

RNase P RNAs from some Archaea are catalytically active

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ABSTRACT The RNA subunits of RNase Ps of Archaea and eukaryotes have been thought to depend fundamentally on protein for activity, unlike those of Bacteria that are capable of efficient catalysis in the absence of protein. Although the eukaryotic RNase P RNAs are quite different than those of Bacteria in both sequence and structure, the archaeal RNAs generally contain the sequences and structures of the bacterial, phylogenetically conserved catalytic core. A spectrum of archaeal RNase P RNAs were therefore tested for activity in a wide range of conditions. Many remain inactive in ionically extreme conditions, but catalytic activity could be detected from those of the methanobacteria, thermococci, and halobacteria. Chimeric holoenzymes, reconstituted from the *Methanobacterium* RNase P RNA and the *Bacillus subtilis* RNase P protein subunits, were functional at low ionic strength. The properties of the archaeal RNase P RNAs (high ionic-strength requirement, low affinity for substrate, and catalytic reconstitution by bacterial RNase P protein) are similar to synthetic RNase P RNAs that contain all of the catalytic core of the bacterial RNA but lack phylogenetically variable, stabilizing elements.

RNase P is an endoribonuclease best known for its role in tRNA biosynthesis, in which it is the enzyme responsible for the removal of 5' leader sequences from transfer RNA precursors (for reviews see refs. 1 and 2). In Bacteria, RNase P consists of two subunits: a large (≈ 140 -kDa, 400-nt) RNA and a small (≈ 14 -kDa, 120-amino acid) protein. Both RNA and protein are required *in vivo* and for optimal activity *in vitro* in reactions at low ionic strength (3, 4). The RNA is the catalytic subunit of the bacterial enzyme; at elevated ionic strength *in vitro*, it is by itself capable of processing pre-tRNAs catalytically; i.e., it is a ribozyme (5). The protein component of the bacterial enzyme alters substrate recognition by directly contacting the leader region of the pre-tRNA (6). The RNase P enzymes of Archaea (formerly archaeobacteria) and Eukarya (the nuclear/cytoplasmic portion of the eukaryotic cell) also contain RNA subunits, but these RNAs have not been shown to be catalytically active. Although it seems likely that the catalytic function of the archaeal and eukaryal enzymes resides in the RNA, the expression of this activity apparently requires the presence of the protein subunits.

RNase P enzymes have been characterized from only two archaeal species: the thermoacidophilic crenarchaeote *Sulfolobus acidocaldarius* (7) and the extremely halophilic euryarchaeote *Haloferax volcanii* (8). The *S. acidocaldarius* RNase P is resistant to micrococcal nuclease treatment and contains a 315-nt RNA that persists after nuclease treatment (9). The enzyme is large (≈ 400 kDa apparent molecular mass) and has a low density in Cs_2SO_4 (1.27 g/cm^3), similar to the densities of the eukaryotic nuclear enzyme and implying a high protein:RNA content. The *H. volcanii* RNase P, on the other hand, resembles the bacterial enzyme in density in Cs_2SO_4

(1.61 g/cm^3 ; ref. 10) and nuclease sensitivity. The *H. volcanii* enzyme contains a 435-nt RNA (8). Neither proteins associated with the archaeal enzymes nor sequences encoding polypeptides with recognizable similarity to eukaryal, mitochondrial, or bacterial RNase P proteins have been identified in archaeal genomes. The RNase P RNAs from *S. acidocaldarius* and *H. volcanii* are similar in size and, to some extent, sequence to those of Bacteria, but these RNAs by themselves could not be shown to be catalytically active after deproteinization or when synthesized *in vitro* (8, 9). This finding and the observation in subsequent tests that the RNase P RNAs from *Methanosarcina barkeri* (11) and *Methanococcus jannaschii* (E.S.H., J.K.H., and J.W.B., unpublished results) also lack activity led to the conclusion that archaeal RNase P RNAs in general, like those of eukaryotes, are not by themselves catalytically active.

We recently developed a well defined secondary structure model of RNase P RNAs from Archaea by using comparative sequence analysis (Fig. 1; ref. 11 and J.K.H., E.S.H., and J.W.B., unpublished data). Nearly all of the conserved sequences and secondary structures in the catalytically active bacterial RNAs (12) are also present in the archaeal RNAs. The structural distinctions between the bacterial and archaeal RNAs have largely disappeared as our understanding of their structures has improved; thus, the dramatic difference in their ability to catalyze pre-tRNA 5' processing was puzzling. A phylogenetic spectrum of archaeal RNase P RNAs were therefore tested for pre-tRNA processing activity in a wide variety of conditions, including ionic conditions outside the range usually considered. We show here that RNase P RNAs from the methanobacteria, thermococci, and extreme halophiles are, like their bacterial homologs, able to process pre-tRNAs in the absence of protein at very high ionic strength. The biochemical properties of the archaeal RNAs are similar to those of synthetic minimal bacterial RNase P RNAs (RNAs that contain only the phylogenetically conserved "core" elements of the bacterial RNAs; refs. 13 and 14), suggesting that the archaeal RNAs contain all of the elements required for substrate recognition and catalysis but are structurally defective in the absence of protein.

