

Characterization of the RNase P RNA of *Sulfolobus acidocaldarius*

THOMAS E. LAGRANDEUR, SYLVIA C. DARR,† ELIZABETH S. HAAS,
AND NORMAN R. PACE*

Department of Biology and Institute for Molecular and Cellular Biology,
Indiana University, Bloomington, Indiana 47405

Received 16 March 1993/Accepted 4 June 1993

RNase P is the ribonucleoprotein enzyme that cleaves precursor sequences from the 5' ends of pre-tRNAs. In *Bacteria*, the RNA subunit is the catalytic moiety. Eucaryal and archaeal RNase P activities copurify with RNAs, which have not been shown to be catalytic. We report here the analysis of the RNase P RNA from the thermoacidophilic archaeon *Sulfolobus acidocaldarius*. The holoenzyme was highly purified, and extracted RNA was used to identify the RNase P RNA gene. The nucleotide sequence of the gene was determined, and a secondary structure is proposed. The RNA was not observed to be catalytic by itself, but it nevertheless is similar in sequence and structure to bacterial RNase P RNA. The marked similarity of the RNase P RNA from *S. acidocaldarius* and that from *Haloferax volcanii*, the other known archaeal RNase P RNA, supports the coherence of *Archaea* as a phylogenetic domain.

RNase P is the ubiquitous endonuclease that cleaves leader sequences from precursors of tRNA to generate their mature 5' ends (1, 6, 15). RNase P is most extensively characterized for members of the *Bacteria*, for instance *Escherichia coli* and *Bacillus subtilis*, in which the enzyme is a ribonucleoprotein composed of a small protein (about 14 kDa) and a large RNA (about 130 kDa). (We use the nomenclature for the primary phylogenetic domains proposed by Woese et al. (22). "*Bacteria*" refers to organisms previously known as eubacteria, "*Archaea*" refers to the phylogenetic group previously called archaeobacteria, and "*Eucarya*" refers to organisms of the eucaryotic nuclear lineage.) The RNA moiety of the bacterial RNase P is the catalytic subunit: in vitro, at high ionic strength, the RNA can perform cleavage in the absence of the protein (9). A phylogenetic comparative analysis of RNase P RNA sequences has been used to infer secondary structures for bacterial RNAs, among which a conserved minimum of sequence and secondary structure is identifiable (4).

RNase P has also been purified from a few members of the *Eucarya*, specifically fungi (12, 13, 23) and vertebrates (3, 8); however, little is known about the structure of those enzymes. The eucaryal RNase P activities copurify with RNAs that have been isolated and sequenced, but the RNAs are not catalytically active in the absence of protein components. The fungal and vertebrate-type RNAs possess little sequence similarity to one another or to bacterial RNase P RNAs, and they seem to lack structural elements present in the bacterial RNAs. The eucaryal RNase P's inspected appear to have a much higher content of protein than do their bacterial counterparts. This conclusion is based on the masses of the holoenzymes relative to the sizes of the putative RNA elements and the low buoyant densities of the holoenzymes in Cs₂SO₄ gradients, nearly that of protein alone.

The RNase P from the third primary evolutionary lineage,

the *Archaea*, offers further perspective on the fundamental properties of RNase P. RNase P from two phylogenetically diverse members of the *Archaea*, the halophile *Haloferax volcanii* (14) and the thermoacidophile *Sulfolobus acidocaldarius* (7), has been examined. An RNA from the *H. volcanii* enzyme has been described previously (14). Despite having a secondary structure that appears highly similar to that of bacterial RNase P RNA, this RNA was not catalytic without protein. The RNase P from *S. acidocaldarius* has intriguing properties similar to those of the eucaryal version of the enzyme, in that it is large and its buoyant density in Cs₂SO₄ is near that of protein. Additionally, it is relatively resistant to nuclease treatment. Although such characteristics might suggest that *S. acidocaldarius* RNase P does not contain an essential RNA, RNA does copurify with the RNase P activity (7). We report here the identification of the *S. acidocaldarius* RNase P RNA and a characterization of its structure and activity.

