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Hypothesis

A 5 S rRNA-like secondary structure in the 7 SL RNA may define a ribosomal binding site of the signal recognition particle

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A new secondary structure model for parts of the 7 SL RNA is proposed which indicates for a stretch of at least 40 bases a strong structural homology to the ribosomal protein L5 binding site of eukaryotic 5 S rRNA. It is suggested that the 5 S rRNA-like structural part of 7 SL RNA mediates binding of the signal recognition particle near to the peptidyl transferase center of the ribosome.

7 SL RNA; 5 S rRNA; Secondary structure; Signal recognition particle; Ribosome binding

1. INTRODUCTION

The SRP is an essential component for the translocation of proteins across the endoplasmic reticulum (for a recent review see [1]). The SRP consists of six polypeptide chains and one 7 SL RNA molecule of about 300 bases [2,3]. The 7 SL RNA consists of two distinct sequence domains. Only the central part of the 7 SL RNA contains 7 SL RNA-specific sequences (S fragment). It is flanked by *Alu* sequences which are middle repetitive in the genome of mammals [4,5].

Recently, secondary structure models have been proposed for the 7 SL RNA based on digestion experiments with single- and double-strand specific nucleases [6] and on the application of the compensatory base change approach [7] for the three known sequences of human [4], *Xenopus* [5] and *Drosophila* [6].

The 7 SL RNA provides support for the six polypeptide chains in the 11 S ribonucleoprotein complex (SRP) [3]. 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 [3,8]. The sites on SRP and on the ribosome, respectively, which interact with each other in the course of this reaction are unknown. It is not even clear whether protein-protein, protein-RNA or RNA-RNA interactions predominate.

Recently, a significant homology in the primary but not in the secondary structure between a stretch of 20 bases in 7 SL RNA and 5 S rRNA was noticed [7]. Here we report a more extended sequence homology between a stretch of at least 40 bases in 7 SL RNA and 5 S rRNA. Moreover, based on these data, a new secondary structure for parts of the 7 SL RNA is proposed which indicates

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Abbreviation: SRP, signal recognition particle

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a striking structural homology to the ribosomal protein L5 binding site of eukaryotic 5 S rRNA. It is suggested that the interaction between the 5 S rRNA-like structural part of the 7 SL RNA and ribosomal protein L5 may be the molecular basis for the transient binding of SRP to the ribosome.

