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Isolation and characterization of two linked mouse U1b small nuclear RNA genes

W.F.Marzluff, David T.Brown, Susan Lobo and Shan-Shan Wang

Department of Chemistry, Florida State University, Tallahassee, FL 32306, USA

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ABSTRACT

A 6.9 kilobase Eco R1 fragment containing genes for two U1 RNAs has been isolated from a library of mouse DNA. The two genes code for an RNA which is very similar, if not identical, to mouse U1b RNA as judged by S1 nuclease mapping. This RNA is one base longer than the mouse U1a RNA, human U1 RNA, and rat U1 RNA and differs in six nucleotide substitutions from rat U1 RNA. The two genes are five kilobases apart and the U1 RNAs are coded for on opposite strands of the DNA with the 5' ends juxtaposed. The sequences flanking the genes are identical for 700 bases 5' to the gene and at least 80 bases 3' to the gene.

INTRODUCTION

The small nuclear RNAs are an abundant class of RNAs present in the nuclei of mammalian cells. The "U" class of small RNAs share a common 5' terminal with an unusual "cap" structure (1). These RNAs are among the most abundant RNAs in mammalian cells (2). They share the property of being precipitated by certain classes of antisera present in patients with lupus erythematosus (3).These RNAs have been conserved across a wide variety of species (4-6). They are coded for by multiple genes (7,8). However in addition to the real genes there are a large number of pseudogenes present in mammalian genomes(9, 10).

The most abundant of the "U" small nuclear RNAs is the U1 RNA. This RNA is a single species in rat (11) and human cells (3). However, in mouse cells there are two electrophoretic variants U1a and U1b, which differ in several nucleotides(3). U1a has a sequence similar, if not identical, to rat U1 RNA (3). The U1 RNAs are synthesized by RNA polymerase II(12-14). We report the isolation of two linked mouse U1 genes which have a sequence identical with mouse U1b RNA. These two genes are transcribed

divergently from opposite strands of the DNA. They are separated by 5000 bases. The 5' and 3' flanking regions are identical suggesting that the genes arose by a novel gene conversion event.

MATERIALS AND METHODS

Isolation of U1 RNA and preparation of U1 cDNA.

The U1 RNA was isolated from mouse myeloma cell nuclei.(7) Nuclei were lysed in 0.5% SDS - 5mM EDTA, the viscous solution adjusted to pH 5 with 1/10 volume of 2M sodium acetate and extracted at room temperature with an equal volume of water-saturated phenol. The RNA was precipitated from the aqueous phase with 2.5 volumes of EtOH. The RNA was fractionated on a 5-20% sucrose gradient and RNA sedimenting between 4S and 8S was collected. This RNA was fractionated on a 10% polyacrylamide gel in 7M urea. The U1 RNA was detected by UV shadowing (15) and recovered by electroelution.

To prepare a specific cDNA probe the RNA was extended with E. coli adenylate transferase(16) and a complementary DNA prepared using RNA-dependent DNA polymerase as previously described for the sea urchin analogue of U1 RNA (5). This cDNA was used to screen a gene library in lambda phage Charon 4A. The library was a random library prepared by partial digestion with Alu I and Hae III of Balb/C mouse sperm DNA(17) and was a gift of Drs. Philip Early and Lee Hood. 10^5 phage were screened with the cDNA probe as previously described(18). Two phage which hybridized to the U1 and U2 cDNA were isolated . One of these was extensively characterized and is discussed in this paper.

DNA Sequencing

The DNA was sequenced by the method of Maxam and Gilbert (19,20). In addition the permanganate cleavage reaction of Rubin and Schmid (21) was used under conditions where it cleaved DNA at thymidine, deoxyguanosine and 5-CH_3 deoxycytidine. The DNA was reacted with potassium permangate (30 gm/ml) for 15 minutes at 20°C . The DNA was labeled at the 5' end using polynucleotide kinase and γ-$^{32}PO_4$-ATP and at the 3' end with the Klenow fragment of DNA polymerase and α-$^{32}PO_4$-dCTP.

S1 Nuclease Mapping

S1 nuclease mapping (22) was performed by the method of

Weaver and Weissman (23) using either purified U1a and U1b RNA or 4-8S nuclear RNA from either mouse myeloma or HeLa cells. In addition S1 nuclease mapping was done with RNA labeled in vivo with $^{32}PO_4$.

Hybridization to $^{32}PO_4$ Labeled RNA

The cloned DNA was digested with appropriate restriction enzymes, resolved by agarose gel electrophoresis, denatured and transferred to nitrocellulose according to Southern (24). The filter was hybridized with the purified $^{32}PO_4$ - labeled U1 RNA in 0.75M Na^+, 50% formamide at 52°C for 48 hours.

Alternatively the cloned U1 DNA was converted to the linear form with Eco R1, denatured and applied to nitrocellulose using a Hybridot apparatus(Bethesda Research Labs) (25). The dot was hybridized to $^{32}PO_4$ labeled RNA for 48 hours. The hybridized RNA was eluted with 99% formamide at 60°C and then analyzed by gel electrophoresis in 7M urea. Duplicate dots were treated with ribonuclease T_1 and the RNA eluted and analyzed by gel electrophoresis.

