Dependence of M1 RNA substrate specificity on magnesium ion concentration

LaNita Nichols and Francis J. Schmidt

Department of Biochemistry, University of Missouri—Columbia, Columbia, MO 65212, USA

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ABSTRACT

We have constructed a plasmid expressing E. coli M1 RNA, the catalytic RNA subunit of ribonuclease P, under the control of a phage T7 promoter. The active M1 RNA species synthesized in vitro by T7 RNA polymerase from this vector was reacted with the tRNA_{Gin} - tRNA_{Leu} precursor RNA (Band K) encoded by phage T4. Only the tRNA_{Gin} moiety of this dimeric precursor RNA contains the 3' terminal C-C-A sequence common to all tRNAs. We observed that protein-free M1 RNA was capable of processing the precursor RNA at the 5' ends of both tRNA sequences. The rate of cleavage of the tRNA_{Gin} sequence was more strongly dependent on [Mg^{2+}] than that of tRNA_{Leu}, increasing severalfold between 100 and 500 mM Mg^{2+}, conditions under which the rate of cleavage at the tRNA-Leu sequence was constant.

INTRODUCTION

The enzyme ribonuclease P is responsible in Escherichia coli for generating 5' termini characteristic of mature tRNA species (1). Studies of tRNA biosynthesis in phage T4-infected cells showed that the presence of the 3' terminal C-C-A sequence in the tRNA precursor substrate is an important determinant of RNase P specificity. This C-C-A sequence, common to all mature tRNAs, is absolutely required for in vivo cleavage of the dimeric tRNA^{Pro} - tRNA^{Ser} precursor RNA species encoded by bacteriophage T4 (2,3). This in vivo substrate specificity of RNase P is reflected, albeit incompletely by the isolated enzyme in vitro. The presence of the 3' terminal C-C-A sequence enhances the rate of precursor RNA cleavage (V_{max}/K_m) by RNase P holoenzyme about threefold relative to the rate of cleavage observed when the precursor species lacking the C-C-A sequence is processed by the enzyme (4).

Subsequent to the discovery that the M1 RNA subunit of RNase P is catalytically active in vitro (5), several investigators reported that the isolated RNA component exhibited a greater requirement for the C-C-A sequence in the tRNA precursor substrate than did the holoenzyme (6,7). This implied that the protein component of RNase P, absolutely required for in vivo catalysis (8),
apparently widened the range of specificity when the reaction was carried out in vitro.

In the present communication we show that M1 RNA is indeed capable of cleaving in vitro tRNA precursor substrates lacking the terminal C-C-A. We further show that M1 RNA substrate specificity is strongly Mg$^{2+}$-dependent. Although M1 RNA catalyzed cleavage of a tRNA precursor sequence lacking C-C-A even in the presence of 20 mM Mg$^{2+}$, the reaction rate increased substantially with higher [Mg$^{2+}$]. In the presence of 500 mM Mg$^{2+}$, kinetic specificity of M1 RNA alone was close to that exhibited by the holoenzyme.

**EXPERIMENTAL PROCEDURES**

**Bacterial and Phage strains and plasmids.**

E. coli B/5 and phage T4A27 (9) were from the collection of W.H. McClain. E. coli FS101, a recA derivative of the rnpA49 strain and plasmid pLN2 have been described previously (10). Plasmids pTZ18R, pTZ18U, pTZ19R, and pTZ19U (11) were from Drs. David Mead and Byron Kemper, University of Illinois.

**Enzymes and Reagents.**

Restriction enzymes were purchased from New England Biolabs or Promega Biotec, Madison, WI. Phage T7 RNA polymerase was from U.S. Biochemicals. Ribonucleotides were from Boehringer-Mannheim. Buffers and stock solutions were prepared in water that had been distilled, deionized, treated with diethylpyrocarbonate and autoclaved.