MATERIALS AND METHODS

RNase P Activity Assays. RNase P RNA assays contained 1.5 nM ^{32}P -labeled *Bacillus subtilis* or *Methanobacterium thermoautotrophicum* pre-tRNA^{Asp}, 50 mM Tris (pH 8), 25 – 300 mM MgCl_2 , 0.1 – 5 M ammonium acetate, 0.1% SDS, and 0.05% Nonidet P-40. Incubations ranged from 2 to 16 h at 30 – 75°C (typically 45°C). Optimal pH was evaluated in activity assays that used Hepes in place of Tris. Reaction products were

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. U42985, AF121773, and AF121774).

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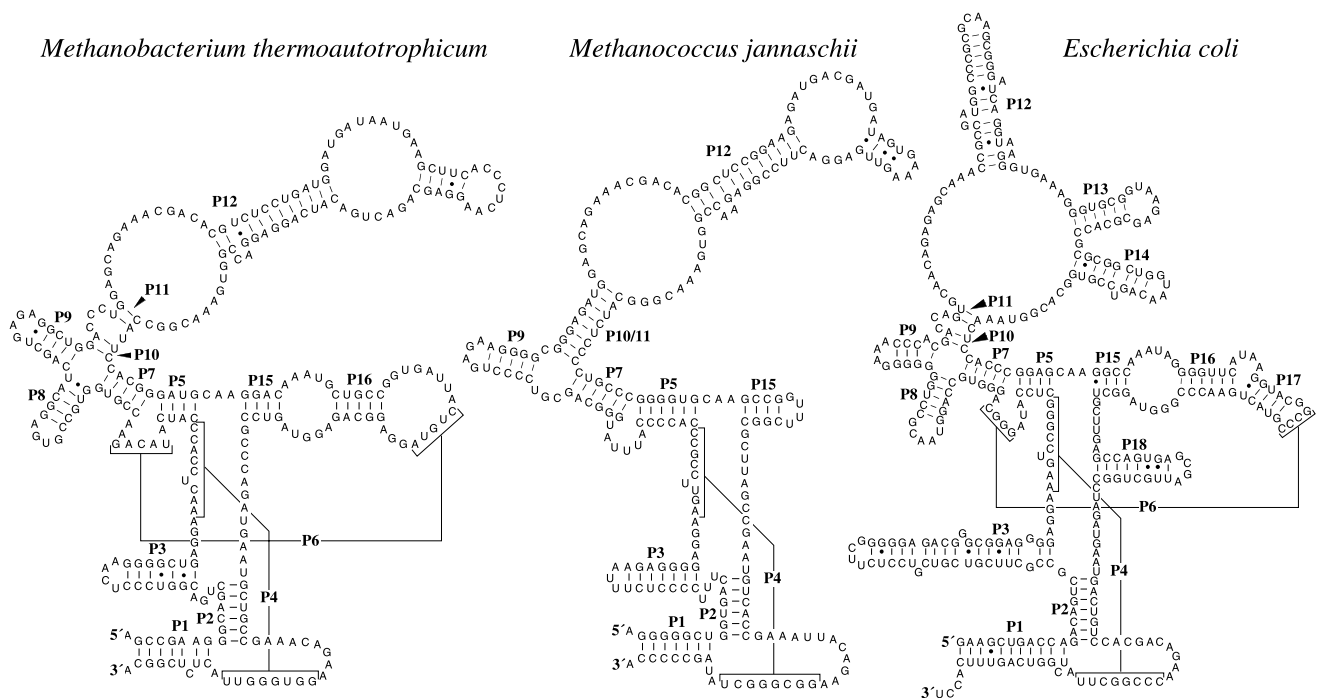


FIG. 1. Secondary structures of the *M. thermoautotrophicum* strain Δ H, *M. jannaschii*, and *Escherichia coli* RNase P RNAs (refs. 11 and 12, and J.K.H., E.S.H., and J.W.B., unpublished data). Helices are numbered P1–P18 as described (24). Helices P4 and P6 are shown with lines and brackets. The 5' and 3' ends of the native archaeal RNAs were determined by primer extension and nuclease S1 protection. Additional RNase P RNA sequences and structures are available on the Ribonuclease P database at www.mbio.ncsu.edu/RNaseP/home.html (25).

separated by electrophoresis on 8 or 12% polyacrylamide/7.5 M urea/TBE buffer (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) gels (SequaGel, National Diagnostics) and visualized by phosphorimager (15).

