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Molecular Cloning and Long Terminal Repeat Sequences of Intracisternal A-Particle Genes in *Mus caroli*

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We isolated DNA clones of intracisternal A-particle (IAP) genes from the genome of an Asian wild mouse, *Mus caroli*. A typical *M. caroli* IAP gene was 6.5 kilobase pairs in length and had long terminal repeat (LTR) sequences at both ends. The size of the LTR was 345 base pairs in clone L20, and two LTRs at both ends of this clone were linked to directly repeating cellular sequences of 6 base pairs. Each LTR possessed most of the structural features commonly associated with the retrovirus LTR. The restriction map of the *M. caroli* IAP gene resembled that of *Mus musculus*, although the *M. caroli* IAP gene was 0.4 kilobase pairs shorter than the *M. musculus* IAP gene in two regions. Sequence homology between the *M. caroli* and *M. musculus* IAP LTRs was calculated as about 80%, whereas the LTR sequence of the Syrian hamster IAP gene was about 60% homologous to the *M. caroli* LTR. The reiteration frequency of the *M. caroli* IAP genes was estimated as 200 to 400 copies per haploid genome, which is at least 10 times the reported value. These results suggest that the IAP genes observed in the genus *Mus* are present in multiple copies with structures closely resembling the integrated retrovirus gene.

Intracisternal A-particles (IAPs) are retrovirus-like structures consistently observed in early embryos (1–3) and in a variety of tumor cells (28) of laboratory mice (*Mus musculus*). Since these particles bud from the endoplasmic reticulum and remain within the cisternae, no infectivity of IAPs has been reported (7, 13). Retrovirus-like intracisternal particles are often observed in rodent cells and sometimes in other mammals (17). Mouse IAPs contain a major protein of 73,000 daltons (22), a reverse transcriptase (29), and polyadenylated RNA molecules (IAP RNAs) (12, 20, 22). Morphologically and biochemically, IAPs have retrovirus-like features, but IAP RNAs have no apparent sequence homology with either type B or type C murine retrovirus RNAs (15), although the RNA of a certain class of retroviruses endogenous to Asian wild mice, *Mus cervicolor*, has been reported to share partial sequence homology with the IAP RNA (4, 11).

DNA sequences homologous to IAP RNAs (IAP genes) are present in ca. 1,000 copies per haploid *M. musculus* genome (14, 20, 24), and these genes appear to be interspersed throughout the chromosomes (14). This great number of IAP genes is at least 20 times more than that of any other type of murine endogenous retrovirus gene. Sequences homologous to *M. musculus* IAP genes were widely distributed in most rodent species and in some mammals (17). The molecularly cloned IAP gene from *M. musculus* was 7.2 to 7.3 kilobase pairs (kb) in length (5, 12) and contained long terminal repeat (LTR) sequences of about 0.35 kb at both ends of the gene (5, 6, 9, 10, 12). IAP genes isolated from Syrian hamsters (*Mesocricelus auratus*) were 7.6 kb in length (18, 25) with LTRs of about 0.35 kb (21) and were repetitively present, as in *M. musculus* IAP genes (18, 25). Recently Lueders and Kuff reported the presence of several hundred IAP genes per haploid genome of two rat species (18). From these results we speculate that from several hundred to one thousand IAP genes with LTR sequences of a distinct class at both ends may be dispersed throughout the genome of the rodent.

One exception, however, is that the reported number of IAP genes in Asian *Mus* species, *M. caroli* and *M. cervicolor*, was found to be 25 to 30 copies per haploid genome (11). We were quite interested in the IAP genes present in these two *Mus* species because the much smaller number of IAP genes seemed to make examination of the structure, expression, and translocation of IAP genes easier. However, the reason for the difference in the number of IAP genes among the three *Mus* species remains unclear. An attempt was made to isolate IAP genes from *M. caroli* and *M. cervicolor*. The cloned *M. caroli* IAP gene was 6.5 kb in length with LTRs of 0.35 kb, but unexpectedly, the number of IAP genes present in *M. caroli* and *M. cervicolor* was found to be as at least 10 times the reported value (11).

MATERIALS AND METHODS

Materials. Restriction endonucleases, T_4 ligase, and terminal deoxynucleotidyl transferase were obtained from Takara Shuzo Co., and digestion of endonucleases was carried out according to the instructions of the supplier. Asian wild mice, *M. caroli* and *M. cervicolor*, were kindly donated by K. Moriwaki of the National Institute of Genetics, Mishima, and Y. Ikawa at the Cancer Institute, Tokyo.

Preparation of DNAs and filter hybridization. Preparation of high-molcular-weight DNAs from livers and kidneys, agarose gel electrophoresis, Southern transfer, filter hybridization, and nick translation were carried out as described previously (20, 25). After hybridization, the filter was washed four times (10 min per wash) in 0.3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate at 65°C.