MATERIALS

Restriction enzymes and other enzymes for DNA sequencing were purchased from Bethesda Research Labs. γ-$^{32}PO_4$-ATP and $^{32}PO_4$ were purchased from ICN. α-$^{32}PO_4$-dCTP was purchased from Amersham.

RESULTS

The mouse small nuclear RNAs were isolated from purified nuclei. RNA sedimenting at 4-8S was purified by sucrose gradient centrifugation. The U1, U2 and U3 RNAs were purified by gel electrophoresis. About 20-50 adenylate residues were added to the RNA using E. Coli poly A polymerase (5,16). A cDNA was made from these RNAs and used to screen a gene library. Four colonies were selected which hybridized strongly to the cDNA. These were screened against cDNA prepared from each of the individual RNAs. Two of the colonies hybridized with U1 cDNA and two with U2 cDNA. A 6.9 kb Eco R1 fragment in the phage pMMU1.10 hybridized to the mouse U1 RNA (Fig. 1A). This fragment has been subcloned and extensively analyzed. We report here that this

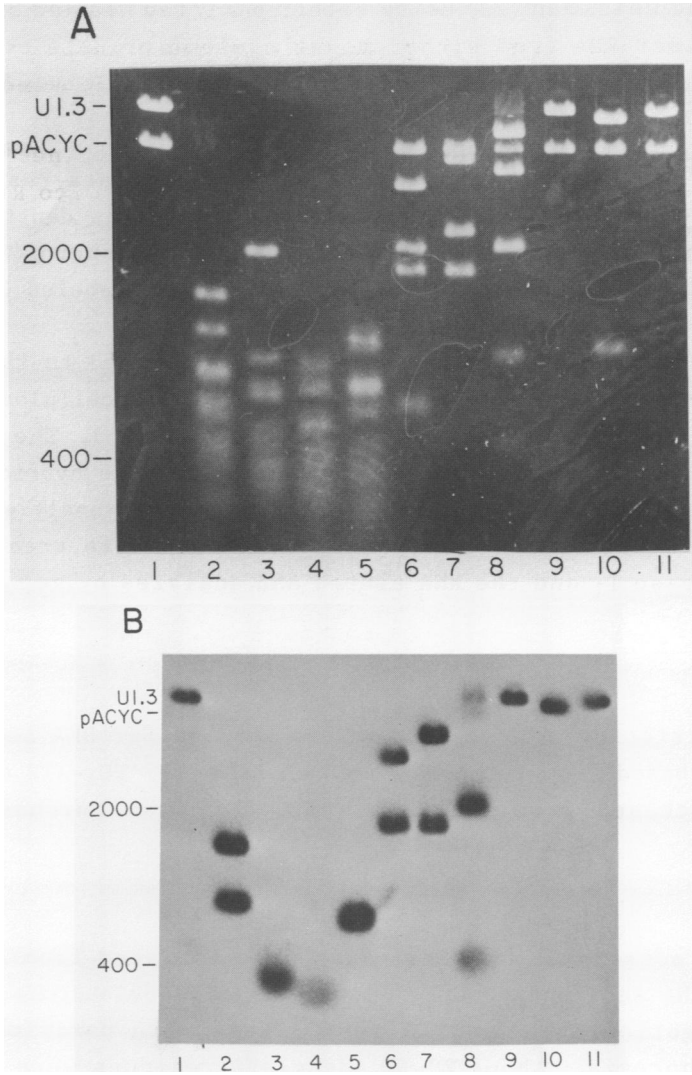

FIG. 1. Two Regions of Homology to U1 RNA in pU1.3
The plasmid pU1.3 was digested with the indicated restriction enzymes. The DNA fragments were transferred to nitrocellulose and hybridized with $^{32}PO_4$-labeled U1 RNA (a mixture of U1a and U1b) as described in Materials and Methods. Left: Ethidium bromide stained gel. Right: Autoradiogram of the filter. The enzymes used were Eco R1 plus: lane 1-none; lane 2-Hinf I; lane 3-Dde I; lane 4-Hae III; lane 5-Alu I; lane 6-Bgl II; lane 7-Sst I; lane 8-Sst II; lane 9- Xba I; lane 10-Pst I; lane 11-Kpn I. The positions of the 2000 bp and 400bp Eco R1-Sst II fragments which were subcloned are indicated.

fragment contains 2 identical U1 RNA genes.

This fragment was subcloned into pACYC 184.(26) Upon digestion of this plasmid , pU1.3, with several restriction enzymes, two fragments were observed which hybridized to the U1 RNA (Fig. 1). The two Eco R1-Sst II fragments which contained U1 RNA genes were subcloned into pACYC 184 from which an Eco R1-Sst II fragment had been removed. The restriction maps of these three clones are shown in Fig. 2. The regions containing the U1 RNA genes which have been sequenced are indicated in Figure 2B.

