Purification, Characterization, and Cloning of the cDNA of Human Signal Recognition Particle RNA 3’-Adenylating Enzyme*

Received for publication, March 2, 2001, and in revised form, April 2, 2001
Published, JBC Papers in Press, April 3, 2001, DOI 10.1074/jbc.M101905200

Karthika Perumal, Krishna Sinha, Dale Henning, and Ram Reddy‡

From the Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030

The 3’-terminal adenylic acid residue in several human small RNAs including signal recognition particle (SRP) RNA, nuclear 75K RNA, U2 small nuclear RNA, and ribosomal 5S RNA is caused by a post-transcriptional adenylation event (Sinha, K., Gu, J., Chen, Y., and Reddy, R. (1998) J. Biol. Chem. 273, 6853–6859). Using the Alu portion of the SRP RNA as a substrate in an in vitro adenylation assay, we purified an adenylation enzyme that adds adenylic acid residues to SRP/Alu RNA from the HeLa cell nuclear extract. All the peptide sequences obtained by microsequencing of the purified enzyme matched a unique human cDNA corresponding to a new adenylation enzyme having homologies to the well characterized mRNA poly(A) polymerase. The amino terminus region of the human SRP RNA adenylation enzyme showed ~75% homology to the amino terminus of the human mRNA poly(A) polymerase that includes the catalytic domain. The carboxyl terminus of the human SRP RNA adenylation enzyme showed less than 25% homology to the carboxyl terminus of poly(A) polymerase, which interacts with other factors and provides specificity. The SRP RNA adenylation enzyme is coded for by a gene located on chromosome 2 in contrast to the poly(A) polymerase gene, which is located on chromosome 14. A recombinant protein for the SRP RNA adenylation enzyme was prepared, and its activity was compared with the purified enzyme from HeLa cells. The data indicate that in addition to the SRP RNA adenylation enzyme, other factors may be required to carry out accurate 3’-end adenylation of SRP RNA.

Most eukaryotic RNA molecules are synthesized as precursor molecules and are subsequently processed. These post-transcriptional processing events include 5’ capping, polyadenylation on the 3’ end of mRNAs, CCA turnover on the 3’ end of tRNAs, and splicing in the case of pre-mRNAs.

Small RNAs are a diverse class of RNAs involved in a variety of important cellular functions (1–4). We recently showed that several small RNAs in human cells contain a single post-transcriptionally added adenylic acid residue on their 3’ ends. These RNAs include SRP0 RNA, 75K RNA, U2 snRNA, and ribosomal 5S RNA (5–9). SRP RNA is the RNA component of the signal recognition particle, which plays an important role in translocation of membrane proteins and secretory proteins (4, 10). SRP RNA is synthesized in the nucleus by RNA polymerase III and is transported through the nucleolus on its way to the cytoplasm (11). Data from our laboratory showed that SRP RNA in the nucleolus is already processed and adenylation on its 3’ end (12). These data indicate that the 3’-end processing and adenylation of SRP RNA are early events in the biogenesis of the signal recognition particle.

The U2 snRNA is a required component for the splicing of pre-mRNA (13, 14). 60–70% of human SRP RNA, 75K RNA, and U2 snRNA molecules contain post-transcriptionally added adenylic acid residues on their 3’ ends (8). In the case of ribosomal 5S RNA, ~10% of the molecules was found to contain this post-transcriptionally added adenylic acid residue (8). The 3’-end adenylation of small RNAs is conserved through evolution because SRP RNA from rodents, amphibians, and insects contain this post-transcriptionally added adenylic acid residue (5, 8, 15).

The functions of this 3’-end adenylation found in the small RNAs are not fully understood. Because this 3’ adenylation occurs in many different RNAs with diverse functions, it is likely that the function of this 3’-adenylic acid residue is related to the metabolism of these RNAs. Data from our lab showed that small RNAs with post-transcriptionally added adenylic acid residue are not substrates for 3’ extension of RNAs by polyuridylation (16). In addition, the post-transcriptionally added 3’-adenylic acid residue has a relatively low turnover (8). These data suggest that one of the functions of this 3’ adenylation is to protect the 3’ ends of RNAs from digestion by exonucleases and also to prevent 3’ extensions by uridylation (16).

