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Nucleotide sequence and structure of cytoplasmic 5S RNA and 5.8S RNA of Chlamydomonas reinhardii

Jean-Luc Darlix and Jean-David Rochaix

Department of Molecular Biology, University of Geneva, 30, quai Ernest-Ansermet, 1211 Geneva 4, Switzerland

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ABSTRACT

Three small RNAs of the cytoplasmic 80S ribosomes of the green unicellular alga Chlamydomonas reinhardii have been sequenced. They include two species of ribosomal 5S RNA, a major and a minor one of 122 and 121 nucleotides respectively, which differ from each other by 17 bases, and also the ribosomal 5.8S RNA of 156 nucleotides. Novel structural features can be recognized in the 5S RNAs of C. reinhardii by a comparison with published 5S RNA sequences. In addition the secondary structure of these small RNA molecules has been examined using a newly developed method based on differential nuclease susceptibility.

INTRODUCTION

The comparative sequence analysis of small ribosomal RNAs can provide interesting insights into the evolutionary relationships between various animal and plant species. Amongst the numerous 5S RNA sequences which have been determined it is possible to distinguish two major classes (1). The 5S RNAs of the first class, which includes animals and fungi, contain around position 40 the oligonucleotide CPyGAU complementary to parts of eukaryotic initiator tRNAs. The 5S RNAs of the second class contain at a similar position either the oligonucleotide CPyGAAC (prokaryotes) or CAGAAC (higher plants and the alga Chlorella) which is thought to interact with part of loop IV (GTΦ loop) in all tRNAs (2). It is interesting to note that among protists, some fall into the first class (Crithidia fasciculata) while others belong to the second class (Chlorella pyrenoidosa). It is therefore of interest to examine 5S RNAs of other protists.

Here we report the sequences of two cytosol 5S RNAs of the green alga Chlamydomonas reinhardii. The sequences are unusual

since they contain the oligonucleotide C A G C A C (U) around position 40 which refers then to the second class. The two 5S RNA species found differ at 17 positions from each other. The sequence of the cytoplasmic 5.8S RNA of C. reinhardii has also been determined and compared to other eukaryotic 5.8S RNA sequences.

RESULTS

a) Sequence of 5S RNA

Upon end labelling of the 5S RNA preparation and electrophoresis of the radioactive RNA on 6 % polyacrylamide gel, two RNA bands were detected. The slower migrating RNA, called I, is estimated to be 3 times more abundant than the second one, called II.

A sequencing gel of 5' end-labelled 5S RNA I is shown in figure 1. The complete nucleotide sequence of both 5S RNA molecules has been worked out by partial enzymatic digestion using 5' and 3' end labelled RNAs. The sequences have been confirmed by means of the wandering spot analysis and are given in figure 2. The nucleotide sequence of 5S RNA I differs by 17 nucleotides from that of 5S RNA II, and is one nucleotide longer. These data demonstrate that both 5S RNA species are unique molecules.

b) Sequence of 5.8S RNA

Sequencing of the 5.8S RNA was carried out by the same methodology used for the 5S RNAs, and the complete nucleotide sequence is reported in figure 3. In addition two G residues, G_{42} and G_{74} appear to be methylated on the ribose moiety (10).

c) Enzymatic approach to the study of 5S and 5.8S RNA secondary structure

To study the structure of small RNA molecules in solution, end-labelled RNA was digested with various RNases under conditions known to stabilize secondary and tertiary structures, i.e. 0.1 M Tris-HCl pH 7,5 and $0^{o}C$ (11). Digestion of the RNA was carried out with RNAses specific for single stranded RNA, namely T_1 RNase and Φ_I RNase, or with RNase III specific for structured RNA (12).

