The nuclear 5S RNAs from chicken, rat and man. U5 RNAs are encoded by multiple genes

Alain Krol*, Hlne Gallinaro⁺, Eliane Lazar⁺, Monique Jacob⁺ and Christiane Branlant^{*†}

*Inst. Biol. Molculaire et Cellulaire du CNRS, Univ. Louis Pasteur de Strasbourg, 15 rue Ren Descartes, 67084 Strasbourg Cedex, and [†]Lab. Gntique Molculaire des Eucaryotes du CNRS, Unit de Biol. Molculaire et de Gnie Gntique, 11 rue Humann, 67085 Strasbourg Cdex, France

Received 31 December 1980

ABSTRACT

Preparations of chicken, rat and human nuclear 5S RNA contain two sets of molecules. The set with the lowest electrophoretic mobility (5Sa) contains RNAs identical or closely related to ribosomal 5S RNA from the corresponding animal species. In HeLa cells and rat brain, we only detected an RNA identical to the ribosomal 5S RNA. In hen brain and liver, we found other species differing by a limited number of substitutions. The results suggest that mutated 5S genes may be expressed differently according to the cell type. The set with the highest mobility corresponds to U5 RNA. In both rat brain and HeLa cells, U5 RNA was found to be composed of 4 and 5 different molecules respectively (U5A, U5B₁₋₄) differing by a small number of substitutions or insertions. In hen brain, no U5B was detected but U5A' differing from U5A by the absence of the 3'-terminal adenosine. All the U5 RNAs contain the same set of modified nucleotides. They also have the same secondary structure which consists of two hairpins joined together by a 17 nucleotide long single-stranded region. The 3' half of the molecule has a compact conformation. Together, the results suggest that U5 RNAs are transcribed from a multigene family and that mutated genes may be expressed as far as secondary structure is conserved. The conformation of U5 RNA is likely to be related to its function and it is of interest to mention that several similarities of structure are found between U5 and U1A RNA.

INTRODUCTION

The cell nucleus contains a set of metabolically stable RNA in the 4-10 S size range (1-4). These small nuclear RNAs (snRNA) were described in the ribonucleoproteins containing heterogeneous nuclear RNA (hnRNPs) (5-8), where they may be partially hydrogen-bonded to premessenger RNAs (9, 10). As hnRNPs are assumed to be the site of premessenger RNA processing, these observations suggest that snRNAs may play a role in such processing. Therefore, it is of great interest to know the primary and secondary structures of snRNA in order to determine whether they have single-

stranded regions complementary to particular sequences of pre-messenger RNA.

The nucleotide sequences of several 7-10S and 4-4.8S snRNAs have already been established (11-15). Recently, we compared the primary structures of U1A RNA (7-10S region) from chicken, rat and human cells (16) and proposed a common model of secondary structure for these three RNAs (17). The 5S RNAs were only roughly characterized by fingerprinting (18). Three types of 5S RNA molecules were observed : 5SI and 5SII had the same fingerprint as ribosomal 5S RNA whereas 5SIII was different.

Using the method for RNA sequencing based on polyacrylamide gel electrophoresis of end-labeled molecules, we analyzed the 5S RNA from HeLa cells, rat brain and hen brain and liver. We report here that nuclear 5S RNAs are more heterogeneous than previously described. In particular, the RNA previously designated as 5SIII (18), G' (19) or U5 (20) is constituted of several types of molecules differing by a limited number of base substitutions or insertions. An experimental study of the secondary structure of these molecules is also described.

MATERIALS AND METHODS

1. Isolation of nuclear 5S RNAs. RNAs were phenol-extracted at 0-4°C pH 7.6 from purified nuclei, nuclear extracts or nuclear residues of rat and hen brain, hen liver and HeLa cells (21, 22). They were fractionated on 15 % polyacrylamide gel, made up in Tris-borate buffer (23).

2. 5'-end labeling. RNAs bearing a 5'-phosphate were dephosphorylated with alkaline phosphatase and labeled in the presence of 100-200 μ Ci of γ - 32 P ATP (Amersham) and 2 U of T4 polynucleotide kinase (24).

3. 3'-end labeling was achieved using 50-100 μ Ci (5'- 32 P)pCp (Amersham) and 2U of T4 RNA ligase under the conditions described by England and Uhlenbeck (25).

