Volume 15 Number 2 1987 Nucleic Acids Research

Plant small nuclear RNAs. II. U6 RNA and a 4.5S_I-like RNA are present in plant nuclei *

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Received September 25, 1986; Revised and Accepted December 18, 1986

ABSTRACT

Two small nuclear RNA species (U6 RNA and a 4.5S_I-like RNA) not described so far for plants were detected in broad bean (Vicia faba L.) nuclei. U6 RNA is 98 nucleotides long, contains Ψ and methylated nucleotides and shows a surprisingly high degree of sequence homology (80%) with its rat counterpart, particularly in the middle part (a 57 nucleotide-long stretch) of the molecule, where it amounts to 98%. The 4.5S_I-like RNA, similar in its structure to 4.5S_I RNA detected so far only in rodent nuclei, is 94 nucleotides long, contains Ψ and an unidentified nucleotide and exhibits 52% overall sequence homology with rat 4.5S_I RNA. A block of 20 consecutive nucleotides at the 5' end of the molecule is conserved between broad bean 4.5S_I-like RNA and rat 4.5S_I RNA. The presence of the two RNA polymerase III internal promoter consensus sequences in 4.5S_I-like RNA suggests that it is an RNA polymerase III transcript.

INTRODUCTION

All eukaryotic cells contain small nuclear RNAs (snRNAs). One class of them comprises the so-called uridylate-rich snRNAs [U-snRNAs; reviewed in (1)]. There are six major molecular species (U1 to U6 RNA) in this group. All of them have been characterized with respect to both their primary and secondary structures. They are 107 (U6 RNA) to 217 (U3 RNA) nucleotides long (2), depending on the molecular species, are capped at their 5' end by $m_3^{2,2,7}$ G (except U6 RNA which has an as yet unidentified non-nucleotide cap), contain modified nucleotides and are transcribed by RNA polymerase II, except U6 RNA which is transcribed by RNA polymerase III (3, 4). They occur in the form of individual ribonucleoprotein complexes [U-snRNPs; reviewed in (5)]; U4 and U6 RNAs are both components of a single U-snRNP (6, 7). Ever increasing evidence indicates that

U1, U2, U5 and U4/U6 snRNPs are involved in the splicing of pre-mRNA (8, 9, 10, 11).

The other class of snRNAs comprises 4.5S and $4.5S_I$ RNAs which have so far been detected only in rodent cells [cf. (1)]. Their primary and secondary structures are known. They are 94 (12) and 98-99 [cf. (13)] nucleotides long, respectively, have pppG at their 5' end and seem to lack modified nucleotides (12, 14). They are RNA polymerase III transcripts [cf. (1)] and occur in the form of RNP complexes that are recognized by anti--La antibodies [(14, 15); cf. (1)] present in the sera of patients with systemic lupus erythematosus. No experimental evidence concerning their possible function has been reported yet.

As far as the plant kingdom is concerned, absolutely nothing is known about the occurrence in plants of snRNAs transcribed by RNA polymerase III. Even with the ubiquitous eukaryotic U-snRNAs [cf. (2)], most of our knowledge of their structure and all our information on their function come from studies of these molecules as obtained from eukaryotes other than plants. Due to methodological difficulties involved in the isolation of plant nuclei from which RNA can be extracted in an undegraded state, earlier results could be achieved only by isolating U-snRNAs from plant material by using either anti-cap antibodies as originally described by Lührmann et al. (16) or cytoplasmic fraction. None of these approaches revealed the entire putative set of U-snRNAs from plant material. By using anti-cap antibodies Krol et al. (17) could isolate U1, U2 and U5 RNAs from pea and determine the entire sequence of five variants of U5 RNA, and the 3' end sequences of U1 and U2 RNAs, and by using a cytoplasmic fraction Skuzeski and Jendrisak (18) were able to isolate and partially sequence a U2-like RNA from wheat.

Recently we developed two methods for the isolation of plant nuclei from which the entire alleged set of snRNAs could be extracted in an undegraded state. The use of these methods permitted us to detect and to partially characterize nucleolar U3 RNA from plant material (19).

As a further exploitation of our method of nuclei isolation here we report for the first time the occurrence in plants of

U6 RNA and of a novel type of plant snRNA very similar to a polymerase III transcript, $4.5S_I$ RNA, shown so far to occur only in nuclei of rodent cells [cf. (1)]. The entire primary and possible secondary structures of both of these molecules are also presented.