The restriction map around each gene for at least 800 bases is identical (Fig. 2). Figure 2A shows the digestion pattern of two 600 base Taq I fragments which are located at either end of the insert in pU1.3, and include the 5' end of the U1b genes. These fragments are identical by this criterion. The Taq I fragment adjacent to each of these fragments has also been analyzed by restriction endonuclease mapping and shows an identical map for an addiional 200 nucleotides(data not shown). The total region of similarity extends 5' to the gene for about 700 bases. The sequence of 400 nucleotides in both pU1.1 (the complete fragment) and pU1.2 has been determined and the sequences are identical in both fragments, which are 5 kb apart in the parent pU1.3 fragment. The sequences are shown in Fig. 3. There is a 165 base region which is similar to the reported rat and human U1 RNA sequence. The sequence shown in Fig. 3 differs from the rat U1 RNA sequence in six nucleotide substitutions, all of which are transitions. The differences are indicated in Fig. 3. In addition there is an extra base inserted at position 79 in the gene we have isolated. The 5' flanking region is striking in that it is very GC rich and does not contain the TATAA box characteristic of most of the regions which flank genes transcribed by RNA polymerase II.

The mouse is unique in that it contains two U1 RNA molecules, which are present in equimolar amounts. These are similar in sequence as judged by their reaction with anti-RNP antibodies and similar, but not identical T1 fingerprints.(3) In addition the mouse U1a species which comigrates with human U1 RNA on electrophoresis apparently has the same nucleotide sequence as rat U1 RNA(3). The reported T1 oligonucleotide differences (3)

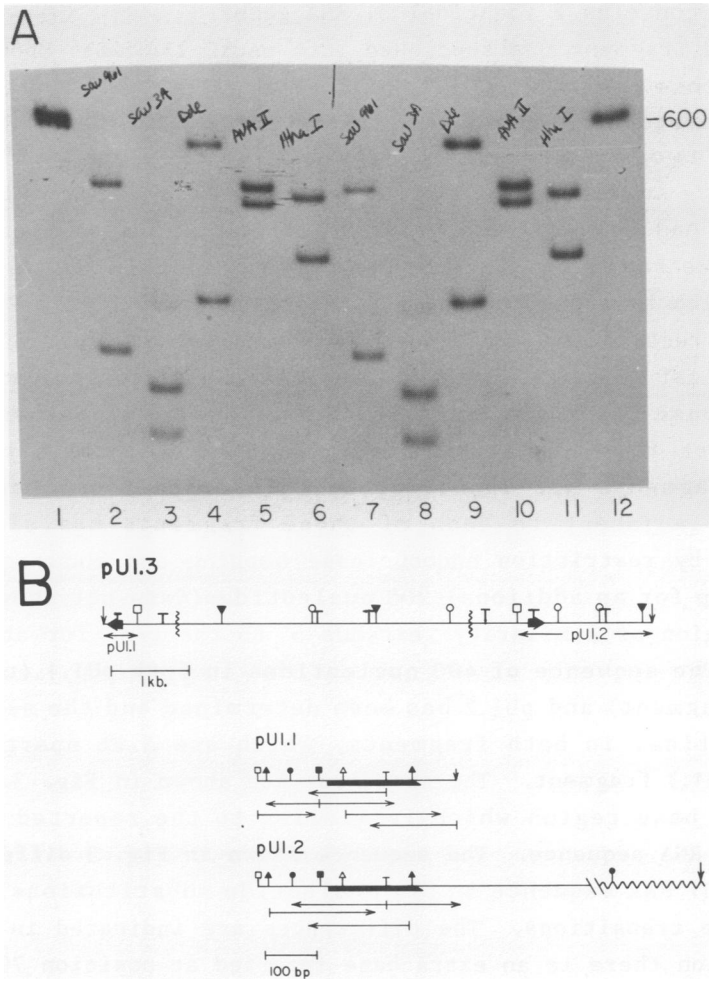

FIG. 2. Restriction map of pU1.1, pU1.2, and pU1.3
A. The 600 base Taq I fragment was purified from the Eco RI - SSt I fragment containing pU1.1(lanes 1-6) and the analogous 600 base Taq I fragment was purified from the Bgl II fragment containing the pU1.2 gene.(lanes 7-12) The fragments were end-labeled, digested with the indicated restriction enzymes, the products analyzed by gel electrophoresis and detected by autoradiography. Lanes 1 and 12-uncut; Lanes 2 and 7-Sau 96I; Lanes 3 and 8-Sau 3A; Lanes 4 and 9- Dde I; Lanes 5 and 10- Ava II; Lanes 6 and 11- Hha I.
B. The restriction map of the 6.9 kb Eco R1 fragment from pU1.3 is shown. The subclones pU1.1 and pU1.2 derived from pU1.3 are indicated. The region of identity extends from 3' to the gene to the ⅓, 700 bases 5' to the U1 gene as judged by restriction

enzyme mapping of end labeled Taq I fragments. The strategy used to sequence pU1.1 and the homologous region of pU1.2 is shown. Only the sites in the regions sequenced are indicated for Dde I, Sau96I and Hha I.
↓ - Eco R1; ⚲-Bgl II; ⚲-Sst II; ⚲-Sst I; ⚲-Taq I; ⚲-Dde I; ⚲-Sau96 I; ⚲-Hha I; ⚲-Sau3A. The limits of the region of identity is indicated by ⚲. The wavy line in the map of pU1.2 is not drawn to scale.

between mouse U1b and U1a are consistent with the changes in the genes we have isolated (see Discussion).