MATERIALS AND METHODS

Isolation of RNase P activity and RNA. RNase P was isolated from *S. acidocaldarius* DSM 639, which in previous RNase P studies (7) was misclassified as *Sulfolobus solfataricus* P1. RNase P activity was purified by the procedure of Darr et al. (7) through a Fractogel TSK DEAE anion-exchange column and then further purified over heparin-Sepharose and fast protein liquid chromatography (FPLC) Mono-Q columns (Pharmacia). RNase P activity, assayed and quantitated as previously described (7), was eluted from the DEAE at ~100 mM NH₄Cl. Fractions containing enzyme activity were pooled and diluted with TMGN buffer (50 mM Tris-HCl, pH 7.6; 10 mM MgCl₂; 5% [wt/vol] glycerol; 0.1 mM Phenylmethylsulfonyl fluoride; 0.1 mM dithiothreitol; 0.05% Nonidet P-40) to a final concentration of ~20 mM NH₄Cl. The diluted pool was loaded onto a heparin-Sepharose column. The column was washed with TMGN-20 (TMGN which additionally contains 20 mM NH₄Cl); washing was followed by a linear gradient of 20 to 600 mM NH₄Cl in TMGN. RNase P activity was eluted from the heparin-Sepharose column at ~300 mM NH₄Cl. Fractions containing

* Corresponding author.

† Present address: Department of Biochemistry, University of Nebraska—Lincoln, Lincoln, NE 68583-0718.

enzyme activity were pooled and concentrated by ultrafiltration with an Amicon PM-10 membrane. The concentrated pool was dialyzed in TMGN-20 and then loaded onto a Mono-Q FPLC column. The column was washed with TMGN-20 and eluted in a linear gradient of 20 to 1,000 mM NH_4Cl in TMGN. RNase P activity was eluted from the Mono-Q column at ~ 380 mM NH_4Cl . Fractions containing RNase P activity were pooled and then adjusted to contain 0.1% sodium dodecyl sulfate (SDS) and 10 mM EDTA. The pool was heated to 70°C for 1 min, and RNA was isolated by extraction first with phenol and then with phenol-chloroform (1:1). The RNA was precipitated in ethanol and resuspended in 10 mM Tris, pH 7.6–1 mM EDTA.

Genomic Southern analysis. *S. acidocaldarius* genomic DNA was digested with restriction endonucleases, separated by electrophoresis through a 1% agarose gel, and then transferred and fixed to a Hybond N filter (Amersham) as described previously (4). The filter was prehybridized (50°C, >4 h) and hybridized (50°C, overnight) in buffer containing 0.02% bovine serum albumin; 0.02% polyvinylpyrrolidone; 0.02% Ficoll; 20 mM NaPO_4 , pH 7.6; 1 mM dithiothreitol; 5 \times SSC (0.75 M NaCl plus 0.075 M sodium citrate); 100 μg of poly(A) per ml; 0.1% SDS; and 100 μg of 16S and 23S *S. acidocaldarius* rRNA per ml, isolated by Sepharose 4B-200 chromatography. The hybridization included 4.7×10^5 dpm of end-labeled probe. After hybridization, the filter was washed twice in 2 \times SSC–0.1% SDS at room temperature and then once in 0.1 \times SSC–0.1% SDS at 50°C for 20 min.

The probe for Southern blots was 5'-end-labeled fragments of partially hydrolyzed RNA extracted from the Mono-Q column chromatography-purified RNase P. The extracted RNA was partially hydrolyzed by incubation at 95°C in 50 mM sodium carbonate, pH 9, from which aliquots were removed at 5, 10, 15, and 20 min. The aliquots were mixed, precipitated in ethanol, and pooled; the RNA fragments were 5' end labeled with T4 polynucleotide kinase and [γ - ^{32}P]ATP.

