Ribonuclease P of Tetrahymena thermophila*

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Ribonuclease P (RNase P) is responsible for the generation of mature 5′ termini of tRNA. The RNA component of this complex encodes the enzymatic activity in bacteria and is itself catalytically active under appropriate conditions in vitro. The role of the subunits in eucaryotes has not yet been established. We have partially purified RNase P activity from the ciliate protozoan Tetrahymena thermophila to learn more about the biochemical characteristics of RNase P from a lower eucaryote. The Tetrahymena RNase P displays a pH optimum and temperature optimum characteristic of RNase P enzymes isolated from other organisms. The \( K_m \) of the T. thermophila enzyme for pre-tRNA\(^{\text{dim}} \) is \( 1.6 \times 10^{-7} \) M, which is comparable to the values reported for other examples of RNase P. The Tetrahymena RNase P is a ribonucleoprotein complex, as supported by its sensitivity to micrococcal nuclease and proteinase K. The buoyant density of the enzyme in Cs\(_2\)SO\(_4\) is 1.42 g/ml, which suggests that the RNA component of the Tetrahymena enzyme comprises a significantly greater percentage of the holoenzyme than that determined for RNase P of other Eucarya or Archaea. The holoenzyme has a requirement for divalent cations displaying characteristics that are unique for RNase P but closely resemble preferences reported for the Tetrahymena group I intron RNA. Puromycin inhibits pre-tRNA processing by the Tetrahymena complex, and implications of the similarities between recognition of tRNA by ribosomal components and RNase P are discussed.

Transfer RNA undergoes extensive processing during the production of the mature molecule. The endoribonuclease ribonuclease P (RNase P)\(^1\) cleaves the nascent transcript (pre-tRNA) to generate the mature 5′ termini of tRNA in both procaryotes and eucaryotes (1, 2). RNase P is an unusual enzyme in that the holoenzyme is a ribonucleoprotein. The RNA and protein subunits of the bacterial RNase P are required for enzymatic functions in vivo (3-5) and under physiologic conditions in vitro (6). In buffers containing high concentrations of monovalent and divalent salts, the RNA subunit (P RNA) displays the catalytic activity of the bacterial enzyme (6). The protein subunit binds to the P RNA in the bacterial holoenzyme and serves to promote product release during the catalytic cycle (7). The RNase P holoenzyme of eucaryotic organisms also exists as a ribonucleoprotein; however, the functional roles of RNA and protein subunits of these enzymes are not well defined. The holoenzyme activities have been characterized in many eucaryotes including mammalian systems (8-10), yeast (11-13), Xenopus (14), spinach (15), potato (16), and Dictyostelium (17). Many studies have shown that RNA and protein molecules are essential for RNase P function in vivo (18, 19) and in vitro (8, 9, 17), but biochemical and genetic analyses of holoenzyme composition have failed to provide clues as to their functional assignments in RNase P.

Our interest in RNase P of eucaryotes is to develop a greater understanding about the catalytic mechanism for the holoenzyme and to learn the manner in which the RNA and protein subunits interact in the holoenzyme to achieve catalytic activity. Phylogenetic analyses of the components of the bacterial RNase P holoenzyme suggest that both the RNA and protein subunit genes change more rapidly than the corresponding ribosomal RNA gene sequences (1, 20). In this regard, the molecular clocks of genes that encode the subunits of RNase P resemble those of genes that encode typical polypeptide enzymes. We have chosen to focus our studies on the RNase P holoenzymes from ciliate protozoa because these organisms comprise an extremely phylogenetically diverse group of organisms (21). Through the examination of the biochemical and molecular properties of homologous enzymes from phylogenetically diverse origins, considerable insight can be obtained relevant to the catalytic mechanism of RNase P and the role of the molecular components in holoenzyme function. We describe in this report our initial purification and characterization of the RNase P from Tetrahymena thermophila.