Examples of partial enzymatic degradation of 5'(^{32}P) 5S RNA are

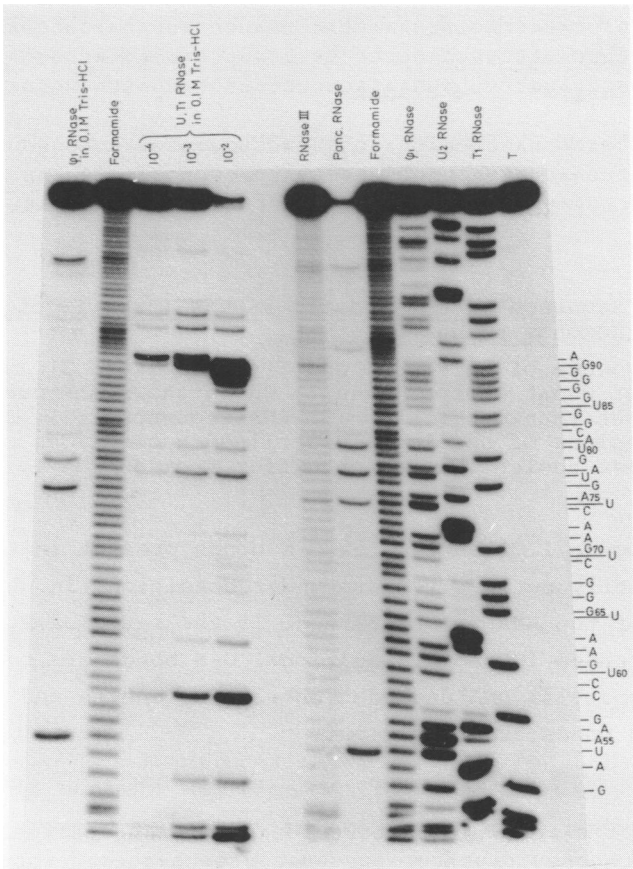

Figure 1 - Sequence and structure study of C. reinhardii cytoplasmic 5S RNA

5S and 5.8S RNAs were prepared from purified 80S ribosomes from the cell wall deficient mutant CW15 (3). Dephosphorylated RNAs were labelled at the 5' end using (γ ^{32}P) ATP (Amersham) and T_4 polynucleotide kinase (P.L. Biochemicals) (4) or at the 3' end with (5' ^{32}P) pCp using T_4 RNA ligase (5). Terminally labelled RNAs were purified by p.a.g.e. in 75 mM Tris-borate pH 8.3, 1 mM EDTA and 7M urea. Sequencing of RNA was carried out following published procedures (6, 7, 8).

For structural study of end labelled RNA, conditions of RNase digestion were 0.1 M Tris-HCl pH 7.5, 30 min at 0°C with T_1 RNase (10^{-2} to 10^{-4} cl per µg of RNA) or Φ_I RNase (0.1 U per µg of RNA). Reactions with E.coli RNase III (9) were conducted in 20 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 150 mM NaCl for 10 min at 37°C.

Electrophoreses were on thin (0.03 x 33 x 40 cm) 6, 8 or 12 % polyacrylamide gels in 75 mM Tris-borate pH 8.3, 1 mM EDTA 7 M urea. A 12 % gel of 5S RNA is shown.

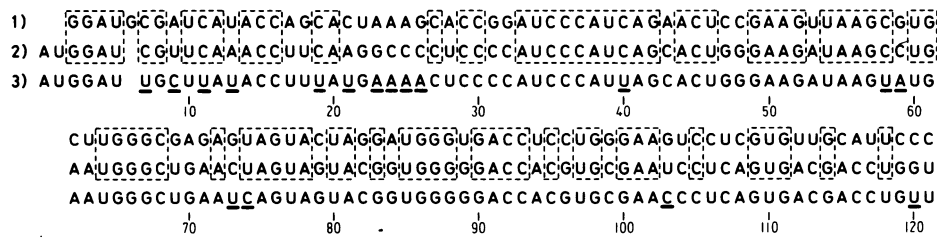

Figure 2 - Sequence of cytoplasmic 5S RNA I and II of Chlamydomonas reinhardii

The sequence of 5S RNAs I and II (line 2 and 3) is aligned with that of wheat embryo (line 1) which shows a sequence homology higher than other known 5S RNA sequences (1). Boxes indicate regions of homology. Underlined nucleotides of 5S RNA II show those nucleotides which differ from 5S RNA I.

shown in figure 1. Clearly, few G-N bonds present in the RNAs are efficiently cut by T_1 RNase under conditions in which the structure of the RNA is maintained. If the ratio of T_1 RNase to RNA is increased 100 fold, additional G-N bonds are cut, but the pattern of partial RNA degradation remains uneven and the majority of G-N bonds are poorly cut.

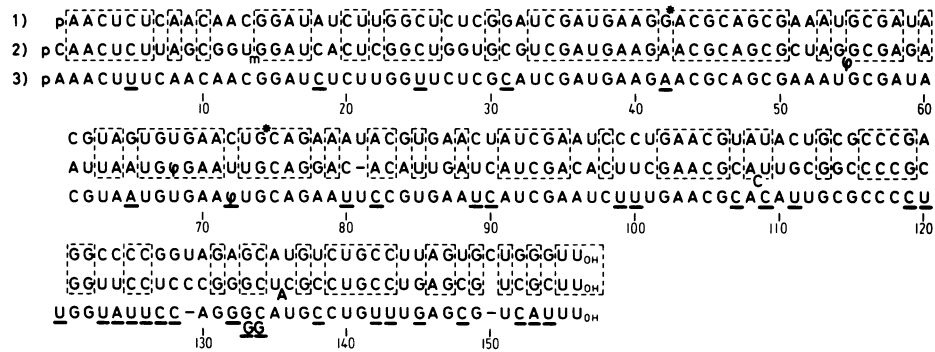

Figure 3 - Nucleotide sequence of 5.8S RNA of Chlamydomonas reinhardii

Sequence of 5.8S RNA of C. reinhardii is shown in line 1, that of chicken in line 2 and that of yeast in line 3. Boxes show the regions of homology between the 5.8S RNAs. Underlined nucleotides of 5.8S RNA of yeast point out to non homologous nucleotides.

