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Polymorphism in an Androgen-Regulated Mouse Gene Is the Result of the Insertion of a B1 Repetitive Element into the Transcription Unit

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The single-copy RP2 gene in mice produces three major mRNAs, the abundances of which are significantly increased in the kidneys by the administration of testosterone. S1 nuclease analysis of the kidney mRNAs indicated that they differ in the lengths of their 3′ untranslated regions as a result of the use of different polyadenylation sites. When the mRNAs from different inbred mouse strains were examined by Northern blot analysis, it was observed that the largest mRNA varies in size, whereas the sizes of the other mRNAs remain the same. In DBA/LiHa and DBA/2J mice, the largest mRNA is approximately 2,150 nucleotides long, whereas the corresponding mRNA in C57BL/6J and BALB/cJ mice is only 1,950 nucleotides in length. All of these strains also have RP2 mRNAs that are 1,450 and 1,350 nucleotides long. By S1 nuclease mapping and comparison of the sequence of cDNA clones representing these mRNAs in DBA/LiHa and C57BL/6J mice, we determined that this size difference or polymorphism observed in the largest mRNA is the result of the insertion of a member of the B1 family of repeats into the 3′ untranslated region of the RP2 gene in DBA mice. This particular B1 repeat is transcribed by RNA polymerase III in vitro, and its transcriptional orientation is opposite to that of the RP2 transcript. The polymorphism described here is evidence for the mobility of B1 repetitive elements within the genome.

The B1 middle repetitive element is the mouse equivalent of the human Alu family of repeats. Its structure and sequence are homologous to the first unit (130 bases) of the dimeric Alu element and it is present in 40,000 to 80,000 copies per genome (16). Another mouse repeat with similar structure but minimal sequence homology to the B1 is the B2 element. Both B1 and B2 repeats have the same approximate size, 130 and 160 base pairs (bp) respectively, are characterized by an A-rich tract at their 3′ ends, are often flanked by short direct repeats at their site of insertion in the genome, and contain putative RNA polymerase III promoter regions (18, 24). In vitro and in vivo transcription of a few individual Alu-type elements by RNA pol III has been reported (6, 10, 17, 37).

Many Alu-type repeats are located within RNA polymerase II (pol II) transcription units; in most cases they are included in intervening sequences and are removed during processing of the primary transcript. Occasionally, repetitive elements are located in the 3′ untranslated region of mRNAs. Ryskov et al. (28) have shown that many cytoplasmic poly(A)$^+$ mouse mRNAs contain B2 elements and that these are oriented in the same transcriptional direction as the gene coding for the mRNA. Because of this orientation, an adventitious polyadenylation signal at the 3′ end of the B2 inserted element becomes available, and it has been shown in one case to be the preferred site for polyadenylation of a pol II transcript (19). There does not seem to be such a specificity to the orientation of B1 repeats found in 3′ untranslated regions (28).

It has been proposed that Alu-type repeats may be mobile in the genome (12, 29, 34). While the actual transposability of these elements has not been demonstrated, two convincing examples of insertion events over evolutionary time have been presented. Grimaldi and Singer (9) described an Alu sequence interrupting a site within highly conserved African green monkey satellite DNA. Kominami et al. (15) presented similar evidence for a B2 element inserted at a locus in the progenitor of the BALB/c mouse strain, but not present at that locus in other inbred or wild mice.

We have recently described a cDNA clone, pMAK-1, which contains a repetitive element and hybridizes to a set of mRNAs in the kidney that are under androgen hormone control (31). The abundance of these mRNAs is increased from basal levels of 0.1% of the poly(A)$^+$ RNA to 0.8% in kidneys of testosterone-induced female C57BL/6J mice. The mRNAs are also inducible by testosterone in the liver and are present in various sizes in other tissues, although not inducible. Evidence from Southern blot hybridizations of mouse genomic DNA indicates that all these mRNAs are the product of a single gene. Berger et al. (1) isolated a cDNA clone termed pMK908, which hybridizes to kidney mRNAs similar to those that we have described. Because of the similarities in the sizes, hormonal induction characteristics, and in vitro translation products of these mRNAs, we have compared unpublished cDNA sequences and determined that pMAK-1 and pMK908 represent mRNAs transcribed from the same gene. A polymorphism identified in the gene encoding these mRNAs has been designated RP2 (7). Although we have previously used the term MAK (mouse androgen kidney) when describing both the mRNAs and the gene (31), we will use the term RP2 from here on to avoid confusion in references to this gene and its mRNAs.

Here we show that at least three mRNAs are produced from the RP2 gene in kidney cells, with the size of the largest RNA varying with the mouse strain. In DBA/2J and DBA/LiHa mice the largest mRNA is 175 nucleotides longer than the corresponding mRNA in C57BL/6J and BALB/cJ

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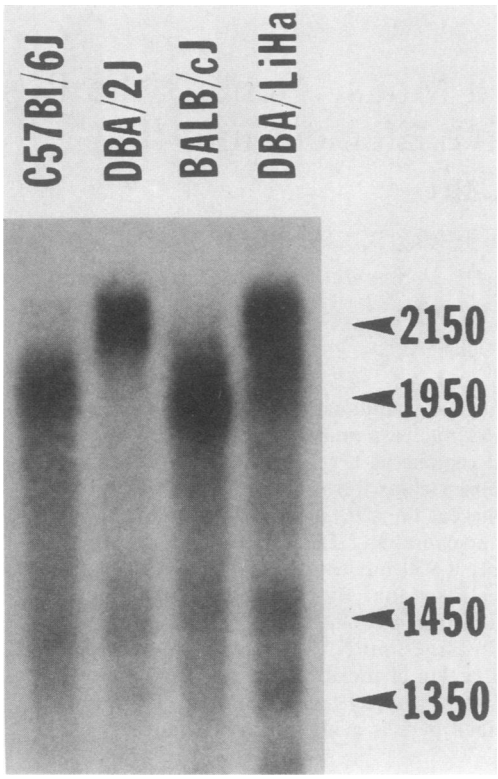

FIG. 1. Northern blot of RP2 RNA from the kidneys of different strains of inbred mice. Glyoxalated kidney mRNA from the indicated mouse strains was probed with the cDNA insert from clone pMAK-4 (see Fig. 3 for a description of this probe). Three major size classes of mRNA are evident in each strain, with the largest mRNAs varying in size by approximately 200 bases. Smaller, nonpolymorphic RNAs are present in each strain, measuring 1,350 and 1,450 nucleotides.

mice. We show that this polymorphism is due to the insertion of a B1 repetitive element in the 3′ untranslated region of the RP2 gene in DBA mice.