Our initial studies were aimed at determining whether mRNA poly(A) polymerase (PAP) is responsible for this 3’ adenylation of small RNAs. The data showed that neither PAP nor tRNA nucleotidyltransferase is involved in carrying out this 3’ adenylation (17). We carried out studies to identify and purify the enzyme responsible for post-transcriptional adenylation of human SRP RNA. This study shows the purification and characterization of a novel 3’-adenylating enzyme that has homology to PAP but it is a product of a distinct gene. A recombinant SRP RNA adenylation enzyme with adenylation activity was obtained and its activity compared with that of the SRP RNA adenylation enzyme purified from HeLa cells.

MATERIALS AND METHODS

Chemicals and Isotopes—[α-32P]ATP was purchased from ICN Biochemicals, Inc. Prepacked columns and chromatography media were purchased from Amersham Pharmacia Biotech. The hydroxyapatite media was from Bio-Rad. The reverse transcription-PCR kit was from Qiagen. Restriction enzymes were from Life Technologies, Inc. The T7 RNA polymerase was from New England Biolabs.

Preparation of Substrate RNAs—Plasmid DNA corresponding to the Alu portion of canine SRP RNA (p7Alu) under the T7 promoter (18) was a gift from Dr. Katharina Strub. For in vitro transcription reactions, a...
DNA template was prepared by PCR to have the desired sequence on the 3′ end of Alu RNA. The U6 snRNA was made by T7 RNA polymerase transcription from a U6 snRNA-containing plasmid linearized by DraI (19). The in vitro transcription of linearized plasmid DNAs with T7 RNA polymerase was performed according to standard protocols (New England Biolabs). All RNA products were purified, fractionated on a polyacrylamide gel, and visualized by PhosphorImager. B. HeLa cell nuclear extract was fractionated consecutively by ammonium sulfate (AS) precipitation, DEAE-Sepharose, hydroxyapatite, gel filtration (Superdex 200), and finally on Source 15Q columns. Fractions containing SRP RNA adenylating activity (assayed as in A) were pooled and subsequently loaded onto the next column. 20 μg each from nuclear extract and ammonium sulfate, 10 μg each from peak activities of DEAE-Sepharose and hydroxyapatite, and 100 ng each from Superdex 200 and Source 15Q fractions were loaded on a 10% SDS-polyacrylamide gel and subjected to electrophoresis. The proteins were visualized by silver staining.

Preparation of HeLa Cell Nuclear Extracts and in Vitro Labeling—Extracts from HeLa cells grown in suspension culture were prepared by the procedure of Dignam et al. (20). The final protein concentration of the extract was 5 mg/ml. The amount of Alu RNA used as substrate for each adenylation assay was ~1 μg (20 pmol), and the in vitro adenylation assays were carried out essentially as described earlier (8). In the case of other RNAs, ~20 pmol of RNA was added into each reaction. For the in vitro labeling of RNAs, 5 μl of 10× in vitro labeling buffer (6 mM each GTP, UTP, and CTP, 250 mM MgCl₂, 60 mM creatine phosphate, and 100 mM Tris-HCl, pH 8.0), 40 μl of nuclear extract, and 50 μCi of [α-32P]ATP were mixed in a total reaction volume of 50 μl and incubated at 30 °C for 3 h. After incubation, labeled RNAs were extracted using the phenol-chloroform procedure, precipitated, and fractionated on 10% polyacrylamide/7 M urea gels. Radiolabeled bands were quantitated with a PhosphorImager densitometer (Molecular Dynamics) using IMAGE QUANT software. The gels were also analyzed by routine autoradiographic methods.

