Post-transcriptional Adenylation of Signal Recognition Particle RNA Is Carried Out by an Enzyme Different from mRNA Poly(A) polymerase*

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A fraction of the signal recognition particle (SRP) RNA from human, rat, Xenopus, and Saccharomyces cerevisiae cells contains a single post-transcriptionally added adenylic acid residue on its 3′-end; in the case of human SRP RNA, over 60% of the SRP RNA molecules contain a nontemplated adenylic acid residue on their 3′-ends (Sinha, K. M., Gu, J., Chen, Y., and Reddy, R. (1998) J. Biol. Chem. 273, 6853–6859). In this study, we investigated the enzyme that is involved in this 3′-end adenylation of SRP RNA. A U1A protein peptide conjugated to albumin completely inhibited the polyadenylation of a 540 mRNA by HeLa cell nuclear extract in vitro; however, the 3′-end adenylation of human SRP RNA or Alu RNA, which corresponds to 5′ and 3′-ends of SRP RNA, was not affected by this U1A peptide conjugate. SRP RNA from mutant strains of S. cerevisiae with a temperature-sensitive mRNA poly(A) polymerase grown at a restrictive temperature of 37 °C also contained a post-transcriptionally added adenylic acid residue just like SRP RNA from wild-type cells and mutant cells grown at permissive temperature of 23 °C. In addition, binding of SRP 9/14-kDa protein heterodimer was required for adenylation of Alu RNA in vitro. These lines of evidence, along with other data, show that post-transcriptional adenylation of SRP and Alu RNAs is carried out by a novel enzyme that is distinct from the mRNA poly(A) polymerase, CCA-adding enzyme, and nonspecific terminal transferase.

Following transcription, eukaryotic precursor RNA molecules undergo various modifications and processing reactions. These modifications include 5′ capping, 3′ polyadenylation, splicing of pre-mRNAs, editing, and modifications on the base, sugar, and phosphate residues. We studied the formation of the 3′-end of several small RNAs, and results showed that a significant fraction of some human small RNAs including SRP (7SL) RNA, nuclear 78K RNA, spliceosomal U2 small nuclear RNA (snRNA)1 and ribosomal 5 S RNA contain a single post-transcriptionally added adenylic acid residue on their 3′-ends (1). This 3′-end adenylation is conserved through evolution, since SRP and U2 snRNAs from Saccharomyces cerevisiae, Xenopus, rat, and human cells contain this nontemplated post-transcriptionally added adenylic acid residue. We also developed an in vitro system in which SRP RNA is accurately processed by the HeLa cell nuclear extract, where three transcriptionally encoded uridylic acid residues are removed on the 3′-end and a single adenylic acid residue is added on the 3′-end (2). These studies also showed that the Alu portion of the SRP RNA or an 87-nucleotide-long RNA containing 5′ and 3′ portions of the SRP/Alu RNA is necessary and sufficient to direct accurate 3′-end processing and adenylation (2). However, not every small RNA contains this post-transcriptionally added adenylic acid residue on its 3′-end. For example, there was no detectable adenylic acid residue on the 3′-end of abundant nuclear RNAs like U1 or U4 snRNAs. These data show that nontemplated adenylic acid residues are specifically added to some cellular RNAs (1).

While the exact function of this 3′-adenylation in SRP RNA or in other small RNAs is not known, several facts point toward the importance of this adenylation. The phenomenon of adenylation is conserved through evolution from yeast to humans. The extent of 3′ adenylation increases through evolution, indicating that this modification confers an advantage. For example, in the case of S. cerevisiae cells only 2–3% of the SRP RNA and U2 snRNA are adenylated, whereas in the case of human cells over 60% of the SRP RNA and U2 snRNA contain this post-transcriptional adenylation. The adenylation occurs very early in the biogenesis of the SRP RNA and is maintained by constant turnover (2). Xu and Cohen (3) provided evidence for RNA degradation mediated by the addition of a short poly(A) tail by poly(A) polymerase. However, in this case, only one adenylic acid residue is added, and over 60% of human SRP RNA molecules contain this adenylation. Therefore, the function of this 3′-adenylation is unlikely to be related to the turnover/degradation of SRP RNA.