4. Partial digestion of end labeled RNA. 3'-end or 5'-end labeled RNAs were partially digested with T1, U2, pancreatic, *Phy* I, *Neurospora crassa* nucleases and with boiling water as previously described (24, 26). In addition, 3'-end labeled RNA were chemi-

cally digested according to Peattie (27).

5. Polyacrylamide gel electrophoresis were carried out on thin polyacrylamide gels (0.5 x 300 x 400 mm) made up in Tris-borate buffer (23).

6. Identification of modified nucleotides. 1 μ g of RNA was digested for 5 h at 37°C with 0.05 U of a mixture of T1 and T2 RNAses (28). The base-modified nucleotides were released as nucleotides 3'-phosphate. The 2'-O-methylated nucleotides gave rise to dinucleotides 3'-phosphate. These mono and dinucleotides were 5'-end labeled and their 3'-phosphates eliminated by digestion with 0.05 μ g of P1 nuclease for 30 min at 37°C, the labeled γ -phosphate of remaining ATP was transferred to glucose by yeast hexokinase (29). The labeled mono and dinucleotides 5'-monophosphate were fractionated by two-dimensional thin-layer chromatography with solvent system A. First dimension, isobutyric acid : concentrated ammonia H_2O (66:1:33, v/v/v) (a). Second dimension, concentrated HCl : isopropanol : H_2O (17.6:68:14.4, v/v/v) (b). Mobilities relative to those of unmodified marker nucleotides with reference to published mobilities allowed identification (30). An additional proof of the identity of base-modified nucleotides was obtained by fractionation in a second system of thin-layer chromatography (B). First dimension, solvent (a). Second dimension, solvent (c), sodium phosphate 0.1 M pH 6.8 : ammonium sulphate : n-propanol (100:60:2, v/w/v) (28).

7. Sequence analysis of the large T1 oligonucleotides. T1 RNase digestion products were 5'-end labeled and fractionated by two-dimensional polyacrylamide gel electrophoresis (24). They were then eluted from the gel. One half was analyzed by the homochromatography procedure (31). For this purpose, the oligonucleotide was digested in boiling water and the resulting products were fractionated by electrophoresis on cellulose acetate followed by homochromatography on DEAE-cellulose. The other half was analyzed by the sequencing gel technique, using partial enzymatic digestions.

8. Preparation and analysis of S1 nuclease partial digestion products. The RNA was partially digested with S1 nuclease under the conditions previously described (16). The resulting fragments were

3'-end labeled by pCp ligation and analyzed by the chemical method for RNA sequencing.

9. Study of RNA secondary structure. The RNA was partially digested with T1, S1 and *Naja oxiana* (32) nucleases, as previously described (17). The resulting products were analyzed by polyacrylamide gel electrophoresis.

RESULTS

1. Fractionation of nuclear 5S RNA. RNA was phenol-extracted from purified nuclei, from a nuclear extract (containing hnRNP, snRNP and soluble material) or from a nuclear residue (containing chromatin, nucleoli, nuclear membranes). They were electrophoresed in 15 % polyacrylamide gels. In each case, for hen brain, rat brain and HeLa cells, two clusters of 5S RNAs (5Sa and 5Sb) were detected, each made of 2, or more, very close bands (Fig. 1). Additional bands were detected in hen liver. Usually, there was little difference between the relative proportions of 5Sa and 5Sb RNA in nuclear extract and residue. However, in certain cases, in particular for HeLa cells, the 5Sa cluster predominated in the residual pellet. This was due to a contamination by ribosomal 5S RNA, as ribosomes bound to the nuclear membranes could not be entirely washed off and sedimented in the nuclear residue with the membranes. The presence of 5Sa RNA in the other fractions could not be attributed to ribosomal contamination since only traces of 28S and 18S were detected (results not shown). As will be discussed, 5Sb RNA corresponds to the RNA 5SIII (18) or G' (19) or U5 (20). U5 RNA seems to be the most currently used designation and we shall adopt it from now on.