For assays of synthetic RNAs, RNase P RNAs were synthesized from cloned genes by using T7 or T3 RNA polymerase, as described by the manufacturer (Promega). For assays of activity in total cellular nucleic acid, proteins were removed

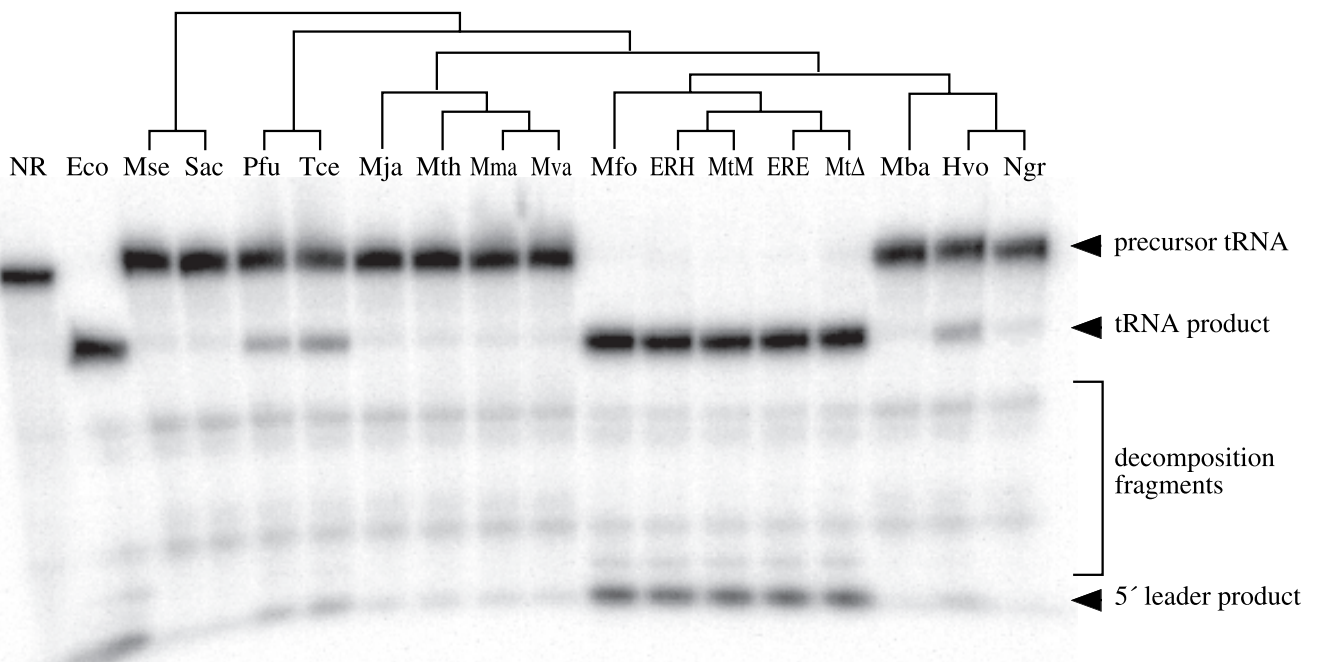


FIG. 2. Catalytic activity of synthetic archaeal RNase P RNAs. Assays contained 4 M ammonium acetate, 300 mM $MgCl_2$, 50 mM Tris-Cl (pH 8), 0.1% SDS, 0.05% Nonidet P-40, 1.5 nM uniformly labeled *B. subtilis* pre-tRNA^{Asp}, and \approx 300 nM RNase P RNA (synthesized from cloned genes by transcription *in vitro*) and were incubated for 3.5 h at 45°C. The location of substrate and product bands are indicated. RNase P RNAs tested were no RNA (NR), *E. coli* (Eco), *M. sedula* (Mse), *S. acidocaldarius* (Sac), *P. furiosus* (Pfu), *T. celer* (Tce), *M. jannaschii* (Mja), *M. thermolithotrophicus* (Mth), *Methanococcus marpaludis* (Mma), *Methanococcus vannielii* (Mva), *M. formicicum* (Mfo), *M. thermoautotrophicum* ER-H (ERH), *M. thermoautotrophicum* Marburg (MtM), *M. thermoautotrophicum* ER-E (ERE), *M. thermoautotrophicum* Δ H (Mt Δ), *M. barkeri* (Mba), *H. volcanii* (Hvo), and *Natronobacterium gregoryi* (Ngr). Activity by the *N. gregoryi* RNA is evident but weak. The phylogenetic relationships between these organisms based on small subunit ribosomal RNA sequences (26) are shown as a cladogram above the gel. Placement of the ER-E and ER-H RNAs, which were cloned from enrichment cultures inoculated with wastewater sludge, are based on similarity to the *M. thermoautotrophicum* strains Δ H and Marburg RNase P RNA sequences.

from archaeal cell lysates (prepared by using a French press and cells resuspended in 50 mM Tris·Cl, pH 8/25 mM MgCl₂/0.1 M ammonium acetate/0.05% Nonidet P-40 as described in ref. 15) by repeated phenol extraction, followed by a phenol:chloroform extraction. Nucleic acids were precipitated with ethanol and resuspended in 10 mM Tris, pH 8/1 mM EDTA/0.1% SDS. Assays contained 10–40 μg/ml cellular nucleic acid.