MATERIALS AND METHODS

1. Isolation and fractionation of nuclear RNAs

Broad bean nuclei isolated by Method I of Kiss et al. (19) were phenol extracted at 65° C as described in (20). The total nuclear RNAs were separated by electrophoresis on 10% polyacrylamide gels (21) containing 8 M urea. The U6 and $4.5S_I$-like RNAs extracted (22) from these gels were satisfactory for sequence analysis. Rat liver nuclear RNAs were isolated according to Reddy et al. (23).

2. Chemicals

RNase A, T1 RNase, U2 RNase, S1 nuclease, alkaline phosphatase, T4 polynucleotide kinase were from Boehringer and RNase Phy M, RNase B. cereus and T4 RNA ligase from Pharmacia. (γ-^{32}P) ATP was obtained from Izinta, Prague.

3. 5' end-labeling

Dephosphorylated RNAs were labeled in the presence of 150-200 μCi of γ-^{32}P ATP and 5 U of T4 polynucleotide kinase (24).

4. 3' end-labeling

Synthesis of (5'-^{32}P) pCp and 3' end-labeling of RNAs were made according to England et al. (25).

5. Fragmentation of broad bean U6 RNA

For limited digestion with S1 nuclease, 0.5 μg of U6 RNA was first preincubated for 10 min at 37° C in 20 μl of 25 mM Na acetate, pH 4.5, containing 50 mM KCl, 1 mM ZnCl and 10 mM $MgCl_2$. After addition of 2.5 U of S1 nuclease, the mixture was incubated for 10 min at 37° C. The digestion was stopped by two subsequent phenol extractions. For limited digestion with RNase A, 0.5 μg of U6 RNA was first preincubated for 10 min at 37° C in 20 μl of 25 mM sodium citrate, pH 5.0, containing 10 mM $MgCl_2$. It was then digested for 10 min at 4° C in the presence of 0.005 ng RNase A. The reaction was stopped by two

subsequent phenol extractions. The resulting S1 nuclease and RNase A fragments were labeled at their 3' and 5' ends, respectively, as described above.

6. Nucleotide sequence analysis

Chemical sequencing of 3' end-labeled RNAs and RNA fragments was carried out according to (26). Partial enzymatic cleavage of 3' and 5' end-labeled RNAs and RNA fragments was performed by RNase T1 (G specific), RNase U2 (A specific), RNase Phy M (U specific), RNase B. cereus (C and U specific) and RNase A (C and U specific when they are 5' to A) according to (27). 2'-O-methylated nucleotides were mapped by limited hydrolysis of end-labeled RNAs in boiling water (28) or in alkali (24). The digests were fractionated on 20% and 10% polyacrylamide gels (21).

RESULTS

1. Survey of plant snRNAs

Fig. 1 shows the polyacrylamide gel electrophoretic pattern of snRNAs from broad bean (Lane B) in comparison to those from rat (Lane A). The molecular species marked U1, U2, U3 and U5 in Lane B have already been identified as U1, U2 and U5 RNAs from pea by Krol et al. (17), and U2 as well as U3 RNAs from broad bean by Kiss et al. (19), whereas those designated U6, "4.5S_I" as well as the material(s) in region X have not been characterized so far.

2. Characterization of broad bean U6 RNA

2.1. Strategy of sequencing broad bean U6 RNA. Broad bean U6 RNA, after purification on polyacrylamide gel and extraction from the gel, proved to be a poor substrate of T4 RNA ligase. Therefore, to confirm the sequence of the molecule, as obtained by chemical sequencing after 3' end-labeling, and to complete it up to the 5' end, it was necessary to partially digest U6 RNA by S1 nuclease and label the fragments at the 3' ends or, alternatively, to digest it by RNase A and label the fragments at the 5' ends. The labeled fragments were fractionated on polyacrylamide gel and those shown in Fig. 2 were sequenced chemically (S1 fragments) and enzymatically (both S1 and RNase A fragments).