Construction and screening of gene libraries. The gene libraries of *M. caroli* and *M. cervicolor* kidney DNAs were prepared as described previously (20, 25). The 13- to 17-kb DNAs prepared by size fractionation on a sucrose gradient

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after partial *Eco*RI digestion were used for in vitro packaging with Charon 4A arms. By using 1.1 μg of insert DNAs and 3.0 μg of Charon 4A arms, the number of independent plaques was found to be 2.0×10^6 for *M. caroli* and 2.8×10^6 for *M. cervicolor*. After amplificaton of the independent plaques, more than 95% of the plaques were determined as recombinants. The gene libraries were screened, and individual clones were plaque purified. Lambda clones containing *M. caroli* IAP genes were digested with *Eco*RI and subcloned into *Eco*RI-digested pBR322.

Reassociation kinetic analysis. Reassociation of ^{32}P-labeled IAP DNAs with total cellular DNAs was carried out (20) in 20 mM Tris-hydrochloride (pH 7.5) containing 1 M NaCl and 1 mM EDTA at 67°C. The rate of reassociation was measured by S1 nuclease (Sankyo Co.) digestion at 45°C for 30 min in 30 mM sodium acetate (pH 4.6) containing 0.15 M NaCl, 1 mM $ZnSO_4$, and 10 μg of heat-denatured calf thymus DNA per ml (type I; Sigma Chemical Co.). A nonrepetitive DNA fraction of BALB/c liver which did not anneal at a C_0t of 200 was prepared by hydroxyapatite chromatography. The single-stranded DNAs, eluted with 0.1 M sodium phosphate (pH 6.8), were recovered as nonrepetitive DNAs. After the total cellular DNAs were annealed with ^{32}P-probes to a C_0t of 10^4, reassociated DNAs were heated in 20 mM Tris-hydrochloride (pH 7.5) containing 0.1 M NaCl and 1 mM EDTA, and thermal denaturation was assayed by S1 nuclease.

DNA sequencing analysis. DNA fragments for sequencing analysis were labeled at the 3′ end with cordycepin-5′-[α-^{32}P]triphosphate (Amersham Corp.) and terminal deoxynucleotidyl transferase (27). The end-labeled fragments were analyzed according to the method of Maxam and Gilbert (19).

RESULTS

Organization of *M. caroli* IAP genes. DNA fragments (probes I, II, and IV, see Fig. 1D) prepared from a cloned *M. musculus* IAP gene, pS81A (5), were used for the detection of IAP genes in the gene libraries. Judging from the average number obtained from each of three experiments in which 1.5×10^4 plaques were screened, the frequencies of reactive plaques with probe I, which contained most of the *M. musculus* IAP gene and a 0.9-kb flanking sequence, were 0.51%, 0.21%, and 0.30% in the BALB/c, *M. caroli*, and *M. cervicolor* gene libraries, respectively. Since nearly all the reactive plaques with probe I were also capable of being hybridized with probe IV generated from probe I and contained no cellular flanking sequence, it may be concluded that these plaques contain at least some sequences homologous to the *M. musculus* IAP gene.

Taking the ratio of recombinant plaques, haploid genome size of *Mus* species, and average length of the insert DNA into consideration (25), the number of IAP genes possessing a certain part of the probe I sequence was calculated as 970 copies per BALB/c, 400 copies per *M. caroli*, and 570 copies per *M. cervicolor* haploid genome. The calculated number of IAP genes in the BALB/c genome was in good agreement with that obtained by reassociation kinetics (14) and filter hybridization (24), whereas 1% of a BALB/c embryo gene library was reported to hybridize with a cloned IAP gene probe (16) (see also Table 1). Among the reactive plaques with probe I, the proportion of the reactive plaques with probe II was 72% in BALB/c, 80% in *M. caroli*, and 84% in *M. cervicolor*. Since about 30% of cloned *M. musculus* IAP genes lacked this sequence (20), nearly 20% of both the *M. caroli* and *M. cervicolor* IAP genes must lack the sequence corresponding to probe II.

From the *M. caroli* gene library, recombinant phages containing the sequence hybridizable with probe I were isolated as clones of *M. caroli* IAP genes. DNAs prepared from plaque-purified clones were digested with *Eco*RI and analyzed by the Southern hybridization technique. *Eco*RI fragments hybridized with probe I were subcloned from Charon 4A into pBR322, and the subclones were aligned according to the common restriction enzyme sites to give preliminary restriction maps of *M. caroli* IAP genes (Fig. 1A). By comparison of a restriction map of a typical *M. musculus* IAP gene (Fig. 1C) with those of *M. caroli* IAP genes (Fig. 1A), a restriction map of a typical *M. caroli* IAP gene was made (Fig. 1B).

