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Isolation and characterization of a cDNA clone encoding the 19 kDa protein of signal recognition particle (SRP): expression and binding to 7SL RNA

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ABSTRACT

Signal recognition particle (SRP) consists of a 7SL RNA molecule and 6 protein subunits. We have isolated and characterized cDNA clones from human liver which encode the 19kDa protein subunit (SRP19). This subunit binds to the RNA directly and mediates binding of a second polypeptide, the 54kDa subunit which is involved in signal sequence recognition. Amino acid sequences deduced from the human cDNA sequence were identical to amino acid sequences of tryptic peptides from canine pancreatic SRP19. In vitro transcription and translation of the human cDNA resulted in a protein product the same size as canine SRP19 which could be immunoprecipitated by an antiserum raised against canine SRP19. SRP19 synthesized in a cell-free system specifically bound to 7SL RNA. The sequence of SRP19 is discussed with respect to its binding to 7SL RNA.

INTRODUCTION

Secreted and membrane proteins contain signal sequences required for targeting to and insertion into the membrane of the endoplasmic reticulum (ER). During this process the signal sequence interacts with receptor molecules. The first cytosolic component which recognizes the signal sequence is the signal recognition particle (SRP). It binds to the signal peptide when it emerges from the ribosome (1-3) and causes pausing in elongation. Elongation continues when SRP comes into contact with its receptor, the docking protein, which is located in the membrane of the ER (4-5). The nascent polypeptide is translocated and the signal sequence may or may not be cleaved by a signal peptidase.

SRP is a ribonucleoprotein particle which has a rod-like shape, 5-6nm wide and 23-24nm long (6). It consists of a 7SL RNA of 300 nucleotides and six different protein subunits of 72, 68, 54, 19, 14 and 9kDa (SRP72, SRP68, SRP54, SRP19, SRP14, SRP9). 7SL RNA from a broad range of organisms has been isolated. While in some cases there is little homology in primary sequence, the secondary structure is nevertheless highly conserved (7-11), suggesting that these RNAs are parts of SRP analogues. 7SL RNA forms a central double stranded rod which is flanked at one end by two small stem loop structures comprising the 3′ and 5′ terminal nucleotides and at the other end by two large stem loops. The nucleotides of the smaller loops and most of the central rod structure show high homology to Alu-like sequences found in the mammalian genome, while the sequences of the larger loops (referred to as S

segment) are specific for 7SL RNA. Treatment of SRP with micrococcal nuclease separates the particle into two subparticles; the S segment and a segment containing the Alu sequences (12).

The protein subunits of SRP can be released from the RNA molecule under non-denaturing conditions. Four of the SRP proteins are released as heterodimers (SRP68/72 and SRP9/14) and two as monomers (SRP19 and SRP54). The heterodimers and SRP19 bind to the RNA molecule directly and binding of SRP54 occurs only if SRP19 is already bound to the 7SL RNA (13). SRP9/14 is bound to the Alu segment, the other protein subunits to the S segment (12). RNA footprinting analysis showed that SRP19 protects the two loop regions of the S segment (14).

SRP has at least three distinct functions which can be associated with the protein subunits: signal recognition, translational arrest and membrane targeting by interaction with the docking protein. SRP54, when bound to SRP19, is involved in signal sequence recognition (1-2, 15), SRP9/14 is required for translational arrest (16-17) and SRP68/72 for protein translocation (15). The functions of the protein subunits have not been assigned to specific polypeptides within each heterodimer.

SRP19 binds to the 7SL RNA, presumably undergoes a conformational change and then mediates binding of SRP54 to the particle (13). SRP54 most likely does not bind directly to the 7SL RNA but only via SRP19. This view is supported by the observation that addition of SRP54 to reassembled SRP did not have an effect on the pattern of nuclease protection (14). Here we describe the isolation and characterization of cDNA clones encoding SRP19.

