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Identification of dynamic sequences in the central domain of 7SL RNA

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ABSTRACT

The human 7SL RNA component of the signal recognition particle can be separated into four major conformers by non-denaturing polyacrylamide gel electrophoresis. We have investigated what sequences give 7SL RNA the property to exist in different conformations. The human 7SL gene 7L30.1 was mutagenized using the random linker insertion approach and twelve mutant genes carrying alterations in the central domain of 7SL RNA were characterized. Mutant RNAs were produced by in vitro transcription of the various templates and their electrophoretic behaviour was determined. Bases between positions 98 and 133 as well as 206 and 251 proved to be necessary for the 7SL RNA to be able to exist in alternative conformations, while changes at the positions 85 to 97, 144 to 166 and 252 to 266 did not abolish this property. The dynamic sequences are located in the "central T" in the secondary structure of the 7SL RNA. They are phylogenetically conserved and include bases which are homologeous to 5S ribosomal RNA. A dynamic core structure composed of the dynamic parts of the 7SL RNA is suggested. An attempt was made to define the different conformers present in the wild-type 7SL RNA. These alternative configurations could play a functional role during the initial stage of protein translocation across the membrane of the endoplasmic reticulum.

INTRODUCTION

In ribonucleoprotein complexes RNA molecules not only provide support for proteins, but also are able to act as enzymes themselves, which has been demonstrated in the case of M1 RNA of RNase P (1) and the self-splicing of precursor ribosomal RNA of Tetrahymena (2). Conformational changes affecting the RNA have been postulated in order to fulfill the function of a ribonucleoprotein complex. Such dynamic changes are suspected to be relatively subtle in small molecules such as tRNA (e.g. 3) or quite extensive in larger

molecules such as ribosomal RNA (4,5) or in messenger RNA (e.g. 6). In the case of the larger RNAs "pseudoknots" (7), "moving bulges" (8) and Z-RNA (9)are being discussed as possibilities to introduce major conformational changes.

Eukaryotic 7SL RNA, 300 nucleotides in length, functions in protein secretion as a component of signal recognition particle or SRP (10). It has a size intermediate between the two size extremes mentioned above and therefore represents a suitable model system to study the role of alternative conformations in the function of the ribonucleoprotein particle.

The secondary structure of the 7SL RNA has been investigated using single strand and double strand specific nucleases (11) and was established after comparing the primary sequences of human, Drosophila and Xenopus 7SL RNAs (12,11,13) using the compensatory base change approach (14). The results of these studies are consistent with the possibility that the 7SL RNA molecule can assume more than one conformation. This is in agreement with the observation that human 7SL RNA can be separated into four major conformers of different mobility by electrophoresis through non-denaturing polyacrylamide gels (14). Moreover, preliminary experiments indicate that the removal of nucleotides between positions 125 and 175 of the 7SL sequence abolishes the ability of the RNA to assume different conformations (14).

In order to define more precisely what sequences confer upon 7SL RNA the potential to assume alternative conformations, we generated a collection of twelve human 7SL genes carrying discrete mutations in the central region of the 7SL coding sequence. These 7SL mutants were recovered after mutagenesis of the human 7SL gene 7L30.1 using the random linker insertion approach (15) and consisted either of simple linker insertions or linker insertions accompanied by small deletions or duplications. Mutant 7SL RNAs were then produced by *in vitro* transcription of the altered 7SL genes; finally the ability of each mutant RNA to adopt different conformations was determined by electrophoresis through a non-denaturing gel. Our results

indicate that the "dynamic sequences" in human 7SL RNA include nucleotides between positions 98 and 133 and 206 to 251. Such sequences are located in the "central T" in the secondary structure of the 7SL RNA and include bases which are homologous to 5S ribosomal RNA. We discuss the possible role of the dynamic sequences of 7SL RNA in relation to SRP function.

MATERIAL AND METHODS

Construction and sequence determination of mutants

Octameric Xho1 linkers were randomly inserted into the human 7SL gene 7L30.1 (16) essentially as described by Heffron et al. (15). Plasmid DNA p7L5'-66 (17) was used as the starting material. 5 µg of DNA were digested with DNAse1 in a buffer containing 1.5 mM MnCl2, 20 mM Tris-HCl pH 7.5. Digestion conditions were chosen to obtain approximately 50% conversion of supercoil to linear molecules. Following phenol extraction and ethanol precipitation, the ends of the DNA were polished with Klenow polymerase and dNTPs and ligated to a 100 fold molar excess of phosphorylated Xho1 linkers (Biolabs, CCTCGAGG) using T4 DNA ligase. The ligation mixture was then digested with Xho1 and the linear plasmid DNA was isolated by electroelution after fractionation through a 0.8% agarose gel. The linear DNA was recircularized by ligation and used to transform E. coli strain DH1. Approximately 2000 ampicillin resistant colonies were scraped off the agar plates, diluted in LB broth and grown to saturation. Plasmid DNA was isolated and the insert DNA was purified by electrophoresis through a 2.5% polyacrylamide/0.7% agarose gel, after digestion with EcoR1 plus Hind3. The DNA fragments containg a mixture of wild-type and mutagenized 7SL genes were recloned between the EcoR1 and Hind3 sites of plasmid vector pUC13. Plasmid DNA was isolated from a bulk culture of ampicillin resistant colonies, digested with with Xho1 and fractionated through a 1.4% agarose gel. The linear DNA was electroeluted, recircularized and transformed into E. coli DH1 cells. Plasmid DNAs from approximately 100 independent colonies were analyzed by restriction enzyme analysis to determine the approximate

position of the linker insertion. Twelve clones carrying insertions in the central domain of the 7SL coding sequence were selected for further analysis.