2. Sequence analysis of 5Sa RNA. The nucleotide sequences of the RNA from the 2 close bands of 5Sa RNA were examined using 3' and 5'-end labeling. In HeLa cells, the 2 RNAs did not contain modified nucleotides. They both had the same nucleotide sequence as human ribosomal 5S RNA (33). The migration of this RNA in 2 bands is to be attributed to the existence of two conformations (34, 35). The situation was the same for rat brain but not for hen, where a microheterogeneity of 5Sa RNA rendered the analyses difficult. Only the 3'-half region could be analyzed. The various substitutions

Figure 1 : Polyacrylamide gel electrophoresis of snRNA from chicken, rat and man. The snRNAs were extracted from hen brain nuclear residue (A) and nuclear extract (B); hen liver nuclei (C); rat brain nuclear residue (D) and nuclear extract (E); HeLa cells nuclear residue (F) and nuclear extract (G).

are indicated in Figure 2. It might be remarked that in liver substitutions of CU dinucleotides occurred thrice from 5Sa₁ to 5Sa₂.

3. Sequence analysis of U5 RNA. Like 5Sa RNA, U5 RNA migrated in two bands (U5A and U5B). In hen liver, other bands were superim-

Figure 2 : Nucleotide sequence of the 3'-terminal regions of 5Sa from hen liver and brain. The sequence is that of 5Sa₁ RNA from liver which is identical to chicken ribosomal RNA (36). Only the mutations in 5Sa₂ RNA from liver and 5Sa from brain are indicated.

posed, making analysis difficult. Therefore, only analysis of U5 RNA from HeLa cells and from hen and rat brains will be reported. The two U5 bands were closer to each other in the case of HeLa cells and rat brain than in the case of hen brain. They could be separated only when a very small amount of RNA was fractionated on the gels. This was the case after 3'-end labeling owing to high specific radioactivity. As a larger amount of material was required for analysis of modified nucleotides and of T1 oligonucleotides, separation could not be achieved and the analyses were performed on a mixture of the two components.

3.1. Identification of modified nucleotides. The RNA was totally digested with T1 and T2 RNases. The mono and dinucleotides were fractionated by thin-layer chromatography as described under Methods. Using solvent system A (Fig. 3), four spots corresponding to modified nucleotides were observed, the same for the RNA of chicken, rat and man. Two of them had the mobilities of the dinucleotides pUm-U and pGm-C or pCm-G. The presence of the dinucleotides Um-U and Gm-C was already observed in 5SIII RNA (18) corresponding to U5 RNA. Two other spots had the mobilities of the mononucleotides p ψ and pD and their identity was confirmed by chromatography in solvent system B. It should be mentioned that the modified nucleotides are labeled with various efficiencies by T4 polynucleotide kinase. Therefore, the method gives on-

Figure 3 : Analysis of modified nucleotides of rat U5 RNA. The RNA was digested as described in Methods. The resulting products were 5'-end labeled and fractionated in the chromatographic system A (30). ATP was not entirely converted into ADP by transfer of its γ - ^{32}P phosphate to glucose by hexokinase. This explains the ATP spot.

ly qualitative informations and does not allow the determination of the number of each modified nucleotide in the RNA molecule.

3.2. Sequence analysis of the large T1 RNase digestion products. After total digestion of human U5 RNA with T1 RNase, the products were 5'-end-labeled and fractionated by two-dimensional electrophoresis (Fig. 4a). The oligonucleotides were analyzed using both homochromatography (Fig. 4b) and sequencing gel techniques (Fig. 4c). Oligonucleotides 1 and 2 both contained three 2'-O-methylated nucleotides as shown by the absence of cleavage by nucleases or boiling water at three different positions. In addition, the sequence of oligonucleotide 1 differed from that of oligonucleotide 2 at a single position, the 5' terminal uridine being replaced by a cytidine (Fig. 4a). This suggests that oligonucleotides 1 and 2 result from a sequence heterogeneity. The fractionation patterns were similar for rat and chicken U5 RNAs. The results of analyses are indicated in Figure 7.

