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Isolation of a Retroviruslike Sequence from the *TL* Locus of the C57BL/10 Murine Major Histocompatibility Complex

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Two retroviruslike sequences have been isolated from the *TL* locus of the major histocompatibility complex of C57BL/10 mice. One sequence (TLev2) hybridizes only with probes derived from the *pol* region of the murine leukemia provirus AKR; the other sequence (TLev1) hybridizes with *gag*, *pol*, and *env* AKR region probes. This 9-kilobase endogenous, *TL* region-associated virus (TLev1) has been further characterized. The TLev1 genome has been shown to contain murine leukemia virus-related sequences bounded by retroviruslike, VL30 long terminal repeats. Hybridization of TLev1-derived probes to mouse genomic digests reveals multiple copies which show distinct patterns compared with those observed with murine leukemia virus probes. The study of TLev1 may prove significant with respect to the interaction of retroviral sequences within the genome, expression of genes within the *TL* locus, and polymorphisms within the major histocompatibility complex.

Studies in our laboratory on the *H-2*-linked resistance to thymoma induction by radiation-induced leukemia virus have led to the identification of two retroviruslike sequences within the *TL* locus of the major histocompatibility complex (MHC) of uninfected C57BL/10 mice (41–44). The *TL*-associated endongenous virus sequences (TLev) have been subcloned for further study. One subclone (TLev1) contains a 9-kilobase (kb) sequence that hybridizes with AKR murine leukemia virus (MuLV)-derived *gag*, *pol*, and *env* gene probes. This subclone was characterized to unequivocally establish the presence of retroviral sequences within the MHC. The results of blot hybridization and sequence analysis suggest that TLev1 is a retroviral element which contains two types of sequences: those which share strong homology with mouse VL30 elements (2, 15, 29, 34) and those which are weakly homologous to MuLV sequences.

The finding of retrotransposable elements among histocompatibility loci may prove significant with respect to the expression of genes within the *TL* locus and polymorphisms within the MHC (42). This report also suggests that study of the TLev1 sequence may provide insight into the evolution or interaction of retroviral elements within the murine genome.

MATERIALS AND METHODS

DNA. The murine C57BL/10 *TL* cluster cosmids (B1.15 and H43) used to isolate TLev1 and TLev2 sequences have been previously described (42, 63). TLev1 was isolated from cosmid B1.15, within a 10-kb *Bgl*II fragment, and subcloned into the *Bam*HI site of pBR322.

Mouse strains used to obtain genomic DNA were all bred at the New York University Medical Center Animal Facility with the exception of AKR/J mice, which were obtained from the Jackson Laboratory, Bar Harbor, Maine. Cellular DNA was obtained from the thymus; thymocytes of young (2 months old) mice were obtained by gentle shearing of the thymus with a forceps. Feral mouse (23) Sc-1 DNA was isolated from the III6A clone provided by T. Pincus, Sloan-Kettering Institute, New York, New York. Infectious clones of NZB xenotropic and an amphotropic virus were gifts of Raymond R. O'Neill, National Institutes of Health. An endogenous VL30 BVL-1 (26) clone was a gift of Micheal Getz, Mayo Clinic Foundation, Rochester, Minn.

DNA preparation. (i) High-molecular-weight genomic DNA. Cells were washed and suspended in buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl and 2 mM EDTA at a concentration of 2×10^7 to 4×10^7 cells per ml. Sodium dodecyl sulfate was added to 0.5% with gentle mixing, followed by incubation at 65°C for 20 min. Proteinase K was added to 200 μg/ml, and samples were gently mixed at 37°C overnight. NaCl was added to 0.4 M, and samples were extracted with phenol (two times), a one-to-one mixture of phenol and chloroform-isoamyl alcohol (24:1) (two times), and chloroform-isoamyl alcohol (24:1) (two times) prior to ethanol precipitation. Samples were dried and suspended in 1 mM Tris chloride (pH 7.5) plus 0.2 mM EDTA. DNA concentrations were determined by the diphenylamine assay (17).

(ii) Cosmid and plasmid DNA. Cosmids were grown to saturation (overnight) in NZCY medium ([grams per liter] NZ amine [Kraft, Inc., Lafayette, N.J.], 10; NaCl, 5; yeast extract, 5; Casamino Acids [Difco Laboratories, Detroit, Mich.], 1; $MgSO_4 \cdot 7H_2O$, 2) plus 100 μg of ampicillin per ml and isolated by the alkali lysis method (3). Plasmids were grown in M9 medium and 50 μg of ampicillin per ml to which uridine (1 mg/ml) was added at an optical density at 550 nm of 0.2 and chloramphenicol (200 μg/ml) at an optical density at 550 nm of 0.5. Plasmids were isolated by a modification of the sodium dodecyl sulfate method (22). Cosmids and plasmids were purified by centrifugation to equilibrium in CsCl-ethidium bromide gradients.

(iii) DNA digests. Genomic DNA (10 μg/50 μl) was digested overnight at 37°C with 2 U of restriction enzyme per μg of DNA in reaction conditions recommended by the supplier (New England BioLabs, Inc., Beverly, Mass.). Completeness of digestion was assayed by adding a 5-μl portion of the reaction mixtures to 200 ng of λ DNA which was subsequently analyzed by minigel electrophoresis. Digestions were terminated by the addition of buffer to a final concentration of 4% Ficoll 400, 0.2 mM EDTA, and 0.02% bromophenol blue.

Blot hybridization analysis. (i) Probes. All probes were

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excised from plasmids with the indicated enzymes, isolated by preparative electrophoresis, and purified by DEAE-Sephacel (Pharmacia, Inc., Piscataway, N.J.) chromatography.

Probes for the various MuLV domains were derived from the molecularly cloned ecotropic provirus pAKR, a gift of Malcolm Martin (National Institutes of Health) as follows. For AKR, two probes were used, an *Eco*RI-*Hin*dIII insert containing the complete viral genome and an ~1-kb mouse (NIH 3T3) flanking sequence (40) and a *Pst*I insert containing one long terminal repeat (LTR) sequence and a complete viral genome (8). For the 5′ LTR-*gag*, a 2.8-kb *Sma*I-*Kpn*I fragment containing an ~500-base-pair (bp) LTR sequence and the entire *gag* gene sequence was used. For the 5′ *pol*, a 1.8-kb *Kpn*I-*Sma*I fragment containing ~55% of the amino-terminal 5′ *pol* gene region was used. For the 3′ *pol*-5′ *env*, a 2.7-kb *Sal*I-*Bam*HI fagment containing ~70% of the carboxyl-terminal end of the *pol* gene and the amino-terminal half of gp70 was used. For 3′ *env*-LTR, a 1.7-kb *Sma*I-*Sma*I fragment containing the carboxyl-terminal half of gp70, the entire p15 E region, and 500 bp of the 3′ LTR was used.

The location of TLev1 virus probes is shown in Fig. 1C. Probes were derived from the subcloned *Bgl*II fragment of cosmid B1.15 (pTLev1). TLev1 is an 8-kb *Xba*I fragment containing the complete viral sequence. TLev1-*Kpn*I is a 0.3-kb fragment showing no cross-hybridization with MuLV sequences.

An endogenous VL30 probe was provided by Michael Getz. The VL30 subclone is an *Xho*I insert of the BVL-1 genomic clone (26) containing a complete VL30 unit minus one LTR.

The class I *H-2* gene probes used in this study include H2III, a *Pst*I insert of pH2III, coding for the 5′-terminal amino acids 63 to 160 of H-2 antigens (56), and H2IIa, an *Sac*I-*Hha*I insert of pH211a, encoding the 3′-terminal amino acids 167 to 352 of H-2 antigens (57).

(ii) Gel electrophoresis and transfer. Agarose gels were run at 5 V/cm for the indicated amount of time. The running buffer contained 40 mM Tris acetate and 2 mM EDTA. DNA was transferred from gels by the method of Southern (55) onto nitrocellulose filters (Schleicher & Schuell, Inc., Keene, N.H.). The filters were baked at 80°C under vacuum prior to hybridization.

(iii) Hybridization. Filters were blocked at 65°C in ~100 ml of 10× Denhardt solution (0.2% Ficoll, 0.2% polyvinylpyrolidone, 0.2% bovine serum albumin) and 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) per filter, with shaking for 3 to 4 h. The filters were air dried and placed in plastic heat-sealable bags. The probes were labeled with [α-^{32}P]dCTP to a specific activity of ~2×10^8 cpm/μg by the nick-translation system of New England Nuclear Corp., Boston, Mass.; ~5×10^6 cpm/ml of hybridization solution was used. After boiling for 10 min along with calf thymus DNA (20 μg/ml of hybridization solution), the probe was added to the hybridization solution (0.02 ml/cm^2 of filter) containing 10× Denhardt solution, 10 mM EDTA, and 2× SSC. The hybridization solution was added to the bag and absorbed by the filter. The filters were incubated for ~16 h in a 68°C water bath and then washed in sequential dilutions of a solution (~200 ml per filter) containing 2× SSC, 0.05% sodium dodecyl sulfate, 20 mM sodium phosphate (pH 7), and 0.06% sodium pyrophosphate. Washes were at 68°C for 20 min in 1× wash solution (four times), 0.5× wash solution (1 time), 0.2× wash solution (one time), and 0.05× wash solution until Cerenkov counts were less than 100 cpm/5 ml. Filters were dried and exposed to Kodak XAR-5 film at −70°C.

