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The mouse intracisternal A particle-promoted placental gene retrotransposition is mouse-strain-specific

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ABSTRACT **Insertion of a single long terminal repeat (LTR) of an intracisternal A particle (IAP) gene into a placenta-expressed cellular gene (mouse IAP-promoted placental gene, MIPP) has recently been found for the mouse strains Swiss and CF-1. To test whether such a retrotransposition event has also taken place in other strains of mouse, genomic DNA and total RNAs were analyzed from the outbred CF-1 strain and five inbred strains of mouse, AKR/J, C57BL/6J, DBA/2J, C3H/HeJ, and BALB/c. Specific DNA fragments flanking the LTR region were isolated from MIPP cDNA and genomic clones and used as hybridization probes. Three transcripts [two minor, 4.4 kilobases (kb) and 2.2 kb; one major, 1.2 kb] were detected. The 4.4-kb and 2.2-kb species were found in all strains of mouse studied. The 1.2-kb transcript (promoted by IAP LTR) is present only in placentas of C3H/HeJ and BALB/c mice. Both of these strains contain an IAP LTR in the MIPP gene. In contrast, there is no IAP LTR in this cellular gene in strains DBA/2J, C57BL/6J, and AKR/J. Thus, the IAP MIPP retrotransposition is strain-specific. The parents of the outbred CF-1 mice used for the present studies were both heterozygous at the MIPP locus. Restriction fragment length polymorphism studies using inbred strains and recombinant inbred mice have further established the linkage between the MIPP gene (*D4Jhu8*) and several loci on distal mouse chromosome 4. The symbol *Ipp* is being used for this gene in all major mouse data bases and in the comparative section of the human genome data base.**

The genes coding for intracisternal A particles (IAPs) are members of a disperse multigene family with 1000 copies per haploid genome in *Mus musculus* (1). They are retrovirus-like (2, 3) with 5′ and 3′ long terminal repeats (LTRs) and coding sequences for the *gag* and *pol* domains (4–6) and resemble the Ty elements of yeast and the *copia* genes of *Drosophila* in their ability to retrotranspose (7). The transposition of IAP genes is a genuine source of genetic variability in the mouse.

In addition to directing their own transcription, IAP LTRs have the capability of promoting transcription of adjacent cellular sequences (8–11). Our previous studies have shown that the expression of a placental-specific transcript is under the direction of an IAP LTR (12). The gene coding for the 1.2-kilobase (kb) message with an open reading frame coding for 202 amino acids has a "solo" IAP LTR positioned directly ahead of the mouse IAP-promoted placental gene (MIPP) exon. In this study, the strain specificity of MIPP gene expression was examined. RNA from placentas of five inbred strains was probed for MIPP expression and the genomic organization of these strains was determined by Southern blot analysis.

MATERIALS AND METHODS

Materials. Restriction endonucleases were obtained from New England Biolabs. Isotopes and nylon membranes (Hybond-N) were purchased from Amersham. Chemicals were purchased from Research Organics and Sigma. Subcloned DNA fragment probes were gel-isolated and then radiolabeled by the procedure of Feinberg and Vogelstein (13).

Animals and Tissue Preparation. Outbred CF-1 mice were obtained from Charles River Breeding Laboratories. Inbred strains AKR/J, C57BL/6J, DBA/2J, and C3H/HeJ were purchased from The Jackson Laboratory. BALB/c mice were kindly provided by Richard Cone (The Johns Hopkins University, Baltimore). To obtain placentas, pairs were mated and the day a vaginal plug was found was designated as day 0.5 of gestation. On day 13.5, females were killed by cervical dislocation and the gravid uteri were removed and cut transversely into individual conceptuses and the individual placentas were dissected away from other tissues.

