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Genetics

Nucleotide sequence of a bean (*Phaseolus vulgaris*) U1 small nuclear RNA gene: Implications for plant pre-mRNA splicing

(RNA processing/ribonucleoprotein complexes)

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ABSTRACT **We have isolated a U1 small nuclear RNA (snRNA) gene from common bean (*Phaseolus vulgaris*). The haploid bean genome contains only one or a few copies of the U1 snRNA gene. The bean and human U1 snRNA genes are 65% homologous but share no significant similarity in the 5′ or 3′ flanking regions. The predicted secondary structure of bean U1 snRNA is identical to that of other U1 snRNAs, and the sequences of the single-stranded regions have been highly conserved. Thus, both the sequence of the single-stranded regions and the secondary structure of U1 snRNA appear to be important for its function. The role of U1 snRNA in pre-mRNA splicing is probably similar in plants and animals.**

Most higher eukaryotic protein-encoding genes are interrupted by intervening sequences that are excised from primary transcripts (pre-mRNAs) by RNA splicing. There has been much recent progress toward understanding the mechanism by which animal pre-mRNAs are spliced (reviewed in refs. 1–3); however, little is known about the mechanism of pre-mRNA splicing in higher plants. The nucleotide sequences surrounding 5′ and 3′ splice sites are quite similar in plant and animal genes (4, 5). In fact, plant pre-mRNAs can be spliced in animal cells and in human nuclear extracts (6–8). However, plant cells appear to be unable to splice human pre-mRNAs (8, 9) and no specific features of the mechanism of pre-mRNA splicing in plants have yet been elucidated.

At least four small nuclear ribonucleoprotein complexes (snRNPs), U1 snRNP, U2 snRNP, U5 snRNP, and U4/U6 snRNP, are involved in animal pre-mRNA splicing (reviewed in refs. 1–3). The 5′ end of U1 small nuclear RNA (snRNA) in U1 snRNP binds to the 5′ splice site region, and U2 snRNP binds to the "branch site" region in the intervening sequence, where the 5′ end of the intervening sequence becomes covalently attached during the splicing process. U1 and U2 snRNAs have been identified in several plant species (10–12). U1 and U2 snRNAs in both plants and animals have a trimethylguanosine cap at their 5′ termini (10, 11, 13). Direct nucleotide sequencing of the 3′ portion of plant U1 snRNAs has shown that they are similar to animal U1 snRNAs in this region (ref. 10; F. Solymosy, personal communication). However, the available sequences are quite limited in extent.

To determine whether U1 snRNP might be involved in pre-mRNA splicing in plants, we isolated a U1 snRNA gene from common bean (*Phaseolus vulgaris*) and determined its nucleotide sequence.† The sequences known to be essential for human U1 snRNA function are identical in bean and animal U1 snRNAs. In addition, other segments of U1 snRNA have been highly conserved between plants and animals, suggesting that they are also important for U1 snRNA function. Furthermore, the predicted secondary structures of bean and animal U1 snRNAs are identical. These data strongly suggest that U1 snRNA plays a similar role in pre-mRNA splicing in plants and animals.

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MATERIALS AND METHODS

Molecular Cloning. A *P. vulgaris* recombinant library was screened by hybridization to a 0.6-kilobase (kb) *Hin*dIII fragment containing the human U1 snRNA gene (14) in 6× SSC (1× SSC = 0.15 M NaCl/0.015 M trisodium citrate, pH 7–8) at 68°C without carrier DNA. Filters were washed sequentially with 3× SSC at 37°C and 0.1× SSC at room temperature. One of several hybridizing phage, PhvU1-5, was analyzed. A single 2.6-kb *Hin*dIII fragment of PhvU1-5 that hybridized to the human U1 probe was subcloned in pBR328 (pPhvU1) for subsequent analyses.

Southern Blot Analysis. *P. vulgaris* genomic DNA was analyzed according to Southern (15). Filters were hybridized in 3× SSC at 68°C with 50 μg of *Escherichia coli* DNA per ml as carrier to bean or human U1 snRNA gene probes. The ^{32}P-labeled probes were prepared by nick-translation of a 310-base-pair (bp) *Ssp* I/*Eco*RV fragment containing the bean U1 snRNA gene (plus 59 and 90 bp, respectively, of 5′ and 3′ DNA) or a 198-bp *Bgl* II/*Rsa* I fragment containing the human U1 snRNA gene (plus 6 and 28 bp, respectively, of 5′ and 3′ flanking DNA). Filters hybridized to the bean probe were washed at 68°C sequentially in 3× SSC and 0.1× SSC, whereas those hybridized to the human probe were washed at 68°C in 3× SSC only.