2. RESULTS

2.1. *Sequence homology between 7 SL RNA and 5 S rRNA*

With a dot matrix program [9] the following sequence alignment for the homologous parts of human 5 S rRNA [10] (positions 65–111) and human 7 SL RNA [4] (positions 222–263) was calculated:

```
          65                                                    111
5 S   rRNA GGCCUGGUUAGUACUUGGAUGGGAGACCGCCUGGGAAUACCGGGUGC
           +  +++ + ++++ + ++++ +      ++++++ +++++ +   +++
7 SL  RNA  GUGCUGAUCAGUAGUGGGAUCG-----CGCCUGUGAAUAGCCACUGC
          222                    243 244                 263
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Neglecting the deletion of five bases between positions 243 and 244 in the 7 SL RNA compared to the 5 S rRNA 30 out of 42 bases are identical for the two RNAs. If no gap in the sequence alignment is allowed for only 20 bases appear to be homologous (see lines above the 5 S rRNA sequence and below the 7 SL RNA sequence) as recently reported by Zwieb [7].

The same degree of homology for a stretch of about 40 bases in the same sequence regions is found by comparison of 5 S rRNA and 7 SL RNA from *Xenopus*, while in *Drosophila* a significant but not as striking homology is found (not shown).

Using these data on the primary structure homologies and the well established general secondary structure of 5 S rRNA ([10–12] and references therein) as a guide the homologous regions of the three 7 SL RNAs can be folded into the secondary structures as shown below (figs 1,3).

2.2. *A new secondary structure for parts of the 7 SL RNA*

Fig.1 shows a scheme for the secondary structure of the human 7 SL RNA according to Gundelfinger et al. [6] and Zwieb [7,13] (EMBL model) and an alternative model, proposed here, for parts of the 7 SL RNA. The proposed alternative secondary structure of 7 SL RNA involves completely new base pairing of the long double-stranded region ranging from positions 87 to 118 and 223 to 262, respectively (helices 7a, 8a and 9a in the EMBL model) into two stem-loop structures (helices 8, 9, 14 and 15 in the new model). All other parts of the 7 SL RNA secondary structure remain the same as in the EMBL model.

For comparison, fig.1 shows a scheme of the secondary structure of human 5 S rRNA and indicates the structural parts of the 7 SL RNA models which are strongly homologous. The completely new folding of the sequence from positions 223 to 262 of the 7 SL RNA also demands a fully new base pairing of the region from positions 87 to 118 because a lot of double-strand specific nuclease cutting sites have been determined in this region [6]. As seen in fig.3 a reasonably good secondary structure for this part of the 7 SL RNA can be constructed with a high degree of base pairs.

The new folding pattern derived initially for the particular sequence of human 7 SL RNA also holds true for *Xenopus* and *Drosophila* 7 SL RNA (fig.3) with slight variations between the three species. Moreover, the newly modelled parts of the 7 SL RNAs from human and *Drosophila* are compatible with the nuclease cleavage data [6] to the same or even greater extent than in the structures proposed before in the literature (figs 2,3).

In [6] it was reported that the distribution of the cutting sites of the different specific enzymes is essentially the same for human and *Drosophila* 7 SL RNA, except for the region surrounding base 230. In this region human 7 SL RNA is digested by single-strand specific enzymes while *Drosophila* 7 SL RNA is cut by double-strand specific RNase. According to the new structure for this part of 7 SL RNA (fig.3) the differences mentioned can be easily explained by the variations in length and stability of helix 14 for human and *Drosophila* 7 SL RNA, respectively. There is no need to propose quite different secondary structures in this