Determination of Native 5' and 3' Ends. Identification of the native 5' and 3' ends of the *M. thermoautotrophicum* ΔH RNase P RNA was performed as described (8). The location of the 5' end was determined by primer extension by using oligonucleotide Mfo145R (CGTGTCGTTTCTGCTCC). The location of the 3' end was determined by S1 nuclease mapping by using a probe DNA fragment that overlapped the 3' end of the RNase P RNA, produced by PCR amplification from genomic DNA by using oligonucleotide primers ΔH138F (GATAATGAAGCTTCACCCTCAAG) and ΔH908RXba (GCTCTAGAGCTCCATCGCGCCTGCG). The downstream priming-site sequence was taken from the complete genome sequence (16).

RNase P RNA-Encoding Genes. RNase P RNA-encoding genes were cloned after PCR amplification from genomic DNAs by using previously described methods (11). The oligonucleotide primers used were ΔH5'Bam (CGGGATCCACCGGGCAAGCCGAAGGGC) and ΔH3'Xba (GCTCTAGACCGGGCATGCCGAGAG) for obtaining genes from *Methanobacterium* spp., Pyro5'Xba (GCTCTAGATAGGCGAGGGGGCTGGGG) and Pyro3'Bam (CGGGATCTAGGCGAGGGGGCTATAG) for obtaining genes from *Pyrococcus* and *Thermococcus* spp., Mja5'Xba (GCTCTAGAGGTAAGGGGGCTGGTG) and Mja3'Bam (CGGGATCCGGTATGGGGGCTATAG) for obtaining genes from *Methanococcus* spp., and Sac5'Bam (CGGGATCTAGGGGAGCCTAACAGG) and Sac3'Xba (GCTCTAGAGGGGAGCCTAACATAACC) for obtaining the gene from *Metallospira sedula*. Primer sequences are based on data from the complete genome sequences of *M. thermoautotrophicum* ΔH (16), *Pyrococcus furiosus* (Utah Genome Center; www.genome.utah.edu), and *M. jannaschii* (17), respectively. The Sac primers were designed based on the published gene sequence of *S. acidocaldarius* RNase P RNA (9).

RNase H Depletions. Reactions (10 μl) containing 100 mM KCl, 10 mM MgCl₂, 20 mM Hepes·KOH (pH 7.4), 1 mM DTT, 0.2 μg of RNase P RNA, and 2 μg of oligonucleotide Mfo145R or Eco145R (GCGGTTTGGCTCTCTGTTGC) were heated to 80°C for 3 min and cooled to 30°C over 30 min; RNase H (2

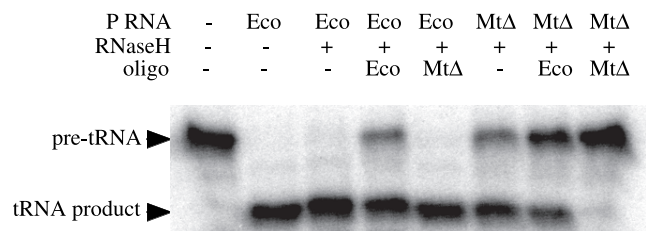


FIG. 3. Oligonucleotide-directed RNase H depletion of RNase P activity. RNase P RNAs were annealed with oligonucleotides complementary to J11/12 in either the *M. thermoautotrophicum* (MtΔ) or the *E. coli* (Eco) RNase P RNA and then digested with ribonuclease H. Reagents included in each reaction are indicated above each lane. Activity by the *M. thermoautotrophicum* RNase P RNA was reduced only in reactions containing the *Methanobacterium*-specific oligonucleotide; reactions lacking the oligonucleotide or containing the *E. coli*-specific oligonucleotide were not inhibited. Activity by the *E. coli* RNase P RNA was reduced only in reactions containing the *E. coli*-specific oligonucleotide; reactions lacking the oligonucleotide or containing the *Methanobacterium*-specific oligonucleotide were not inhibited.