**EXPERIMENTAL PROCEDURES**

Cloning of T. thermophila Pre-tRNA\(^{\text{Gln}}\) Template—T. thermophila nuclei were isolated as described previously (22). Genomic DNA was extracted from sonicated nuclei according to standard procedures (23) to serve as a template for the polymerase chain reaction amplification (24) of the T. thermophila tRNA\(^{\text{Gln}}\) and 5′-flanking DNA (25). Amplification of tRNA\(^{\text{Gln}}\) was accomplished using Vent DNA polymerase (New England Biolabs Inc.) according to manufacturer’s instructions and the following synthetic DNA primers (Operon Technologies, Inc.): TGLN3: 5′-CGGAATTCGGTCTCGTGGAGGTCCCACTGGGATTCG-3′ and TGLN5: 5′-CCGGATCTCATTACGACTATAGTACGTTGTTGCG-3′. The resulting DNA amplification product was digested with EcoRI and BamHI restriction endonucleases (New England Biolabs) and cloned into pSP65 (Promega) using T4 DNA ligase (New England Biolabs) to produce the plasmid pTGLN1. The DNA sequence of the cloned insert was verified by double-stranded cycle sequencing procedures (26) using Vent(exo-) DNA polymerase (New England Biolabs).

Preparation of Precursor tRNA Substrate—The plasmid pTGLN1 was linearized with Bsal (New England Biolabs) and transcribed in vitro using purified T7 RNA polymerase (27) to generate a pre-tRNA for use in 5′-end-processing studies. The \(^{32}P\) internally labeled pre-tRNA substrate was generated in a 20-μl transcription reaction that contained 1-2 μg of linearized template DNA, 0.5 mM GTP, 0.5 mM CTP, 0.5 mM UTP, 1.25 mM ATP, 50 mM Tris-HCl (pH 8.0), 10 mM MgCl\(_2\), 10 mM dithiothreitol, 50 μCi of \(^{32}P\)ATP, and T7 RNA polymerase and proceeded for 2 h at 37 °C. The reaction was terminated with the *This work was supported by National Institutes of Health Grant RO1 GM47854. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: RNase P, ribonuclease P; P RNA, RNA subunit of RNase P; pre-tRNA, precursor tRNA; SLB, sample loading buffer; MN, micrococcal nuclease; M1 RNA, catalytic RNA subunit of the E. coli RNase P; MOPS, 3-(N-morpholino)propanesulfonic acid.
addition of an equal volume of sample loading buffer (SLB: 40 mM EDTA, 8 mM urea, 0.2% xylene cyanol, and 0.2% bromphenol blue). The product was resolved on a denaturing 15% polyacrylamide gel, and the full length pre-tRNA was detected by autoradiography. The precursor tRNA was eluted from the gel slice in elution buffer (0.25 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA) overnight at 4°C. The transcribed pre-tRNA was ethanol-precipitated from the eluate and resuspended in 75 μl of H2O. Unlabeled pre-tRNA was generated following the procedure of Latham et al. (28).

Specific Activity Measurements—The RNase P activity was monitored throughout the purification and characterization by assaying the ability of fractions to cleave 32P internally labeled T. thermophila pre-tRNA. The reaction mixture (20 μl) at a flow rate of 0.2 ml/min with a 480-ml linear gradient from 25 mM KCl to 500 mM KCl. RNase P activity was eluted at approximately 250 mM KCl. Fractions containing RNase P activity were pooled and concentrated in an Amicon stirred cell concentrator fitted with an Amicon YM-10 membrane. This activity (11.2 ml) was dialyzed against HCB7 buffer (1.5 liters) for 3 h, and an aliquot of the dialysate (2.25 ml) was applied to a 10-ml spermagine-agarose (Sigma) column (1.5 cm diameter). The RNase P activity eluted sequentially with 3 volumes of HCB7 buffer and 140 ml of HCB7 buffer containing 250 mM KCl. RNase P was eluted with a 240-mM linear gradient of KCl from 250 mM to 1 mM in HCB7 buffer. The activity began eluting at approximately 300 mM KCl, and the active fractions were pooled (24 ml) and concentrated (5.5 ml). The spermagine-agarose-eluted RNase P was dialyzed against HCB7 buffer (1.6 liters) for 3 h.