Cloning and sequencing of the *S. acidocaldarius* RNase P RNA gene. Preparative restriction digests of genomic DNA were resolved by electrophoresis through a 1% agarose gel, and the region of the gel containing the RNase P RNA gene was excised on the basis of Southern blot information. DNA was recovered from the gel slice by treatment with NaI and purified with glass powder (20). The size-selected DNAs were cloned into pBluescript KS(-) (Stratagene) and screened by colony hybridization (18) with the same probe used for the Southern analysis. Following sequence determination and identification of the *S. acidocaldarius* gene, a clone of the gene with smaller 5' and 3' flanking sequences was constructed by subcloning a restriction fragment into a pUC19 derivative, placing the gene immediately downstream of a T7 RNA polymerase promoter.

Double-stranded plasmid DNAs were sequenced by the dideoxynucleotide termination method (19) with Sequenase version 2.0 (U.S. Biochemicals). Parts of the gene were sequenced from restriction fragment subclones or deletion subclones generated with exonuclease III and S1 nuclease (11, 18).

Northern (RNA) analysis of cellular RNAs. Total cellular RNAs (20 μg) or size-enriched RNAs (5 μg) were separated by electrophoresis through a 4% polyacrylamide–8 M urea gel; transferred to a Hybond N filter by electroelution in 25 mM NaPO_4 , pH 6.8; and then fixed to the filter by UV irradiation. Hybridization was performed as described for the Southern analysis, with hybridization and final wash temperatures of 65°C. The probe was in vitro-synthesized,

uniformly ^{32}P -labeled, complementary RNA from a clone containing the RNase P gene.

Size-enriched RNAs were from total RNA (10 mg) fractionated on a Sepharose 4B-200 column in 100 mM NaCl–10 mM Tris, pH 8.0–1 mM EDTA–0.1% SDS. Fraction samples were diluted in water and transferred to a Hybond N filter by vacuum slot blotting (18). The RNA was fixed to the filter and probed as for the Northern analysis. RNase P RNA-containing fractions were combined for the size-enriched RNA pool.

5' and 3' end determination. Primer extension analysis was performed with size-enriched RNAs (described above) to determine the 5' end of the *S. acidocaldarius* RNase P RNA. An oligonucleotide complementary to nucleotides 120 to 102 of the *S. acidocaldarius* RNase P RNA was 5' end labeled with polynucleotide kinase and [γ - ^{32}P]ATP, annealed to the RNA in 20 mM Tris, pH 8.0–30 mM KCl by heating at 90°C for 2 min and slowly cooling over 15 min to room temperature, and extended with avian myeloblastosis virus reverse transcriptase (Seikagaku) in 50 mM Tris, pH 8.0–10 mM dithiothreitol–10 mM MgCl_2 –100 μM each deoxynucleoside triphosphate at 45°C for 20 min. The reaction products were resolved by electrophoresis through an 8% polyacrylamide–8 M urea gel, and sizes were determined by comparison to nonrelated sequencing reaction products used as markers.

The 3' end was identified by S1 nuclease protection. The probe was a 339-bp *Bst*NI-*Eco*RV restriction fragment that overlapped 103 bp of the 3' end of the *S. acidocaldarius* RNase P RNA gene. The fragment was 3' end labeled by filling in the *Bst*NI-generated single-nucleotide recessed end with the Klenow fragment of DNA polymerase I and [α - ^{32}P]TTP. The labeled fragment was hybridized to size-selected *S. acidocaldarius* RNA under conditions which favor formation of an RNA-DNA duplex and treated with S1 nuclease (18). The reaction products were resolved as described above.

RNase P activity assays. ^{32}P -labeled precursor tRNA^{ASP} from *B. subtilis* and RNase P RNAs were transcribed in vitro with T7 RNA polymerase from linearized plasmid template DNAs as previously described (16). The *S. acidocaldarius* RNase P RNA prepared in vitro contained three and nine extra nucleotides at its 5' and 3' ends, respectively. Extra sequences at the termini of bacterial RNase P RNAs have no influence on catalytic activity (4).

The buffer for the assay with RNase P RNA alone contained 25 mM MgCl_2 ; 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–NaOH, pH 8.0; 0.05% Nonidet P-40; and NH_4Cl ranging from 0.1 to 3 M. Assays included 50 nM precursor tRNA^{ASP} and either 5, 50, or 500 nM RNase P RNA. Reaction mixtures were incubated at 37 or 65°C for 1 h; reactions were stopped by adding an equal volume of 100% formamide loading buffer, and the products were resolved by denaturing polyacrylamide gel electrophoresis. Reaction products were detected by autoradiography.