RNA Isolation and Northern Blots. Total cellular RNA was prepared from tissues and cells according to the single-step acid guanidium thiocyanate–phenol/chloroform extraction procedure of Chomczynski and Sacchi (14). RNA was denatured in 50% (vol/vol) dimethyl sulfoxide/2.2 M formaldehyde/20 mM sodium phosphate, pH 6.8, by heating at 65°C for 30 min. The denatured samples were separated on 1.5% agarose gels containing 2.2 M formaldehyde and 20 mM sodium phosphate (pH 6.8), stained in ethidium bromide in 0.05 M sodium phosphate (pH 6.8), destained in the same buffer, photographed, and transferred directly to a nylon membrane. Prehybridization was carried out at 42°C in 5× SSPE (1× SSPE = 0.18 M NaCl/10 mM sodium phosphate, pH 7.7/1 mM EDTA)/50% (vol/vol) formamide/5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinylpyrollidone/0.02% Ficoll/0.02% bovine serum albumin)/0.1% SDS/sheared denatured salmon sperm DNA (20 ng/ml). After 12 h, the prehybridization solution was removed and replaced with a fresh solution of 5× SSPE/50% formamide/2× Denhardt's solution/0.1% SDS/denatured salmon sperm DNA (20 ng/ml), and the radiolabeled probe at 1–3 × 10^6 cpm/ml. Hybridization was carried out at 42°C for 12 h. The filters were washed for two 15-min periods in 2× SSPE/0.1% SDS at room temperature and for two 15-min periods in 0.1× SSPE/0.1% SDS, once at room temperature and once at 50°C. As a control for loading and transfer, the filters were stained in 0.04% methylene blue to visualize the RNA by the method of Herrin and Schmidt (15).

Isolation of DNA and Southern Blots. High molecular weight DNA was prepared from mouse tails or livers. The tissue was minced on dry ice, transferred to 50 mM Tris·HCl, pH 7.5/0.1 M NaCl/25 mM EDTA/1% SDS/proteinase K (150 μg/ml), and digested overnight at 55°C. The tissue digest was extracted once with buffer-saturated phenol; once with phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol); and once with chloroform/isoamyl alcohol, 24:1 (vol/vol). The

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Abbreviations: LTR, long terminal repeat; IAP, intracisternal A particle; RFLP, restriction fragment length polymorphism; RI, recombinant inbred; SDP, strain distribution pattern; MIPP, mouse IAP-promoted placental gene.

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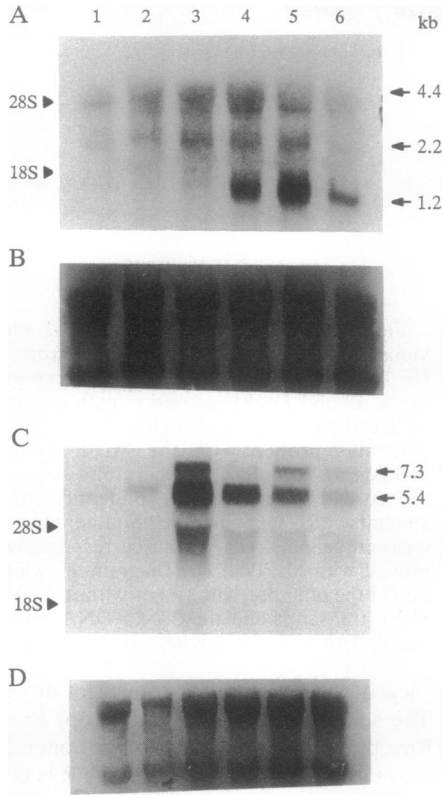

FIG. 1. Northern blot analysis of MIPP and IAP transcripts among laboratory mouse strains. Total RNA (25 μg) from 13.5-day placentas of each strain was loaded and probed with MIPPd4 (*A*) and 1.4-kb IAP *Bam*HI fragment (*C*). (*B* and *D*) Methylene blue staining of corresponding filters to monitor loading and transfer. Sizes of hybridizing transcripts are noted by arrows and the position of rRNA migrations are indicated. Lanes: 1, AKR/J; 2, C57BL/6J; 3, DBA/2J; 4, C3H/HeJ; 5, BALB/c; 6, CF-1.

DNA was precipitated with 2 vol of ice-cold 95% ethanol, spooled onto a glass rod, and washed twice in ice-cold 80% ethanol. After drying under vacuum, the DNA was resuspended in 10 mM Tris·HCl/0.1 mM EDTA, pH 7.5. The DNA was digested for 16 h with a 5-fold excess of restriction endonuclease according to manufacturer's recommended