3.3. Sequence analysis of 3'-end-labeled U5 RNA and of partial digestion products. The RNA and the fragments released upon limited digestion with S1 nuclease were 3'-end-labeled and sequenced as described in Methods (Fig. 5 and 6). These analyses showed that nuclear U5 RNA constituted a family of closely related RNA. In rat brain and HeLa cells, the band U5A contained a single molecular species whereas 3 or 4 types molecules respectively were present in the band U5B. These molecules differed by a limited number of nucleotide substitutions or insertions (Fig. 7). The situation was different for hen brain U5 RNA. Both U5A and U5B were homogeneous and U5B only differed from U5A by the absence of the 3'-terminal adenylic acid. Therefore, U5B from hen brain does not correspond to U5B from rat brain and HeLa cells. Therefore, the U5 RNAs from hen brain will be designated as U5A and U5A'.

Combining the results of the various experiments, as described above, we could determine : 1) the complete nucleotide sequence of U5A RNA of HeLa cells, rat brain and hen brain ; 2) the complete sequence of one of the HeLa cells U5B RNA and the 3'-terminal sequence of another of these RNAs ; 3) the 3'-terminal sequence of three rat brain U5B RNAs ; 4) the complete sequence of U5A' from chicken brain (Fig. 7).

Figure 4 : Analysis of T1 RNase digestion products.

(a) Fractionation of the T1 RNase digestion products from HeLa cells U5 RNA by two-dimensional polyacrylamide gel electrophoresis.

(b) Analysis of T1 oligonucleotide 1 by homochromatography.

(c) Sequencing gel of T1 oligonucleotide 1. Digestions were with Pancreatic (P), U2 and *Neurospora crassa* (N.C.) RNases and with boiling water (L).

Figure 5 : Sequencing gel of 3'-end labeled chicken U5A RNA. The RNA was chemically digested according to Peattie (27).

Figure 6 : Sequencing gels of 3'-end labeled S1 digestion fragments from rat U5 RNAs. (a) S1 fragment 1 ; (b) S1 fragment 2 of three different species of U5B RNA.

When the chemical method for RNA sequencing was applied to U1A RNA (16), a peculiar pattern of digestion was observed which corresponded to the cleavage of the cap structure ($3m^{227}$ GpppAmUm) by hydrazine. The same pattern of digestion was observed upon analysis of U5 RNAs (Fig. 6a), suggesting that these RNAs also have a cap structure of the type XpppAmUm where X is an undetermined nucleotide. The existence of a cap structure in U5 RNA was already mentioned (20).

All the U5 RNAs contained the same modified nucleotides. Three of them were detected in the large T1 oligonucleotide 1 and were located at positions 38, 42 and 46 in all cases. Chemical sequencing

Figure 7 : The nucleotide sequence of U5 RNAs from chicken brain, rat brain and HeLa cells. The sequence of chicken brain U5A RNA is given, only the substitutions and insertions observed in HeLa cells U5A and U5B RNAs are indicated. The substitutions and insertions in partially sequenced rat brain and HeLa cells U5B RNAs are also indicated. N is an undetermined nucleotide, X might be ψm. The T1 and S1 digestion products used to establish the sequences are indicated.

methods showed that nucleotides 38 and 42 were cleaved by reagents specific for G and U respectively. Consequently, they correspond to 2'-O-methylguanylic acid and 2'-O-methyluridylic acid in agreement with the characterization of the two dinucleotides pGm-C and pUm-U upon direct analysis (Fig. 3). Nucleotide 46 was resistant to cleavage by any of the chemical reagents and therefore, could not be identified in this way. The identity of nucleotide 46 could not be deduced either from the direct analysis of modified nucleotides since no labeled dinucleotide was observed that could correspond to pXm-C. There are two possible explanations : 1) either Xm-Cp cannot be labeled at its 5'-end by polynucleotide kinase and, therefore, is not detected, or, 2) pXm-C is not detected in chromatographic system A because it comigrates with one of the four unmodified nucleotides. It should be mentioned that chromatographic system B was not informative in this respect, since no data of the literature indicates the mobilities of dinucleotides of known sequence in this system. The existence of ψ m at position 46 would be compatible with the two sets of results since ψ is not cleaved by the chemical reagents under the conditions of sequence analysis (16, 27) and p ψ m-C has the same mobility as pG in chromatographic system A (30).

In addition to nucleotide 46, nucleotide 54 was the only other nucleotide which was not cleaved by chemical reagents. Therefore, it should correspond to p ψ observed upon direct analysis of modified nucleotides. The presence of pD was also observed by this latter method but pD could not be localized on sequencing gels as it behaves like pU in the chemical as well as in the enzymatic reactions (16).