A tRNA affinity column was constructed by coupling 40 μg of biotinylated tRNA32P-16S pre-tRNA (Promega) to 8 mg of streptavidin Dynabeads M-280 (Dynal, Inc.) in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.4 mM KH2PO4, pH 7.3) with shaking at room temperature for 1 h. After binding, the beads were washed twice with HCB7 buffer containing 1 mM NaCl and washed twice with HCB7 buffer and 140 ml of HCB7 buffer. HCB7 buffer containing 250 mM KCl had been recovered from the spermagine-agarose fractionation was diluted 2-fold in HCB7 buffer and bound to the resin for 1 h at 4°C with shaking. The bound RNase P activity was removed after harvesting the tRNA-containing streptavidin beads with a Dynal magnetic particle concentrator. The beads were subsequently washed five times with 1 ml of HCB7 buffer. The RNase P activity was eluted with 200 μl of HCB7 buffer containing 1 mM KCl.

Buoyant Density Experiments—Cesium sulfate step density gradients were generated using 1.6-ml layers of 25, 31, and 37% Cs2SO4 (w/v) in buffer G (50 mM Tris-HCl (pH 8.0), 100 mM KCl, 10 mM MgSO4, 10 mM glycerol) as described previously (31). A 500-μl sample of concentrated, pool ed RNase P activity was applied to the top of the gradient and was resolved on a denaturing 15% polyacrylamide gel, and the active fractions were collected by centrifugation at 35,000 rpm for 23 h at 18°C in a Beckman SW 50.1 rotor. Fractions of 200 μl were drawn from the top of the gradient. A portion of each sample was dialyzed against HCB7 buffer for 2 h at 4°C and an RNase P activity assay was completed immediately thereafter. The refractive index of each fraction was measured and the density was calculated as described previously (32). The protein and RNA standards were determined in separate experiments. The buoyant density for the Escherichia coli RNase P standard could not be accurately determined with the step density gradients described above. For the E. coli RNase P, step density gradients were generated using 1.6-ml layers of 31, 37, and 45% Cs2SO4 (w/v) in buffer G (50 mM Tris-HCl (pH 7.5), 300 mM KCl) with 40% glycerol (25 μl of each layer was removed and terminated with the addition of SLB, followed by immediate cooling to 0°C) and the initial velocity of the cleavage reaction over a range of temperature and pH values. The enzyme activity and the buffering components were preincubated for 5 min at the temperature of assay before the pre-tRNA substrate was added to the mixture to initiate the assay. Aliquots of the reaction were removed and terminated with the addition of SLB, followed by immediate cooling to 0°C. The optimal temperature was
determined in MBB buffer. The optimal pH was determined at 37 °C in a buffer containing 9 mM MgCl₂ and 50 mM MOPS for the pH range of 6.3–7.8. The effects of monovalent salts (NaCl, CsCl, KCl, and NH₄Cl) and divalent salts (CaCl₂, MgCl₂, MnCl₂) on RNase P function were as- 
sessed using activity diluted in HB buffer (15 mM K-HEPES (pH 7.0), 10% glycerol, 0.1% Tween 20) such that upon dilution, the maximum 
concentrations of MgCl₂ and KCl were 0.015 and 0.125 mM, respec-
tively. The salt(s) and the appropriate amount of water were pre-warmed prior to the addition of enzyme. The enzyme mix was then preincubated at 37 °C for 5 min prior to the addition of pre-tRNA Gln 
substrate in MBB buffer, which was also pre-warmed to 37 °C. The initial velocity of the 
processing reaction was determined as described above.

The apparent Kₘ was determined by measuring the velocity of the reaction as a function of pre-tRNA Gln concentration using RNase P 
activity enriched through spermine-agarose chromatography. The ass-
says were performed at 37 °C in MBB buffer with pre-tRNA Gln used as the 
substrate.

