Volume 13 Number 17 1985 Nucleic Acids Research

The secondary structure of the 7SL RNA in the signal recognition particle: functional implications

Christian Zwieb

European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg, FRG

Received 7 June 1985; Revised 9 August 1985; Accepted 16 August 1985

ABSTRACT

The secondary structure of the 7SL RNA in the signal recognition particle was determined by applying both a theoretical and an experimental approach. The compensatory base change approach was taken comparing the published sequences of human, Drosophila and Xenopus 7SL RNA's. The deduced secondary structure was confirmed by post-labeling of an RNase V1-nicked dog SRP with P32-pCp and RNA-ligase and analysis of the labeled RNA-fragments by non-denaturing/denaturing 2D polyacrylamide gel electrophoresis. Two interesting features in the secondary structure were revealed: Firstly, bases at positions 122 to 127 of the human 7SL RNA are not only able to pair with bases at positions 167 to 170, but also with a single-stranded region of the bases at positions 223 to 228, suggesting an alternative base pairing scheme for the 7SL RNA in all three organisms. In agreement with this finding, four different conformations were identified after transcription of the 7SL RNA from the genomic human clone. The involvement of the particular basepairing interaction postulated was confirmed by the analysis of a 7SL RNA deletion mutant (Sma1-409). Secondly, a significant sequence homolgy of the paired bases at positions 236 to 255 and 104 to 109 in 7SL RNA with bases in 5S ribosomal RNA at the positions 84 to 110 was noticed, suggesting that 5S ribosomal and 7SL RNA interact with the same target during protein biosynthesis.

These findings are summarized by proposing a mechanism for the translational arrest of protein synthesis by the signal recognition particle using specific sequences and an alternative configuration in the 7SL RNA.

INTRODUCTION

The signal recognition particle (SRP) is one of the few small ribonucleoprotein complexes with an established functional role, namely its involvement in the secretion of proteins (1-4). In a heterologous in vitro translation system (wheat germ translation system / dog pancreas SRP) SRP binds strongly to polysomes translating a protein with a signal sequence (5). After about 70 amino acids of the N-terminal portion of the

protein have been synthesized further translation is inhibited by the SRP. Only when the complex of polysomes, SRP and arrested protein interacts with components of the rough endoplasmic reticulum is the translational arrest released and the synthesis of the protein completed (6,7).

The SRP can be isolated in intact and functionally active form from the pancreas of dogs as an 11S particle. It consists of six proteins of 72, 68, 54, 19, 14 and 9kd and one 7S RNA-molecule of about 300 bases (8,9). A crude mapping of two sets of proteins (14,9kd and 72,68,54,19kd) to particular parts of the RNA has been achieved (10). Also RNA secondary structure models have been suggested for human and Drosophila 7SL RNA which are based on partial enzymatic digestion patterns (11).

Sequences in the 5'- and the 3'-domain of 7SL RNA are very similar to the middle repetitive Alu-sequences found in the genome of mammals. Only the central part of the RNA contains 7SL RNA specific sequences (12,13). It was therefore suggested that Alu-sequences represent processed 7SL RNA-genes (14).

In this paper the compensatory base change approach (15) was used in order to establish the secondary structures of the 7SL RNA's of human, Drosophila and Xenopus refering to the three published 7SL RNA sequences of these organisms (13,11,14). This approach has proven to be very powerful in the analysis of large rRNA molecules, especially if a sequence homology of about 70 percent is found between two species (16). In this case the two sequences are similar enough for an unambigious alignment, but also show enough sequence variability in order to be able to establish the secondary structure by defining a large number of compensatory base changes.

To confirm the theoretically deduced secondary structure experimentally, the approach of identifying interacting fragments by non-denaturing/denaturing 2D polyacrylamide gel electrophoresis was chosen because, in contrast to the use of partial enzymatic digestion (e.g. 17) or chemical modification (e.g. 18) of the RNA, this approach gives direct evidence as to wether an RNA fragment is base paired or not (19). A labeling procedure for the 7SL RNA in the SRP had to be developed in order to be able to detect the interacting fragments and to

identify them after gel electrophoresis.

The important structural but also dynamic properties of various RNA's as being part of ribonucleoprotein complexes have been emphasized recently by several independent findings. For example it has been shown that ribosomal RNA precursors of Tetrahymena can undergo a highly specific splicing reaction and are able to circularize the spliced intron without the activity of any protein (20). In the case of RNase P, it has been shown that its RNA component is sufficient to act as an enzyme in the processing reaction of the 5'-termini of tRNA-precursors (21). Also the ribosomal RNA's exist in different alternative conformations which possibly represent necessary intermediate stages during ribosome assembly and/or the translocation of tRNA and mRNA during protein biosynthesis (22). Therefore the possibility that 7SL RNA is a dynamic molecule was considered and investigated by using non-denaturing polyacrylamide gel electrophoresis and site-directed mutagenesis.

MATERIAL AND METHODS

Digestion and Labeling of SRP

1ml (0.8 A260) of gradient purified SRP (23) was dialysed at 4°C into a buffer containing 60mM HEPES pH 7.5, 24mM $MgCl_2$, 62.5mM KCl and 4mM DTT. RNase V1 (PL-Biochemicals, 1 to 10µl of a 1:1000 dilution of a stock of 700 units/ml) was added to 42µl of dialysed SRP and incubated at 25°C for 20min. The sample was put on ice to terminate the digestion reaction after which it was transfered to another tube containing 10µl of lyophilized P32-pCp (Amersham, 100µCi). 2µl of 250µM ATP, 2,7 units of T4 RNA-ligase (PL-Biochemicals) and water were added to make up a buffer containing 50mM HEPES pH 7.5, 20mM $MgCl_2$, 50mM KCl and 3.3mM DTT for a total reaction volume of 50µl. The sample was incubated overnight at 4°C.