For sequence analysis mutant and wild-type plasmids were retransformed into E. coli AB 1157 cells. Hind3-EcoR1 fragments were prepared from isolated plasmid DNA and introduced into the replicative form of M13 mp9 DNA (18) which had been digested with Hind3 plus EcoR1. After ligation with T4 DNA ligase E. coli 1718 cells were transformed and white plaques were isolated. Single stranded M13 DNA was prepared from 2 ml cultures and used for sequence determination (19).

Transcription of wild-type and mutant DNAs

A HeLa-cell lysate was prepared as described by Manley (20). Transcription was carried out essentially as described previously (14) in a volume of 25 µl, containing 10 µl lysate, 1.6 µg caesium chloride purified plasmid DNA, the four NTPs (50 µM each), 4 mM of creatine phosphate and 10 uCi of P32-αGTP (Amersham). Incubation was for 1 hr at 30°C. The reaction was stopped by putting the sample on ice and mixing it with 150 µl phenol/chloroform and 150 µl TNM/SDS-buffer (25 mM Tris-HCl pH 7.8, 30 mM NaCl, 10 mM MgCl2, 0.5 % SDS). After centrifugation the water phase was removed, 150 µl 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 300 µl phenol/chloroform, the water phase was removed and extracted twice with chloroform. 15 µl 4 M Na-acetate pH 8.3 and 800 µl 95 % ethanol were added to precipitate the RNA by leaving the sample at -70 C for 2 hrs. The pellet was collected by a 10 min. spin, washed with 95 % ethanol and dissolved in 200 µl TNM/SDS. 10 µl 4 M Na-acetate and 600 µl ethanol were added for a second precipitation and wash step. The pellet was dissolved in 200 µl of PB/SDS (90 mM Tris-borate, 2.5 mM EDTA with 0.2 % SDS).

Polyacrylamide gel electrophoresis of transcribed RNAs

A 5 µl aliquot of transcribed RNA was mixed with 5 µl of PB/SDS containing 35 % glycerol and bromophenol blue and was separated on a 1 mm thick 7 % (acrylamide:bisacrylamide 19:1)

polyacrylamide gel in PB- buffer at 20 mA at room temperature or on a 7 % (29:1) polyacrylamide gel in PB-buffer containing 7 M urea at 40 mA. RNA separated on non-denaturing PB-gels was eluted from the crushed gel piece by the addition of 300 µl extraction buffer (20 mM Tris-HCl pH 7.8, 1 % SDS, 2 mM EDTA, 300 mM Na-acetate), 300 µl phenol/chloroform and shaking at room temperature for 4 hrs. 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 and analyzed further on urea containing polyacrylamide gels.

Scanning of autoradiograms

Autoradiograms from transcribed RNA which had been separated on non-denaturing polyacrylamide gels were performed after fixation of the gels in 50% formamide and drying of the fixed gel. The x-ray film was scanned using a Helena Model 1053 Quick Scan apparatus.

RESULTS

Construction and determination of the primary structure of 7SL RNA mutants

Linker insertion mutations were introduced into a derivative of the human 7SL RNA gene 7L30.1 (16) called 7L5′-66 (17) as described in Material and Methods. The successful insertion of the linker DNA introduced a recognition site for Xho1, a sequence not present in p7L5′-66. The approximate locations of the insertions were determined by digesting the mutant plasmid DNAs with Xho1 plus Hind3 or EcoR1 and by analyzing the resulting fragments on a polyacrylamide gel (22). Twelve different mutants having alterations in the central domain of the 7SL RNA were characterized further. Their sequences were determined after insertion of the Hind3-EcoR1 recombinant fragments into M13 mp9 RF DNA (18) and dideoxy sequencing of the single stranded DNAs (19).

