JOURNAL OF VIROLOGY, Feb. 1988, p. 472–478
0022-538X/88/020472-07$02.00/0

Vol. 62, No. 2

# Mouse Mammary Tumor Proviruses from a T-Cell Lymphoma Are Associated with the Retroposon L1Md

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Received 17 July 1987/Accepted 4 November 1987

**Four Charon 4A clones containing mouse mammary tumor virus (MMTV) proviruses and their cellular flanking sequences were obtained from partial *Eco*RI libraries of a C57BL/6 T-cell lymphoma with both endogenous and newly acquired MMTV proviruses. The cellular flanking sequences of three of four MMTV proviruses contained DNA homologous to the 3′ end of the long interspersed retroposon L1Md. Two of the three proviruses were newly acquired in the lymphoma DNA, and these MMTV proviruses appeared to be 5 kilobases downstream and in the same transcriptional orientation as the L1 sequence. The third provirus was endogenous *Mtv-9* and was located less than 500 base pairs from the 3′ end of L1. Seven additional clones containing MMTV proviruses were isolated from partial *Mbo*I libraries of a B6 T-cell lymphoma. Five of the seven clones contained L1 elements in the cellular DNA flanking MMTV DNA. At least two clones (including one with the *Mtv-8* provirus) had multiple L1 copies flanking the MMTV provirus, and one clone contained a single MMTV long terminal repeat directly integrated into a truncated L1 sequence. Although the frequencies of B1 and L1 in random library clones were similar, only one MMTV-containing clone hybridized to the abundant repetitive element B1. These data suggest a nonrandom association between MMTV and L1Md.**

The mouse mammary tumor virus (MMTV) is involved in the induction of breast cancer in mice (19) although the mechanism of this event is not well understood. High mammary cancer incidence strains, such as GR and C3H, have a breast cancer incidence of greater than 90%, and these strains transmit virus (also called exogenous or milk-borne MMTV) through the milk to their offspring (19). Low-incidence strains, such as C57BL/6 (B6) and BALB/c, lack the milk-transmitted virus but harbor the endogenous MMTV proviral copies common to most inbred strains of mice (15, 36, 37). Mammary tumors from high tumor incidence strains contain additional integrated copies of MMTV DNA (3, 17).

The MMTV provirus, like those of other retroviruses, structurally resembles transposable elements (22, 27) which can activate expression of genes in or near their integration sites (21, 33, 34). In the C3H strain, transcription of a unique gene known as *int-1* is detectable in mammary tumors which contain an MMTV provirus within 20 kilobases (kb) of this gene (32, 33). Two other loci have been identified as common MMTV integration sites in BR6 (C57BL × RIII) and Czech II mammary tumors (14, 34, 35).

Some murine T-cell lymphomas also contain additional copies of MMTV DNA (4, 6, 30). Several factors distinguish these lymphomas from MMTV-induced mammary tumors. Acquired MMTV proviruses in B6 T-cell tumors originate from endogenous MMTV DNA and are transcribed at high levels, although MMTV proteins and virus are undetectable (6, 7). Molecular cloning of integrated MMTV copies from B6 lymphomas reveals extensive restriction enzyme polymorphism in the acquired MMTV proviruses (7), and in both GR and B6 lymphomas, proviruses have deletions within the $U_3$ region of the MMTV long terminal repeat (LTR; 25, 31; L. Hsu and J. P. Dudley, unpublished data). Additional MMTV proviruses in B6 lymphomas map to at least two different chromosomes which lack the endogenous MMTV loci of this strain (7). No common regions of MMTV integration have been identified in lymphomas.

Retroviral integration appears to occur without specificity for host sequences (43). To investigate the nature of MMTV integration sites in T-cell lymphomas further, molecular clones of MMTV proviruses and their cellular flanking DNA were characterized. Restriction mapping and hybridization analyses indicated common regions of cellular DNA flanking the proviruses. One such region was the repetitive retroposon L1Md (38, 40, 47). Full-length copies of this repeat are approximately 7 kb long and have two overlapping open reading frames of 1,137 and 3,900 base pairs (bp), similar to those found in retroviruses (26, 29). Within the mouse genome, the 3′ end of L1 is almost 10 times more abundant (85,000 copies) than the 5′ end (10,000 copies) (26), indicating that most L1 elements are truncated with respect to their 5′ ends (38, 40). L1 elements have a structure similar to that of the I elements which control hybrid dysgenesis in *Drosophila melanogaster* (10).