Analysis of Interacting Fragments by Non-denaturing/Denaturing Polyacrylamide Gel Electrophoresis

RNA-fragments were separated on a 1mm thick, 40cm long 10% polyacrylamide gel containing Tris-HCl pH 7.8, SDS and EDTA as described (19). An equal volume of half concentrated reservoir buffer containing 35% glycerol and bromophenol blue was added

directly to the nicked and labeled SRP and loaded into a 1cm wide slot of the gel. Electrophoresis was at 4°C with a cooling fan until bromophenol blue had traveled for 25cm. When desired, an equal volume of phenol/chloroform was added to the labeled SRP before electrophoresis, spun for 5min., and the RNA from the water phase was precipitated by adding 2 volumes of 95% ethanol and incubated at -20°C for 4hrs. The pellet was washed with 95% ethanol and dissolved in half concentrated reservoir buffer. To digest the nicked and labeled SRP with proteinase K a 5µl aliquot was added to 15µl reservoir buffer containing 2µl proteinase K (Sigma, 5mg/ml). Incubation was for 15min. at 25°C after which the sample was loaded directly onto the gel. After electrophoresis an 8mm wide strip was cut out of the gel and polymerized into the top of a 20% , 40cm long (acrylamide: bisacrylamide 29:1) polyacrylamide gel containing Tris-borate, EDTA (PB-buffer (24)) and 7M urea. Electrophoresis was performed at 1600V using a thermoplate flushed with water heated to 60°C (25). After autoradiography of the two-dimensional gel radioactive spots were cut out and the RNA was eluted from the crushed gel piece by shaking overnight in 300µl of 0.5M ammonium acetate, 0.1% SDS, 0.1mM EDTA at 4°C. The eluted RNA was precipitated with ethanol, washed and dried. Aliquots of the sample were digested partially with RNase T1 (G-specific) or RNase U2 (A-specific) for sequence identification in a 20% or 15% sequencing gel (26).

Construction of the Deletion Mutant Sma1-409

Plasmid p7L30.1 (27) was digested to completion with the restriction endonuclease Sma1 (Boeringer) at the only site and incubated with exonuclease Bal31 (Boeringer) for 1 to 20min. to remove 5 to 100 base pairs from both ends of the DNA. After ligation with T4 DNA-ligase (New England Biolabs) and transformation of competent (28) Escherichia coli AB 1157 cells the presence of a deletion in miniprep-DNA was confirmed by screening with Sma1. The approximate size of the deletion was judged by the size of a Hind3, EcoR1-fragment covering the area of the deletion (29).

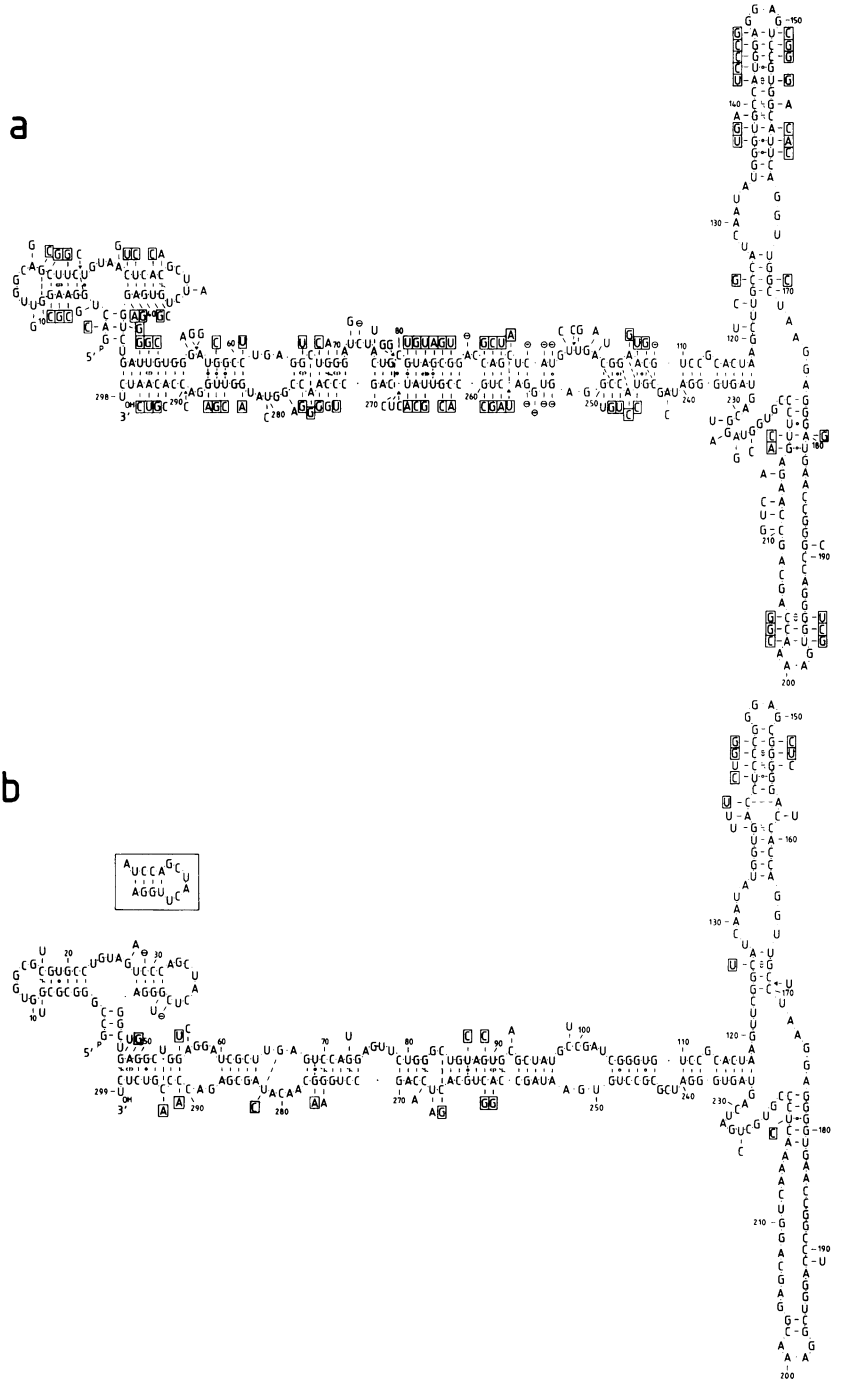
Transcription of 7SL RNA in a HeLa Cell Lysate