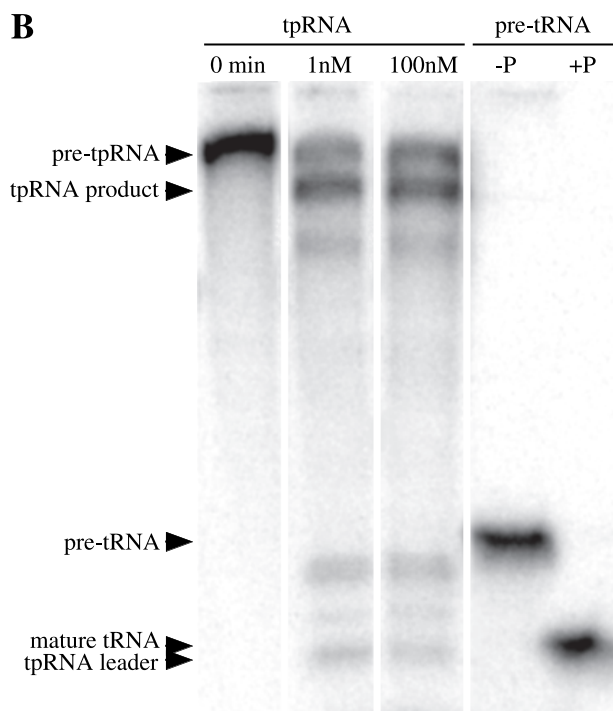
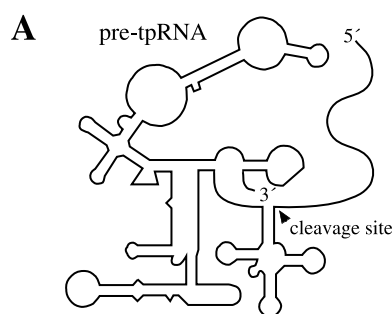


FIG. 4. Intramolecular cleavage by a unimolecular substrate:enzyme RNA. (A) The secondary structure of the conjugated *B. subtilis* pre-tRNA^{ASP}:*M. formicicum* (circularly permuted) RNase P RNA (pre-tpRNA) is shown diagrammatically. (B) Unreacted pre-tpRNA is shown in the first lane. Lanes 2 and 3 are the same RNA, incubated in parallel, at 1 and 100 nM; the extent of cleavage is ≈65% in both cases. The three other weaker cleavage products representing either Mg²⁺-sensitive sites or miscleavages were not characterized. Lanes 4 and 5 are the trans-cleavage analogs of lanes 1 and 3, in which the *B. subtilis* pre-tRNA^{ASP}, but not the *M. formicicum* RNase P RNA, is radiolabeled.

units; Life Technologies, Grand Island, NY) was added and incubated for 1 h at 30°C. The remaining RNase P activity was assessed as described above.

Unimolecular Enzyme:Substrate RNAs. Unimolecular enzyme:substrate RNAs were constructed by using RNase P RNA sequences from *Methanobacterium formicicum* and *M. barkeri* and the *B. subtilis* tRNA^{ASP}. Full-length RNase P RNA-encoding sequences were amplified from cloned genes by PCR by using primers ΔH5'Bam and ΔH3'Xba for *M. formicicum* or Msb5'Bam (CGGGATCCATGCCGAGAGAGGCTGG) and Msb3'RBam (CGGGATCCATGCCGAGT-GAGGCACG) for *M. barkeri*; this DNA was phosphorylated with ATP and T4 polynucleotide kinase and treated with T4 DNA ligase to generate circular genes. Secondary amplifications from these DNAs with primers Mfo261FBg/IITP (GAA-GATCTAGGTAAGTTCGCATAGATG) and Mfo260RHind-TP (CCCAAGCTTCTGCCTCATAACAGGATTC) for *M. formicicum* or MsbTP2505' (CGTCTAGAAACGCATAGCCG-AATG) and MsbTP2503' (AACTGCAGAACAACCGGG-