Purification of the SRP RNA Adenylation Activity—The HeLa cell extract prepared by the method of Dignam et al. (20) was used as the starting material. Solid ammonium sulfate was added to 40% (w/v) saturation to the HeLa cell extract. The mixture was kept on ice, occasionally shaken for 30 min, and then centrifuged at 3,000 × g for 30 min. The pellet was discarded and the supernatant was brought to 65% saturation with solid ammonium sulfate and again centrifuged at 3,000 × g for 30 min. The resulting pellet was dissolved in 10 ml of buffer A (20 mM Tris-HCl buffer, pH 8.2, with 50 mM KCl, 5 mM dithiothreitol, 2 mM EDTA, and 10% glycerol) and dialyzed at 4 °C overnight against the same buffer. The resulting material was applied to a DEAE-Sepharose CL-6B column (Amersham Pharmacia Biotech) pre-equilibrated with buffer A. The elution was achieved with a linear gradient of buffer A and buffer B containing 1 mM KCl. Samples containing SRP/Alu RNA adenylating activity were pooled and loaded onto a hydroxyapatite (Bio-Rad) column. The elution was carried out with a linear gradient of 10 mM sodium phosphate buffer, pH 6.8, 0.5 mM dithiothreitol and the same buffer containing 500 mM sodium phos-
phosphate. After hydroxypatite chromatography, a size-exclusion chromatography system, Superdex 200 column from Amersham Pharmacia Biotech, was used. Fractions were collected and assayed for SRP/Alu RNA adenylation activity. Active fractions were pooled and loaded onto a strong anion exchanger (Source 15Q column from Amersham Pharmacia Biotech). The bound proteins were eluted in a linear gradient of 0.1–1.0 m NaCl and the same buffer containing 400 m M NaCl. All column purifications were carried out using an AKTA fast protein liquid chromatography system from Amersham Pharmacia Biotech.

**Sequence of the Purified Protein Bands**—The purified protein obtained after the Source 15Q column was subjected to microsequencing at the Protein Chemistry Core Facility (Baylor College of Medicine, Houston, TX).

**Reverse Transcription-PCR and Cloning into the Expression Vector**—Using partial cDNA sequences from GenBank (accession numbers BE781551 and AK024249), primers were designed with 5’ overhangs containing restriction enzyme sites (NcoI on the 5’ end and HindIII on the 3’ end). Using the Qiagen One-Step reverse transcription-PCR kit, a >2-kilobase product was obtained from a HeLa cell total RNA preparation. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined. This product was digested with NcoI and HindIII and inserted into the pPROEX-HTa vector (Life Technologies, Inc.). Positive clones were checked for the insert, and then the DNA sequence was determined.
RNA was also adenylnated on its 3’ end (Fig. 1A, lane 1). The Alu RNA is a domain in the SRP RNA corresponding to its 5’ and 3’ ends (9, 21). When the HeLa cell extract was supplemented with wild-type Alu RNA, the 3’ end of Alu RNA was adenylnated (Fig. 1A, lane 2). However, mutant Alu RNA that cannot bind SRP 9/14 proteins (18, 22) was not adenylnated in vitro (Fig. 1A, lane 3). The 3’-end analysis of labeled SRP RNA and Alu RNA showed that a single adenylic acid residue is added to the 3’ end of these RNAs, which is identical to the single A addition on the 3’ end of SRP RNA in vivo (8, 16). This 3’ adenylation of exogenously added Alu RNA by the HeLa cell extract and by purified fractions was used as an assay to purify the SRP/Alu RNA adenylnating enzyme.

Purification of the Adenylnating Enzyme—To purify and obtain the adenylnating enzyme that adds a single A residue to the 3’ end of SRP RNA, the HeLa cell nuclear extract was fractionated through several columns. The adenylnating enzyme was sequentially fractionated through ammonium sulfate precipitation and chromatography through DEAE-Sepharose, hydroxyapatite, Superdex 200, and Source 15Q columns (Fig. 1B and Table I). The fractions containing peak adenylnation activity were pooled and fractionated on a 10% polyacrylamide/SDS gel. The pooled fraction from the Source 15Q column contained a major protein of ~80 kDa (Fig. 1B, lane 7). The SRP RNA adenylnating activity eluted on the Superdex 200 column with an apparent molecular mass of 190–220 kDa. This suggests that the native enzyme functions in a multisubunit complex, which is larger than the 80-kDa monomer. Table I shows the enrichment of SRP RNA adenylnating activity and yield of human SRP RNA adenylnating enzyme at different steps of purification.