Mapping of U1 snRNA 5′ and 3′ Termini. S1 nuclease analyses were performed as described (16) using total *P. vulgaris* RNA and 5′-end-labeled 700-nucleotide (nt) *Rsa* I/*Eco*O 109 or 3′-end-labeled 260-nt *Bst*NI/*Eco*RI fragments from pPhvU1 as probes. To provide an accurate indication of molecular size, the latter probe was subjected to partial DNA sequence analysis (17).

DNA Sequence Analysis. DNA fragments from pPhvU1 were subcloned into M13mp18 or M13mp19 (18) and their DNA sequences determined (19).

RESULTS

Structure and Number of *Phaseolus* U1 snRNA Genes. *Phaseolus* U1 recombinant phage PhvU1-5 contained ≈14 kb of bean DNA, including a 2.6-kb *Hin*dIII fragment to which the human U1 snRNA gene hybridized. A partial restriction map of this 2.6-kb *Hin*dIII fragment is illustrated in Fig. 1.

We analyzed genomic *Phaseolus* DNA by Southern blotting (15) using as probe a 310-bp nick-translated *Ssp* I/*Eco*RV fragment containing the bean U1 snRNA gene. To avoid hybridization between the probe and U1 snRNA genes in

Abbreviations: snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein complex; nt, nucleotide(s).

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†This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03563).

carrier DNA, *E. coli* DNA was used as carrier. As shown in Fig. 2, in each digest the probe hybridized strongly to one genomic DNA fragment. The genomic *Hin*dIII fragment identified corresponds to the 2.6-kb *Hin*dIII fragment containing the U1 snRNA gene in bacteriophage PhvU1-5. The probe also hybridized to the single expected 1.6-kb fragment in an *Xba* I plus *Eco*RV digest of bean genomic DNA (data not shown). Two hybridizing *Sau*3AI fragments are evident in Fig. 2 because of a *Sau*3AI site within the U1 snRNA gene. Thus, pPhvU1-5 corresponds to the strongly hybridizing bands in Fig. 2.

To estimate the number of copies of the U1 gene in *Phaseolus* DNA, we included linearized pPhvU1 DNA corresponding to 1, 5, 10, and 20 copies of the U1 snRNA gene per diploid genome (Fig. 2). Although the accuracy of such reconstructions is only approximate, there appear to be two to four copies of the U1 snRNA gene per diploid bean genome. Only the single U1 snRNA gene was present in the 14 kb of bean DNA contained in pPhvU1-5 (data not shown). Therefore, either there is only one U1 snRNA gene per haploid bean genome or there are a few copies with a duplication unit larger than 14 kb that includes at least the ≈10-kb genomic *Pst* I fragment (Fig. 2).

The bean U1 probe also hybridized very weakly to several other fragments in *Phaseolus* DNA. To characterize these fragments, we performed Southern blot analysis of bean DNA using as probe a fragment of the human U1 snRNA gene containing virtually only the transcribed region (data not shown). The human U1 snRNA gene hybridized strongly to the major *Hin*dIII, *Eco*RI, and *Xba* I/*Eco*RV fragments identified by the bean U1 snRNA gene probe. Some, but not all, of the minor fragments also hybridized very weakly to the human U1 snRNA gene probe. Because these minor fragments hybridize so weakly to the very short bean (310 bp) and human (198 bp) U1 snRNA gene probes used, they must be quite different from the U1 snRNA gene and are unlikely to even be U1 snRNA pseudogenes. It seems probable that this hybridization is nonspecific, possibly resulting from G+C-rich regions within the bean and human U1 snRNA genes.

5′ and 3′ Termini of *Phaseolus* U1 snRNA. The 5′ terminus of *Phaseolus* U1 snRNA was defined by S1 nuclease mapping using a 5′-end-labeled 700-nt *Rsa* I/*Eco*O 109 fragment from pPhvU1 as probe. As shown in Fig. 3*A*, bean RNA protected a 140-nt fragment of this probe, consistent with a 5′ terminus of bean U1 snRNA identical to that of all other U1 snRNAs.

