In eukaryotes, dozens of posttranscriptional modifications are directed to specific nucleotides in ribosomal RNAs (rRNAs) by small nucleolar RNAs (snorRNAs). We identified homologs of snoRNA genes in both branches of the Archaea. Eighteen small sno-like RNAs (sRNAs) were cloned from the archaeon *Sulfolobus acidocaldarius* by coimmunoprecipitation with archaeal fibrillarin and NOP56, the homologs of eukaryotic snoRNA-associated proteins. We trained a probabilistic model on these sRNAs to search for more sRNAs in archaeal genomic sequences. Over 200 additional sRNAs were identified in seven archaeal genomes representing both the Crenarchaeota and the Euryarchaeota. snoRNA-based RNA processing was therefore probably present in the last common ancestor of Archaea and Eukarya, predating the evolution of a morphologically distinct nucleolus.

Ribosomal biogenesis in Eukarya occurs in the nucleolus. Several nucleolar proteins (NOPs), including fibrillarin, Nop56, and Nop58, and dozens of snoRNAs are involved in this process (1). The snoRNAs fall into two major classes: C/D box and H/ACA box RNAs. The C/D box snoRNAs are efficiently recruited from other Pediatric Oncology Group (POG) institutions before treatment began. Informed consent was obtained, and procedures approved by the Committee on Human Research at the University of Vermont and other POG institutions were followed. Peripheral blood was separated and the mononuclear cell fraction was obtained for the T cell cloning assay within 12 to 24 hours of its collection. The T cell cloning assay and analysis have been described [see B. A. Finette et al., Mutat. Res. 308, 223 (1994)].

12. Mutation frequencies in subjects at diagnoses, remission, and relapse were compared with normal controls by the nonparametric Kruskal–Wallis test. Pairwise differences between groups were assessed by Mann–Whitney tests, with a Bonferroni adjustment for multiple comparisons. Linear regression analysis was used to examine the relationship between the logarithm of MFI (lmFI) and months since diagnosis.

**Homologs of Small Nucleolar RNAs in Archaea**

**Arina D. Omer,** Todd M. Lowe, Anthony G. Russell, Holger Ebbhardt, Sean R. Eddy, Patrick P. Dennis

In eukaryotes, dozens of posttranscriptional modifications are directed to specific nucleotides in ribosomal RNAs (rRNAs) by small nucleolar RNAs (snorRNAs). We identified homologs of snoRNA genes in both branches of the Archaea. Eighteen small sno-like RNAs (sRNAs) were cloned from the archaeon *Sulfolobus acidocaldarius* by coimmunoprecipitation with archaeal fibrillarin and NOP56, the homologs of eukaryotic snoRNA-associated proteins. We trained a probabilistic model on these sRNAs to search for more sRNAs in archaeal genomic sequences. Over 200 additional sRNAs were identified in seven archaeal genomes representing both the Crenarchaeota and the Euryarchaeota. snoRNA-based RNA processing was therefore probably present in the last common ancestor of Archaea and Eukarya, predating the evolution of a morphologically distinct nucleolus.

Ribosomal biogenesis in Eukarya occurs in the nucleolus. Several nucleolar proteins (NOPs), including fibrillarin, Nop56, and Nop58, and dozens of snoRNAs are involved in this process (1). The snoRNAs fall into two major classes: C/D box and H/ACA box RNAs. The C/D box snoRNAs are efficiently recruited from other Pediatric Oncology Group (POG) institutions before treatment began. Informed consent was obtained, and procedures approved by the Committee on Human Research at the University of Vermont and other POG institutions were followed. Peripheral blood was separated and the mononuclear cell fraction was obtained for the T cell cloning assay within 12 to 24 hours of its collection. The T cell cloning assay and analysis have been described [see B. A. Finette et al., Mutat. Res. 308, 223 (1994)].