In the course of U5 RNA sequencing, we observed that the 21 nucleotides at the 3' end of the molecules were hardly cleaved by enzymes even under the denaturing conditions used in the sequencing method. We previously made the same observation for the 3'-terminal region of U1A RNA (16) and this could be explained by the presence of a very stable hairpin (17). As will be developed in the next section, the same explanation may be given for U5 RNA.

4. The conformation of U5 RNA

4.1. Experimental study. 3'-end labeled U5A and U5B RNA from HeLa cells and U5A RNA from rat brain were partially digested

with T1, S1 and *Naja oxiana* nucleases under the non denaturing conditions previously used for the study of the structure of U1A RNA (17). Briefly, these conditions were selected in order to 1) stabilize RNA conformation, 2) favour enzyme activities, 3) allow S1 and T1 nucleases to cleave nucleotides in single-stranded regions and allow *Naja oxiana* nuclease to cleave nucleotides involved in secondary or tertiary interactions.

The various digests were fractionated by polyacrylamide gel electrophoresis (Fig. 8). Nucleotides preferentially digested under these non denaturing conditions were identified by comparison to fragments released by T1 RNase or boiling water under denaturing conditions.

Superimposable results were obtained for the three RNAs that we analyzed, indicating that their secondary structure is the same. As hen brain U5A RNA has the same primary structure as rat brain U5A RNA, its secondary structure should also be the same and was not studied.

4.2. Possible secondary structure. On the basis of the data of the common primary structure of U5A RNA from hen and rat brain, a model insuring maximum base-pairing could be built (Fig. 9). It is made of two hairpins (I and II) joined together by a single-stranded region (d). In addition to the top loop b, there are two bulge loops (a and c) in hairpin I. This model corresponds to a stable structure, with a free energy of -25 Kcal according to the thermodynamic values established by Tinoco *et al.* (37).

As shown in Figure 9, the model agrees with the experimental data of digestion of single-stranded regions by T1 and S1 nucleases and of structured regions by *Naja oxiana* nuclease. The presence of the stable hairpin II at the 3'-end explains the absence of enzymatic digestion even under denaturing conditions. In addition, the model is compatible with the sequences of the various U5B RNAs as shown in Figure 7. Most of the substitutions are found in the loop of the 3'-terminal hairpin II or in the free single-stranded region d adjacent to this hairpin. Another is in bulge loop a. The G inserted in two of the rat U5B RNAs slightly decreases the stability of hairpin II. The terminal A which is missing in chicken U5A' RNA is not included in this hairpin. Finally, only a substitution of C by U (in HeLa cells U5B RNA) is

Figure 8 : Experimental study of HeLa cells U5B RNA secondary structure. The RNA was partially digested with T1 (T1 Mg^{++}), S1, and *Naja oxiana* (N.ox.) nucleases in the non-denaturing conditions previously described (17). The resulting fragments were fractionated by polyacrylamide gel electrophoresis and were identified by their mobilities relative to those of products released by statistical digestion with T1 RNase (T1) and in boiling water (L).

Figure 9 : The secondary structure of U5 RNAs. The sequence is that of chicken brain U5A RNA. The differences existing in other U5 RNA species are indicated ○, ◐ T1 RNase, ◑ S1 nuclease, ◒ *Naja oxiana* RNase.

found in the stem of hairpin I and transforms a G.C into a G.U bond.

4.3. Informations on tertiary structure. The various single-stranded regions of U5 RNA were not cleaved at the same rate by S1 and T1 nucleases (Fig. 8 and 9). We may distinguish : 1) the very sensitive regions b and d, 2) the sensitive region c, 3) the regions a and e, hardly cleaved by the enzymes. The first group corresponds to well accessible regions probably located at the surface of the molecule. In contrast, the third group corresponds to regions buried in the molecule or involved in tertiary interactions.