MATERIALS AND METHODS

Restriction enzymes, other DNA modifying enzymes and tRNA from calf liver were from Boehringer Mannheim. The capping reagent ^{7}mG(5')ppp(5')G was from Pharmacia. L-^{35}S-methionine and radiolabelled nucleotides were from Amersham; Entensify was from DuPont. The vector pGEM2 and SP6 RNA polymerase were obtained from Promega. Radiolabelled protein standards were from Amersham and RNA molecular weight markers were from BRL.

Protein sequencing

Canine SRP proteins were separated on preparative SDS polyacrylamide gels (10-15%) and electroeluted (18). Purified SRP19 protein was digested with trypsin/TPCK, and tryptic peptides were fractionated by reverse phase HPLC on a C-4 column. Individual peaks were collected and sequenced on an ABI gas phase sequenator without further fractionation.

Screening and clone selection

A human liver cDNA library cloned into the expression vector pEX627 (19) was screened with an antiserum against SRP19 (20). Identification of positive colonies was carried out essentially as described (21).

In order to verify the authenticity of the selected clones anti SRP19 antibodies were affinity purified on fusion proteins and tested for their binding to canine pancreatic SRP19. Proteins from selected colonies were separated on 7% SDS polyacrylamide gels, blotted onto

nitrocellulose filter, stained with Ponceau-S and the area containing the fusion protein was cut out. Filter-bound protein was then used for affinity purification of anti-SRP19 antibodies. Antibodies affinity purified on the fusion proteins were then tested for their ability to recognize SRP19 in a Western blot analysis.

Affinity purification of antibodies

The anti SRP19 antibodies were affinity purified on SRP19 protein or fusion proteins blotted onto nitrocellulose. Antibodies bound to the 19kDa protein were eluted with 0.2M glycine-HCl, pH 2.8 at 0°C and the solution immediately neutralized with 1M Tris base. Affinity purified antibodies were used in a Western blot analysis without further dilution.

Radiolabelled probes

cDNA fragments were isolated from agarose gels and radiolabelled by extension of sequences randomly primed with oligonucleotides (22).

Screening of lambda gt10 library

A radiolabelled PstI-HindIII fragment of the partial clone p19.8 selected by antibody screening was used to screen a human liver cDNA library cloned into bacteriophage lambda NM1149. This library was kindly provided by C. Schneider. The cDNA library was plated out in Escherichia coli Q358 cells. In total 8x105 plaques were screened. Plaques were blotted onto nitrocellulose and probed using standard techniques (23). A positive plaque was identified (p19i), the cDNA was excised and subcloned into the EcoRI site of pGem2.

Sequencing of cDNAs

cDNA fragments of p19.8 were excised from pEX627, subcloned into M13 vectors (24) and sequenced on both strands. Fragments of p19i (EcoRI-XbaI; XbaI-HindIII; HindIII-EcoRI; fig. 1A) were excised from pGem2 and sequenced in M13 vectors. To eliminate the formal possibility that two XbaI sites or two HindIII sites respectively should occur in close proximity, these restriction sites were sequenced on the full-length p19i cDNA. In these cases, sequencing reactions were primed using oligonucleotides corresponding to cDNA sequences located upstream and downstream of these restriction sites. Sequencing reactions were carried out in the presence of ^{35}S-dATP using the Sequenase sequencing kit (United States Biochemical) and following the recommendations of the manufacturers.

In vitro transcription and translation

A cDNA clone encoding full length SRP19 was constructed by ligating the EcoRI-XbaI fragment of p19i to the XbaI-PstI fragment of p19.8 (fig. 1A). This cDNA, designated pSRP19, was inserted into the plasmid pGem2. For in vitro transcription the plasmid was linearized at a PvuII site located 50bp downstream of the insert DNA. A transcript encoding the N-terminal 68 amino acids was generated by linearizing pSRP19 at the XbaI site in the middle of the coding region. Plasmid DNA digested with XbaI was made blunt using T4 DNA polymerase as described (25). The *in vitro* transcription reaction was carried out as described earlier (26), except that SP6 polymerase was used instead of T7 polymerase. Transcripts were translated in wheat germ lysate (27).