12. Mutation frequencies in subjects at diagnoses, remission, and relapse were compared with normal controls by the nonparametric Kruskal–Wallis test. Pairwise differences between groups were assessed by Mann–Whitney tests, with a Bonferroni adjustment for multiple comparisons. Linear regression analysis was used to examine the relationship between the logarithm of MFI (lmFI) and months since diagnosis.

**Homologs of Small Nucleolar RNAs in Archaea**

**Arina D. Omer,** Todd M. Lowe, Anthony G. Russell, Holger Ebbhardt, Sean R. Eddy, Patrick P. Dennis

In eukaryotes, dozens of posttranscriptional modifications are directed to specific nucleotides in ribosomal RNAs (rRNAs) by small nucleolar RNAs (snorRNAs). We identified homologs of snoRNA genes in both branches of the Archaea. Eighteen small sno-like RNAs (sRNAs) were cloned from the archaeon *Sulfolobus acidocaldarius* by coimmunoprecipitation with archaeal fibrillarin and NOP56, the homologs of eukaryotic snoRNA-associated proteins. We trained a probabilistic model on these sRNAs to search for more sRNAs in archaeal genomic sequences. Over 200 additional sRNAs were identified in seven archaeal genomes representing both the Crenarchaeota and the Euryarchaeota. snoRNA-based RNA processing was therefore probably present in the last common ancestor of Archaea and Eukarya, predating the evolution of a morphologically distinct nucleolus.

Ribosomal biogenesis in Eukarya occurs in the nucleolus. Several nucleolar proteins (NOPs), including fibrillarin, Nop56, and Nop58, and dozens of snoRNAs are involved in this process (1). The snoRNAs fall into two major classes: C/D box and H/ACA box RNAs. The C/D box snoRNAs are efficiently precipitated with antibodies against fibrillarin. Most C/D box snoRNAs target specific ribose methylations within rRNA, whereas most H/ACA box RNAs target specific conversions of uridine to pseudouridine within rRNA (2).

The general mechanism of C/D box snoRNA-targeted ribose methylation has been well established. Each snoRNA contains a 9- to 21-nucleotide (nt)–long sequence, located 5' to the D or D' box motif, that is complementary to an rRNA target sequence. Methylation is directed to the RNA nucleotide that participates in the base pair 5 nt upstream from the start of the D or D' box. It is likely that most, if not all, eukaryotic ribose ribose methylations are guided by snoRNAs. In the yeast *Saccharomyces cerevisiae*, methylation guide snoRNAs have been assigned to all but four of the 55 rRNA ribose methylation sites (3).

SnoRNAs, which are apparently ubiquitous in Eukarya, have not been found in Bacteria or Archaea. However, the rRNA of the archaeon *Sulfolobus solfataricus* (5) has been shown to contain 67 ribose methylation sites, a number similar to that found in eukaryotes (4). Even though Archaea are unicellular prokaryotic organisms that lack a nucleolus, their genomes encode homologs to the essential eukaryotic nucleolar proteins, fibrillarin and NOP56/58 (5, 6). On the basis of these observations, we decided to examine Archaea for the presence of sno-like RNAs (sRNAs).

To isolate sRNAs from the archaeon *Sulfolobus acidocaldarius* (Sac), we cloned the *S. acidocaldarius* homologs of the eukaryotic fibrillarin and NOP56/58 proteins, designated aFIB and aNOP56, using sequence information from a related species, *S. solfataricus* (7). The cloned genes were expressed in *Escherichia coli*, and the recombinant proteins were purified and used to raise polyclonal antibodies in rabbits. The two antibody preparations were each highly specific and recognize single polypeptides of the predicted size in total *S. acidocaldarius* cell extracts (Fig. 1A). The antibodies were used to monitor the size distribution of particles containing aFIB and aNOP56 in a glycerol gradient fractionation of partially purified cell lysate (Fig. 1A) (8). Both aFIB and aNOP56 sedimented as a large heterogeneous complex.