Among seven clones, a full copy of the IAP gene was present in clones L3, L20, and L21. Two clones, L1 and L26, must both be the deletion type since the DNA prepared from these clones hybridized weakly with probe II but strongly with probe I and IV and had common restriction maps with the 3′ end of the *M. caroli* IAP gene. Since the 5′ end of four clones (L15, L13, L26, and L1) was interrupted by a Charon 4A arm, it cannot be said for certain that absence of the 5′ LTR sequence in these clones results from the deletion occurring at the beginning or some accidental cloning artifact.

The restriction maps inside the *M. caroli* IAP genes were mutually homologous but not always identical among the clones L3, L21, L15, L20, and L13. Also, each sequence positioned inside a particular IAP gene was highly homologous to the corresponding sequence in other IAP clones. The sequences outside the IAP genes were so heterogeneous, as reported in other IAP gene clones (16, 20, 25), that these genes must be located dispersedly on many chromosomes.

Although the *M. caroli* IAP gene was 0.5 kb shorter than the *M. musculus* gene in two regions, a and b, the restriction maps of the two IAP gene families closely resembled each other.

Sequence organization of LTRs and their flanking regions. As shown in the *M. musculus* and Syrian hamster IAP genes (9, 10, 21), the *M. caroli* IAP gene seemed to have LTR sequences at both ends. The presence of the LTR sequence of the *M. caroli* IAP gene was supported by an experiment in which the putative LTR regions of all the cloned *M. caroli* IAP genes cross-hybridized with each other (data not shown).

To elucidate the fine structure of the *M. caroli* LTR sequence, the nucleotide sequence of the LTR regions of the clone, L20, was determined. A restriction map and sequencing strategy of the L20 clone are shown in Fig. 2A, and the sequence of the LTRs and their flanking regions are presented in Fig. 2B. The length of the LTRs was 345 base pairs (bp), but one base substitution was observed at position 215. At the ends of the LTR, an inverted repeat sequence of 3 bp, 5′TGT---ACA3′, was observed. This inverted sequence was identical to that found in the Syrian hamster LTR (21) but was one base shorter than that of the *M. musculus* LTR (9, 10).

The sequence CATAAAA, starting at position 194, corresponded to the so-called "TATAA" box presumed to be a promotor sequence for RNA polymerase II. The sequence CCAAT, corresponding to another presumed promotor, the "CAT" box, was observed at intervals of 35 bp upstream from the TATAA box. As was pointed out previously, the distance between the CAT and TATAA boxes was nearly always the same among the LTRs cloned from the two *Mus*