A HeLa-cell lysate was prepared as described by Manley

(30). Transcription was carried out in a volume of 25µl with 10µl lysate, 1.6µg cesium chloride purified plasmid DNA, four NTP's (50µM each), 4mM of creatine phosphate and 10µCi of P32-α GTP (Amersham). Incubation was for 1hr at 30°C. The reaction was stopped by putting the sample on ice and mixing it with 150µl phenol/chloroform and 150µl TNM/SDS-buffer (25mM Tris-HCl pH 7.8, 30mM NaCl, 10mM $MgCl_2$, 0.5% SDS). After centrifugation the water phase was removed, 150µl TNM/SDS- buffer was added to the phenol phase, the centrifugation was repeated and the water phase was removed. The two combined water phases were re-extracted with 300µl phenol/chloroform, the water phase was removed and extracted twice with chloroform. 15µl 4M Na-acetate pH 8.3 and 800µl 95% ethanol were added to precipitate the RNA by leaving the sample at -70°C for 2hrs. The pellet was collected by a 10min. spin, washed with 95% ethanol and dissolved in 200µl TNM/SDS. 10µl 4M Na-acetate and 600µl ethanol were added for a second precipitation and wash step. The pellet was dissolved in 200µl of PB/SDS (Tris-borate, EDTA with 0.2% SDS). Transcription efficiency was judged by the amount of P32-αGMP incorporated into ethanol precipitable material and was between 2 and 5%.

Analysis of the Transcribed RNA of p7L30.1 and Sma1-409

A 20µl aliquot of transcribed RNA was mixed with 20µl of PB/SDS containing 35% glycerol and bromophenol blue and was separated on a 7% (acrylamide:bisacrylamide 19:1) polyacrylamide gel in PB-buffer at 20mA at room temperature or on a 7% (29: 1) polyacrylamide gel in PB-buffer containing 7M urea at 40mA. RNA separated on PB-gels was eluted from the crushed gel piece by the addition of 300µl extraction buffer (20mM Tris-HCl pH 7.8, 1% SDS, 2mM EDTA, 300mM Na-acetate), 300µl phenol/chloroform and shaking at room temperature for 4hrs. After ethanol precipitation the RNA was washed once with 80% and once with 95% ethanol and dissolved in a small volume of 0.01% SDS. The RNA was either analyzed by polyacrylamide gel electrophoresis or subjected to fingerprint analysis using RNase T1. 3µl of RNase T1 (Calbiochem, 1mg/ml) were added to 10µl of sample containing 20µg of carrier tRNA and incubated at 37°C for 20min. The


a
b

resulting T1-oligonucleotides were separated using a two-dimensional PEI-fingerprint system (31).

RESULTS

Determination of the Secondary Structure of the 7SL RNA of Human, Drosophila and Xenopus using the Compensatory Base Change Approach

In Fig. 1 the secondary structures of Drosophila (a) and human (b) 7SL RNA's are presented as the basic structures. They are drawn in the way which is supported by compensatory base changes in related species (Drosophila vs human in Fig. 1a; human vs Xenopus in Fig. 1b). No problems in constructing very similar secondary structures for all three organisms were encountered. The structure essentially consists of a central rod of the bases at positions 48 to 118 paired to bases at positions 233 to 299. This central part is flanked by a small 5'-domain defined by the pairing of bases at positions 1 to 3 and 44 to 46 and a larger domain of 113 bases defined by an interaction between bases at positions 114 to 118 and 233 to 237. The region of the bases at the position 134 to 163 can be considered as a good example for how the compensatory base change approach works. This region forms a perfect stem and loop structure in Drosophila (see Fig. 1a). An almost identical structure for the equivalent RNA-region can be proposed for human and Xenopus which is supported by A-U to G-C or G-C to C-G compensations at the top of the stem structure. On the other hand an unpaired structure for most of the bases at positions 182 to 215 is suggested, because a possible pairing in this region is clearly discounted, while a small stem and loop in the middle of this region between bases 195 to 197 and 202 to 204 is supported by

Fig. 1
The secondary structure of Drosophila 7SL RNA with base changes in human 7SL RNA (a) and of human 7SL RNA with base changes in Xenopus 7SL RNA (b). Base changes in support of the secondary structure are boxed in. Additional or lost base pairs in the 'outside' sequence are indicated by dashed lines in the case of GC- and AU- or by broken circles in the case of GU-interactions. Inserted bases are shown by ▲, lost bases by ⊖. The numbering refers to the 'inside' sequence. The boxed-in insert in Fig.1b shows the Xenopus structure for the bases at position 27 to 41.

compensatory base changes. The bases at positions 110 to 118 and 233 to 240 are included in the secondary structure model as being paired, although no compensations are found in this highly conserved region.

Confirmation of the 7SL RNA Secondary Structure by Non-Denaturing/Denaturing Polyacrylamide Gel Electrophoresis

To confirm the suggested secondary structure presented above, interacting RNA-fragments were analyzed by non-denaturing/denaturing 2D polyacrylamide gel electrophoresis after a mild digestion of the 7SL RNA in SRP. A gradient purified SRP-preparation was dialysed into 1.2X RNA-ligase buffer containing in addition 62.5mM KCl. The RNA was digested at 25°C for 20min. after the addition of a suitable amount of RNase V1 (see Material and Methods). Labeling with P32-pCp and RNA-ligase was accomplished in 1X ligase buffer containing 50mM KCl at 4°C. Because RNA-ligase will not only label the natural 3'-end (position 299), but also 3'-OH groups created after cutting with RNase V1, radioactive landmarks can be introduced into the 7SL RNA at sites sensitive for RNase V1. Although the incorporation was not very efficient (about 0.5 to 10% , depending on the extent of digestion with RNase V1) due to the presence of 50mM KCl and a protection of the RNA by the proteins of the SRP a clear autoradiogram of the two-dimensional gel was obtained after an overnight exposure and enough radioactivity could be recovered from the prominent spots for identification of the already endlabeled RNA-fragments by using RNase T1 and RNase U2 in the sequencing reactions.