This paper characterizes the L1 repeats linked to MMTV proviruses in B6 T-cell lymphomas. The frequency of L1 copies is approximately three times that expected in randomly selected clones containing murine DNA. This frequency is made more striking by the presence of a single B1 repeat (23, 24; the *Alu*I sequence equivalent [18]) in the cellular DNAs of these clones. The data suggest that integration of MMTV proviruses is not random with respect to mouse DNA.

## MATERIALS AND METHODS

**Restriction enzyme digestions, nick translations, Southern blotting, and hybridization analysis.** Restriction enzyme digestions were performed as recommended by the manufacturer (New England BioLabs or Bethesda Research Laboratories). Procedures for Southern blotting (42), nick translation, and hybridization analysis have been described previously (6).

**Molecular clones.** Isolation of the phage clones AACl 1, AACl 2, AACl 6, and AACl 7 containing MMTV proviruses has been previously described (7). Each of the clones was derived from a partial *Eco*RI library of the B6 lymphoma P3C which contains additional MMTV proviruses. Subclone

p2-12 (probe A) was obtained by transferring the 1.5-kb *Eco*RI-*Hind*III fragment 5′ to the AACl 1 provirus into plasmid vector pUC9 (44). Subclones of the L1 repeat in M13mp phages were generously provided by M. Comer, C. Hutchison, and M. Edgell and transferred into pUC9 before DNA purification on CsCl gradients containing ethidium bromide. Clones containing the mouse repetitive sequence B1 (38) were obtained from D. Endean and O. Smithies, University of Wisconsin, Madison. Mm31 contains a 1,050-bp *Hin*dIII insert in pBR322, and Mm35 contains a 2,500-bp *Eco*RI-*Hin*dIII insert in pBR322 (23, 24). The pVC217 probe (a 1.35-kb *Eco*RI-to-*Bgl*II fragment immediately 3′ to the *Mtv-8* provirus provided by G. Peters) (36) and the GR-40 clone (20) (from N. Hynes) were used to confirm the presence of *Mtv-8* in AACl 14.

The phage clones AACl 8, AACl 9, AACl 10, AACl 11, AACl 12, AACl 13, and AACl 14 were obtained by using the lambda vector EMBL3 (12). High-molecular-weight DNA from the cell line E102P4C (16) was extracted as described previously (6), partially digested with *Mbo*I, and treated with calf intestinal alkaline phosphatase (25 U/ml) before gradient fractionation. Approximately 15- to 20-kb DNA fragments were precipitated with ethanol for ligation to EMBL3 DNA (Promega Biotech) doubly digested with *Eco*RI and *Bam*HI. Ligated DNA was packaged by using Packagene extracts (Promega Biotech). Packaged phage were plated at approximately $10^4$ phage per 150-mm (diameter) petri dish and screened with an MMTV LTR probe (28).

**Restriction enzyme mapping.** Mapping of the EMBL clones was accomplished by using partial digestions and Southern blotting before hybridization with probes for the ends of linear DNA fragments. This is a modification of the method of Smith and Birnstiel (41). Maps obtained by this method were confirmed by subcloning and/or single and double digestions of recombinant phage DNA.

**Dideoxynucleotide sequencing analysis.** Nucleotide sequencing analysis was performed by the method of Sanger et al. (39). Reactions were analyzed on 8% acrylamide buffer gradient gels containing 7 M urea as described by Biggin et al. (1). Plasmid DNA (1 to 2 μg) purified by ethidium bromide-CsCl gradient centrifugation was incubated in 0.2 N NaOH–0.2 mM EDTA at 22°C for 5 min, neutralized by addition of 0.2 M ammonium acetate, pH 4.5, and precipitated with ethanol. The plasmid was added to 25 ng of 17-mer primers from nucleotides 1273 to 1289 of the MMTV LTR (22) or from nucleotides 4848 to 4864 of the L1Md sequence of Loeb et al. (26). Samples were heated for 3 min at 100°C, cooled in ice water for 10 min, and then incubated at 37°C for 5 min before dideoxynucleotide sequencing. With these primers, the sequence was obtained from both strands. Primers were synthesized by the Oligonucleotide Synthesis Facility at the University of Texas at Austin.