MATERIALS AND METHODS

Clones. cDNA clones were isolated from libraries prepared from the poly(A)$^+$ cytoplasmic RNA from kidneys of mice induced with subcutaneous testosterone pellets. The DBA/LiHa kidney cDNA library was a gift from Roger Ganschow (25). The first clone, pMAK-1, was isolated from this library by using a genomic restriction fragment that contained a B1 repeat as the probe (31). All subsequent screenings were done with the unique 300-bp *Pvu*II-*Rsa*I fragment or the overlapping 350-bp *Bgl*I-*Rsa*I fragment from pMAK-1 as probes (see Fig. 3). The C57BL/6J kidney library was kindly made available by Kenneth Paigen (G. Watson, M. Felder, L. Rabinow, K. Moore, C. Labarca, C. Tietze, G. VanderMolen, and K. Paigen, unpublished data). Sequencing of the cDNA clones was done by the method of Maxam and Gilbert (22).

RNA analysis. RNA was isolated from mouse kidneys as previously described (31). Glyoxalated total kidney RNA (10 μg) was electrophoresed for 5 h at 150 V on 1.4% agarose gels and blotted to nitrocellulose. Filters were baked for 2 h at 80°C in a vacuum oven, and residual glyoxal was removed by washing in 20 mM Tris (pH 8.0) at 100°C. Prehybridization and hybridization were done by the method of Thomas (33). The stringency of the washes was 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 50°C. RNA sizes were determined by comparison with stained ribosomal RNAs and glyoxalated single-stranded DNA markers.

The probe for the S1 nuclease analysis of the 3′ region of the large RP2 mRNA was the 553-bp BglI-*Pvu*I fragment from cDNA clone pMAK-1, which contains the 3′ end sequence representative of DBA mouse RP2 RNA plus 126 bp of flanking pBR322 vector sequence. The 3′ region of the 1,450-nucleotide RP2 mRNA was analyzed with the adjacent 350-bp *Rsa*I-BglI fragment from the pMAK-1 insert. These fragments were 3′ end labeled with the Klenow fragment of DNA polymerase I and strand separated (21). S1 nuclease analysis was performed by the method of Berk and Sharp (2) as modified by Weaver and Weissmann (35). Total kidney RNA (10 μg) was hybridized to excess probe at 68°C for 2 h. Nonhybridized DNA probe was subsequently digested with 400 U of S1 nuclease (Sigma Chemical Co.) per ml at 21°C for 1 h. The resulting protected end-labeled DNA fragments were electrophoresed on 8% acrylamide–50% urea denaturing gels and autoradiographed.

In vitro transcription. Reactions were performed on circular plasmids (1 μg) with [^{32}P]UTP as described previously (20). The nuclear extract used in the experiments was an S30 fraction of HeLa cells optimized for RNA polymerase II transcription (5) and was the generous gift of Donal Luse. The transcription products were electrophoresed on 5% polyacrylamide–7 M urea gels and autoradiographed.

Primer extension reactions. Primer extension of the B1 transcript was done by hybridizing the 15-base oligonucleotide 5′-CTTTAGCAGGGTCTT-3′, which was end labeled with [γ-^{32}P]ATP by polynucleotide kinase, to unlabeled in vitro synthesized transcription products at 47°C for 8.5 h. The hybrids thus formed were ethanol precipitated, and the pellets were rinsed once with cold 70% ethanol. Reverse transcription was performed with Moloney murine leukemia virus reverse transcriptase by following the protocol recommended by the manufacturer (Bethesda Research Laboratories). Products of the reaction were electrophoresed on a 5% polyacrylamide–50% urea gel and autoradiographed.

RESULTS

A subfragment of a mouse genomic clone containing repetitive sequences was used to screen a kidney cDNA library made from the RNA of testosterone-induced DBA/LiHa mice. One of the cDNA clones isolated was shown to contain, in addition to a repetitive region, sequences that hybridize to several RNAs with an abundance that is regulated by testosterone in the kidneys (31). This clone was named pMAK-1. A restriction fragment from this clone which lacks the repetitive sequence was subsequently used to rescreen the same DBA/LiHa cDNA library, and eight more overlapping clones were identified. Previous analysis of RNA homologous to pMAK-1 in our laboratory had shown two major size classes of RP2 mRNA 1,600 and 2,200 nucleotides in length (31). Since this work was limited to RNA prepared from C57BL/6J mice and our cDNA was prepared from DBA/LiHa mice, we compared the RP2 RNA hybridization patterns from these and several other strains on a Northern blot. Kidney mRNA from DBA/LiHa, DBA/2J, C57BL/6J, and BALB/cJ inbred mice was glyoxalated, electrophoresed under conditions to achieve maximum separation of bands, blotted, and probed with the entire insert from cDNA clone pMAK-4. The results (Fig. 1)