Sequence analysis. Sequencing was performed by the dideoxy chain termination method of Sanger et al. (51). The end terminal regions of TLev1 were sequenced by cloning an *Xba*I-*Sal*I fragment from the 5′ end and an *Xba*I-*Pst*I fragment from the 3′ end (see Fig. 1C) in both orientations with M13mp10W and M13mp11W vectors (New England BioLabs). Regions of the 5′ and 3′ recombinant vectors were spliced out with the enzyme *Sst*I to extend the sequence from within the fragments. To complete the 5′ terminus of the LTR sequences, *Taq*I and *Hpa*II digests of a 5′ *Pst*I-*Sst*I fragment and a 3′ *Kpn*I-*Sst*I fragment were ligated to the *Acc*I site of M13mp10W. *Taq*I and *Hpa*II cut between the *Xba*I-*Sst*I sites within the LTR sequence. By screening the two fragment libraries with an LTR sequence probe, clones overlapping and extending into the 5′-terminal region of the LTR sequences were obtained. Greater than 95% of the LTR regions were sequenced in both directions, and each nucleotide was sequenced at least twice. An internal *Hin*dIII fragment, located ~2.5 kb before the 3′ end of the TLev1 (see Fig. 1C), was cloned into the *Hin*dIII site of M13mp10W. Sequences were compiled and analyzed by the programs of Pustell and Kafatos (47), modified by R. Smith and M. Plotnick, New York University Medical Center, New York, N.Y.

RESULTS

Retroviral sequences occur within the C57BL/10 *TL* locus. Figure 1A indicates the positions of the two sequences within the *TL* locus which have been shown to hybridize with MuLV-derived probes (42). The two virus sequences are separated by ~20 kb and occur in opposite orientation to the adjacent *TL* genes. TLev1 and TLev2 appear unrelated by restriction enzyme analysis. Since the TLev2 subclone apparently contains a defective viral genome, the studies presented here focus on the TLev1 sequence.

Blot hybridization analysis, performed under stringent conditions, localized MuLV hybridizing sequences within the TLev1 subclone. Digestion of an 8-kb *Xba*I fragment of TLev1 with both *Hpa*I and *Bam*HI yielded four fragments (Fig. 2, lane 1); the size and position of the restriction fragments are shown in Fig. 1C. Electrophoretic resolution of the individual TLev1 *Hpa*I-*Bam*HI digests, followed by blot hybridization with MuLV probes (described in Materials and Methods), yielded the results shown in Fig. 2. The 3.4-kb *Xba*I-*Hpa*I fragment, located at the 5′ end of TLev1, predominantly hybridized with the 5′ LTR-*gag* probe (Fig. 2, lane 2). Since the TLev1 LTR regions do not share homology with AKR sequences, as shown by sequence analysis and hybridization experiments (data not shown), it is presumed that the observed hybridization reflects homology with AKR *gag*-related sequences. The 5′ *pol* probe showed strong hybridization to the 1.4-kb *Hpa*I-*Hpa*I fragment. Additional weak hybridization (difficult to see in Fig. 2) was observed to the 3.4-kb *Xba*I-*Hpa*I fragment and the 1.1-kb *Hpa*I-*Bam*HI fragment (Fig. 2, lane 3) to which the 3′ *pol*-5′ *env* probe hybridized more intensely (Fig. 2, lane 4). A probe derived from the 3′ region of the AKR virus, 3′ *env*-LTR, hybridized to the 2.0-kb *Bam*HI-*Xba*I fragment, located within the corresponding region of the TLev1 genome (Fig. 2, lane 5). TLev1 sequences did not hybridize with *H-2* gene probes (Fig. 2, lane 6 and 7), as expected from their map position relative to the class I *TL* genes (Fig. 1A).

The hybridization results show that MuLV probes mapped

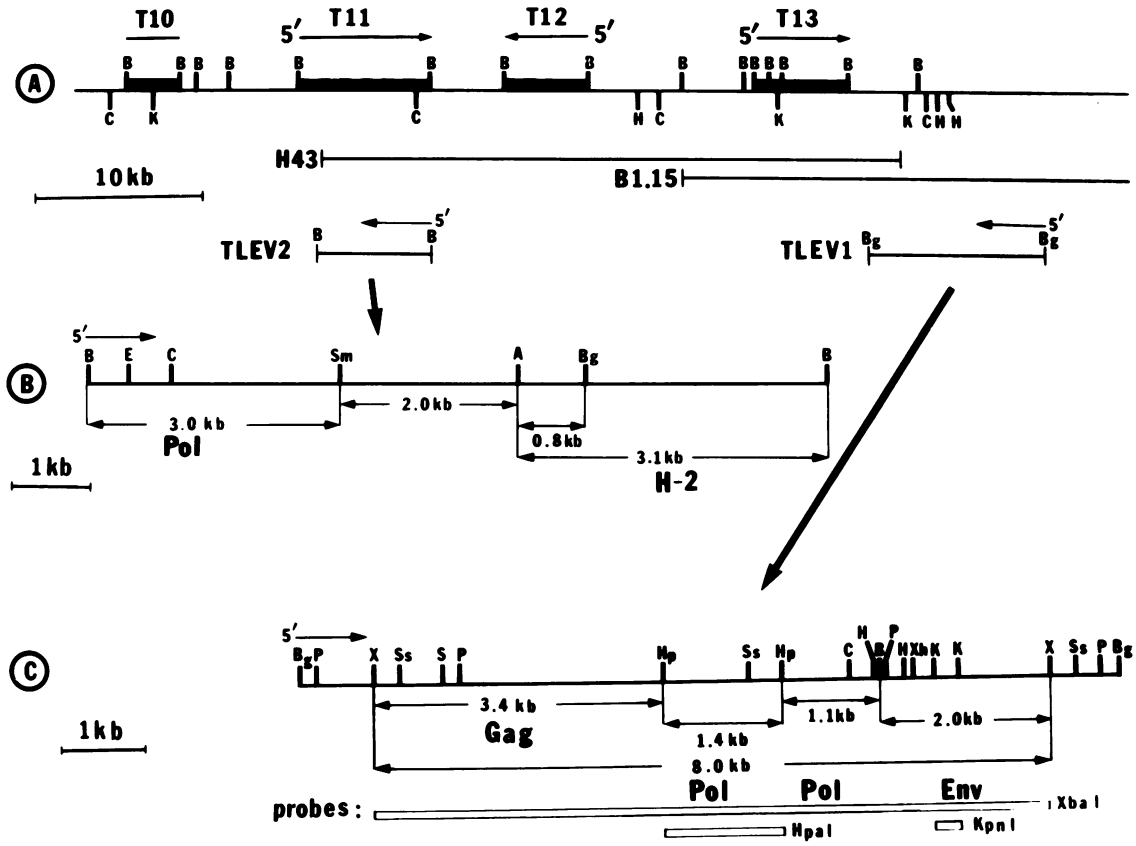

FIG. 1. Position of retroviruslike sequences within the *TL* locus of the C57BL/10 murine MHC. (A) A map of the *TL* region where retrovirus sequences are located is shown. The location of class I genes within *Bam*HI fragments are shown by solid lines. H43 and B1.15 represent overlapping cosmids within the *TL* locus (63). (B) Partial restriction map of TLev1. (C) Partial restriction map of TLev2. Note that the kilobase scale is expanded in panels B and C and the orientation of TLev sequences has been reversed. Symbols: A, *Acc*I; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; K, *Kpn*I; P, *Pst*I; S, *Sal*I; Ss, *Sst*I; Sm, *Sma*I; X, *Xba*I; Xh, *Xho*I.

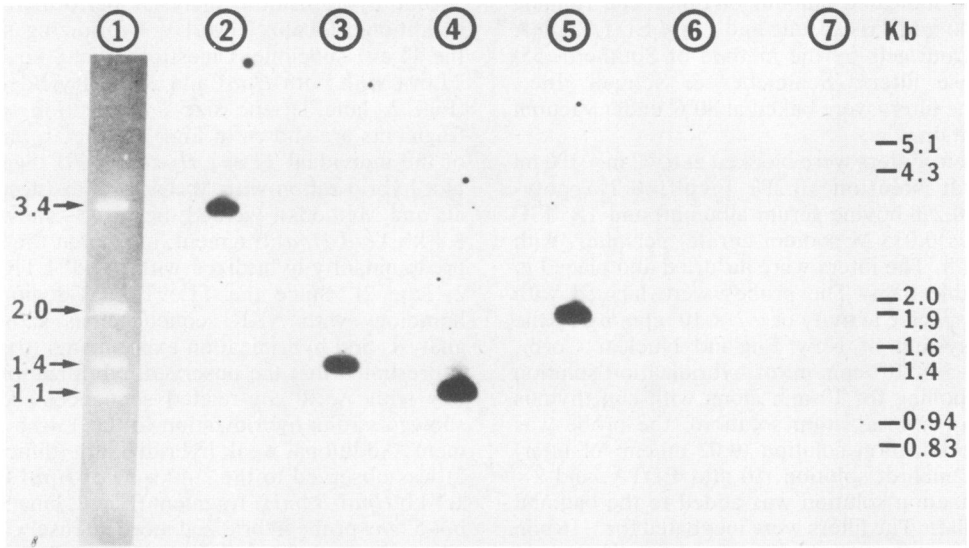

FIG. 2. Location of MuLV-related sequences in restriction fragments of TLev1. Blot hybridization analysis is shown for individual lanes from 1% agarose gel containing the 8-kb *Xba*I fragment of TLev1 (125 ng) digested with both *Hpa*I and *Bam*HI (Fig. 1C). The conditions for DNA digestion and Southern blot hybridization and the description of probes are presented in Materials and Methods. Electrophoresis was at 5 V/cm for ~18 h. Hybridization of AKR-derived probes is shown as follows. Lanes: 2, 5′ LTR-*gag*; 3, 5′ *pol*; 4, 3′ *pol*-5′ *env*; 5, 3′ *env*-LTR. Hybridization to *H-2* probes are shown in lanes 6, and 7 (H2III and H2IIa, respectively). Lane 1 shows the ethidium bromide-stained digestion pattern (markers in kilobases). The indicated markers on the right represent the mobility of λ *Hin*dIII-*Eco*RI fragments. Exposure was for 2 days.