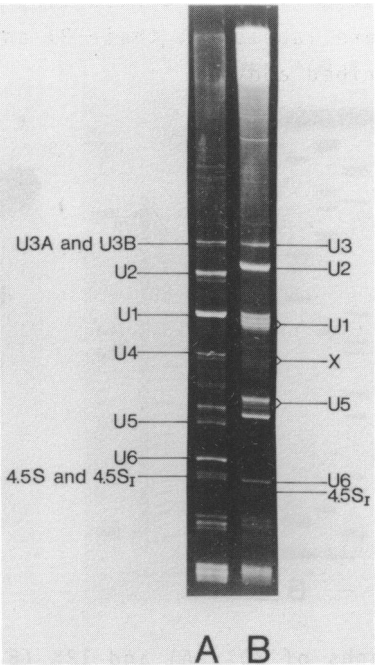

Fig. 1. Electrophoretic patterns of low-molecular-weight RNAs extracted by the hot phenol/SDS method from rat liver nuclei (A) isolated according to (23) and from broad bean nuclei (B) isolated by Method I as described (19). For more details see Materials and Methods.

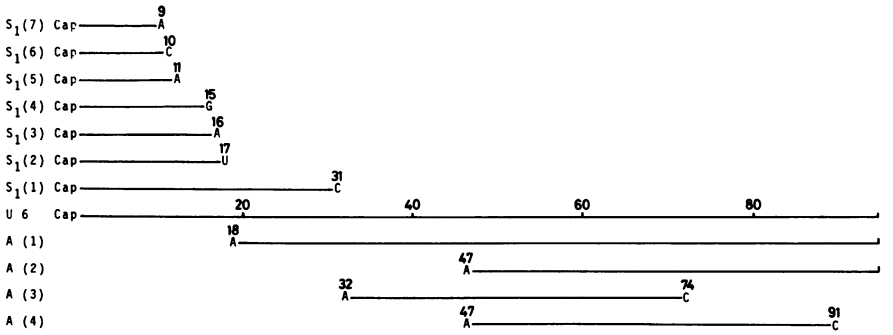

Fig. 2. Strategy of sequencing broad bean U6 RNA. The fragments selected after partial digestion of broad bean U6 RNA by S1 nuclease [S1(1) to S1(7)] or RNase A [A(1) to A(4)] and labeled at the 3' end (S1) or 5' end (A) were sequenced enzymatically (A) and enzymatically as well as chemically (S1) to determine the complete sequence (U6) of the molecule.

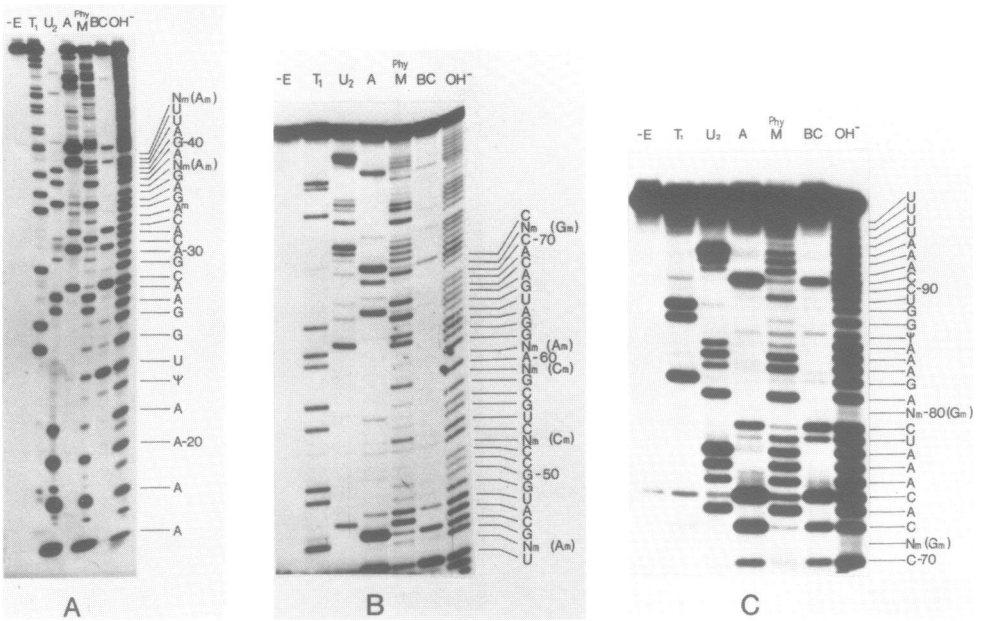

Fig. 3. Autoradiographs of 20% (A) and 12% (B and C) sequencing gels as obtained after electrophoresis of enzymatic digests of 5' end-labeled RNase A fragment 1 [A(1) in Fig. 2] from broad bean U6 RNA. The 2'-O-methylated nucleosides (indicated in paranthesis) were determined by chemical sequencing of 3' end--labeled U6 RNA (not shown). For the enzymatic digestions T1 RNase (T1), U2 RNase (U2), pancreatic RNase (A), Phy M RNase (Phy M) and B. cereus RNase (BC) were used. The lanes obtained after partial alkaline hydrolysis (OH^-) and without enzyme treatment (-E) are also shown. For more details see Materials and Methods.