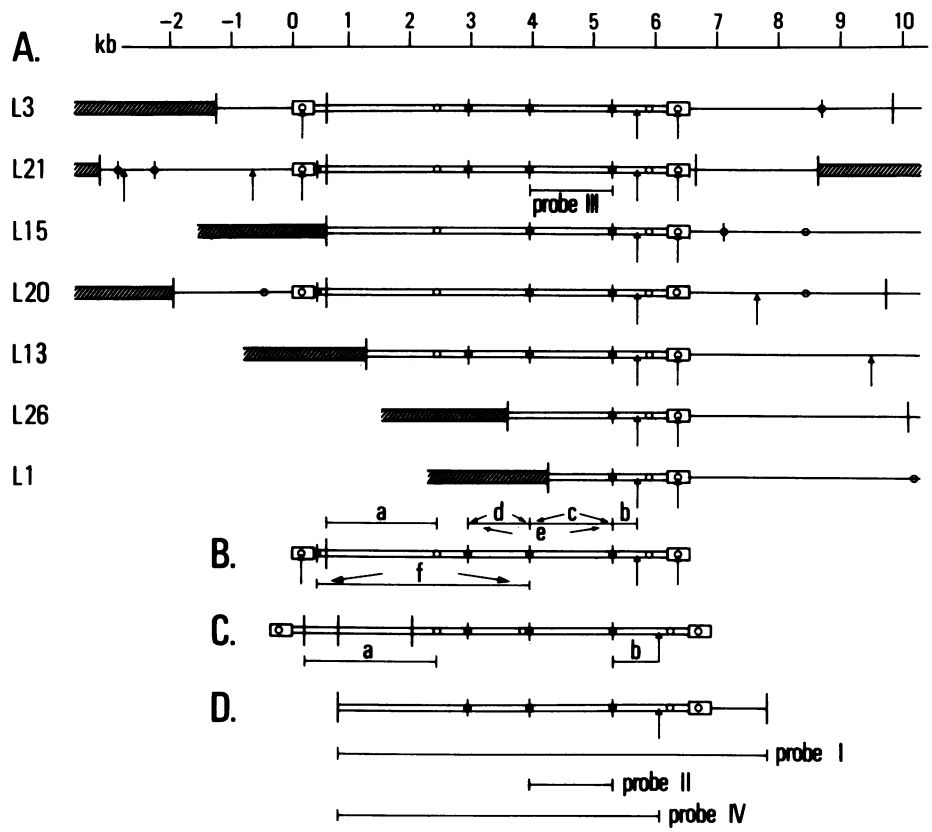


FIG. 1. Restriction map of cloned *M. caroli* IAP genes. (A) Relative positions of the restriction enzyme sites were determined by digestion of subcloned DNAs with *Eco*RI (|), *Bam*HI (●), *Hin*dIII (↑), and *Pst*I (○). These cloned sequences were aligned according to the common restriction enzyme sites. The linkage of each *Eco*RI fragment was determined by *Bam*HI, *Hin*dIII, or *Pst*I digestion of the lambda DNA and hybridization with probe I. A restriction map of a typical *M. caroli* IAP gene (B) was deduced by comparison of a restriction map of a *M. musculus* IAP gene (C) with those of *M. caroli* IAP genes (A). The 5′ end of the gene is situated on the left. (D) A restriction map of a *M. musculus* IAP gene, pS81A (5), whose fragments were used as probes. Symbols: ——, flanking cellular sequences in IAP clones; □, LTR; ▨, Charon 4A arm.

species and the Syrian hamster genes (21). A typical polyadenylation signal, AATAAA, was present and situated at intervals of 65 bp downstream from the TATAA box, and a presumed polyadenylation site, CA, was situated at intervals of 10 bp downstream from the polyadenylation site.

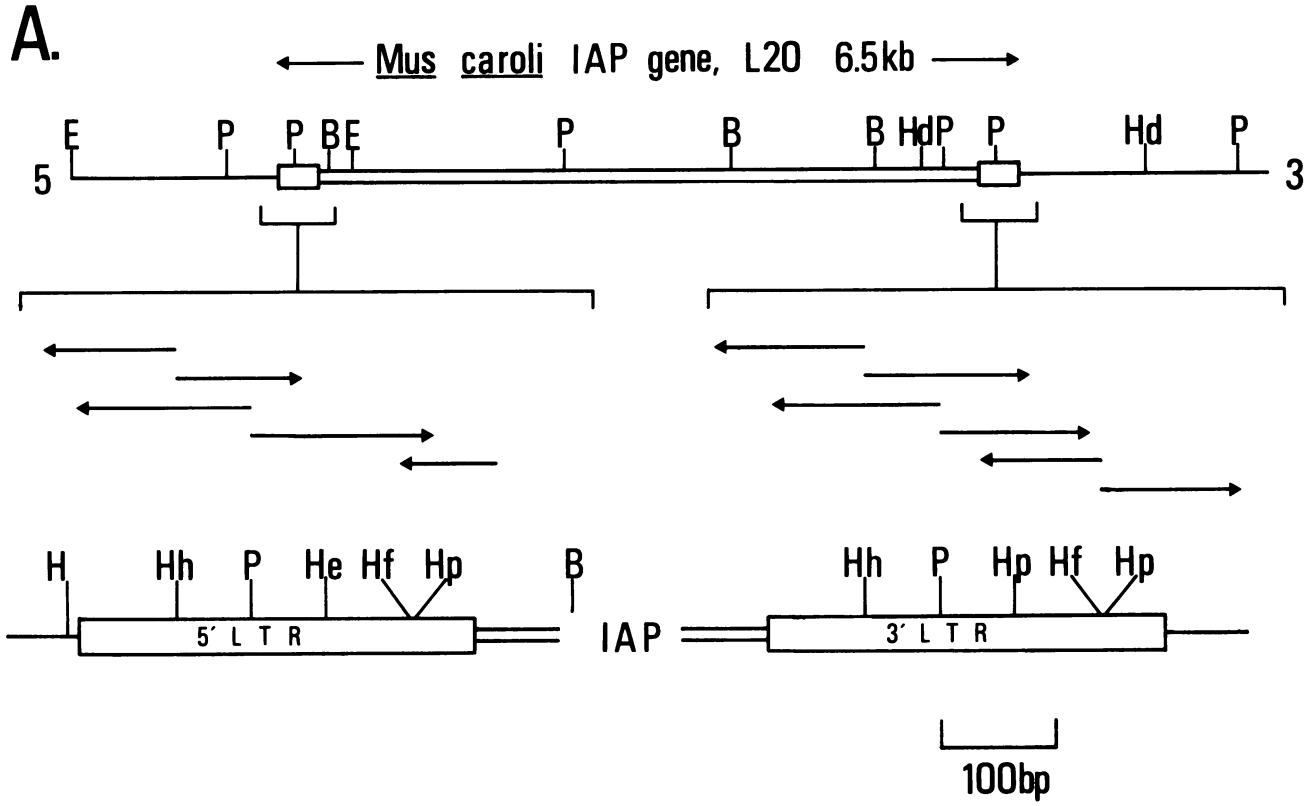
The LTRs at both ends of the IAP gene were linked directly to repeating cellular sequences of 6 bp. The purine-rich sequence of 17 bp was positioned immediately upstream from the 3′ LTR. As in the case of other IAP LTRs so far sequenced, these results indicate that the LTR sequence of the *M. caroli* IAP gene contains most of the structural features common to the retrovirus LTR (26). Since the length of the LTR was 0.35 kb and the distance between the two *Pst*I sites in the LTR, mapped at positions 148 to 153, was 6.10 kb, the size of a typical *M. caroli* IAP gene, L20, was calculated as 6.45 kb.