Immunopreciptation

SRP19 was immunoprecipitated from the cell-free translation mixture with an anti SRP19 polyclonal antiserum essentially as described (28). When denaturing conditions were required, the translation mixture was adjusted to 0.5%SDS, heated at 95°C for 5min and subsequently cooled on ice. Prior to the addition of antiserum, the reaction mixture was adjusted to 0.1% SDS with buffer (50mM Tris-HCl pH7.5, 150mM NaCl, 1% NP40, 4µg/ml phenylmethylsulfonyl fluoride). Washing was carried out as under native conditions.

RNA binding assay

SRP was isolated from canine pancreas as described by Walter and Blobel (13) and the proteins were extracted with phenol (12). 7SL RNA was precipitated with ethanol and dissolved in water.

In vitro transcript from pSRP19 was translated at 25°C for 30min in a wheat germ cell-free system. A 50µl aliquot of the translation reaction was adjusted to 0.3M KOAc. 7SL RNA (1µg/assay), tRNA (1µg/assay) or water were added and incubation was continued at 25°C for 30min. 1/10 of the reaction volume was removed to monitor for total protein synthesis (T). Ribosomes and aggregated material were removed from the remainder by centrifugation in a Beckman Airfuge at 20psi for 15min. An aliquot of the ribosomal pellet (P) and the supernatant (S) were set aside and the remainder of the supernatant was applied to a DEAE sepharose column (100-150µl bead volume) equilibrated with 0.3M KOAc, 100mM Tris-HCl, pH 7.5. The column was washed with 4 column volumes of 0.3M KOAc, 100mM Tris-HCl, pH7.5 and then eluted with 2M KOAc, 100mM Tris-HCl, pH 7.5. Fractions from the flow through and from the washing steps were combined (F) and precipitated with 10% w/v trichloroacetic acid (TCA) . The fractions of the eluate (E) were also precipitated with 10% TCA. The samples were then analysed by polyacrylamide gel electrophoresis and autoradiography.

SDS-polyacrylamide gelelectrophoresis

Proteins were separated on 10-15% Laemmli-type SDS polyacrylamide gels (29) and radiolabelled bands were visualized using Entensify.

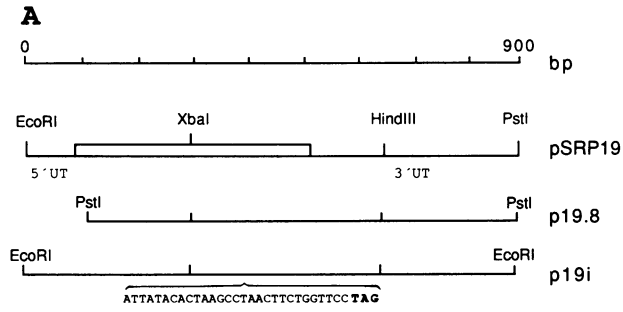
Northern Blot Analysis

Polyadenylated mRNA was isolated from a postribosomal supernatant of HeLa cells as described (28). 10µg of Hela mRNA and SP6 transcript from 15ng of pSRP19 DNA linearized with PvuII were separated on 1% agarose gels containing formaldehyde as described (25). RNA was transferred to Genescreen filters and probed with radiolabelled full-length pSRP19 cDNA as described (25).

RESULTS

Immunoisolation of an SRP19 specific cDNA clone

A human liver cDNA library (0.5×10^6 clones) cloned into the expression vector pEX627 (19) was screened with an anti SRP19 antiserum. The library was kindly provided by K.