There are several well-studied enzymes that add adenylic acid residues to the 3′-end of RNAs. The most characterized among these enzymes are the CCA-adding enzyme to the 3′-end of transfer RNAs and the mRNA poly(A) polymerase responsible for poly(A) formation in mRNAs (4, 5). In addition to polyadenylation of mRNAs, poly(A) polymerase is known to be responsible for the addition of one adenylic acid residue, few adenylic acid residues, or poly(A) to the 3′-end of many other RNAs including human telomerase RNA (6) and some stable small RNAs (7, 8). The CCA addition is carried out by a tRNA-specific nucleotidyltransferase, and the same enzyme adds both C and A residues to the 3′-end of tRNAs (9). In fact, Weiner and co-workers (10) have shown that the CCA-adding enzyme contains a single active site that contacts the same tRNA structure while adding both the C and A residues. The tRNA nucleotidyltransferase covalently linked to the tRNA substrate was capable of adding both C and A residues (11). Since the SRP-adenylating enzyme adds only an A residue but not a C residue (1), 3′-adenylation of SRP RNA is not mediated by tRNA nucleotidyltransferase.

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1 The abbreviations used are: snRNA, small nuclear RNA; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; SRP, signal recognition particle; RNP, ribonucleoprotein.
The mRNA poly(A) polymerase specifically adds a poly(A) tail to the mRNAs in the presence of specificity factors. In the absence of specificity factors, poly(A) polymerase is capable of adding multiple adenylic acid residues to any RNA containing 3′ OH groups. While the S. cerevisiae contains a single poly(A) polymerase (12), the human cells contain several isoforms of poly(A) polymerase (13, 14). However, all these isoforms of human poly(A) polymerase are closely related, and specific polyadenylation is dependent on AAUAAA signal and specificity factors (13). Stable RNAs with 1–7 adenylic acid residues on their 3′-ends accumulate in 3′ exonuclease-deficient Esche- richia coli and in yeast strains (7, 8, 15). In E. coli strain lacking tRNA nucleotidyltransferase, poly(A) polymerase participates in the incorporation of A residue into the defective tRNA-C-C in order to maintain functional tRNA (16). Tyrosine tRNA in chicken mitochondria contains a short poly(A) tail out of which a single adenylic acid is retained before CCA is added to obtain a functional tyrosine tRNA. This short poly(A) tail in this tRNA is added by poly(A) polymerase (17). The mitochondria ribosomal RNAs are also known to contain a poly(A) tail (18, 19). The enzyme involved in the addition of these adenylic acid residues to the stable RNAs is the poly(A) polymerase (7, 8, 18, 20). In addition, there are several other instances, including bacteriophage oop RNA (21), where 3′ adenylyl residues are added by poly(A) polymerase. Further, poly(A) polymerase surprisingly adds only a single adenylic acid residue in vitro under conditions of limiting ATP concentrations (22). Therefore, it appeared likely that the enzyme responsible for the addition of adenylic acid residue to the 3′-end of SRP RNA may be mRNA poly(A) polymerase. Several lines of evidence obtained in this study show that mRNA poly(A) polymerase is not involved in the 3′ adenylation of SRP RNA; therefore, a novel adenylation enzyme is responsible for the addition of a single adenylic acid residue to the 3′-end of SRP RNA.

MATERIALS AND METHODS

Chemicals and Isotopes—[α-32P]ATP and [α-32P]UTP were purchased from Amersham Pharmacia Biotech. All other chemicals were obtained from Sigma. Tag DNA polymerase was purchased from Life Technologies, Inc. T7 RNA polymerase and all other restriction enzymes were obtained from New England Biolabs. The TA-Cloning Kit was from Invitrogen, and the PCR DNA product purification kit was from QIAGEN. The BSA-U1A peptide and free U1A peptide were obtained through Research Genetics, Inc. (Huntsville, AL).

Preparation of Substrate RNAs—Plasmid DNA containing the Alu portion of canine SRP RNA (pAlu) under the T7 promoter was a gift from Dr. Katharina Strub (23). This Alu sequence was altered by PCR in order to insert a DraI site on its 3′-end and was cloned into pUC19 vector by insertion into EcoRI and HindIII sites. The Alu RNA was transcribed by T7 RNA polymerase from the DNA template linearized by DraI, and the transcribed RNA contained three uridylic acids on the 3′-end. DNA template to prepare mutant Alu RNA was constructed by PCR-mediated mutagenesis, cloned into pUC19 vector, and transcribed by T7 RNA polymerase from the plasmid DNA template linearized by DraI.