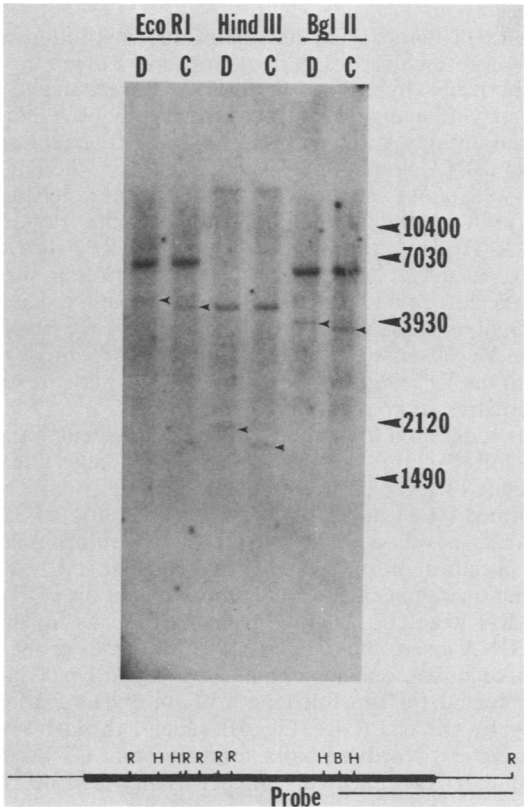

FIG. 2. Southern blot hybridization analysis of DNA from two polymorphic strains of mice. Genomic DNA was prepared from livers of C57BL/6J (C) and DBA/2J (D) mice, digested with the restriction enzymes indicated above the gels, electrophoresed, blotted, and probed with the 457-bp *Bgl*I-*Rsa*I fragment from the 3' end of clone pMAK-1E that is diagrammed below the figure. The thick line represents the cDNA sequence; the thin line represents flanking pBR322 vector sequence. Polymorphic bands are evident in each pair of lanes as indicated by the small arrows, and in each case the band in the DBA/2J lane is larger by an amount equal to the difference in the sizes of the largest RP2 mRNAs. Symbols: B, BglI; R, *Rsa*I; H, *Hin*fI.

indicate a size difference in the largest mRNA between strains. Both C57BL/6J and BALB/cJ mouse RNAs have a broad band of RP2 RNA migrating at a size of 1,950 nucleotides. In DBA/LiHa and DBA/2J mouse RNAs, this band migrates with a size approximately 200 nucleotides longer, or 2,150 bases in total length. All four strains share smaller nonpolymorphic RP2 RNA species which migrate as a doublet at 1,350 and 1,450 nucleotides. Stained 18S and 28S ribosomal RNA bands and glyoxalated single-stranded DNA homologous to the probe were used as size markers.

Several explanations could account for the dramatic size difference in the largest RP2 mRNAs between mouse strains. We have already established that RP2 is a single-copy gene, and so the difference could reflect differential processing of the primary transcript in these mouse strains, or perhaps a DNA polymorphism. To further explore this latter possibility, the DNA from two mouse strains exhibiting the size difference was analyzed by Southern blot hybridization (32). Genomic DNA from livers of C57BL/6J and DBA/2J mice was digested with the enzymes *Bgl*II, *Hin*dIII, and *Eco*RI. When the DNA was electrophoresed, blotted, and probed with a 3'-end-specific probe from cDNA clone pMAK-1E, two bands were observed in each lane (Fig. 2). An intensely hybridizing nonpolymorphic band represents an exon that is separated from a polymorphic exon by an intervening sequence containing *Bgl*II, *Hin*dIII, and *Eco*RI restriction sites. A shorter length of the cDNA probe fragment hybridized to the polymorphic exon than to the nonpolymorphic one, explaining the difference in intensity. The significant observation is that in all cases the polymorphic restriction fragment in DBA/2J mice was larger than the equivalent fragment in C57BL/6J mice by approximately 200 bases, an amount corresponding to the difference in size of the largest RP2 mRNAs in these strains. Similarly sized polymorphic bands were previously reported for *Hin*dIII-digested genomic DNA hybridized with pMK908 (7).

To further characterize this polymorphism, which is evident at both the genomic level and within the RP2 mRNAs, RP2 cDNA clones were isolated from a C57BL/6J mouse kidney cDNA library for comparison with those from the

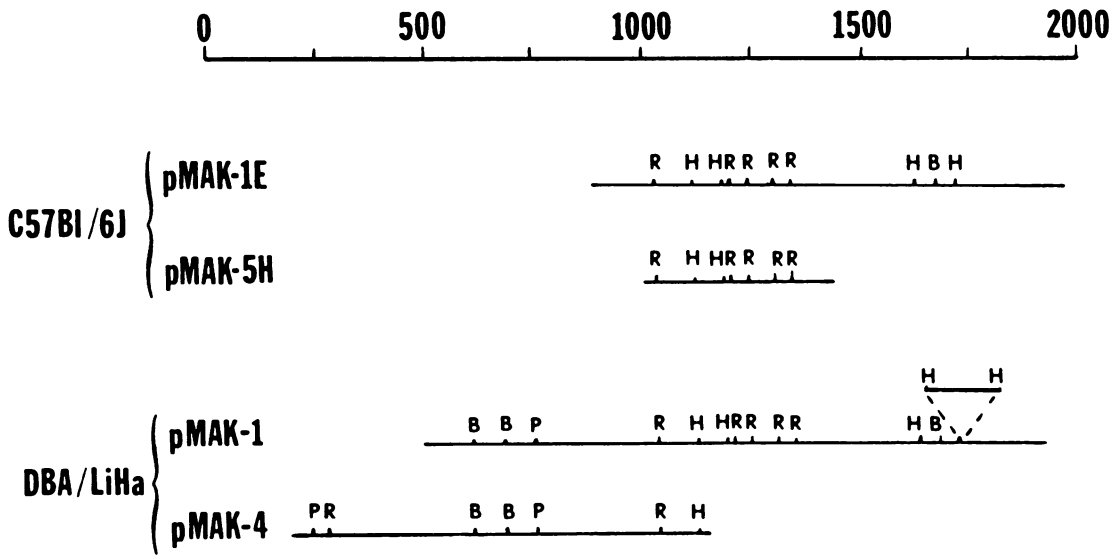

FIG. 3. Alignment of cDNA clones from DBA/LiHa and C57BL/6J mice. DBA/LiHa clone pMAK-1 contains a 175-bp insertion in the 3' untranslated region not found in C57BL/6J clone pMAK-1E. Symbols: R, *Rsa*I; B, *Bgl*I; P, *Pvu*II; H, *Hin*fI.