Heterologous reconstitutions contained 12 nM RNase P RNA and equimolar or a 2-, 5-, 10-, or 50-fold molar excess of *E. coli* or *B. subtilis* RNase P protein (kindly provided by B. Pace). The plasmid containing the *H. volcanii* RNase P RNA-encoding gene was a gift of C. Daniels (Ohio State University). Reconstitutions were typically in 50 mM Tris, pH 8.0–30 mM MgCl_2 –100 mM NH_4Cl –0.05% Nonidet P-40. RNase P RNA and protein were preincubated together in reaction buffer at 37°C for 10 min; preincubation was followed by the addition of precursor tRNA^{ASP} and incubation

at 37, 45, or 65°C for 30 min. Reactions were stopped and products were detected as described above.

Nucleotide sequence accession number. The nucleotide sequence presented in this report has been submitted to GenBank under accession number L13597.

RESULTS

S. acidocaldarius RNase P was purified more than 5,000-fold by Trisacryl-DEAE and heparin-Sepharose chromatographies. The enzyme was further purified by Mono-Q column chromatography. Activity from that column was eluted in a sharply defined peak (data not shown), but there was little total protein or nucleic acid present, so the extent of purification was not determined. The Mono-Q column chromatography-purified enzyme was inactivated by freezing, while less purified RNase P activity was not. The loss of activity after freezing was not simply a result of exposure to low temperature (−20°C), since Mono-Q column chromatography-purified RNase P stored in 50% glycerol at this temperature retained activity.

RNA from the Mono-Q column chromatography-purified RNase P was used as a probe in a Southern analysis of *S. acidocaldarius* genomic DNA. The probe hybridized to single fragments in each of several restriction digests, indicating that the RNase P RNA is encoded by a single-copy gene in *S. acidocaldarius* (data not shown). Two independent restriction fragments were cloned and gave overlapping sequences. The sequence was examined for a potential RNase P RNA gene by searching for the 11-nucleotide sequence 5'-GAGGAAAGUCC-3', which is nearly 100% conserved in bacterial RNase P RNAs (10). A sequence identity of 10 of the 11 nucleotides was found (nucleotides 36 to 43, Fig. 1A).

This potential gene was tested for expression by Northern analysis of total cellular RNA. The antisense RNA probe hybridized to a single band of approximately 320 nucleotides (Fig. 2A). The 5' end of the RNA, first predicted on the basis of its similarity to the bacterial RNA, was determined by primer extension analysis (Fig. 2B). The approximate 3' end of the RNA, also predicted, was determined by S1 nuclease analysis (Fig. 2C). The 5' and 3' end determinations resolved the length of the *S. acidocaldarius* RNase P RNA to be 315 nucleotides, shown in Fig. 1A.

Numerous attempts to detect catalytic activity by *in vitro* transcripts of *S. acidocaldarius* RNase P RNA in the absence of other components of the holoenzyme were made. Various combinations of different RNA concentrations, ionic conditions, and reaction temperatures failed to produce any cleavage of pre-tRNA by RNA alone. Attempts with RNA extracted from purified holoenzyme were likewise unsuccessful. We also attempted to reconstitute activity of the *S. acidocaldarius* RNA with bacterial RNase P protein from *B. subtilis* or *E. coli*. No activity was detected in reactions with a range from equimolar to 50-fold molar excess protein/RNA ratios. The RNase P RNA from the archaeon *H. volcanii* was previously reported to be rendered catalytic by the *B. subtilis* RNase P protein (14). We, however, were unable to reproduce this result with either the *S. acidocaldarius* or *H. volcanii* RNA. Control reconstitutions with *E. coli* RNase P RNA and either *E. coli* or *B. subtilis* RNase P protein were successful, demonstrating that the lack of activity in the heterologous reconstitutions was not due to inactive bacterial protein.