2.2. Determination of modified nucleosides in broad bean U6 RNA. Sequencing gels for enzymatic digests of a 5' end-labeled RNase A fragment (A/1 in Fig. 2) are shown in Fig. 3. It can be seen that in positions 22 (Fig. 3A) and 86 (Fig. 3C) alkaline digestion was fully effective but RNase Phy M, RNase B. cereus and RNase A cleaved only slightly. Upon chemical sequencing of the 3' end-labeled U6 RNA (not shown) no cleavage was obtained at these positions. Taken together, the above data indicate that broad bean U6 RNA is likely to have Ψ in positions 22 and 86.

The nucleotide in position 34 was sensitive to alkaline digestion and also to cleavage by RNase Phy M but not to that

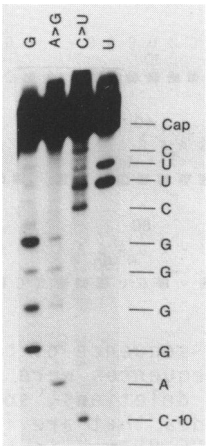

Fig. 4. Autoradiograph of a 20% sequencing gel as obtained after electrophoresis of chemical degradation products of 3' end-labeled S1 nuclease fragment 6 [S1(6) in Fig. 2] from broad bean U6 RNA. For more details see Materials and Methods.

by RNase U2 despite its sensitivity to diethylpyrocarbonate in the chemical sequencing reaction (not shown). These data suggest that broad bean U6 RNA has N^6-methyladenosine in position 34. Rat U6 RNA has also N^6-methyladenosine in this position of the aligned sequence (Fig. 5).

2'-O-methylated nucleotides in positions 38, 44, 53, 59, 61, 71 and 80 (gaps in the sequencing ladder obtained after alkaline hydrolysis; Fig. 3A-C) could easily be determined from the results of chemical cleavage (not shown).

2.3. Unusual cap structure in broad bean U6 RNA. Since no 5' end-labeling was obtained after treatment of broad bean U6 RNA with alkaline phosphatase, followed by incubation with (γ-^{32}P) ATP and polynucleotide kinase (not shown), we conclude that broad bean U6 RNA has a cap at its 5' end. The cap does not seem to consist of the canonical $m_3^{2,2,7}$ G triphosphate, a general characteristic of all U-snRNAs other than U6 RNA (2), because, as shown in Fig. 4, no characteristic cleavage pattern of canonical cap structure was obtained when fragment S1/6, shown in Fig. 2, was sequenced chemically and the cleavage products were fractionated by electrophoresis on a 20% polyacrylamide gel. Whereas the canonical $m_3^{2,2,7}$ G cap structure is

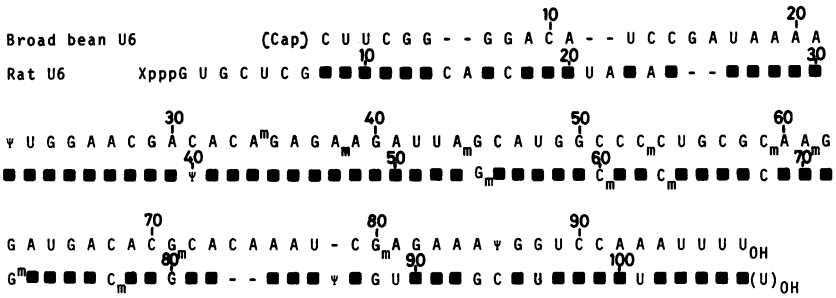

Fig. 5. Comparison of the sequence of broad bean U6 RNA with that of rat U6 RNA (2). Sequences were aligned for maximum homology. Dashes indicate deletions, solid squares in the sequence stand for nucleotides that are identical in rat U6 RNA with those in broad bean U6 RNA. X is an unidentified non--nucleotide (2) and (Cap) stands for a non-analyzed structure.

preferentially attacked on its second and mainly on its third phosphate by the nucleophylic anhydrous hydrazine [cf. Fig. 4 in (29)], in the case of broad bean U6 RNA a strong cap-specific cleavage was obtained in all reaction columns (Fig. 4). No attempt was made to identify the cap structure in broad bean U6 RNA. In other U6 RNAs sequenced so far it seems to be an unidentified non-nucleotide (2).