The specificity of the purified adenylnating enzyme was checked at various stages of enzyme purification (Fig. 2). All the fractions up to the final purification were able to adenylnate the Alu RNA. However, the pooled fractions from the hydroxyapatite column adenylnated not only the wild-type Alu RNA (Fig. 2, lanes 4 and 5) but also adenylnated the mutant Alu RNA (Fig. 2, lane 6). This is in contrast to the specificity observed in nuclear extracts where mutant Alu RNA was not adenylnated (Fig. 1A, lane 3). These data show that some of the specificity observed in nuclear extracts was lost as the purification progressed through various chromatography steps. However, some RNAs such as U4 and U6 RNAs were not adenylnated by this partially purified enzyme (Fig. 2, lanes 7 and 8), indicating that this enzyme is not adenylnating RNAs nonspecifically.

Isolation of Human cDNA for SRP RNA Adenylnating Enzyme—To obtain the cDNA sequence for the SRP RNA adenylnating enzyme, several partial peptide sequences were obtained from the purified 80-kDa enzyme from the Source 15Q column fraction (Fig. 1B, lane 7). A search of the available protein/expressed sequence tag data banks yielded a perfect match to a unique cDNA with a reading frame for an ~80-kDa protein. One of the proteins that copurified with the SRP RNA adenylnating enzyme up to the Superdex 200 column (Fig. 1B, lane 6), upon microsequencing, turned out to be a previously characterized 78-kDa protein, GRP-78/BIP (23).

The cDNA sequence corresponding to this new adenylnating enzyme has been submitted to GenBank™ with the accession number AY029162. This cDNA sequence contains a 2,211-nucleotide open reading frame followed by an ~1,800-nucleotide 3’-untranslated region. The consensus polyadenylation signal AAUAAA and the cleavage site are not found in the currently characterized expressed sequence tag data bank clones. This indicates that this probably is a partial cDNA sequence and this mRNA contains a longer, as yet uncharacterized, 3’-untranslated region. There is a 166-nucleotide long 5’-untranslated region, which contains an in-frame upstream terminator codon corresponding to ~94 to ~96 and a purine at the ~3 position of the initiator ATG codon, constituting an adequate Kozak consensus sequence. The transcription initiation site of this mRNA has not yet been determined.

This new SRP RNA adenylnating enzyme is 736 amino acids in length, and comparison with human PAP (Fig. 3) shows that the amino terminus (amino acids 1–490) has ~75% identity, and the carboxyl terminus is highly divergent with only ~25% identity. Although the catalytic domain and the nuclear localization signals are conserved between these two enzymes, the regulatory domains seem to be different (Fig. 4C). The three consensus and four nonconsensus cyclin-dependent kinase phosphorylation sites (underlined in red in Fig. 3) identified in human PAP (24) are not well conserved in the SRP adenylnating enzyme. But there are potential cyclin-dependent kinase phos-
phorylation sites in the SRP RNA adenylylating enzyme in the same vicinity that conform to the consensus S/TP\textsubscript{X}R and the nonconsensus S/TPXX sequence motifs. The cyclin recognition motif (SKIRILVG) present in PAP (25) is also present in the SRP RNA adenylylating enzyme (Fig. 4).

Unique Gene for SRP RNA Adenylylating Enzyme—A search of the available human genome sequences using the partial cDNA sequence identified two overlapping high throughput genomic sequencing phase entries that contained the entire gene for the SRP RNA adenylylating enzyme. The gene for this enzyme is located on chromosome 2, whereas the gene for PAP is on chromosome 14 (26). The exon-intron organization (Fig. 5) was identified using a BLAST algorithm to align the cDNA sequence against the genomic sequence. The SRP RNA adenylylating enzyme gene has 21 exons encoding the protein sequence. Exons coding for the 5'- and 3'-untranslated regions have not been included because the untranslated regions are incomplete. The exons 1–15 between PAP and the SRP adenylylating enzyme are highly conserved (Fig. 5), and these data indicate that both of these genes originated from a common ancestral gene. Because *Saccharomyces cerevisiae* has only the PAP gene (27) and contains no distinct gene for SRP RNA adenylylating enzyme, it is likely that this new gene for the adenylylating enzyme arose from duplication of the PAP gene and acquired divergence in the regulatory domains while keeping the catalytic domain largely intact (see Figs. 3, 4, and 5).