**Isolation of RNA Species Synthesized in vivo and in vitro.**

The phage T4 $[^{32}P]$tRNA$^{Gln}$ - tRNA$^{Leu}$ precursor RNA (Band K; see ref. 12) was prepared by infecting E. coli FS101 at 42°C with phage T4A27 at a multiplicity of 15 plaque forming units per cell as described previously (3). At $t=4$ min. post infection, $[^{32}P]$phosphate (1 mCi mL$^{-1}$) was added and incubation continued for a further 11 min. $[^{32}P]$ Band K RNA was prepared by direct phenol extraction of the culture, followed by electrophoresis in 10% and 20% polyacrylamide gels (12). Native E. coli M1 RNA was prepared from a culture of E. coli FS101 carrying pLN2 (10) by gel electrophoresis in 5% polyacrylamide gel containing 7 M urea. The M1 RNA band was visualized by UV shadowing, electroeluted and further purified by chromatography on a column (0.5 ml bed volume) of Whatman DE52 DEAE-cellulose. RNA was eluted from the column by application of preheated (65°C) 10 mM Tris-C1, pH 8.0/1 mM EDTA/1.5 M NaCl. The RNA was recovered by precipitation with an equal volume of 2-propanol at room temperature. The RNA was dissolved and reprecipitated with ethanol and the pellet washed at room temperature with 70% (v/v) ethanol. The recovered
RNA was redissolved in neutralized, diethylypyrocarbonate-treated water at a concentration of -1 μM.

**In vitro** transcription reactions were done in 100-130 μL of a solution containing 2-5 μg linearized plasmid DNA; 500 μM each of ATP, CTP, GTP and UTP; 40 mM Tris Cl, pH 8.0; 8 mM MgCl₂; 25 mM NaCl; 2 mM spermidine-HCl; 10 mM dithiothreitol (freshly prepared from 1 M stock); 1-2 U human placental RNase inhibitor ("RNAsin ™", Promega-Biotec, Madison, WI); and 85-120 U T7 RNA polymerase. Transcription was allowed to proceed at 37° for 90 min at which time the solution was extracted with phenol and made 300 mM in K-acetate (pH 5.0) followed by addition of 3 volumes absolute ethanol. The RNA pellet was redissolved in 10 M urea containing bromphenol blue and xylene cyanol FF, heated at 55° for 2 min. and applied to a 5% polyacrylamide/7 M urea gel as described above. RNA was visualized by UV shadowing and purified as described above for the native M1 RNA species.

**Construction of T7 promoter plasmids containing the M1 RNA gene.**
Plasmid pLN2 was digested with EcoRl, KpnI and SnaBl. The 650 bp KpnI-SnaBl fragment containing the promoter region and the M1 RNA structural gene was purified from low-melting point agarose. This fragment was cut with AluI and the AluI - SnaBl fragment spanning nucleotides +6 to +384 of the M1 RNA gene was ligated into the SmaI site of plasmid pTZ18R (11). The cloned M1 RNA sequence obtained by this construction was in the antisense orientation relative to the T7 promoter so the insert was recloned as an EcoRI - HindIII fragment into plasmid pTZ19R (11). Orientation was confirmed by determining the orientation of the internal SmaI site at nucleotide +290 of the M1 RNA gene (14, 15) relative to the polylinker EcoRl and BamHI sites and by dideoxynucleotide sequencing of the insert.

**RNA processing reactions.**
The conditions used for RNase P holoenzyme reactions were as described by Robertson, et al. (1). RNA catalysis was carried out by preincubating the catalyst (0.01 - 1.0 pmole per assay point) at 37° for 30 min in 62 mM Tris Cl, pH 7.6/125 mM NH₄Cl/MgCl₂. Then 3-6 fmol of tRNA precursor in 0.25 volumes H₂O were added and incubation continued. The reaction was stopped by addition of 5 μL aliquots to 2 μL of 10 M urea/500 mM EDTA/0.25% (w/v) bromphenol blue/0.25% (w/v) xylene cyanol FF, followed by freezing. The reaction products were separated by electrophoresis on a sequencing gel and analyzed by autoradiography. Quantitation was by cutting out bands from the dried gel and counting in scintillation fluid or by densitometry of the autoradiogram.
RESULTS

Synthesis of active M1 RNA in vitro.