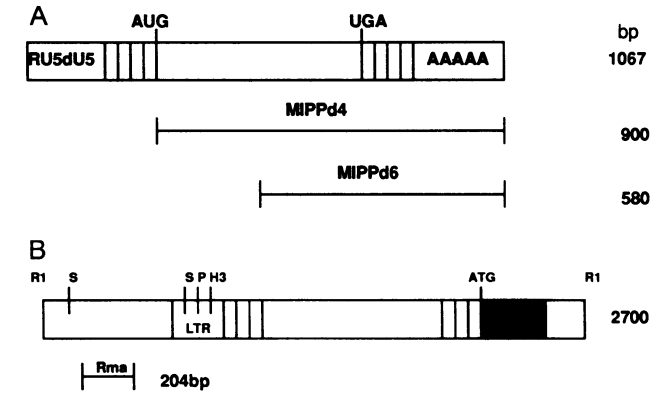

FIG. 2. Schematic representation of MIPP mRNA and MIPP gene. (*A*) MIPP cDNA clone. The untranslated regions are represented by open vertical bars. Hybridization probe MIPPd4 consists of the MIPP cDNA sequence without the LTR and 5′ untranslated region and probe MIPPd6 contains only the 3′ cDNA sequence used in RFLP studies. (*B*) A 2.7-kb *Eco*RI restriction fragment of a genomic MIPP clone showing the position of the IAP LTR insertion. Exon (solid box) and intron (open box) regions. RI, *Eco*RI; S, *Sty* I; P, *Pst* I; H3, *Hin*dIII. Hybridization Rma 1 probe, consisting of a 204-bp *Pvu* II–*Rma* I unique DNA fragment 5′ to the IAP LTR.

buffer conditions and subjected to electrophoresis on 1% agarose gel in 40 mM Tris acetate/1 mM EDTA. Southern blot analysis was performed as follows: prehybridization was carried out at 65°C for 2 h in 5× SSPE/5× Denhardt's solution/0.5% SDS/sheared denatured salmon sperm DNA (20 ng/ml). Hybridization was carried out at 65°C for 12 h in the same solution with the addition of the denatured labeled probe at 1×10^6 cpm/ml.

RESULTS

Detection of MIPP-Specific Transcripts in Certain Strains of Mouse. We have shown (12) that MIPPd4 detects three transcripts in placental RNA from outbred CF-1. The major transcript is a 1.2-kb MIPP-specific RNA species that we have found to be promoted by a solo IAP LTR. Two larger 4.4-kb and 2.2-kb MIPP-related species were also present but at a much reduced hybridization intensity. To help determine when the proposed IAP retrotransposition event occurred and whether these RNA species are present in different

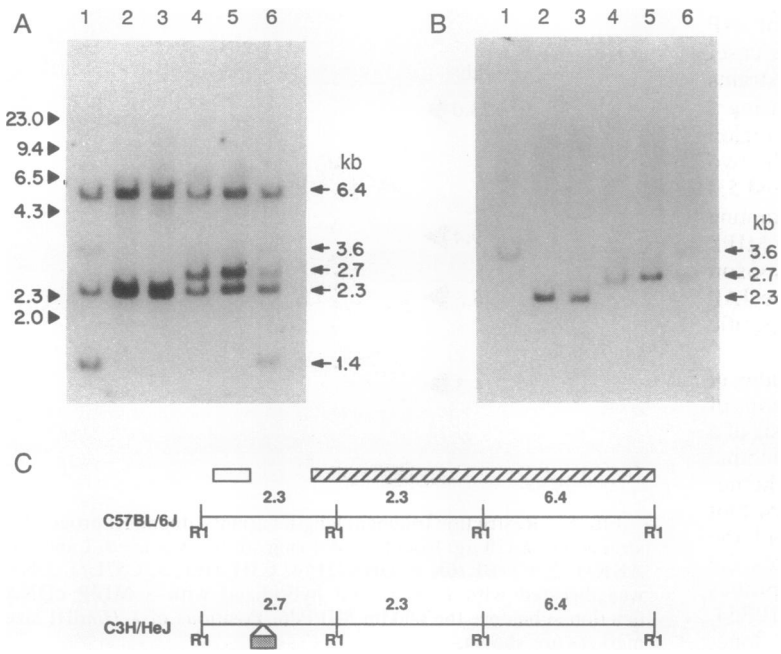

FIG. 3. Southern blot of genomic DNAs from six laboratory mouse strains digested with *Eco*RI and probed by the MIPPd4 probe (*A*) and the Rma probe (*B*). (*C*) Hybridization region detected by MIPPd4 (hatched box) and by Rma probe (open box) and insertion sequence (stippled box). (*A*) The positions of a *Hin*dIII-digested λ marker are shown at the left. The sizes of the hybridizing bands in both blots (*A* and *B*) are indicated by the corresponding arrows at the right. Lanes: 1, AKR/J; 2, C57BL/6J; 3, DBA/2J; 4, C3H/HeJ; 5, BALB/c; 6, CF-1.