An example of an autoradiogram of a two-dimensional gel is given in Fig. 2a. In this case a rather mild RNase V1 digest was used, therefore some undigested 7SL RNA, labeled at its natural 3'-end, is still left undigested at the top left of the gel. A diagonal created by RNA fragments being without a partner under the conditions of the first dimension gel and a row of six spots below the undigested 7SL RNa can be seen. RNA-sequencing revealed that the five upper spots of the six all were labeled at the natural 3'-end. In addition a horizontal row of four spots with the same mobility as spot 5 is detected as being part of an interaction and RNA-seqencing actually showed that these

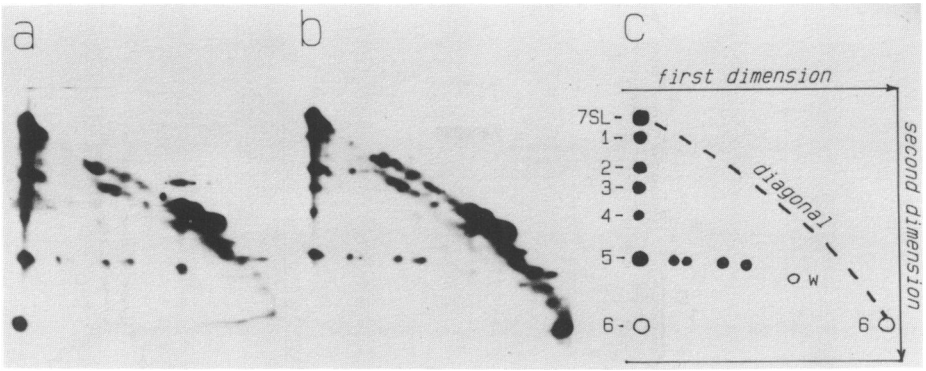


Fig. 2 Non-denaturing/denaturing 2D polyacrylamide gel electrophoresis of RNase V1-nicked and P32-pCp labeled 7SL RNA (a). The sample was extracted with phenol/chloroform prior to loading onto the gel (b). (c) Key diagram for figures (a) and (b). w marks the position of an unidentified RNA-fragment.

horizontal spots contain the identical sequences as spot 5 covering the bases from position at about 235 to 299 in the 7SL RNA. This is exactly the RNA-fragment which is predicted as being base paired with bases at positions 48 to 118 in the secondary structure model of the 7SL RNA (see Fig. 1). No partner for the labeled RNA-fragment was found presumbaly because bases inside the region at position 48 to 118 were not labeled in the first place or were already degraded by RNase V1.

Fig. 2b shows the autoradiogram derived from the same digest, but after phenol/chloroform extraction of the sample prior to loading onto the first dimension gel. It will be noticed that spot 6 is now no longer interacting with the 7SL RNA (which is positioned at the top left of the gel) but becomes a part of the diagonal (bottom right corner of the gel). This was confirmed by sequencing the RNA in both spots and by proving that they are identical. The RNA in spot 6 was found to consist of bases at the positions 1 to about 40 therefore containing the 5'-end of the 7SL RNA. An identical pattern as shown in Fig. 2a was derived when the sample was digested with proteinase K, but not phenol extracted (data not shown). It is therefore concluded that phenol treatment is destroying weak RNA-RNA interactions in the 7SL RNA. This is also in agreement with the observation that