The 3′ terminus of bean U1 RNA was mapped using a 3′-end-labeled 260-nt *Bst*NI/*Eco*RI fragment from pPhvU1 as probe. As shown in Fig. 3*B*, bean RNA protected an ≈147-nt fragment of this probe, consistent with a total length of bean U1 snRNA of ≈156 nt. However, because of secondary structure in this region, we were unable to define the 3′ terminus of bean U1 snRNA precisely.

Nucleotide Sequence of the *Phaseolus* U1 snRNA Gene and Predicted Secondary Structure of Bean U1 snRNA. The DNA sequence of a 605-bp segment that includes the bean U1 snRNA gene is shown in Fig. 4. The 5′ and 3′ termini of the RNA were assigned by S1 nuclease mapping described above and comparison with other U1 snRNAs (see below). The predicted secondary structure of bean U1 snRNA, based on the sequence of bean U1 snRNA and the secondary structure of other U1 snRNAs (21, 22), is shown in Fig. 5. The predicted structure includes four stem–loop structures, a single-stranded 5′ region, and a single-stranded region between stems III and IV, and is identical to the experimentally determined secondary structures of chicken, rat, and human U1 snRNAs (21) and that predicted for *Drosophila* U1 snRNA (22). The sequences of the single-stranded 5′ end and the "domain A" Sm antigen binding site (23–25) are identical among bean and all animal U1 snRNAs (refs. 22 and 26–28; see Fig. 6). The human and bean U1 snRNA genes are 65% homologous; however, there is no significant sequence homology in the 5′ and 3′ flanking regions. In particular, there are no sequences flanking the bean U1 gene that are similar to the vertebrate snRNA transcriptional enhancer (29–31), the "−50" promoter element (32, 33), or a downstream sequence required for formation of 3′ ends of some snRNAs and histone mRNAs (34–36).

DISCUSSION

We have isolated a U1 snRNA gene from the common bean *P. vulgaris* and have determined its nucleotide sequence. We have not yet been able to demonstrate expression of this gene in transgenic plants. We find that the haploid bean genome most probably contains only one, or at most a few, copies of the U1 snRNA gene. Therefore, it is probable (but not certain) that the U1 snRNA gene that we isolated is an expressed gene. The small number of U1 snRNA genes in the