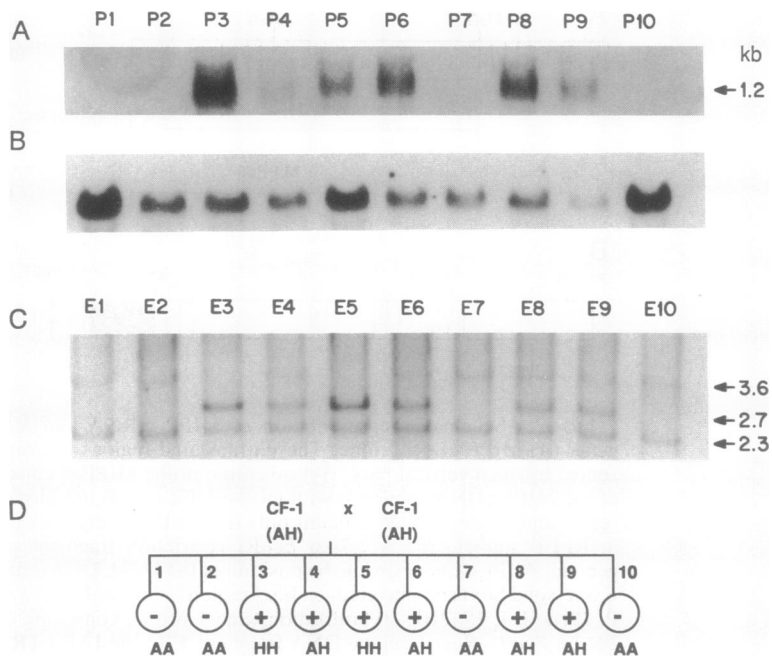

FIG. 4. Segregation of the two CF-1 alleles. Individual conceptuses (numbered 1–10) from a CF-1 × CF-1 mating were analyzed for MIPP expression (placenta, P) and *Eco*RI restriction pattern (embryo, E). (*A*) Northern blot analysis of total RNA (25 μg) from 10 placentas probed with the MIPP-specific probe MIPPd4. (*B*) Identical Northern blot stripped and reprobed with a γ-actin probe to identify differences in amounts of RNA per lane. (*C*) Southern blot of *Eco*RI-digested genomic DNA from corresponding embryos probed with MIPPd4. (*D*) Diagram showing segregation of the offspring with (+) or without (−) the 1.2-kb MIPP transcript and the 2.7-kb DNA fragment containing IAP LTR. A, AKR/J; H, C3H/HeJ.

strains of mouse, placental RNA from five inbred strains (AKR/J, C57BL/6J, DBA/2J, C3H/HeJ, and BALB/c) was analyzed. Total cellular RNAs were prepared from 13.5-day placentas from these inbred strains and also from the outbred CF-1 strain. The RNAs were electrophoresed, blotted onto a nylon filter, and probed with the MIPPd4 (see Fig. 2*A*). The filters were stained with methylene blue to monitor the RNA loading and transfers (Fig. 1*B*). The results of the Northern blot analysis (Fig. 1*A*) revealed that although low levels of the 4.4-kb and 2.2-kb transcript can be seen in all of the strains, only two inbred strains, C3H/HeJ and BALB/c, express the LTR-promoted 1.2-kb MIPP transcript characterized in the outbred CF-1 strain. Even upon overexposure of the autoradiogram, the 1.2-kb transcript could not be seen in AKR/2J, C57BL/6J, or DBA/2J mice. Thus the 1.2-kb placental transcript is mouse-strain-specific. In contrast, the two MIPP-related 4.4-kb and 2.2-kb species are conserved for all mouse strains studied.

The strain specificity observed for the IAP-LTR-driven transcript may be the result of a strain-specific retrotransposition event or a strain difference in the strength of IAP LTR promoter. To determine whether the latter is the case, equal amounts of placental RNAs from these inbred strains were probed for IAP gene expression (Fig. 1*C*) by using a 1.4-kb *Bam*HI fragment that is specific for the internal region of the IAP gene (16). All placental RNAs contained the two major IAP transcripts with measured sizes of 7.2 kb and 5.4 kb. DBA/2J contains the highest amount of IAP RNA among the stains tested, yet it does not express the 1.2-kb MIPP transcript. Therefore, a strain-specific retrotransposon event, rather than a difference in the strength of the IAP promoter, is likely to be responsible for the strain-specific MIPP transcription.

