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Functional, developmentally expressed genes for mouse U1a and U1b snRNAs contain both conserved and non-conserved transcription signals

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ABSTRACT

Four genes that encode mouse U1a1, U1b2 and U1b6 snRNAs have been isolated from a mouse genomic DNA library. They all appear to be functional U1 genes since they are accurately transcribed into full length, capped snRNAs upon injection into Xenopus oocytes. A mouse pseudogene that is not transcribed in Xenopus oocytes was also isolated from the mouse genomic library. DNA sequence analysis of the 5' and 3' flanking regions of the functional genes revealed the presence of three highly conserved sequence elements that have been shown to be required for transcription initiation or 3' end formation in other U1 genes. Each of these U1 RNA genes also contains non-conserved sequences in the 5' flanking region that could function in their controlled expression during development.

INTRODUCTION

U1 small nuclear RNAs (U1 RNAs) are very abundant in eucaryotic cell nuclei (reviewed in references 1 and 2) where they are components of nuclear RNP particles that participate in the splicing of messenger RNA precursors (3-7). In mice there are two types of U1 RNA called U1a and U1b RNA (3). Moreover, two variants of U1a RNA and six variants of U1b RNA have been described (8,9).

The genes encoding U1b RNAs in mice are subject to developmental control (8). U1a RNAs are synthesized in all tissues but U1b RNAs are synthesized only in fetal tissues and in adult tissues such as thymus, spleen and testis, that retain significant numbers of undifferentiated stem cells (8). Furthermore, U1b genes are transcribed in relatively undifferentiated, malignant cell lines like teratocarcinoma, lymphoma, fibrosarcoma and Friend cells, but not in cell lines like cultured mouse kidney cells or C127 and 3T3 cells that retain differentiated phenotypes (8-10). Consequently, U1b RNA is considered an "embryonic" RNA that may be synthesized only in undifferentiated cells.

The function of the developmental control of U1 gene expression is unknown. As discussed elsewhere, sequence differences between U1a and U1b RNAs could allow the formation of an alternative stem loop structure that

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might alter RNP formation and function (8,9) in splicing (reviewed in reference 11). Thus, differential accumulation of U1a and U1b snRNPs could influence the pattern of gene expression during development (8).

The mechanism of developmental control of U1 gene expression is unknown. Sequences in the 5' flanking regions of the Xenopus embryonic xU1b gene are responsible for the preferential expression of these genes in certain tissues (12; E. Lund, C. J. Bostock and J. E. Dahlberg, submitted). Similar sequences in the promoters for mouse U1b genes might play a role in their developmental expression.

We are studying mouse U1 genes in order to identify mechanisms for their developmental expression. In this report we present the structures of functional genes for mouse U1a1, U1b2 and U1b6 RNA. Each gene contains highly conserved DNA sequence elements in its 5' and 3' flanking region. Similar sequences occur in the flanking regions of other mammalian, avian and amphibian snRNA genes and have been shown to function as promoter and maturation elements for the synthesis of U1 RNA (12-23). In addition, each of the mouse U1 genes contains sequences (including GC boxes, CAAT boxes and direct and inverted repeats) in its 5' flanking region that could function as additional promoter elements. We discuss these findings with respect to their possible role in the developmental expression of mouse U1 genes.

MATERIALS AND METHODS

Isolation and Sequence Anaysis of Mouse U1a and U1b Genes

The protocol for the isolation of mouse U1 coding sequences has been described in detail elsewhere (24). Smaller DNA fragments were subcloned into pAT153 and subsequently into the M13 vectors mp8 and mp9 (25).

The cloned DNA was sequenced using the dideoxy chain termination method (26). The mouse U1a pseudogene in clone pU1-ψ325 was sequenced using the chemical cleavage method of Maxam and Gilbert (27).

Transcription Assays and Analysis of RNA

Presumptive U1 genes were tested for template activity by injection of supercoiled U1 DNA into stage VI *X. laevis* oocytes in the presence of [γ-^{32}P-GTP] as previously described (15,28). Total nucleic acids were extracted from pooled oocytes after 20 hours of incubation at 18°C and analyzed by electrophoresis in 12% (30:0.8) polyacrylamide gels that contained 7 M urea (12). In some experiments, putative mouse U1a and U1b RNAs were eluted from the 12% polyacrylamide gels and further purified by electrophoresis in 15% (19:1) non-denaturing polyacrylamide gels (10). Individual mouse