For the polyadenylation of yeast CYC1 pre-mRNA with yeast extract, the plasmid DNA containing the CYC1 gene (a gift from Dr. Walter Keller) was linearized with XhoI restriction enzyme, and capped CYC1 pre-mRNA was synthesized in vitro with T7 RNA polymerase including [α-32P]UTP as the labeled nucleotide. The run-off CYC1 pre-mRNA transcript was 194 nucleotides long, containing a few nucleotides down-stream of the AAUAAA polyadenylation signal. This precleaved RNA was used as a substrate for polyadenylation by yeast extract in vitro, and this method of RNA preparation is identical to that described by Keller’s laboratory (24). For the polyadenylation of SV40 late pre-RNA from HeLa cell nuclear extract, the plasmid DNA containing SV40 late pre-mRNA (kindly provided by Dr. Susan Berget) was linearized with HpaI, and capped precleaved RNA was transcribed with SP6 RNA polymerase including [α-32P]UTP as a labeled nucleotide. The in vitro transcription with T7 RNA polymerase was performed according to standard protocol (New England Biolabs). All RNA products were purified by fractionation on a 10% polyacrylamide/7 M urea gel, extracted from the gel, and purified by precipitation with ethanol. The concentration of the RNAs, whenever necessary, was determined by optical density measurements at 260 nm.

Growth Conditions for Wild-type and Temperature-sensitive Yeast Strains—The wild-type yeast cells were grown with shaking in YEPD medium at 30°C to an A600 between 2 and 6. The temperature-sensitive mutants for poly(A) polymerase pap1-2, pap1-5, and pap1-7 (kindly provided by Dr. Walter Keller; see Ref. 25) were grown in YEPD medium to a concentration of 1–3 × 105 cells/ml at 23°C, and then the cultures were divided into two halves, and one half was incubated with shaking at 37°C for 6 h. The other half of the cultures was left to grow at 23°C for 6 h. The yeast cells were harvested by centrifugation at 1000 × g for 5 min and then used either for preparation of whole cell extracts or isolation of total RNA according to published procedures (26, 27).

Polyadenylation Assay in Yeast Whole Cell Extract—The polyadenylation reaction was done according to Minvielle-Sebastia et al. (25). Briefly, the 25-μl reaction volume contained 40% (v/v) extract, 1.6 μM Heps-KOH (pH 7.9), 0.016 mM EDTA, 4 mM potassium chloride, 1 mM dithiothreitol, 1.6% glycerol, 2% polyethylene glycol, 75 mM potassium acetate, 2 mM ATP, 20 mM creatine phosphate, creatine kinase (0.2 mg/ml), 0.01% Nonidet P-40, and precleaved CYC1 RNA (~50,000 cpm). The reaction was carried out for 60 min at 30°C for extracts from mutant cells and at 30°C for extracts from wild-type cells grown at 23°C. Yeast cells grown at 37°C were harvested by centrifugation at 1000 × g for 5 min and then used either for preparation of whole cell extracts or isolation of total RNA according to published procedures (26, 27).

Preparation of HeLa Cell Nuclear Extract and in vitro Labeling—Extracts were prepared from HeLa cells grown in suspension culture by the procedure of Dignam et al. (28). The final protein concentration of the extract was 5 mg/ml. The nuclear extract was fractionated with ammonium sulfate as described by Englard and Seifler (29). For in vitro labeling of RNAs, 5 μl of 10X in vitro labeling buffer (6 mM concentrations each of GTP, UTP, and CTP, 250 μM ATP, 10 mM dithiothreitol, 200 mM KCl, 60 mM creatine phosphate, and 100 mM Tris-Cl, pH 8.0, 40 μM of nuclear extract or equal amounts of protein from ammonium sulfate fractions, and 50 μCi of [32P]ATP were mixed in a total reaction volume of 50 μl and incubated at 30°C for 3 h. The amount of in vitro synthesized Alu RNA or mutant Alu RNA used as substrate for adenylating assay was ~1 μg (20 pmol). Labeled RNAs were extracted using the phenol/chloroform procedure, purified, and fractionated on 10% polyacrylamide, 7 M urea gels.