The results of the sequence analysis are summarized in Figure 1. In most cases (clones 1, 2, 3, 4, 8, 9 and 10) a simple linker insertion into the various regions of the central domain of the 7SL RNA can be observed. The number of base

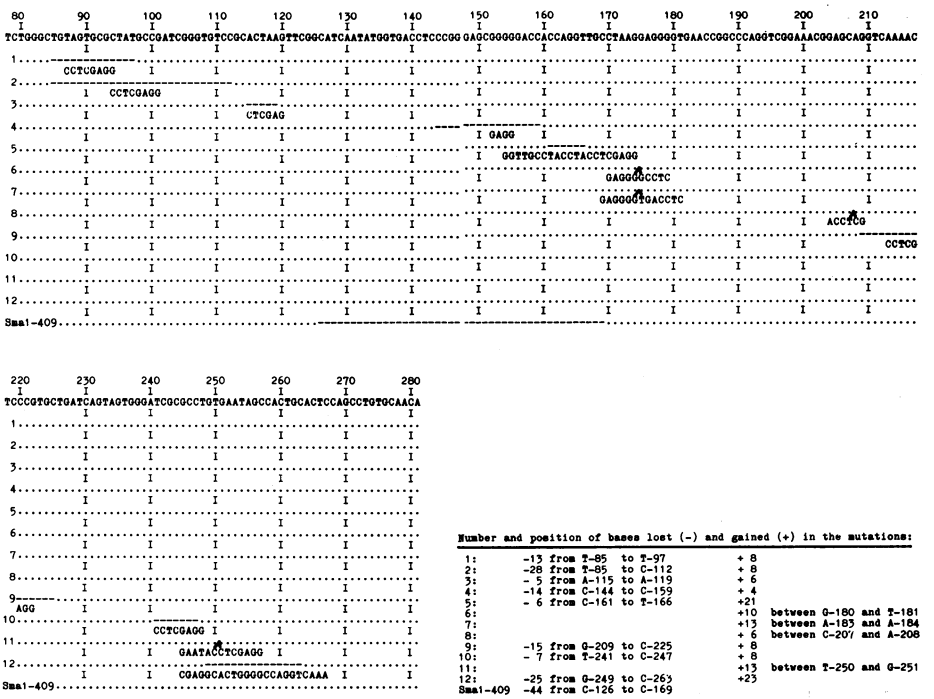

Fig. 1: Characterization of mutants. The wild-type sequence of the central part of the human 7SL DNA between positions 78 and 281 is shown at the top. The twelve linker insertion mutants are listed below and numbered 1 to 12 according to the position of the mutation in the sequence. - indicates bases lost in the mutant clones. The number and position of bases lost and gained in the twelve linker insertion mutants 1 to 12 and in the deletion mutant Sma1-409 (14) are summarized at the bottom right of the Figure.

pairs lost from the wild-type DNA after DNase1-treatment and polishing of the ends varies from none (in the mutants 7 and 8) to 28 base pairs (in mutant 2). In five cases (mutants 5, 6, 7, 11 and 12) non-linker sequences have been inserted which in the mutants 6 and 7 appear to contain 7SL RNA specific sequences. Figure 1 also contains the sequence of a 44 base pair deletion mutant (Sma1-409) which was constructed previously (14).

Transcription of wild-type and mutant clones

Purified DNA of plasmid 7L5'-66 or the various mutants was transcribed *in vitro* in a HeLa cell extract in the presence of

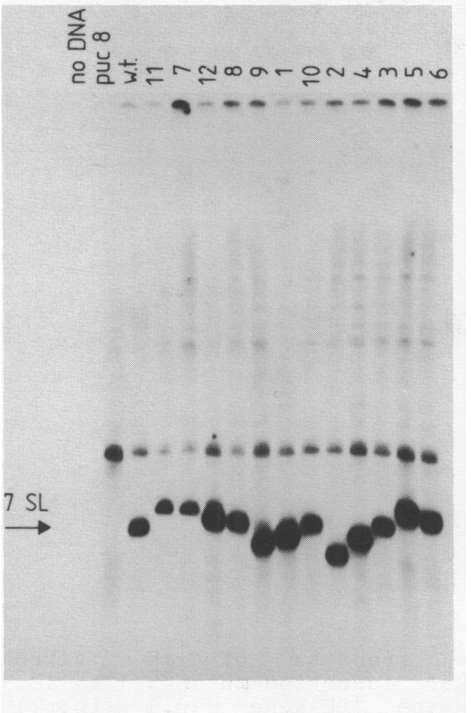

Fig. 2: Autoradiogram of the products of in vitro transcription of 7SL Xho1-linker insertion mutants 1 to 12, plasmid pUC8 (pUC8) and wild-type 7SL-gene 30.1 (w.t.) separated on a denaturing polyacrylamide gel. The position of isolated 7SL RNA is indicated.

32P-αGTP (20). The products of transcription were analyzed by polyacrylamide gel electrophoresis as described in Material and Methods. Analysis under denaturing conditions showed that all constructions were transcribed efficiently producing RNAs of the expected size, which is dependent on the number of bases gained or lost in the mutant genes (Figure 2) . An RNA-fragment larger than 7SL RNA was always observed in minor amounts and represents a vector- specific transcript.

Analysis of the conformers of wild-type and mutant RNAs

The ability of the wild-type and the mutant RNAs to adopt alternative conformations was determined by electrophoresis of the deproteinized transcription reactions through non-denaturing polyacrylamide gels. Figure 3 shows a representative example of this analysis.