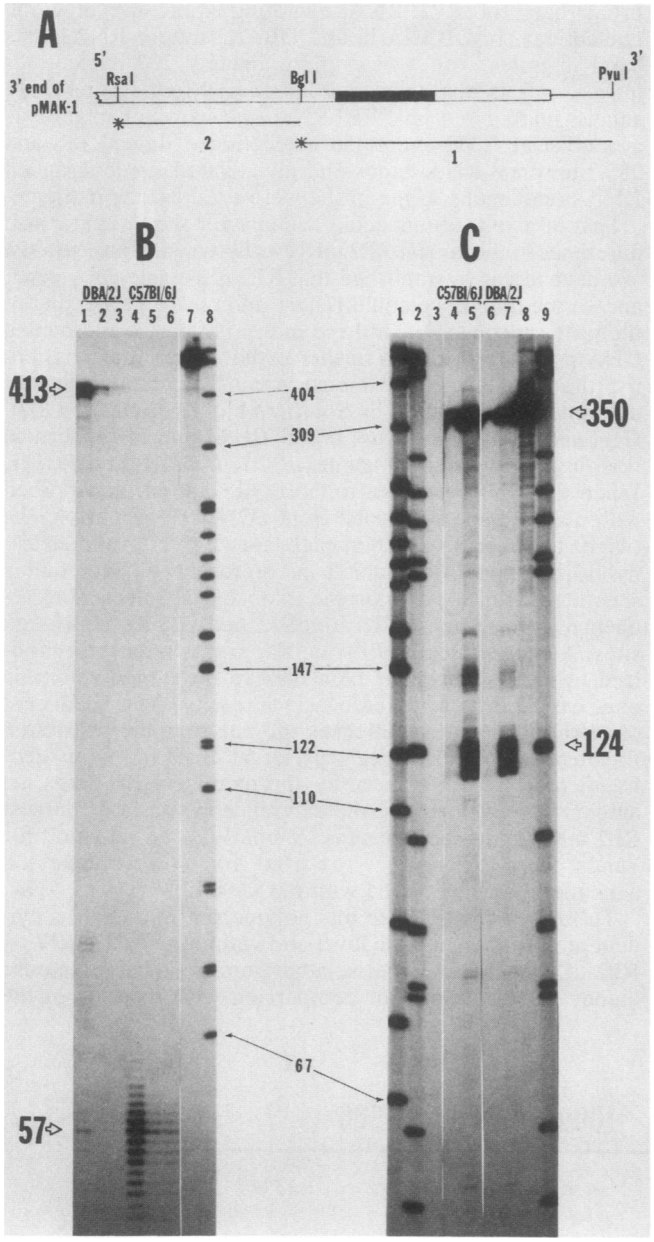

FIG. 4. S1 nuclease analysis of the 3′ ends of RP2 RNAs. (A) Map of the 3′ end of DBA/LiHa cDNA clone pMAK-1 and two S1 nuclease probes derived from it. The cDNA insert is presented as a bar with the repetitive region shaded. Flanking vector DNA is represented as a single line. Probe 1 (553 bases) and probe 2 (350 bases) are indicated below the map. (B) S1 nuclease data with probe 1 hybridized to C57BL/6J kidney RNA shows that the site of the insertion is 57 bases from the end-labeled *Bgl*I site. DBA/2J kidney RP2 RNA, which contains the inserted element, hybridizes fully to the cDNA sequence in probe 1. The mice were either induced with testosterone for 4 to 5 days before kidney RNA was prepared or not induced. Lanes: 1 and 4, induced females; 2 and 5, uninduced males; 3 and 6, uninduced females; 7, probe 1 alone (undigested); 8, end-labeled DNA size markers. (C) S1 nuclease analysis with probe 2 shows a polyadenylation site for the 1,450-nucleotide RP2 mRNA 124 bases from the end-labeled *Rsa*I site in both DBA/2J and C57BL/6J mice. Lanes: 1, 2, and 9, DNA size markers; 3, yeast tRNA (negative control); 4 and 6, uninduced females; 5 and 7, induced females; 8, probe 2 alone (undigested).

DBA/LiHa library. The alignment and restriction maps of four representative cDNA clones are shown in Fig. 3. Southern blot hybridization analysis and extensive restriction enzyme mapping indicated that a repetitive element is located entirely within the 175-bp *Hin*fI fragment at the 3′ end of cDNA clone pMAK-1, obtained from the DBA/LiHa library (data not shown). It was hybridization to this repeat that allowed the original isolation of this clone. Clone pMAK-1E, representing the longest RP2 RNA from C57BL/6J mice, has only one *Hin*fI restriction site in the same region and does not contain any repetitive sequences. An obvious explanation for the size difference or polymorphism we observed is the insertion of a repetitive element within the RP2 transcription unit of DBA mice, resulting in the creation of an additional *Hin*fI site.

The suggestion that the size difference evident in the larger RP2 mRNAs on Northern blots is actually due to the presence of the repetitive sequence within the mRNA was confirmed by S1 nuclease analysis. An additional S1 nuclease analysis was performed which provided information on the structure of one of the two smaller mRNAs. Two restriction fragments located at the 3′ end of the cDNA clone pMAK-1 were chosen (Fig. 4). Probe 1 spans the 3′ end of the cDNA insert in clone pMAK-1. Since this probe is from a DBA mouse clone and contains the repetitive sequence, it is protected for the full length of its cDNA portion (413 bases) by DBA/2J RNA (Fig. 4B, lanes 1 through 3). cDNA mapping and Northern blots suggested that C57BL/6J mice do not carry the repeated sequence in their RP2 mRNA, and this was confirmed by a strong signal at 57 bases, the site of the insertion of the repeat (lanes 4 through 6).