Comparison of LTR sequences derived from three rodent species. Recently the nucleotide sequences of the LTR region of two *M. musculus* IAP genes, MIA14 (10) and rc-*mos* (9), have been reported. Furthermore, we determined the LTR sequences of two Syrian hamster IAP genes, H10 and H18 (21). These LTRs were about 350 bp in size and possessed all the features commonly observed in the retrovirus LTR.

To elucidate the interrelationship of the IAP genes cloned from three phylogenetically distant rodent species, the sequence homology among these LTR was investigated (Fig. 3). The sequence homology between two LTRs was calculated as the percentage of the number of identical nucleotides divided by that of all the nucleotides from which deleted or inserted nucleotides were eliminated. The LTR sequence of L20 was about 80% homologous to both rc-*mos* and MIA14 (data not shown) LTRs but about 60% homologous to H10. This value of 60% was the same as the degree of homology between H10 and two *M. musculus* LTR sequences (21).

As was previously pointed out (21), highly homologous regions between L20 and H10 LTR were dispersed on the LTRs. One region was situated between the 5′ end and the CAT box, and another was situated almost between the polyadenylation signal and polyadenylation site.

Estimation of the number of *M. caroli* IAP genes. Since the number of either the *M. caroli* or *M. cervicolor* IAP genes calculated by the frequency of the positive spots was at least 10 times the reported value (11), we attempted to estimate the gene number by reassociation kinetic analysis. In addition to probe II, a probe III, corresponding to probe II in the *M. caroli* IAP gene, was generated from the L21 clone; both these probes were used for the analysis. A typical kinetic profile and summary of the kinetic analysis are shown in Fig. 4 and Table 1, respectively. Based on the $C_0t_{1/2}$ value of the nonrepetitive sequence prepared from the liver DNA of



B.

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                -40       -30       -20       -10         1        10        20        30        40        50
5'LTR                                  GGTCACAAGGAAAATGGCCACTCACAAAATTGTAAGGAGCCGTCCTCACATTCGCCATTATAAGATGGCACTGACACCTG
                                                          ************************************************
3'LTR                    TGGGTGGCCTATTGCTCTTAAATAAAAGAAAAGGGGGAGATGTAAGGAGCCGTCCTCACATTCGCCATTATAAGATGGCACTGACACCTG
                                                  ==================

                60        70        80        90       100       110       120       130       140       150
5'LTR  TGTTCTAAGTAGTAAACAAACCATCTGCACATGCGCTGGGGTAGTTTCCCACCCCATGTGCTCTGCCTTTCCCGTGATGACAACTCTGGCTGATGGGCTG
       ************************************************************************************************
3'LTR  TGTTCTAAGTAGTAAACAAACCATCTGCACATGCGCTGGGGTAGTTTCCCACCCCATGTGCTCTGCCTTTCCCGTGATGACAACTCTGGCTGATGGGCTG

                160       170       180       190       200       210       220       230       240       250
5'LTR  CAGCCAATCAGGGAGTGACACTCCGAGGCGGAGGACAGTCCTCCATAAAAGGGAGGGGGCTCCGGCTCTGTCGCTCTCTCTCTGGCTTTCTCCACTGGCT
       ********************************************************** ************************************
3'LTR  CAGCCAATCAGGGAGTGACACTCCGAGGCGGAGGACAGTCCTCCATAAAAGGGAGGGGGCTCCGACTCTGTCGTCCTCTCTCTGGCTTTCTCCACTGGCT

                260       270       280       290       300       310       320       330       340       350
5'LTR  CCTGATGGGGTAAGCAATAAAGTTTGTACTGCAAAGGATTCCGGTTGCGGTGTGCGTGTTCTTACCAGGGGGACACACAAAAGCGGCGGGCAACAAATGG
       ********** ********** ************************************************************************ ---
3'LTR  CCTGATGGGGTAAGCAATAAAGTTTGTACTGCAAAGGATTCCGGTTGCGGTGTGCGTGTTCTTACCAGGGGGACACACAAAAGCGGCGGGCAACACAAAA