The U1 DNA fragments hybridize to both the mouse U1a and U1b RNA efficiently (see Fig. 5). Using S1 nuclease mapping however, the two RNAs can be distinguished and U1b RNA is colinear with the gene we have isolated by this criterion. Fig. 4A shows a low resolution S1 nuclease experiment using the U1 gene and the total mouse small nuclear RNA. The DNA was labeled at the 5' end with $\gamma^{32}PO_4$-ATP at the Hha site at position 156. The RNA was hybridized at different temperatures. At low temperatures two components are present, one the length expected from the mature U1 RNA and the second one shorter, about 80 bases long, corresponding to cleavage at the position of the base insertion in the gene we have isolated. At high temperatures only the full-length RNA protected the DNA. We stress that the difference in these experiments is in the hybridization conditions, not the S1 nuclease conditions. Therefore at the higher temperature the mismatched RNA was not capable of hybridizing to the DNA fragment. Since the large fragment was not protected by HeLa cell RNA (unpublished results) we interpret the protection of the large fragment to protection by U1b RNA. A high resolution S1 nuclease analysis is shown in Figure 4B. For this experiment the DNA was labeled at the Taq I site at position 117. The internal cleavage site is at precisely the site where there are base changes between the gene we have isolated and the U1a RNA (Fig. 4C), although the first G - A mismatch is not cleaved.

The 3' region of the molecule has also been studied by S1 nuclease mapping. The DNA was labeled at the 3' end with α-$^{32}PO_4$ dCTP and the Klenow fragment of DNA polymerase I at the Sau 3A site at nucleotide 30. Again there is complete protection of this fragment by mouse small nuclear RNA and purified mouse

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      150         140         130         120         110
    CCG CGGCGAGGGA GGGAGCGTGG ATACGGTGTC AGCAGAGCGC AAGAATGTCG

      100          90          80          70          60
        TGTGCGACGA GGGAGACCCG AGATCGGCTA GGGCTAAGTG ACCGTGTGTT

       50          40          30          20          10
        AAGAGTGTAG CGGCGACGGG TGATGAGCCG GGGCCGGGCA GTGGGAAAGC

                10          20          30          40          50
U1B     ATACTTACCT GGCAGGGGAG ATACCATGAT CATGAAGGTG GTTTTCCCAG
RAT U1                                     C

                60          70          80          90         100
U1B     GGCGAGGCTC ACCCATTGCA CTTTGGGGTG TGCTGACCCC TGCGATTTCC
RAT U1           T  T           CC  -A

               110         120         130         140         150
U1B     CCAAATGCGG GAAACTCGAC TGCATAATTT GTGGTAGTGG GGGACTGCGT
RAT U1

               160                     10          20          30
U1B     TCGCGCTCTC CCCTG       ATTTTTGTGG TGCTAAAAGT TAGATGCATT

                40          50          60          70          80
        CTGCTCTTCT CATGTCTCTT TACATGTTGT TTGTGAGGCA TGGCACGAAT TC
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Fig. 3. Sequence of pU1.1 and pU1.2.
The sequence of pU1.1 and pU1.2 is shown. The 165 bases corresponding to the U1 RNA start at base 1. The differences in sequence between pU1.1 and the rat U1 RNA are indicated. The pU1.1 and pU1.2 were identical in sequence in the region shown. The pU1.2 sequence only extends 70 bases 3' to the gene. The Eco R1 site 3' to the gene(nucleotide 75-81) is an artificial site due to attachment of the linker during construction of the library.

U1b RNA but not by U1a RNA or HeLa cell RNA (Fig. 5). The DNA is protected to the expected 3' end of the RNA by the U1b RNA. This evidence combined with the 5' mapping indicates that the U1b RNA is colinear with this gene by S1 mapping.