2.4. Complete primary structure of broad bean U6 RNA. The complete sequence of broad bean U6 RNA was established by using the sequences of all the individual fragments shown in Fig. 2. Sequences of the overlapping regions as well as the sequence as determined by subjecting intact, 3' end-labeled U6 RNA to base--specific chemical degradation, served to corroborate our results.

In Fig. 5 the primary structure of broad bean U6 RNA is compared and aligned for maximum sequence homology with that of rat U6 RNA (2). It can be seen that broad bean U6 RNA is 8 nucleotides shorter than the shorter variant of rat U6. Seven nucleotides present at the 5' end, right after the cap, of rat U6 RNA are missing from broad bean U6 RNA as also is a Ψ residue (position 86 in rat U6 RNA). In addition, there are two compensatory deletions and additions, respectively, in the two molecules: The dinucleotides CA and UA at positions 14-15 and 21-22,

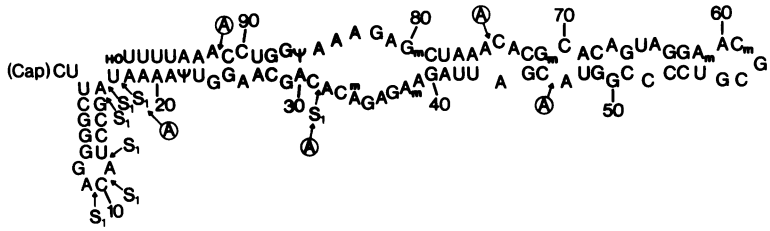

Fig. 6. Possible secondary structure of broad bean U6 RNA. The sites of cleavage by nuclease S1 and pancreatic RNase are marked by S1 ⟶ and Ⓐ⟶, respectively.

respectively, of rat U6 RNA are missing from the corresponding positions of the aligned broad bean U6 RNA molecule, but a GA addition occurs in positions 15-16 and a CA addition in positions 74-75 of broad bean U6 RNA compared to the aligned rat U6 RNA.

Apart from these differences, sequence homology between the two molecules is surprisingly high (80%), particularly in the middle part (nucleotides 17 to 73 in broad bean U6 RNA and nucleotides 26 to 82 in rat U6 RNA) of the molecules, where it is 98%, disregarding differences in methylation pattern. The overall number of 2'-O-methylated nucleosides is 7 in broad bean U6 RNA and 8 in rat U6 RNA, four of them being at identical positions, distributed in the most conserved central region of the molecules. One of the 2 and 3 pseudouridines in broad bean U6 RNA and rat U6 RNA, respectively, also coincides in position, and so does N^6-methyladenosine (position 34 in broad bean U6 RNA).

2.5. Possible secondary structure of broad bean U6 RNA. Fig. 6 represents a possible secondary structure of broad bean U6 RNA. This structure, developed to establish maximum base pairing, is very similar to those of mouse U6 RNA (30) and rat U6 RNA (31). It is consistent with the preferential cleavage sites of RNase A and S1 nuclease. The overall structure has a free energy of ΔG=-15.8 Kcal/mol (32).

3. Characterization of broad bean $4.5S_I$-like RNA

3.1. Strategy of sequencing broad bean $4.5S_I$-like RNA. Broad bean $4.5S_I$-like RNA, after purification on polyacrylamide gel

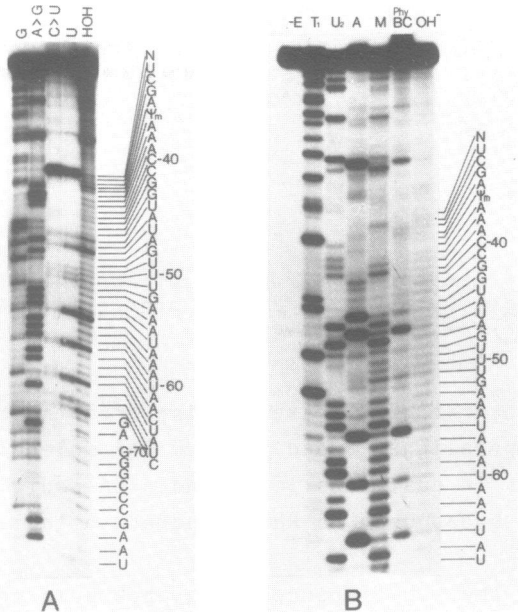

Fig. 7. Autoradiographs of 10% sequencing gels as obtained after electrophoresis of chemical degradation products (A) and enzymatic digests (B) of 3' end-labeled $4.5S_I$-like RNA from broad bean nuclei. In chemical sequencing (A) the RNA was partially hydrolyzed in boiling water (HOH) to get the ladder. In enzymatic sequencing (B) T1 RNase (T1), U2 RNase (U2), pancreatic RNase (A), Phy M RNase (Phy M) and B. cereus RNase (BC) were used. The lanes obtained after partial alkaline hydrolysis (OH^-) and without enzyme treatment (-E) are also shown. For more details see Materials and Methods.