We constructed a derivative of pTZ19R containing the M1 RNA gene. The T7 promoter resident in the pTZ vectors then was used to direct in vitro transcription of the cloned M1 RNA sequence (nucleotides +6 to +384; refs. 14,15) from linearized pTZ19-M1. The structure of the template is shown in Fig. 1. The product RNA migrated as a single band in denaturing polyacrylamide gels at a mobility consistent with its predicted chain length of 434 nucleotides. Note that the RNA species so produced is longer than native M1 RNA itself (377 nucleotides) since polylinker sequences in the vector were also transcribed from these constructions by the T7 RNA polymerase.

Figure 1. Structure of pTZ19 M1, a derivative of pTZ19R which places the M1 RNA sequence under the control of a promoter for T7 RNA polymerase. The antisense strand is shown; however single-stranded phage DNA from this construct contains the sense strand (11). The start point of transcription, transcribed polylinker and EcoRI site used for linearization are shown. The M1 RNA sequence is given in refs. 14 and 15.

Figure 2. Catalysis of precursor RNA cleavage by native and synthetic M1 RNA species. Catalytic RNA was preincubated for 20 min in reaction buffer. Reaction was initiated by addition of substrate (~ 6 fmol) and stopped after 20 min. The products were separated in an 8% polyacrylamide sequencing gel and visualized by autoradiography. In each experiment, Lane 1 contained no M1 RNA. Lane 2, 0.6 fmol of M1 RNA; Lane 3, 1.2 fmol; Lane 4, 3 fmol; Lane 5, 6 fmol; Lane 6, 60 fmol. Experiment a, native M1 RNA. Experiment b, synthetic M1 RNA.
Figure 3. Products produced by M1 RNA cleavage of dimeric pre-tRNA\textsuperscript{Gln}\textsuperscript{Leu} varied with time of incubation and [Mg\textsuperscript{2+}]. The RNA species shown in the autoradiogram have been characterized by Guthrie (12) and by Subbarao et al (11). Lane (a), products of M1 RNA cleavage of precursor RNA in 500 mM MgCl\textsubscript{2} for 1 hr. at 37°. Lane (b), pre-tRNA\textsuperscript{Gln}\textsuperscript{Leu} was incubated without M1 RNA for 1 hr. in 20 mM MgCl\textsubscript{2}. Lane (c), as in b, except that incubation was for 20 hr. Lane (d), incubation of precursor RNA with synthetic M1 RNA at 37°, 1 hr., in 20 mM MgCl\textsubscript{2}. Lane (e), as in (d), but for 20 hr. Identity of bands: Band K, pre-tRNA\textsuperscript{Gln}\textsuperscript{Leu}; Band 1, mature tRNA\textsuperscript{Leu}; Band 2, immature tRNA\textsuperscript{Gln}; Band 3, mature tRNA\textsuperscript{Gln}.

**Reaction of catalytic RNA with tRNA precursor species.**

The synthetic and native M1 RNAs were used to cleave several tRNA precursors in vitro. Among these precursors was the dimeric tRNA\textsuperscript{Gln}\textsuperscript{Leu} precursor from phage T4-infected E. coli. The two tRNA sequences in this substrate differ in their degree of 3' end maturation: the tRNA\textsuperscript{Leu} sequence has a 3' C-C-A terminus encoded in the DNA while the tRNA\textsuperscript{Gln} moiety must be posttranscriptionally modified at the 3' end after separation from the tRNA\textsuperscript{Leu} sequence. Biosynthesis of tRNA\textsuperscript{Gln} requires additional processing to remove a U from the 3' end of the tRNA sequence and to synthesize the C-C-A terminus (12).