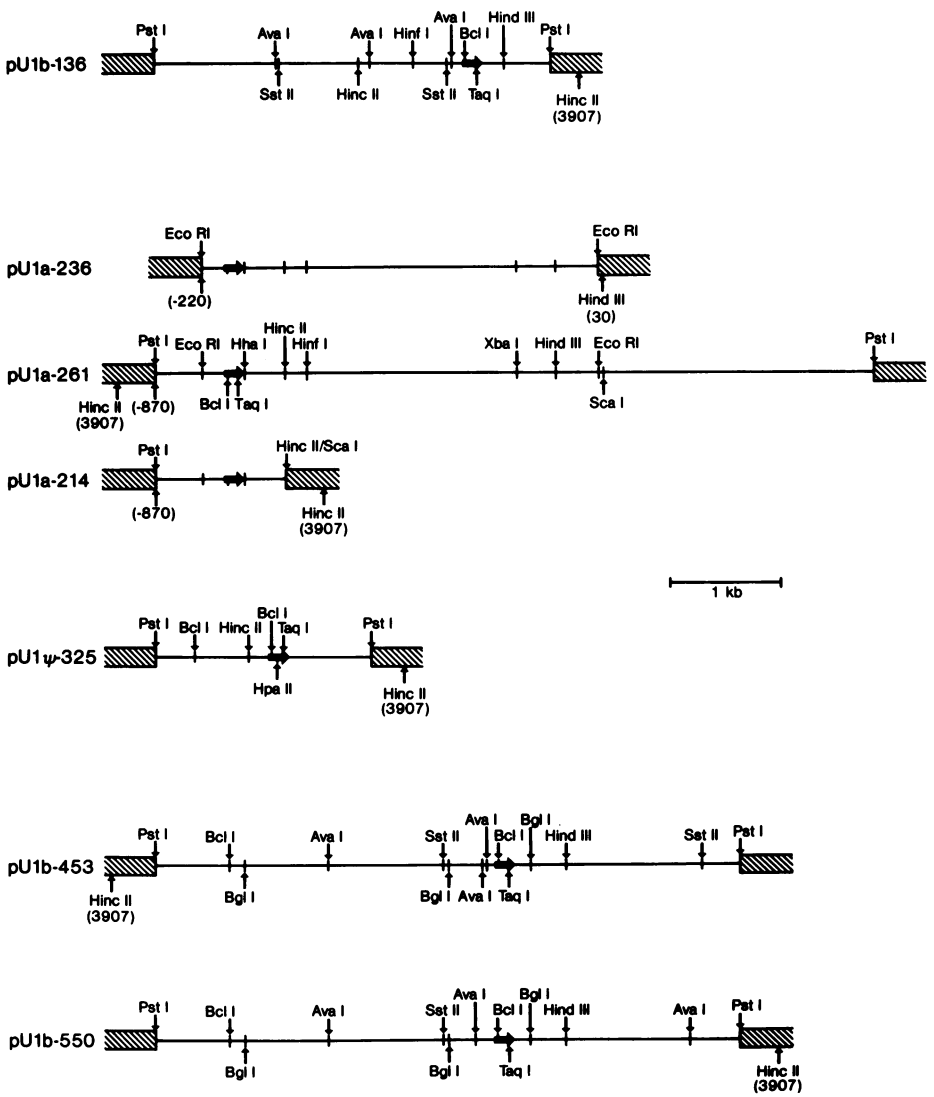
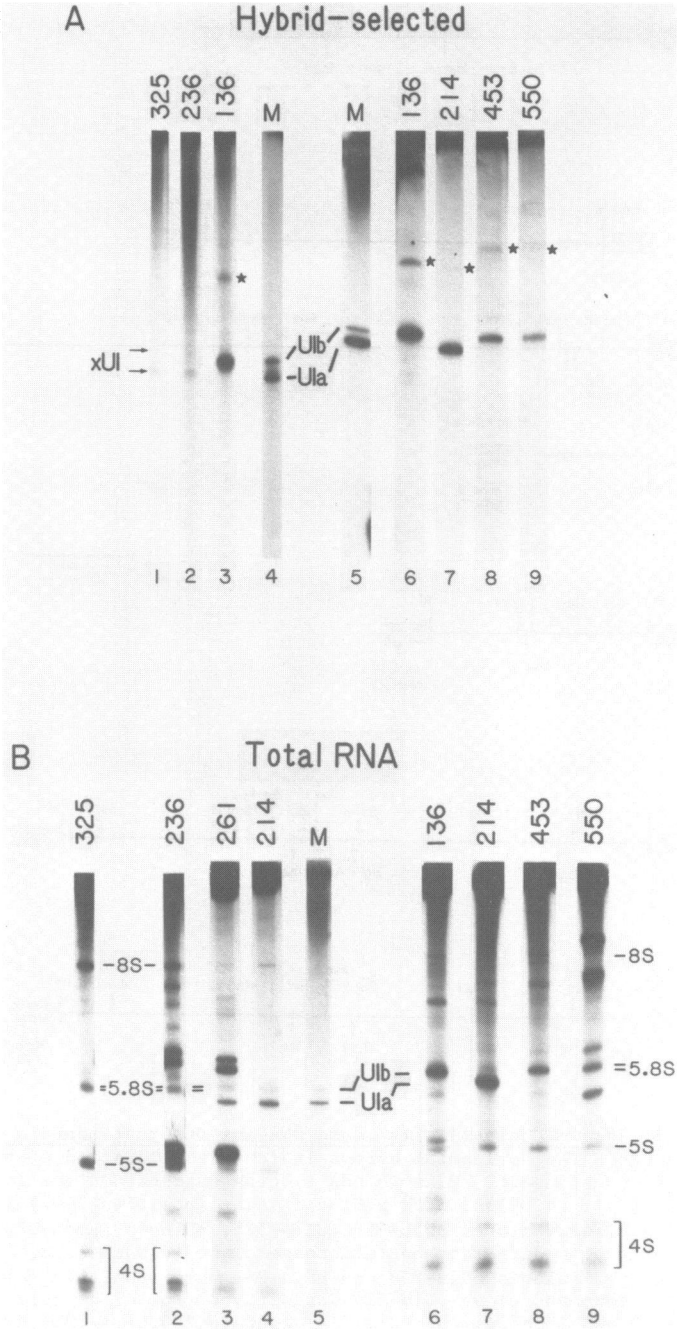