To detect RNAs that associate with aFIB- and aNOP56-containing complexes, we immunoprecipitated aliquots from gradient fractions with either antibody to aFIB or antibody to aNOP56. Total RNA was extracted with phenol from the supernatants and the pellets, and a portion from each was 3' end-labeled with [*P]*-cytidine-5',3'-bis-phosphate (pCp) and displayed by denaturing polyacrylamide gel electrophoresis (Fig. 1, B and C). The most abundant RNAs that were coimmunoprecipitated appeared as a family of bands ranging in length from about 50 to 70 nt. This size class of RNAs, which is substantially shorter than eukaryotic C/D box snoRNAs, was invisible when total cellular RNA was labeled with pCp. To obtain cDNA clones, we gel-purified the RNAs precipitated from fraction 5 with antibody to aFIB and from
after electroelution was end-labeled and displayed on an 8% denaturing polyacrylamide gel (right).

An aliquot of the pooled RNA was end-labeled and displayed on an 8% denaturing polyacrylamide gel. The positions of tRNA and sRNA are indicated on the left. The precipitated RNA recovered from fraction 5 was separated on an 8% denaturing polyacrylamide gel, recovered by electroelution, and used as a template for RT-PCR cloning (right). (B) Aliquots from every other gradient fraction between 2 and 20 were simultaneously analyzed by Western blotting for the presence of aFIB and aNOP56 with the two antibodies prepared against the recombinant proteins expressed and purified from E. coli. The positions of 30S and 50S ribosomal subunits in the gradient are indicated. In the control, the aFIB antisomomal subunits in the gradient are precipitated with antibody to aFIB (right). (C) Aliquots from fractions 6 to 8 and 10 to 13 with antibody to aNOP56, ligated them to the oligonucleotide AO30, used them as template for reverse transcription polymerase chain reaction (RT-PCR), and cloned them (9).

A total of 104 clones from the two immuno precipitated RNA pools were sequenced. From these, one or more representatives of 18 different sequences that exhibited features characteristic of eukaryotic C/D box snoRNAs were recovered (Table 1) (2). Other clones contained small fragments of S. acidocaldarius 16S, 23S, and 5S rRNAs. The snoRNA-like clones contained well-defined C and D box motifs located near their 5' and 3' ends, respectively, and recognizable internal C' and D' box motifs, giving the RNAs a dyad repeat structure characteristic of eukaryotic methylation guide snoRNAs (10).

Primer extension analysis was used to confirm the presence of sRNAs within total RNA extracted from S. acidocaldarius. Each sRNA primer was designed to overlap the D box motif, the adjacent guide region, and a portion of the C' box motif. Extension products were obtained for sR1 to sR17 (clone sR18 was identified later and not tested); a subset of these is illustrated in Fig. 2. The lengths of the products for all sRNAs were within 2 nt of the 5' ends of the cDNA, except for sR3, 4, 6, and 8, which were between 3 and 5 nt longer than the respective cDNA clones (11).

To find additional homologs of our cloned S. acidocaldarius sRNA genes, we ran BLASTN on each cDNA clone against the nonredundant nucleotide database (12) and recovered two weak hits against sequences in other Sulfolobus species: Sac-sR3 had a hit near the Sulfolobus shibatae top6B topoisomerase II gene (score = 40.1 bits, expectation value = 0.038), and Sac-sR1
had a hit that partially overlapped with the \textit{S. solfataricus} aspartate aminotransferase gene (score = 38.2 bits, expectation value = 0.15). Although these candidates contained canonical C and D boxes, their authenticity as true sRNAs remained questionable because of their low scores. We tested for the presence of the Sac-sr1 homolog by primer extension analysis using \textit{S. solfataricus} RNA as a template. A product with a length similar to that of Sac-sr1 was detected (Fig. 2C) and was designated Sso-sr1. Primer extension products for cloned \textit{S. acidocaldarius} RNA sr1 to sr17 and the apparent \textit{S. solfataricus} sr1 homolog demonstrate the existence of archaeal snoRNA-like C/D box sRNAs.