**Determination of L1 and B1 frequencies and statistical analysis.** Approximately 1,000 to 1,500 phage were plated onto 100-mm (diameter) dishes and transferred to nitrocellulose in triplicate. Each set was hybridized to an L1, B1, or lambda probe. Only plaques which hybridized to the lambda probe were scored. Nitrocellulose transfers were marked to facilitate alignment of plaques which hybridized to both the L1 and B1 probes. The statistical analysis was performed by using the binomial expansion.

## RESULTS

**A common repetitive element in the flanking DNAs of three Charon 4A clones containing MMTV proviruses.** A number of mouse T-cell lymphomas contain additional MMTV proviruses (4, 6, 30). Several proviruses and their adjacent flanking DNA sequences from a B6 T-cell lymphoma were cloned and characterized previously (7). Two of these proviruses from AACl 1 and AACl 2 were acquired in the lymphomas, whereas AACl 6 and AACl 7 contained the 3′ ends of the endogenous *Mtv-17* and *Mtv-9* proviruses, respectively (7; unpublished data). To detect common regions within the AACl 1 and AACl 2 proviral integration sites, the cellular DNA flanking the MMTV proviruses was subcloned. The DNA immediately adjacent to the provirus in clones AACl 1 and AACl 2 was found to be unique and did not cross-hybridize to the other clones (7; unpublished data). However, a 1.5-kb *Eco*RI-*Hin*dIII fragment upstream of the AACl 1 provirus (Fig. 1; probe A) hybridized to two of the three other Charon 4A clones, AACl 2 and AACl 7 (containing *Mtv-9*; Fig. 2). The cross-reactive region in AACl 2 was localized to a 2.1-kb *Pst*I-*Eco*RI fragment and, in AACl 7, to a *Pst*I fragment of 5.9 kb (Fig. 2, lanes 5 and 11). The AACl 6 clone (containing the 3′ portion of the *Mtv-17* provirus and approximately 13 kb of flanking cellular DNA) did not hybridize to probe A.

Hybridization analysis revealed that probe A contained repetitive sequences. Total B6 liver DNA was labeled by nick translation and hybridized to Southern blots of probe A, AACl 1, and AACl 2 DNAs digested with *Hae*III, *Eco*RI, *Bam*HI, *Pst*I, and *Hin*dIII. Results of this experiment (data not shown) indicated that probe A contains highly repeated sequences. Moreover, the pattern of hybridization on AACl 1 and AACl 2 DNAs with probe A (Fig. 2) was identical to that observed for total cellular DNA probes. This indicated that the only repeated sequences in AACl 1 and AACl 2 were homologous to the 1.5-kb probe A subclone.

**The repetitive element in AACl 1, AACl 2, and AACl 7 is part of the retroposon L1Md.** The Charon 4A clones were tested for homology to a variety of cellular repetitive sequences. Only probes specific for the 3′ portion of the retroposon element L1Md (*Bam*540 and R; 2, 8, 28, 48; Fig. 3) hybridized to AACl 1, AACl 2, and AACl 7 DNAs (Fig. 1). DNAs from the three Charon 4A clones were digested with the enzymes *Bam*HI, *Eco*RI, and *Pst*I, subjected to Southern analysis, and hybridized to a probe for the *Bam*540

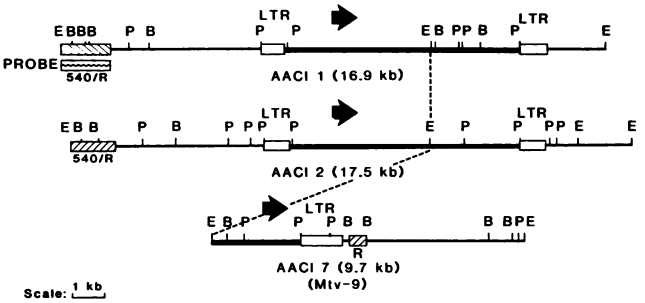


FIG. 1. Restriction maps of Charon 4A clones containing MMTV proviruses. The maps of these clones have been reported previously; the size of the cloned insert is shown in parentheses next to the clone number. The box containing a wavy line under AACl 1 represents the 1.5-kb *Eco*RI-*Hin*dIII fragment subcloned into pUC9 (probe A). Open boxes represent the viral LTRs. Hatched boxes represent sequences which have homology to probe A. Large arrows indicate the transcriptional orientation of each provirus. The central *Eco*RI site in each provirus is connected by a dashed line. Restriction enzyme sites are abbreviated as follows: E, *Eco*RI; B, *Bam*HI; P, *Pst*I.