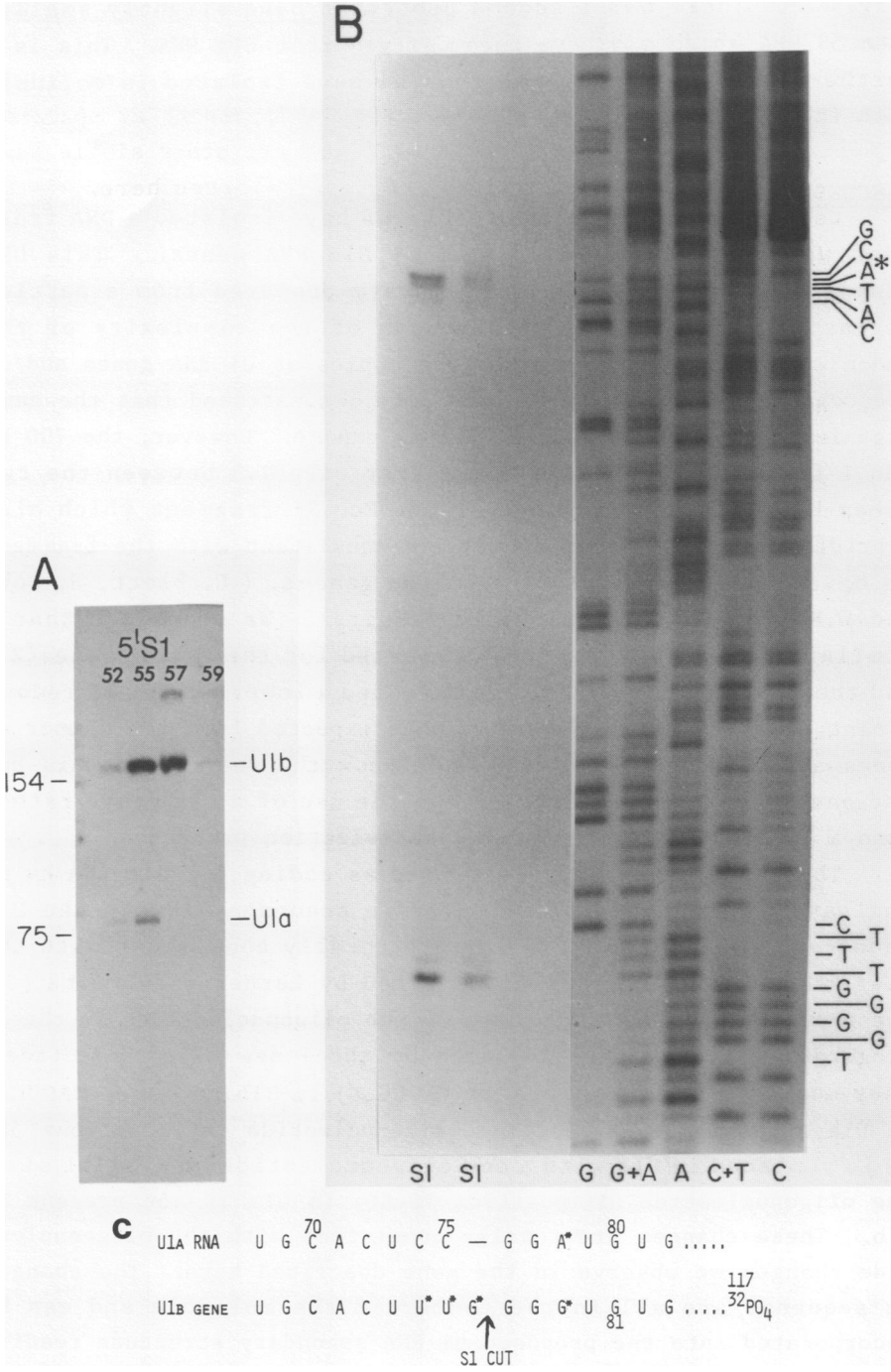
S1 nuclease mapping has also been used to determine whether the DNA we have isolated will select $^{32}PO_4$-labeled U1b RNA. $^{32}PO_4$-labeled small nuclear RNA was hybridized with the pU1.1 and pU1.3 plasmid. Both U1a and U1b RNA were efficiently selected by this plasmid. Digestion of the hybrids with RNase T_1 did not affect either the U1a or U1b RNA (Fig. 6A). However, when $^{32}PO_4$ labeled nuclear RNA was hybridized in solution to pU1.2 DNA and the hybrids digested with nuclease S1 only U1b RNA was protected

(Fig. 6B). There was a second protected band slightly smaller than 5S RNA which may have been derived from U1a RNA. This is a further indication that the gene we have isolated is colinear with the U1b RNA.

DISCUSSION

Using a homologous cDNA probe we have isolated a DNA fragment which contains two identical U1b RNA genes. This DNA fragment was isolated from a library prepared from a partial digest of mouse sperm DNA. Because of the complexity of the genomic blots due to repeated gene copies of U1 RNA genes and/or pseudogenes, we have not conclusively demonstrated that the same organization is present in the mouse genome. However, the 700 bp Taq I fragment located in the center of pU1.3 between the two genes hybridizes to a single large Eco R1 fragment which also hybridizes to pU1.1. This result is consistent with the fragment we have cloned being present in the genome. (C. Blatt, S. Lobo and W.F. Marzluff, unpublished results). We note also that a similar organization has been reported for the rat U1 genes(27) and the mouse U2 genes.(28) We selected a lower number of recombinant phage in our screen than were expected from the number of genes and pseudogenes.(9) We don't know the reason for this but the one procedural difference was the use of a DNA probe rather than a labeled RNA probe during the selection procedure.

The genes have been identified as coding for U1b RNA by S1 nuclease mapping. The differences in sequence between the U1b gene reported here and U1a RNA are totally consistent with the oligonucleotide differences reported by Lerner and Steitz (3). These authors showed that three of the oligonucleotides in the T1 fingerprint of U1a were replaced by three new oligonucleotides. They suggest that the heptamer ($ACCU_3G$) in U1b replaces CACUCCG in U1a at positons 69-75. The oligonucleotide at positions 58-68 is replaced in U1b with a new oligonucleotide of similar size. The oligonucleotide ATG(position 78-80) in U1a is not present in U1b. These changes are totally consistent with the oligonucleotide changes we observe in the gene described here. The changes in sequence are all in the center of the molecule and can be incorporated into the proposed U1 RNA secondary structure readily


A
5'S1
52 55 57 59
154 –
75 –
–U1b
–U1a
B
G
C
A*
T
A
C
–C T
–T T
–G G
–G G
–T
S1 S1 G G+A A C+T C
C
U1A RNA U G C A C U C C — G G A* U G U G .....
U1B GENE U G C A C U U* U* G* G G G* U G U G 32PO4
S1 CUT

(29,30). The change at nucleotide 33 is not apparent in the fingerprint of U1b RNA presented by Lerner and Steitz(3).