We compared the catalytic efficiency of native and synthetic M1 RNA species on this precursor RNA. In the experiment shown in Fig. 2., serial dilu-
Figure 4. Increased concentration of monovalent cation did not facilitate M1 RNA cleavage at the 5' end of tRNA^Gln in 50 mM Tris Cl, pH 7.6, 50 mM MgCl_2. M1 RNA (0.1 pmol) was preincubated at 37° for 20 min. Then substrate was added and the reaction allowed to proceed for 20 min. Lane 1, the reaction mixture contained 500 mM NH_4Cl; Lane 2, 200 mM NH_4Cl; Lane 3, 100 mM NH_4Cl; Lane 4, 50 mM NH_4Cl; Lane 5, 20 mM NH_4Cl; Lane 6, 10 mM NH_4Cl. Lane 7, the reaction mixture contained no M1 RNA. Lane 8, the reaction was carried out in 50 mM Tris Cl, pH 7.6/100 mM MgCl_2.

ections of native and synthetic M1 RNAs were reacted with a constant amount of tRNA^Gln - tRNA^Leu precursor RNA. The results show that detectable cleavage of precursor RNA occurred at the same concentrations of M1 RNA. We conclude that the abilities of native and synthetic M1 RNAs to cleave precursor RNA do not differ by more than twofold under these conditions. The data presented here do not allow detailed comparisons of K_m and k_cat values since the specific activity of the substrate was not known. However, results of Guerrier-Takada and Altman (13) also indicate that native and synthetic M1 RNA species have similar kinetic constants.

In the experiments shown in Fig. 3., the products of reaction were examined in more detail. Guthrie (12) showed that cleavage of tRNA^Gln - tRNA^Leu precursor RNA by RNase P holoenzyme produced three RNA species: the mature tRNA^Leu sequence, the tRNA^Gln sequence (lacking mature C-C-A) and an intermediate product, designated immature tRNA^Gln, which resulted from RNase P action at the interstitial (tRNA^Leu) sequence only and therefore contained 5' precursor-specific sequences. All three of these RNAs could be observed when
Figure 5. Initial rate of pre-tRNA\textsuperscript{Gln} - tRNA\textsuperscript{Leu} cleavage depended on [Mg\textsuperscript{2+}] concentration. The precursor RNA was reacted with M\textsubscript{1} RNA at varying Mg concentrations. a, [Mg\textsuperscript{2+}] = 20 mM; b, [Mg\textsuperscript{2+}] = 50 mM; c, [Mg\textsuperscript{2+}] = 100 mM; d, [Mg\textsuperscript{2+}] = 500 mM. Aliquots were taken at t = 0 (lane 1); t = 2 min. (lane 2); t = 5 min (lane 3); t = 10 min. (lane 4); t = 30 min (lane 5); t = 4 hr. (lane 6).

We reacted the tRNA\textsuperscript{Gln} - tRNA\textsuperscript{Leu} precursor RNA with either native or synthetic M\textsubscript{1} RNA. Typical results are shown in Figs. 2 and 3. The RNAs produced by cleavage with synthetic M\textsubscript{1} RNA were of identical mobility with those produced by cleavage with RNase P holoenzyme (12) and are assumed to be identical. All reactions produced mature tRNA\textsuperscript{Leu} (Species 1 in Fig. 3.); this molecule resulted from enzymatic cleavage at the 5' end of the tRNA\textsuperscript{Leu} sequence in precursor RNA. Two tRNA\textsuperscript{Gln} species were produced by the processing reaction as well: immature tRNA\textsuperscript{Gln} (band 2 in Fig. 3.) contained 5' precursor-specific sequences, while mature tRNA\textsuperscript{Gln} (band 3 in Fig. 3.) resulted from a second endonucleolytic cleavage at the 5' end of tRNA\textsuperscript{Gln}. In buffers containing 20 mM Mg\textsuperscript{2+}, immature tRNA\textsuperscript{Gln} was the only tRNA\textsuperscript{Gln} species observed after 1 hr. incubation, indicating that M\textsubscript{1} RNA cleaved the tRNA precursor only once, at the 5' end of the tRNA\textsuperscript{Leu} sequence. In buffers containing higher [Mg\textsuperscript{2+}], a second tRNA\textsuperscript{Gln} product was also observed, corresponding in mobility to the fully processed tRNA\textsuperscript{Gln}. The ability of M\textsubscript{1} RNA to carry out the cleavage at the 5' end of tRNA\textsuperscript{Gln} was enhanced with higher [Mg\textsuperscript{2+}] so that at 500 mM Mg\textsuperscript{2+} the products of the reaction were predominantly mature tRNA\textsuperscript{Gln} and tRNA\textsuperscript{Leu}.
Figure 6. $[\text{Mg}^{2+}]$ dependence of the rate of cleavage at the 5' end of mature tRNA\textsuperscript{Gln}. The pre-tRNA\textsuperscript{Gln}-tRNA\textsuperscript{Leu} species was reacted with Ml RNA in the presence of 20 mM MgCl\textsubscript{2} for 60 min. to completely process the tRNA\textsuperscript{Leu} sequence. Then MgCl\textsubscript{2} was added and the extent of reaction measured. The products were separated on a sequencing gel and detected by autoradiography.