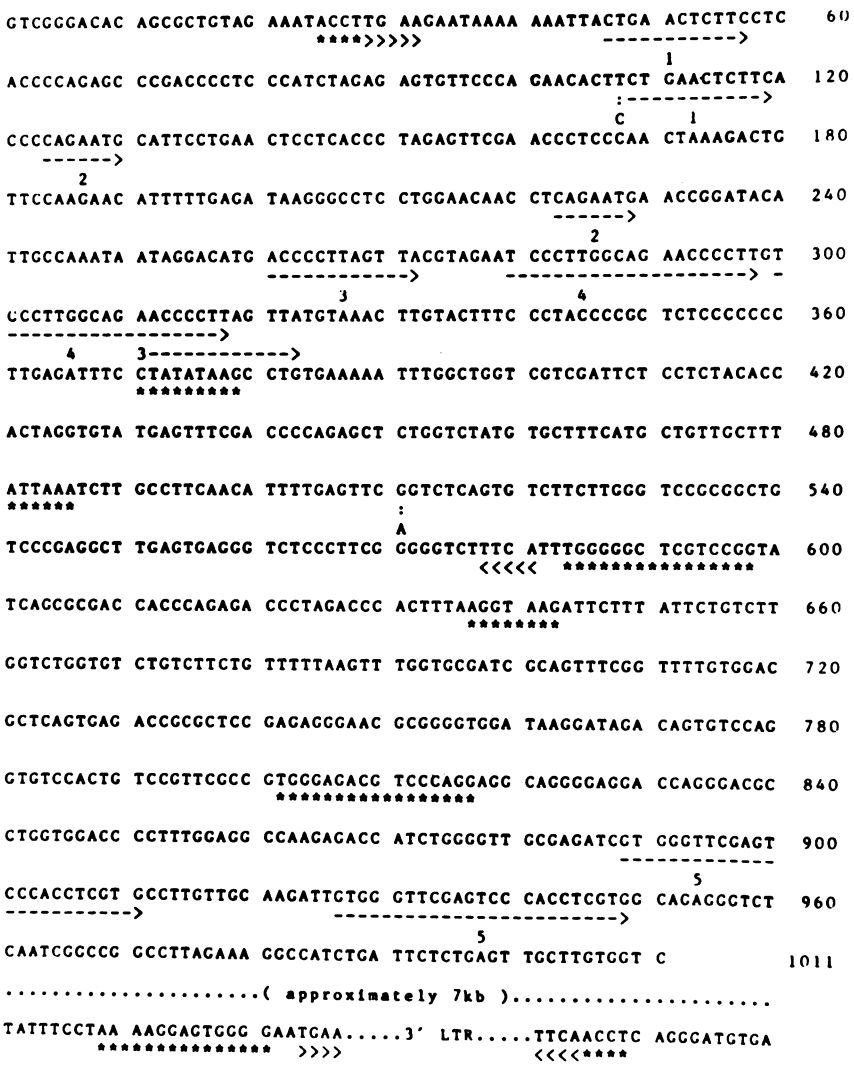

FIG. 3. Nucleotide sequence of the LTR regions of TLev1. Sequence analysis was performed as described in Materials and Methods. Symbols: ****, structural elements defined within the text; —→ direct repeat sequences; >>>>, <<<<, inverted repeat sequences. A T-C and a G-A transition are shown at positions 168 and 571, respectively. Numbers on the right refer to sequential nucleotides.

within TLev1 in the order *gag-pol-env*, indicating a shared sequence and structural homology with AKR MuLV. The hybridization experiments also suggested that the AKR and TLev1 sequences were not strongly homologous. When cross-hybridization experiments were performed with various concentrations of AKR and TLev1 genomes on a Southern blot, 50 to 100 times more DNA was required to see signals equivalent to those observed when the individual virus sequences were self-hybridized (data not shown). Restriction endonuclease site analysis also suggested a distant relationship between TLev1 and MuLV sequences. Restriction sites, such as *Bgl*II, *Sma*I, *Pvu*II, and *Eco*RI, which are found in many MuLV proviruses and endogenous MuLV DNAs (8, 9, 27, 33, 36), are absent from the TLev1 sequence, precluding the categorization of TLev1 among prototypic MuLV proviruses.

LTR sequences are present in TLev1. An attempt was made to determine whether LTR regions exist within the TLev1 subclone. The presence of LTR sequences would formally characterize TLev1 as a retroviruslike element as well as delineate the endpoint of the virus and augment the possibility that TLev1 may be capable of functional interactions with the host genome.

The strategy for sequencing the end termini of TLev1 is described in Materials and Methods. The nucleotide sequence for the 5'-terminal region of TLev1 is shown in Fig. 3. The 3'-terminal sequence was identical to nucleotides 29 to 581 of the 5' LTR with the exception of two transitions at nucleotides 108 (T-C) and 511 (G-A); the points of sequence divergence define the boundaries of the LTR units. In addition to the terminal redundancy, the sequences can be characterized as LTRs by the following criteria (see references 13, 58, 61 for reviews): (i) inverted repeats (TGAA. . .TTCA), ending with the dinucleotides TG. . .CA, are found at the borders of the LTR sequences; (ii) a 4-bp duplication of host genomic DNA (ACCT) occurs at the host-virus junction sites of the 5' and 3' LTR; (iii) the 3' boundary of the 5' LTR (bp 581) is followed by two T residues and then a site (bp 584 to 598) homologous to the first 15 bp of an 18-bp $tRNA^{Pro}$ negative strand primer binding site, found at the corresponding position of most mammalian type C retroviruses (11); (iv) the 5' boundary of

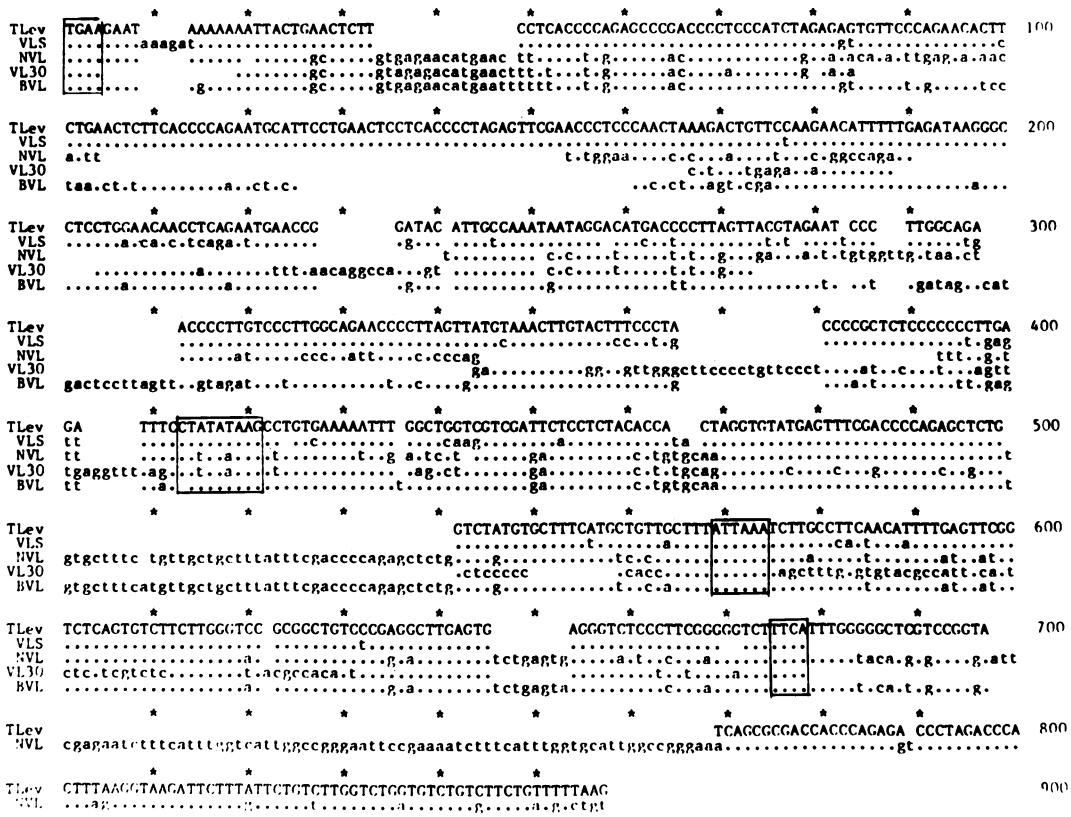

FIG. 4. Comparison of TLev1 and VL30 LTR sequences. A computer-assisted alignment of VL30 LTR sequences is presented. Numbers on the right refer to the relative position of nucleotides. Spaces represent gaps introduced to align the sequences. The following sequences are compared. TLev1 (TLev, 553 bp); VLS-1 (VLS, 556 bp), a detached LTR sequence isolated from a BALB/c mouse library (50); NVL-3 (NVL, 498 bp), an LTR sequence of an expressed VL30 element cloned from a pseudovirion isolated from NIH 3T3 cells (45); the VL30-3 clone (VL30), isolated from a BALB/c library (31); BVL-1 (BVL, 601 bp), the LTR of a VL30 clone isolated from a BALB/c library (26). The inverted repeat sequences, TATA box, and polyadenylation signals were boxed to provide reference points. ...., Homology with TLev1, lower-case letters denote nonhomologous nucleotides.

the 3′ LTR is directly preceded by two adenine residues, which follow a 13-bp purine-rich region thought to be a primer site for the positive strand synthesis of proviral DNA; (v) putative transcriptional regulatory sites include a TATA box at nucleotides 371 to 389 and a polyadenylation signal at nucleotides 481 to 486; (vi) four sets of short direct repeat sequences, observed in the region spanning nucleotides 47 to 323, may be related to the larger repeat units observed in the U3 region of many retrovirus species.