and extraction from the gel, could conveniently be labeled by pCp and T4 RNA ligase at the 3' end. The entire sequence of broad bean $4.5S_I$-like RNA could be determined by combining the data obtained from chemical and enzymatic sequencing of the 3' end-labeled intact molecule. Representative sequencing gels as obtained by using the chemical method and the enzymatic one for a portion of broad bean $4.5S_I$-like RNA are presented in Fig. 7A and Fig. 7B, respectively.

3.2. Determination of modified nucleosides in broad bean $4.5S_I$-like RNA. In broad bean $4.5S_I$-like RNA there were only two nucleotides, one in position 31 and the other one in position 36 (Fig. 8), that behaved differently from the four common

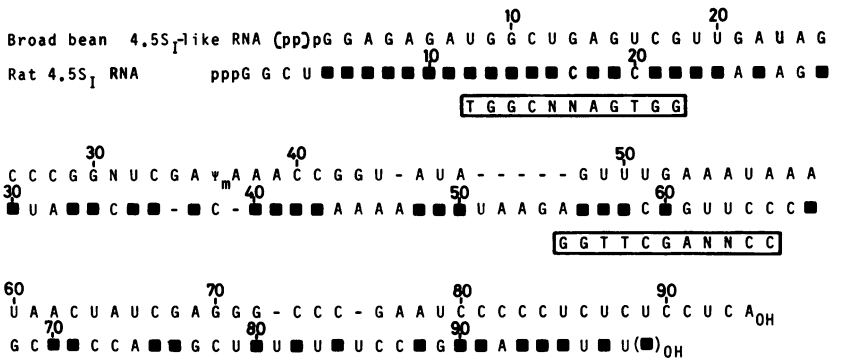

Fig. 8. Comparison of the sequence of broad bean 4.5S$_I$-like RNA with that of rat 4.5S$_I$ RNA (14). Sequences were aligned for maximum homology. Dashes indicate deletions, solid squares in the sequence stand for nucleotides that are identical in rat 4.5S$_I$ RNA to those in broad bean 4.5S$_I$-like RNA. The two RNA polymerase III internal promoter consensus sequences (33) are boxed. N is an unidentified nucleotide (see Results). The 5' end of broad bean 4.5S$_I$-like RNA contains at least one phosphate group.

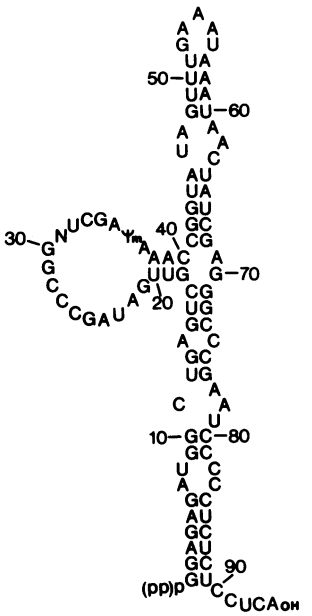

Fig. 9. Possible secondary structure of broad bean 4.5S$_I$-like RNA.

nucleotides towards the chemicals and enzymes used in sequencing. The nucleotide at position 31 strongly reacted with both anhydrous (C reaction) and aqueous (U reaction) hydrazine (Fig. 7A). In the enzymatic sequencing reactions it was hardly cleaved by either RNase A, or RNase B cereus or RNase Phy M. We suggest that it is a modified pyrimidine. The nucleotide at position 36 was cleaved neither after alkaline hydrolysis (Fig. 7B) nor ofter hydrolysis in boiling water (Fig. 7A),indicating a 2'-O-methylation of the ribose. As this nucleoside was also refractory to chemical cleavages (Fig. 7A) we suggest that it is Ψ_m.