MIPP Gene Structure Is Found Only in Certain Strains of Mouse. The presence of a solo IAP LTR located in front of the MIPP exon was confirmed (12) by sequence analysis of a MIPP cDNA clone from CF-1 strain and a MIPP genomic clone isolated from a Swiss mouse library. To test whether this genomic configuration is strain-specific, Southern blot analysis of DNA from these five inbred strains and the outbred CF-1 strain was performed. Fig. 2 illustrates the DNA organization of the cloned MIPP gene and the probes used for the hybridization studies. The first probe, MIPPd4, is a DNA fragment derived from the MIPP cDNA clone, containing nearly the entire MIPP transcript downstream of the LTR. The second is a 204-base-pair (bp) *Pvu* II–*Rma* I fragment (Rma probe, Fig. 2*B*) that lies in a noncoding unique region upstream of the LTR. The MIPP LTR is contained in a 2.7-kb *Eco*RI fragment from the genomic clone (12). When genomic DNAs from the different strains were digested with *Eco*RI and probed with MIPPd4, a 2.7-kb band was seen in the 1.2-kb MIPP expressing strains C3H/HeJ, BALB/c, and the outbred CF-1, but not in C57BL/6J and DBA/2J mice (Fig. 3*A*). The latter two strains display a hybridization band of 2.3 kb. The MIPPd4 probe contains nearly the entire transcript, which accounts for the other hybridizing bands. The 204-bp *Pvu* II–*Rma* I Rma fragment reveals a simpler

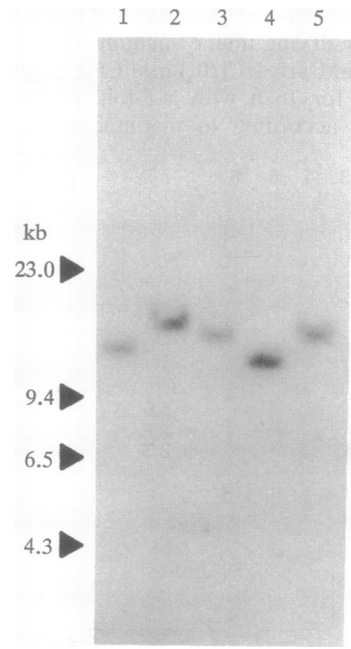

FIG. 5. Restriction fragment length variants among RI progenitor strains. DNA (10 μg) from the following strains was used. Lanes: 1, AKR/J; 2, C57BL/6J; 3, DBA/2J; 4, C3H/HeJ; 5, C57L/J. DNA was digested with *Hin*dIII and hybridized with a MIPP cDNA deletion subclone, the 600-bp MIPPd6. Positions of λ *Hin*dIII size markers are shown.

Table 1. SDPs of *D4Jhu8* and chromosome 4 loci for RI strain set AKXL

Locus	5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38
D4Jhu8		A	A	L	L ×		L	L	L	A	L	A ×	L		L	A	A	L
Pmv-19		A	A	L	A	L	L	L	L	A	L	L	L	A	L	A	A	L
Mpmv-19		A	A	L	A	L	L	L	L	A	L	L	L	A	L	A	A	L

Alleles of *D4Jhu8* are defined by RFLPs detected by the MIPPd6 probe (see text). The SDPs of the other loci are as described by Blank *et al.* (19). Recombinations are indicated by a ×.

hybridization pattern. In *Eco*RI-digested DNA, a 2.7-kb band is seen in C3H/HeJ and BALB/c, whereas a 2.3-kb band is seen in C57BL/6J and DBA/2J (Fig. 3*B*). The absence or presence of the 392-bp IAP LTR in the MIPP gene could explain the size difference between the two hybridizing *Eco*RI bands. *Eco*RI-digested DNA from the AKR/J strain contains neither the 2.3-kb nor the 2.7-kb fragments. Instead a larger 3.6-kb hybridizing band is found. This band (along with the 2.7-kb band) is also present in the DNA of the outbred CF-1 mice.