Examination of the rates of cleavage of double-stranded regions by *Naja oxiana* nuclease also shows differences of sensitivity. Whereas the two strands of hairpin II were cleaved at the same rate, the 5' strand of hairpin I was cleaved more rapidly than the 3' strand. In particular, this was the case of the sequence U-U-U close to the hairpin-loop and of the sequence C-U-C at the bottom of the hairpin-stem. A high sensitivity to *Naja oxiana* nuclease of regions of the 5' strand close to the hairpin loop was already observed for hairpins I, II and III of U1A RNA (17). As previously mentioned, this could result either from enzyme specificity or from a greater accessibility of this region of the hairpins. It is striking that such specific cleavages were not observed for the 3'-terminal hairpins of either U5 RNA or U1A RNA. This might be accounted for by tertiary interactions in the 3' half of the molecules. In the case of U5 RNA loops a and e are probably involved and the differences of sensitivity of enzymes along the free region d might also be related to such tertiary interactions.

DISCUSSION

In agreement with an early work of Ro-Choi *et al.* (18), two classes of 5S RNA were found in our preparations of hen and rat brain and of HeLa cell nuclei. The situation might be more complex in other cell types as shown by the example of hen liver where more snRNA species which might be variants of the major ones or not were detected.

5Sa RNAs (121 nucleotides) were structurally identical to ribosomal 5S RNA or closely related to it. This raises the problem of

contamination of nuclei by ribosomes. Previous controls of our standard preparation of rat brain nuclei showed that such contamination was low (38, 39). In the experiments reported here, except for HeLa cells where a large fraction of 5Sa RNA was likely to be of cytoplasmic origin, we observed that the nuclear preparations contained stoichiometrically much less 28S, 18S and 5.8S ribosomal RNAs than expected from the amount of 5Sa RNA. The existence of variants of ribosomal 5S RNA in the nucleus (5Sa₂ in hen liver, 5Sa in hen brain) which were never described in the cytoplasm, the apparent absence of ribosomal 5S RNA in the nucleus of chicken brain are other arguments in favor of the nuclear localization of transcripts of the 5S gene family (40-41). This might be related to the peculiar metabolism of ribosomal 5S RNA since only a small amount of its precursor is incorporated into ribosomes (19). Nevertheless, more evidence is certainly required to demonstrate the presence of 5Sa RNA in the nucleus and it would be of particular interest to know whether it is localized in the nucleolus or in the nucleoplasm.

The very existence of 5Sa₂ from hen liver and 5Sa from hen brain indicates that chicken 5S RNA are encoded by a multigene family and that some of the genes are mutated. In *Drosophila melanogaster* (42) and *Xenopus* (40, 41) mutated 5S genes were observed and the corresponding 5S RNA were not detected in the ribosomes. Our results show that such mutated 5S RNA genes may be expressed, that the expression may vary according to the cell type and that the corresponding RNA might be localized in the nucleus.

U5 RNAs (117 nucleotides) are considered as nuclear species and seem to be localized in the nucleus at least preferentially. Several U5 RNA species were found in HeLa cells and rat brain (U5A and U5B). This shows that like 5S RNAs, U5 RNAs are transcribed from a multigene family in both cell types, that mutations occurred in certain of these genes and that the mutated genes were expressed. In this respect, it is worth mentioning that the different substitutions or insertions did not modify the secondary structure or the post-transcriptional modifications which both might be peremptory for a functional RNA.

In hen brain, no such variant RNA were detected. U5A' RNA differed from U5A RNA by the absence of the 3' terminal adenylic acid.

Whether this is due to a deletion in the gene at this position, to early arrest of transcription or to a different process of maturation remains to be determined.

The complete conservation of the sequence of U5A RNA from chicken to rat is of interest since U1A RNAs from the same two animal species differ at 6 positions (16). It will be important to know if other chicken cells have RNAs similar to U5B from HeLa cells or rat brain. RNAs from hen liver are currently investigated.

The existence of multiple forms was not described yet for small nuclear RNA with the exception of the nucleolar U3 RNA (13). In our comparative study of U1A RNA from chicken, rat and man (16), we found no evidence for sequence heterogeneity.

A comparison between the structures of the various snRNAs may be of interest as it might allow the determination of common, functionally important features. In this discussion, we shall restrict our comparison to U5 and U1A RNA, which were the only snRNAs whose secondary structure was experimentally studied.