DISCUSSION

Despite its undetectable catalysis *in vitro*, there are sound reasons to associate the identified RNA with RNase P. First, the RNA was uniquely isolated from highly purified RNase P activity, suggesting that it is a subunit of that enzyme. Second, the *S. acidocaldarius* RNA contains a majority of nucleotides that correspond to conserved residues in bacterial RNase P RNAs. Since there is little sequence conservation among bacterial RNase P RNAs, it is difficult to identify with certainty RNase P RNAs simply on the basis of sequence. However, there are several, key, conserved nucleotides, most notably a contiguous sequence of 11 nucleotides that are nearly universal in *Bacteria*. The *S. acidocaldarius* RNA has that sequence (nucleotides 34 to 46), with a single difference, a C instead of a G (nucleotide 43). In bacterial RNase P RNAs this nucleotide is base paired, and the equivalent pairing is maintained by a compensatory change in the *S. acidocaldarius* sequence, to pair nucleotides 43 and 294. The *Sulfolobus* sequence additionally contains a number of other conserved residues, viewed in the reference frame of the secondary structure. Finally, the identified RNA is strongly indicated as the *S. acidocaldarius* RNase P RNA at the level of higher-order structure. As shown in Fig. 1, the RNA can be modeled with a secondary structure that is strikingly similar to the bacterial RNase P RNA. Helical elements occur in the folded *S. acidocaldarius* structure at all except one of the relative positions, in which they occur in the well-proven bacterial structure (Fig. 1B). However, two of the proposed *S. acidocaldarius* helical elements, indicated in Fig. 1A by italics, differ substantially in gross structure and sequence from their putative bacterial counterparts. Similar variation occurs in the RNase P RNA of *H. volcanii* (Fig. 1C). Any of the differences between the archaeal RNAs and the bacterial RNAs might be responsible for the archaeal RNase P RNAs lacking demonstrable catalytic activity in the absence of protein.

We were unable to obtain RNase P activity by adding bacterial RNase P proteins to the *S. acidocaldarius* RNase P RNA, nor could we verify reconstitution results previously reported for *H. volcanii* RNA. The protein components of these two archaeal RNase P's remain uncharacterized, so it is unknown whether they may be similar to the bacterial proteins. The buoyant density of the *S. acidocaldarius* holoenzyme in Cs₂SO₄ gradients is 1.27 g/ml, nearly that of protein alone (7). This is much lighter than that of the bacterial holoenzyme, which in the case of the *E. coli* RNase P is 1.55 g/ml of Cs₂SO₄. On the basis of the size of the *S. acidocaldarius* holoenzyme (ca. 400 kDa [7]) in comparison to the size of the RNA (ca. 104 kDa), it is expected that about 70% of the mass of the enzyme is composed of protein or other non-RNA substances, such as lipid or carbohydrate. This contrasts with the bacterial version of the enzyme, which typically is 90% RNA and only 10% protein. The *S. acidocaldarius* RNase P is nevertheless still lower in buoyant density than expected from its protein content (7). It is possible that the holoenzyme contains a low-density component such as lipid or that the protein components are modified in a manner that reduces density. The size of the native *S. acidocaldarius* RNase P RNA, revealed by Northern analysis of cellular RNA, is as expected from the sequence on the basis of comparisons to the bacterial RNA.

It has been suggested on the basis of some sequence and other comparisons (17) that the extremely thermophilic, sulfur-metabolizing members of the *Archaea* (*Crenarchaeota*, [22]) such as *S. acidocaldarius* might be evolutionarily-

FIG. 1. Secondary structures of the *S. acidocaldarius* and other RNase P RNAs. (A) *S. acidocaldarius* RNase P RNA. The sequence in roman type aligns well with the bacterial consensus minimum (B) (5), while the italicized sequence represents a putative structure that cannot be aligned well with the bacterial sequences. The italicized sequence does, however, agree with potential structure seen in the *H. volcanii* RNase P RNA (2). The nucleotides indicated with asterisks potentially pair to form a helix that occurs in most bacterial RNase P RNAs (5). (B) Bacterial consensus minimum (5). Absolutely conserved nucleotides in bacterial RNase P RNAs are shown in the consensus structure in uppercase letters; nucleotides that are not invariant, but are conserved in at least 90% of the available sequences, are shown in lowercase letters. Nucleotides that are less than 90% conserved are shown by solid circles. Nucleotides that are not present in all sequences, but are absent in less than 10% of the available sequences, are indicated by open circles; those that are absent in more than 10% of the sequences are not shown. (C) *H. volcanii* RNase P RNA (2).