Approximately 450 nucleotides after the TLev1 5′ LTR have been sequenced. This region, which is not homologous with MuLV sequences and does not display an open reading frame, may prove to be part of a long untranslated leader sequence (13). Within this region, at nucleotides 637 to 643, a sequence homologous to a donor mRNA splice site (52) was observed. Two other features of this region have been noted. A 16-bp sequence, located ~200 bp (nucleotides 802 to 818) from the tRNA primer binding site, is homologous to a sequence found in the same relative position in Moloney MuLV (53), Moloney sarcoma virus (59), Abelson MuLV (48), Akv MuLV (25), and FBJ murine osteosarcoma virus (59). A 23-bp direct repeat sequence occurs at nucleotides 889 to 911 and 927 to 948. Although the significance of these sequences is not clear, they may play a role in functions of the leader region, such as dimer linkage of RNA, mRNA splicing, and packaging of RNA into virions (13).

The TLev1 LTR is homologous to VL30 LTR sequences. Comparison of the TLev1 LTR with MuLV and various other retrovirus LTR sequences revealed no significant homologies. Approximately 80% of the TLev1 LTR sequence shared less than 50% homology with MuLV LTRs; the highest homologies were ~55% over regions of 40 to 50 bp. Homology was observed with LTR sequences of murine VL30 elements, a retroviruslike gene family encoding 30S mRNA molecules which appear to contain signals for virion packaging, replication, and proviral integration but lack functional replicative genes and genes for viral structural proteins; VL30 elements can be transmitted as pseudovirions. (2, 29, 35).

In Fig. 4, the TLev1 5′ LTR is aligned with four reported VL30 LTR sequences. The highest degree of homology was observed between TLev1 and the "solo" detached VL30 LTR sequence (VLS-1), isolated by Rotman et al. (50); approximately 90% homology was observed, with only 12 gaps introduced to align the sequences. The aligned sequences of TLev1 and the other VL30 LTRs presented in Fig. 4 showed lesser although significant homologies: BVL-1, ~80%, NVL-3, ~70%; VL30, ~65%. The major differences among the VL30 LTRs involved insertions and deletions of sequences. For example, the NVL, VL30, and BVL LTRs contained 15-bp insertions relative to TLev1 and VLS at positions 34 to 48. The NVL and BVL LTRs contain

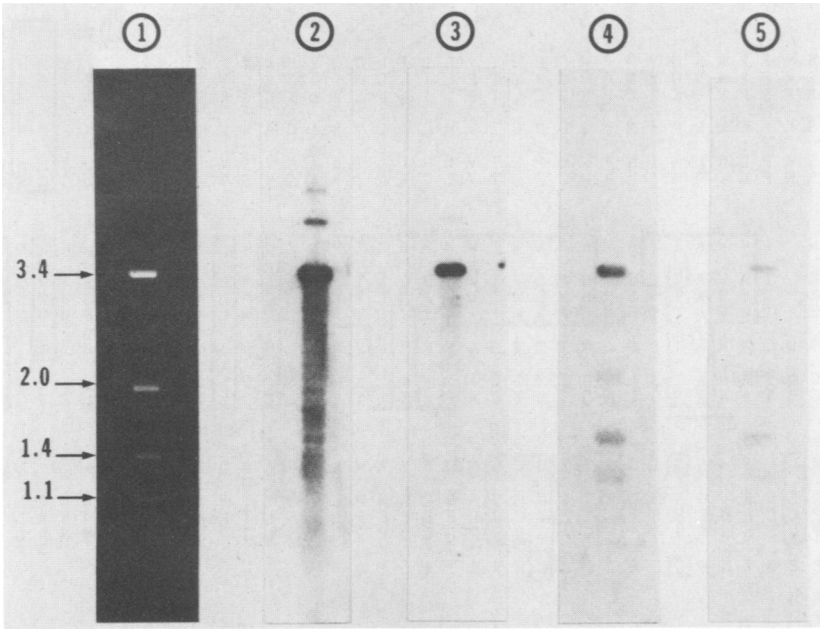

FIG. 5. Hybridization of TLev1 with VL30 and AKR probes. A blot hybridization of the *Hpa*I-*Bam*HI digest of the 8-kb *Xba*I fragment of TLev1 was performed as described in the legend to Fig. 2. Lane 1 shows the ethidium bromide-stained digestion pattern of 100 ng of the TLev1 *Xba*I fragment. Lanes 2 and 3 were hybridized with the excised *Xho*I insert of BVL-1 VL30 (26). Lanes 4 and 5 were hybridized with the *Pst*I insert of AKR. Lanes 2 and 4 contain 100 ng of the TLev1 digest. Lanes 3 and 5 contain 10 ng of the TLev1 digest. Filters were exposed overnight. Markers (in kilobases) are given at the left.

42 bp insertions at positions 499 to 541, which differ only by the addition of a single adenine nucleotide in the BVL sequence. In the region spanning positions 100 to 200, NVL, VL30, and BVL LTRs show 40- to 50-bp deletions relative to TLev1 and VLS. The sequences of TLev1 and NVL extend beyond the 3′ border of the 5′ LTR. Here again considerable homology is observed, with the exception of a 72-bp insertion (positions 698 to 768) in the NVL-3 LTR, which encompasses duplications of the inverted repeat and tRNA primer binding site (45).

Regulatory sequences, such as the TATA box and polyadenylation signals, appear to be conserved within all LTR sequences. Interestingly, the BVL and NVL LTRs contain a primer binding site for $tRNA^{Gly}$ instead of $tRNA^{Pro}$. Itin and Keshet (32) have recently found primer binding sites for three tRNA species within the VL30 gene family. The similarity between two of the VL30 LTR sequences does not appear to correlate with the species of the tRNA primer binding site found adjacent to their 5′ LTRs. As previously observed (30, 45, 50), each of the VL30 LTR sequences have the same inverted repeat sequence at their 5′ and 3′ borders. TLev1 and VLS share 12 of 12 while NVL, BVL, and VL30 share 11 of 12 nucleotides of the 3′ inverted repeat with eight murine type C retroviruses and Feline sarcoma virus (11). Only the first four bases of the 3′ inverted repeat sequence are found at the 5′ inverted repeat of the VL30 LTRs.

The 5′ region of TLev1 hybridized with an endogenous VL30 clone. To determine whether internal TLev1 sequences also share homology with VL30 elements, an *Hpa*I-*Bam*HI digest of the TLev1 *Xba*I fragment (Fig. 1C and Fig 2) was hybridized with an endogenous VL30 sequence isolated by Hodgson et al. (26). Figure 5 shows the results obtained when 100 and 10 ng of the TLev1 digest is hybridized with the *Xho*I insert of the VL30 BVL-1 clone (Fig. 5, lanes 2 and 3, respectively). The only strong hybridization observed occurred between the VL30 sequence and the 3.4-kb *Xba*I-*Hpa*I fragment of TLev1. While the *Xba*I-*Hpa*I fragment contained the 5′ LTR, which has been shown to be homologous by sequence analysis, an internal *Sal*I-*Hpa*I TLev1 fragment also showed strong hybridization with the VL30 clone (data not shown), suggesting that some homology is shared between the 5′ region of TLev1 and VL30 sequences. In agreement with the results shown in Fig. 5, no hybridization was observed when the following Tlev1 fragments (Fig. 1C) were used to probe the VL30 clone: *Hpa*I-*Hpa*, *Hin*dIII-*Kpn*I, *Kpn*I-*Kpn*I (data not shown).

For comparison, hybridization of the TLev1 digest with AKR MuLV is shown in Fig. 5, lanes 4 and 5. While the AKR viral probe hybridized to all four fragments of TLev1, the degree of hybridization was less than that observed with the VL30 probe; the VL30 sequence hybridized more intensely to 10 ng compared with AKR hybridization to 100 ng of the TLev1 digest.