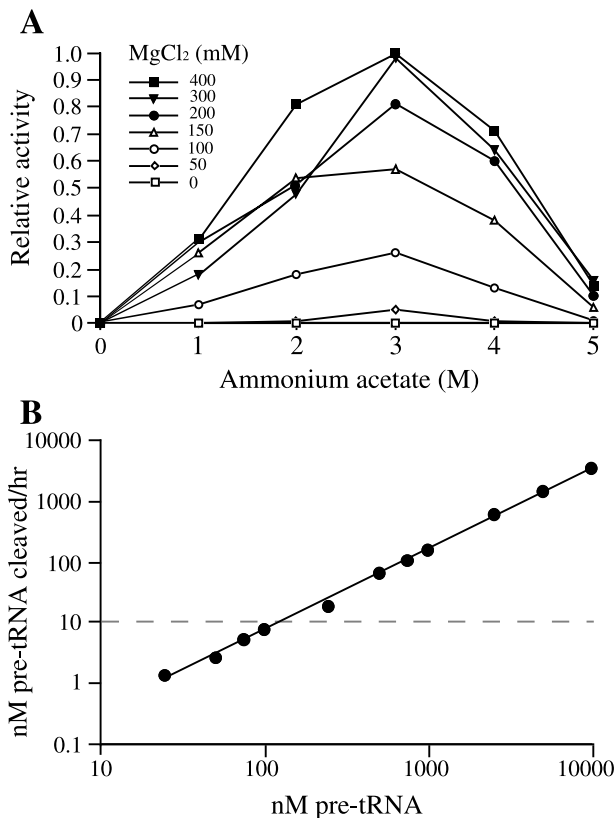


Fig. 5. Activity of the *M. thermoautotrophicum* RNase P RNA as functions of ammonium acetate, MgCl₂, and substrate concentration. (A) Activity of the *M. thermoautotrophicum* RNase P RNA as functions of ammonium acetate and MgCl₂ concentration. Reactions contained 20 nM substrate RNA and 1 nM RNase P RNA. Maximal activity in this assay represents 47% cleavage. (B) Activity of the *M. thermoautotrophicum* RNase P RNA as a function of substrate concentration. Reactions were performed under optimal conditions (see *Materials and Methods* and A). Reactions contained 10 nM RNase P RNA and were incubated for 2.5 h; points above the dashed horizontal line represent multiple turnovers over the course of the incubation. All data points in both A and B are represented by reactions with less than 50% cleavage, within the linear range of enzyme concentration and time for the reaction (data not shown).

AGAGTCCG) for *M. barkeri* generated circularly permuted RNase P RNA genes. Circularly permuted DNAs were cloned immediately downstream of the tRNA^{Asp} from *B. subtilis* in pDW128 (18). Radiolabeled enzyme:substrate RNAs were generated from linearized plasmids by using T7 RNA polymerase and [α -³²P]GTP.

Reconstitution of Chimeric Holoenzymes. RNase P reconstitution assays were conducted in 50 mM Tris-Cl (pH 8), 100 mM ammonium acetate, 25 mM MgCl₂, 1.5 nM *B. subtilis* pre-tRNA^{Asp}, 20–200 μ g/ml RNase P RNA, 18 μ g/ml nonsense transcript of the *M. thermoautotrophicum* RNase P RNA gene (included as a “decoy” for traces of nuclease activity in the RNase P protein preparation), and 0–10 μ g/ml *B. subtilis* RNase P protein (a gift from C. Fierke, Duke University, Durham, NC). Reactions containing archaeal RNase P RNA and negative controls containing no RNase P RNA were incubated at 37°C for 16 h. Reactions containing *E. coli* RNase P RNA were incubated at 37°C for 5 min. Cleavage products were separated by electrophoresis in denaturing 8% acrylamide gels followed by phosphorimager.

RESULTS AND DISCUSSION

In preliminary experiments, lysates from a variety of archaeal cultures were capable of 5' processing either the *B. subtilis* or

M. thermoautotrophicum pre-tRNA^{Asp} (15). Removal of protein from these lysates in many cases eliminated catalytic activity, but those of the methanobacteria and the thermococci (*P. furiosus* and *Thermococcus celer*) retained RNase P activity (data not shown). This activity was resistant to exhaustive phenol extraction and the inclusion of SDS (1%) in the reaction buffer, supporting the belief that catalysis was independent of protein. RNAs from the extreme halophiles *H. volcanii* and *Natronobacterium gregoryi* purified by organic extraction failed to process pre-tRNA, but the activity present in cell lysates was resistant to the inclusion of SDS in the reaction buffer, suggesting that the RNA was responsible for this activity.

The convincing proof that ribozymes, including bacterial RNase P RNA, are catalytically proficient in the absence of associated proteins came from experiments with RNAs transcribed *in vitro* (19). Synthetic RNAs, transcribed from cloned RNase P RNA-encoding genes, from a wide phylogenetic range of archaeal species were therefore tested for pre-tRNA 5'-processing activity; those of the methanobacteria, the extreme halophiles, and thermococci, but not other Archaea, were found to contain RNase P activity (Fig. 2). The catalytic proficiency of these synthetic RNAs indicates that they do not depend on protein for activity, nor are posttranscriptional modifications such as pseudouridylation or 2'-O-methylation essential for activity.