Figure 1: Plasmid clones with mouse DNA inserts that contain U1 snRNA coding sequences. The horizontal arrows indicate the location of the single U1 sequence in each insert. Plasmid DNA is crosshatched and the location of the Hind III (position 30) site or a Hinc II site (position 3907) in pAT153 is indicated. Plasmid clones pU1b-136, pU1ψ-325, pU1b-453 and pU1b-550 were each subcloned from a different bacteriophage clone (clones 1, 3, 4, and 5 respectively; 24). Clones pU1a-236 and pU1a-261 were subcloned from the same bacteriophage clone and clone pU1a-214 was derived from clone pU1a-261. Clones pU1a-236 and pU1a-261 (or pU1a-214) contain 220 bp (-220) or 870 bp (-870) of mouse DNA upstream from the U1 coding sequence, respectively.


A
Hybrid–selected
325
236
136
M
M
136
214
453
550
xUl
Ulb
Ula
1 2 3 4 5 6 7 8 9
B
Total RNA
325
236
261
214
M
136
214
453
550
-8S-
=5.8S=
-5S-
4S
Ulb
Ula
-8S
=5.8S
-5S
4S
1 2 3 4 5 6 7 8 9

Ula and Ulb RNAs were eluted from the 15% gels and analyzed by RNase T1 fingerprinting (29) using homomix C (30).

Purified Ula and Ulb RNAs were also prepared by hybrid selection (28) followed by one-dimensional polyacrylamide gel electrophoresis. The gel-purified snRNAs were characterized by RNAse T1 fingerprinting.

RESULTS

Cloned Restriction Enzyme Subfragments of Mouse DNA that Contain Sequences Homologous to U1 snRNA

When a mouse genomic library in lambda bacteriophage Charon 28 was screened with a cDNA probe derived from chicken U1 RNA, five clones were isolated (24). The approximately 15 kb of mouse DNA insert in each phage clone contained only one U1-type coding sequence.

Plasmid subclones pUlb-136, pU1ψ-325, pUlb-453 and pUlb-550, derived from bacteriophage clones number 1, 3, 4 and 5 respectively, contain U1-specific restriction enzyme subfragments of the phage clone inserts (Figure 1). The three subclones designated pUla-236, pUla-261 and pUla-214 were all derived from bacteriophage clone number 2 and contained the same U1 sequence. The plasmid subclones depicted in Figure 1 are referred to below as clones 136, 236, 261, 214, 325, 453 and 550.

Transcription Assays in Xenopus Oocytes

U1 Gene Transcripts. To determine which constructs contained functional U1 genes, they were microinjected into the nuclei of X. laevis oocytes together with α-^{32}P-GTP (Methods). After 20 hours of incubation, total RNA was extracted from pooled oocytes and labeled U1 RNAs were identified by polyacrylamide gel electrophoresis of hybrid selected U1 RNAs (Figure 2A) or

Figure 2: Synthesis of mouse U1 snRNA in Xenopus laevis oocytes. (A) Electrophoretic analysis of hybrid selected mouse U1 RNAs that were synthesized in Xenopus oocytes. Total RNA was extracted from oocytes that had been microinjected with clones 325, 236, 136, 453, or 550. U1 RNAs were purified by hybrid selection using a human U1 gene immobilized on nitrocellulose and were analyzed by electrophoresis in a 12% denaturing polyacrylamide gel. Marker mouse RNA (M) was purified by hybrid selection from F9 cell RNA (lane 4) or L cell RNA (lane 5); the mobilities of endogenous X. laevis U1 RNAs are indicated (xU1). The samples in lanes 1-4 and 5-9 were electrophoresed in separate gels. The U1-related RNAs (stars) are molecules with 5' or 3' extensions that probably accumulated as a result of the heterologous nature of the transcription system (28). (B) Total RNAs from oocytes that had been microinjected with DNA of mouse U1 clones. Samples in lanes 1, 2, lanes 3-5 and lanes 6-9 were electrophoresed in separate gels. Samples in lanes 4 and 7 were from two independent injections and are included twice for comparisons with neighboring samples. M (lane 5) corresponds to hybrid selected mouse L cell U1 RNAs.