The overall base composition of U5 RNA is very different from that of U1A RNA (16). U5 RNA contains 34 % uridine as compared to 23 % in U1A RNA. Its G + C / A + U ratio is about 0.7 against about 1.4 for U1A RNA. In respect of base composition, it might be emphasized that the ribosomal 5S RNA or the nuclear 5Sa RNA are much closer to U1A RNA than to U5 RNA. In spite of the difference in base composition, there are several structural similarities between U5 and U1A RNA, especially in the 3'-end region. 1) Both RNAs bear a very stable hairpin at their 3'-end. The top of the loop has the sequence Py-Py-Py-G in both RNAs. 2) Both 3'-end hairpin are followed by a long single-stranded region (17 and 13 nucleotides in U5 and U1A RNA, respectively), both containing 77 % of A and U. The sequences PyAA(U)₆G and UAA(U)₃G are found in the single-stranded region of U5 and U1A RNA, respectively. 3) At the 5'-end of the free single-stranded regions, starts a hairpin. The foots of the hairpin are made of four base-pairs including two G.C bonds in both cases. These four base-pairs are followed by a bulge loop of six nucleotides on the 3'-side of the hairpin. Both bulge loops contain the sequences C-U-C and Pu-A-C. 4) Evidences for tertiary interactions were shown in the 3'-end region of U5 and U1A RNA by the study of accessibility to nucleases. The bulge-

loop and the loop of the 3'-end hairpin were involved in these interactions in both U5 and U1A RNA.

The other part of the RNA molecules have a quite different structure. However, top loop b of hairpin I from U5 RNA bears similitudes to top loop b of hairpin II from U1A RNA. They are made of 11 and 10 nucleotides, respectively and are rich in pyrimidines. The last nucleotide (at the 3'-end) is a C. The 6th nucleotide starting from the 5'-end is a 2'-O-methylated nucleotide (Um and Am). These two loops are very accessible at the surface of both molecules. It may also be remarked that the 2 molecules have the sequences Am-Um-A-C-U-Py at their 5'-end and the sequence C-U-Pu at their 3'-end.

It is likely that the common characteristics of U5 and U1A RNAs are related to a common function which remains to be determined. It was postulated for U1A RNA that the long single-stranded regions which are not closed by base-pairing may hybridize to premessenger RNA *in vivo* whereas the very accessible hairpin-loops may associate with proteins. This might also be true for U5 RNA. However, it must be underlined that the long single-stranded region of U1A RNA which contains the sequence ACCU and was assumed to participate in the splicing mechanism (43, 44) has no counterpart in U5 RNA.

†To whom correspondence should be addressed.

ACKNOWLEDGEMENTS

This work was supported by grants from the "Centre National de la Recherche Scientifique", the "Institut National de la Santé et de la Recherche Médicale" and the "Délégation Générale à la Recherche Scientifique et Technique". We are indebted to Dr J.P. Ebel for his help and support along this work. We are grateful to Dr P. Carbon and S. Vassilenko for their generous gift of *Naja oxiana* nuclease. The excellent technical assistance of B. Muller and L. Kister is acknowledged.

REFERENCES

1. Larsen C.J., Galibert F., Lelong J.C. & Boiron M. (1967) C.R. Acad. Sci. Paris, 264, 1523-1526.
2. Prestayko A.W. & Busch H. (1968) Biochim. Biophys. Acta, 169, 327-337.