Probe 2 (Fig. 4A) distinguishes the nonpolymorphic 1,450-nucleotide RP2 mRNA from the larger RNAs. When probe 2 was hybridized to kidney RNA from polymorphic strains of mice (Fig. 4C, lanes 4 through 7), a broad signal in the region of 124 baes was detected in each case. This indicates that in both strains some RP2 mRNAs protect only the first 124 bases of the 350-base probe from S1 nuclease digestion. The full length of the probe is protected by the large RP2 RNAs from both polymorphic strains. The probe does not produce a stable hybrid with the 1,350-nucleotide mRNA, which is expected to terminate very near to the labeled end of the fragment.

In an effort to more clearly define the nature of the insertion element and differences between the RP2 mRNAs, DNA sequence analysis was performed. The sequence of the 3′ ends of three cDNA clones representing different size classes of MAK RNA is presented in Fig. 5. pMAK-1 (representing DBA/LiHa RNA) and pMAK-1E and pMAK-5H (clones from the C57BL/6J library [Fig. 3]) are aligned for maximum homology. The complete 3′ ends of two different-length mRNAs are present in clones pMAK-5H and pMAK-1E, as evidenced by the presence of poly(A) tracts preceded in each case by polyadenylation signals: AGTAAA for pMAK-5H and ATTAAA for pMAK-1E. Although these sequences differ slightly from the commonly found consensus polyadenylation signal AATAAA (27), both of these sequences have been documented as being functional polyadenylation signals for other mRNAs (3, references in reference 36). The location of the polyadenylation site used in the mRNA complementary to pMAK-5H is in excellent agreement with the S1 nuclease analysis results for the 1,450-nucleotide RP2 RNA. In addition, the distance from this site to the polyadenylation site used to produce the polymorphic large RP2 mRNAs (525 bases in C57BL/6J mice and 700 bases in DBA mice) agrees with the size differences

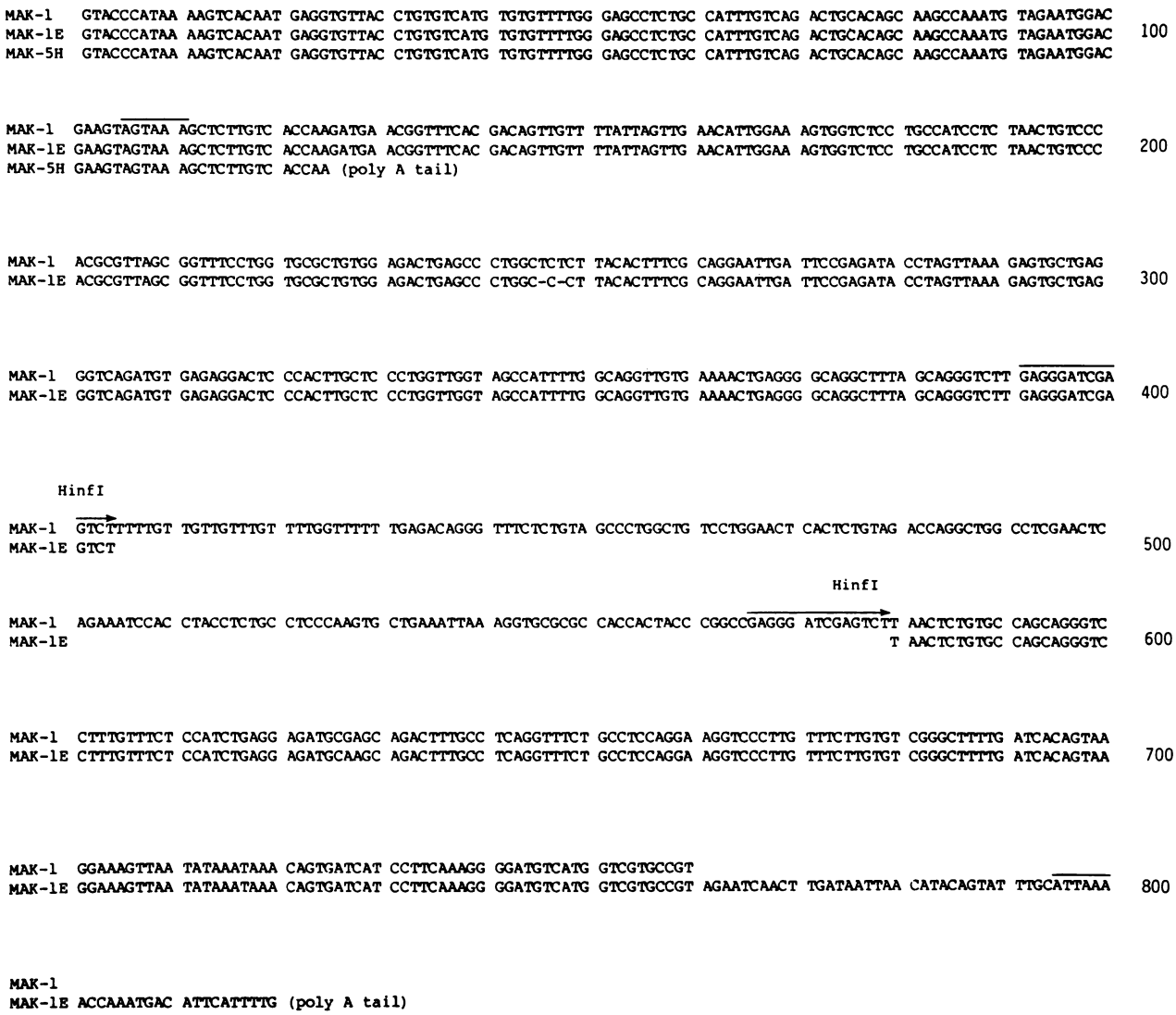

FIG. 5. Sequence of the 3′ untranslated region of pMAK cDNA clones. DNA sequence from the 3′-most *Rsa*I site is presented for the 3′ ends of clones pMAK-1, pMAK-1E, and pMAK-5H. This region encompasses two functional polyadenylation signals (overbars) for RP2 transcripts, and the inserted B1 repetitive element is flanked by short direct repeats (overlined arrows). Relevant restriction sites are indicated.

observed on Northern blots. Sixty bases plus the poly(A) tail were presumably lost from the 3′ end of pMAK-1 during construction of the cDNA.