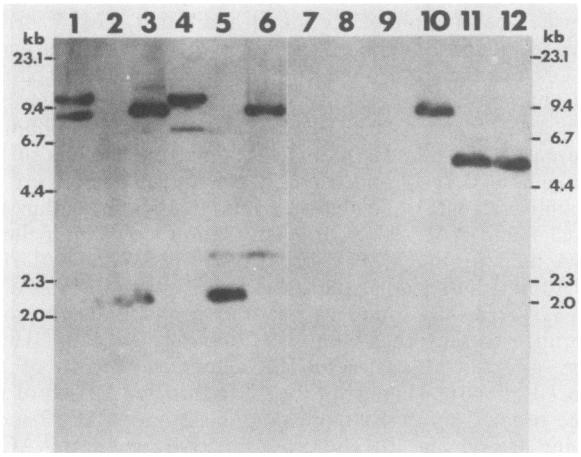


FIG. 2. Southern blots of DNAs from clones AACI 1, AACI 2, and AACI 7. Cloned DNA (1 μg) was digested singly or doubly with restriction enzymes and subjected to electrophoresis on 0.8% agarose gels before Southern transfer. Blots were hybridized to probe A (Fig. 1). Lanes 1, 4, 7, and 10 contain DNAs digested with *Eco*RI, lanes 2, 5, 8, and 11 contain *Eco*RI-*Pst*I-digested DNAs, and lanes 3, 6, 9, and 12 contain *Pst*I-digested DNAs. Lane 1 contains two bands, one of which represents the 5′ *Eco*RI fragment of AACI 1. The smaller band at approximately 9 kb represents a deletion which accumulates in large-scale preparations of this clone. Similar faint bands, which represent deletions of AACI 2, can be seen in lanes 4 to 6.

region of L1Md (8, 26). This region includes the 3′ portion of the 3,900-bp open reading frame within the 7-kb repeat (Fig. 3). In both AACI 1 and AACI 2, *Bam*540 hybridization was localized to a *Bam*HI fragment of approximately 600 bp (Fig. 1). This *Bam*HI fragment is located within a 2.1-kb *Eco*RI-*Pst*I fragment in clones 1 and 2 and confirms hybridization data obtained with probe A (Fig. 2) and total cellular DNA probes. The Charon 4A clone AACI 7 (containing *Mtv-9*) did not hybridize with the *Bam*540 probe.

Similar hybridizations were performed with an L1Md R (26) probe. This region lies 3′ to the *Bam*540 region and does not include open reading frame sequences (26; Fig. 3). These experiments indicated that L1 DNA was localized to a 1.4-kb *Pst*I-*Bam*HI fragment in both clones AACI 1 and AACI 2. This homology was within 2.2-kb *Bam*HI and 10.5-kb *Pst*I fragments in AACI 1 and 2.6-kb *Bam*HI and 10.5-kb *Pst*I

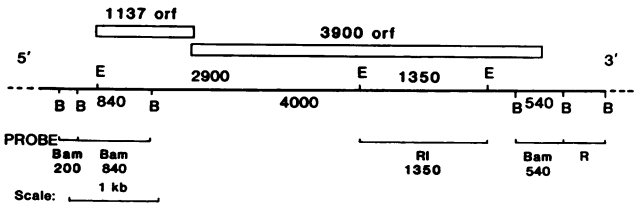


FIG. 3. Organization and consensus map of retroposon L1Md. The open boxes above the restriction map represent the open reading frames (orf) of L1Md described by Loeb et al. (26). The sizes of these orf regions and the distances between restriction sites are given in base pairs. The regions of L1 used as probes are delineated as thin lines under the restriction map. The 3′ ends of these elements often contain A-rich regions. The restriction enzyme sites shown (*Eco*RI [E] and *Bam*HI [B]) are the consensus of several different L1 elements.

fragments in AACI 2. These results place L1Md approximately 4.5 to 5.0 kb upstream of the MMTV proviruses in clones AACI 1 and AACI 2 (Fig. 1). If the positions of *Bam*540 and R correspond to those observed in the sequence of Loeb et al. (26), these results suggest that L1Md and MMTV are in the same transcriptional orientation.

Homology of the R probe to the AACI 7 insert (containing *Mtv-9*) was localized to a 0.5-kb *Bam*HI fragment within a 5.5-kb *Pst*I fragment. Southern blotting experiments with additional enzymes indicated that the R region is located just 3′ to the *Mtv-9* provirus (data not shown). If the *Mtv-9* LTR is 1.3 kb, the L1 R region is within a few hundred bases of the LTR.