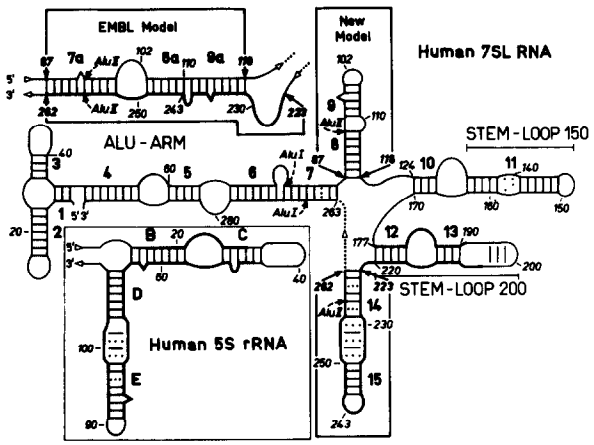

Fig.1. Schematic diagrams for the secondary structures of human 7 SL RNA and for large parts of the human 5 S rRNA. Two alternative models are shown for the central sequence domain of 7 SL RNA. The double-stranded regions of 7 SL RNA are numbered from the 5′-end. The designation of the helices in the 5 S rRNA model is taken from [10,12]. Thick lines indicate homologous parts of the primary and/or secondary structure of 7 SL RNA and 5 S rRNA. GC, AU and GU base pairs are represented by full lines, non-standard base pairs by dotted lines. The limits of the sequence homologies between the 7 SL RNA and the left and right monomers of the human *Alu* consensus sequence [4,5] are indicated by *Alu*I and *Alu*II, respectively.

region for the 7 SL RNAs of human and *Drosophila* as has been done in [6].

2.3. *The 5 S rRNA-like part of the new secondary structure of 7 SL RNA*

A comparison of the secondary structures of human, *Xenopus* and *Drosophila* 5 S rRNAs with the proposed new secondary structures of 7 SL RNAs from the three species indicates a striking homology between the helix D/internal loop/helix E structural part of 5 S rRNA with the helix 14/internal loop/helix 15 structure of 7 SL RNA (fig.3).

The identity of nearly all bases which constitute the mentioned internal loops in 5 S rRNA and 7 SL RNA is remarkable. The $A_{74}GUA_{77}$ and $G_{99}AA_{101}$ sequence strings are invariant residues in nearly all eukaryotic 5 S rRNA species and are believed to be involved in distorted double-helical structures [14–16]. It is reasonable therefore to propose a similar 'higher-order structure' with several non Watson-Crick base pairs also for the internal loop between helices 14 and 15 in 7 SL RNA. An interesting difference between 5 S rRNA and 7 SL RNA is the deletion of five bases in the hairpin loop around helix 15 of 7 SL RNA compared to the corresponding part of 5 S rRNA. Helix 15 of 7 SL RNA therefore looks like a trun-

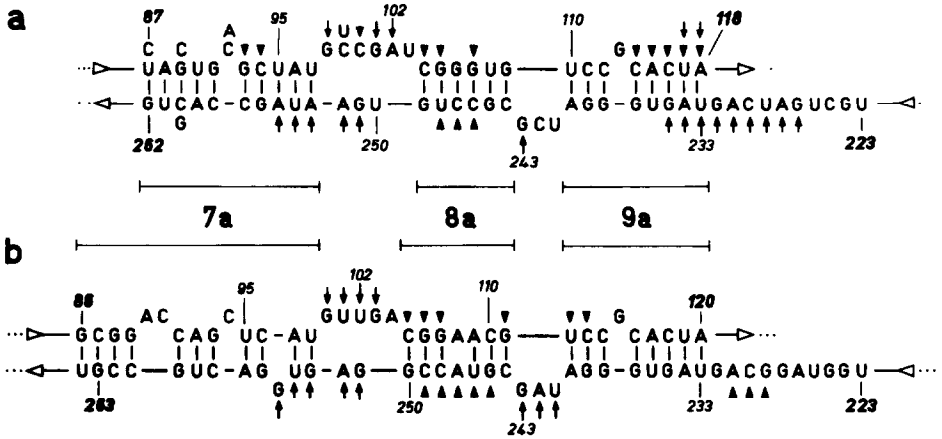

Fig.2. EMBL models of the secondary structure for (a) human, *Xenopus* and (b) *Drosophila* 7 SL RNA according to [7,13] for the indicated sequence stretches. Single-strand specific and double-strand specific cutting sites for human and *Drosophila* 7 SL RNA [6] are indicated by arrows and triangles, respectively. The human 7 SL RNA is shown with base changes in *Xenopus* 7 SL RNA. See fig.1 for explanation of other symbols.

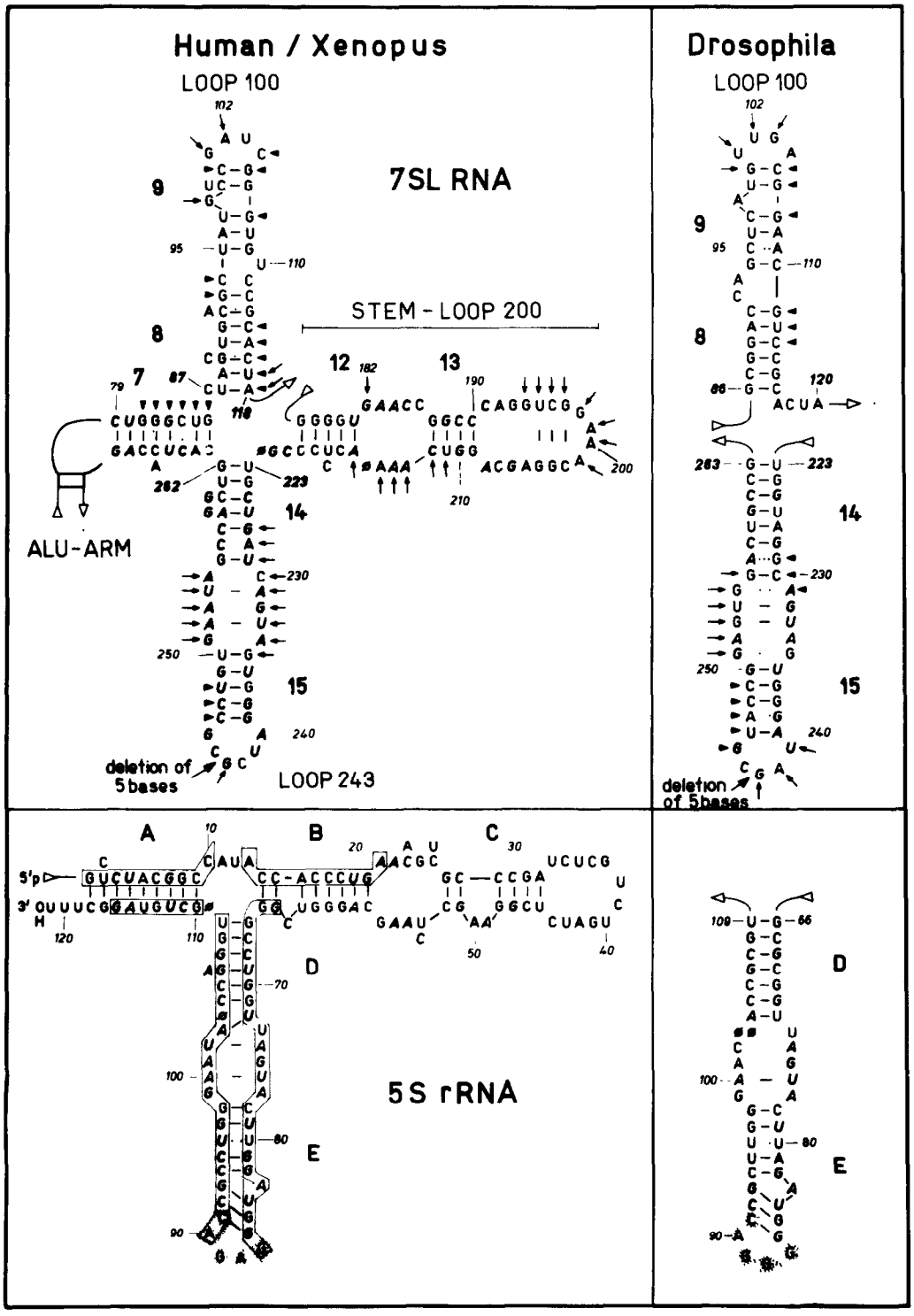
Human / Xenopus
LOOP 100
7SL RNA
STEM - LOOP 200
ALU-ARM
deletion of 5 bases
LOOP 243
Drosophila
LOOP 100
deletion of 5bases
5S rRNA

Fig.3. Secondary structure models for human, *Xenopus* and *Drosophila* 7 SL RNA for its central sequence domain compared with the 5 S rRNA secondary structures of the three species. For clarity only parts of the 7 SL and 5 S rRNA structures from *Drosophila* are drawn. Italics indicate identical bases in the 7 SL and 5 S rRNAs if their sequences are aligned within the same secondary structure frame. Single base deletions which follow from this alignment are indicated by Ø. Note the deletion of 5 bases from the 7 SL RNA when compared to the 5 S rRNA (bases stippled). The ribosomal protein L5 binding site in the 5 S rRNA secondary structure according to [18] is boxed. Other indications as in the legends to figs 1 and 2.

cated helix E of 5 S rRNA, a feature which also exists in some 5 S rRNA species [10].