Polyadenylation Assay in HeLa Nuclear Extracts—A standard polyadenylation reaction for precleaved SV40 late pre-mRNA was followed exactly (20). The nuclear extract (5 μl) contained 20 mM creatine phosphate, 2 mM ATP, 0.6 mM MgCl2, 0.5% PEG, 0.15 mM ddithiothreitol, 40% (v/v) HeLa cell nuclear extract, and labeled substrate RNA (~50,000 cpm). The reaction mixture was incubated for 45 min at 30°C. Inhibition of poly(A) polymerase activity in HeLa cell nuclear extract by bovine serum albumin-conjugated U1 peptide was carried out according to Gundersen et al. (31). Extraction, purification, and analysis of RNA was carried out as described above.

Determination of 3′-End Adenylate Content of SRP RNA from S. cerevisiae—An oligonucleotide, Oligo 1 (5′-gattagttagtgtacactaatgaatctaa*′-3′), with 3′ cordycepin (a*) was ligated to RNAs purified from the yeast cells. A yeast SRP RNA-specific oligonucleotide (SRP RNA-[35S]-373), 5′-gptgtaggtaggtgctcggggg-3′ and an oligonucleotide (Oligo 2) complementary to Oligo 1 were used for RT-PCR amplification. If the 3′-end nucleotide of the RNA is an adenylic acid, a BglII restriction site would be created in the RT-PCR product. Internally labeled RT-PCR products were subjected to BglII digestion and fractionated on a nondenaturing 10% polyacrylamide gel; the gel was dried and subjected to autoradiography or PhosphorImage analysis. Further quantification was done using the Molecular Dynamics system with the ImageQuant software.

RESULTS

Fractionation of SRP-adenylating Enzyme—In our attempts to characterize and purify the SRP adenylating activity, the HeLa cell nuclear extracts were subjected to ammonium sul fate precipitation. Four fractions comprising 0–31%, 31–50%, and 51–70% ammonium sulfate precipitates and the 70% ammonium sulfate supernatant were obtained. These fractions were dialyzed, and equal amounts of protein from each fraction were used to assay SRP RNA in the nuclear extract activity. Since there is endogenous SRP RNA in the nuclear extract that may get adenylated (see Fig. 1, lanes 1 and 4), we used Alu RNA as the
in vitro adenylation of SRP RNA. Therefore, we tested this possibility using our U1A peptide-albumin conjugate. Since the U1A peptide corresponding to 103–119 amino acids conjugated to albumin, interacts with the catalytic site of human poly(A) polymerase, resulting in complete inhibition of mRNA poly(A) polymerase activity. We reasoned that if poly(A) polymerase is involved in SRP RNA adenylation, then U1A peptide-albumin conjugate should also inhibit adenylation of SRP RNA. Therefore, we tested this possibility using our in vitro system for the 3'-end adenylation of SRP/Alu RNA. Fig. 2A shows the effect of U1A peptide-albumin conjugate on the adenylation of SV40 late mRNA. When compared with the starting material (Fig. 2A), there was significant poly(A) formation on the 3'-end of the SV40 mRNA when incubated with HeLa cell nuclear extract (Fig. 2A, lane 2). U1A peptide conjugated to albumin completely inhibited the polyadenylation of this mRNA (Fig. 2A, lanes 3–5). Albumin alone, U1A peptide alone, or an unrelated peptide conjugated to albumin did not inhibit the polyadenylation of this SV40 mRNA (Fig. 2A, lanes 6, 7, and 8, respectively). These data are as expected and are completely consistent with the data published by Mattaj and associates (31). Adenylation of human SRP RNA and Alu RNA was studied under the same conditions. The adenylation of SRP RNA and Alu RNA in the presence of U1A peptide-conjugate (Fig. 2B, lanes 2–4) was the same in the control (Fig. 2B, lane 1) or when incubated in the presence of albumin alone, U1A peptide alone, or an unrelated peptide conjugated to albumin (Fig. 2B, lanes 5, 6, and 7, respectively). These data show that under conditions where mRNA poly(A) polymerase is completely inhibited, the 3' adenylation of SRP and Alu RNA is unaffected. These data are consistent with the suggestion that the addition of adenylc acid residue on the 3'-end of human SRP RNA is facilitated by poly(A) polymerase, which is required for the completion of mRNA processing.
SRP RNA is carried out by an enzyme distinct from mRNA poly(A) polymerase.