To determine if these sRNAs might guide ribose methylation as in eukaryotes, we examined the sRNAs for potential guide sequences by comparison with \textit{S. acidocaldarius} tRNA (13). Regions complementary to tRNA and adjacent to the D or D' boxes were identified for 14 of the sRNAs (Table 2). Using the D/D' box + 5 nt rule, we predicted the locations of potential ribose methyl modifications in tRNA and experimentally tested for some of these sites using the deoxyribonucleotide triphosphate (dNTP) concentration-dependent primer extension assay (3, 14). In this assay, ribose 2'-O-methyl sites cause characteristic pauses that are displayed in the reverse transcriptase reactions at low but not at high dNTP concentrations. We identified characteristic pauses at six predicted sites of methylation in \textit{S. acidocaldarius} tRNA (Table 2). Several examples are shown in Fig. 3. Both Sac-sr1 and Sso-sr1 were predicted to target methylation to position U52 in the respective 16S rRNAs; pause sites were detected at this position in both rRNAs. Two of the sRNAs, sr10 and sr14, exhibit strong complementarities to \textit{S. solfataricus} tRNAs (Table 2). The target nucleotide for sr14 is C34, the anticodon “wobble” base, which is commonly ribose methylated in eukaryotes (15). Not all eukaryotic C/D box snoRNAs containing complementary regions participate in ribose methylation (i.e., U3 and U8), so methylation guide function should not be assumed for all archaeal C/D box sRNAs. Gene disruption systems for \textit{S. acidocaldarius} and most other Archaea are currently not available; consequently, we were not able to verify loss of predicted methylation sites upon disruption of sRNA genes. However, our evidence suggests that many of the positions predicted may be true methylation guides.

Fig. 2. Detection and 5' end mapping of sRNAs from \textit{S. acidocaldarius} and \textit{S. solfataricus}. Primers specific for the D box guide region of Sac sr1 to sr17 were 5' end-labeled with \gamma-P-ATP and polynucleotide kinase and used in extension reactions with total RNA (10 μg) isolated from \textit{S. acidocaldarius} as template. (A) The extension products obtained with Sac sr1 and sr2 specific oligonucleotide primers were run alongside a 32P-DNA sequence ladder generated with the same primers and Sac sr1 or sr2 cDNAs as template. (B) The extension products obtained with Sac sr8, sr14, sr3, sr5, sr6, sr16, and sr10 specific primers and run with the DNA sequence ladder generated with Sac sr8 cDNA clone. The main sr8 extension product is 3 nt longer than the 5' end of the sr8 cDNA clone. For each extension reaction, the major extension product (>50%) and the approximate positions of the 5' terminal nucleotide in the corresponding cDNA clone (*) are indicated beside the lane. (C) The primer extension reaction was as in (A), except that the primer was specific to Sso sr1 and total RNA from \textit{S. solfataricus} was used as template. The DNA ladder was generated with Sac sr1 primer and the Sac sr1 cDNA clone as template. The Sac and Sso primers are complementary to the same region but differ at two internal positions.

Table 2. Annotations of \textit{S. acidocaldarius} sRNAs.