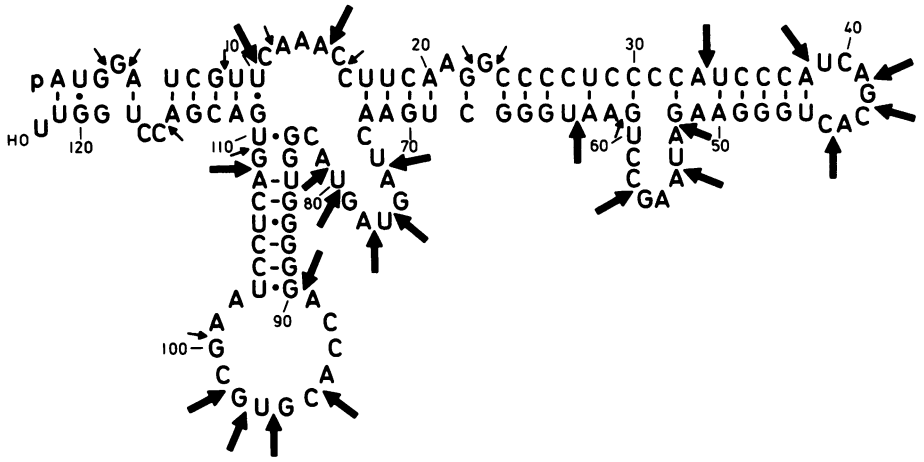

Figure 4 - A model structure for cytoplasmic 5S RNA I of C. reinhardii

Part of the results of T_1 and Φ_I RNase digestion of 5S RNA in high ionic conditions are presented in figure I. A large arrow indicates a high susceptibility, a small one a reduced susceptibility. No arrow suggest a tight structure with an uncertainty on C-C bonds which are not cleaved by T_1 and Φ_I RNases.

Φ_I RNase was shown to cut preferentially Pu-Pu, Pu-U and U-Pu bonds, less well U-Pyr bonds and very poorly C-N bonds in single-stranded RNA (13). The pattern of partial degradation of end-labelled RNA is very uneven and only a minority of the phosphodiester linkages are hydrolyzed by Φ_I RNase in high salt and at $0^{\circ}C$. Increasing the Φ_I RNase to RNA ratio as well as the temperature causes an increase in the susceptible bonds but does not modify the extreme uneveness of the partial RNA degradation pattern (figure 1).

Susceptibility of C. reinhardii 5S and 5.8S RNAs to T_1 and Φ_I RNases in high salt are summarized in figures 4 and 5.

Digestion of 5S and 5.8S RNAs with E.coli RNase III was done in high salt in order to maximize the specificity of the enzyme, which should then degrade only single stranded loops in structured RNA (12). Under these ionic conditions almost every phosphodiester linkage of 5' end-labelled 5S RNA is susceptible to degradation by RNase III (see figure 1). Some bonds

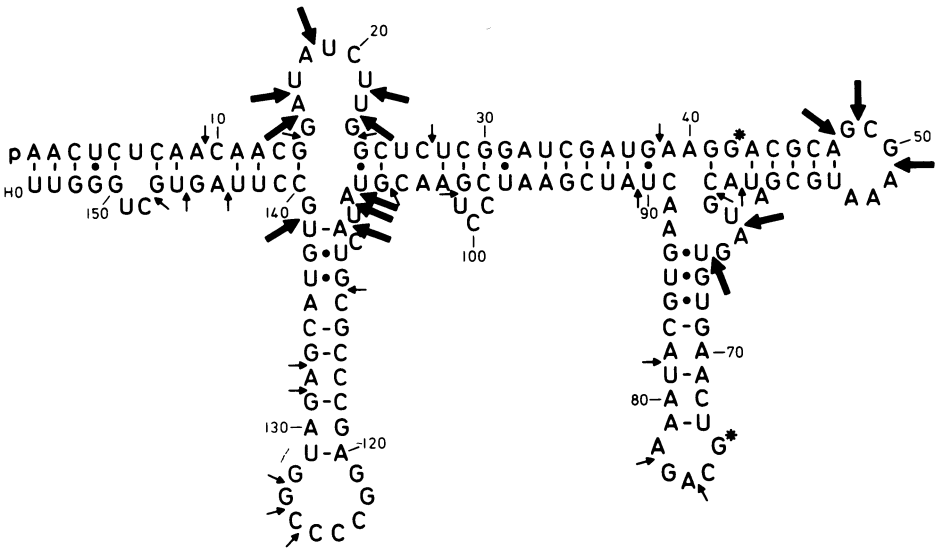

Figure 5 - A model structure for the 5.8S RNA of C. reinhardii
For details of the structure study, see figures 1 and 4.