**Adenylation of Yeast SRP RNA in Poly(A) Polymerase Mutants**—While over 60% of the human SRP RNA molecules contain non-template encoded adenylic acid residue on their 3′-ends (1), approximately 2–3% of the *S. cerevisiae* SRP RNA molecules contain this post-transcriptionally added adenylic acid.² Keller’s laboratory (25) has characterized the *S. cerevisiae* poly(A) polymerase mutants, and we wanted to see whether adenylation of SRP RNA in these temperature-sensitive mutants is affected when the poly(A) polymerase is inactivated by a shift from permissive to nonpermissive temperature. Fig. 3 shows the data obtained with the yeast mutants. The yeast cell extracts prepared from wild-type cells (Fig. 3A, lane 1) and all three yeast *pap* mutant cell lines grown at the permissive temperature of 23 °C (Fig. 3A, lanes 2, 4, and 6, respectively) were capable of adenylylating a yeast CYC1 RNA. However, all three yeast mutants when grown at the nonpermissive temperature of 37 °C were totally inactive for poly(A) polymerase, and no polyadenylated mRNA was detectable in an in vitro assay for polymerase activity (Fig. 3A, lanes 3, 5, and 7, respectively). These data are consistent with published reports by Keller and colleagues (25).

Total RNA was isolated from these mutant yeast cells grown either at permissive or restrictive temperature, and the presence of the adenylic acid residue on the 3′-end of yeast SRP RNA was tested by a newly developed oligonucleotide ligation/RT-PCR/BglII digestion method (2). In this method, DNA derived from RT-PCR of RNAs containing a 3′ adenylic acid residue would be cleaved by BglII restriction enzyme and yield two smaller fragments. In the case of wild type *S. cerevisiae* cells, 2–3% of the SRP RNA was digested by the BglII restriction enzyme (Fig. 3B, lane 4). In the case of yeast mutant *pap1-5* grown at the permissive temperature of 23 °C (lane 6), or grown at nonpermissive temperature of 37 °C (lane 8), 2–3% of the DNA was cleaved by BglII restriction enzyme. Identical results were obtained with two other *pap* mutants, namely *pap1-2* and *pap1-7* (Fig. 3C). These data show that under conditions of restrictive temperature, where yeast mRNA poly(A) polymerase is inactivated, the SRP RNA is adenylylated to the same extent as in the wild-type cells or cells grown at permissive temperature (Table I). Thus, these results obtained with the yeast system are consistent with the results obtained with HeLa cell extracts (Fig. 2) and support the conclusion that an enzyme distinct from the mRNA poly(A) polymerase is involved in the 3′ adenylylation of SRP RNA.

**Table I**

Quantitation of 3′-end-adenylated SRP RNA from *S. cerevisiae* *pap* mutants

| Source of RNA | Percentage of adenylated SRP RNA (%) |
|--------------|--------------------------------------|
| Wild-type strain | 2.5 |
| Mutant Strains | |
| *pap1-2* grown at 23 °C | 2.8 |
| *pap1-2* grown at 37 °C | 2.2 |
| *pap1-5* grown at 23 °C | 1.7 |
| *pap1-5* grown at 37 °C | 1.9 |
| *pap1-7* grown at 23 °C | 3.3 |
| *pap1-7* grown at 37 °C | 3.5 |

² K. Perumal, J. Gu, and R. Reddy, unpublished data.
SRP 9/14-kDa Protein Is Required for the Adenylation of SRP RNA—It is known that purified poly(A) polymerase and terminal transferases add nucleotides to any RNA containing 3'-OH groups. We wanted to see the specificity of this 3' adenylation on the SRP/Alu RNA. The substitution of SRP/Alu RNA 24GUA26 → AGG in the mutant Alu RNA is shown in Fig. 4A. It is known that substitution of these three nucleotides in the loop near the 5' end of SRP/Alu RNA abolishes the binding of SRP 9/14-kDa proteins (23, 33). The 3'-end sequences of the Alu RNA and mutant Alu RNA are identical, and if the adenylation occurs by poly(A) polymerase or by a nonspecific terminal transferase, one would expect both of these RNAs to be adenylated. Fig. 4B shows the results obtained with this mutant Alu RNA. In the case of nuclear extract used as a control, the SRP RNA can be visualized as the adenylated RNA (Fig. 4B, lane 1). While Alu RNA was adenylated in lane 2, there was no detectable adenylation when the reaction mixture was supplemented with mutant Alu RNA (lane 3). These data show that RNA alone is not a suitable substrate for 3' adenylation, and binding of 9/14-kDa protein to form a ribonucleoprotein complex is necessary before 3' adenylation of SRP/Alu RNA can take place. These data are consistent with our earlier results, which showed that adenylated Alu and SRP RNAs were immunoprecipitable with anti-9/14-kDa protein antibodies (2) and provide evidence for the specificity of adenylation reaction and provide additional line of evidence for the involvement of a novel adenylation enzyme distinct from mRNA poly(A) polymerase or a nonspecific terminal transferase.