g. The reaction was carried out in 20 mM MgCl\textsubscript{2}, lane 1, $t = 0$; lane 2, $t = 60$ min.; lane 3, $t = 61$ min; lane 4, $t = 62$ min.; lane 5, $t = 65$ min.

b. Ml RNA was allowed to react for 60 min. in 20 mM MgCl\textsubscript{2}. Then MgCl\textsubscript{2} was added to 50 mM final concentration. lane 1, $t = 0$; lane 2, 60 min. after initiation of the reaction in 20 mM MgCl\textsubscript{2}; lane 3, 15 sec. after Mg\textsuperscript{2+} addition; lane 4, 1 min. after Mg\textsuperscript{2+} addition; lane 5, 5 min after Mg\textsuperscript{2+} addition; lane 6, 15 min. after Mg\textsuperscript{2+} addition.

c. As in b, except that $[\text{Mg}^{2+}]$ was raised to 100 mM.

d. As in b, except that $[\text{Mg}^{2+}]$ was raised to 500 mM.

This altered distribution of Ml RNA cleavage products was shown to be an effect of $[\text{Mg}^{2+}]$ rather than a generalized effect of ionic strength. Changes in $[\text{NH}_4^+]$ at constant $[\text{Mg}^{2+}]$ had no effect on the distribution of tRNA\textsuperscript{Gln} species (Fig. 4.). In the presence of 5 mM spermidine, increasing $[\text{Mg}^{2+}]$ altered the products produced by the RNA reaction but the effect of $[\text{Mg}^{2+}]$ was apparent at lower concentrations so that significant amounts of mature tRNA\textsuperscript{Gln} were produced at 20 mM Mg\textsuperscript{2+} (data not shown). In separate experiments (data not shown) RNase P holoenzyme was used to cleave the tRNA\textsuperscript{Gln} - tRNA\textsuperscript{Leu} precursor RNA. Variation of the $[\text{Mg}^{2+}]$ from 3 to 20 mM had no effect on the distribution of products. Thus, the the effect of Mg\textsuperscript{2+} on substrate specificity was characteristic only of catalysis by Ml RNA alone.

We examined the effect of $[\text{Mg}^{2+}]$ kinetically. The effect of $[\text{Mg}^{2+}]$ on initial rate of the tRNA\textsuperscript{Leu} cleavage by synthetic Ml RNA is shown in Fig. 5. Note the rate of disappearance of the dimeric substrate is a measure of the rate of precursor cleavage at the tRNA\textsuperscript{Leu} sequence. The results are similar.
to those reported for the rate of cleavage of the monomeric E. coli tRNA\(^{\text{Tyr}}\) precursor RNA by Ml RNA as a function of [Mg\(^{2+}\)] (16); the rate of reaction was maximal at 100 mM Mg\(^{2+}\). Increasing [Mg\(^{2+}\)] from 100-500 mM had no detectable effect on the rate of the cleavage at the 5' end of tRNA\(^{\text{Gln}}\).