Comparison of the 3′ sequences of clones pMAK-1 and pMAK-1E revealed that the inserted element in the DBA clone is 175 bases long and is flanked by 14-bp direct repeats. The sequence of the insert is highly homologous to the B1 family of Alu-type repeats (18). The B1 element is followed by a characteristic A-rich tract and is situated in the opposite transcriptional orientation to the RP2 transcript. This B1 repeat plus one 14-bp direct repeat are precisely missing from the C57BL/6J cDNA sequence.

An alignment of the B1 element inserted in the DBA RP2 gene with the B1 family consensus sequence (13) is shown in Fig. 6. There are 10 mismatches over 133 bases, or 92% homology. Two of these mismatches occur within the presumed RNA pol III split promoter. Mismatches between two sequences are designated by an asterisk between them. A comparison of the RP2 B1 sequence with a consensus RNA pol III promoter sequence composed by Galli et al. (8) from functional tRNA and ribosomal RNA genes shows six mismatches over 22 bases, with five of those mismatches occurring in the first half of the promoter. The polymerase typically initiates transcription 10 to 15 bases upstream of the first half of the promoter. Evidence exists that the distal half of the promoter element alone may be sufficient for the transcription of Alu-type repeats (26). Other investigators have previously established that selected Alu-type elements are capable of being transcribed by RNA pol III (6, 10, 37).

To determine whether the B1 element present in the RP2 gene is capable of being transcribed by RNA polymerase III, in vitro transcription and primer extension analyses were performed. RNA pol III transcription is known to terminate at runs of four Ts (4). The first such sequence that would be encountered by a polymerase initiating at the beginning of

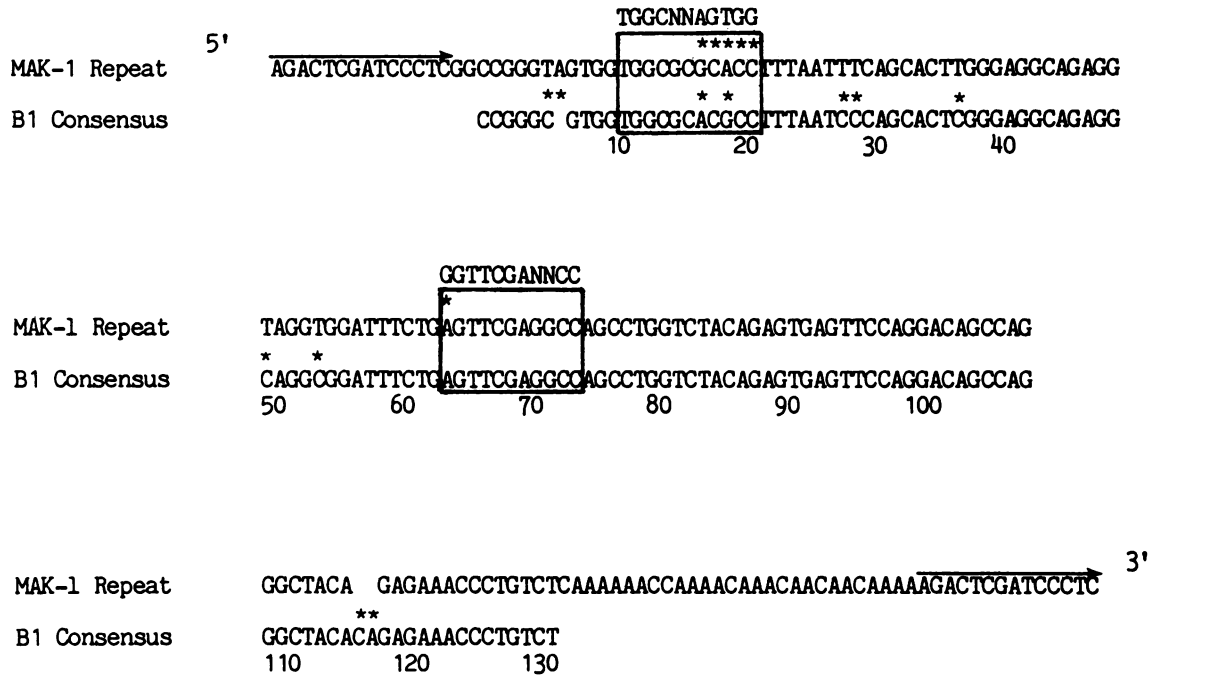

FIG. 6. Alignment of DBA RP2 inserted element with the B1 consensus sequence (12). Asterisks indicate mismatched bases. The putative RNA pol III split promoter is boxed and compared with the polymerase III promoter consensus sequence (8) above. The 14-bp direct flanking repeats are overlined with arrows.

the B1 repeat is found at position 361 in Fig. 5. Since it is the sense strand of the RP2 RNA that is presented in this figure and the B1 repeat has the opposite orientation within this transcript, the termination signal is ncessarily represented in this case by four As. The next possible termination signal is at position 9 in Fig. 5.