                360       370       380       390       400       410
5'LTR  TGCCG

3'LTR  TGGTAGAAATATATATAGGAGGCCTAATATGACAGAATATAGACCCCATACAAGGTTTTA
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FIG. 2. (A) Restriction map and sequencing strategy for the terminal regions of the *M. caroli* IAP gene, L20. Top diagram is a restriction map of the L20 with LTRs (boxed). Expanded restriction maps of the LTR regions are shown below. The arrows indicate the extent and direction of the sequence determined. Restriction enzyme abbreviations: B, *Bam*HI; E, *Eco*RI; Hd, *Hin*dIII; He, *Hae*III; Hf, *Hin*fI; Hh, *Hha*I; Hp, *Hpa*II; P, *Pst*I. (B) Nucleotide sequences of LTRs and their flanking regions in L20. LTR sequences of the strand having the same polarity as *M. musculus* IAP RNA (20) are presented. Asterisks indicate nucleotide identity in the LTR region. The CAT box, TATAA box, polyadenylation signal, and polyadenylation site are boxed. Cellular direct repeats of 6 bp immediately outside the LTRs are underlined. Sequences corresponding to the primer-binding site and purine-rich region are indicated by dashed and double-dashed underlining, respectively.

```
                10        20        30        40        50        60        70        80        90       100
L20     TGTAAGGAGCCGTCCTCACATTCGC......CATTATAAGATGGCACTGACAC.CTG.TGTTCTAAGTAG...TAAACAAACCATCTGCACATGCGC.TG
        ***  *******  * *********      *  ** ******** ******  *** ********** *   ********  ****** **** **
rc-mos  TGTTGGGAGCCGCGCCCACATTCGC......CGGTACAAGATGGCGCTGACATCCTG.TGTTCTAAGTGG...TAAACAAATAATCTGCGCATGTGCCAA
        *** ******** **** *  ****      * *** ******** ******  *** *** * *  * *   ***
H10     TGTGAGGAGCCGCCCTCGCTATCGCTATTGCCGTTAGAAGATGGCGCTGACAT.CTGCTGT.C.AGTTGGAGTTAA(43bp).................

                110       120       130       140       150       160       170       180       190       200
L20     GGGTAGTTTCCCACCCCATGTGCTCTGCCTTTCCCGTGATGACAACTCTGGCTGATGGGCTG.CAGCCAATCAGGGAGTGACAC.TCCGAGGCGGAGGAC
        *****  **   **  * ************** ******* * *****  *** ***** *** ********************** ********* ****
rc-mos  GGGTATCTTATGACTACTTGTGCTCTGCCTTCCCCGTGACGTCAACT..GGCCGATGG.CTG.CAGCCAATCAGGGAGTGACACGTCCGAGGCGAAGGAG
                  **   *  *** ***** *******  * ** **  **  **** **   *  ******  **    **** ** *  ***** *
H10     .............CCGTTTAAGCTGTGCCTCTCCCGTGGCGTCATCTGGGGT.GATGTGCAAACCACCAATCCCGGCTCTACACGTCTCACTCGGAGCTC

                210       220       230       240       250       260       270       280       290       300
L20     AGTCCTCCATAAAAGGGAGCGGGCTCCGGCTCTGTCG.............CTCTCTCTCTGGCTTTCTCCACTGGCTCCTGATGGGGTAAGCAATAAA
        * * **** *** ********** * **  * * ***                ******  **     **  * ********** *  ***** ******
rc-mos  AATGCTCCTTAAGAGGGAGGGGGTTTCG.TTTTCTCGCTCTCTTGCTTCTTGCTCTCTTGCT..TCCTCGACCCTGGCTCCTGAAGATGTAAGAAATAAA
         *** *****   *** * *       *                **** ****       *  *     *       * *** ****
H10     CTAGGCTTATATAAGGGGCTGGGTTTCT.TAGCTGGG.............GTCTCCCTCT..AAGAAGCTGATCATCTATCTCTCAAGATGCATTAAA

                310       320       330       340       350       360       370
L20     GTTTGTACTGCA.AAGGATTCCGGTTGCGGTGTGCGTGTTCTTACCAGG..GGGACACACAAAAGCGGCGGGCAACA
        * ** * * *** *** **** ***     **** ******** ** **  **     * **  *** *    ****
rc-mos  GCTT.TGCCGCAGAAG.ATTCTGGTC....TGTG.GTGTTCTT.CCTGGCCGGTCGTGAGAA.CGCGTCTAATAACA
        * ** ******* ****** *  **     *    * **  ** * *   **   * **   ****  **   ***
H10     GCTT.TACTGCAGAAGGATCCGAGTG....TTCT.GCGTCGTT.CTTGC.TCG.CGAGACGGTAGCGCGGG...ACA
```

FIG. 3. Comparison of LTR sequences of *M. musculus*, *M. caroli*, and Syrian hamster IAP genes. LTR sequences of rc-*mos* (*M. musculus*) (9) and H10 (Syrian hamster) (21) are described. LTRs positioned at the 5′ end of each IAP gene are presented. Asterisks indicate nucleotide identity with L20. Dots indicate the absence of a nucleotide. The regulatory signals for transcription are boxed. A directly repeating sequence (43 bp in H10) is underlined, and the size is enclosed in parentheses.