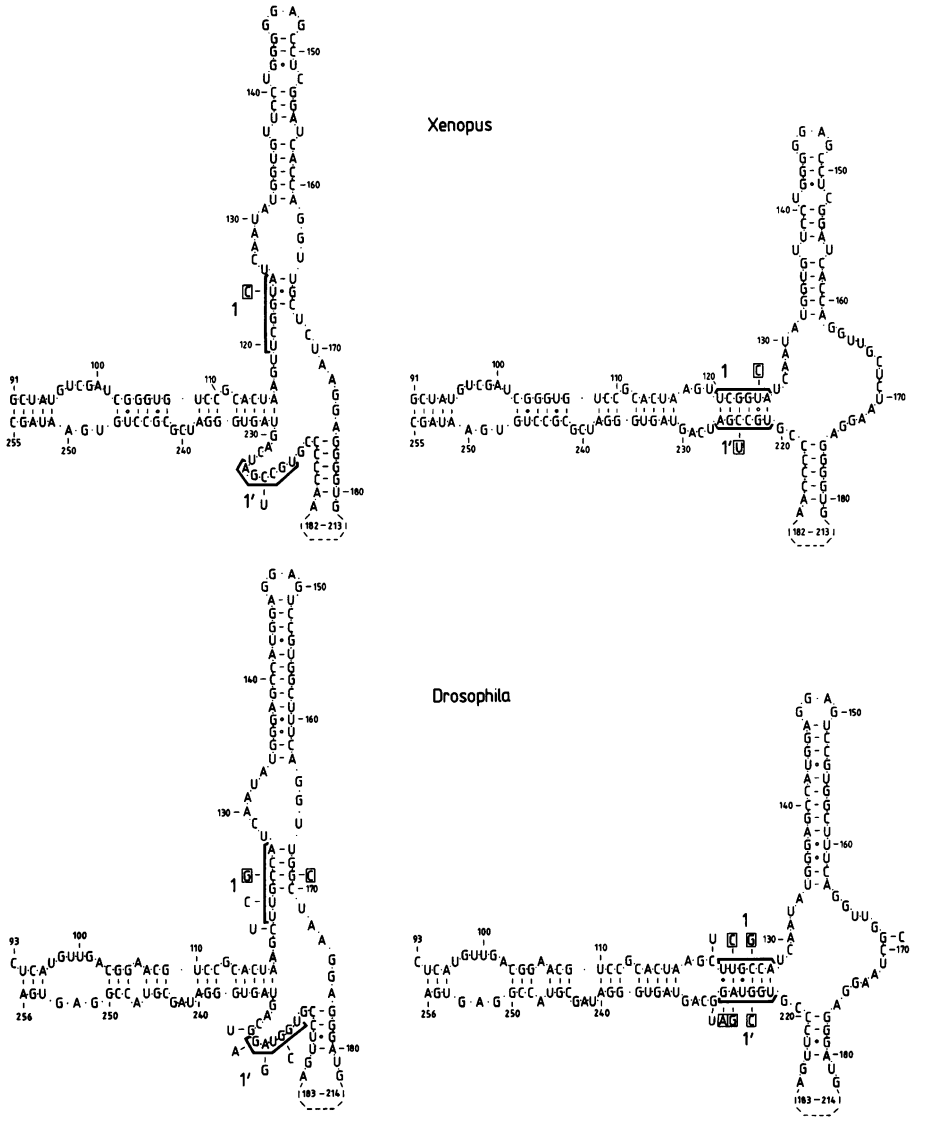


Fig. 3 Possible alternative base pairing in Xenopus and Drosophila 7SL RNA. Compensatory base changes in human 7SL RNA in support of the alternative base pairing scheme are shown like in Fig. 1.

after more extensive digestion of the RNA with RNase V1 and extraction with phenol/chloroform some RNA-RNA interactions are lost after two-dimensional gelelectrophoresis in comparison with

a digested sample which is loaded directly (data not shown).

A Possible Alternative Base Pairing in the 7SL RNA of all Three organisms

During the construction of the secondary structure model of the human 7SL RNA it was noticed that is possible to account for an alternative base pairing for the bases at positions 124 to 127. Their interaction with bases at positions 167 to 170 is confirmed by one compensatory base change (G-C to C-G) in Drosophila (see Fig. 1). On the other hand it is also possible to pair bases at the positions 122 to 127 with the otherwise single stranded region of the bases at positions 223 to 228 as is shown in Fig. 3. The same alternative interaction exists in the 7SL RNA of Xenopus because there is not much sequence variation between human and Xenopus in this particular region of the 7SL RNA. Nevertheless two base changes namely U to C at position 226 and C to U at position 126 can be easily explained if the alternative conformation also exists. In this case a helix between bases 122 to 127 and 223 to 228 of identical stability in human and Xenopus 7SL RNA is present. An even better alternative interaction can be drawn for Drosophila 7SL RNA, but only the same six base pairs present in human and Xenopus are supported by several compensating base changes. From these findings it is concluded that every 7SL RNA molecule has the potential to exist in two different conformations which are interchangable, by using a "switch"- structure consisting of the bases at positions 122 to 127, 167 to 170 and 223 to 228.

Analysis of 7SL RNA conformers of the Wild-Type and the Deletion Mutant Sma1-409

7SL RNA was transcribed and labeled in a HeLa cell lysate using P32αGTP and DNA of p7L30.1 (27). A deletion mutant (Sma1-409) was constructed by digesting p7L30.1-DNA to completion at a unique site for the restriction endonuclease Sma1. Its cutting site corresponds to bases at the position 145 in the 7SL RNA (see Fig. 1). The linear DNA was then partialy digested with exonuclease Bal31, religated and transformed into competent Escherichia coli AB1157 cells. Several deletions of approximately 100 to 300 base pairs in size were isolated, one (Sma1-409) being a deletion of about 50 base pairs as was judged

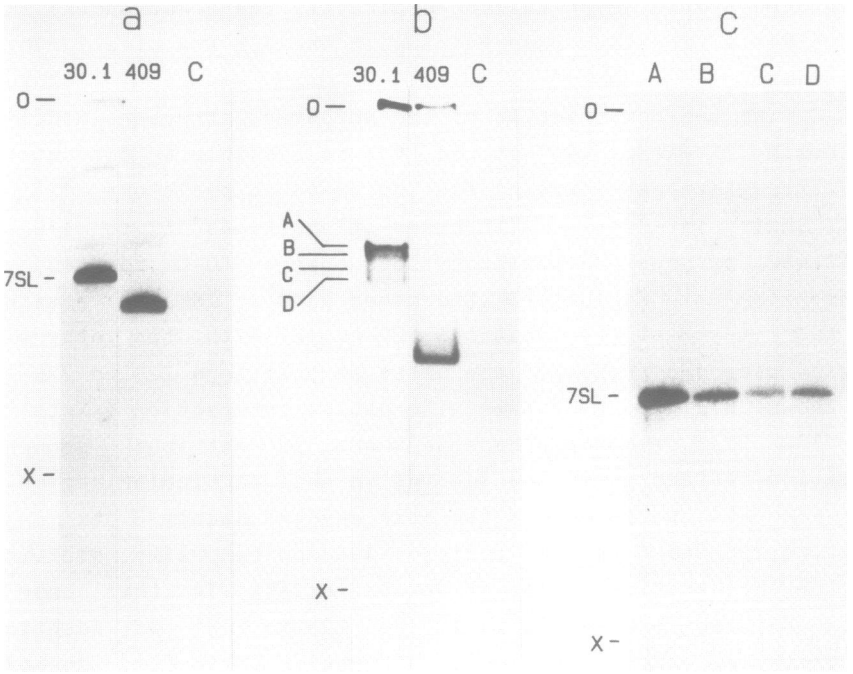


Fig. 4 Analysis of wild-type 7SL RNA and Sma1-409 on a 7% 7M urea polyacrylamide gel (a), and on a non-denaturing 7% Tris-borate/EDTA polyacrylamide gel (b). (c) re-run of the different conformers A, B, C and D in urea. x: xylenecyanol marker; 7SL: dog 7SL-RNA, run in parallel and stained with ethidium bromide.

by the reduced size of a Hind3, EcoR1-fragment covering the area of the deletion. Both the wild-type DNA and the mutant Sma1-409 are faithfully transcribed with equal efficiency and yield stable transcripts. This is shown by electrophoresis of an aliquot of the phenol extracted RNA on a denaturing gel (Fig.4a). The fingerprints of the wild-type and the deletion mutant RNA using RNase T1 show that all the expected T1-oligonucleotides are present in the wild-type 7SL RNA, but characteristic T1-oligonucleotides (e.g. CAUCAAUAUG at positions 125 to 134 and UUG at positions 165 to 167) are missing in the deletion mutant Sma1-409. The deletion starts at position 125 or 126 because oligonucleotide 24 (UUCG) is present and oligonucleotide 3-10 (CAUCAAUAUG) is absent in the Sma1-409