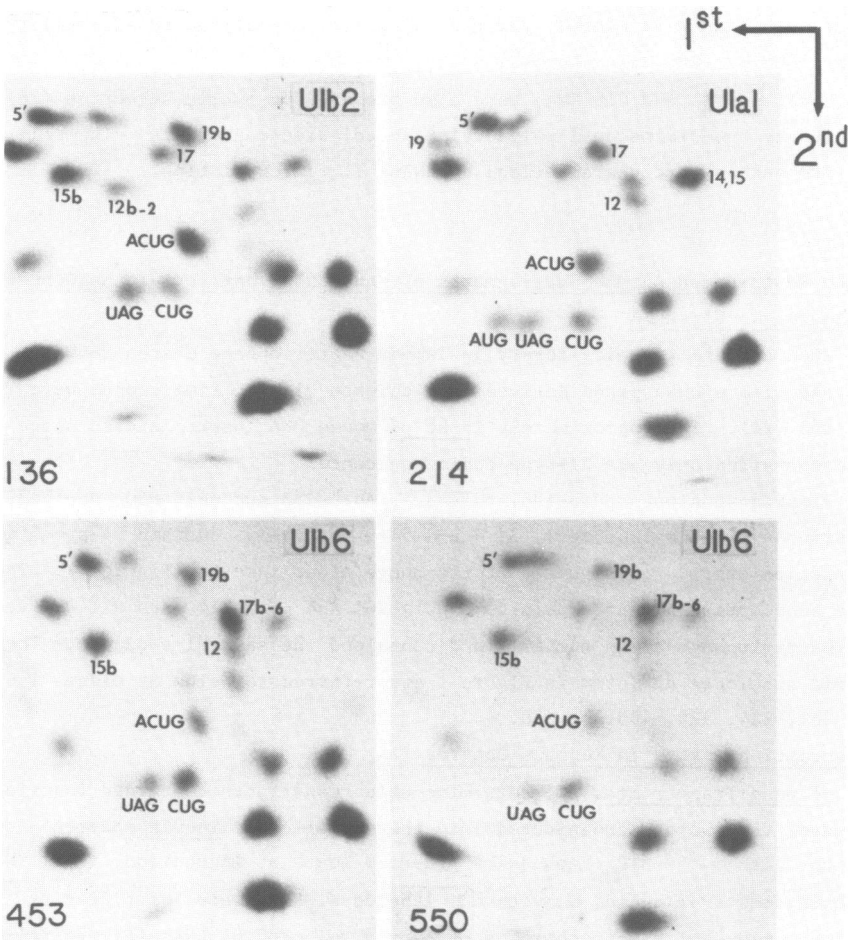


Figure 3: RNAse T1 fingerprint analysis of mouse U1 RNAs synthesized in X. laevis oocytes. Panel 214: U1a1 RNA transcribed from clone 214. A U1a1-specific oligonucleotide (AUG) is present. 5' denotes the capped 5' terminus of U1a1 RNA (m3GPPPAmUmCΨΨACCUG). Oligonucleotide 17 is derived from the 3' terminus of U1a1 RNA and retains a phosphate residue at the 3' end (CUCUCCCCUGp). This phosphorylated overlap nucleotide has also been observed in transcripts of human U1 genes in Xenopus oocytes (15), resulting from cleavage of a primary transcript that extends slightly beyond the 3' terminus of the mature coding sequence. Panels 136, 453 and 550: U1b RNAs transcribed from clones 136, 453 or 550. In all three fingerprints U1b-specific oligonucleotides 15b (CACUUUG) and 19b (CUCACCCAUUG) are present and the U1a1-specific oligonucleotide AUG is absent (8,9). The 5' oligonucleotide for the U1b RNAs is identical to that of U1a1 RNA. The fingerprint in panel 136 contains two U1b2-specific features: oligonucleotide 12b-2 (AUCAUG) and a 2:1 molar ratio of ACUG to CUG (9). In panels 453 and 550 the fingerprints contain two U1b6-specific features: oligonucleotide 17b-6 (CCCCCCUGp) and an ACUG to CUG molar ratio of 1:2 (9).

total RNAs (Figure 2B). RNAs that comigrated in gels with mouse U1 RNA were recovered from oocytes that had been injected with clones 136, 214, 261, 453 or 550. Two clones, 325 and 236, did not support U1 RNA accumulation (Figure 2A, lanes 1 and 2; Figure 2B, lanes 1 and 2). As discussed below, 325 is a pseudogene, so the lack of transcription is not surprising. However, 236 is a subclone of the same gene that gave rise to two active subclones, 214 and 261 (cf lane 7, Figure 2A and lanes 3, 4 and 7, Figure 2B). Since these transcriptionally active subclones have 870 bp of upstream flanking region sequences, whereas clone 236 has only 220 bp, the lack of transcription of clone 236 appears to be due to the absence of one or more essential promoter sequence elements located upstream from position -220.