**Isolation of additional MMTV clones from partial *Mbo*I libraries.** Retroposon L1Md maps approximately 5 kb upstream and in the same orientation to two newly acquired MMTV proviruses located on separate mouse chromosomes (7). The right-hand LTR of the endogenous provirus *Mtv-9* also is flanked by the L1 R region. Moreover, Fanning et al. have reported that endogenous provirus *Mtv-8* is integrated into the 5′ portion of the L1Md repeat (9). To confirm the high frequency of L1 repeats in the cellular DNA flanking MMTV proviruses, seven additional clones were isolated from partial *Mbo*I libraries of B6 lymphoma DNA. Restriction maps of clones AACI 8, AACI 9, AACI 10, AACI 11, AACI 12, AACI 13, and AACI 14 were constructed by digestions with *Kpn*I, *Sal*I, and *Eco*RI, followed by South-

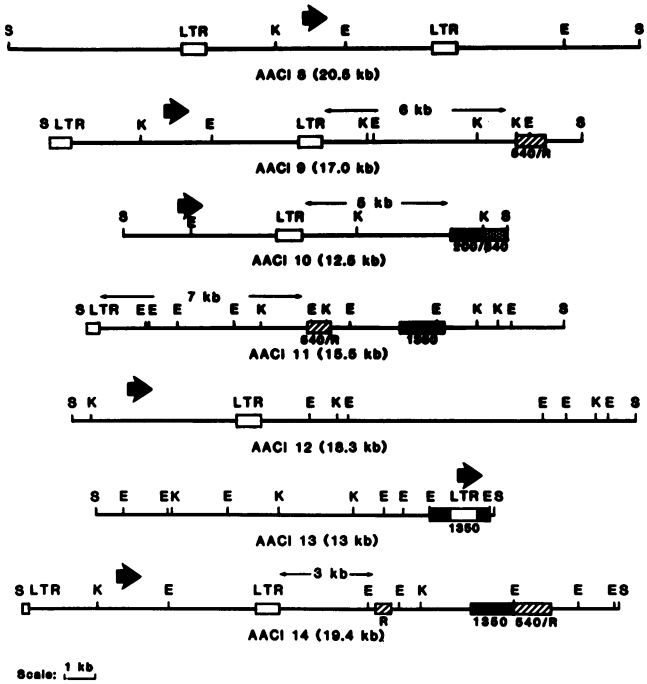


FIG. 4. Restriction enzyme maps of EMBL clones containing MMTV proviruses. The open boxes represent MMTV LTRs, and the boldface arrows show the transcriptional orientation of the proviruses. The smaller arrows indicate the approximate distance between the viral LTRs and the closest L1 elements. The size of each insert is given next to the clone designation and is flanked by *Sal*I sites which are contributed by the EMBL3 vector. The 540 and R regions of L1 are shown as hatched boxes, the 1350 regions are filled boxes, the 840 region is a double-hatched box, and the 200 region is a stippled box. A slash between *Bam*540 and R regions (i.e., 540/R) indicates that the order of these elements is not known. Restriction enzyme sites are given as follows: E, *Eco*RI; K, *Kpn*I; S, *Sal*I.

ern blotting and hybridizations with MMTV-specific probes (Fig. 4).

Four of the seven clones hybridized to probes for the 3′ end of the L1 repeat. In AACl 9, homology to the L1 R region was localized to a 2.2-kb *Sal*I-*Kpn*I fragment, whereas hybridizations with AACl 11 DNA showed that the R region is located on a 1.2-kb *Eco*RI fragment within a 2.1-kb *Kpn*I fragment (Fig. 4). Another clone, AACl 14, contained L1 R sequences on 1.0- and 2.1-kb *Eco*RI fragments; the homology was limited to a 10.5-kb *Kpn*I fragment and a 6.4-kb *Sal*I-*Kpn*I fragment (Fig. 4). *Bam*540 and R region probes gave identical hybridization patterns to Southern transfers of either AACl 9 or AACl 11 DNA, suggesting that *Bam*540 and R are linked within these clones (Fig. 4).

