with 100 mM NH4Cl. Using the semisynthetic monomeric substrate, E. coli tRNA with A3C appended to the 5' terminus, optimal ionic conditions for in vitro cleavage by P-RNA were determined to vary from 400 to 250 mM MgCl2 when the NH4Cl concentration was increased from 0.6 to 2.0 M (Gardiner et al., 1985). The B. subtilis precursor tRNA(Ala)(Fig. 5) also requires high salt conditions for effective processing.

Our data indicate that a high ionic strength buffer is not necessary for the processing of some tRNA precursors by the catalytic RNA alone. This ability to be processed at low ionic strength can exist in monomeric as well as multimeric tRNA precursor substrates. The RNase P protein is believed to act as an ionic shield to reduce the anionic repulsion between the substrate and the catalytic RNA (Reich et al., 1988). It is possible that substrates differ in the amount of ionic shielding required or that the protein component of RNase P may be necessary to induce a more favorable conformation in some substrates at low ionic strength that otherwise can only be caused by the presence of a high ionic strength buffer.

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