Fingerprint Analysis of Mouse U1 snRNAs Transcribed In Vivo. The U1-type snRNAs encoded by the plasmid clones were isolated by elution from polyacrylamide gels and analyzed by RNase T1 fingerprinting (Figure 3). Comparison of our data to the published sequences of mouse U1a and U1b RNA variants (8,9) reveals that the RNA transcribed from clone 214 (or 261) is U1a1, the RNA transcribed from clone 136 is U1b2 and the RNAs transcribed from both clones 453 and 500 are U1b6. Since each U1 snRNA variant was full length and contained the predicted 5' and 3' termini (see legend to figure 3), we conclude that each mouse U1-type gene was accurately transcribed in Xenopus oocytes.

Other transcription units. Analyses of total radiolabeled RNAs from injected oocytes revealed the presence of active transcription units in addition to the functional U1 genes in some of the clones. The RNA products of these additional transcription units appeared as predominant bands in the total RNAs from oocytes injected with clone 136, 236, 261 or 550, whereas no additional RNA species labeled to this extent were seen in the RNAs transcribed from clones 214 or 453 (Figure 2B). These RNAs did not have extensive sequence homology with U1 RNA because they were not hybrid selected (Figure 2A).

RNase T1 fingerprint analysis (data not shown) of several of the additional RNAs confirmed that they are not related to U1 RNA and demonstrated that the transcripts of the different clones are not identical. Furthermore, the additional RNAs transcribed from an individual clone appear to be related, presumably as products of the same coding sequence. A similar observation has been made regarding the transcription of an Alu sequence located in the human U2 RNA gene repeat (23).