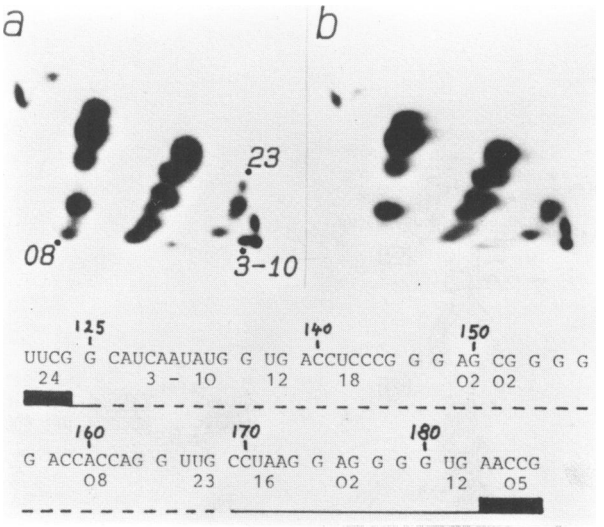


Fig. 5 RNase T1-fingerprint of wild-type 7SL RNA (a) and SmaI-409 (b). T1-oligonucleotides present in p7L30.1 but not in SmaI-409 are indicated in (a). The region of 7SL covering the area of the deletion is shown below. The two characteristic T1-oligonucleotides 24 and 05 are shown by black bars and are present in the deletion mutant. The dashed line covers the area of the deletion, while the solid line covers a region containing no characteristic T1-oligonucleotides.

mutant proving that the mutant RNA has lost the stem-loop structure of the bases approximately between positions 125 and 175 (Fig. 5). Interestingly when the transcribed RNA's were loaded onto a non-denaturing gel containing Tris-borate and EDTA, four bands can be resolved in the case of the wild-type 7SL RNA, while the mutant RNA gives rise to one only band (Fig 4b). Re-electrophoresis of the four different bands (A, B, C and D) proves that they represent four different conformers of 7SL RNA (Fig. 4c). It is therefore concluded that the removal of the stem-loop structure between the bases at the positions 124 and 170 in the 7SL RNA deprives the RNA of the capability to exist in different conformations.

Localization of Homologous Sequences between 7SL RNA and 5S Ribosomal RNA in their Secondary Structures

An extensive sequence homology (16 out of 20 bases being identical) was noticed between human 7SL RNA and human 5S

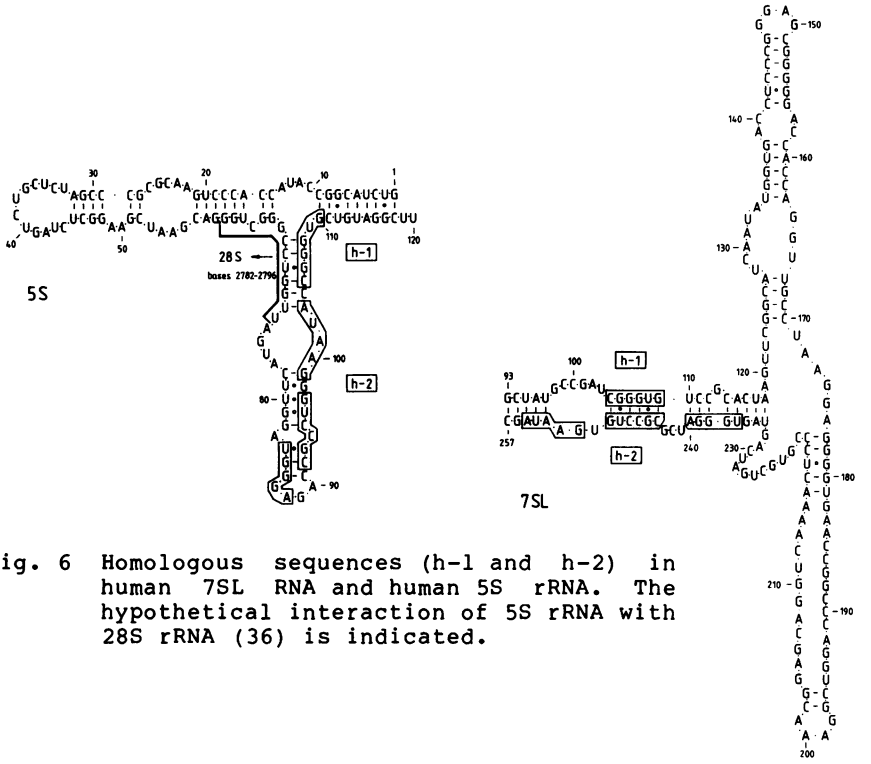


Fig. 6 Homologous sequences (h-1 and h-2) in human 7SL RNA and human 5S rRNA. The hypothetical interaction of 5S rRNA with 28S rRNA (36) is indicated.

ribosomal RNA. The same degree of homology is found between Xenopus 7SL and 5S rRNA, while in Drosophila the homology is not so striking (14 out of 22 bases being identical), but still significant by using a 'compare' computer program (32, 33). As is shown in Fig. 6 the sequences between the very much conserved bases at positions 236 and 255 in 7SL RNA are to a large extent identical to the bases at positions 84 to 103 in 5S rRNA. Even the spacing (one triplet and one single base) between the three blocks of identical sequences is conserved in the human and Xenopus RNA's (but not in the Drosophila RNA's). In addition the sequence CGGGUG (h-1 in Fig. 6) occurs in 7SL RNA and 5S rRNA. In 7SL RNA this sequence is located at positions 104 to 109 and is paired to CGCCUG at positions 244 to 249 which is part of h-2. In 5S rRNA h-1 is located at positions 105 to 110 and interacting with non-homologous sequences of the bases at positions 67 to 72 (CCUGGU). Interestingly the equivalent region

of Escherichia coli 5S rRNA was found to be in an interaction with a particular RNA region of the large ribosomal subunit as is indicated in Fig. 6 (16). The functional implications of these findings will be discussed below.