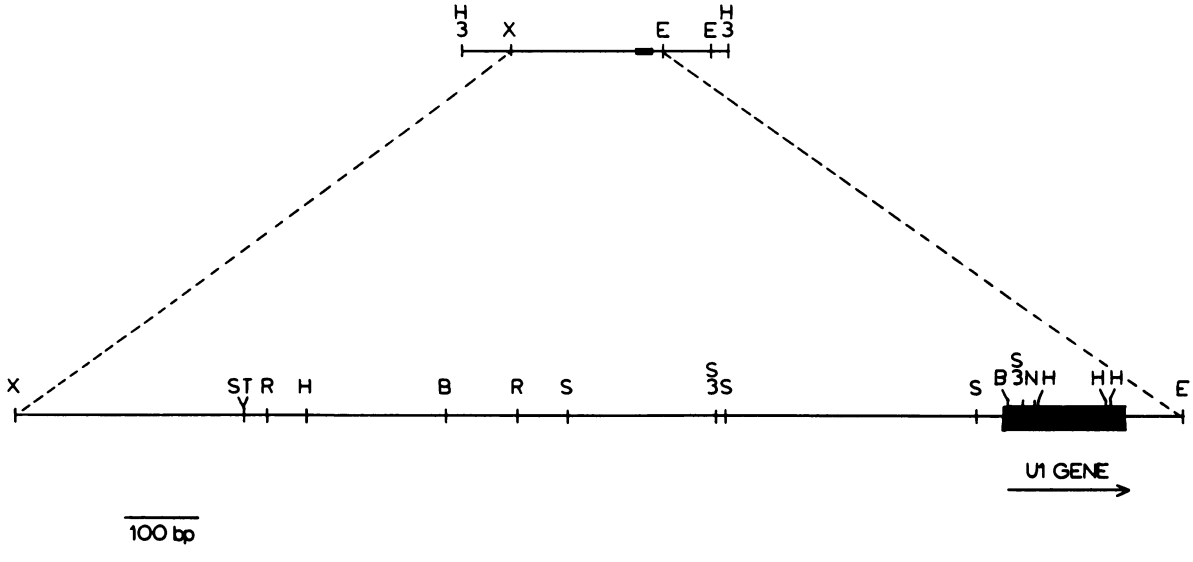

FIG. 1. Restriction map of the bean U1 snRNA gene region. The 2.6-kb *Hin*dIII and 1.6-kb *Xba* I/*Eco*RV fragments were mapped by Southern blotting (15) and partial restriction endonuclease digestion (20). E, *Eco*RV; B, *Bst*NI; H, *Hae* III; H3, *Hin*dIII; N, *Nco* I; R, *Rsa* I; S, *Ssp* I; S3, *Sau*3AI; X, *Xba* I.

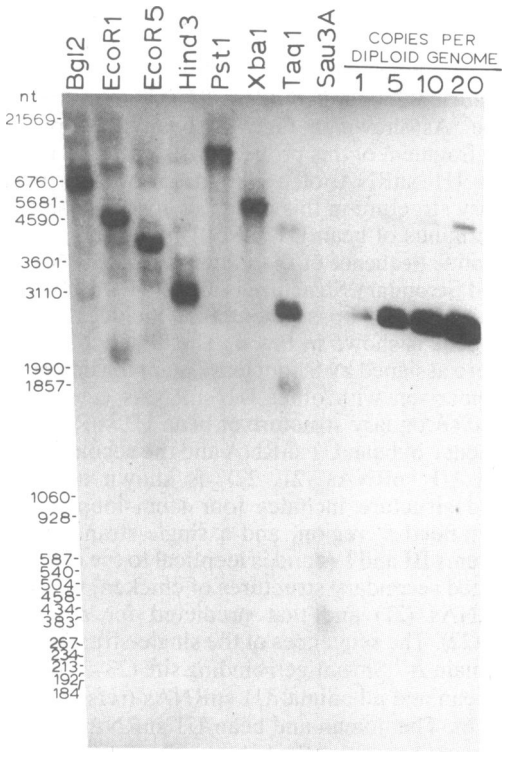

FIG. 2. Southern blot analysis of bean genomic DNA. *P. vulgaris* DNA was isolated from embryo tissue and 10-μg aliquots were cleaved with the restriction endonucleases indicated, electrophoresed in a 0.8% agarose gel, and transferred to nitrocellulose (15). The hybridization probe was the bean U1 snRNA gene fragment described in the text. Lanes 1, 5, 10, and 20, pPhvU1 plasmid DNA corresponding to 1, 5, 10, and 20 copies, respectively, of the U1 snRNA gene per diploid genome, assuming 4 pg per bean diploid genome.