The 650-bp *BglI-RsaI* fragment from the 3′ end of pMAK-1 that contains the B1 repeat plus some flanking pBR322 sequence was subcloned into the *SmaI* site in pUC8. This plasmid (pBR650) as well as the entire pMAK-1 cDNA was transcribed in vitro (Fig. 7A). RNA synthesis incorporating [^{32}P]UTP was carried out in the absence of α-amanitin or at low (0.5- μg/ml) or high (150-μg/ml) concentrations of this inhibitor. Transcription of the pMAK-1 cDNA clone gives a major signal at 550 nucleotides and a weaker signal at 215 nucleotides (lane 5), which correlate with transcription initiating at the 5′ end of the B1 repeat and terminating at the two signals just described. Since transcription terminating at these signals was detected in the presence of the low α-amanitin concentration (lane 5) but was eliminated at the high concentration (lane 6), the transcription can be attributed to RNA pol III. Supporting data was obtained with the pBR650 subclone containing the same B1 repeat (lane 9). The termination signal which gives the 215-nucleotide transcript was included in this subclone and is recognized by the polymerase. Runs of Ts present in the vector serve as secondary termination signals giving other bands at 260 and approximately 650 bases. Again, these signals are all sensitive to high α-amanitin concentrations (lane 10). The controls showing that low concentrations of α-amanitin inhibit RNA pol II transcription are in lanes 13 and 14, which contain linearized DNA carrying the adenovirus major late promoter (*Hin*dIII p*Sma*-F). The high concentration of α-amanitin eliminates transcription by RNA pol III as shown in control lanes 11 and 12, which contain a functional Alu repeat promoter (E plasmid).

The bands resulting from the in vitro transcription of the B1 repeat in pMAK-1 are compared with the results of in vitro transcription performed on clone pMAK-1E in Fig. 7B. The major difference between these two cDNAs is the absence of the B1 repeat in pMAK-1E (Fig. 3). As expected, the signals at 550 and 215 nucleotides generated from the RNA pol III promoter in the B1 repeat of pMAK-1 (lanes 5 and 6) were not detected when pMAK-1E was transcribed (lanes 3 and 4).

Figure 8 shows the result of a primer extension experiment confirming that the transcripts made in vitro originated at the 5′ end of the B1 element. A 15-base oligonucleotide, CTTTAGCAGGGTCTT, was synthesized to be homologous to the unique sequence immediately downstream (transcriptional orientation) of the repetitive element (beginning at position 376 in the sequence in Fig. 5). The sequence is complementary to the B1 transcript, and in a primer extension assay it will direct the synthesis of DNA to the origin of the pol III transcript. An in vitro transcript was prepared from the pBR650 subclone in the presence of the low concentration (0.5 μg/ml) of α-amanitin and four unlabeled nucleotides. In vitro transcripts were also prepared from the pUC8 vector alone as a negative control. The RNA products of both of these reactions were then hybridized to the ^{32}P-5′-end-labeled oligonucleotide, and Moloney murine leukemia virus reverse transcriptase was used to extend the primer to the 5′ end of the B1 transcripts. Primer extension of the in vitro transcripts generated from the pBR650 subclone gives a strong signal at 191 bases, which is absent in the control lane. This is consistent with initiation of RNA pol III transcription at the beginning of the B1 insert. A much weaker signal at 202 bases may represent a low level of upstream initiation. Extra bands present in both experimental and control lanes are most probably due to the binding of this short oligonucleotide to RNA molecules present in the nuclear extract used for the in vitro transcriptions.

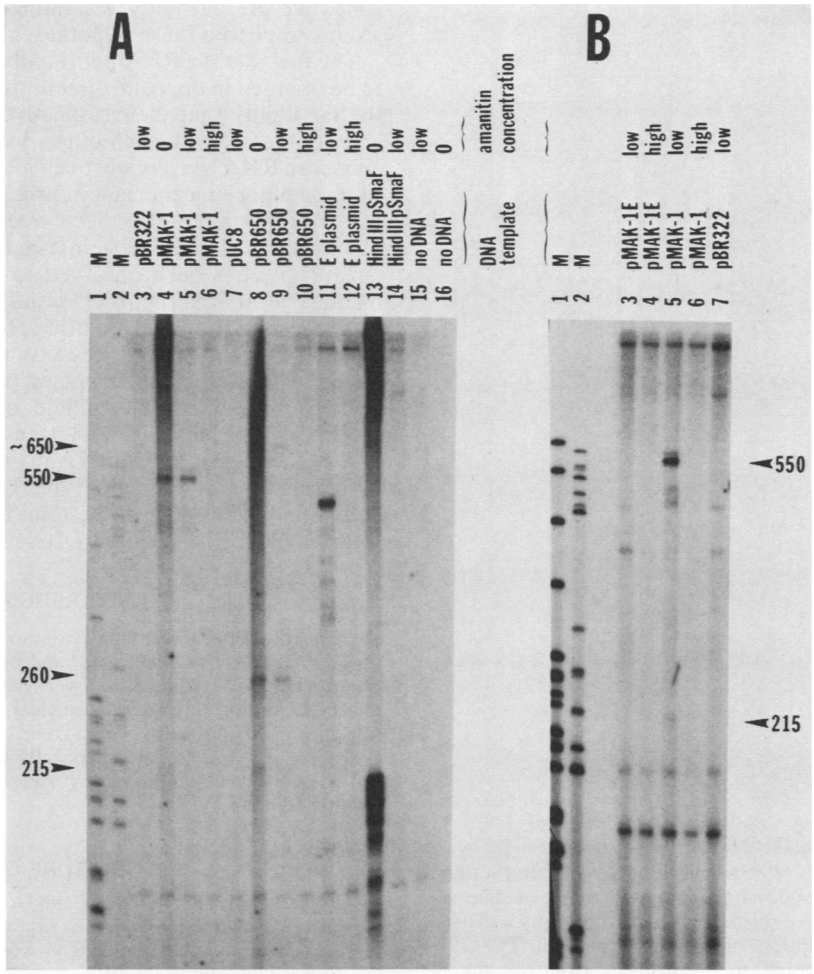

FIG. 7. Analysis of RNA polymerase III transcription of the B1 element in DBA mice. (A) In vitro transcription products are shown for the B1 repeat in pMAK-1 (lanes 4 through 6) and the same B1 repeat subcloned into pUC8 (pBR650: lanes 8 through 10). End-labeled DNA size markers were run in lanes 1 and 2. Transcripts of the two vectors alone in the presence of the low concentration of α-amanitin were included as negative controls (lanes 3 and 7). Controls for modulation of RNA pol III activity (lanes 11 and 12) and RNA pol II activity (lanes 13 and 14) by α-amanitin were also included. The uniform signal seen at 160 bases is probably due to end labeling of RNA endogenous to the nuclear extract (lanes 15 and 16). (B) In vitro transcription of essentially equivalent cDNA clones both with (pMAK-1; lanes 5 and 6) and without (pMAK-1E; lanes 3 and 4) the inserted B1 element is shown. Transcription of the pBR322 vector alone is shown in lane 7. Concentrations of α-amanitin used in each reaction are indicated. End-labeled DNA size markers were run in lanes 1 and 2.