The *Eco*RI 1350 region (2, 26; Fig. 3) also was used in Southern hybridization experiments. In AACl 11, the 1350 sequences were localized to 2.3- and 2.8-kb *Eco*RI fragments which did not contain *Bam*540 or R regions. Therefore, it appears that these regions are unlinked to the 1350 sequence in AACl 11 DNA and that they represent two distinct truncated copies of L1. In AACl 14 DNA, hybridizations with the 1350 probe indicate linkage of the 1350, *Bam*540, and R regions within a 6.4-kb *Sal*I-*Kpn*I fragment (Fig. 4). Because the R probe also detects a 10.5-kb *Kpn*I fragment which contains the 3′ half of the MMTV provirus, the data suggest that there are at least two truncated L1 elements 3′ to the provirus in AACl 14. Restriction mapping and hybridization analysis indicate that AACl 14 contains the endogenous *Mtv-8* provirus (20, 37; data not shown).

AACl 13 was unusual, since the MMTV LTR and 1350 probes hybridized to an identical *Eco*RI fragment of 1.9 kb (Fig. 4). No other MMTV or L1Md probes tested hybridized to this clone. Sequencing analysis of a 1.9-kb subclone (Fig. 5) revealed that the MMTV LTR is inserted between nucleotides 5033 and 5034 of the 3.9-kb L1 open reading frame described by Loeb et al. (26; Fig. 3). The data also indicate that the orientation of the 1350 element is opposite to that of the LTR. Preliminary experiments indicate that the 1350 sequence continues uninterrupted on the other side of the LTR (data not shown), suggesting that viral integration was not accompanied by deletion of cellular DNA.

**The MMTV provirus in AACl 10 is linked to an L1 5′ end.** All recombinant phage clones were hybridized to probes which detect the 5′ ends of the L1Md element. Only AACl 10 DNA demonstrated homology to these probes. Homology of AACl 10 DNA to *Bam*840 was localized to a 0.8-kb *Sal*I-*Kpn*I fragment within a 2.3-kb *Kpn*I fragment (Fig. 6A, lanes 4 and 5), indicating that the *Bam*840 sequences were at one end of the phage insert. The AACl 10 insert also hybridized to a 200-bp probe representing L1 DNA immediately 5′ to *Bam*840 sequences (Fig. 3 and 6B). Since L1 elements usually are truncated at their 5′ ends, these data suggest that the MMTV provirus in AACl 10 is in the same transcriptional orientation and approximately 5 kb upstream of a full-length L1Md element.

Restriction maps of two clones, AACl 8 and AACl 12, which contain no L1Md elements in their flanking DNAs also are shown (Fig. 4). The MMTV provirus in AACl 12 lacks the virtually ubiquitous internal *Eco*RI site (15, 17).

**Representation of other repetitive sequences in the MMTV proviral clones.** Because the L1 copy number approaches $10^5$ (38, 40), the Charon 4A and EMBL clones were screened with probes specific for other sequences of equivalent or greater copy number. One such element, the mouse B1 sequence (38), has homology to and abundance similar to that of the human *Alu*I elements (18). DNA from each of the clones was digested with *Eco*RI, subjected to Southern blotting, and hybridized to a B1 probe. Only AACl 6 (containing *Mtv-17*) showed homology to the B1 probe (Fig. 7, lane 3). Approximately 27% of random *Mbo*I clones hybridized to the B1 probe, whereas the L1 R probe detected 24% of the total number of clones. This is in good agreement with results reported by others for partial *Eco*RI libraries (26, 45). The frequency of library clones containing both L1 and B1 was approximately 6%, suggesting a random association between L1 and B1.

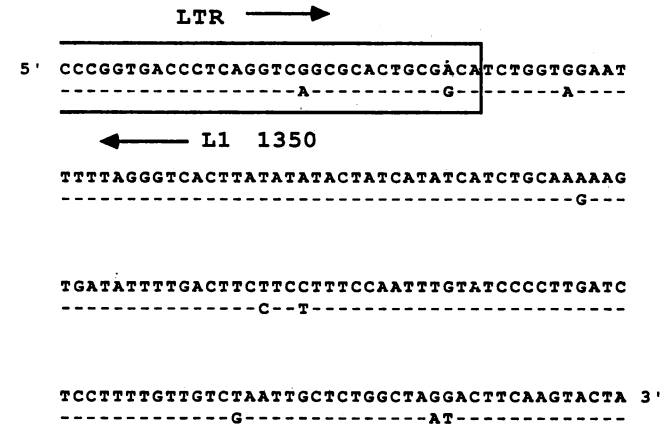


FIG. 5. Dideoxynucleotide sequencing analysis of the MMTV LTR-L1Md junction in AACl 13. The transcriptional orientations of the MMTV LTR and L1 sequences are given by arrows. Sequences included within the box delineate the 3′ end of the MMTV LTR. Nucleotides different from the endogenous *Mtv-8* sequence of Kennedy et al. (22) and the L1 sequence of Loeb et al. (26) are indicated by letters, whereas identical nucleotides are indicated by dashes.