DISCUSSION

Refined secondary structure models for the 7SL RNA in the signal recognition particle of human, Xenopus and Drosophila are suggested by using the compensatory base change approach. Essentially identical secondary structures can be drawn for the 7SL RNA of these three organisms, and it is likely that the 7SL RNA sequences of other eucaryotes published in the future will not change the basic structural features. These include a central rod containing the bases at positions 48 to 118 and 233 to 299, a small domain containing the 44 bases of the 5'-end and a larger domain containing the stem-loop of bases 134 to 163. Important differences to the secondary structure of human and Drosophila 7SL RNA's published previously (11) are a more open structure of the bases at positions 182 to 215, a new base pairing between bases at positions 196,197 and 202,203, an open structure for the bases at positions 121,122 and 172,173, and a correction in the interaction between bases at positions 177 to 181 and 216 to 220.

Speculations about the three-dimensional arrangement of the 7SL RNA seem to be somewhat preliminary, but it is possible that the single-stranded loops around positions 15 and 35, respectively, interact in a similar fashion to the dihydroU- and the TΨC-loop in tRNA's, because base pairing is possible, the bases in the two loops are conserved and the loss of single-stranded bases at one side in the RNA is compensated by opening up the stem at the other side of the interaction. By coaxial stacking of the helix composed of the bases at position 28 to 31 and 40 to 43 and the helix of the bases 1 to 3 and 44 to 46 a tRNA-like RNA-part without an anticodon stem and loop is created (see Fig.1b). As simple model building has shown (unpublished results) such additional tertiary interactions are certainly necessary to fold the secondary structure of the 7SL RNA into a SRP that fits to the shape and the size visualized in the

electron microscope (34). Such a view also implies that the basic structure of the SRP is built up by the 7SL RNA itself, while most of the six SRP-proteins will bind to certain regions of the RNA and only a few will be bound by protein-protein interactions only.

It was shown that by nicking double-stranded regions of the RNA in the SRP with RNase V1 and labeling the liberated 3'-OH-groups with P32-pCp and RNA-ligase radioactive landmarks can be introduced into RNase V1 sensitive sites of an unlabeled ribonucleoprotein particle. Because the incorporation of radioactive pCp is not very efficient, the possibility that a minor subfraction of the SRP-preparation is labeled preferentially cannot be excluded. Also some small RNA-species of unknown nature (e.g. w in Fig. 2c) which are not very abundant but which are labeled quite efficiently make the analysis of interacting fragments after a more extensive digestion difficult. Nevertheless the basic features of the theoretically deduced secondary structure of 7SL RNA were confirmed. Clearly the RNA-part consisting of bases at the positions 235 to 299 is shown to be in a strong interaction which is not lost after phenol/chloroform treatment. The 5'-end of the RNA (bases at positions 1 to 40) is only loosely attached because its binding is abolished after extraction of the sample with phenol/chloroform. The two proteins of 9kd and 14kd (10) are probably not responsible for stabilizing the attachment of the 5'-end to the rest of the 7SL RNA because it seems unlikely that a peptide possibly increasing the stability of the interaction is protected against proteinase K-digestion in the SDS containing buffer.

A particulary interesting feature of the secondary structure includes an alternative base pairing for the bases at the positions 122 to 127. They can interact with the bases at the positions 167 to 170 or with bases at the positions 223 to 228. Both interactions are supported by compensating base changes therefore making it likely that every RNA-molecule is able to exist in at least two different conformations. No topological difficulties are encountered (using simple model building) to "switch" from one conformer to the other. Actually

four different forms of 7SL RNA are found experimentally after the separation of the transcribed human 7SL RNA on a non-denaturing polyacrylamide gel. It should be noted that two conformers of 7SL RNA were identified previously in the analysis of the distribution of small RNA's in different fractions of HeLa-cells (35). Although it is not yet possible to correlate any of these observed conformers to a particular secondary or tertiary structure of the RNA, it is likely that the quite conserved bases of the single-stranded regions at position 182 to 194 and 205 to 215 are part of the "switch", when the three-dimensional structure of the RNA is considered. The stem-loop structure consisting of the bases at the position 124 to 170 containing the dynamic sequence UCGGCA around position 125 is responsible for the capacity of the RNA to exist in different conformations. This is clearly demonstrated by the analysis of the transcribed RNA of the deletion mutant Sma1-409 which shows one single band on a non-denaturing polyacrylamide gel. It is likely that the bases at the positions 134 to 163 (Fig. 1b) from the area of the deletion do not have dynamic properties because the primary sequence of this part of the RNA is highly variable while its secondary structure is highly conserved.

A second feature of the 7SL RNA is the strong sequence homology of the bases at the positions 236 to 255 to bases at positions 84 to 103 in 5S ribosomal RNA. These bases cover the most conserved area of the 7SL RNA, when human, Xenopus and Drosophila are compared. In addition h-1 is base paired to homologous sequences in the 7SL RNA (positions 244 to 249) but paired to non-homologous sequences in the secondary structure of the 5S rRNA (positions 67 to 70). These non-homologous sequences of 5S rRNA have been found to base pair with a region in Escherichia coli 23S rRNA using the analysis of interacting fragments by 2D gel electrophoresis (16). An equivalent interaction between 5S rRNA and the 28S rRNA can be confirmed in eucaryotes by sequence comparison (36). It is suggested that 5S rRNA uses the exposed bases at position 60 to 72 in order to interact with the 23S or 28S rRNA, respectively, during the initiation of protein synthesis (37,38, and unpublished results). If this is true the bases at positions 103 to 109 in