The results in Fig. 5 also indicated that the rate of cleavage at the 5' end of the tRNA\(^{\text{Gln}}\) sequence in precursor RNA also increased with increasing [Mg\(^{2+}\)]. We assessed the effect of [Mg\(^{2+}\)] on the initial rate of tRNA\(^{\text{Gln}}\) cleavage as shown in Fig. 6. In this experiment, precursor RNA and synthetic Ml RNA were reacted in the presence of 20 mM [Mg\(^{2+}\)], conditions under which only the tRNA\(^{\text{Leu}}\) cleavage occurred; then the [Mg\(^{2+}\)] was increased and the rate of appearance of mature tRNA\(^{\text{Gln}}\) was measured. This protocol insured against potentially complicating effects of the enzyme acting at both cleavage sites of the dimeric precursor RNA.

The initial rates of cleavage at the tRNA\(^{\text{Leu}}\) (Fig. 5) and tRNA\(^{\text{Gln}}\) (Fig. 6) 5' termini were determined and are shown in Fig. 7 as a function of [Mg\(^{2+}\)]. It is apparent that the rate of reaction to generate mature tRNA\(^{\text{Gln}}\) was more strongly [Mg\(^{2+}\)]-dependent than that to generate mature tRNA\(^{\text{Leu}}\). Fig. 7 shows that the rate of cleavage increased several fold between 100 mM and 500 mM [Mg\(^{2+}\)], conditions under which the rate of tRNA\(^{\text{Leu}}\) cleavage was nearly constant.

**Figure 7.** Differential effects of [Mg\(^{2+}\)] on initial rate of Ml RNA cleavage of tRNAs with and without C-C-A. Initial velocities of cleavage were determined from the data in Figs. 4 and 5 and are normalized to the maximum rate observed in each case. Open bars: Rate of tRNA\(^{\text{Leu}}\) cleavage (i.e., with C-C-A). Hatched bars: Rate of tRNA\(^{\text{Gln}}\) cleavage (i.e., without C-C-A).
Studies of the biosynthetic pathway and enzymatic specificity of tRNA processing in phage T4-infected cells (2-4) have pointed out an important determinant of E. coli RNase P activity: the enzyme has specificity for tRNA precursors containing the C-C-A sequence characteristic of the 3' end of mature tRNAs. Synthesis of this sequence is rate-limiting for the in vivo biosynthesis of T4 of tRNA_{Ser} and the corresponding precursor RNA is not processed by RNase P until this sequence is added enzymatically (2,3). This absolute in vivo requirement for the C-C-A sequence is not necessarily true for monomeric tRNA precursors, as the 5' ends of tRNA_{Pro} and tRNA_{Gln} can be synthesized in vivo in the absence of 3' terminal C-C-A synthesis; however, these 5' maturation may not be carried out only by RNase P (3,4).

In vitro, purified RNase P holoenzyme reflects partially this in vivo specificity, exhibiting a threefold preference in rate \( \frac{V_{\text{max}}}{K_m} \) for cleavage of the precursor RNAs containing the 3' C-C-A sequence over those containing other sequences (4). Subsequent to the discovery that the M1 RNA subunit of RNase P is catalytically active in vitro (5), two groups examined the dependence of processing by the isolated RNA on the presence of the C-C-A sequence in the substrate (6,7). The substrate specificity of the isolated RNA catalyst was observed to be greater than that exhibited by the holoenzyme: processing by M1 RNA of tRNA precursors lacking the 3' C-C-A sequence occurred only to a very slight extent under conditions where these precursors were extensively cleaved by the holoenzyme. Thus, the addition of the protein component of RNase P holoenzyme to M1 RNA seemed to result in a lowered specificity of the enzyme, enabling it to carry out a nonphysiological reaction in vitro (6). This situation was somewhat paradoxical, as the protein component of RNase P is absolutely required for tRNA processing in vivo (2,8), a situation where processing by the holoenzyme was presumably more specific than it is in vitro (4). In the present work we have characterized the activity of purified and synthetic M1 RNA on a dimeric precursor RNA and shown that enzymatic specificity for a tRNA precursor sequence containing C-C-A is strongly Mg\(^{2+}\)-dependent. At low [Mg\(^{2+}\)], M1 RNA catalysis strongly preferred the cleavage site at the 5' end of tRNA_{Leu}, the tRNA containing an intact C-C-A sequence, although the 5' end of tRNA_{Gln} was also cleaved very slowly (Fig. 2). At higher [Mg\(^{2+}\)], cleavage specificity was less strongly dependent on the C-C-A sequence.