B

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    EcoRI
AGTGAATTCCGGAAACTCAGAGCCGGGTTCCTCCCGGGTTTCTGCCGGGTTTCTCCCTGC
GGCTCCTGGGTTGTTGAGACTCTTGTGAAGATGGCTTGCGCTGCCGCGCGGTCCCCGGCC
                              MetAlaCysAlaAlaAlaArgSerProAla   10

GACCAGGACAGGTTTATTTGTATCTATCCTGCTTATTTAAATAATAAGAAGACCATCGCA
AspGlnAspArgPheIleCysIleTyrProAlaTyrLeuAsnAsnLysLysThrIleAla   30

GAGGGAAGGCGAATCCCCATAAGTAAGGCTGTTGAAAATCCTACAGCTACAGAGATTCAA
GluGlyArgArgIleProIleSerLysAlaValGluAsnProThrAlaThrGluIleGln   50

GATGTATGTTCAGCAGTTGGACTTAACGTATTTCTTGAGAAAAATAAAATGTACTCTAGA
AspValCysSerAlaValGlyLeuAsnValPheLeuGluLysAsnLysMetTyrSerArg   70

GAATGGAATCGTGATGTCCAATACAGAGGCAGAGTCCGGGTCCAGCTCAAACAGGAAGAT
GluTrpAsnArgAspValGlnTyrArgGlyArgValArgValGlnLeuLysGlnGluAsp   90

GGGAGCCTCTGCCTTGTACAGTTCCCATCACGTAAGTCAGTAATGTTGTATGCAGCAGAA
GlySerLeuCysLeuValGlnPheProSerArgLysSerValMetLeuTyrAlaAlaGlu  110

ATGATACCTAAACTAAAAACAAGGACACAAAAAACAGGAGGTGCTGACCAAAGTCTTCAA
MetIleProLysLeuLysThrArgThrGlnLysThrGlyGlyAlaAspGlnSerLeuGln  130

CAAGGAGAGGGAAGTAAAAAAGGGAAAGGAAAGAAAAAGAAGTAACCTAGTATCAGCATC
GlnGlyGluGlySerLysLysGlyLysGlyLysLysLysLysEnd                 144

AAGTATGTGGTACTACTGTAAGAGACATGAATGGAGACTTCTAATTTGTATCGGAGGGAA
ACAGAAGCTTTTTGTTTGCATCATTTAACTGAACTGTGAACCCTTGTGCCTCTCATCTTT
ATCATCGGAGTTGACAGTGAAACAAATTTACATCAGAAGTTTGCATCTCGCGTATATGCC
GTATAAAAGAATTTTTTTGTCTTTCAATGCAGTTTTTTGGAAGAAAATATTTTTAAATGG
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TTAAAATAAACATTATGTCCACCTTTTAAGTTAATGAAATAAAATTTGAAACTGAAAAAA
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Fig.1A SRP19 cDNA clones. p19.8 is a partial clone immunoisolated from an expression library of human liver cloned into the PstI site of the plasmid vector pEX627. p19i was selected from a human liver cDNA library cloned into the EcoRI site of lambda phage NM1149 using p19.8 as a hybridization probe. Apart from additional 5´sequences, p19i contains an insert of 32 bp not present in p19.8 (inserted sequence). This insert contains an inframe TAG stop codon. pSRP19 was constructed by ligating the 5´sequences of p19i to the 3´sequences of p19.8 at the unique XbaI site. The box in pSRP19 shows the coding region. UT indicates the untranslated regions. The number of bases is shown on the top scale.
Fig.1B Nucleotide sequence and deduced amino acid sequence of pSRP19. Subclones of p19.8 and p19i were ligated into M13 bacteriophage vectors and sequenced by dideoxy sequencing. The 5´EcoRI site is indicated. Amino acid sequences obtained from 5 tryptic peptides of canine pancreas SRP19 are underlined.