Recombinant Protein for SRP RNA Adenylylating Enzyme—Using HeLa cell total RNA, a cDNA clone was obtained by reverse transcription-PCR, and a cDNA sequence corresponding to amino acids 1–736 (Fig. 3) was inserted into a pPROEX-HTa expression vector. The cDNA was sequenced and was found to be in frame with the histidine tag and corresponded to the full-length cDNA. The recombinant protein was expressed in *E. coli* DH5α cells, and the protein was purified using a nickel-nitrilotriacetic acid affinity column. Analysis of this recombinant protein on an SDS-polyacrylamide gel (Fig. 6) showed one major protein band. The major band in lanes 1–6 

**Fig. 6. Analysis of the recombinant SRP RNA adenylylating enzyme.** The human cDNA for the SRP RNA adenylylating enzyme was cloned into the pPROEX-HTa vector in frame with the histidine tag and expressed in *E. coli*. The bacterial pellet was lysed, and the recombinant protein was purified by affinity chromatography using nickel-nitrilotriacetic acid (Qiagen) columns. The bound protein was eluted with 100 mM imidazole and dialyzed, and an aliquot of the purified recombinant protein was fractionated on a 10% SDS-polyacrylamide gel (lane 1). Lane 2, the affinity-purified protein digested with recombinant tobacco etch virus protease to remove the histidine tag. The proteins were stained with Coomassie Brilliant Blue. M, molecular mass markers.

Recombinant Enzyme Shows Adenylylation Activity in Vitro—The recombinant protein was assayed for *in vitro* adenylylation activity using the conditions recommended for *E. coli* PAP (Fig. 7). Alu RNA, U6 RNA, and yeast tRNA were used as substrate RNAs. As expected, the endogenous SRP RNA and supplemented Alu RNA were adenylylated by the 3' addition of a single adenylic acid residue in the HeLa nuclear extract (lane 1). Both *E. coli* PAP (lane 7) and the recombinant human SRP RNA adenylylating enzyme (lanes 2–6) showed polyadenylation activity. The recombinant SRP RNA adenylylating enzyme added multiple adenylic acid residues to all of the RNA substrates tested. This apparent lack of specificity and regulation in limiting the number of adenylic acid residues added to the 3' end of Alu RNA show that accurate and regulated adenylylation of human SRP/Alu RNA *in vitro* may require other components. Although the most purified preparation of the SRP RNA adenylylating enzyme from HeLa cells contained an ~80-kDa protein as the major band, other proteins were also present when more sample was loaded on the gel and stained (data not shown). It is worth noting that the substrate for the accurate adenylylation in *vitro* is an RNA-protein complex (17).

**Fig. 7. The in vitro adenylylation activity of the recombinant human SRP RNA adenylylating enzyme.** The recombinant enzyme (Fig. 6, lane 1) and *E. coli* poly(A) polymerase purchased from Life Technologies, Inc. were used to test for adenylylation activity *in vitro*. The conditions used were those recommended for the assay of *E. coli* poly(A) polymerase. Lane 1, HeLa cell nuclear extract incubated in the presence of [α-32P]ATP and T7-transcribed Alu RNA. The substrate RNA and the enzyme used for each of the in vitro adenylylation assays are indicated above the lanes. Lanes 2, 4, and 6 were incubated with 0.1 μg of recombinant enzyme and [α-32P]ATP; lanes 3 and 5 were incubated with 0.3 μg of recombinant enzyme and [α-32P]ATP. Incubation was at 30 °C for 60 min; lane 7 was incubated with 1 unit of *E. coli* PAP. The labeled RNAs were purified and fractionated on a 10% polyacrylamide gel and subjected to autoradiography. NE, nuclear extract.