Seven mutant RNAs (2, 3, 8, 9, 10, 11 and Sma1-409) migrated as a single homogeneous species, while the wild-type 7SL RNA and the remainder six mutant RNAs (1, 4, 5, 6, 7 and 12) were separated into more than one electrophoretic

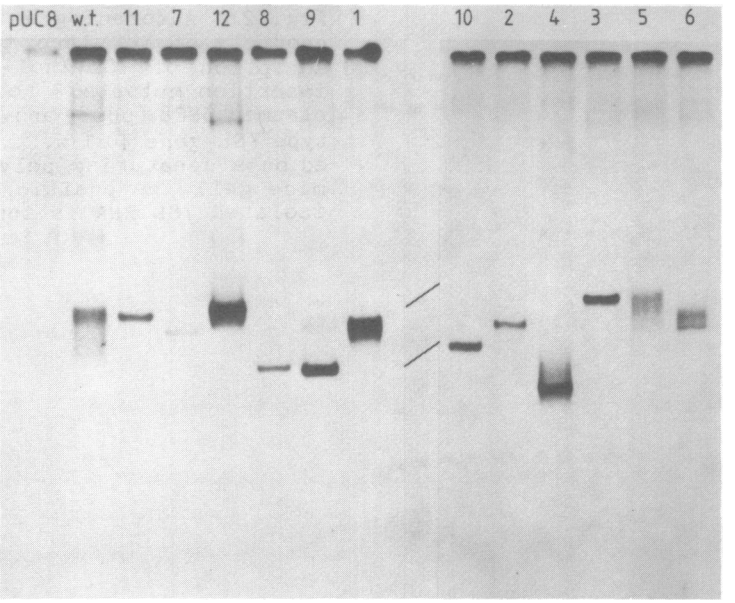

Fig. 3: Autoradiogram of the products of *in vitro* transcription of 7SL XhoI-linker insertion mutants 1 to 12, plasmid pUC8 (pUC8) and wild-type 7SL-gene 30.1 (w.t.) separated on non-denaturing polyacrylamide gels.

component. To establish that the latter RNAs represented different conformers and were not derived from RNA degradation, we isolated the various RNA species from the non-denaturing polyacrylamide gel and rerun them under denaturing conditions. The results of this analysis (data not shown, but see Figure 2) indicated that the non-homogeneous migration of 7SL RNA and of the six mutant RNAs (1, 4, 5, 6, 7 and 12) under native conditions reflected the presence of different conformers within each RNA species.

In order to roughly quantitate the amounts of different conformers within each RNA species and distinguish them more clearly from each other, the autoradiograms of non-denaturing polyacrylamide gels were scanned with a densitometer. The result of this analysis are shown in Figure 4.

At least four different conformers (labeled A, B, C and D in Figure 4) can be distinguished in the wild-type RNA. A

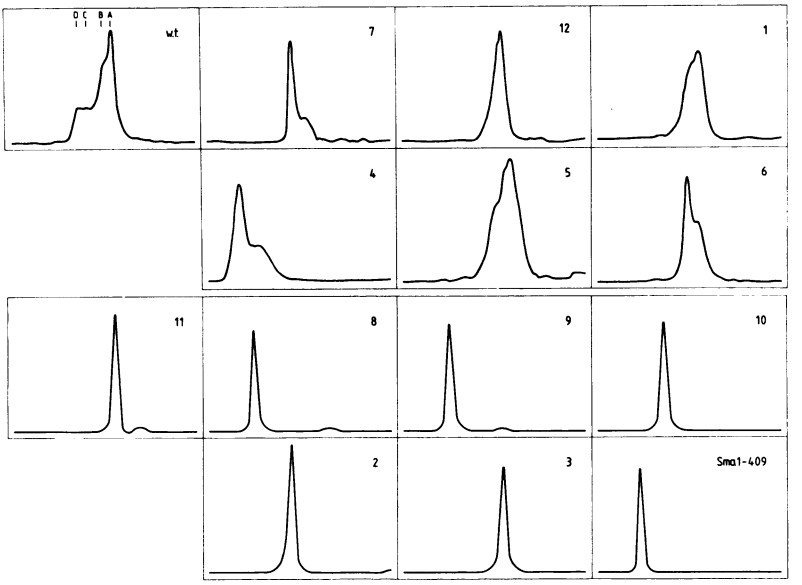

Fig. 4: Density scans of the autoradiograms of transcribed wild-type (w.t.) or mutant RNAs 1 to 12 and deletion mutant Sma1-409 (14) which had been separated on non-denaturing polyacrylamide gels. Direction of migration is from right to left. A, B, C and D designate the different conformers of the wild-type transcript.

similar pattern is seen in the RNA transcribed from clone 5. In contrast the RNA from the mutants 2, 3, 8, 9, 10, 11 and Sma1-409 appears as a single peak. Therefore in all these mutants the bases which are necessary for the 7SL RNA to exist in alternative conformations must have been altered. Most of the other mutant RNAs exist in what appears to be two different conformers, and the relative amounts of each conformer vary in each mutant RNA. A summary of this analysis is presented in Figure 5. The existence of single or multiple forms is indicated and also the relative low or high mobility of the conformers in respect to the four conformers A, B, C and D of the unmutated 7SL RNA is shown. It can be seen, for example, that the mutation in 3 causes the RNA to travel with a mobility being very similar to the mobility of the A-conformer, while the alteration in 10 causes the RNA to adopt a mobility being similar to the mobility of the D- conformer.