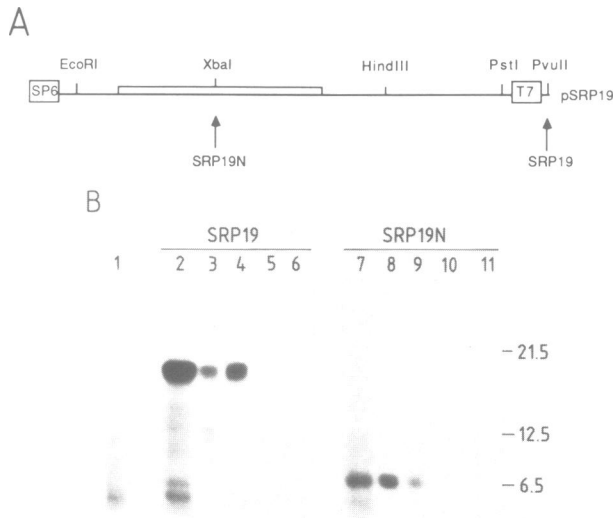

Fig.2A SRP19 polypeptides expressed from pSRP19. pSRP19 was ligated between the EcoRI site and the PstI site of the polylinker region of plasmid vector pGem2. For the generation of full-length SRP19, the cDNA was linearized at the PvuII site prior to transcription and for the expression of an N-terminal polypeptide (SRP19N), the cDNA was linearized at the unique XbaI site. The open box in pSRP19 represents the coding region; SP6 and T7 the respective promoters.
Fig2B Immunoprecipitation of SRP19. pSRP19 was transcribed from the SP6 promoter of pGem2 and the transcripts were translated in a wheat germ cell free translation system in the presence of 35S methionine and analysed on 10-15% SDS polyacrylamide gels. SRP19 protein was expressed either as a full-length product (lanes 2-6) or as an N-terminal fragment, containing only the N-terminal 68 amino acids (SRP19N, lanes 7-11). *In vitro* translation products were synthesized in the absence (lane 1) or in the presence (lanes 2 and 7) of added SRP19 mRNA. Lanes 3-4 and 8-9: products immunoprecipitated with rabbit anti SRP19 antiserum under native (lanes 3 and 8) or under denaturing (lanes 4 and 9) conditions. Lanes 5-6 and 10-11: products after immunoprecipitation with non immune serum under native (lanes 5 and10) or under denaturing (lanes 6 and 11) conditions. Molecular weights in kDa are indicated on the right.

Stanley. Six positive clones were found and these were further analysed: fusion proteins from these clones were separated by polyacrylamide gel electrophoresis and tested by Western blot analysis with affinity purified anti SRP19 antibodies. Antibodies affinity purified onto fusion proteins from the selected colonies were tested for their ability to recognize authentic SRP19 protein. One clone (p19.8, fig.1A) remained positive throughout these tests and was sequenced. It contained an open reading frame coding for 139 amino acid residues.

Five tryptic peptides of canine SRP19 were sequenced and compared with the amino acid sequence deduced from p19.8. Although the peptides were from canine SRP19, identical sequences were found in the selected human cDNA clone (see fig. 1B). This demonstrated that

the selected clone coded for human SRP19 and that the determined sequences are identical in the human and the canine protein.

Isolation of a full-length cDNA clone encoding SRP19

In order to obtain a full-length cDNA clone, a cDNA library from human liver was screened with the radiolabelled 507bp PstI-HindIII fragment of the p19.8 cDNA. One positive clone (p19i, fig.1A) was selected and sequenced. At the 5′ terminus, this cDNA contained 92bp more than p19.8. It contained an ATG codon 12bp upstream of the 5′ end of the p19.8 sequence which is positioned in the same frame as the open reading frame of p19.8. The sequence of 80bp upstream of this ATG does not contain ATG codons.

Apart from the additional 5′ sequence, p19i contains a fragment of 32 bp which is not present in p19.8. The sequence of this insert contains an inframe TAG stop codon (fig.1A). Two lines of evidence suggest that the insert in p19i is not part of the authentic pSRP19 mRNA: firstly, expression of p19i results in a protein considerably smaller than SRP19 isolated from canine pancreas (not shown). Secondly, protein analysis of SRP19 revealed peptide sequences which are encoded by nucleotide sequences located downstream of the TAG codon (fig.1B). It is possible that p19i is a cDNA clone derived from an aberrantly spliced RNA. The occurrence of aberrantly spliced mRNAs has been described previously, but their physiological role remains unclear (30-32).