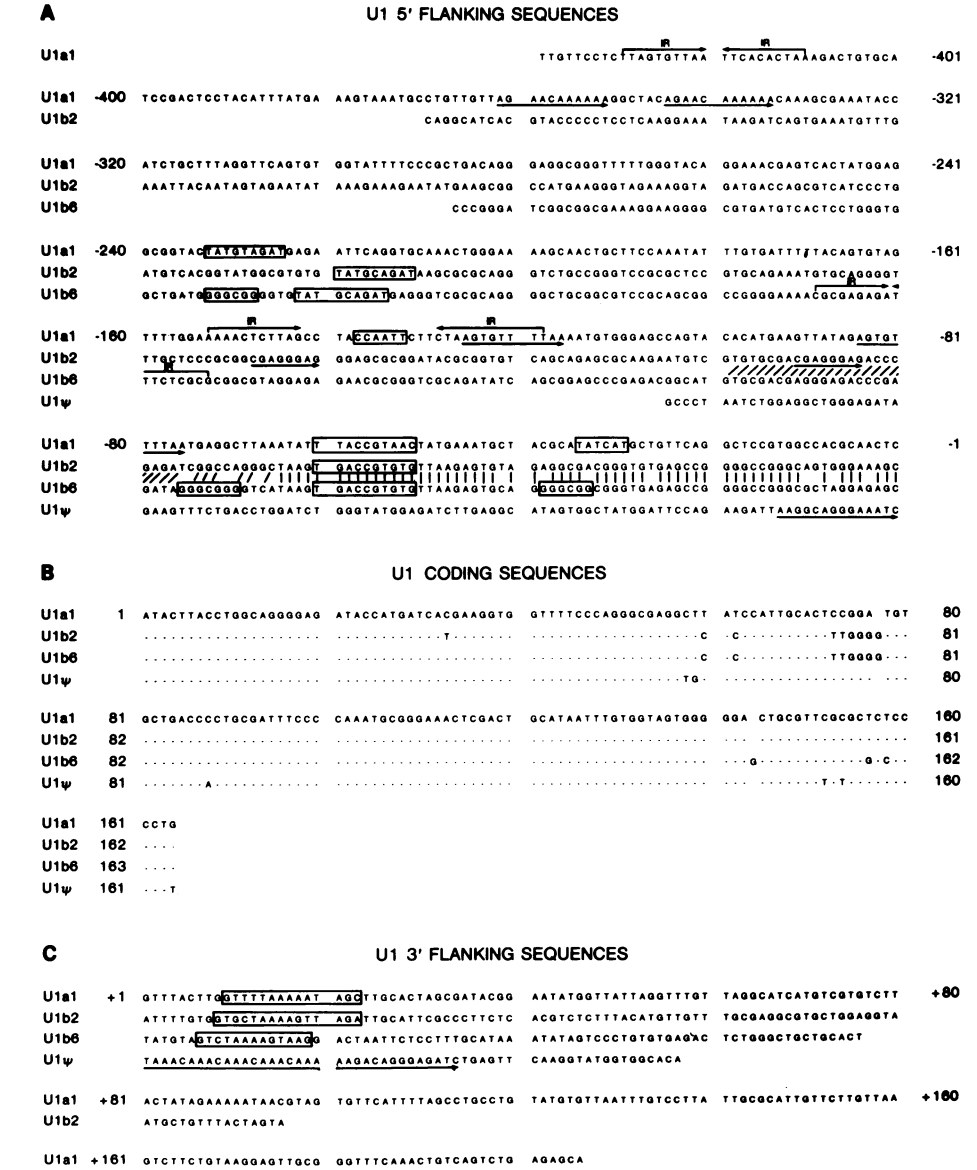


Figure 4: DNA sequence analysis of cloned mouse U1 genes. DNA sequences are presented for the noncoding strands of the U1a1 gene in clone 214, the U1b2 gene in clone 136, the U1b6 gene in clone 453, and the U1 pseudogene in clone 325. (A) 5' flanking sequences. Position -1 is the first nucleotide upstream from the coding sequence. Homologies with the sequence immediately above are indicated by vertical or diagonal lines. Direct repeats are underlined with arrows. Inverted repeats are overlined with arrows and designated

IR. Conserved and non-conserved promoter sequence elements are boxed. (B) Coding sequences. Position 1 is the potential cap site of the snRNA. (C) 3' flanking sequences. Position +1 is the first nucleotide downstream from the coding sequence. A conserved sequence element required for 3' end formation of the snRNA is boxed. An A-rich sequence immediately downstream from the U1 pseudogene is solid underlined. A sequence which occurs both in the 5' (figure 5A) and 3' proximal flanking regions of the U1 pseudogene is underlined with an arrow.

DNA Sequence Analysis of Cloned Mouse U1 Genes

The DNA sequences of the coding and flanking regions of the four mouse U1 genes were determined and are presented in Figure 4.

Coding Regions. Clones 214, 136 and 453 contain full-length, perfect coding sequences for mouse U1a1, U1b2 and U1b6 snRNAs respectively (Figure 4B, 8,9). The coding sequence of the U1b2 gene in clone 136 is identical to the coding sequences of two closely spaced (within 6.9 kb), functional mouse U1b genes that were isolated and sequenced by Marzluff and coworkers (13,31). The 15 kb fragment of mouse genomic DNA we isolated contained only one U1b2 gene, suggesting that it may be different from previously isolated U1b2 genes.

Clone 325 contains a pseudogene whose origin may have been the insertion of a cDNA copy of a U1a snRNA at a staggered break in genomic DNA (32-34). There are six substitutions in the coding region relative to the mouse U1a1 gene (Figure 4B), an A-rich tract immediately downstream of the coding sequence (Figure 4C) and an imperfect direct repeat (5'-AAGGCAGGGAAATC-3') flanking the first base of the coding region and the 3' terminus of the A-rich tract (Figures 4A, 4C). Moreover, the DNA sequence of the U1 coding region and the immediate flanking DNA in clone 325 is virtually identical to that reported by Piechaczyk et al. (34) for a presumptive mouse U1 pseudogene. Our observation that the U1 coding sequence in clone 325 is not transcribed supports the conclusion that it is a U1 pseudogene.

3' Flanking Regions of Mouse U1 Genes. Accurate formation of the 3' end of human U1 and U2 snRNAs requires the presence of a sequence element in the 3' flanking region of the gene that has the consensus GTTYN(0-3)AAARRYAGA (20,21; H. E. Neuman de Vegvar, E. Lund, and J. E. Dahlberg, in press). Each of the functional mouse U1 genes contains a sequence element that exhibits extensive homology with the consensus sequence (Figure 5C) and is positioned 7-10 nucleotides from the 3' terminus of the U1 coding region (Figure 4C). This conserved sequence element is not present in the 3' flanking region of the U1 pseudogene.