more closely related to *Eucarya* than to the other branch of *Archaea* (*Euryarchaeota*), which includes *H. volcanii*. The RNase P RNAs from *S. acidocaldarius* and *H. volcanii* have similar structures, distinct from those of characterized bacterial and eucaryal RNase P RNAs. Thus, the nature of the archaeal RNase P RNAs supports the coherence of the

phylogenetic domain of *Archaea*, in agreement with rRNA sequence comparisons (21).

ACKNOWLEDGMENTS

We thank James W. Brown for graphic assistance and for providing the bacterial minimum consensus structure and Charles Daniels for discussions and provision of a plasmid containing the *H. volcanii* RNase P RNA gene.

This work was supported by Public Health Service grant GM34527 from the National Institute of General Medical Sciences and Department of Energy grant FG02-92ER2088 to N.R.P. and by a fellowship from the Boehringer-Mannheim Co. to T.E.L.

REFERENCES

- Altman, S. 1989. Ribonuclease P: an enzyme with a catalytic RNA subunit. *Adv. Enzymol. Relat. Areas Mol. Biol.* **62**:1-36.
- Armbruster, D., E. S. Haas, J. W. Brown, and C. J. Daniels. 1993. Unpublished data.
- Baer, M., T. W. Nilsen, C. Costigan, and S. Altman. 1990. Structure and transcription of a human gene for H1 RNA, the RNA component of human RNase P. *Nucleic Acids Res.* **18**:97-103.
- Brown, J. W., E. S. Haas, B. D. James, D. A. Hunt, J. Liu, and N. R. Pace. 1991. Phylogenetic analysis and evolution of RNase P RNA in proteobacteria. *J. Bacteriol.* **173**:3855-3863.
- Brown, J. W., and N. R. Pace. 1992. Ribonuclease P RNA and protein subunits from bacteria. *Nucleic Acids Res.* **20**:1451-1456.
- Darr, S. C., J. W. Brown, and N. R. Pace. 1992. The varieties of ribonuclease P. *Trends Biochem. Sci.* **17**:178-182.
- Darr, S. C., B. Pace, and N. R. Pace. 1990. Characterization of ribonuclease P from the archaeobacterium *Sulfolobus solfataricus*. *J. Biol. Chem.* **265**:12927-12932.
- Doria, M., G. Carrara, P. Calandra, and G. P. Tocchini-Valentini. 1991. An RNA molecule copurifies with RNase P activity from *Xenopus laevis* oocytes. *Nucleic Acids Res.* **19**:2315-2320.
- Guerrier-Takada, C., K. Gardiner, T. Marsh, N. Pace, and S. Altman. 1983. The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. *Cell* **35**:849-857.
- Haas, E. S., D. P. Morse, J. W. Brown, F. J. Schmidt, and N. R. Pace. 1991. Long-range structure in ribonuclease P RNA. *Science* **254**:853-856.
- Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**:351-359.
- Krupp, G., B. Cherayil, D. Frendewey, S. Nishikawa, and D. Söll. 1986. Two RNA species co-purify with RNase P from the fission yeast *Schizosaccharomyces pombe*. *EMBO J.* **5**:1697-1703.
- Lee, J.-Y., and D. R. Engelke. 1989. Partial characterization of an RNA component that copurifies with *Saccharomyces cerevisiae* RNase P. *Mol. Cell. Biol.* **9**:2536-2543.
- Nieuwlandt, D. T., E. S. Haas, and C. J. Daniels. 1991. The RNA component of RNase P from the archaeobacterium *Haloflex volcanii*. *J. Biol. Chem.* **266**:5689-5695.
- Pace, N. R., and D. Smith. 1990. Ribonuclease P: function and variation. *J. Biol. Chem.* **265**:3587-3590.
- Reich, C., K. J. Gardiner, G. J. Olsen, B. Pace, T. L. Marsh, and