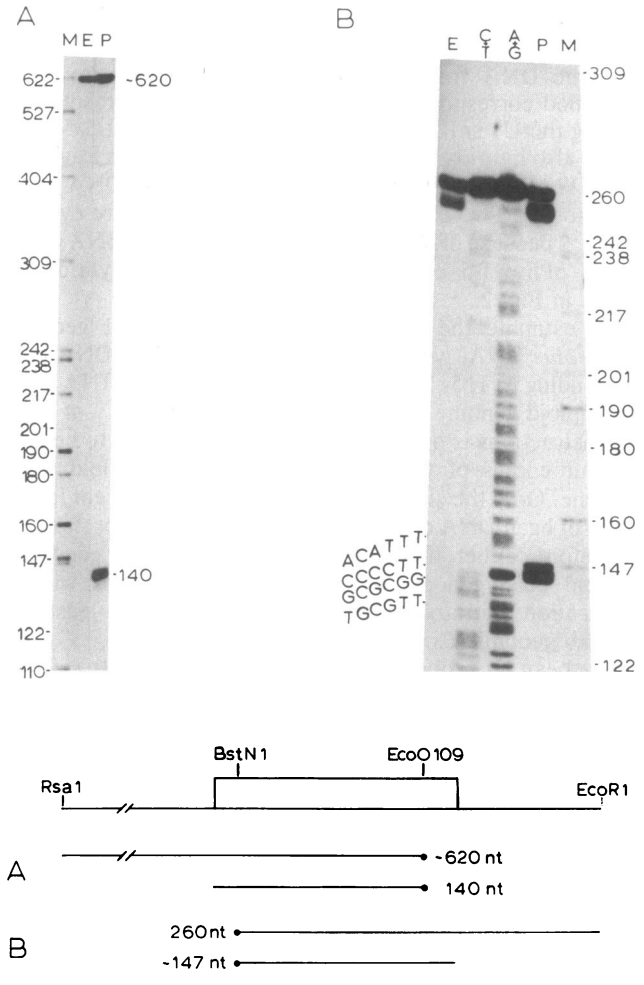

FIG. 3. S1 nuclease mapping of *Phaseolus* U1 snRNA 5′ and 3′ termini. S1 nuclease probes are described in the text. (*A*) U1 snRNA 5′ terminus. Lanes: M, molecular size standard [from *Msp* I digestion of pBR322; sizes (in nucleotides) at left]; E, *E. coli* RNA; P, *Phaseolus* RNA. (*B*) U1 snRNA 3′ terminus. Lanes are the same as in *A*, except C+T and A+G are pyrimidine and purine Maxam and Gilbert (17) sequencing ladders, respectively, of probe. Note that although the "antisense" strand of the probe was sequenced, the sequence of the "sense" strand is displayed.

bean genome differs from the human genome, which contains at least 30 true U1 snRNA genes plus numerous pseudogenes (14) arranged both within a large multicopy repeated domain and as unique gene copies (37). (The unique or low number of copies of the U1 snRNA gene in common bean is not universal in plants; the soybean genome contains a large number of U1 snRNA genes; unpublished data.)

Bean and human U1 snRNA genes are 65% homologous. As shown in Fig. 6, most base differences among bean and other U1 snRNAs occur in base-paired "stem" regions, with compensatory changes such that the overall secondary structures of the various U1 snRNAs are essentially identical (Fig. 5; refs. 21 and 22). Furthermore, single-stranded regions known to be essential for the function of human U1 snRNAs are completely conserved in bean U1 snRNA. These include the 5′ end, which base pairs with 5′ splice sites (38, 39), and the "Sm binding site," necessary for assembly of the snRNA into a snRNP (23–25). As shown in Fig. 6, the four single-stranded loop regions are also highly conserved. Loop I has the most differences, with bean U1 snRNA differing at three consecutive bases from all animal species shown. Loop II is highly conserved among bean U1 snRNA and animal U1 snRNAs. Furthermore, U1 loop II (5′ AUUGCACU 3′) is identical at seven of eight bases to part of loop IV of U2 snRNAs (secondary structure as shown in ref. 40) from animals (5′ AUUGCAGU 3′; refs. 41–45) and plants (5′ GUUGCACU; refs. 10–12 and 46). This sequence might therefore interact with proteins common to U1 and U2 snRNPs. Bean U1 snRNA loop II (5′ AUUGCACU 3′) is also complementary to a single-stranded region (5′ AGUGUAGU 3′) in loop I (secondary structure model as shown in ref. 40) or between loops I and II (secondary structure model of Keller and Noon, ref. 47) of human and broad bean (46) U2 snRNAs and thus might be involved in interactions between U1 and U2 snRNAs during pre-mRNA splicing.

Most U1 loop III sequences conform to the consensus CAA$\overset{A}{G}$UG, although this conservation is obscured by the alignment shown in Fig. 6. In plants, U1 loop III (5′ CAAGU 3′) is complementary to loop III (5′ GCUUG 3′) in U2 snRNAs of animals (41–45) and plants (10, 12, 46). These sequences might therefore also be involved in interaction between U1 and U2 snRNAs. However, each of the animal U1 snRNAs shown in Fig. 6 differs from plant U1 snRNAs at one or more bases in loop III, making the U1 and U2 snRNA loop III sequences less complementary in animals.

Loop IV is also highly conserved among plant and animal U1 snRNAs. Although U1 snRNA loop IV is only 4 bases long, 12 consecutive bases in loop IV and stem IV are conserved among plant and animal U1 snRNAs, whereas the nucleotide sequences of the other stems are not. This suggests that some of the bases in stem IV may have a function other than maintenance of U1 snRNA secondary structure.