The predicted sequence of an internal TLev1 fragment corresponded to the amino-terminal region of the MuLV gp70 protein. To more precisely determine the degree of homology between the TLev1 and AKR genomes, a small, 0.35-kb, *Hin*dIII internal fragment of TLev1 was sequenced. A 125-bp region of 70% nucleotide identity was the longest homologous sequence observed between the AKV genome and the TLev1 *Hin*dIII fragment (data not shown). The region of homology occurred within the first 300 bp of the AKV *env* gp70 gene. Similar degrees of homology were found with the corresponding regions of two other ecotropic viruses, Moloney MuLV and Friend MuLV. Lesser homologies were found with the gp70 gene of xenotropic and dualtropic viruses. The amino-terminal two-thirds of the gp70 gene is the most variable region of MuLV genomes; the heterogeneity appears to correlate with the ecotropic,

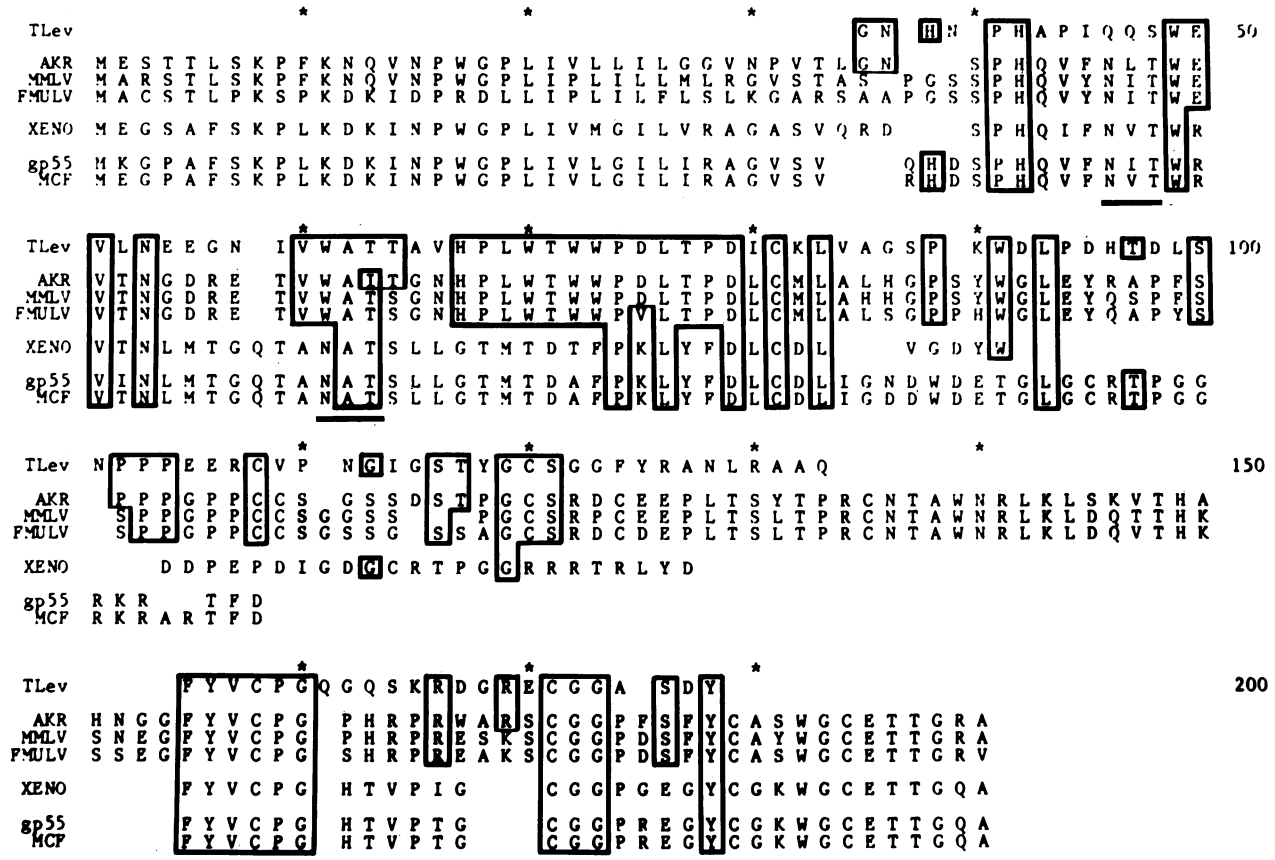

FIG. 6. Homologies of a predicted TLev1 amino acid sequence corresponding to the amino-terinal region of MuLV gp70. The predicted amino acid sequences of the corresponding regions from several MuLVs were aligned with the aid of computer data and comparisons with previous alignments reported in the literature (28, 49). Numbers on the right refer to relative positions of amino acids. Spaces represent gaps introduced for alignment. The standard one-letter amino acid abbreviations are used (Eur. J. Biochem. **138**:31, 1984). Homologies of the TLev1 amino acids with other virus sequences are boxed. Horizontal spacing separates classes of retroviruses. Retrovirus class: ecotropic, AKV (AKR) (39), Moloney MuLV (MMLV) (53), Friend MuLV (FMULV) (37); xenotropic (XENO) (46); Dualtropic, spleen focus-forming virus gp55 (1) and MCF 247 (MCF) (28). Potential glycosylation sites (Asn-X-Thr or Ser) are underlined.

xenotropic, or dualtropic class of the MuLVs (28, 48). Due to the difficulty in aligning nucleotide sequences within the amino-terminal gp70 gene, the predicted amino acid sequences were determined and aligned for the regions of several MuLV sequences showing homology with TLev1. Figure 6 shows the comparison of the predicted TLev1 *Hin*dIII sequence with the amino-terminal region of three ecotropic, one xenotropic, and two dualtropic *env* genes. The homology between aligned sequences of TLev1 and MuLVs was 49% with ecotropic MuLVs and 27% with xenotropic and dualtropic MuLVs; several insertions or deletions or both were required to align the sequences. Amino acid identities between TLev1 and MuLV sequences are boxed.

The amino acid sequence of TLev1 from position 30 to 60 showed a similar degree of homology to those of all three classes of MuLV. Segments of this region (positions 43 to 48) were quite heterogeneous, having amino acids which require at least two nucleotide substitutions. TLev1 lacked the potential glycosylation site which appears conserved within this region of the three MuLV classes. The region of amino acids spanning positions 50 to 100 showed relatively strong homology to the ecotropic *env* sequences. The amino acid tryptophan, designated by a single codon, was conserved at five positions within this region. Both TLev1 and the ecotropic MuLV sequences lacked the potential glycosylation site found within the xenotropic and dualtropic *env* sequences.

At amino acid positions 120 to 154, TLev1 showed no homology with other MuLV sequences but resembled xenotropic and dualtropic *env* genes in having a large deletion relative to the ecotropic viruses. The remaining TLev1 amino acids showed relatively similar homology to all classes of MuLV sequences. The similarities observed, between the TLev1 *Hin*dIII fragment and MuLV *env* gene sequences support the hybridization data which indicate a relatedness between the TLev1 and MuLV genomes.

Comparison of TLev1, MuLV, and VL30 sequence organization in the mouse genome. Probes derived from TLev1 hybridized to multiple sequences within the mouse genome. Figure 7 compares a blot hybridization of C57BL/10 mouse genomic digests with MuLV AKR (Fig. 7, panel 1), TLev1 (Fig. 7, panel 2), and an *Xho*I insert of an endogenous VL30 element, BVL-1 (26) (Fig. 7, panel 3). In lane A of each panel, *Eco*RI digests are shown. While *Eco*RI sites do not occur within TLev1, AKR (8, 9), or the BVL-1 VL30 sequence (26), a conserved *Eco*RI site does occur in MuLV xenotropic viruses (33, 35), and *Eco*RI sites have been found

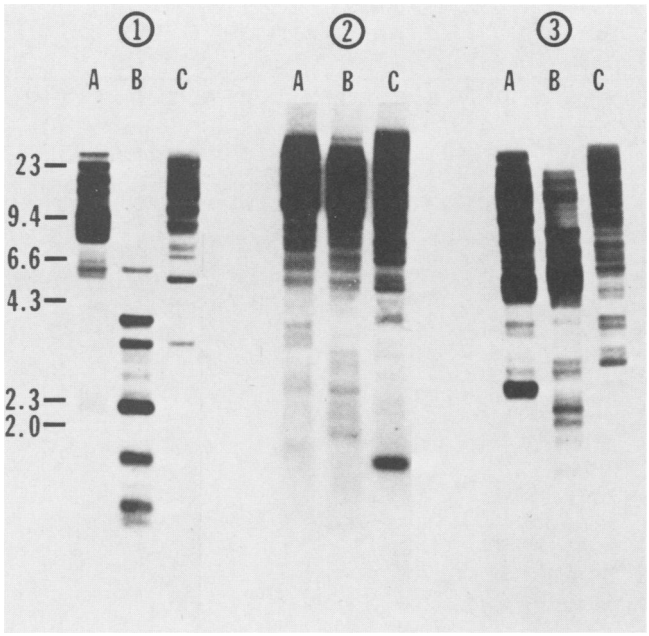

FIG. 7. Blot hybridization analysis of C57BL/10 genomic DNA with AKR and TLev1 and with BVL-1 VL30 probes. C57BL/10 thymus DNA (10 μg per lane) was digested with *Eco*RI (lane A), *Bgl*II (lane B), or *Hpa*I (lane C) and electrophoresed through to a 0.7% agarose gel at 5 V/cm for ~18 h. Conditions for DNA digestion and Southern blot hybridization and the description of probes are presented in Materials and Methods. Hybridization results with the indicated probes are shown as follows. Panel: 1, AKR; 2, TLev1; 3, BVL-1 VL30 *Xho*I insert. Note that the AKR probe used in this experiment was an *Eco*RI-*Hin*dIII insert which contains mouse genomic flanking sequences (40). Similar results were obtained, however, when a *Pst*I insert, which cuts within the LTR sequence, was used (8). Size markers (in kilobases) represent the mobility of λ *Hin*dIII fragments. Exposure was overnight with an intensifying screen.

in other endogenous VL30 clones (34, 45). The hybridization patterns revealed many high-molecular-weight bands, reflecting the multiplicity of cross-hybridizing virus sequences and the limited number of *Eco*RI sites in the viral genomes. It appears that the TLev1 sequence hybridized to a greater number of bands within the 9.4- to 23-kb size range than the AKR and BVL probes.