**Statistical analysis.** The probability of obtaining linkage of L1 and MMTV proviruses by chance was calculated. Assuming that the frequency of L1 R is 24% in random clones (26, 45; see above; M. Edgell, personal communication), the probability of isolating 6 of 11 MMTV-positive clones with L1 R is 0.028.

## DISCUSSION

The nature and location of L1Md sequences flanking several MMTV proviruses have been determined by restriction mapping and hybridization analysis. Such analyses indicate that retroposon L1Md is located within 7 kb of the MMTV LTR in 8 of the 11 clones obtained from partial *Eco*RI or *Mbo*I libraries of a B6 T-cell lymphoma. Of the eight clones containing L1Md, AACl 1 and AACl 2 contained L1 approximately 5 kb upstream and in the same transcriptional orientation as the MMTV provirus, AACl 10 had L1 approximately 5 kb downstream and in the same transcriptional orientation, and AACl 13 had a solitary MMTV LTR inserted directly into L1 1350 in the opposite transcriptional orientation. Three other clones contained L1 flanking the provirus, but their orientation was not determined. In two of these cases, AACl 11 and AACl 14 (containing *Mtv-8*), at least two separate L1 elements flanked MMTV DNA. In view of the large number of these elements (about 85,000 [26]) in the mouse genome, it can be calculated (on the basis of a mouse genome size of $3 \times 10^9$ bp [28]) that L1 DNA is present approximately every 35 kb. Excluding MMTV proviral sequences, approximately 112 kb of cellular DNA has been cloned, and thus one would expect 3.2 copies

of L1. The observed value of 10 elements is considerably higher than expected in random mouse DNA. The fact that MMTV proviruses are inserted in nonrandom sites in mouse DNA also is supported by the presence of a single B1 element in cloned flanking DNA (23, 24). Although the frequency of L1 capture in random library clones may be higher (because of its larger size) than that of B1 capture, a 490-bp region of L1 (R) also is represented in 6 of 11 clones containing MMTV proviruses, and 1 clone (AACl 14) has two distinct R elements. The probability of this event is 0.028, a value which does not account for the presence of multiple R elements in AACl 14, other portions of L1 in MMTV flanking DNA, the distance of R from the MMTV provirus, or the presence of a high percentage of MMTV DNA in cloned inserts. This finding is inconsistent with the idea that retroviruses integrate without specificity for host DNA (43).

Nonrandom distribution of L1 sequences has been reported in the genome of rats (13). Several factors appear favorable for L1 integration, including high A + T content, the presence of the hexanucleotide TACTCA, and a stretch of alternating purine-pyrimidine residues (13). The association of MMTV proviruses with L1, but not B1, elements is consistent with the notion that L1 elements are nonrandomly dispersed in mammalian genomes.

The transcriptional activity of integration sites may affect the distribution of MMTV and L1. L1Md is transcribed into discrete cytoplasmic polyadenylated transcripts which are detectable in B6 lymphomas with or without acquired MMTV proviruses (5). In contrast to cytoplasmic transcripts, a wide range of L1 transcripts is present in the nuclear RNAs of these and other murine cell types (5, 8), probably representing readthrough transcription from adjacent cellular promoters (47). Transcriptional activity is thought to be essential for L1 dispersal (46, 47). High levels of transcription are detectable from acquired but not endogenous MMTV proviruses in B6 lymphomas, and no readthrough transcripts between MMTV and L1Md have been observed (7). MMTV transcription is affected by the proviral integration site (11).