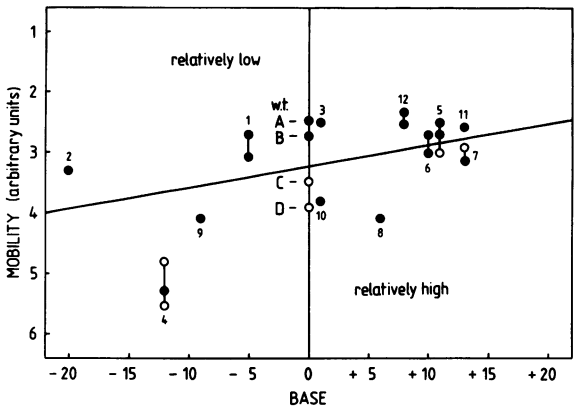

Fig. 5: Behavior of wild-type (w.t.) and mutant 7SL RNAs 1 to 12 on non-denaturing polyacrylamide gels. The mobility of the mutant RNAs in relation to the number of bases gained or lost in the various mutations is indicated. Conformers present in major amounts are shown by solid dots. Conformers present in minor amounts are shown by open circles. RNA conformers located above the diagonal line are considered as having a relatively low electrophoretic mobility, while conformers located below the diagonal line are considered as having a relatively high electrophoretic mobility. Different conformers of the same clone are connected by a vertical line. The four conformers of the wild-type 7SL RNA (w.t.) are marked A, B, C and D.

Localization of the mutations in the central part of the secondary structure of human 7SL RNA

Figure 6 shows the central part of the human 7SL RNA secondary structure. It contains the interactions deduced previously using the compensatory base change approach (14). Four additional base pairs between the bases at positions 187 to 190 and 209 to 212 are included, because they are supported by compensatory base changes but were not noticed previously. The locations of the various linker insertion mutations and of the deletion mutant Sma1-409 are shown. As mentioned above, wild-type sequence are often deleted and the linker is simply inserted. In contrast, in the the mutants 6, 7, 8 and 11 no wild-type sequences are lost and bases are only added at the locations indicated. A special situation can be noticed in the case of the mutations 6 and 7 which contain tandem repeats of 7SL RNA specific sequences, flanking the linker DNA. In both

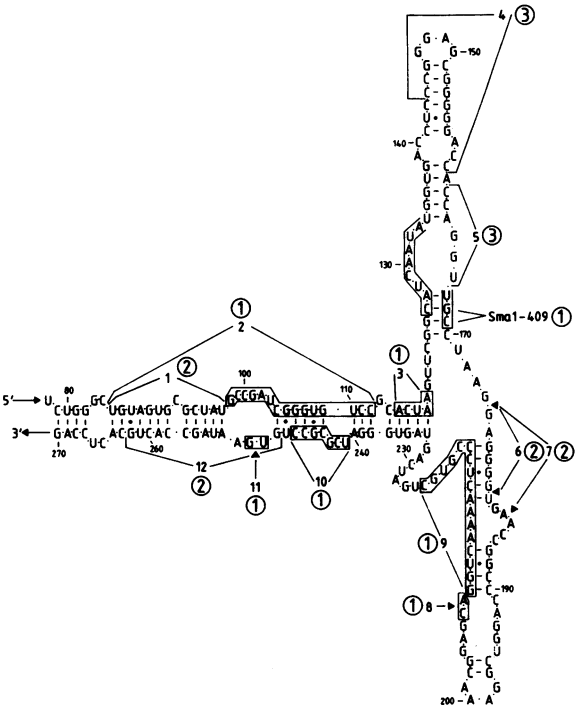

Fig. 6: Localization of mutants 1 to 12 and deletion mutant Sma1-409 (14) in the secondary structure of the central part of the 7SL RNA. The sequences necessary for the ability of 7SL RNA to exist in more than one conformer are shown in boxes. Each mutant is marked with an encircled number which indicates the number of observed RNA conformers. In the case of mutants 6 and 7 two possibilities exist for inserting the additional bases into the wild-type 7SL RNA as is indicated by the two arrows because 7SL RNA specific sequences have been duplicated (see text).

mutants the possibility of an interaction between bases around position 180 and 218 in the secondary structure is retained.