The most striking difference between the TLev1 and AKR hybridization patterns was observed in *Bgl*II digests of mouse genomic DNA (Fig. 7, lane B). While *Bgl*II cut just outside the TLev1 genome, many MuLV proviral DNAs contain more than one *Bgl*II site (25), and *Bgl*II sites have been found in VL30 elements (35, 45). TLev1 clearly hybridized with multiple bands within the 9.4- to 23-kb range which do not appear to hybridize or hybridize weakly with the AKR probes (Fig. 7, panels 1 and 2, lane B). Conversely, the AKR probes showed intense hybridization with several lower-molecular-weight bands to which TLev1 probes may hybridize to a far lesser extent if at all. When a xenotropic MuLV sequence, NSF-Th-1 (7), was used as a hybridization probe, the overall patterns were similar to those found with the AKR-derived probes (data not shown). The data suggest that the mouse genome contains multiple sequences that, like TLev1, lack *Bgl*II sites and that cross-hybridize only weakly with MuLV DNA. While TLev1 and BVL-1 hyridization patterns were less distinct (Fig. 7, panels 2 and 3, lane B), TLev1 sequences apparently hybridized to a greater proportion of high-molecular-weight bands than BVL-1, while BVL-1 sequences hybridized to a greater proportion of bands within the 4- to 6-kb region.

TLev1 hybridization to an *Hpa*I digest of genomic DNA (Fig. 7, panel 2, lane C) revealed an intense band of 1.4 kb which corresponded to sequences hybridizing with the internal *Hpa*I fragment of the TLev1 genome (Fig. 1C). Even though the TLev1 1.4-kb *Hpa*I fragment cross-hybridized with the AKR *pol* gene (Fig. 2), the most highly conserved of the MuLV genome (12), the AKR probe did not appear to hybridize to the 1.4-kb *Hpa*I fragment within the genome (Fig. 7, panel 1, lane C). BVL-1 VL30 sequences also did not hybridize to a 1.4-kb *Hpa*I genomic band (Fig. 7, panel 3, lane C); however, this was expected since the TLev1 *Hpa*I fragment did not hybridize with the BVL-1 VL30 subclone (data not shown).

The distinct differences between the AKR and TLev1 hybridizations support the observations that MuLV and TLev1 genomes are distantly related. While it is difficult to analyze the multiple band patterns, the TLev1 and BVL-1 blot hybridizations appear more similar than TLev1 and AKR. Since the TLev1 genome is ~4 kb larger than the BVL-1 subclone, it is possible that the additonal hybridizing sequences present on TLev1 obscure what would otherwise be similar band patterns. The VL30 probe does appear to hybridize to a greater number of bands within the 2- to 4-kb range of the genomic digests, suggesting that the BVL-1 clone contains sequences which are not present in TLev1.

Distribution of TLev1 sequences among mouse strains. A 0.3-kb *Kpn*I probe, derived from the 3′ end of the TLev1 subclone (Fig. 1C), was utilized to examine the distribution and heterogeneity of TLev1 sequences among various mouse strains. The TLev1 *Kpn*I probe does not hybridize with AKR ecotropic MuLV DNA, NSF-Th-1 or NZB xenotropic MuLV DNA (46), amphotropic MuLV DNA (10), or a subcloned endogenous VL30 sequence, BVL-1 (26) (data not shown). Digestion of genomic DNA with *Kpn*I, followed by blot hybridization with the *Kpn*I probe, should reveal a 0.3-kb band corresponding to the genomic TLev1 sequence. Additional bands of different sizes signify cross-hybridizing sequences with altered 0.3-kb *Kpn*I sites. The results of such an experiment are presented in Fig. 8, panel 1. A 0.3-kb *Kpn*I band was observed in the DNA of all mouse strains, including Sc-1 wild mouse DNA (Fig. 8, lane f). Numerous faint bands were also observed, many of which appear to be shared by the various mouse strains. The relatively high intensity of the 0.3-kb *Kpn*I band suggests that the *Kpn*I sites are conserved among multiple TLev1-like sequences. An indication of the number of TLev1 cross-hybridizing sequences in the genome is given in Fig. 8, panel 4, in which *Bgl*II, which cut outside TLev1, was used to digest the genomic DNA. Figure 8, panel 2 shows the blot hybridization analysis of *Sst*I digests probed with the *Kpn*I probe. The internal 4-kb *Sst*I sequence of TLev1 (Fig. 1C) which hybridizes to the *Kpn*I probe was found in all mouse strains tested. Again, the intensity of the 4-kb *Sst*I band indicated conserved sites within multiple TLev1 cross-hybridizing sequences. Multiple faint hybridizing bands, some of which appear conserved and some of which appear polymorphic, were also present among the mouse strains. Digestion wiht the enzyme *Bam*HI yielded a more complicated *Kpn*I hybridization pattern (Fig. 8, panel 3). Since *Bam*HI cut once within TLev1, *Kpn*I hybridization localized the 3′ integration site of the virus sequence. The multiple bands observed may either reflect different integration sites of the various "virus" sequences or internal fragments of

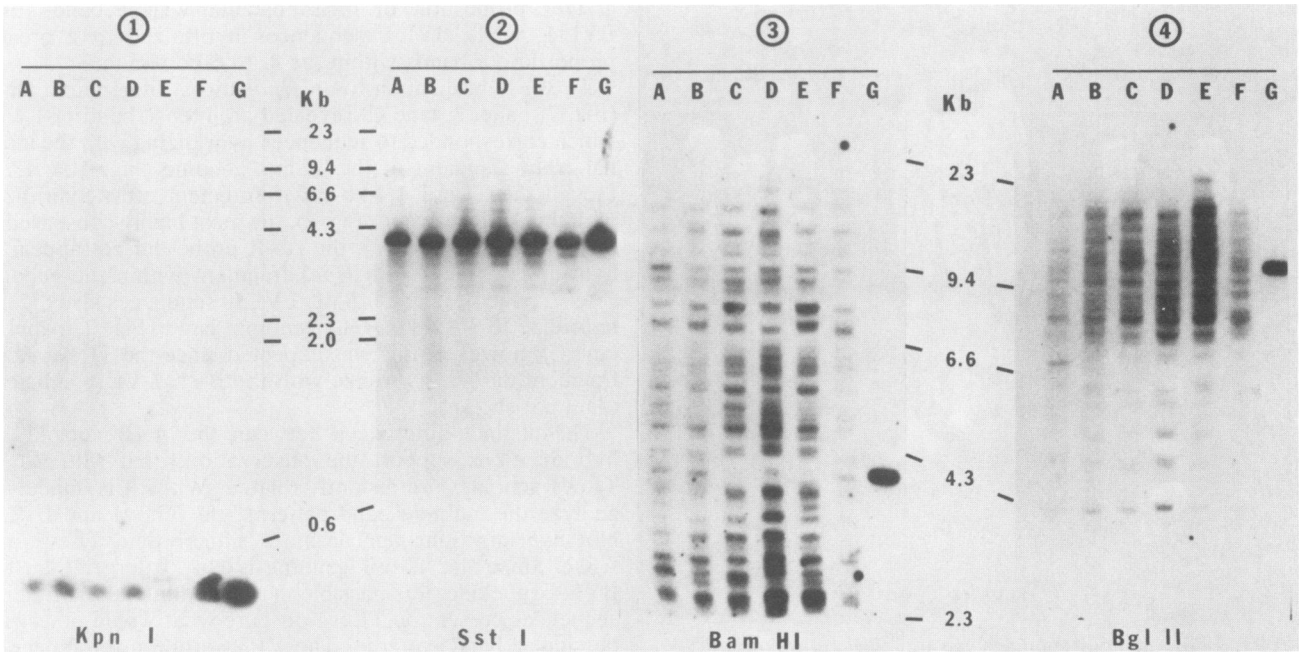

FIG. 8. Blot hybridization analysis of genomic DNA from various mouse strains with an internal TLev1 *Kpn*I probe. Cellular DNA (5 μg per lane) was digested with the following endonucleases. Panels: 1, *Kpn*I; 2, *Sst*I; 3, *Bam*HI; 4, *Bgl*II. Digested samples were electrophoresed through 0.8% agarose gels at 5 V/cm for 16 (panels 1 and 2) or 48 (panels 3 and 4) h. Lanes: A, C57BL/10; B, AKR/J; C, BALB/cBy; D, DBA/2J; E, A/J; F, Sc-1; G, B1.15 cosmid DNA (2 ng). Conditions for DNA digestion and Southern blot hybridization are presented in Materials and Methods. The indicated size markers represent the mobility of λ *Hin*dIII fragments. Exposure was for 5 days.