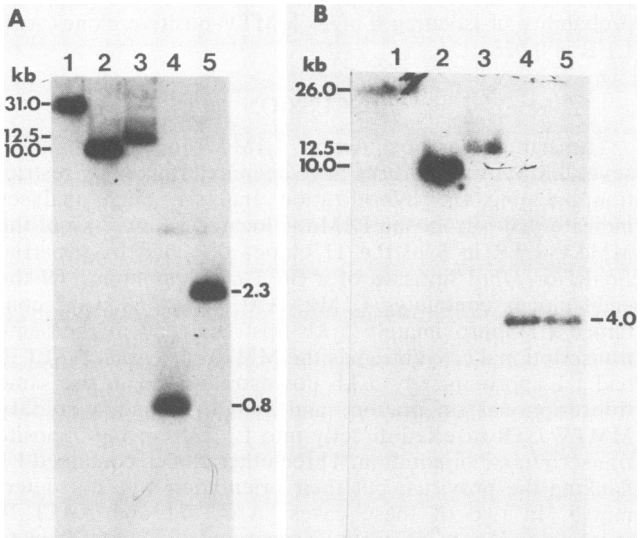


FIG. 6. Hybridization of AACl 10 DNA to L1 5′ probes. Cloned DNA (1 μg) was digested with restriction enzymes, subjected to electrophoresis in agarose, and transferred to nitrocellulose. DNA was digested with *Eco*RI (lane 1), *Eco*RI and *Sal*I (lane 2), *Sal*I (lane 3), *Sal*I and *Kpn*I (lane 4), or *Kpn*I (lane 5). DNA was hybridized to a probe for the L1 *Bam*840 region (panel A) or the L1 200 region (panel B).

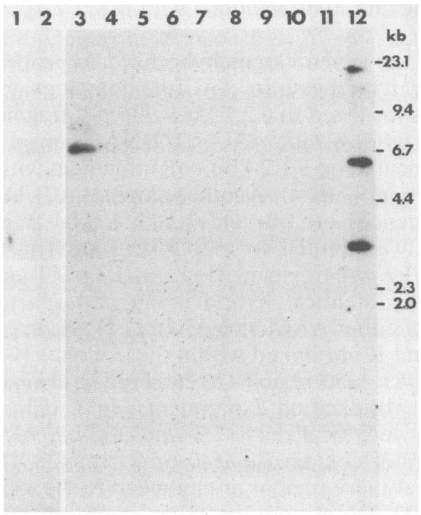


FIG. 7. Hybridization of Charon 4A and EMBL clones to the mouse B1 probe. Cloned DNA (1 μg) was digested with *Eco*RI (lanes 1 to 4) or *Sal*I (lanes 5 to 11) and subjected to Southern blotting after agarose gel electrophoresis. The sources of DNA were as follows (lanes): 1, AACl 1; 2, AACl 2; 3, AACl 6; 4, AACl 7; 5, AACl 8; 6, AACl 9; 7, AACl 10; 8, AACl 11; 9, AACl 12; 10, AACl 13; 11, AACl 14; 12, 50 ng of uncut Mm31 DNA.

Both MMTV and L1Md are RNA polymerase II transcripts (38, 43, 47), whereas mouse B1 elements are transcribed by RNA polymerase III (18, 47). In view of other data presented here, retrotransposons, such as MMTV, and retrotransposonlike elements (L1Md) may integrate preferentially into regions of active RNA polymerase II transcription. This would explain the apparent nonrandom distribution of L1Md elements and MMTV proviruses and the relative paucity of murine B1 sequences. It also suggests that the regions of MMTV integration in T cells are actively transcribed in T cells. Other loci containing active polymerase II transcription units contain multiple L1Md elements (46, 48). For example, eight L1Md elements have been described within the beta-globin locus (ca. 65 kb), yet only one B1 element resides in the same region (45, 46).

The endogenous provirus *Mtv-8* (unit II) reportedly is integrated into the *Bam*840 region of L1Md (9). This result was not confirmed by hybridizations with L1 5′ probes for *Bam*840 or the A type L1 200 sequence (Fig. 6). This discrepancy may indicate the presence of several truncated L1 copies at the *Mtv-8* integration site (Fig. 4) and explains why unique cellular flanking probes have been obtained from this region (36). Two other proviruses, *Mtv-9* (in AACl 7) and an LTR-only provirus in AACl 13, also have L1 sequences adjacent to the MMTV LTR. Since LTR-only and endogenous virus transcripts are not detectable in most T-cell lymphoma lines (7), it is possible, as suggested by Fanning et al. (9), that L1 has a negative or damping effect upon the transcription of MMTV proviruses within a few hundred bases.

## ACKNOWLEDGMENTS

This work was supported by Public Health Service grant CA 34780 from the National Cancer Institute and by grant F-956 from the Robert Welch Foundation.

I acknowledge Ann Glasgow and Charmaine Fabritius for technical assistance and Henry Bose and Bill Folk for helpful suggestions on the manuscript. I also thank Lilian Hsu for the sequencing analysis.

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