In Figure 6 the bases necessary for the 7SL RNA to exist in more than one conformation are shown as boxed in regions. They were identified as follows:

The RNA from mutant 1 shows two conformers in about equal amounts which have a mobility corresponding to the A- or B-conformer of the wild-type RNA. In contrast, the RNA from clone 2 which contains a larger deletion having lost 20 bases between U-85 and C-112, clearly shows only one conformer. We

therefore conclude that the RNA containing the bases between positions 98 and 112 must have dynamic properties. Mutant 3 contains a very small alteration in the most conserved part of the 7SL RNA and causes the RNA to be in one A-like conformer. In Sma1-409 44 bases are deleted causing the loss of the stem and loop structure around position 150 . The RNA from this clone can exist only in one conformer (14). Only five bases of the interaction between bases around positions 125 and 168 are deleted in the Sma1-409 mutant. Nevertheless, this interaction is totally abolished, because it cannot exist without a hairpin loop. No mutations were recovered between positions 134 to 143, but it is unlikely that these bases have dynamic properties because a phylogenetically conserved secondary structure is required for the upper part of the 150-stem while the primary sequence is very variable when human, Xenopus and Drosophila 7SL RNAs are compared (14). In fact, the RNAs of the two mutations 4 and 5, also being located at the top of the 150 stem and loop, are able to exist in more than one conformer. The RNA from clone 4 appears as three conformers, which all have a relatively high mobility. As was mentioned earlier, 5 is the mutation which has a behavior very close to the wild-type RNA. Mutations 6 and 7 behave very similarly in showing two conformers each and contain a tandem repeat of bases necessary for the interaction between bases at the positions 180 and 218. Because no mutation was located between positions 182 to 206 we do not know if this region has dynamic properties (but see discussion below). The three mutations 8, 9 and 10 clearly belong to the group of alterations causing the RNA to be in one conformer. In these three cases a RNA-form with a mobility resembling a high mobility C- or D-form is adopted. The mutation of clone 11 contains an insertion of thirteen bases between U-250 and G-251. Its RNA has a relatively low mobility (see Figure 5). The RNA from the mutant 12 again retains the ability of existing in two conformers which are related to the A- or B-forms.

In summary, nucleotides between positions 98 to 133 and 206 to 251 are necessary for the 7SL RNA to be able to assume alternative conformations, while changes at positions 85 to 97,

144 to 166 and 252 to 266 do not abolish this property. The dynamic sequences form a T-like shape in the secondary structure of the central domain of the 7SL RNA.

In Figure 6 the bases at positions 221 to 232 are shown as being single stranded. It has been shown previously (14) that this part can also base pair with a region around position 125. It should also be mentioned that bases from the dynamic part of the molecule at the positions 104 to 109 (h-1) and 236 to 255 (h-2) show a significant sequence homology with bases of the equivalent 5S ribosomal RNA of human, Xenopus and Drosophila 7SL RNA (14).

In the discussion we will show that it is possible to correlate the mobility effects of the various mutations with particular secondary or tertiary structure features. Also the functional implications of a dynamic 7SL RNA molecule will be discussed below.

DISCUSSION

Construction and analysis of mutations in the central domain of 7SL RNA

We have analyzed the effect that thirteen different mutations in the central domain of human 7SL RNA exert on the ability of the RNA to adopt different conformations. Conformers of mutant and wild-type RNAs were separated on non-denaturing polyacrylamide gels. Two groups of mutants can be distinguished. The first group contains mutations in the RNA which totally abolish its ability to exist in more than one conformer. The bases altered by these mutations must therefore be necessary for this property. They are located in the "central T" in the secondary structure of the 7SL RNA which consists of a core of the dynamic parts of the molecule (see below). The second group consists of mutant RNAs which still can be in at least two alternative electrophoretic forms. These alterations do not disrupt completely the dynamic properties of the 7SL RNA, but make the mutant RNAs behave somewhat differently from wild-type RNA. They are located outside of the dynamic core structure; typical examples are the mutations located in the upper part of the stem-loop structure

around position 150 (clones 4 and 5). It is interesting to note that the other arm of the "T" (bases 176 to 232) consists mainly of dynamic sequences, with perhaps the exception of a few bases around position 200. The dynamic sequences coincide with the bases which are phylogenetically conserved (14). It is plausible that the selective pressure is higher on the conservation of sequences which have multiple interactions, compared to the conservation of an isolated stem-loop structure. Interestingly, also viroid RNAs contain a centrally located conserved secondary structure feature with eight base pairs and one buldged base which might adopt an alternative conformation (24).

We also observe that certain mutations cause the RNA to adopt a mobility similar to the mobility of the A- or B-conformer, while others alter the RNA-conformation such that a relatively high mobility form is adopted, resembling more a C- or D-conformer. In several cases overlapping mutations (Sma1-409, 4 and 5 or 6 and 7), mutations located next to each other (8 and 9) or located on opposite strands in the secondary structure of the RNA (1 and 12) have the same effect onto the mobility and the capability of the RNA to occur in more than one conformer. These mutations therefore support the proposed secondary structure model of the 7SL RNA (14). It also should be noted that very small alterations, such as those of the mutants 3 and 10, can have a profound effect on the RNA-mobility. We assume that such a mobility change is reflecting a major change of the conformation of the RNA. For example, the RNA from the mutation 3 exists as a single A-like conformer while the RNA from the mutation 10 behaves like an D-conformer. These findings make it likely that a small change in one part of the RNA can induce more drastic changes in some other parts of the molecule which are not directly affected by the alteration. It can be suspected, that also many other medium size or large RNA molecules might show major structural changes triggered by a small alteration in the base pairing scheme.