A TRANSCRIPTION ACTIVATOR/ENHANCER

Mouse U1a1	T A T G T A G A T	-225
Mouse U1b2	T A T G C A G A T	-212
Mouse U1b6	T A T G C A G A T	-215
Human U1, HU1-1	T A T G T A G A T	-212
Human U2	C A T G C A A A T	-214

B 5' END FORMATION

Mouse U1a1	T T A C C G T A A C	-52
Mouse U1b2	T G A C C G T G T G	-52
Mouse U1b2 (M)	T G A C C G T G T G	-53
Mouse U1b6	T G A C C G T G T G	-52
Mouse U2	G G A C C G T G A G	-52
Human U1, HU1-1	T G A C C G T G T G	-52
Human U2	T C A C C G C G A C	-50

C 3' END FORMATION

Mouse U1a1	+10	G T T T T - - A A A A A T A G C
Mouse U1b2	+ 9	G T G C T A - A A A G T T A G A
Mouse U1b2 (M)	+11	G T G C T A - A A A G T T A G A
Mouse U1b6	+ 7	G T C T - - - A A A A G T A A G
Mouse U2	+20	G T C A G - - A A A A A T A G A
Human U1, HU1-1	+11	G T T T C - - A A A A G T A G A
Human U2	+19	G T T T C C T A A A A G T A G A

Figure 5: Conserved sequence elements in the 5' flanking region of functional mammalian U1 and U2 genes. Sequences from the genes characterized in this study are designated mouse U1a1, U1b2 or U1b6. Sequences from the mouse U1b2 gene isolated by Marzluff et al. (13) are designated U1b2 (M). Human U1 sequences (HU1-D) are from Lund and Dahlberg (44). Human U2 sequences are from Ares et al. (18) and mouse U2 sequences are from Nojima and Kornberg (45). (A) Transcription activator/enhancer. (B) Sequence required for 5' end formation of U1 and U2 RNAs. (C) Sequence required for 3' end formation of U1 and U2 RNAs.

Conserved Sequence Elements in the 5' Flanking Regions of Mouse U1 genes. The DNA sequence of the proximal 5' flanking region of the U1b2 and U1b6 genes is highly conserved from position -100 to -1 (Figure 4A). The two U1b sequences exhibit 87% homology in this region and are in perfect register from

position -65 to -1. The homology between the U1b genes and the U1a1 gene in the 100 proximal 5' flanking nucleotides is significantly lower (about 45%).

Regardless of the degree of sequence homology in the region -100 to -1, each functional U1 gene contains a sequence element that has been shown to be essential for the accurate initiation of transcription at the 5' end of human U1 RNA (15,17). This element contains an invariant core sequence ACCGT and is located at position (-62 to -50) in mouse and human U1 and U2 genes (Figure 4A, 5B). The element was not found in the U1 pseudogene.

Another highly conserved sequence element in each of the functional mouse U1 genes matches the consensus YATGYARAT and is located more than 200 nucleotides upstream from the cap site (Figures 4A, 5A). This sequence has been shown to be part of a transcription activator that is required for efficient expression of U1 and U2 genes (12,15,17-19,23). Unlike the sequence element required for transcription initiation, the distance of the presumptive activator from the cap site is variable, ranging from 212 nucleotides (U1b2, Figures 4A and 5A) to 225 (U1a1). In clone 236, the 5' flanking DNA of the U1a1 coding sequence ends at position -220 (Figure 1). Thus, the lack of expression of the U1a1 gene following microinjection of clone 236 into Xenopus oocytes is probably due to the absence of the transcription activator sequence.

Non-conserved Sequence Elements in the 5' Flanking Regions of mouse U1 genes. In addition to the conserved sequences described above, the upstream flanking regions of the mouse U1 genes contain sequence elements (Figure 4A) that frequently occur in promoters for RNA polymerase II, as reviewed by Dynan and Tjian (35). Short direct repeats and closely spaced inverted repeats occur in the 5' flanking regions of the functional mouse U1 genes (Figure 4A). The sequences and positions relative to the cap site of the repeats are not conserved among the mouse U1 genes.

The U1a1 gene contains a TATA-like element TATCAT (36) at position -35 and a "CAAT box" core element (CCAAT; 37) at position -138. The CCAAT element is flanked by an inverted repeat. The promoter region of the U1b6 gene contains three "GC box" core elements (GGGCGG; 38,39), one of which occurs immediately upstream of the transcription activator (position -233; Figure 4A). GC boxes are not observed in the U1a1 or U1b2 genes. All of the observed GC boxes are in the same 5'-3' orientation.

DISCUSSION

In this study we have isolated, sequenced and expressed a mouse U1a1 gene and two U1b genes, U1b2 and U1b6. Both the U1a1 and U1b6 genes are the

first of their types to be cloned and characterized. A mouse U1a pseudogene was also characterized. The U1a1 and U1b genes are considered to be functional because they are transcribed into the encoded mouse snRNAs following microinjection into Xenopus oocytes. The identities of these transcripts were confirmed by RNA fingerprint analysis; in all cases, the snRNAs are appropriately capped, faithful copies of their respective coding sequences.