**DISCUSSION**

This study was initiated to see whether poly(A) polymerase is involved in the adenylation of a nontemplate-encoded adenylic acid residue in several stable small RNAs. This possibility was very likely, since poly(A) polymerase is known to be involved in the addition of short oligo(A) stretches found on the 3'-end of stable RNAs in *S. cerevisiae* and *E. coli* strains deficient in 3'-exonuclease. We used adenylation of SRP/Alu RNA to test this possibility, since an accurate in vitro adenylation system for SRP/Alu RNA is available (2). Data obtained in this study provide convincing evidence that poly(A) polymerase or a nonspecific terminal transferase is not involved in the adenylation of SRP RNA.

The following four lines of evidence are presented to show that an enzyme distinct from mRNA poly(A) polymerase is involved in the SRP adenylation: 1) the SRP adenylylating activity and poly(A) polymerase fractions in different ammonium sulfate fractions; 2) under conditions where poly(A) polymerase in HeLa cell nuclear extract was completely inhibited by U1A peptide conjugated to albumin, there was no effect on the adenylation of SRP RNA; 3) under conditions where yeast poly(A) polymerase was inactive in three different temperature-sensitive strains, there was no effect on the adenylation of SRP RNA; and 4) the adenylation of SRP RNA was dependent on the intact SRP 9/14-kDa protein heterodimer binding site. As detailed in the Introduction, our previous results showed that CCA-adding enzyme is not involved in the adenylation of SRP RNA. These lines of evidence provide definitive evidence that an enzyme distinct from the mRNA poly(A) polymerase is involved in the adenylation of SRP RNA.

*S. cerevisiae* genome is known to contain a single copy of the poly(A) polymerase (12). In the yeast *pop* mutant strains that we used in this study, it is clear that poly(A) polymerase was inactive when maintained at restrictive temperatures (Fig. 3A). The SRP RNA belongs to the class of stable RNAs with low turnover (34). Therefore, one has to consider whether the adenylation found in SRP RNA from cells grown at restrictive temperatures could be due to RNA synthesized under permissive conditions and remain intact due to low RNA turnover. This is unlikely because the cells were maintained at the restrictive temperature of 37 °C for 6 h, which is more than two generation times, and the 3' adenylic acid residue in SRP RNA is known to turn over independent of the SRP RNA turnover (2). In addition, there was no reduction in the extent of adenylation between control and mutant cells (Fig. 3, B and C). Therefore, these data are supportive of the conclusion that yeast poly(A) polymerase is not involved in the adenylation of SRP RNA.

The poly(A) polymerases, CCA-adding enzymes, and polynucleotide phosphorylases belong to a superfamily of nucleotidyltransferases (35), and all members of this superfamily possess a conserved sequence motif corresponding to the active site.
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(36). Since SRP-adenylating enzyme catalyzes the transfer of nucleotide to the 3′-end of SRP RNA, its properties are consistent with being a member of the superfAMILY of nucleotidyldtransferases. This study shows that this SRP-adenylating enzyme is different from CCA-adding enzyme and poly(A) polymerase. The SRP-adenylating enzyme is not sensitive to micrococcal nuclease treatment, indicating that it is a protein enzyme and not a ribonucleoprotein (2).

Does each RNA have an adenylating enzyme of its own? In addition to SRP RNA, other small RNAs, including 7SK, U2, and 5 S RNAs contain a nontemplated adenylic acid on their 3′-ends. Since our data show that SRP/Alu RNA specific 9/14-kDa proteins are required for SRP RNA adenylation, it is logical to ask which enzyme(s) are involved in the adenylation of these RNAs. The SRP 9/14-kDa protein associates with the SRP and Alu RNAs and is an integral part of SRPs (23); however, this protein heterodimer is not part of ribosomal 5 S, U2, or 7SK RNPs. One possibility is that these RNAs have a common adenylating enzyme that recognizes diverse RNPs that may have some common structural motif(s). Development of in vitro systems capable of accurately adenylating other small RNAs and purification of the enzyme responsible for adenylation SRP RNA is necessary to answer these questions.

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