Not only the helix D/internal loop/helix E structural motif of 5 S rRNA exists in the 7 SL RNA structure. With some variation in detail the structural features of helix A and the helix B/internal loop/helix C motif of 5 S rRNA are also seen in the 7 SL RNA structure (helix 7 and helix 12/internal loop/helix 13 motif), again with a remarkable conservation of bases in the internal loops (fig.3).

The described parts of the 5 S rRNA structure coincide nearly with the binding site for ribosomal protein L5 from rat liver ribosomes [17,18] as indicated in fig.3.

Taken together about three-quarters of the secondary structure of 5 S rRNA resembles the described parts of the new 7 SL RNA structure. The largest differences between both secondary structures are seen for their hairpin loops around helices 13 and 15 in 7 SL RNA and helices C and E of 5 S rRNA, respectively, and for their multibranched loops.

3. DISCUSSION

The extensive sequence homologies between large parts of 5 S rRNAs and 7 SL RNAs indicate that a part of the S fragment of the 7 SL RNA may have evolved from 5 S rRNA genes. Both 7 SL RNA genes [19,20] and 5 S rRNA genes (see, e.g. [21]) are transcribed by RNA polymerase III. Interestingly the homologous sequence stretch of 7 SL RNA/DNA from *Xenopus* roughly coincides with the binding region of 5 S RNA/DNA for transcription factor IIIA [22,23]. We cannot therefore exclude a role of this region in the regulation of the transcription of the 7 SL RNA genes as well. However, experimental data have so far detected only one strong internal 7 SL promoter in its *Alu* sequence part [20]. Nevertheless, as outlined above, large parts of 7 SL RNA and 5 S rRNA are also strongly homologous at the secondary structure level.

Recently, it was reported that human 7 SL RNA can be separated into four major conformers by non-denaturating polyacrylamide gel electrophoresis [7,13]. It was concluded in [13] that alternative conformations of the 7 SL RNA might be required for the function of the SRP. Moreover, from the electrophoretic behaviour of mutant human 7 SL RNA the bases between positions 98 and 133 as well as 206 and 251 have been proved to be necessary for the 7 SL RNA to be able to exist in alternative conformations [13]. The new secondary structure of 7 SL RNA for its central part proposed here involves about two-thirds of the mentioned 'dynamic sequences' [13]. It is conceivable that alternative base pairing schemes correspond to the different conformations. Our new model may in fact be one of them. This model would be compatible with some of the mutant 7 SL RNAs described in [13] but other conformations for particular mutants are possible as well (S.B., unpublished). We speculate that the suggested new 7 SL RNA structure may be one of the alternative conformers in which the 7 SL RNA exists during different functional states of SRP. There is a striking structural homology between the ribosomal protein L5 binding site of 5 S rRNA [17,18] and its counterpart in the new 7 SL RNA model (figs 1,3). Therefore, it is reasonable to assume that the 5 S rRNA-like part of 7 SL RNA competes with the 5 S rRNA for binding to protein L5. The binding of the SRP in this manner should disturb the balanced interactions of the 5 S rRNA-protein L5 complex with its surroundings and thereby lead to a distortion in the (unknown) function(s) of the 5 S rRNA-protein L5 complex. This complex is located near to the peptidyl transferase center [24,25] and may play a dynamic role in protein biosynthesis [26,27]. Whether the suggested

binding of SRP to ribosomal protein L5 exists and whether it influences the rate of elongation by competition with the 5 S rRNA remain to be investigated.

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