The RNA from mutation 11 shows only one conformer although it is located "inside" mutation 12 which shows clearly two conformers. This could be explained by the fact that mutant 11

is located in a single stranded region of the RNA which is not altered by mutation 12. Interestingly, the altered region in mutant 11 is located at the position of the secondary micrococcal nuclease cut which affects the function of the the signal recognition particle in an *in vitro* assay (23). It is possible that the bases around position 252 are important for the RNA to adopt different conformers and also to exhibit SRP-activity. From this and other results we conclude that alternative conformations might be required for the function of the signal recognition particle (see discussion below).

Correlation of the alternative conformers of 7SL RNA with particular secondary and tertiary structure features

The dynamic sequences identified above are part of a dynamic core structure which is presented in Figure 7. In addition to the interactions seen in the secondary structure of Figure 6, it contains two interactions, the "switch" between bases at positions 122 to 128 and 222 to 228, which was proposed previously (14), and an additional interaction between the bases at positions 218 to 223 and 235 to 240. In contrast to the former interaction, the latter cannot be confirmed by compensatory base changes because the bases in this area are highly conserved in human, Xenopus and Drosophila 7SL RNAs. Since in the RNA from mutant 3 the bases around position 115 have been altered and can no longer interact with the bases around positions 238, and since the RNA has a low mobility, we suggest that in the A- or B- conformer of the 7SL RNA this interaction is also not taking place (see also Figures 5 and 6). In contrast, a C/D conformer would contain the 115/238 interaction. This is supported by the observations that C- or D-like conformers are obtained either by deleting the bases around position 220 (mutation 9) or by inserting bases at position 210 (mutation 8) or, quite interestingly, also by destroying the interaction between bases at positions 106 to 109 and 244 to 249 (mutation 10). The suggested base pairing schemes of the A/B- and the C/D-form of the 7SL RNA are also shown in Figure 7. We cannot make any predictions about the nature of the structural differences between the A- and the B-form or between the C- and the D-form, but this might be a

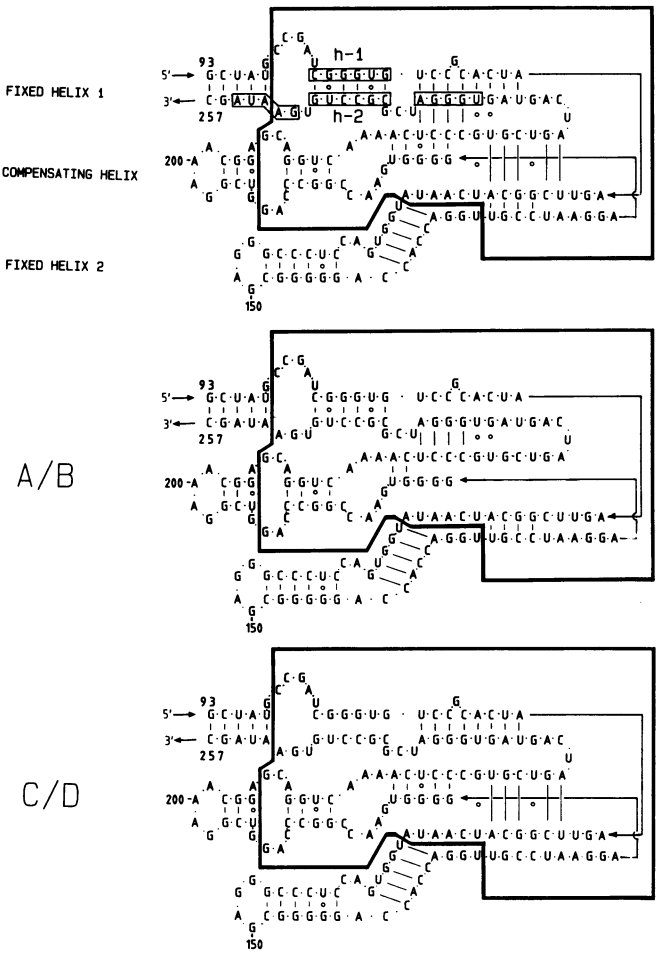

Fig. 7: The dynamic core of the 7SL RNA. Bases being involved in alternative conformer formation are located inside the field defined by thick black lines. The path of the RNA chain is indicated by thin black lines. In the top panel of the Figure possible base pairs are shown (see text). The position of the 5S ribosomal RNA homologeous sequences h-1 and h-2 (14) is shown in boxes. The suggested base pairing scheme in A- or B-like and C- or D-like conformations is shown in the middle and the bottom panel respectively.

reflection of the lack of information about the base pairing scheme in the stem and loop structure around position 200. It can also not be excluded that features at the 5'-end or in other regions of the 7SL RNA which are not part of our analysis

will in some mutant cases contribute to these structural differences. In addition, the alterations introduced by our mutations might still be too extensive to define the differences between the A- and the B- or between the C- and the D-conformer.