There is no significant homology between the bean and animal U1 sequences 5′ and 3′ to the U1 snRNA genes, and

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GATCTTAGAATATTGTGATGGCAGTTTGCAGCCACTAATTCGCAGAATGCAGCAGGTTTAGTTCAAGGTCTTGTTATGGACATGTGAACTCAACTAGTAT 100

GGGATGGTCATGTTTTTTTTGACATTCAATTCAATAACTAGTGTATTAATGAAACGCTGATTCTTGAAGGCAAATTCTTTTTACTTTGAGTATAGTTTTA 200

CCGAATCCTTTTTCTTTAATTTAAACCAAGCACTCAACGGCTTTGAAACCATTCTATGTTAGCCTTCAAAAACTAAATCCCACATTGAAAATATTTCTTA 300

TAGAGTCATGGAAAATAGACTACAAATATAAGATTTGTCACCCTGAGTTCCATACTTACCTGGACGGGGTCAATGGATGATCTATAAGGTCCATGGCCTA 400
                                                    5'

GGGAAGTGACCTTCATTGCACTCAGAAGGGGTGCTACTCTAAGGTCTGTCCAAGTGATGGAGCCTACGTCATAATTTGTGGTAGTGGGGGCCTGCGTTCG 500

CGCGGCCCCTTACATTTTAGTCAATTGTTGTTTGACTTTTGTATTTCATTGTCATCTGTATATATCTTCCTAATGGGATTTCTATTAGCCATATAATGAG 600
          3'

ATATC 605
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FIG. 4. Nucleotide sequence of the bean U1 snRNA gene region. The sequence shown extends from the *Sau*3AI site 5′ of the gene to the *Eco*RV site 3′ of the gene (see Fig. 1). The U1 snRNA gene is underlined. The 3′ terminus of bean U1 snRNA is uncertain.

we have not yet experimentally identified the sequences required for transcription initiation, termination, and processing of bean U1 snRNA. However, the sequences surrounding the bean U1 snRNA gene exhibit considerable homology to those surrounding two cloned U1 snRNA genes from soybean (unpublished data). The promoters of mammalian and *Xenopus* U1 snRNA genes consist of at least two parts, a "proximal element" ≈50 bp 5′ of the 5′ terminus of the RNA, and an enhancer-like element 230–280 bp upstream of the 5′ terminus (29–33, 48). The conserved enhancer-like element consists of a G+C-rich region similar to the SP1 transcription factor binding site (49), followed by an octomer (ATGCAAAT) found in other enhancer elements (50–52). However, no sequences similar to either the mammalian or the *Xenopus* proximal promoter element or to the entire vertebrate snRNA enhancer occur upstream of the bean U1 snRNA gene, although sequences similar to either the G+C-rich region or the conserved octomer of the vertebrate snRNA enhancer are present. It remains to be determined whether these sequences have a role in transcription of the bean U1 snRNA gene.

Human U1 snRNA is apparently synthesized as a U1 snRNA precursor from which several nucleotides are removed from the 3′ end to generate mature U1 snRNA (53). The 12 bases 15–26 nt downstream of the mature U1 snRNA 3′ terminus are required for biosynthesis of mature human pre-U1 snRNA, either by termination of transcription or by RNA processing (34–36). A similar sequence occurs downstream of other animal U1 and U2 snRNA genes (37) and is similar to those required for 3′ end formation of histone