In the present experiments, as in others (5-7), M1 RNA usually was present in large molar excess over substrate. This condition was used here for
two reasons. Besides the obvious one of increasing the rate of reaction, the condition of excess catalyst reduces the Michaelis-Menten equation to a pseudo-first order reaction whose rate is dependent on substrate concentration alone. The validity of this assumption was verified in control experiments (not shown) in which tenfold increases or decreases in the concentration of Ml RNA did not affect either the overall rate of precursor cleavage or the relative distribution of products. The results in Fig. 6, therefore, can be regarded as reflecting the relative rate of RNA catalysis absent complications of competitive inhibition by the mature tRNA Leu sequence present in the reaction mixture. We conclude from all the data obtained that the two cleavage sites in T4-encoded tRNA Gln - tRNA Leu precursor RNA are differentially RNA affected by changes in [Mg^{2+}]. The tRNA Leu cleavage rate was maximal at 100 mM [Mg^{2+}], while the rate of tRNA Gln cleavage increased substantially as [Mg^{2+}] was raised from 100 to 500 mM (Fig. 7).

The kinetic mechanism by which Mg^{2+} activates cleavage of the tRNA Gln sequence could not be determined with the in vivo-labeled substrate; however, it seems likely that the effect of [Mg^{2+}] is exerted on the interaction of substrate and catalytic RNAs. First, previous investigations indicated that bulk tRNA lacking the C-C-A terminus had a higher K^I for inhibiting the Ml RNA reaction than did intact tRNA (6). This implies a difference in enzyme-inhibitor association. Secondly, in parallel experiments, Green and Vold (19) have observed that the preference of B. subtilis P-RNA for a pre-tRNA His species containing C-C-A over one lacking C-C-A can be ascribed to a difference in K_m. The relative K_m values vary with [Mg^{2+}] in a manner consistent with that reported here for the overall rate of Ml cleavage. However, processing of precursor tRNAs with different sequences may be affected differently by changes in [Mg^{2+}].

All characterized RNA catalytic events require Mg^{2+} (20). Two observations about the self-splicing of group I introns may be relevant to understanding the role of Mg^{2+} in RNase P RNA-substrate RNA interactions. First, Inoue et al. (21) observed that the site of cyclization of the Tetrahymena rRNA intron depended on [Mg^{2+}] so that different sites were selected under high (25 mM) or low (5 mM) [Mg^{2+}] conditions. Secondly, Been and Cech (22) found that a genetically engineered mismatch in the internal guide sequence of the Tetrahymena rRNA intron could be corrected by raising [Mg^{2+}]. They concluded that higher [Mg^{2+}] promoted base pairing between the mismatched sequences. We hypothesize that similar effects operate in the present case, and high [Mg^{2+}] promotes interaction (not necessarily Watson-Crick type) be-
tween substrate and catalytic RNAs. Interaction in the presence of high Mg$^{2+}$ occurs despite the lack of optimal sequence information in the substrate.

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*To whom correspondence should be addressed at: Department of Biochemistry, M121 Medical Sciences Building, University of Missouri–Columbia, Columbia, MO 65212, USA

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