The extent of activity for all of the active archaeal RNase P RNAs was quite low; initial assays included large molar excesses (in the range of 100:1) of RNase P RNA over substrate and incubation for several hours, but often resulted in only partial processing of the substrate. The potential for contamination by catalytically active bacterial RNase P RNAs was therefore a significant concern. Previous descriptions of catalytic activity by the *H. volcanii* RNA (8) and reconstitution of chimeric RNase P holoenzymes from archaeal and bacterial components (8, 10) were previously discounted as the result of contamination, because the amount of activity recovered was very small and the results could not be reproduced (9). Three observations argued against the possibility that the catalytic activity reported here might result from contamination by bacterial RNase P RNA rather than from the archaeal RNA: (i) correlation of the presence or absence of activity from RNA extracted from cells and RNAs synthesized *in vitro* from genes cloned from the same species (data not shown), (ii) the unusual biochemical properties of the activity (see below), and (iii) the precise electrophoretic comigration of activity with the archaeal RNA (data not shown). The direct experimental evidence that the observed activity resides in the archaeal RNase P RNA comes from oligonucleotide-directed RNase H depletion experiments (Fig. 3) and intramolecular cleavage of unimolecular enzyme:substrate RNAs (Fig. 4). In the first instance, pretreatment of the *M. thermoautotrophicum* Δ H RNase P RNA with RNase H in the presence of an *M. thermoautotrophicum*-specific oligonucleotide, but not an *E. coli*-specific oligonucleotide, reduces RNase P activity. However, the *E. coli*-specific oligonucleotide and RNase H reduced the activity of the *E. coli*, but not *M. thermoautotrophicum*, RNase P RNA. In the second case, cleavage of the tethered *M. formicicum* RNase P RNA:*B. subtilis* pre-tRNA^{Asp} was independent of concentration, indicating cleavage was *in cis*. The *M. formicicum* RNase P RNA:*B. subtilis* pre-tRNA^{Asp} required conditions similar to the native *M. thermoautotrophicum* RNA (see below) for optimal activity. The analogous enzyme:substrate RNA based on the *M. barkeri* RNase P RNA, an RNase P RNA that is not catalytically active, does not self-cleave (data not shown).

The reaction conditions required by the archaeal RNase P RNA activity are unusual. Both the native and synthetic *M. thermoautotrophicum* RNase P RNAs were found to require 300 mM MgCl₂ and 3 M ammonium acetate for maximal

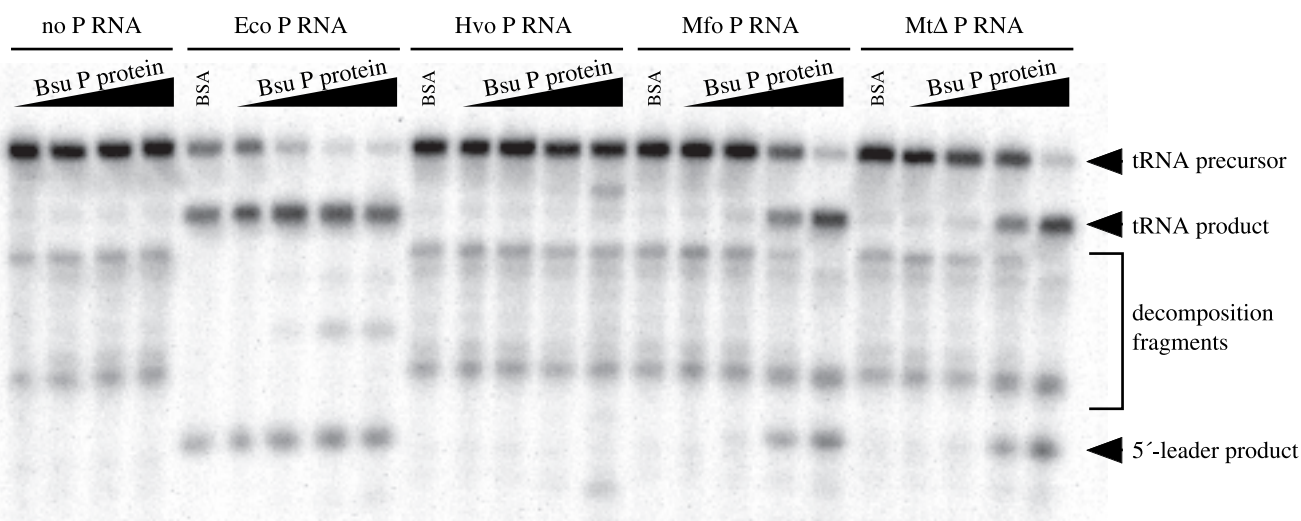


Fig. 6. Reconstitution of active chimeric holoenzymes. RNase P RNAs from *E. coli* (Eco, 38 $\mu\text{g/ml}$), *H. volcanii* (Hvo, 170 $\mu\text{g/ml}$), *M. formicicum* (Mfo, 60 $\mu\text{g/ml}$), or *M. thermoautotrophicum* ΔH (Mt Δ , 33 $\mu\text{g/ml}$) were assayed for RNase P activity in the presence of 0, 0.1, 1, or 10 $\mu\text{g/ml}$ *B. subtilis* RNase P protein (increasing protein concentration indicated by black wedges above the reaction lanes). Specific RNase P cleavage products are indicated by black arrowheads. The *E. coli* RNase P RNA is somewhat active by itself under these conditions, but activity is enhanced by the inclusion of the *B. subtilis* RNase P protein. The *M. thermoautotrophicum* and *M. formicicum* RNase P RNAs are not active in the absence of protein under these conditions but were activated by inclusion of the *B. subtilis* RNase P protein. Correct processing by the *H. volcanii* RNase P RNA was not observed under these conditions in either the presence or absence of the *B. subtilis* RNase P protein. However, a specific inappropriate cleavage was generated at the highest concentration of protein; the nature of these products and the apparent miscleavage have not been determined.