Restriction Sites Specific for the MIPP IAP LTR Are Present in C3H/HeJ but not in C57BL/6J Mice. Several restriction sites occur once in the IAP LTR of the cloned MIPP gene (Fig. 2*B*). Genomic DNA from C3H/HeJ mice (which express the 1.2-kb LTR-driven transcript) and C57BL/6J mice (which do not express the 1.2-kb transcript) was digested with each of these LTR-specific restriction enzymes (*Hin*dIII, *Pst* I, or *Sty* I) and probed with either MIPPd4 or Rma. The results of this Southern blot analysis revealed that in each case the C3H/HeJ allele, and not the C57BL/6J allele, carried the additional restriction site diagnostic for the presence of the MIPP IAP LTR (data not shown).

Segregation of the Two CF-1 Alleles. Data from the Southern blot hybridization analysis of *Eco*RI-digested genomic DNA suggest that the parent CF-1 mouse harbors two alleles: one that resembles the allele present in strains that do not express MIPP (e.g., AKR/J), and another allele characteristic of MIPP-expressing strains (e.g., C3H/HeJ and BALB/c) (Fig. 3). To confirm the heterozygosity of the CF-1 mouse, individual conceptuses from a CF-1 × CF-1 mating were analyzed for placental expression of MIPP and *Eco*RI Southern blot hybridization pattern. A Northern blot of total RNA from each of 10 placentas was probed with MIPPd4. Whereas 6 of the 10 placentas were positive for the 1.2-kb LTR-driven transcript, the remaining 4 showed no evidence of MIPP-specific expression (Fig. 4*A*). Although all strains contain the 2.2-kb and 4.4-kb MIPP-related species (data not shown), DNA isolated from the embryos of each conceptus were probed with MIPPd4 and confirmed that the MIPP-expressing conceptuses carried both homozygous (AA or HH) and heterozygous (AH) alleles. Since both types of homozygotes (A and H) are seen in the offspring (Fig. 4 *C* and *D*), these results indicate that the two CF-1 alleles segregate and the CF-1 parents of this particular cross were both heterozygous.

Mapping of the MIPP Gene to the *Ipp* Locus on Chromosome 4. The 580-bp MIPPd6 fragment (MIPPd6, Fig. 2*A*) was found to detect restriction fragment length polymorphisms (RFLPs) among inbred strains of mouse, including the progenitor strains of recombinant inbred (RI) mice. RI strains are derived by crossing two highly inbred strains and inbreeding their offspring to homozygosity by brother × sister matings until the F_{20} generation and beyond is reached. Strains of an RI strain set are developed in parallel from the F_2 generation. RI strains are useful in linkage analysis because, during inbreeding, unlinked genes are randomized with respect to progenitor origin, while linked genes tend to stay together and become fixed with respect to progenitor origin. Therefore, comparison of the strain distribution patterns (SDPs) of alleles of an unmapped locus to those of previously mapped loci can infer chromosomal location and give an estimate of linkage (17–19). Likewise, a comparison of the SDP of the RFLPs detected by the MIPPd6 fragment and the SDPs of alleles of other loci within RI strain sets can indicate the location of its corresponding locus (provisionally named *D4Jhu8*). Digestion with *Hin*dIII distinguishes the following fragments in RI progenitor strains: A, AKR/J, 11 kb; B, C57BL/6J, 13 kb; D, DBA/2J, 12.1 kb; H, C3H/HeJ, 10.3 kb; L, C57L/J, 11.8 kb (Fig. 5). The SDPs of the MIPPd6 fragment were determined in three RI strain sets (AKXL, AKXD, and BXH). These SDP comparisons (Tables 1–3) indicated linkage (Table 4) between *D4Jhu8* and several loci on distal mouse chromosome 4.

DISCUSSION

In this study, we have shown that only certain strains of mouse carry the MIPP IAP LTR and conclude that this is the reason that MIPP gene expression is strain-specific. We have proposed (12) that the presence of the LTR is the result of an IAP retrotransposition event. This event must have been a recent one since, of the inbred strains studied, only C3H/HeJ and BALB/c possessed the MIPP LTR. Based on the known geneology of inbred strains, it is possible that this retrotransposition occurred in the ancestry of Bagg's Albino mice (20).