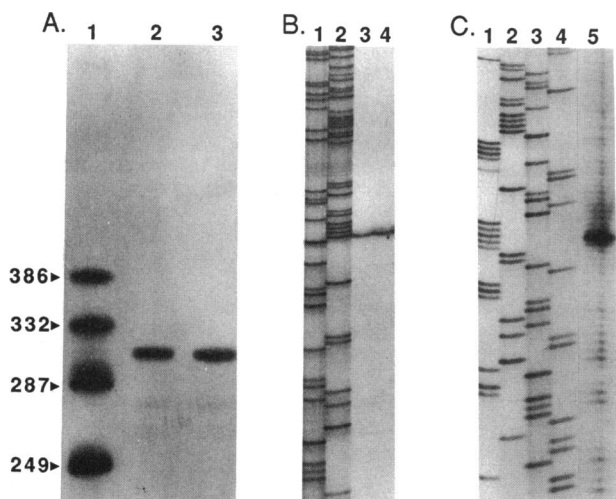


FIG. 2. Analysis of the native *S. acidocaldarius* RNase P RNA. (A) Northern blot analysis of *S. acidocaldarius* RNase P RNA. Total cellular or size-fractionated RNAs were resolved by polyacrylamide gel electrophoresis and transferred to a charged nylon membrane as described in Materials and Methods. Size markers are ^{32}P -labeled RNAs of defined size prepared by *in vitro* transcription. Hybridization was carried out with a ^{32}P -labeled complement of the *S. acidocaldarius* RNase P RNA, prepared by *in vitro* transcription of the cloned gene. Lanes: 1, nucleotide size marker RNAs; 2, total cellular RNA; 3, size-fractionated RNA. (B) 5' end determination by primer extension. Primer extension by avian myeloblastosis virus reverse transcriptase was carried out with a ^{32}P -labeled primer complementary to nucleotides 120 to 102 of the RNA as described in Materials and Methods, and the products were resolved on an 8% polyacrylamide sequencing gel. Double-stranded plasmid containing the cloned gene was sequenced by dideoxynucleotide termination with the same end-labeled primer. Lanes: 1 and 2, sequence corresponding to A and U residues in the RNA, respectively; 3 and 4, primer extension products of size-fractionated RNA from one-fourth and half of the primer extension reaction, respectively. (C) 3' end determination by S1 nuclease protection. A 3'-end ^{32}P -labeled restriction fragment from a 1-kb clone containing the gene was denatured and annealed to size-fractionated RNA and treated with S1 nuclease, as described in Materials and Methods. Reaction products were resolved on an 8% polyacrylamide sequencing gel. 5' end ^{32}P -labeled-primer dideoxynucleotide sequencing reactions of plasmid Bluescript KS(-) were used as markers to determine the size of the labeled probe after S1 nuclease digestion. Lanes: 1 to 4, size marker sequencing reactions; 5, S1 nuclease protection products.

- N. R. Pace. 1986. The RNA component of the *Bacillus subtilis* RNase P: sequence, activity, and partial secondary structure. *J. Biol. Chem.* **261**:7888–7893.
17. Rivera, M. C., and J. A. Lake. 1992. Evidence that eukaryotes and eocyte prokaryotes are immediate relatives. *Science* **257**: 74–76.
 18. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 19. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
 20. Vogelstein, B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA* **76**:615–619.
 21. Woese, C. R. 1987. Bacterial evolution. *Microbiol. Rev.* **51**:221–271.
 22. Woese, C. R., O. Kandler, and M. L. Wheelis. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci. USA* **87**:4576–4579.
 23. Zimmerly, S., V. Gamulin, U. Burkard, and D. Söll. 1990. The RNA component of RNase P in *Schizosaccharomyces* species. *FEBS Lett.* **271**:189–193.