| Sac | Ab* | PE Conf† | Guide | Target‡ | Match§ | Notes|| |
|-----|-----|----------|-------|---------|--------|-------|
| sr1 | F   | +        | D     | 165 U52 | 11/0   | CDP, Mod |
| sr2 | F   | +        | D     | 235 C1914 | 11/0  | CDP |
| sr3 | F   | +        | D     | 235 G2739 | 10/0  | No pause |
| sr4 | N   | +        | D     | 235 G1995 | 10/0  | No pause |
| sr5 | F, N | +        | D     | 165 G1056 | 12/0  | CDP, Mod |
| sr6 | F   | +        | D     | 235 G2266 | 9/1   | No pause |
| sr7 | F   | +        | D'    | 235 G2649 | 9/0   | No pause |
| sr8 | N   | +        | D'    | 235 U2692 | 10/0  | No pause |
| sr9 | F   | +        | D'    | 165 G326 | 12/1  | CDP |
| sr10| F   | +        | D'    | tRNA Gly-CCC C50 | 12/0 | CDP |
| sr11| F   | +        | D'    | 235 A2618 | 10/2  | No pause |
| sr12| F   | +        | D'    | 235 G1114 | 11/0  | CDP |
| sr13| N   | +        | D'    | 235 G2855 | 10/1  | No pause |
| sr14| F   | +        | D'    | 235 G2999 | 11/1  | CDP |
| sr15| F   | +        | ?    | tRNA Gln-UUG U34 | 10/0 | CDP |
| sr16| N   | +        | ?    | ?        | ?      | ? |
| sr17| F   | +        | ?    | ?        | ?      | ? |
| sr18| F   | +        | D'    | 235 G140  | 9/1   | ? |

*The precipitations from which the respective sRNAs were recovered: F, antibody to srlF; N, antibody to sRNPs. †The presence of the sRNA in total cellular RNA was verified by primer extension. ‡The position of the guide within the sRNA (D or D' box associated) and the predicted site of methylation in the target RNA are indicated. §The number of matches and mismatches in the complementarity between the guide and target sequence are indicated. Proposed complementarity to tRNA targets is based on the following criteria: Watson-Crick base pair at position -5 (site of methylation); a minimum of eight base pairs with no more than two GU base pairs; one mismatch permitted at positions other than -5. ||CDP,” dNTP concentration-dependent primer extension pause observed at predicted site of methylation, indicating likely ribose 2'-O-methyl; "No Pause," no pause was detected at either high or low dNTP concentrations; "Mod," known site of nucleotide modification of unknown type in \textit{S. acidocaldarius} 16S rRNA (13). Guides without notation were not experimentally examined.
these sRNAs function as guides for ribose methylation, as in eukaryotes.

We next asked whether sRNAs are found in other Archaea. We retrained a previously developed eukaryotic snoRNA search program with the verified S. acidocaldarius snoRNA genes (3, 16) and used it to screen the available archaeal genome sequences. We first searched the genome sequence of the closely related archaeon S. solfataricus (17). The program identified dozens of sRNA candidates, each of which had the potential to target a modification to a particular position in rRNA. We designed primers complementary to the top scoring candidate sRNAs and performed primer extensions on S. solfataricus total RNA to detect stable RNAs. Ten candidates (Sso sR1 to sR10), all ranking within the top 13 candidates by score, generated products of the anticipated size, 2 to 6 nt upstream of the predicted C box. An alignment of the 10 verified S. solfataricus sRNAs, plus three high-scoring, untested sRNA predictions (sR11 to sR13), is available (18). Six predicted target ribose methylation sites were assayed with the dNTP concentration-dependent primer extension assay (Fig. 3), and four showed reverse transcription pauses characteristic of ribose methylation (18). Three additional target site predictions are known to be modified at the homologous position in S. acidocaldarius 16S rRNA (13, 18).

Sulfolobus is a member of the Crenarchaeota, one of the two main phyla of Archaea; the other phylum, the Euryarchaeota, is evolutionarily distant. Complete genome sequences are available from archaean species covering a wide range of genera, including both the Crenarchaeota (Aeropyrum pernix) and the Euryarchaeae (Methanococcus jannaschii, Archaeoglobus fulgidus, Methanobacterium thermoautotrophicum, Pyrococcus horikoshii, Pyrococcus abyssi, and Pyrococcus furiosus) (5, 17). In searching these genomes for guide sRNAs, we found strong candidates in six of the seven species (18).