**DISCUSSION**

This study presents data on the purification of a new 3'-adenylylating enzyme from HeLa cells. This purified enzyme is capable of adding adenylic acid residues to SRP/Alu RNA. The cDNA for this enzyme has been isolated, and interestingly, this enzyme is highly homologous to the well characterized mRNA PAP (Figs. 3 and 4). However, the SRP RNA adenylylating enzyme is the product of a distinct gene located on chromosome 2.
The homology between these two enzymes is ~75% in the amino-terminal region (Figs. 3 and 4). However, the carboxyl terminus is highly divergent and shows only 25% homology between these two enzymes. It is not surprising that the amino terminus is conserved because this region contains the catalytic domain. The carboxyl terminus of the PAP contains the domain that interacts with other factors including the cleavage and polyadenylation specificity factor, which confers the specificity for the enzyme (28). Therefore, it is reasonable to expect divergence in the carboxyl terminus of the SRP RNA adenylating enzyme.

The recombinant SRP RNA adenylating enzyme expressed in E. coli was capable of adenylating RNAs in vitro. However, there was no substrate specificity, and it added multiple adenylate residues (Fig. 7). This is not very surprising in light of what we already know about other purified enzymes. PAP in vivo and in nuclear extracts exhibits substrate specificity and adenylates only mRNAs containing the polyadenylation signal.

However, purified PAP adenylates any RNA with a 3′-OH group (29, 30). Similarly, the capping enzyme guanylyltransferase caps only RNAs transcribed by RNA polymerase II in vivo. However, purified guanylyltransferase caps any RNA with appropriate 5′ phosphates (31, 32). In both cases, the specificity and regulation of enzymatic activity is caused by a multiprotein complex involved in these biological reactions. From the results obtained with the recombinant SRP RNA adenylating enzyme, it is very likely that other factors are necessary to carry out accurate and regulated adenylation in vitro. In this context, it is worth noting that the ribonucleoprotein complex consisting of Alu RNA bound to SRP 9/14 proteins is the required substrate for adenylation in vitro (17). Therefore, it may be necessary to use Alu RNA complexes with SRP 9/14 proteins to obtain accurate 3′-end adenylation in vitro. It is also possible that in addition to adenylating SRP RNA, this new adenylating enzyme is an mRNA poly(A) polymerase for some cellular mRNAs.

The enzymatic activity of the SRP RNA adenylating enzyme may be regulated by other post-translational modifications such as phosphorylation. The PAP, for example, is highly phosphorylated and regulated through its phosphorylation (33). Hyperphosphorylation of PAP inhibits adenylation activity, and the dephosphorylated enzyme is more active (33). Several consensus and nonconsensus phosphorylation sites are present in the S/T-rich domain at the carboxyl terminus of the SRP RNA adenylating enzyme, and the corresponding region in PAP is highly phosphorylated. Therefore, it is very likely that the SRP RNA adenylating enzyme is phosphorylated in vivo, and its activity may be regulated by phosphorylation. Further work is needed to experimentally verify this possibility.

The cDNA sequence for the SRP RNA adenylating enzyme is a partial sequence, and further work is needed to characterize the complete cDNA sequence in the 5′- and 3′-untranslated regions. The available 1,768 nucleotides of the 3′-untranslated region show no AAUAAA polyadenylation signal. Although the human PAP cDNA was isolated several years ago, the complete 5′-untranslated region has not been characterized, and the transcription initiation site is not yet known. Our unpublished data indicate that there is a long 5′-untranslated region in the case of the SRP RNA adenylating enzyme. It will be interesting to characterize the complete cDNA sequence of the PAP and SRP RNA adenylating enzyme because genomic organization (Fig. 5) suggests that the SRP RNA adenylating enzyme may have arisen by duplication of the PAP gene and subsequent divergence in the carboxyl terminus region.
Purification, Characterization, and Cloning of the cDNA of Human Signal Recognition Particle RNA 3′-Adenylylating Enzyme
Karthika Perumal, Krishna Sinha, Dale Henning and Ram Reddy

doi: 10.1074/jbc.M101905200 originally published online April 3, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M101905200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 18 of which can be accessed free at http://www.jbc.org/content/276/24/21791.full.html#ref-list-1