Expression of SRP19 *in vitro*

To construct a cDNA clone encoding SRP19, 5′ sequences of p19i were ligated to 3′ sequences of p19.8 at the unique XbaI site to produce a clone designated pSRP19 (fig.1A).

The pSRP19 cDNA was cloned into the plasmid vector pGem2. The resulting construct was linearized at its unique PvuII site to allow *in vitro* transcription from the SP6 promoter, and transcripts were translated in wheat germ lysate. The translation products were immunoprecipitated with anti-SRP19 antibodies under native and denaturing conditions (fig. 2, lanes 3 and 4). Preimmune serum failed to precipitate SRP19 either under native or denaturing conditions (fig.2, lanes 5 and 6).

pSRP19, linearized at the unique Xba I site in the middle of the coding region, produced a shortened transcript which encoded the N-terminal 68 amino acids. Translation of this truncated mRNA resulted in a protein of 7kDa (SRP19N) (fig.2, lane7). SRP19N could be immunoprecipitated specifically with anti SRP19 antiserum. Incidentally, we noted that in contrast to the full-length product, precipitation of SRP19N was more efficient under native than under denaturing conditions (fig.2, lanes 8 and 9). No SRP19N was precipitated with preimmune serum (fig. 2, lanes 10 and 11).

The size of SRP19 expressed from pSRP19 was compared to that from canine pancreas. The major 35S-labelled translation product from pSRP19 showed identical electrophoretic mobility as SRP19 from canine pancreas, visualized by silver staining (fig.3).

The protein expressed from pSRP19 specifically binds to SRP 7SL RNA

Chromatography on DEAE Sepharose is a step in the purification of SRP because of the high

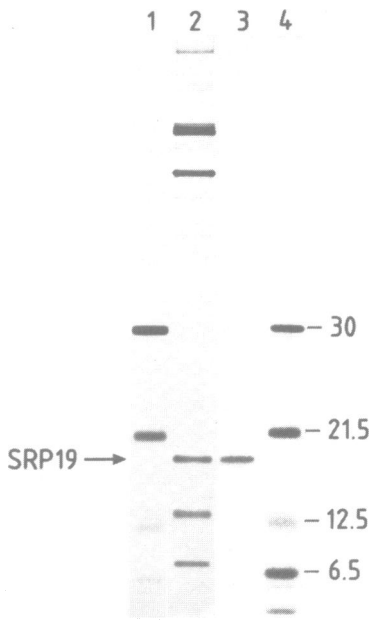

Fig.3 SRP19 from canine pancreas is of the same MW as SRP19 expressed from pSRP19. mRNA derived from pSRP19 was translated in wheat germ lysate in the presence of ^{35}S methionine. 4µl of the translation reaction was separated on a 10-15% SDS polyacrylamide gel and labelled proteins were visualized by autoradiography (lane3). Proteins from canine pancreas SRP were separated on the same gel and visualized by silver staining (37) (lane 2). Lanes 1 and 4: molecular weight markers; lane 1: visualized by silver staining; lane 4: visualized by autoradiography.

affinity of the 7SL RNA to the DEAE resin. Purified SRP proteins do not bind to DEAE resin in 0.3 M KOAc (13). Therefore the binding of SRP19 protein to the 7SL RNA can be monitored by retention of the RNA/SRP19 complex on DEAE sepharose and the flowthrough of free SRP19 protein.

Since SRP19 synthesized in a cell-free system might non-specifically aggregate, we tested binding to 7SL RNA first in a cotranslational assay. Transcripts of pSRP19 were translated in wheat germ lysate in the presence or absence of canine 7SL RNA. The reaction mixture was adjusted to 0.3M KOAc and chromatographed on DEAE sepharose. After centrifugation to remove aggregates and ribosomes we observed that even in the absence of 7SL RNA a large proportion of SRP19 was non specifically retained by the resin. In order to apply more stringent conditions in the binding reaction, we used a posttranslational test system: SRP19 was expressed in wheat germ lysate, the reaction mixture was adjusted to 0.3M KOAc, the ribosomes were removed by centrifugation and the supernatant was incubated either in the absence of added RNA or in the presence of tRNA or 7SL RNA (fig. 4). In the absence of 7SL RNA