Functional implications of a dynamic 7SL RNA

The analysis of thirteen mutations located in the central domain of the human 7SL RNA shows that a central core of dynamic sequences exists which has a T-like shape in the secondary structure of the RNA. Two ends of the "T" namely the long double stranded region ("fixed helix 1" in Figure 7) of the 7SL RNA and the top of the stem loop around position 150 ("fixed helix 2") do not have dynamic properties, while the second arm of the "T" (the stem-loop around position 200, "compensating helix") is involved in conformational changes. The center of the dynamic core contains highly conserved sequences in which the sequence ACUCCCGUGCUGAUCAGUAGUGGGA at positions 216 to 240 might play the role of an "organizer" of alternative configurations. It should be noted that the sequence CUCCCGUGCUGA from the "organizer" was never found to be accessible either to single or double strand specific nucleases and must therefore be hidden in the dynamic core structure. Bases at the positions 107 and 108 have been found to be accessible both to single strand specific as well as to double strand specific nucleases, which is in agreement with a dynamic involvement of this part of the 7SL RNA (11).

The dynamic property of the central part of the 7SL could play a role during transcription and/or the assembly of the signal recognition particle. Also, the possibility exists that alternative forms are adopted during the function of the SRP in the translocation of polypeptides across the membrane of the endoplasmic reticulum. This latter possibility is supported by the observation that a sequence homology exists between bases located in the dynamic core structure in 7SL RNA and 5S rRNA(h-1 and h-2), suggesting that both molecules may interact with the same or a similar target on the 26S and/or 18S ribosomal RNA (14). In this context it is worth mentioning that only the eukaryotic 5S rRNAs (and not the prokaryotic 5S

rRNAs) possess an extra "moving bulge" (8) at the location of the h-1 and h-2 sequences (Figure 7) which would enable this part of the 5S rRNA to move away from its "normal" location and make this place available for the homologous 7Sl RNA sequences. In support of a dynamic involvement of 7SL RNA in SRP- function is also the fact that mutation 11 abolishes the appearance of alternative conformations. As was mentioned above, a cut of the RNA by micrococcal nuclease at the position in which the RNA is altered in mutant 11 also affects the activity of SRP in an in vitro protein translocation assay (23).

We have shown that the technique of site directed mutagenesis and the analysis of the properties of altered molecules, can provide valuable information about the structure, dynamics and function of 7SL RNA. In connection with functional studies of specifically altered particles this approach will prove to be useful in the understanding of the role of the SRP in protein translocation.

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REFERENCES

1. Guerrier-Takada, C., and Altman, S. (1984) Science (Washington, D.C.) 223, 285-286
2. Chech, 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
3. Ehrenberg, M., Rigler, R. and Wintermeyer, W. (1979) Biochemistry 18, 4588-4599
4. 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 Chan, Y-L., Gutell, R., Noller, H. and Wool, I. (1984) J. Biol. Chem. 259, 224-230
5. Klein, B.K., Staden, A., and Schlessinger, D. (1985) Proc. Acad. Sci. USA, 82, 3539-3542
6. Lee, K.A.W., Guertin, D. and Sonnenberg, N. (1982) J. Biol. Chem. 258, 707-710

7. Pleij, C.W.A., Rietveld, K. and Bosch, L. (1985) Nucl. Acids Res. 13, 1717-1731
8. De Wachter, R., Chen, M.-W., Vandenberghe, A. (1984) Eur. J. Biochem. 143, 175-182
9. Hall, K., Cruz, P., Tinoco, I.Jr., Jovin, T.M. and van de Sande, J.H. (1984) Nature (London) 311, 584-586
10. Walter, P., and Blobel, G. (1982) Nature (London) 299, 691-698
11. Gundelfinger, E.D., DiCarlo, M., Zopf, D., and Melli, M. (1984) EMBO J. 3, 2325-2332
12. Ullu, E., Murphy, S., and Melli, M. (1982) Cell 29, 195-202
13. Ullu, E., and Tschudi, C. (1984) Nature (London) 312, 171-172
14. Zwieb, C. (1985) Nucl. Acids Res. 13, 6105-6124
15. Heffron, F., So, M. and McCarthy, B.J. (1978) Proc. Acad. Sci. USA, 75, 6012-6016
16. Ullu, E., and Weiner, A.M. (1984) EMBO J. 3, 3303-3310
17. Ullu, E., and Weiner, A.M. (1985) Nature (London) 318, 371-374
18. Messing, J. and Vieira, J. (1982) Gene, 19, 269-276
19. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Nat. Acad. Sci. USA 74, 5463-5467
20. Manley, J.L. (1984) in: Transcription and Translation, ed. Hames, B.D. and Higgins, S.J. IRL Press, Oxford
21. Peacock, A.C., and Dingman, C.W. (1968) Biochemistry 7, 668-674
22. Maniatis, T., Fritsch, E.F., and Sambrock, J. (1982) in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory
23. Gundelfinger, E.D., Krause, E., Melli, M., and Dobberstein, B. (1983) Nucl. Acids Res. 11, 7363-7374
24. Keese, P. and Symons, R.H. (1985) Proc. Acad. Sci. USA, 82, 4582-4586