BALB/c mice, the number of genes hybridizable with either probe II or III was calculated as 690 per BALB/c or 180 per *M. caroli* haploid genome.

A heterologous combination of the probe and driver DNAs made reassociation much slower than the homologous combination. Thus one possible reason for the discrepancy in Table 1 may possibly be the difference in sequence homology between the probe and driver DNAs used for the kinetic analysis. It is also possible that the discrepancy may arise from the peculiarities of the particular probe used for the analysis. Instead of the cDNA prepared from IAP RNA (11), a part of the cloned IAP gene was used as the probe.

A discrepancy resulting from misidentification of the *Mus* species is unlikely since the reported results for the total Southern hybridization analysis of IAP genes in *M. caroli* and *M. cervicolor* (4) genomes are quite similar to those in Fig. 5. Stated simply, the chromosomal DNA of *M. caroli*

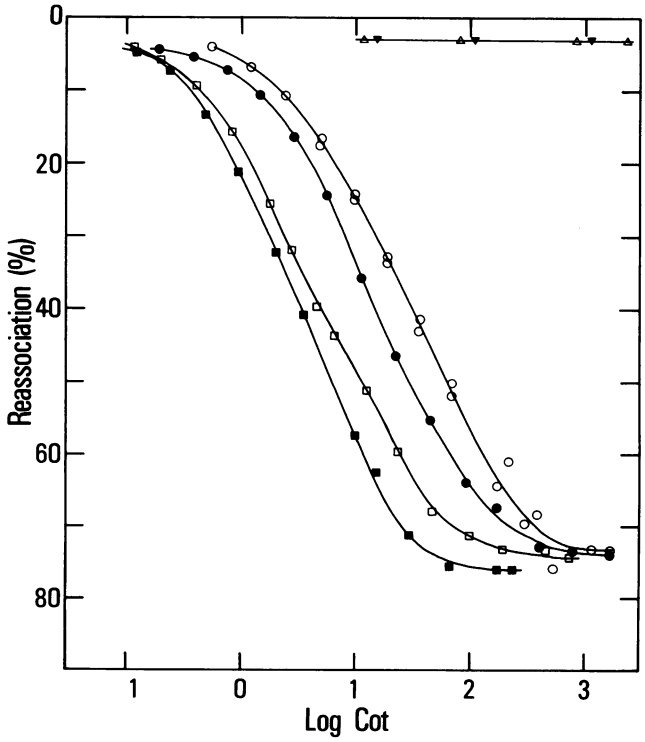

FIG. 4. Reassociation kinetics of ^{32}P-labeled probe I or III with BALB/c and *M. caroli* liver DNA. Nick-translated probe II DNA (1.4-kb *Bam*HI fragment from pS81A; see Fig. 2D) hybridized with BALB/c DNA (■), *M. caroli* DNA (○), or calf thymus DNA (▼). Nick-translated probe III (1.35-kb *Bam*HI fragment from L21; see Fig. 2A) hybridized with BALB/c DNA (□), *M. caroli* DNA (●), or calf thymus DNA (△).

TABLE 1. Number of IAP genes in the genome of three *Mus* species estimated by three different methods

Species (strain)	No. by reassociation kinetics with:			No. by plaque hybridization with:			No. by filter hybridization with fragments of cloned IAP gene
	Probe II	Probe III	cDNA	Probe II	Probe I	Fragment of cloned IAP gene	
M. musculus (BALB/c)	6.9×10^{2}[a]	4.8×10^{2}[a]	1.1×10^{3}[b]	7.0×10^{2}	9.7×10^{2}	1.9×10^{3}[c]	9.3×10^{2}[d]
M. caroli	0.8×10^{2}[a]	1.8×10^{2}[a]	0.3×10^{2}[e]	3.2×10^{2}	4.0×10^{2}	ND[f]	ND
M. cervicolor	1.8×10^{2}[a]	3.3×10^{2}[a]	0.3×10^{2}[e]	4.8×10^{2}	5.7×10^{2}	ND	ND

[a] Calculated from $C_0t_{1/2}$ of reassociation compared with $C_0t_{1/2}$ of 2.2×10^{3} (moles per second per liter) found for reannealing of nonrepetitive BALB/c liver DNA.
[b] Reference 14.
[c] Reference 16. The number of IAP genes was calculated from the frequency of the reactive plaques, as described in the text.
[d] Reference 24.
[e] Reference 11.
[f] ND, Not determined.

and *M. cervicolor* was digested with *Hin*dIII plus *Bam*HI (lanes A and B) or *Bam*HI (lanes D and E) and analyzed by the blotting hybridization method by using probe IV (lanes A and B) or probe III (lanes D and E). Three bands (1.0, 1.4, and 2.4 kb) commonly observed both in lanes A and B corresponded to fragments d, c, and e, respectively, on the map of the *M. caroli* IAP gene (Fig. 2B). A 3.5-kb band observed only in lane A seemed to correspond to fragment f. This 3.5-kb band and a 2.6-kb band in lane B characteristic of the *M. cervicolor* IAP gene are described in that article (4).