5S rRNA have to become single-stranded and might be used to assure that the next step in protein synthesis, namely elongation, is not yet occurring. It is conceivable that h-1, in conjunction with h-2 is used by the 7SL RNA of the SRP to interact with the same or a similar target in the large ribosomal subunit like 5S rRNA. h-1 and h-2 are probably base paired for some time because compensations between Drosophila and human 7SL RNA exist in this region if one allows for one base "slippage" only (see Fig. 1a). It is therefore suggested that this interaction exists and is broken upon binding of the SRP to the ribosome in order to fulfill its function. In such a case, topological constraints created by exposing h-1 and h-2 could cause the RNA in the SRP to adapt an alternative conformation by using the "switch"-structure located in the neighbourhood to the homologous sequences. A mechanism of the action of the SRP involving specific sequences of its RNA and an alternative base pairing can therefore proposed as follows: If a signal sequence emerges from the ribosome during elongation, SRP binds strongly to polysomes possibly involving the h-sequences and influencing elongation by interacting with a similar target on the ribosome as does 5S rRNA. At the same time the SRP would adopt an alternative conformation using, among others, the two base pairing possibilities of the sequence UCGGCA around position 125. Only in this particular conformation would the complex of ribosomes and SRP be able to interact specifically with membrane components succeeded by the release of SRP and further synthesis of the protein into the membrane of the rough endoplasmic reticulum. By this mechanism it is assured that SRP by itself is not able to interact in a functionally active way with the membrane in which case it would destroy its own function by occupying specific binding sites in the membrane.

Further studies relating to the functional role of the 7SL RNA in the SRP will have to consider the nature of its alternative base pairing possibility but also the involvement of specific parts of the SRP in defined functional steps during its interaction with the translating ribosome and the membrane.

ACKNOWLEGEMENTS

I am grateful to Bernhard Dobberstein for support and to Elisabetta Ullu, Eckart Gundelfinger, Joachim Lipp and Wolfgang Lauer for inspiring discussions. I also like to thank Christopher Marshallsay for excellent technical assistance, Lucia Plefka for her patience with the figures and Graham Warren for critical reading of the manuscript.

REFERENCES

1. Warren, G., and Dobberstein, B. (1978) Nature (London) 273, 569-571
2. Walter, P., Ibrahimi, I., and Blobel, G. (1981) J. Cell Biol. 91, 545-550
3. Walter, P., Gilmore, R., and Blobel, G. (1984) Cell 38, 5-8
4. Hortsch, M., and Meyer, D. (1984) Biol. cell. 91, 557-561
5. Walter, P., and Blobel, G. (1981) J. Cell Biol. 91, 557-561
6. Meyer, D., Krause, E., and Dobberstein, B. (1982) Nature (London) 297, 641-650
7. Gilmore, R., Blobel, G., and Walter,P. (1982) J. Cell Biol. 95, 463-469
8. Walter, P., and Blobel, G., (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 7112-7116
9. Walter, P., and Blobel, G. (1982) Nature (London) 299, 691-698
10. Gundelfinger, E.D., Krause, E., Melli, M., and Dobberstein, B. (1983) Nucl. Acids Res. 11, 7363-7374
11. Gundelfinger, E.D., DiCarlo, M., Zopf, D., and Melli, M. (1984) EMBO J. 3, 2325-2332
12. Jelinek, W.R., and Schmid, C.W. (1982) Ann. Rev. Biochem. 51, 813-844
13. Ullu, E., Murphy, S., and Melli,M. (1982) Cell 29, 195-202
14. Ullu, E., and Tschudi, C. (1984) Nature (London) 312, 171-172
15. Fox, G., and Woese, C.R. (1975) Nature (London) 256, 505-507
16. Glotz, C., Zwieb, C., Brimacombe, R., Edwards, K. and Koessel, H. (1981) Nucl. Acids Res. 3287-3306
17. Vassilenko, S.K., Carbon, P., Ebel, J.P., and Ehresmann,C. (1981) J. Molec. Biol. 152, 699-721
18. Noller, H.F. (1974) Biochemistry 13, 4694-4703
19. Ross, A., and Brimacombe, R. (1979) Nature (London) 281, 271-276
20. Cech, T.R., Tanner, N.K., Tinoco, I.,Jr., Weir, B.R., Zuker, M., and Perlman, P.S. (1983) Proc. Acad. Sci. U.S.A. 80, 3903-3907
21. Guerrier-Takada, C., and Altman, S. (1984) Science (Washington, D.C.) 223, 285-286
22. Brimacombe, R., Maly, P., and Zwieb, C. (1983) in: Progress in Nucl. Acids Res. 28, 1-48 ed. Cohn, W.E. Academic Press, New York
23. Meyer, D., and Dobberstein, B. (1980) J. Cell Biol. 87, 498-502

24. Peacock, A.C., and Dingman,C.W. (1968) Biochemistry 7, 668-674
25. Ansorge, W., and Barker, R. (1984) J. Biochem. Biophys. Meth. 9, 33-47
26. Donis-Keller, H., Maxam, A.M., and Gilbert, W. (1977) Nucl. Acids Res. 4, 2527-2538
27. Ullu, E., and Weiner, A.M. (1984) EMBO J. 3, 3303-3310
28. Dagert, M., and Ehrlich, S.D. (1979) Gene 6, 23-28
29. Maniatis, T., Fritsch, E.F., and Sambrock, J. (1982) in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory
30. Manley, J.L. (1984) in: Transcription and Translation, ed. Hames, B.D., and Higgins, S.J. IRL Press, Oxford
31. Volckaert, G., and Fiers, W. (1977) Analyt. Biochem. 83, 228-239
32. Erdmann, V.A., Wolters, J., Huysmans, E., Vandenberghe, A., and Wachter, R.,De., (1984) Nucl. Acids Res. 11, r133-r166
33. Maizel, J.V.,Jr., and Lenk, P.L. (1981) Proc. Nat. Acad. Sci. U.S.A. 78, 7665-7669
34. Andrews, D.W., Walter, P., and Ottensmeyer, F.P. (1985) Proc. Nat. Acad. Sci. U.S.A. 82, 785-789
35. Zieve, G., and Penman, S. (1976) Cell 8, 19-31
36. Clark, C.G., Tague, B.W., Ware, V.C., and Gerbi, S. (1984) Nucl. Acids Res. 12, 6197-6220
37. Dahlberg, J.E., Kintner, C., and Lund, E. (1978) Proc. Nat. Acad. Sci. U.S.A. 75, 1071-1075
38. Zwieb. C., and Dahlberg, A.E. (1984) Nucl. Acids Res. 12, 7135-7152