The pseudogene (clone 325) cannot be transcribed in Xenopus oocytes. The DNA sequence of this pseudogene and its flanking regions is nearly identical to a mouse U1 pseudogene characterized by Piechaczyk et al. (34). Our observation that this gene is not transcribed in oocytes confirms the speculation of Piechaczyk et al. that this sequence is indeed a pseudogene.

The developmental expression of several genes in both mammals and amphibians is mediated by trans-acting transcription factors that bind to promoter sequence elements in cis with the regulated genes (40,41; reviewed in reference 42). We expect that similar factors and promoter elements play a role in the developmental control of expression of mouse U1 genes. DNA sequence analysis of a functional U1a1 gene and two functional U1b genes (U1b2 and U1b6) was undertaken to determine if these genes contain promoter elements that could serve as potential binding sites for regulatory, trans-acting factors. In addition to sequences that are conserved among snRNA genes, the mouse U1 genes contain several other sequence motifs that could serve as transcription factor binding sites and function in modulation of gene expression.

Conserved Sequence Elements. The DNA sequences immediately upstream from the U1b2 and U1b6 coding regions are 87% homologous from position -100 to -1. The homology between this region in the U1a1 gene and the U1b genes is approximately 45%. Consequently, in this proximal 5' flanking region the U1b genes are much more similar to each other than they are to the mouse U1a1 gene. Indeed, the 5' flanking regions that we have sequenced in both U1b genes exhibit more homology with the 5' flanking regions of a rat U1 gene (14) and a functional human U1 gene (15,44) than with the 5' flanking region of the mouse U1a1 gene.

Each mouse U1 gene contains three highly conserved transcription signals, two in the 5' and one in the 3' flanking region, that have been observed in other functional mammalian U1 and U2 genes (Figure 5). In this regard, the three genes closely resemble other snRNA genes and the U1a1 gene is not unusual. Moreover, the promoters of the mU1a1 and mU1b2 genes have recently

been shown to function with equal efficiencies when transfected into mouse L cells (D. McKenzie, personal communication).

The conserved sequences in the 5' flanking regions are an activator element positioned more than 200 bp upstream from the snRNA coding sequence, and a sequence required for accurate initiation of transcription located between position -60 and -50. An element required for 3' end formation is positioned 7-10 nucleotides downstream from the coding regions. The activator element appears to be required for the expression of the U1a1 gene because a clone (236) with only 220 bp of upstream DNA lacks this sequence and is not efficiently transcribed in oocytes (Figure 2).

Non-conserved Sequence Elements. In addition to the highly conserved activator element and the sequence required for initiation, each of the functional mouse U1 genes we analyzed contains additional, non-conserved sequence elements in its 5' flanking region that could serve as cis-acting transcription signals for RNA polymerase II (Figure 4A). Several unique direct repeat sequences and closely spaced inverted repeat sequences occur upstream from each U1 coding sequence. Repeated sequences of this type are observed in the promoter regions of other functional U1 genes (12,15,23).

Two of the mouse U1 genes contain 5' flanking sequence elements that have been observed in the promoters of other functional genes and have been shown to be essential for the transcription of these genes by RNA polymerase II (35). Two of these elements, CCAAT and TATCAT (TATAA-like), occur in the 5' flanking region of the U1a1 gene (Figure 4A). A third element, GGGCGG (GC box), occurs three times in the 5' flanking region of the U1b6 gene. Multiple GC boxes have also been observed in the 5' flanking regions of chicken U1 and U2 genes (22). Conceivably, these sequence elements might influence the expression of U1 genes by regulating overall promoter function (see below).

Developmental Expression of mouse U1 Genes

The developmental control of expression of mouse U1 genes (8) appears to resemble the developmental expression of *X. laevis* U1 and U4 genes (Lund and Dahlberg, submitted) and 5S ribosomal RNA genes (reviewed in ref. 42). The mechanism for the developmental control of expression of *X. laevis* 5S genes has been elucidated in a series of elegant studies leading to the conclusion that oocyte 5S genes become inactive in somatic cells when transcription factors are limiting (43).

A similar regulation scheme might be utilized by the promoters for the

mouse U1 genes in controlling the developmental expression of mouse U1a and U1b genes. For example, if transcription factors had higher affinities for U1a than U1b promoter sequences, limitation of factors (perhaps in adult tissues) would result in preferential expression of U1a genes. In contrast, if there were excess factors in embryonic tissues or adult tissues with persistent stem cells (e.g. testis, spleen), both types of mouse U1 genes would be transcribed efficiently.

Alternatively, differential expression of mouse U1a and U1b genes might be mediated by the levels of trans-acting factors that recognize specific sequence elements in either the 5' or 3' flanking regions of one type of gene but not the other. Thus, the transcription of an entire family of genes could be controlled coordinately if expression was dependent on these factors.

In order to differentiate between models for control of transcription, homologous transcription systems must be developed for mouse U1 genes. These systems could then be optimized to reproduce the phenomenon of developmental control.

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