The S1 nuclease assay is not capable of distinguishing single base differences (it did not cleave the G-A mismatch at position 79, Figure 4C) so it is possible that there are other single base changes between the U1b RNA and the genes reported here. It is also of interest to note that T_1 ribonuclease was not capable of cleaving the U1a RNA-U1b gene hybrid. Thus this enzyme cannot be used to detect small changes in RNA sequence either. Since the U1b genes are probably repeated in the mouse genome it is not possible to conclude that these genes are expressed.

Study of DNAs which share sequence homology with mammalian small nuclear RNAs has revealed a large number of potential pseudogenes in addition to the expected large number of actual genes (9, 10). Most of the genes or pseudogenes reported thus far have not been closely linked to other small nuclear RNA genes. In contrast sea urchin small nuclear RNAs are found in tandemly repeated units (5) while the chicken U1 genes are not closely linked (12). The two genes we report here are arranged in an unusual manner in that the genes are on opposite strands. In most repeated genes studied thus far, e.g. globins(31), α-fetoprotein and serum albumin (32), and heavy chain constant genes(33), all of the genes are coded by the same strand of the DNA and hence are in the same orientation. Divergently oriented genes are found in the histone genes of Drosophila (34), yeast

Fig. 4. S1 Mapping of the 5' End.
A. The 400 base pair fragment in pU1.1 was cut with Hha I (at position 156) and labeled with polynucleotide kinase. The DNA was denatured and hybridized to mouse small nuclear RNA at different temperatures. The hybrids were digested with S1 nuclease and the resistant DNA analyzed by polyacrylamide gel electrophoresis in 7M urea. The size of the protected fragments is indicated. They were estimated using pBR322 digested with Hinf I.
B. The pU1.1 was cut with TaqI (base 117) and hybridized to mouse small nuclear RNA(left lane S1) and purified U1 RNA(right lane S1) at 55°C. The S1-resistant RNA was analyzed on a sequencing gel beside the DNA sequence. The sequence of the complementary strand is indicated. The A* marks the first nucleotide of the RNA.
C. The sequence of the U1b RNA and the rat U1 RNA are aligned. The position of the major cut by S1 nuclease is shown.

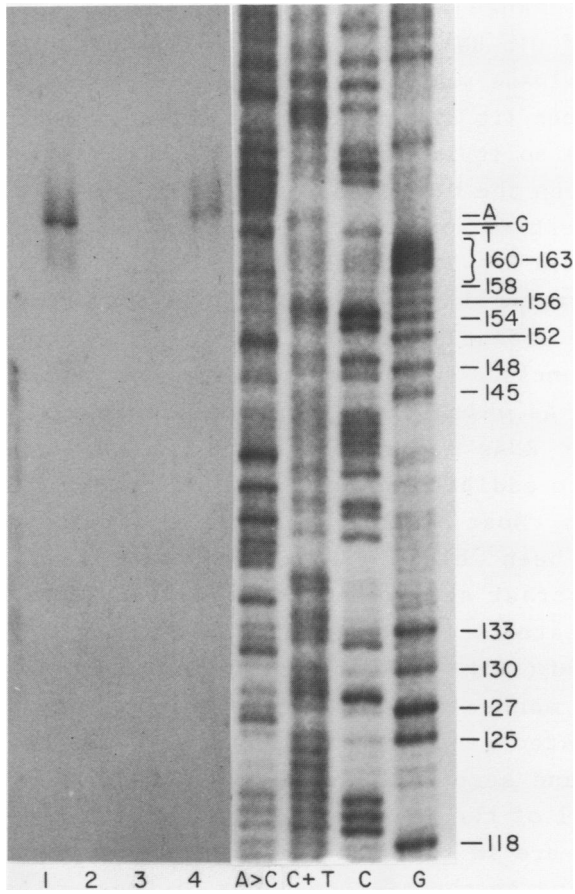


Fig. 5. S1 Mapping of the 3' End
The 400 base pair insert from pU1.1 was digested with Sau 3A and labeled at the 3' end with the Klenow fragment of DNA polymerase. The fragment was hybridized with total mouse small nuclear RNA, HeLa cell RNA, and U1 RNA. The S1-resistant DNA fragments were analyzed on a sequencing gel next to the sequence of the 3' end of the DNA. The numbers indicated the position of cytosines in the RNA sequence. The sequence of the complementary strand in the region of the 3' end of the RNA is indicated. Lane 1-mouse SnRNA; Lanes 2-tRNA; Lane 3-HeLa SnRNA; Lane 4-mouse U1 RNA.