3.3. Structure of the 5' end of broad bean 4.5S_I-like RNA. Broad bean 4.5S_I-like RNA could be labeled at its 5' end only after treatment with alkaline phosphatase, indicating that its 5' end is phosphorylated. By analogy with rat 4.5S_I RNA we assume that its 5' end bears a triphosphate.

3.4. Complete primary structure of broad bean 4.5S_I-like RNA. In Fig. 8 the complete sequence of broad bean 4.5S_I-like RNA, as obtained by chemical and enzymatic sequencing of the 3' end-labeled intact molecule, is compared and aligned for maximum sequence homology with that of rat 4.5S_I RNA (2). It is 4 nucleotides shorter than the shorter variant of its rat counterpart and the sequence homology between these two molecules is 52% amounting to 95% with respect to the first 20 nucleotides at the 5' end of broad bean 4.5S_I-like RNA. Broad bean 4.5S_I-like RNA, similarly to rat 4.5S_I RNA, contains the two RNA polymerase III internal promoter consensus sequences (33). The first one is present in a basically unaltered form (one mismatch out of 11 nucleotides) between nucleotides 8 and 18, whereas the second one (between nucleotides 47 and 57) somewhat deviates from the consensus sequence (four mismatches out of 11 nucleotides). The above two consensus sequences are present also in rat 4.5S_I RNA at identical positions of its sequence aligned to that of broad bean 4.5S_I-like RNA. A basic difference between these apparently related molecules is that rat 4.5S_I RNA has a track of four (or five) Us at its 3' terminus whereas the 3' end nucleoside of broad bean 4.5S_I-like RNA is A preceded by a track of 15 pyrimidines.

3.5. Possible secondary structure of broad bean 4.5S_I-like RNA. Fig. 9 represents a possible secondary structure of broad bean 4.5S_I-like RNA. This structure was developed to establish maximum base pairing. It is very similar to that of rat 4.5S_I RNA (34) and has a free energy of ΔG=-8.2 Kcal/mol (32).

DISCUSSION

This is the first report on the occurrence of U6 RNA and a 4.5S_I-like RNA in plant nuclei.

Failure to detect until now U6 RNA in plant material may have been due to any of the following three reasons: (i) low concentration of the molecule in plant nuclei, (ii) lack of a canonical cap structure in plant U6 RNA, similar to all other eukaryotic U6 RNAs studied so far [cf. (2)], which made its isolation using anti-$m_3^{2,2,7}$ G antibodies impossible (17), and (iii) inherent inefficiency of labeling the molecule at its 3' end by pCp and RNA ligase. Difficulties in 3' end-labeling have been reported earlier for 4.5S_{III} RNA (= U6 RNA) from Novikoff hepatoma (35). Myslinski et al. (36) were unable to detect U6 RNA in Drosophila perhaps because of inefficient 3' end-labeling of the putative molecule.

Resistance of broad bean U6 RNA to accept pCp upon incubation with T4 RNA ligase may be due either to a very stable double-stranded structure of the molecule at its 3' end region or to a particular nucleotide sequence of its 3' end region that is not well accepted by RNA ligase, or both. Our data seem to lend support to both of these possibilities: six nucleotides at the 3' end of broad bean U6 RNA seem to be base-paired (Fig. 6) and the sequence of this region is UUUU, a stretch of nucleotides that is a particularly poor substrate for T4 RNA ligase (25).

By using the methods and approach described in this paper we overcame these difficulties.

Comparison of the primary structures of U-snRNAs from phylogenetically distant species helps identify functionally essential sequences expected to be highly conserved in Nature. This approach has led to some significant discoveries: (i) the establishment of domain A in U1, U2, U4 and U5 RNAs (37), a

probable protein binding site (38), (ii) the finding of a sequence between nucleotides 30 and 50 in U5 RNA which is highly conserved in all eukaryotic U5 RNAs including those from dinoflagellates (39), and pea (17) and, strangely enough, in avocado sunblotch viroid (40) and (iii) the discovery of two conserved blocks of nucleotides in nucleolar U3 RNA isolated from phylogenetically distant species such as, rat (41), Dictyostelium (42) and broad bean (19), supporting the notion that these sequences may indeed be involved in pre-rRNA processing as originally suggested by Bachellerie et al. (43).