Inhibition of RNase P by Puromycin—

Puromycin (Sigma) was prepared for use as described previously (10). The concen-
tration of puromycin diluted in 0.1 M HCl was determined spectrophotome-
trically (ε₂₅₀ = 1.95 × 10⁴ M⁻¹ cm⁻¹) (34). RNase P activity was diluted in HB buffer and preincubated at 37 °C for 5 min with puromycin in MBB buffer that contained a final concentration of 20 mM NaCl. The activity assay was initiated with the addition of pre-tRNA Gln 
substrate, and the reaction was incubated at 37 °C for a further 30 min. The assays were analyzed as described above.

RESULTS
RNase P from T. thermophila was purified nearly 700-fold by 
the chromatographic steps summarized in Table I. The enzyme activity cleaved the 98-nucleotide pre-tRNA Gln substrate into a 75-nucleotide mature tRNA and a 23-nucleotide 5'-leader se-
quence (Fig. 1A). The E. coli RNA subunit (M1 RNA) and 
holoenzyme also cleaved this substrate RNA to generate the same 
fragments observed with the T. thermophila enzyme (data not shown). The enzyme activity was stable through 
DEAE-Sepharose chromatography in this purification scheme 
when stored in HCB7 buffer at 4°C or at 20°C. T. thermophila are rich in protease and nuclease activities (35, 36); 
therefore, co-purifying substances that inactivate RNase P may 
account for the loss of activity observed in the purification 
procedures. A contaminating nuclease activity co-purified with RNase P activity through the DEAE-Sepharose step and was detected by the appearance of an anomalous cleavage 
product of pre-tRNA Gln in activity assays (data not shown). 
RNase P was resolved from this contaminating nuclease by 
chromatography on spermine-agarose. RNase P was effectively 
recovered in three fractions following spermine-agarose chro-
matography (Fig. 1B) but was unstable if not concentrated immediately.² In a concentrated form (0.48 mg/ml total protein), the RNase P enriched through spermine-agarose fractionation was stable in HCB7 buffer at 4 °C or at -20 °C for months. Specific activity measurements for this enzyme puri-
fication fluctuated during steps such as Affi-Gel heparin and spermine-agarose, which apparently concentrate inhibitors of 
the activity. The inclusion of these fractionations was necessary because they enabled RNase P to be further enriched by affinity chromatography using an immobilized tRNA resin. RNase P enriched through tRNA-agarose chromatography was 
analyzed by SDS-polyacrylamide gel electrophoresis and 
Coomassie blue staining to assess the degree of purity. One 
polyepitope of an estimated molecular mass of 36 kDa was 
visible by this procedure (data not shown). Several RNA species 
copurify with RNase P activity through spermine-agarose, as 
determined by the appearance of an anomalous cleavage 
product of pre-tRNA Gln in activity assays (data not shown). The biochemical properties of the T. thermophila RNase P 
were determined in order to compare this enzyme's characteristics to RNase P from other organisms. The optimal tempera-
ture for the Tetrahymena RNase P was 40 °C under standard

² H. L. True and D. W. Celander, unpublished observations.
The pH dependence of the reaction varied with the buffer used in the assay. The optimal pH for the activity in MOPS buffer is about 6.8 (Fig. 4B). The enzyme had a higher pH optimum (pH 7.5) in reaction buffers that contain 50 mM Tris-HCl rather than 50 mM MOPS.

To determine the behavior of the enzyme in different ionic conditions, the pre-tRNA processing activity was assessed in assay conditions (Fig. 4A). The pH dependence of the reaction varied with the buffer used in the assay. The optimal pH for the activity in MOPS buffer is about 6.8 (Fig. 4B). The enzyme had a higher pH optimum (pH 7.5) in reaction buffers that contain 50 mM Tris-HCl rather than 50 mM MOPS.