(35) and mice(Sittman, Graves, and Marzluff unpublished results) although in these cases the divergently oriented genes are not identical but code for different histone proteins. The second unusual property of these genes is the large stretches of iden-

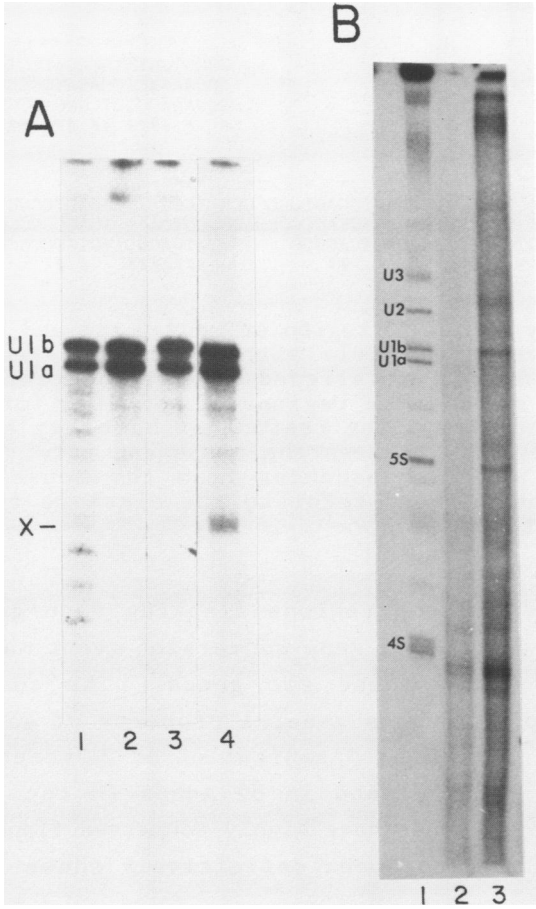


Fig. 6. Hybridization of $^{32}PO_4$-RNA to pU1.1

A. Mouse small nuclear RNA labeled with $^{32}PO_4$ was hybridized to pU1.1 or pU1.3 and immobilized on nitrocellulose dots. Some of the dots were treated with RNase T_1 prior to elution of the RNA. The eluted RNA was analyzed by polyacrylamide gel electrophoresis. The minor bands in lanes 1 and 2 are probably due to random digestion by RNase T_1.
Lane 1-RNA selected by pU1.1 and resistant to RNase T_1; Lane 2-RNA selected by pU1.1; Lane 3- RNA selected by pU1.3 and resistant to RNase T_1; Lane 4- RNA selected by pU1.3. The band X is selected by a repeated sequence in pU1.3 but is sensitive to T_1.

B. The $^{32}PO_4$-labeled RNA was hybridized to pU1.2 as described in Materials and Methods. The hybrids were treated with nuclease S1 and the resistant RNA analyzed by polyacrylamide gel electrophoresis. Lane 1: Total small nuclear RNA from mouse myeloma cells; Lane 2: RNA treated with S1 nuclease; Lane 3: RNA protected by pMU1.2 and resistant to S1 nuclease.

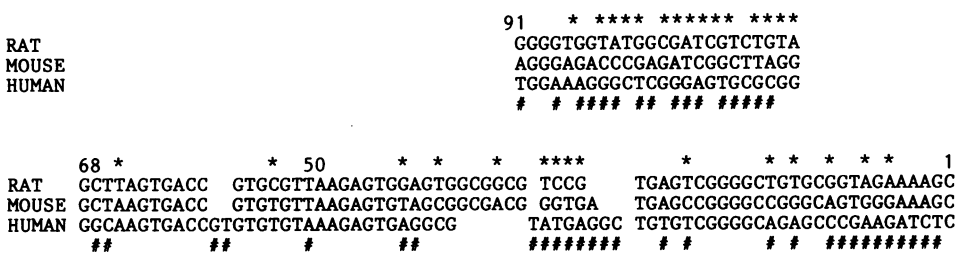


FIG. 7 Homology in the 5' region of mouse, rat and human U1 genes
The sequences directly flanking the U1 genes in mouse, rat(30) and human(8) are aligned to give the maximum homology. The sequences share large regions of homology for the first 70 bases 5' to the gene. The regions of homology are aligned to give maximum overlap between the mouse and rat. The difference in the human and rat sequences from the mouse sequence are indicated. The numbers refer to the distance from the first nucleotide in the RNA.

tical sequences in the regions flanking each gene. This is suggestive of an unusual gene conversion event maintaining the flanking regions of these two genes. The conservation of flanking region may be a general property of genes for small nuclear RNAs. A similar organization of U1 genes has been reported in the rat (27) and in U2 genes in the mouse(28). In addition, human U1 genes have highly conserved flanking sequences although they have not been definitively shown to be closely linked (8).

The sequence flanking the 5' end of the mouse U1b genes has extensive homology with the sequences 5' to the rat and human U1 genes. Figure 7 compares the 90 bases 5' to the mouse, human (8) and rat (27) genes. The first 70 bases are highly homologous among all three species with the exception of a group of 5 bases at about position 35 and the first 10 bases between the human and rodent species. The boundary of the region of homology is 70 bases 5' to the gene and is very sharp. The high degree of sequence conservation (85%) suggests a functional role for this region. This region includes the presumptive promoter region assuming the first nucleotide of the RNA is the site of initiation of transcription. This region does not show any features of the typical promoter region for RNA polymerase II. However since

these RNAs have different cap structure than mRNAs, it is possible they utilize a different type of promoter. Alternatively there could be a precursor at the 5' end of the gene and the sequence conservation could reflect the conservation of a particular RNA structure. There is indirect evidence for much larger precursors for U1 RNA (36), and the product of transcription of human U1 RNA genes in a cell-free system initiates 183 nucleotides upstream from the first nucleotide of the RNA(13).