activity (Fig. 5A), well above the requirements of any characterized bacterial RNase P RNA. Mn^{2+} could efficiently replace Mg^{2+} and was effective at lower concentrations (100 mM) but at the expense of a dramatic increase in nonspecific hydrolysis of substrate, product, and presumably enzyme. Ca^{2+} , Zn^{2+} , and Cu^{2+} were unable to promote cleavage. The Mg^{2+} requirement was not reduced by increases in ammonium acetate concentration nor *vis a versa*. The high ionic strength may be required for stabilization of the RNA structure, and the high Mg^{2+} may be required for further specific stabilization and/or to overcome poor binding of catalytically involved Mg^{2+} . The RNase P RNA synthetic transcript from *H. volcanii* also required very high ionic strength for activity (4 M ammonium acetate and 300 mM MgCl_2) for maximal activity. The optimal temperatures and pH for these RNase P RNAs was 45–50°C and 8.0, respectively. However, the upper bounds of these assays are limited by the extent of nonspecific hydrolysis in these reactions at elevated temperatures and pH, although high temperature or pH alone did not allow optimal catalysis at lower ionic strength or Mg^{2+} concentrations.

Poor catalysis by the archaeal RNase P RNAs, even in optimal conditions, seems to result at least in part from poor affinity for substrate. Substrate cleavage rates increase linearly with substrate concentration up to at least 10 μM (Fig. 5B), implying that the K_m of the reaction is in excess of $\approx 40 \mu\text{M}$. This result may occur in part because of the extreme conditions required to obtain activity; the K_m of the *E. coli* RNase P RNA-catalyzed reaction is also quite high under these far-from-optimal conditions (2.2 μM , as opposed to 74 nM in reactions containing 1 M ammonium acetate and 25 mM MgCl_2). The V_{max} of the reaction catalyzed by the archaeal RNA could not be determined, but given the high K_m and respectable catalytic rate at high substrate concentrations, there is no evidence for a defect in V_{max} or k_{cat} . At the highest substrate concentrations, each mole of archaeal RNase P RNA processed more than 100 mol of substrate during the 2.5-h reaction (2.2 min^{-1}).

The protein component or components of the archaeal RNase P have yet to be identified despite the availability of several complete archaeal genome sequences and RNase P

protein sequences from a wide variety of Bacteria and the yeast nucleus and mitochondrion (as well as some of the human sequences; refs. 20–23). Because of the similarity of the archaeal and bacterial RNase P RNAs, we attempted to reconstitute chimeric holoenzymes from archaeal RNase P RNA and bacterial RNase P protein (Fig. 6). Catalytic activity by the *Methanobacterium* RNase P RNAs was reconstituted at low ionic strength and Mg^{2+} concentration by using the *B. subtilis* RNase P protein, indicating the functional interaction of these heterologous subunits. It seems likely, therefore, that the structure and function of the bacterial and archaeal protein components are, at least in part, similar in the archaeal and bacterial enzymes. RNase P activity at moderate ionic strength absolutely depended on the presence of the protein, unlike the *E. coli* or *B. subtilis* RNase P RNAs, which can process this substrate at rates of $\approx 20\%$ of that of the reconstituted enzymes in these conditions. We did not detect enhanced activity of the *M. sedula*, *H. volcanii*, *M. barkeri*, or *M. vannielii* RNAs in the presence of the bacterial protein under these conditions, although analogous reconstitution of *H. volcanii* RNA and *B. subtilis* protein has been reported (8).

The catalytic proficiency of archaeal RNase P RNAs is much lower than their bacterial homologs, but their ability to process pre-tRNA at any rate implies that they contain all of the sequences and structures necessary for substrate recognition and catalysis. The catalytic competence of the archaeal RNAs is consistent with our understanding of the conserved features of the archaeal and bacterial RNase P RNAs; these RNAs are remarkably similar in both sequence and structure, and the invariably present sequences and structures of the catalytically active bacterial RNAs are present in the archaeal RNAs as well (except those of *Methanococcus* and *Archaeoglobus*). Synthetic RNase P RNAs that contain only these core bacterial sequences and structures are catalytically active; however, these RNAs are structurally deficient, with biochemical properties similar to those of the active archaeal RNAs (13, 14). The archaeal RNAs apparently depend on the protein component(s) of the holoenzyme for structural integrity but not for essential catalytic function. The exception to this generality is the RNase P RNAs of *Methanococcus* and *Archaeoglobus*, both

of which lack core structural features (P8 and L15) that are directly involved in substrate recognition in Bacteria. These RNAs are not catalytically active, even in the presence of the bacterial protein. How the holoenzymes compensate for the lack of core RNA structures is not known.

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