Some features of the primary structure of broad bean U6 RNA point to the importance of highly conserved sequences in U-snRNAs. First, this is the plant U-snRNA species that gives the highest degree of overall sequence homology with its mammalian equivalent. By taking sequences aligned for maximum sequence homology, pea U5A RNA exhibits 53% overall sequence homology with rat U5 RNA, and the 3' end parts sequenced so far in pea U1 RNA, pea U2 RNA, broad bean U2 RNA and broad bean U3 RNA are 64%, 54%, 52% and 48% homologous, respectively, with their rat counterparts. This shows that the function of U6 RNA in eukaryotic cell nuclei, although not yet specified, is more dependent on a highly conserved overall primary structure than that of the other major U-snRNAs. Secondly, the highest degree of sequence homology between broad bean U6 RNA and rat U6 RNA is localized in the middle part of the molecule. The region in mammalian U6 RNA proposed to be involved in base pairing with a given sequence in U4 RNA in three different models (6, 7, 44) is also located in the middle segment of the molecule. This finding implies that plant nuclei are likely to contain U4 RNA. Although all efforts to detect U4 RNA in plants have failed so far (17, 19), we suggest on the basis of electrophoretic mobility data that region X in Fig. 1 contains (variants of) plant U4 RNA. Thirdly, the fact that seven nucleotides are missing from the 5' end of broad bean U6 RNA in comparison with rat U6 RNA suggests that unlike in the case of U1 and U2 RNAs (8, 9, 45, 46) it is the rest of the molecule, rather than its 5' end, which is of functional importance in U6 RNA.

The other small nuclear RNA whose occurrence we discovered

in broad bean nuclei is a $4.5S_I$-like RNA. This RNA species was designated as such because it (i) is uncapped just like rat $4.5S_I$ RNA (34), (ii) exhibits from nucleotide position 1 to 20 a striking sequence homology with rat $4.5S_I$ RNA and (iii) contains at least the first internal promoter consensus sequence (33) of RNA polymerase III, similar to rat $4.5S_I$ RNA.

It differs, however, from this molecular species in that (i) it contains the second internal promoter consensus sequence (33) of RNA polymerase III in an imperfect form, (ii) its 3' end is an oligopyrimidine track rather than an oligo U, and (iii) it has two modified nucleosides in its primary structure, whereas rat $4.5S_I$ RNA was reported to have none (14). It should be noted, however, that in contrast to the above finding (14), Hendrick et al. (47) have found Ψ in mouse $4.5S_I$ RNA, although its position has not been determined.

Considering the above data we argue that broad bean $4.5S_I$--like RNA is an RNA polymerase III transcript. Deviations from the consensus sequence in the second block of the RNA polymerase III internal promoter in broad bean $4.5S_I$-like RNA do not seem to invalidate this assumption, because in some of the Ro-RNAs, a group of cytoplasmic small RNAs (Y-RNAs) of HeLa and rodent cells, this sequence was not evident either in spite of their being experimentally proven RNA polymerase III transcripts (48).

Our findings presented in this paper strongly suggest that the structural organization of and perhaps even the gene pattern for small nuclear RNAs transcribed by RNA polymerase III are more universal in eukaryotes than was previously thought: they may apply to the plant kingdom as well.

If $4.5S_I$ RNA has a similar function in the plant cell nucleus as in rodent nuclei,such as participation in pre-mRNA processing, it can be assumed that it will exhibit the same structural organization, i.e. it will occur in the form of RNP particles, as established for rodents (47). In rodents $4.5S_I$ RNP reacts with anti-La antibodies (47). It would be worth testing whether or not La antigens are present in plant cells as well.

Rodent $4.5S_I$ RNA has been reported to exhibit sequence homology with the rodent equivalent (B2) of moderately repetitive

Alu type 2 sequences [cf. (49)]. Recently (50), a different set of moderately repetitive sequences has been detected in rat, that also shows a high degree of sequence homology with rat $4.5S_I$ RNA. Plant DNAs are known to contain a surprisingly high percentage of repetitive sequences (51) but to the best of our knowledge no Alu-related sequences have been reported so far for plant DNA. It would be interesting to see whether or not Alu-related sequences occur in the plant genome. If they do, one might infer that plants have a reverse transcriptase that permits retroposition similar to what has been described for 7SL RNA and Alu type 1 sequences in mammals (52).

ACKNOWLEDGEMENTS

Thanks are due to Mr. B. Dusha and Mr. T. Kószó for preparing the photographs and drawings, respectively, and to Mrs. Mária Doktor for typing the manuscript. This work has been supported by grants KKA 8/1/400/2/85/1/118 and OKKFT(Tt)/1986 from the Hungarian Academy of Sciences.

*Part I is Reference No. 19.

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