The mouse is the only mammalian species in which more than one U1 RNA has been reported. In the human (3) and rat (11) cells studied, there is only one major Ul RNA. We have also observed only one U1 RNA in Chinese hamster ovary cells (our unpublished results). This RNA has an identical mobility with U1a. Since U1a and U1b are present in equal amounts in the mouse cells we have studied, it is likely that there are multiple copies of both of these genes in the mouse genome. The mouse is thus far the only mammalian species known to contain a U1b RNA. This gene could be present in other species but simply not expressed in tissues thus far examined. Alternatively it could have arisen in the mouse and subsequently spread by gene duplication. To distinguish among these possibilities it will be necessary to survey numerous tissues from different mammalian, particularly rodent, species and to determine whether there are any latent genes present in the genome of other mammals. The S1 nuclease assay may provide a sensitive method to assay for these RNAs in other species.

ACKNOWLEDGEMENTS

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REFERENCES

1. Weinberg, R.A. and Penman, S. (1968) J. Mol. Biol. 38 289-306.
2. Ro-Choi, T.S., Yang. C., Henning, D., McCloskey, T. and Busch, H.(1975) J.B.C. 250 3921-28.
3. Lerner, M.R. and Steitz, J.A. (1979) PNAS USA 76 5495-99.
4. Branlant, C., Krol, A., Ebel, T.P., Lazar, E., Gallinaro, H., Jacob, M., Sriwidada, T. and Jeanteur, P.(1980) NAR 8 4143-54.
5. Card, C. O., Morris, G.F., Brown,D. T. and Marzluff, W. F. (1982) NAR 10 7677-88.

6. Lerner, M. R., Boyle, J. A. Mount, S. M., Wolin, S. L. and Steitz, J. A. (1980) Nature 283 220-24.
7. Marzluff, W. F., White, E., Benjamin, R., and Huang, R.C. (1975) Biochemistry 14 3715-24.
8. Manser, T. and Gesteland, R. F. (1982) Cell 29 257-264.
9. Denison, R., VanArsdell, S., Bernstein, L. B. and Weiner, A. (1981) PNAS 78 810-814.
10. Denison, R. A. and Weiner, A. M. (1982) Molecular and Cellular Biology 2 815-28.
11. Busch, H. (1982) Ann. Rev. Biochem. 51 617-59.
12. Roop, D. R., Kristo, P., Stumph,W. E., Tsai, M. J. and O'Malley, B. W. (1981) Cell 23 671-80.
13. Murphy, J.T., Burgess, R.R., Dahlberg, J.E., and Lund, E. (1982)Cell 29 265-74.
14. Morris, G.M. and Marzluff, W.F.(1983) Biochemistry 22 645-53.
15. Hussur, S.M. and Whitlock, H. W. (1974) Anal. Biochem. 59 162-64.
16. Sippel, A. (1973) Eur. J. Biochem. 37 31-40.
17. Maniatis, T., Hardison, R. C., Lacy, E., Laver, J., O'Connell, C., Quon, D., Sim, G.K. and Efstratiadis, A. (1978) Cell 15 687-701.
18. Sittman, D.B., Chiu, I-M., Pan, C-J., Cohn, R.H., Kedes, L.H., and Marzluff, W.F.(1981) Proc. Nat. Acad. Sci. 78 4078-82.
19. Maxam, A. M. and Gilbert, W. (1977) PNAS 74 560-564.
20. Maxam, A. M. and Gilbert, W. (1980) Methods in Enzymology 65 499.
21. Rubin, C. M. and Schmid, C. W. (1980) Nuc. Acids Res. 8 4613-19.
22. Berk, A. J. and Sharp, P.A. (1977) Cell 12 721-32.
23. Weaver, R. and Weissman, C. (1979) Nuc. Acids Res. 7 1175-93.
24. Southern, E. M. (1975) J. Mol. Biol.98 503-15.
25. McKnight,G. S. and Palmiter, R. D. (1979) J. Biol Chem. 259 9050-58.
26. Chang, A.C.Y. and Cohen, S.N. (1978) J. Bacteriol. 134 1141-47.
27. Natanake, I., Nagasu, N., Itoh, Y., Tani, T., Okano, K. Koga, N., Okada, N. and Oshima, Y. (1983) Nuc. Acids Res. 11 1791-1801.
28. Nojima, H. and Kornberg, R.D.(1983) J. Biol. Chem. 258 8151-55.
29. Krol, A., Branlant, C., Lazar, E., Gallinaro, H. and Jacob, M. (1981) NAR 9 841-58.
30. Mount, S.M. and Steitz, J.A.(1981) Nuc. Acids Res. 9 6351-58.
31. Laver, J., Shen, C.K.J. and Maniatis, T. (1980) Cell 20 19-130.
32. Ingram, R. S., Scott, R. W. and Tilghman, S. M. (1981) PNAS 78 4694-4698.
33. Early, P.W., Davis, M. M., Faback, D. G., Davidson, N. and Hood, L.(1979) PNAS 76 857-61.
34. Lifton,R. P., Goldberg, M. L., Karp, R. W. and Hogness, D. S. (1977) Cold Spring Harbor Symp. Quant. Biol. 42 1047-1051.
35. Hereford, L. M., Fahrner,K., Woolford, J. and Rosbash, M. (1978) Cell 18, 1261-1271.
36. Elicieri, G. L. (1979) Nature 279 80-81.