"viruses" which have altered *Bam*HI sites. Many of the *Bam*HI bands observed among the various strains appear to be similar. Should these represent virus integration sites, the implication would be that many TLev1-like sequences have been stably integrated into the murine genome prior to the divergence of inbred strains; the similar hybridization patterns observed in *Bgl*II digests appear to support this interpretation.

DISCUSSION

The TLev1 genome. The evidence presented demonstrates the existence of retroviruslike sequences within the *TL* locus of the C57BL/10 mouse. Sequence analysis and blot hybridization data indicate that the TLev1 retroviral element is related to both MuLV and VL30 genomes. Interactions between MuLV and VL30 sequences are well established. The derivation of Harvey and Kirsten strains of murine sarcoma virus involves interactions between MuLV, rat VL30, and cellular oncogene sequences (18). Apparent MuLV-VL30 recombinants have been isolated from a BALB/c mouse library (30), and discrete regions of homology between AKV proviral and rodent VL30 genes have been previously reported (21).

The TLev1 genome is related to VL30 elements by sequence analysis of LTR regions and the relatively strong hybridization observed between the 5′ region of TLev1 and a VL30 probe. The TLev1 genome differs from the BVL-1 VL30 clone with respect to size and lack of hybridization within the 3′ half of the Tlev1 sequence. The 8.5-kb TLev1 sequence is more similar in size to MuLV genomes than to endogenous rodent VL30 elements, which are reported to be 5 to 6 kb in length (15, 21, 34, 35). Blot hybridization analysis of mouse genomic digests also indicates differences between the BVL-1 VL30 clone and TLev1. It is difficult to characterize TLev1 with respect to VL30 elements on the basis of comparison with a single VL30 sequence, since this retroviruslike family appears quite heterogeneous (35). The 0.3-kb *Kpn*I probe, which does not hybridize with MuLV sequences or with the BVL-1 VL30 clone, might hybridize with other members of the VL30 gene family. The *Kpn*I probe resembles VL30 sequences in that it hybridizes to multiple copies in the genomes of all mouse strains tested (15, 34) and in its ability to hybridize to rat, but not hamster, DNA (34) (data not shown). The TLev1 *Kpn*I sequence may be derived from a VL30 endogenous element or a different multiple gene family. Sequence analysis of the *Kpn*I fragment and analysis of genomic clones, which have been selected by *Kpn*I probe hybridization, for the presence of other retroviral sequences should provide insight into the nature of the *Kpn*I fragment and the derivation of the TLev1 genome.

While TLev1- and MuLV-cloned sequences cross-hybridize under conditions of high stringency, genomic blot hybridizations, restriction enzyme maps, and titration experiments (not shown) indicate that the TLev1 and MuLV genomes are not strongly homologous. Sequence analysis of a TLev1 *Hin*dIII fragment which corresponds to the MuLV *env* region indicates that short regions (125 bp) of ~70% homology can account for the hybridization data. Based on these results, it is unlikely that the TLev1 genome reflects a recent recombination event between MuLV and VL30 sequences, which would be expected to yield a molecule having a stronger homology to MuLV sequences.

Giri et al. (21) reported a hybridization study between rodent VL30 elements and MuLV genomes. While mouse VL30 elements hybridized to AKV-MuLV under conditions of reduced stringency (3× SSC), rat VL30 elements like TLev1 showed hybridization at conditions of high stringency (0.1× SSC). The BVL-1 VL30 sequence, which hybridizes to the 5′ region of TLev1, also hybridizes within the 5′ region

of a rat VL30 clone (21), raising the possibility that TLev1 may be more closely related to rat VL30 elements.

The evolutionary relationship between MuLV proviruses and VL30 genes has been discussed in reports by Giri et al. (21) and Itin and Keshet (30). The origin of TLev1 is currently unknown. TLev1 may have evolved from a recombinant event between VL30 and MuLV sequences and subsequently diverged and dispersed in the genome, or it may represent a unique retrovirus entity. The TLev1 genome and related sequences may reflect a closer association or a more prevalent interaction between MuLV and VL30 sequences than previously considered.

Implication of retroviruslike sequences within the *TL* locus of the MHC. The expression of TL antigens shows phenotypic variation with respect to genotype, thymocyte differentiation, and leukemic transformation (4, 19). Normal thymocytes of C57BL/10 mice do not express TL antigens on their cell surfaces, while leukemic cells do display TL surface antigens (19). Whether the TLev sequences play a role in the phenotypic expression of TL antigens is a crucial question. Viral genomes are known to affect the expression of cellular genes in their vicinity. The enhanced expression of c-*myc* by avian leukosis virus is an example of proviral activation of flanking cellular DNA sequences (24). Inactivation of cellular genes by the insertional mutagenesis of retroviruses has also been documented (5, 14, 62). Since both the TLev and the *TL* genes cross-hybridize with multiple sequences in the mouse genome, study of their functional relationship presents a difficult problem. Attempts to correlate the presence of TLev1 with TL^- versus TL^+ phenotypes of various mouse strains will require low-copy probes from cellular flanking sequences. The derivation of *TL* gene-specific probes from the *Tl1* to *Tl3* region of the C57BL/10 mouse will allow study of their expression. TLev1 has been shown to contain two LTR sequences. The LTR regions of retroviruses contain elements which regulate the expression and replication of the viral genome. LTRs also contain enhancer regions, which are responsive to tissue-specific factors (16, 38), and regions which interact with viral *trans*-acting transcriptional activators (6, 54). It is possible that normally quiescent endogenous retrovirus sequences may be activated by factors produced under conditions of cellular transformation or virus infection. In this regard, it is important to note that the induction of VL30 mRNA by epidermal growth factor has been observed (20), evoking the possibility that the expression of TLev1 sequences can also be induced by factors affecting the proliferative state of the cell. Investigations are under way to determine whether TLev1 LTR sequences are capable of promoting transcription in normal versus leukemic thymocytes.

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LITERATURE CITED

1. **Amanuma, H., A. Katori, M. Obata, N. Sagata, and Y. Ikawa.** 1983. Complete nucleotide sequence of the gene for the specific glycoprotein (gp55) of Friend spleen focus-forming virus. Proc. Natl. Acad. Sci. USA **80**:3913–3917.
2. **Besmer, P., U. Olshevsky, D. Baltimore, D. Dolberg, and H. Fan.** 1979. Virus-like 30S RNA in mouse cells. J. Virol. **29**:1168–1176.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Boyse, E. A.** 1984. The biology of Tla. Cell **38**:1–2.
5. **Breindl, M., K. Harbers, and R. Jaenish.** 1984. Retroviral-induced lethal mutation in collagen 1 gene of mice is associated with an altered chromatin structure. Cell **38**:9–16.
6. **Broome, S., and W. Gilbert.** 1985. Rous sarcoma virus encodes a transcriptional activator. Cell **40**:537–546.
7. **Buckler, C. E., M. D. Hoggan, H. W. Chan, J. F. Sears, A. S. Khan, J. L. Moore, J. W. Hartley, W. P. Rowe, and M. A. Martin.** 1982. Cloning and characterization of an envelope-specific probe from xentropic murine leukemia proviral DNA. J. Virol. **41**:228–236.
8. **Chattopadhyay, S. K., M. W. Cloyd, D. L. Linemeyer, M. R. Lander, E. Rands, and D. R. Lowy.** 1982. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. Nature (London) **295**:25–31.
9. **Chattopadhyay, S. K., M. R. Lander, E. Rands, and D. R. Lowy.** 1980. Structure of endogenous murine leukemia virus DNA in mouse genomes. Proc. Natl. Acad. Sci. USA **77**:5774–5778.
10. **Chattopadhyay, S. K., A. I. Oliff, D. L. Linemeyer, M. R. Lander, and D. R. Lowy.** 1981. Genomes of murine leukemia viruses isolated from wild mice. J. Virol. **39**:777–791.
11. **Chen, H. R., and W. C. Barker.** 1984. Nucleotide sequences of the retroviral long terminal repeats and their adjacent regions. Nucleic Acids Res. **12**:1765–1778.
12. **Chiu, I.-M., R. Callahan, S. R. Tronick, J. Scholm, and S. A. Aaronson.** 1983. Major *pol* gene progenitors in the evolution of oncoviruses. Science **223**:364–370.
13. **Coffin, J.** 1982. Structure of the retroviral genomes, p. 261–368. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), Molecular biology of tumor viruses: RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Copeland, N. G., K. W. Hutchison, and N. A. Jenkins.** 1983. Excision of the DBA ecotropic provirus in dilute coat-color revertants of mice occurs by homologous recombination involving the viral LTRs. Cell **33**:379–387.
15. **Courtney, M. G., P. K. Elder, D. L. Steffen, and M. J. Getz.** 1982. Evidence for an early evolutionary origin and locus polymorphism of mouse VL30 DNA sequences. J. Virol. **43**:511–518.
16. **DesGroseillers, L., E. Rassart, and P. Jolicoeur.** 1983. Thymotropism of murine leukemia virus is conferred by its long terminal repeat. Proc. Natl. Acad. Sci. USA **80**:4203–4207.
17. **Dische, Z.** 1955. Color reactions of nucleic acid components, p. 287–290. *In* E. Chargaff and J. N. Davidson (ed.), The nucleic acids. Academic Press, Inc., New York.
18. **Ellis, R. W., D. DeFeo, T. Y. Shih, M. A. Gonda, H. A. Young, N. Tsuchida, D. R. Lowy, and E. M. Scolnick.** 1981. The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature (London) **292**:506–511.
19. **Flaherty, L.** 1981. Tla-region antigens, p. 35–37. *In* M. Dorf (ed.), The role of the major histocompatibility complex in immunobiology. Garland Publishing, Inc., New York.
20. **Foster, D. N., L. J. Schmidt, C. P. Hodgson, H. L. Moses, and M. J. Getz.** 1982. Polyadenylated RNA complementary to a mouse retrovirus-like multigene family is rapidly and specifically induced by epidermal growth factor stimulation of quiescent cells. Proc. Natl. Acad. Sci. USA **79**:7317–7321.
21. **Giri, C. P., C. P. Hodgson, P. Elder, M. G. Courtney, and M. J. Getz.** 1983. Discrete regions of sequence homology between cloned rodent VL30 genetic elements and Akv-related MuLV provirus genomes. Nucleic Acids Res. **11**:305–319.
22. **Godson, G. N., and D. Vapnek.** 1973. A simple method of preparing large amounts of ϕX174 RF1 supercoiled DNA. Biochim. Biophys. Acta **299**:516–520.

23. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology **65**:128–134.
24. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1981. ALV-induced lymphoid leukosis: activation of a cellular *onc* gene by promoter insertion. Nature (London) **290**:475–479.
25. **Herr, W.** 1984. Nucleotide sequence of AKV murine leukemia virus. J. Virol. **49**:471–478.
26. **Hodgson, C. P., P. K. Elder, T. Ono, D. N. Foster, and M. J. Getz.** 1983. Structure and expression of mouse VL30 genes. Mol. Cell. Biol. **3**:2221–2231.
27. **Hoggan, M. D., C. E. Buckler, J. F. Sears, H. W. Chan, W. P. Rowe, and M. A. Martin.** 1982. Internal organization of endogenous proviral DNAs of xenotropic murine leukemia viruses. J. Virol. **43**:8–17.
28. **Holland, C. A., J. Wozney, and N. Hopkins.** 1983. Nucleotide sequence of the gp70 gene of murine retrovirus MCF 247. J. Virol. **47**:413–420.
29. **Howk, R. S., D. H. Troxler, D. Lowy, P. H. Duesberg, and E. M. Scolnick.** 1978. Identification of a 30S RNA with properties of a defective type C virus in murine cells. J. Virol. **25**:115–123.
30. **Itin, A., and E. Keshet.** 1983. Apparent recombinants between virus-like (VL30) and murine leukemia virus-related sequences in mouse DNA. J. Virol. **47**:178–184.
31. **Itin, A., and E. Keshet.** 1983. Nucleotide sequence analysis of the long terminal repeat of murine virus-like DNA (VL30) and its adjacent sequences: resemblance to retrovirus proviruses. J. Virol. **47**:656–659.
32. **Itin, A., and E. Keshet.** 1985. Primer binding sites corresponding to several tRNA species are present in DNAs of different members of the same retrovirus-like gene family (VL30). J. Virol. **54**:236–239.
33. **Joseph, D. R.** 1981. Comparison of restriction endonuclease maps of unintegrated proviral DNAs from four xenotropic murine leukemia viruses. J. Virol. **40**:963–970.
34. **Keshet, E., and A. Itin.** 1982. Patterns of genomic distribution and sequence heterogenity of a murine "retrovirus-like" multigene family. J. Virol. **43**:50–58.
35. **Keshet, E., Y. Shaul, J. Kaminchik, and H. Aviv.** 1980. Heterogeneity of 'virus-like' genes encoding retrovirus-associated 30s RNA and their organization within the mouse genome. Cell **20**:431–439.
36. **Khan, A. S., W. P. Rowe, and M. A. Martin.** 1982. Cloning of endogenous murine leukemia virus-related sequences from chromosomal DNA of BALB/c and AKR/J mice: identification of an *env* progenitor of AKR-247 mink cell focus-forming proviral DNA. J. Virol. **44**:625–636.
37. **Koch, W., G. Hunsmann, and R. Friedrich.** 1983. Nucleotide sequence of the envelope gene of a Friend murine leukemia virus. J. Virol. **45**:1–9.
38. **Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host-specific activation of transcription by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA **79**:6453–6457.
39. **Lenz, J., R. Crowther, A. Straceski, and W. Haseltine.** 1982. Nucleotide sequence of the Akv *env* gene. J. Virol. **42**:519–529.
40. **Lowy, D. R., E. Rands, S. K. Chattopadhyay, C. F. Garon, and G. L. Hager.** 1980. Molecular cloning of infectious integrated murine leukemia virus DNA from infected mouse cells. Proc. Natl. Acad. Sci. USA **77**:614–618.
41. **Meruelo, D.** 1980. *H-2D* control of leukemia susceptibility: mechanism and implications. J. Immunogenet. **7**:81–90.
42. **Meruelo, D., R. Kornreich, A. Rossomando, C. Pampeno, A. L. Mellor, E. H. Weiss, R. A. Flavell, and A. Pellicer.** 1984. Murine leukemia virus sequences are encoded in the murine major histocompatibility complex. Proc. Natl. Acad. Sci. USA **81**: 1804–1808.
43. **Meruelo, D., and J. Kramer.** 1981. *H-2D* control of radiation leukemia virus induced neoplasia: evidence for interaction of viral and *H-2* genomic information. Transplant. Proc. **13**: 1858–1862.
44. **Meruelo, D., A. Rossomando, M. Offer, J. Buxbaum, and A. Pellicer.** 1983. Association of endogenous viral loci with genes encoding murine histocompatibility and lymphocyte differentiation antigens. Proc. Natl. Acad. Sci. USA **80**:5032–5036.
45. **Norton, J. D., J. Connor, and R. J. Avery.** 1984. Unusual long terminal repeat sequence of a retrovirus transmissible mouse (VL30) genetic element: identification of functional domains. Nucleic Acids Res. **12**:3445–3460.
46. **O'Neill, R. R., C. E. Buckler, T. S. Theodore, M. A. Martin, and R. Repaske.** 1985. Envelope and long terminal repeat sequence of a cloned infectious NZB xenotropic murine leukemia virus. J. Virol. **53**:100–106.
47. **Pustell, J., and F. C. Kafatos.** 1982. A convenient and adaptable package of DNA sequence analysis programs from microcomputers. Nucleic Acids Res. **10**:51–59.
48. **Reddy, E. P., M. J. Smith, and A. Srinivasan.** 1983. Nucleotide sequence of the Abelson murine leukemia virus genome: structural similarity of its transforming gene product to other *onc* gene products with tyrosine-specific kinase activity. Proc. Natl. Acad. Sci. USA **80**:3623–3627.
49. **Repaske, R., R. R. O'Neill, A. S. Khan, and M. A. Martin.** 1983. Nucleotide sequence of the *env*-specific segment of NFS-Th-1 xenotropic murine leukemia virus. J. Virol. **46**:204–211.
50. **Rotman, G., A. Itin, and E. Keshet.** 1984. 'Solo' large terminal repeats (LTR) of an endogenous retrovirus-like gene family (VL30) in the mouse genome. Nucleic Acids Res. **12**:2273–2282.
51. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
52. **Seif, I., G. Khoury, and R. Dhar.** 1979. The genome of human papovavirus BKV. Cell **18**:963–977.
53. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293**:543–548.
54. **Sodrodski, J. G., C. A. Rosen, and W. Haseltine.** 1984 Transacting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science **225**:381–386.
55. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–518.
56. **Steinmetz, M., J. B. Frelinger, D. Fisher, T. Hunkapiller, D. Pereira, S. M. Weissman, H. Uehara, S. Nathenson, and L. Hood.** 1981. Three cDNA clones encoding mouse transplantation antigens: homology to immunoglobulin genes. Cell **24**: 125–134.
57. **Steinmetz, M., A. Winoto, K. Minard, and L. Hood.** 1982. Clusters of genes encoding mouse transplantation antigens. Cell **28**:489–498.
58. **Temin, H. M.** 1981. Structure, variation and synthesis of retrovirus long terminal repeat. Cell **27**:1–3.
59. **Van Beveren, C., F. Van Straaten, T. Curran, R. Meuller, and I. M. Verma.** 1983. Analysis of FBJ-MuSV provirus and c-Fos (mouse) gene reveals that viral and cellular fos gene products have different carboxy termini. Cell **32**:1241–1255.
60. **Van Beveren, C., F. Van Straaten, J. A. Galleshaw, and I. M. Verma.** 1981. Nucleotide sequence of the genome of a murine sarcoma virus. Cell **27**:97–108.
61. **Varmus, H. E.** 1982. Form and function of retrovial proviruses. Science **216**:812–820.
62. **Varmus, H. E., N. Quintrell, and S. Ortiz.** 1981. Retroviruses as mutagens: insertation and excision of a nontransforming provirus alter expression of a resident transforming virus. Cell **25**:23–26.
63. **Weiss, E. H., L. Golden, K. Fahrner, A. L. Mellor, J. J. Devlin, H. Bullman, H. Tiddens, H. Bud, and R. A. Flavell.** 1984. Organization and evolution of class I gene family in the major histocompatibility complex of the C57BL/10 mouse. Nature (London) **310**:650–655.