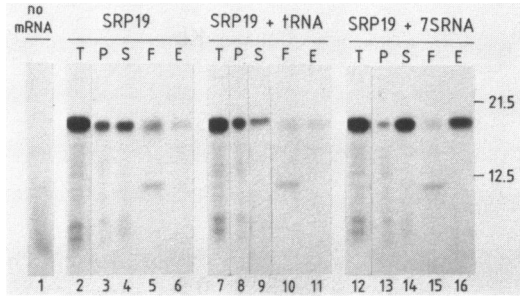

Fig.4 RNA binding. SRP19 mRNA derived from *in vitro* transcription was translated in wheat germ lysate and the radiolabelled protein was incubated either in the absence of canine pancreas 7SL RNA (lanes 2-6), in the presence of tRNA (lanes 7-11) or in the presence of 7SL RNA (lanes 12-16). After incubation, ribosomes were separated from the reaction mixture and the supernatant was applied to a DEAE sepharose column as described in materials and methods. T total *in vitro* translation reaction; P ribosomal pellet after centrifugation; S soluble proteins after centrifugation; F flow through from DEAE column in 0.3M KOAC; E eluate from DEAE column (2M KOAc). Lane 1 shows an in vitro translation reaction in the absence of added SRP19 mRNA. The molecular weights in kDa are indicated on the right.

a large proportion of labelled SRP19 cosedimented with the ribosomal fraction (fig. 4, lanes 3 and 8), whereas in the presence of 7SL RNA most of SRP19 was recovered in the supernatant (fig.4, lane 14). It is not clear whether the sedimentation of SRP19 is caused by aggregation of the protein or by an interaction with ribosomes in the absence of 7SL RNA: whichever is the case, this effect could be inhibited specifically by the addition of 7SL RNA. In the absence of 7SL RNA most SRP19 was collected in the flow through fraction of the DEAE sepharose column (fig. 4, lanes 5 and 10). In the presence of 7SL RNA, SRP19 was collected in the eluate fraction (fig.4, lane 16), indicating that it was bound to 7SL RNA.

SRP19 mRNA from HeLa cells is of a similar size as the *in vitro* transcript derived from pSRP19

In order to determine the size of the authentic SRP19 mRNA isolated from mammalian cells, polyadenylated mRNA isolated from HeLa cells and mRNA derived from *in vitro* transcription of pSRP19 was separated on agarose gels, blotted and probed with a radiolabelled probe of the full-length pSRP19 cDNA (fig.5). Under stringent washing conditions (0.1xSSC, 0.1%SDS, 65^{o}C), a single band of 0.9kb was detected in HeLa mRNA. This is approximately the same size as the mRNA transcribed from the SP6 promoter (fig.5, lanes 2 and 3). The comparison of the *in vitro* transcript with the authentic mRNA demonstrates that pSRP19 encodes near full-length SRP19 mRNA.

Under less stringent washing conditions (0.2xSSC, 0.1% SDS, 55^{o}C) an additional RNA band could be identified in HeLa cells of about 5kb. This RNA species was not detected using a radiolabelled probe missing large parts of the 3′ untranslated region (EcoRI-HindIII fragment,

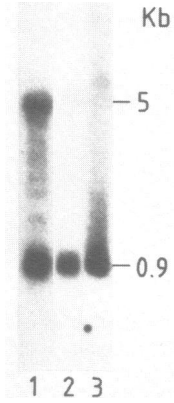

Fig. 5 Northern Blot Analysis. Polyadenylated mRNA from HeLa cells and RNA obtained from *in vitro* transcription of pSRP19 were separated on 1% agarose/formaldehyde gels, transferred to genescreen filters and probed with radiolabelled pSRP19 cDNA. Lane 1 and 2: mRNA from HeLa cells; lane 3: *in vitro* transcription mixture. Lane 1: hybridization after washing in 0.2xSSC, 0.1%SDS at 55°C; lanes 2 and 3: hybridization after washing in 0.1xSSC, 0.1% SDS at 65°C. The washing steps were carried out for 1h each.

fig. 1A) indicating that the cross-hybridization was due to sequences within the 3′ untranslated region (data not shown).