DISCUSSION

The RP2 gene is present in a single copy in mice and has several interesting features. Its expression is regulated by testosterone in the kidney (1, 31), and the gene produces multiple mRNAs which differ in the lengths of their 3′ untranslated regions as a result of the alternate use of at least two polyadenylation signals. RP2 may therefore be added to the growing list of genes that produce multiple mRNAs from the same primary transcription unit.

A B1 repetitive element was inserted in the 3′ untranslated region of the RP2 gene during the evolution of DBA inbred mice. This insertion event serves to increase the size of the largest RP2 transcript from 1,950 to 2,150 nucleotides. The sizes of the two small RP2 mRNAs (1,350 and 1,450 nucleotides) are unaffected by the B1 repeat, since the insertion event occurred 3′ to their polyadenylation sites. The high copy number, highly conserved sequence homology, and dispersed nature of the individual members of Alu, B1, and B2 repetitive families have prompted questions of how these sequences became established in their respective genomes. It has been proposed that RNA pol III transcripts from Alu-type repeats may occasionally be reverse transcribed into DNA and inserted back into random breakpoints in the genome (12, 29, 34). This theory is supported by the fact that nearly all the Alu-type repeat sequences found to date (including the B1 insert in the mouse RP2 gene) are flanked by short (6- to 20-bp) direct repeats similar to those created by the insertions of known procaryotic and eucaryotic transposable elements. If only a few of the many repetitive elements are actually transcriptionally active and have been seeding copies of themselves into other locations, this might serve to explain the high degree of sequence conservation (>80%) generally observed between family members. Along these lines, Kalb et al. (13) have been able to divide a collection of B1 elements into two subfamilies on the basis of analysis of specific base differences. The B1 repeat within the RP2 gene falls into one of these subfamilies because it shares five of six diagnostic nucleotides with that group. Data presented here indicate that this particular B1 repeat in

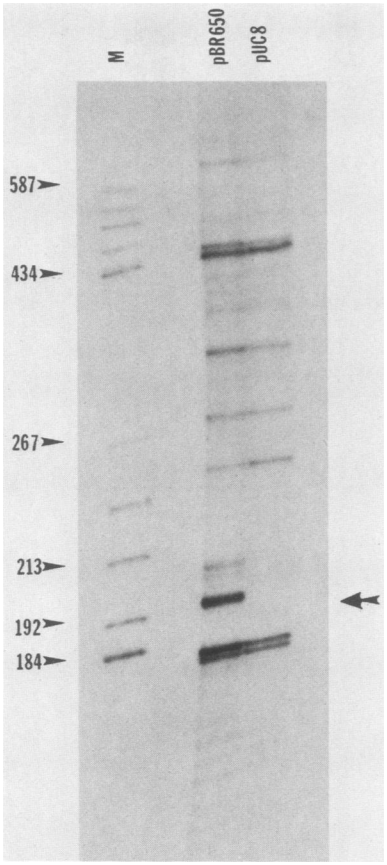

FIG. 8. Primer extension of in vitro-synthesized B1 transcripts. An end-labeled oligonucleotide complementary to the unique sequence just 3′ to the repetitive region of the B1 transcripts was used to prime reverse transcription of the B1 template. The 191-nucleotide product corresponds to the distance from the site of hybridization to the 5′ end of the B1 inserted element in the pBR650 subclone. Primer extension of the transcription products of the vector alone (pUC8) is a negative control.

the RP2 gene can be transcribed by RNA pol III in vitro. If transcription is indeed required for the mobility of Alu-type repeats, then some recently inserted copies might be expected to retain transcriptional activity. The insertion within the RP2 gene in DBA mice has probably occurred recently enough in evolutionary time that the RNA pol III transcription signals have been functionally conserved.

This unusual incidence of an active RNA pol III promoter existing within an RNA pol II transcription unit invites speculation about potential interactions in vivo between these promoters and any transcriptional regulatory elements. Three possibilities can be considered. First, there may be no effect of one promoter on the other, as evidence from genomic blots (unpublished data) indicates that they are separated from each other by at least 5 kilobases in the genome. Second, transcription from one promoter may enhance expression from the other, by way of opening up a chromosomal domain to various transcription factors. It is possible, for example, that increased transcription from the RP2 pol II promoter in the presence of testosterone would allow increased transcription by RNA pol III of an otherwise hormone-insensitive B1 element. Third, expression from the two promoters may actually be antagonistic. That is to say, increased transcription from the pol II promoter may inhibit expression of the pol III promoter located inside the pol II gene. We are currently exploiting the polymorphic mouse strains to pursue these hypotheses further.

The fact that the RNA pol II and pol III promoters happen to be oriented in opposite directions to each other also raises the possibility that their respective RNA products may be able to interact with each other. Inhibition of translation by antisense RNA has already been identified and characterized as a regulatory mechanism in procaryotes (23, 30). Eucaryotic systems have also been manipulated to exhibit this phenomenon (11). In these instances, translation of particular mRNAs has been observed to be inhibited by complementary strand pairing that includes the 5′ terminus. In at least one case, however, hybrid-arrested translation of interleukin-2 in *Xenopus* oocytes was achieved with equal efficiency when the complementary oligonucleotides were derived from the 5′ end, the middle, or the 3′ end of the coding sequence (14). The B1-complementary sequence retained as part of the largest RP2 mRNA may hybridize to B1 RNA in vivo, effectively removing the largest transcript (which amounts to about half of the total RP2 transcripts) from the translatable mRNA pool in DBA mice.

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