The searches of the M. jannaschii (Mja) and A. fulgidus (Afu) genomes gave eight and four strong sRNA hits, respectively; guide regions in most of these candidates exhibit complementarity to tRNA (18). The presence of all eight Mja sRNAs was confirmed by primer extension analysis on M. jannaschii total RNA (18). We attempted to verify seven of the ribose methylation sites predicted by the Mja sRNAs. Five sites showed concentration-dependent pauses indicative of ribose methylation, and the two other sites showed concentration-independent pauses, inconclusive for ribose methylation (18). An example pause site predicted by Mja-sR6 at position C2034 in 23S rRNA is shown (Fig. 3D). The D box guide region of Mja-sR8 predicts methylation of the anticodon wobble base for the intron-containing precursor of tRNA-Met. We did not test any tRNAs for ribose modifications, although the wobble base in tRNA-Met is known to be ribose-methylated within another hyperthermophilic crenarchaeon (19). The search of the Aeropyrum pernix (Ape) genome produced 23 candidate sRNAs (18). There were no strong sRNA hits in the genome of M. thermoautotrophicum (20).

The genomes of three Pyrococcus species have been sequenced: P. horikoshii (Pho), P. furiosus (Pfu), and P. abyssi (Pab) (17). These related sequences enabled us to infer support for sRNA predictions using comparative sequence analysis. From separate genome searches followed by comparative analysis, we identified 57 groups of homologous Pyrococcus sRNA genes (21). Forty-seven groups were found in all three species, eight were found in only two species, and two were unique to single species. Examples of two of these groups, sR3 and sR4, are illustrated (Fig. 4), and the complete set is available online, as are the alignment, annotation, and genomic distribution of the candidate sRNAs found in P. horikoshii (18).

We asked whether predicted sRNA methylation sites occurred at homologous rRNA positions in different archaeal genera. We view our site predictions with caution, as the sRNA complementarities are short and few have been experimentally tested. Nonetheless, on the basis of an sRNA multiple alignment (22), a total of 19 predicted methylation sites were conserved between two or more genera. Figure 4A shows 16S Um52, a confirmed modification in Sulfolobus, which we predict is guided by sR1 in Sulfolobus and by sR4 in Pyrococcus. However, Sulfolobus sR1 and Pyrococcus sR4 also have dissimilar D’ associated guide sequences that are predicted to target methylation to nonhomologous positions (16S Um33 in S. solfataricus and 16S Am361 in Pyrococcus). Figure 4B shows that the predicted guide sequences for a site in 23S rRNA (Sac U2692, Ape U2714, and Pho U2673) contain four separate nucleotide substitutions that are matched by compensatory substitutions in

---

**Fig. 3.** Detection of 2’-O-ribose methylation sites in rRNA. Positions of ribose methylation in rRNA were detected with the dNTP concentration-dependent primer extension pause assay (3, 14). Total RNA from S. acidocaldarius (A to C) and M. jannaschii (D) was used as template. The sequence ladder produced was generated from either DNA or RNA templates with the same primers used in the pause reactions. The position of pausing is indicated on the right along with the position of the methylated nucleotide; the pause characteristically occurs 1 nt upstream of the modification. When a sequence ladder is generated from DNA template, as in (A), the pause may occur 1 nt upstream of the modification or directly at the modification. The sequence of the sRNA guide and the complementary rRNA target are shown below each panel; the site of methylation in rRNA is in the base pair (boxed) positioned 5 nt upstream of the start of the D or D’ box.