*4 H. L. True and D. W. Celander, unpublished observations.*
the presence of various monovalent and divalent salts. The T. thermophila enzyme was inhibited significantly, even at low concentrations, by all monovalent salts tested (Fig. 4C). Pre-tRNA\textsuperscript{Gln} processing was not detected for T. thermophila RNase P preparations equilibrated in HCB\textsuperscript{7} buffer lacking divalent cations (data not shown). Fig. 4D illustrates that the pre-tRNA processing activity displayed by RNase P preparations equilibrated in buffer containing an optimal concentration of MgCl\textsubscript{2} (5 mM) was approximately 30% greater than that observed by RNase P preparations equilibrated in HCB\textsuperscript{7} buffer containing an optimal concentration of MnCl\textsubscript{2} (2 mM). When the divalent salt concentration is increased 5-fold relative to the optimal concentration of each divalent cation, RNase P activity is reduced by approximately 30–50% (Fig. 4D). Pre-tRNA\textsuperscript{Gln} processing activity was not detected for RNase P preparations equilibrated in buffer containing CaCl\textsubscript{2} as the sole divalent salt at any concentration tested (Fig. 4D).

The inability of Ca\textsuperscript{II} to support RNase P function prompted us to investigate whether processing buffers containing Ca\textsuperscript{II} in the presence of Mg\textsuperscript{II} or Mn\textsuperscript{II} could support holoenzyme activity. The addition of Ca\textsuperscript{II} to processing buffer containing suboptimal concentrations of Mn\textsuperscript{II} stimulated holoenzyme pre-tRNA\textsuperscript{Gln} processing activity to levels greater than that observed for RNase P equilibrated in buffers containing the optimal concentration of Mn\textsuperscript{II} only (Fig. 5). This stimulatory effect was not observed for RNase P equilibrated in buffer containing Ca\textsuperscript{II} and Mg\textsuperscript{II}. Equimolar amounts of Ca\textsuperscript{II} neither stimulated nor inhibited holoenzyme activity in buffer mixtures that contain suboptimal concentrations of Mn\textsuperscript{II} (data not shown). Calcium\textsuperscript{II} inhibited RNase P function when present at a molar excess greater than 4-fold relative to Mn\textsuperscript{II} (Fig. 5; data not shown).

Polyamine supplementation has been demonstrated to enhance the ability of RNase P to generate mature tRNA in some systems in vitro (37, 38). The inclusion of spermidine in processing assay buffers did not support pre-tRNA cleavage by the T. thermophila RNase P in the absence of divalent ions. Furthermore, spermidine did not enhance activity in processing assays when buffers contained divalent ions at concentrations insufficient for optimal holoenzyme activity (data not shown).

The apparent \( K_m \) for the processing of pre-tRNA\textsuperscript{Gln} by partially purified RNase P was determined by an Eadie-Hofstee plot to be 160 nM (Fig. 6).

Puromycin is a potent inhibitor of ribosome function by virtue of its ability to interfere with tRNA binding at the A site (39). To assess whether puromycin inhibits the T. thermophila RNase P, the pre-tRNA\textsuperscript{Gln} processing assay was done in the presence of 1–8 mM puromycin. A concentration of 2 mM puromycin...
mycin in the activity assay resulted in approximately 50% inhibition of cleavage of pre-tRNA substrate (Fig. 7).

**DISCUSSION**

This study describes the isolation and characterization of RNase P from a ciliate protozoan. *T. thermophila* was chosen as a candidate organism for these studies because the organism represents one of the few ciliate protozoa that can be grown under axenic conditions. Many of the unique properties of the RNase P from *T. thermophila* were observed during our attempts to purify the activity from crude whole organism lysates. RNase P from other eucaryotic organisms has been purified by chromatography on successive anion and cation exchange resins (11, 13, 14, 16, 17). We were unable to adsorb the RNase P from *T. thermophila* to either phosphocellulose or SP-Sepharose cation exchange resins under any condition tested, which suggests that the enzyme has very strong anionic character. The application of hydrophobic resins, glycerol gradient sedimentation, and Cs₂SO₄ density gradient centrifugation, each of which had been successfully used during the purification of other eucaryotic RNase P enzymes (9, 31), failed to afford us either substantial or stable enrichment of the *T. thermophila* enzyme. Other techniques that also proved unsuccessful in the purification of the RNase P from *T. thermophila* include the use of dye-coupled resins and tRNA-agarose resin (14). The enzyme activity could be adsorbed to the dye-coupled resin Cibacron blue under mildly acidic conditions (pH 6.0); however, the enzymatic activity was unstable if the activity was maintained under these conditions for prolonged periods of time (72 h). The chromatographic behavior of the *T. thermophila* enzyme during purification suggests that the RNase P enzyme from ciliate protozoa may differ substantially from RNase P enzymes from other eucaryotic organisms.