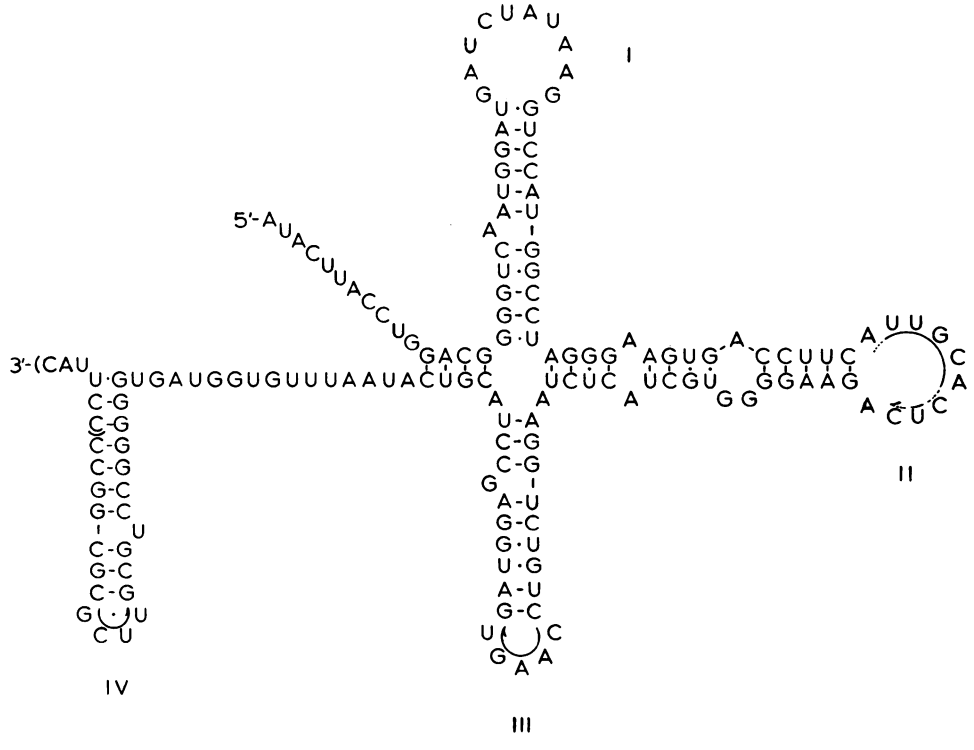

FIG. 5. Predicted secondary structure of bean U1 snRNA. Roman numerals denote stem–loop structures. Lines in the loops indicate bases that are conserved among U1 snRNAs. Dotted lines indicate partial conservation. Parentheses indicate uncertainty regarding the location of the 3′ terminus.

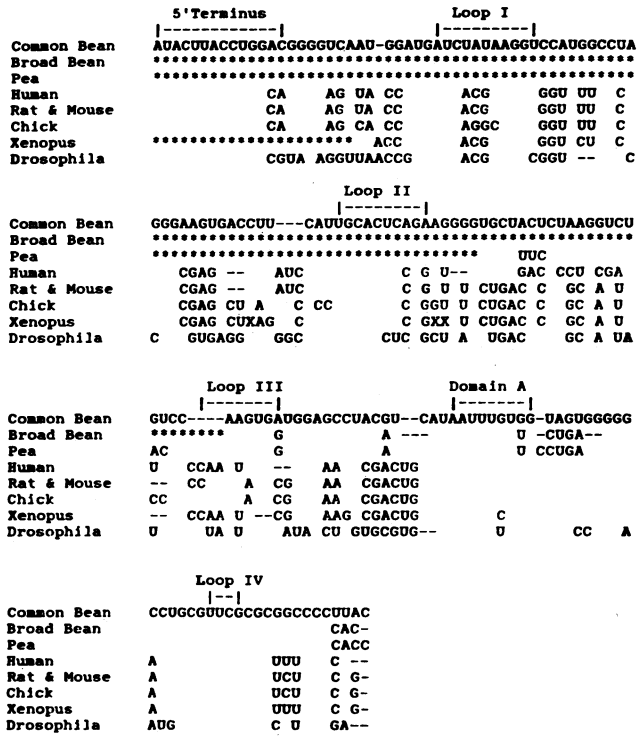

FIG. 6. Comparison of U1 snRNA sequences. Only bases that differ from common bean U1 snRNA are indicated for the other U1 snRNAs. Dashes indicate gaps introduced to maximize homology. Asterisks indicate sequences not determined. References for the sequences are as follows: broad bean, F. Solymosy (personal communication); pea, ref. 10; human, ref. 26; rat, ref. 26; mouse, ref. 27; chicken, ref. 26; *Xenopus*, ref. 28; *Drosophila*, ref. 22.

mRNAs. However, this sequence does not occur 3′ to the bean U1 snRNA gene.

The remarkable conservation of secondary structure and sequences of single-stranded regions of U1 snRNAs between plants and animals suggests that these elements are crucial for the function of U1 snRNA and also that U1 snRNPs play a similar role in pre-mRNA splicing in plant and animal cells. As shown in Fig. 5, the 5′ terminus of bean U1 snRNA is apparently single stranded, and pea U1 snRNA has a trimethylguanosine 5′ cap that is exposed in snRNP (10). These data suggest that plant U1 snRNA 5′ termini are available for base-pairing with 5′ splice sites. U1 snRNP and other snRNPs from a wide range of animal species are precipitated by human anti-Sm autoantisera (38). Plant U1 snRNAs contain putative Sm antigen binding sites (24, 25), and fungal, yeast, and plant snRNPs can be immunoprecipitated by both human anti-Sm and anti-(U1) RNP autoimmune antisera (54). Thus, both the RNA and proteins of U1 snRNPs appear to be highly conserved.

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