DISCUSSION

In this report we describe the cloning and sequencing of the cDNA encoding human SRP19. The authenticity of the cDNA was confirmed by the following criteria:

(i) an SRP19 cDNA clone was selected from an expression library using an anti-SRP19 antiserum. Antibodies affinity purified on the recombinant protein recognized the authentic SRP19 protein (retro-blot analysis). Antibodies raised against SRP19 protein specifically immunoprecipitated the protein expressed *in vitro* from the plasmid pSRP19.

(ii) amino acid sequences obtained from peptides of SRP19 were identical to amino acid sequences deduced from the cDNA sequence.

(iii) SRP19 from canine pancreas is a basic protein (13). The protein encoded by pSRP19 contains 28 basic residues and 14 acidic residues, the positively charged residues being clustered at the C-terminal tail of the molecule.

(iv) the protein expressed from pSRP19 is identical in size to SRP19 isolated from canine pancreas

(v) the protein expressed from pSRP19 specifically binds to 7SL RNA from canine pancreas.

The sequences of the tryptic peptides from canine pancreatic SRP19 represent about one third of the total SRP19 sequence. The same amino acids are found in the sequence deduced from

the human cDNA sequence, indicating a high degree of homology between the human and the canine protein.

When expressed in a wheat germ cell-free translation system, the protein encoded by pSRP19 migrates at the same position in SDS-polyacrylamide gels as SRP19 protein isolated from canine pancreas (fig.3). This provides evidence that pSRP19 encodes full-length SRP19 protein. SRP19 expressed from pSRP19 can be bound to 7SL RNA *in vitro*, showing that no posttranslational modification which is not performed in the wheat germ cell-free expression system, is required for specific RNA binding .

Since SRP19 is an RNA-binding protein it was interesting to compare it with other RNA binding proteins characterized from different RNPs. A sequence comparison of proteins bound to heterogeneous nuclear RNA (hnRNA), mRNA and pre-rRNA has revealed a consensus sequence for RNA binding proteins; the motif is rich in aromatic residues which are preceded by positively charged amino acids (33-34). This RNP consensus sequence may be directly involved in RNA binding: in the case of single stranded nucleic acids it has been shown that aromatic residues intercalate with the nucleotide bases (35) and the positively charged residues might then interact electrostatically with the carbohydrate moiety of the RNA (34). The RNP consensus sequence cannot be a prerequisite for RNA binding per se, since it is not found in ribosomal proteins (34). We have not found a sequence in SRP19 which would resemble the RNP consensus sequence and therefore the sequences of this protein which bind to the RNA have to be determined experimentally. SRP19 contains a very basic C-terminal domain of 7 lysine residues interrupted by 2 glycine residues. Due to the hydrophilic nature of this domain, the C-terminal tail of the molecule is most probably exposed on the molecule. It is possible that this positively charged domain interacts with the 7SL RNA.

It is conceivable that different types of RNA-protein interactions exist; for example binding of a protein could occur to single stranded RNA, to double stranded RNA or to hairpin structures. Each one of these interactions could require a different type of binding site within the respective proteins. The RNA structure which is recognized by both the yeast mRNA-poly(A) binding protein and hnRNP binding proteins appears to be single stranded RNA and the recognition by the protein is sequence specific (36). RNA footprinting analysis demonstrated that SRP19 binds to two distinct sites, corresponding to two loops located in the S segment of the 7SL RNA (15). It is likely that in the folded molecule these ends are in proximity to each other. In this case they could be recognized either by a single or by two separate protein domains. The cDNA is now amenable to site specific mutations and the binding assay allows a subsequent functional analysis in order to define sequences required for RNA binding.

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