Despite its unique properties that were revealed during purification, the RNase P of *T. thermophila* possesses many enzymatic properties that are shared with RNase P from other organisms. The *Tetrahymena* RNase P displays a pH optimum and temperature optimum characteristic of RNase P enzymes isolated from other organisms. The *Kₘ* of the *T. thermophila* enzyme for pre-tRNA³⁰⁰ is 1.6 × 10⁻⁷ M, which is comparable to the values reported for other examples of RNase P, 2.5 × 10⁻⁷ M for Sulfolobus acidocaldarius RNase P (40), 2.3 × 10⁻⁷ M for Saccharomyces cerevisiae mitochondrial RNase P (13), 4.2 × 10⁻⁸ M for E. coli RNase P (41, 42), 2.0 × 10⁻⁷ M for Bacillus subtilis RNase P (7), and 2.4 × 10⁻⁷ M for Dictyostelium discoideum RNase P (17).

RNase P enzymatic activities from other sources are mildly stimulated by low concentrations (< 100 mM) of monovalent salts; however, RNase P activity from these organisms is inhibited when the enzyme activity assays are performed in the presence of elevated concentrations (> 100 mM) of monovalent cations.
The Tetrahymena enzyme activity is reduced by the presence of monovalent salts in the reaction mixture. The molecular significance of this remarkable ionic strength effect on the Tetrahymena RNase P activity remains unclear. It is possible that monovalent salts alter the holoenzyme conformation, displace a required divalent cation, or alter the manner in which the enzyme interacts with substrates and products during the catalytic cycle.

Almost all RNase P enzymes require divalent cations for their activity (1, 2, 13, 37, 38). One reason for this metal ion requirement stems from the hydrolytic mechanism of the pre-tRNA processing reaction. Divalent metal ions are presumed to have two roles during the hydrolytic cleavage of the scissile bond in a pre-tRNA substrate: one metal ion acts as a Lewis acid to generate a suitable nucleophile from water, and the other metal ion stabilizes the negative charge that develops at the phosphodiester bond during nucleophilic attack (43, 44). Divalent cations also can serve structural roles in catalytic function, possibly participating in folding enzyme components into a conformation required for activity (45) or allowing for the substrate to fit into the active site (46). The Tetrahymena RNase P, like RNase P from other organisms, possesses an absolute divalent metal ion requirement for activity. The divalent cations Mg(II), Mn(II), and Ca(II) were found to stimulate other RNase P activities to varying degrees (13, 37, 38). The RNase P activity from T. thermophila was supported by either Mg(II) and Mn(II) but not by Ca(II). This remarkable divalent ion preference displayed by the Tetrahymena RNase P is more closely related to the corresponding divalent ion preferences observed with the large ribosomal RNA self-splicing group I intron RNA from T. thermophila (47) than to the corresponding divalent ion preferences observed with RNase P from other organisms.