The solo IAP LTR arrangement present in the mouse strains expressing the 1.2-kb transcript presumably arose from a germ-line retrotransposition of a complete IAP element with the subsequent removal of the body of the IAP element after homologous recombination between the two IAP LTRs. The presence of the solo LTR was detected by Southern blot analysis of *Eco*RI-digested DNA. It is conceivable that some mouse strains have the complete IAP element at the MIPP locus. Whether the unique 3.6-kb *Eco*RI hybridization band seen in strain AKR/J represents such an organization or is simply an RFLP has not been determined. It would be interesting to examine other mouse strains to see whether the intact IAP element is present and whether this configuration still allows for MIPP expression.

MIPP is one of only a few germ-line retrotranspositions described in the mouse. One of the renin genes, *Ren-2*, contains an IAP genome (MIARN) in its 3′ flanking region

Table 2. SDPs of *D4Jhu8* and chromosome 4 loci for RI strain set AKXD

Locus	1	2	3	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28
D4Jhu8		A ×		D	D ×	D	D	A	D	A	D	D	D	D		D	D	A		D	A	D	D		
Ck-1	D	D	D	D	A	D	D	A	D	A	D	D	D	D		D	D	A	A	D	A	D	D	D	D
Lymc-1	D	D	D	D	A	D	D	A	D	A	D	D	D	D	D	D	D	A	A	D	A	D	D	D	D

For details, see Table 1.

Table 3. SDPs of *D4Jhu8* and chromosome 4 loci for RI strain set BXH

Locus	2	3	4	6	7	8	9	10	11	12	14	19
Pmv-19	H		H	B	B	B	H	H	H	B	B	H
				×								
Scl	H		H	H	B	B	H	H	H	B	B	H
								×				
D4Jhu8	H		H	H	B	B	H	B	H	B	B	H
					×						×	
Ck-1	H		H	H	H	B	H	B	H	B	H	H
Mpmv-19	H		H	H	H	B	H	B	H	B	H	H

For details, see Table 1.

(21) and the angiotensinogen gene is also closely linked to an IAP element (22). The renin and the angiotensinogen IAP transpositions also represent recent events as they are limited to certain strains. However, they must have occurred at different times and within a different ancestry than that of the MIPP retrotransposition, as their pattern of distribution is different. Any direct effect to the renin or angiotensinogen gene by the IAP transposition remains to be determined.

Compared to the other mouse germ-line IAP retrotranspositions, MIPP is unique in that it acts as a promoter, directing the expression of a gene transcript with an open reading frame of 202 amino acids. The peptide encoded by the MIPP gene has homology to the C terminus of proteins encoded by the *Drosophila* kelch gene (L. Cooley, personal communication) and a family of vaccinia virus genes (23). The kelch gene appears to be necessary for growth of the oocyte during oogenesis in *Drosophila* (24). Since MIPP expression is limited to certain strains of mouse, the MIPP gene product by itself may not fulfill a critical function. Two larger MIPP-related transcripts are found in all of the inbred strains examined. One of these may encode a kelch-like gene. It seems likely that the MIPP LTR may have transposed into an intron of this larger gene without disrupting the reading frame. Because the IAP LTR is active in the placenta, this transposition now causes tissue-specific expression of a truncated kelch-like transcript. Since the protein reading is conserved, it is possible that this truncated version may still retain its functional properties.

IAP genes are a potentially important cause of genetic variability in mice. Since germ-line IAP retrotransposition events are relatively stable and can be inherited in classical Mendelian fashion, they contain information that can be useful in phylogenetic analysis. Such a study has been reported (17) using known integration sites of endogenous retroviruses. Transposed IAP elements can be considered as a type of transgene. Cases like MIPP, therefore, also present us with the opportunity to examine other phenomena, e.g., parent-specific expression and DNA methylation patterns (imprinting).

Table 4. Linkage of *D4Jhu8* with chromosome 4 loci

Locus	No. SDP differences/no. total strains compared	Recombination distance	95% limits
Ck-1	4/30	4.17	1.00–14.24
Pmv-19	4/26	5.00	1.17–18.28
Mpmv-19	4/26	5.00	1.17–18.28
Lymc-1	5/26	6.76	1.82–24.01

Data from Tables 1–3 were analyzed by Bayesian statistics as described by Silver (20) and Taylor (18). The recombination distance is the estimated percent recombination in a single meiosis. The 95% limits are the lower and upper recombination percentages at a 95% confidence limit.

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