such as A_{20} - A_{21}, C_{31} - C_{32}, A_{50} - A_{51}, U_{74} - A_{75} adn G_{90} - A_{91} are more susceptible than others and should be located within loops according to the specificity of the nuclease (12). The locations of the nucleotide bonds with an increased susceptibility to RNase III are indeed at unpaired bases within double-stranded RNA as indicated by the proposed scheme for the secondary structure of 5S RNA (figure 4).

DISCUSSION

In figure 2 the sequences of the cytoplasmic 5S RNAs of C. reinhardii are compared to each other and are also aligned with the corresponding wheat embryo sequences (2). Calculations of percent homology (identical residues : average length) reveal that the two 5S RNAs show 86 % homology with each other, and 62% homology with the 5S RNA of wheat embryo. Comparison with the 5S RNA of another protist, Crithidia fasciculata, shows an homology not greater than 55 % (14). As already pointed out by Mac Kay et al. (14), these data argue in favor of the protistan diversity.

Further evidence for protistan diversity is found in residues 42-45 of C. reinhardii 5S RNAs. This tetranucleotide is believed to be involved in complementary interactions with sequences in loop IV of tRNAs (15). All previously sequenced 5S RNAs show either C^{Py}_{A} G A A C (Bacteria, plants), or CPy - G A U (fungi, animals C. fasciculata) in this position (1).

The cytoplasmic 5S RNAs of C. reinhardii are unique in showing $\frac{C}{U}$ A G C A C, and furthermore differ from the chloroplast 5S RNA which has the tetranucleotide G A A C in this position (J.-L. Darlix and J.-D. Rochaix, unpublished data). The major and minor 5S RNA species of C. reinhardii differ in 17 nucleotides from each other and most of the divergence occurs in the 5' portion of the molecule. This 5S RNA sequence divergence is quite remarkable compared with the 5S RNA heterogeneity found in other organisms, e.g., wheat (16), Xenopus laevis (17), mammals (19, 20) where sequence heterogeneity is limited to 1-5 bases, or 15 bases in the case of Xenopus borealis (18). The significance of this sequence heterogeneity is not clear. It could be that in C. reinhardii the two 5S RNA species correspond to two functionally distinct groups of ribosomes, a hypothesis which may be tested.

The sequence of the 5.8S RNA of C. reinhardii is aligned with those of chicken and yeast, and calculated homologies reveal that 5.8S RNA of C. reinhardii shows 78,5 % and 63,5 % homology with the 5.8S RNA of yeast and chicken, respectively. It is difficult to draw any conclusion from the phylogenetical point of view since 5.8S RNAs of yeast and chicken show a homology as high as 78,5 % (1). It is interesting to note that the 5.8S RNA of C. reinhardii contains three G A A Py tetranucleotides between positions 69 and 97. Yeast 5.8S RNA contains four such tetranucleotides in the corresponding region (21). These sequences could theoritically interact with parts of loop IV in eukaryotic tRNAs (1).

We have constructed a secondary structure model for the major species of cytoplasmic 5S RNA of C. reinhardii based on proposed models for 5S RNAs (16, 22, 23), and on the differential susceptibilities of the nucleotide bonds towards T_1 and Φ_I RNases in 0.1 M Tris-HCl pH 7,5. The proposed structure

model agree well with the nuclease susceptibility of the RNA molecule except for the following nucleotide bonds : A_{33} - U_{34} should be less hydrolyzed whereas G_{100} - A_{101} - A_{102} should be more according to the model.

Single stranded regions of 5S RNA molecules have been located around positions 14, 28, 44, 59, 70 and 95 (2), and the unpaired regions in the model proposed are at the same places except for the one around position 28 which is absent (see figure 4) in the model for the cytoplasmic 5S RNA.

Similarly we have constructed a secondary structure model for C. reinhardii 5.8S RNA based on the proposal of Nazar et al. (24) and on the relative susceptibilities of the nucleotide bonds towards T_1 and Φ_I RNases in 0.1 M Tris-HCl pH 7,5 (see figure 5). The structure model presented for C. reinhardii 5.8S RNA is very similar to that of Hela cell 5.8S RNA (25), and is in good agreement with the nuclease susceptibility of the RNA except for two single stranded loops. These two loops are located at positions 74-78 and 121-128 and are poorly cut by the nucleases in contrast to what is predicted from the structure model, unless they are involved in a tertiary structure and then protected from nuclease digestion.

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