3. Weinberg R.A. & Penman, S. (1968) *J. Mol. Biol.*, **38**, 289-304.
4. Hellung-Larsen P., Frederiksen S. & Plesner P. (1971) *Biochim. Biophys. Acta*, **254**, 78-90.
5. Deimel B., Louis C. & Sekeris C.E. (1977) *FEBS Lett.*, **73**, 80-84.
6. Northemann W., Scheurlen M., Gross V. & Heinrich P.C. (1977) *Biochem. Biophys. Res. Commun.*, **76**, 1130-1137.
7. Guimont-Ducamp C., Sri-Widada J. & Jeanteur P. (1977) *Biochimie*, **59**, 755-758.
8. Gallinaro H. & Jacob M. (1979) *FEBS Lett.*, **104**, 176-182.
9. Flytzanis C., Alonso A., Louis C., Krieg L. & Sekeris C.E. (1978) *FEBS Lett.*, **96**, 201-206.
10. Jelinek W. & Leinwand L. (1978) *Cell*, **15**, 205-214.
11. Busch H. (1976) *Perspectives in Biology and Medicine*, Summer 1976, 549-567.
12. Reddy R., Henning D. & Busch H. (1979) *J. Biol. Chem.*, **254**, 11097-11105.
13. Reddy R., Henning D. & Busch H. (1980) *J. Biol. Chem.*, **255**, 7029-7033.
14. Epstein P., Reddy R., Henning D. & Busch H. (1980) *J. Biol. Chem.*, **255**, 8901-8906.
15. Horada F., Kato N. & Nishimura S. (1980) *Biochem. Biophys. Res. Commun.*, **95**, 1332-1340.
16. Branlant C., Krol A., Ebel J.P., Lazar E., Gallinaro H., Jacob M., Sri-Widada J. & Jeanteur P. (1980) *Nucleic Acids Res.* **8**, 4143-4154.
17. Branlant C., Krol A., Ebel J.P., Gallinaro H., Lazar E. & Jacob M. (1980) submitted for publication.
18. Ro-Choi T., Reddy R., Henning D. & Busch H. (1971) *Biochem. Biophys. Res. Commun.*, **44**, 963-972.
19. Zieve G. & Penman S. (1976) *Cell*, **8**, 19-31.
20. Lerner M.R. & Steitz J.A. (1979) *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 5495-5499.
21. Stévenin J., Gallinaro H., Gattoni R. & Jacob M. (1977) *Eur. J. Biochem.*, **74**, 589-602.
22. Gattoni R., Stévenin J. & Jacob M. (1980) *Eur. J. Biochem.*, **108**, 203-211.
23. Sanger F. & Coulson R. (1978) *FEBS Lett.*, **87**, 107-110.
24. Branlant C., Krol A., Machatt M. & Ebel J.P. (1979) *FEBS Lett.*, **107**, 177-181.
25. England E. & Uhlenbeck O. (1978) *Nature*, **275**, 560-561.
26. Krupp G. & Gross H. (1979) *Nucleic Acids Res.*, **6**, 3481-3490.
27. Peattie D. (1979) *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 1760-1764.
28. Silberklang M., Gillum A.M. & Rajbhandary U.L. (1979) *Methods in Enzymology*, **59**, 58-109.
29. Brown R.S., Rubin J.R., Rhodes D., Guilley H., Simoncsits A. & Brownlee G.G. (1978) *Nucleic Acids Res.*, **5**, 23-36.
30. Nishimura S. (1979) *Cold Spring Harbor Labor. (ed. Schimmel, Söll, Abelson)* 551-552.
31. Brownlee G.G. & Sanger F. (1969) *Eur. J. Biochem.*, **11**, 395-399.
32. Vassilenko S. & Babkina V. (1965) *Biokhimiya*, **30**, 705-712.
33. Matten L., Amaldi F. & Attardi G. (1969) *Biochemistry U.S.A.*, **8**, 4989-5005.
34. Forget B. & Weissmann S. (1967) *Nature (London)*, **213**, 878-882.
35. Weinberg R. & Penman S. (1968) *J. Mol. Biol.*, **38**, 289-304.
36. Brownlee G.G. & Cartwright E.M. (1975) *Nucleic Acids Res.*, **2**, 2279-2288.

37. Tinoco I., Borer P., Dengler B. & Levine M. (1973) *Nature New Biol.*, 246, 40-41.
38. Gallinaro H., Stévenin J. & Jacob M. (1975) *Biochemistry U.S.A.*, 14, 2547-2554.
39. Devilliers G., Stévenin J. & Jacob M. (1977) *Biol. Cellulaire*, 28, 215-220.
40. Korn L.J. & Brown D. (1978) *Cell*, 15, 1145-1156.
41. Peterson R., Doering J. & Brown D. (1980) *Cell*, 20, 131-141.
42. Tschudi C. & Pirrotta V. (1980) *Nucleic Acids Res.*, 8, 441-451.
43. Lerner M., Boyle J., Mount S., Wolin S. & Steitz J. (1980) *Nature*, 283, 220-224.
44. Rogers J. & Wall R. (1980) *Proc. Natl. Acad. Sci. U.S.A.*, 77, 1877-1879.