The number of IAP genes in the *M. caroli* genome as calculated by the kinetic analysis should be corrected as 230 copies per haploid genome, since about 20% of the *M. caroli* IAP genes seemed to lack the region corresponding to probe II. Similarly, the corrected number of IAP genes thus determined must be more than 390 per haploid *M. cervicolor* genome since about 84% of the *M. cervicolor* IAP genes were estimated to have the probe II region. From these results, it may be concluded that the number of IAP genes was 230 to 400 per *M. caroli* and 390 to 570 per *M. cervicolor* haploid genome.

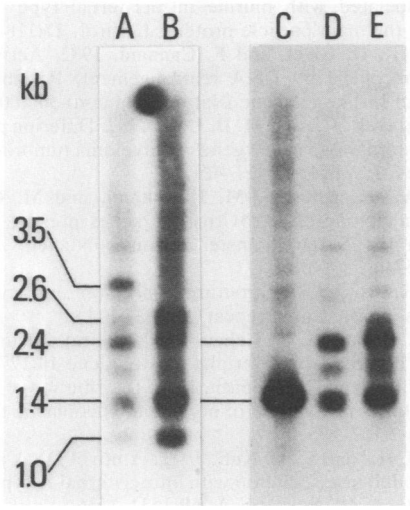

FIG. 5. Blot hybridization analysis of liver DNAs from *M. caroli*, *M. cervicolor*, and *M. musculus* (BALB/c). Cellular DNA (2 μg) was digested with *Bam*HI plus *Hin*dIII (lanes A and B) and *Bam*HI (lanes C, D, and E) and analyzed by the Southern blotting method with ^{32}P-labeled probe IV (lanes A and B) or probe II (lanes C, D, and E). DNA fragment size was estimated from the *Hin*dIII digest of wild-type lambda DNA. *M. caroli* DNA, lanes A and D; *M. cervicolor* DNA, lanes B and E; BALB/c, lane C.

DISCUSSION

This article describes the cloned *M. caroli* IAP gene which was 6.6 kb in length and contained LTRs possessing most of the structural features commonly associated with retrovirus LTR. The number of IAP genes present in *M. caroli* and *M. cervicolor* was calculated as 230 to 400 and 390 to 570 copies per haploid genome, respectively. These values are at least 10 times greater than those reported (11). If our estimation is correct, the number of IAP genes in the four rodent species analyzed is from several hundred to one thousand copies per haploid genome. This suggests that the IAP genes observed in most rodents and in some mammals have structures quite similar to the integrated retrovirus genes with LTR sequences of a distinct class and are present in multiple copies.

The biological significance of the IAP genes is still not clear. A comparison of the *M. musculus* IAP LTR sequences with those of the Syrian hamster gives the impression that the ancestors of the IAP gene were present in the common progenitors of rodents before their divergence and achieved their particular development after species differentiation. Also, the emergence of the IAPs at a very early stage in the embryogenesis of the rodent (1–3) suggests their having some significant but unidentified roles in embryogenesis and development.

Recently, amplification of a particular subset of IAP genes (24) and activation of a proto oncogene, c-*mos*, due to the integration of an IAP gene close upstream from the gene (9, 23) have been observed in mouse myeloma cells. Furthermore, the integration of an IAP gene into the intron of an actively transcribed immunoglobulin gene has been reported to cause inactivation of the gene (8, 10). This strongly indicates that some IAP genes in *M. musculus* still have the ability to translocate.

Since the presence of a extracellular phase in the life cycle of the IAP gene (7, 13) has not yet been proved, the translocation of the IAP gene most likely occurs intracellularly, as in the manner of procaryotic and eucaryotic transposable elements. IAP gene integration seems to take place

by way of reverse transcription and the formation of intermediate DNA as in the integration of the retrovirus gene, since a great number of IAPs with IAP RNA and reverse transcriptase can always be observed in mouse myeloma or hybridoma cells. The conserved primer-binding site complementary to the sequence located at the 3′ end of phenylalanine tRNA was observed in both *M. musculus* and Syrian hamster IAP genes (21). However, neither a linear nor a circular form of the intermediate DNA has been detected in the mouse myeloma cell lines studied (24; data not shown).

The LTR sequences of IAP genes seem to be functional since the IAP LTR sequences studied to date have all had the structural features commonly observed in retrovirus LTR (26). That is, the considerable number of IAP genes present in the rodent genome can act as endogenous mutator genes capable of giving rise to insertion mutations. The integration of an IAP gene might result in genetic disease such as cancer since integration is a genetic change. But for the activation of an IAP gene in translocation, such a change does not seem necessary in all cases. Rather an epigenetic change such as induction either by a hormone or a tumor promoter may possibly play a role in bringing about activation.

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