---

**Fig. 4.** Taxonomic distribution of the 57 groups of homologous sRNAs found in the genomes of P. horikoshii (Pho), P. furiosus (Pfu), and P. abyssi (Pab). The related sequences enabled us to infer support for sRNA predictions using comparative sequence analysis. From separate genome searches followed by comparative analysis, we identified 57 groups of homologous Pyrococcus sRNA genes (21). Forty-seven groups were found in all three species, eight were found in only two species, and two were unique to single species. Examples of two of these groups, sR3 and sR4, are illustrated.
23S rRNA, strong evidence that this sRNA/ rRNA interaction is evolutionarily conserved. In nearly all cases, the intergenera sequence similarity between sRNAs that predict methylation at a homologous site is limited to the interacting guide region. In only one instance, we detected some end-to-end sequence similarity between two sRNAs from different archaeal genera: Pho-sr39 and Mja-sr6 (Fig. 4C). Moreover, the guide sequences can be either both in the same position (i.e., both D box associated) or in different positions (i.e., one D’ and the other D box associated; see Fig. 4B). Therefore, simple relationships of homologous sRNAs with homologous methylation sites are not obvious, and it remains uncertain whether sRNA guide sequences directing methylation to a homologous site are related to each other by common ancestry or by sequence convergence.

In general, all the archaeal sRNAs we identified are small, usually 50 to 60 nt in length, whereas human and yeast methylation guide snoRNAs average roughly 75 and 100 nt, respectively (65°C) has no easily recognizable sRNAs. In Bacteria, there is a low abundance of 2'-O-methylation and pseudouridylation in tRNA, and neither a fibrillarin homolog nor C/D box sRNAs have been described. Nonetheless, the existence of sRNA-directed modifications in bacterial stable RNAs remains a possibility.

The number of sRNAs revealed by the search program seems to correlate with the optimum growth temperature of the organism: Pyrococcus species (95°C) have more than 50 putative sRNAs, whereas M. thermoautotrophicum (65°C) has no easily recognizable sRNAs. This may imply that a larger number of methylation modifications in rRNA might be required to fold or stabilize rRNA at high temperature (4) or that sRNAs are easier to recognize in hyperthermophiles because their gene features are more canonical.

For eukaryotes, sRNAs do not act solely on rRNA. A number of cellular and viral RNAs transit through the nucleolus during maturation and at least one of these, the spliceosomal snRNA U6, is a substrate for sRNA guide-directed methylation (23). Three cloned, verified Sac sRNAs (Table 2) do not appear to target any known stable RNAs (18), and several archaeal sRNAs exhibit complementarity to various tRNAs. Four of the sRNAs we identified (the Pyrococcus sr40 genes and Afu sr3) reside within the intron of the genes encoding tRNA-Trp. Our program detected these putative genomic sRNAs because they appeared to be capable of targeting methylation to sites within tRNA (18). However, Daniels and co-workers (24) have independently identified these sRNAs and suggest that the D’ and D box guides are targeting methylation to positions C34 and C39 within the intron-containing precursor tRNA. These observations suggest that both ribosomal and nonribosomal RNAs may be substrates for sRNA guide-directed methylation in Archaea.

Thus, it appears that an RNA-based guide mechanism for directing specific RNA 2'-O-ribose methylations was an established feature in the common ancestor of Archaea and Eukarya (5). In Bacteria, there is a low abundance of 2'-O-methylation and pseudouridylation in tRNA, and neither a fibrillarin homolog nor C/D box sRNAs have been described. Nonetheless, the existence of sRNA-directed modifications in bacterial stable RNAs remains a possibility.

**References and Notes**


6. A clone (Sso cosm id number 33) containing the alf and anNOPS genes from *S. solfataricus* was provided by M. A. Ragan and C. W. Sensen. We used Southern hybridization to identify a 5-kb Xba I restriction fragment containing the corresponding genes from *S. acidocaldarius*; the fragment was cloned (pPD 1238), and its sequence was determined. The 16S RNA sequences from *S. acidocaldarius* and *S. solfataricus* are 76% identical; the alfB and anNOPS predicted amino acid sequences are 76% and 65% identical, respectively. The *S. acidocaldarius* alfB protein is 45% and 46% identical to the yeast and human proteins, and anNOPS protein is 32% identical to the yeast and human proteins. Our choice of the name anNOPS is arbitrary, because the anNOPS sequence is also similar to eukaryotic NOP5/ NOP58; the eukaryotic EIF4/NO5/P058.
proteins appear to be paralogs that arose by a gene duplication in Eukarya. The GenBank accession numbers for the aNOP56 and aFIB protein sequences from S. acidocaldarius are YF201092 and YF201093, respectively.