The behavior of the Tetrahymena RNase P in the presence of different divalent cations suggests that two types of divalent cation binding sites exist in the holoenzyme, sites whose occupancy is necessary for catalysis (catalytic sites) and sites whose occupancy is required for an auxiliary function (structural sites). When RNase P equilibrated in buffers containing only one type of divalent ion, Mg(II) and to a lesser extent Mn(II) can fulfill the roles of both catalytic and structural sites. Calcium(II) is deficient in one of these roles, since holoenzyme activity was not evident in buffers containing this metal ion only. The results from the enzymatic activity assays for the buffers that contain mixtures of Ca(II) with either Mg(II) or Mn(II) suggest that Ca(II) can compete for, and in some cases function in, a site that is normally occupied by Mg(II) or Mn(II) in an active holoenzyme. Divalent cations have been rigorously shown to possess similar roles in catalysis by a shortened form of the Tetrahymena group I intron RNA (47). Unlike the metal ion studies completed with the group I intron RNA, however, we know little about the exact role of these metal ions in relation to Tetrahymena RNase P function. Metal ions may participate directly in catalysis in the Tetrahymena enzyme or may promote binding of the pre-tRNA substrate to the enzyme, as observed for the bacterial enzyme (37, 38, 43). The purified components of the holoenzyme must be obtained to rigorously test these roles and other functions of divalent metal ions for the Tetrahymena RNase P.

Structural aspects of transfer RNA are recognized by at least three types of macromolecules in the cell: RNase P, aminoacyl tRNA synthetase, and the ribosome. The sensitivity that both ribosomes and the Tetrahymena RNase P display toward puromycin suggests that the antibiotic may interfere with tRNA binding events in both macromolecules (39). Puromycin also has been shown to inhibit RNase P holoenzyme activity from mouse and bacteria (10, 48) and the catalytic activity of the E. coli M1 RNA (48). Since an RNA-catalyzed reaction can be inhibited by puromycin, Vioque suggested that similar substrate recognition mechanisms exist for ribosomal RNA and for the E. coli M1 RNA (48). The inhibitory effects of puromycin on Tetrahymena RNase P function could not be alleviated by arginine, an amino acid which can bind to RNA (49) and inhibit puromycin binding to ribosomes (50). This suggests that while there may be similarities in the recognition of tRNA and puromycin by ribosomes and RNase P, there are still fundamental differences in their recognition or affinity.

The Tetrahymena RNase P is composed of both RNA and protein. The buoyant density of the Tetrahymena RNase P is greater than that reported for HeLa (human) nuclear RNase P (ρ = 1.28 g/ml (31)), the Xenopus laevis RNase P (ρ = 1.34 g/ml (14)), the S. cerevisiae mitochondrial RNase P (ρ = 1.28 g/ml (13)), the spinach chloroplast RNase P (ρ = 1.28 g/ml in CsCl (15)), and the D. discoideum RNase P (ρ = 1.23 g/ml (17)) as eucaryotic representatives and is greater than that reported for the S. acidocaldarius RNase P (ρ = 1.27 g/ml (40)) as an Archaea representative. All of these enzymes, including the Tetrahymena RNase P, display buoyant densities on Cs$_2$SO$_4$ gradients that are considerably less than that observed with the RNase P from E. coli (ρ = 1.55 g/ml (8, 51)). The buoyant density of the E. coli enzyme corresponds to an enzyme composed of 10% protein and 90% RNA, in accordance with its known 119-amino-acid protein (52) and a 377-nucleotide RNA (53, 54). The buoyant density of the Tetrahymena RNase P presumably corresponds to a holoenzyme composed of about 50% protein and 50% RNA (55).

The RNase P holoenzyme from T. thermophila possesses RNA and protein components that are essential for catalytic activity in vitro. RNA and protein of RNase P from other organisms have been shown to play essential, although not always well understood, roles in holoenzyme function. In the bacterial holoenzymes, the RNA subunit harbors the catalytic activity (6), while the protein subunit serves to promote product release during the catalytic cycle (7). The division of labor is not so clearly established for examples of RNase P from organisms of either Eucarya or Archaea. The genetic isolation of mutants in RNase P function has provided important clues that point to the in vivo relevance of both protein and RNA subunits in pre-tRNA processing in S. cerevisiae (18, 19). Eucaryotic P RNA has not been demonstrated to maintain the ability to cleave substrate pre-tRNA in the absence of its protein counterpart(s). Whether similar observations will hold true for the P RNA subunit of Tetrahymena may be addressed once the candidate RNA subunit(s) for the RNase P holoenzyme of this organism is isolated.

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