9. P. Wu, J. S. Brockenbrough, A. C. Metcalf, S. Chen, J. P. Aris, J. Biol. Chem. 273, 16453 (1998). Briefly, the primer AO30 (5′-CTGACATCTGGACCCGGG-3′) was 5′ phosphorylated with T4 polynucleotide kinase and adenosine triphosphate (ATP) and blocked at the 3′ end with terminal deoxynucleotidyl transferase (Gibco BRL) and dideoxycytidine triphosphate (ddCTP). The modified oligo was then ligated to gel-purified sRNA for 16 hours at 4°C. The ligation products were reverse transcribed with Thermoscript RT (Gibco BRL) at 55°C for 30 min, with AO31 (5′-CCCGATTCGATCTGGACCCGGG-3′) as primer. The RNA template was hydrolyzed with ribonuclease H, and the cDNA strand was extended with deoxy-ATP with terminal deoxynucleotidyl transferase. The extended cDNA strand was used as template for PCR (95°C denaturation, 65°C hybridization, 72°C extension, 30 cycles) with AO31 and AO32 (5′-CCGGATCCAGATCTCGAG-3′) as primers. The DNA products were cleaved with Pst I and Xho I, ligated between their sequences were determined.


11. Southern hybridizations confirmed the existence of single-copy genes for sR1, sR2, sR5, and sR13 in S. acidocaldarius genomic DNA. Genomic clones of the single-copy genes for sR1, sR2, sR5, and sR13 in S. acidocaldarius were isolated and sequenced. In both cases, the sRNA sequences overlap the 3′ end of the corresponding aspartate aminotransferase genes. The translation termination codons UAA for S. acidocaldarius and UAG for S. solfataricus fall within the D box guide regions in the two sRNAs (Fig. 4A).


13. G. J. Olsen et al., J. Mol. Evol. 22, 301 (1985); D. A. Stahl, K. R. Luehrs, C. R. Woese, N. R. Pace, Nucleic Acids Res. 9, 6129 (1981); E. Darms, P. Londei, P. Cammarano, A. Vandenbergh, R. De Wachter, Nucleic Acids Res. 11, 4667 (1983); P. Durovic and P. P. Dworkin, Mol. Microbiol. 13, 229 (1994). As a result of strain misidentification, the 16S RNA sequence published in the first paper is now recognized to be from S. acidocaldarius and not S. solfataricus as originally indicated. The two originally published 5′ ends of the 16S RNA sequence are identical and both are derived from S. solfataricus. The S. solfataricus SS5 sequence has been published more recently along with a brief documentation of the strain misidentification problem [P. M. Ajuh, P. Londei, P. Dworkin, Biochimie 77, 721 (1995)] as primers. The DNA products were cleaved with Pst I and Xho I, ligated between their sequences were determined.

14. B. E. H. Maden, M. E. Corbett, P. A. Heeney, K. Pugh, J. Biol. Chem. 273, 77 (1998). This assay is not further primer extension success rate with archaeal species appear to be paralogs that arose by a gene duplication in Eukarya. The GenBank accession numbers for the aNOP56 and aFIB protein sequences from S. acidocaldarius are YF201092 and YF201093, respectively.

15. A pivotal step in the initiation of T cell immunity is the presentation of antigenic peptides by MHC II molecules, which are expressed on DCs. In general, MHC II molecules bind peptides formed in endocytic organelles (J). In antigen-presenting cells (APCs) such as B lymphocytes, MHC II molecules in late endosomal and lysosomal compartments (collectively termed MHC II compartments) together with other components required for antigen processing. These include the invariant (I) chain that targets MHC II from the Golgi to