JOURNAL OF VIROLOGY, July 1985, p. 251–255
0022-538X/85/070251-05$02.00/0

Vol. 55, No. 1

Proviral Genome of Radiation Leukemia Virus: Molecular Cloning of Biologically Active Proviral DNA and Nucleotide Sequence of Its Long Terminal Repeat

M. JANOWSKI,[1]* J. MERREGAERT,[1] J. BONIVER,[2] AND J. R. MAISIN[1]

Departement de Radiobiology, Centre d'Etude de l'Energie Nucléaire (C.E.N./S.C.K.), Boeretang 200, B-2400 Mol,[1] and Laboratoire d'Anatomie Pathologique, B-23, Université de Liège au Sart-Tilman, B-4000 Liège,[2] Belgium

Received 12 December 1984/Accepted 25 February 1985

The proviral genome of a leukemogenic and thymotropic C57BL/Ka mouse retrovirus, RadLV/VL$_3$(T+ L+), was cloned as a biologically active *Pst*I insert in the bacterial plasmid pBR322. Its restriction map was compared with those, already known, of two nonthymotropic and nonleukemogenic viruses of the same mouse strain: the ecotropic BL/Ka(B) virus and the xenotropic constituent of the radiation leukemia virus complex. Differences were observed around the *gag-pol* gene junction, in the *pol* gene, and in the *env* gene. Moreover, the nucleotide sequence of the RadLV/VL$_3$(T+ L+) long terminal repeat revealed the existence of two copies of a 43-base-pair sequence, of which BL/Ka(B) possesses only one copy.

Radiation leukemia virus (RadLV) is an in vivo-passaged murine leukemia virus (MuLV) complex, isolated from thymic lymphomas of X-ray-irradiated C57BL/Ka mice (24). It can be propagated in vitro from established cultures of RadLV-induced lymphomas, retaining its thymotropic (T+) and leukemogenic (L+) properties after reinjection into C57BL/Ka hosts (9, 10). One of these cultures was termed BL/VL$_3$. It releases RadLV/VL$_3$, a viral complex of which the RadLV/VL$_3$(T+ L+) entity occurs in a 1,000-fold excess over a xenotropic constituent. The proviral genome of RadLV/VL$_3$(T+ L+) is the subject of the studies described in the present note. C57BL/Ka mice harbor at least three classes of nonthymotropic, nonleukemogenic retroviruses replicating, in contrast with the T+ L+ entity, in cultured fibroblasts of the appropriate host range. Well characterized are a B-tropic ecotrope, BL/Ka(B), an N-tropic ecotrope, BL/Ka(N), and a xenotrope, BL/Ka(X) (31, 32). The T+ L+ entity is believed not to be originally present in the germ line of C57BL/Ka mice, but rather to have evolved by recombinational events between endogenous viruses (1, 2).

There seems to be a close correlation between the ability of some MuLVs to replicate in the thymus and their leukemogenicity (7, 9, 12). Studies on in vivo- or in vitro-derived recombinants between some oncogenic and nononcogenic retroviruses have indicated that the U3 region of the proviral long terminal repeat (LTR) determines the growth rate in fibroblasts (38), the thymotropicity (14), the oncogenic potential (30, 33, 38), and the disease specificity (6). The tandem repeats in that region (14, 29) could play a role in the enhancement of expression of a cellular gene (29) by a mechanism which is not yet understood. In the case of RadLV/VL$_3$(T+ L+), it is not known if the LTR displays features which, compared with those of nononcogenic viruses such as AKR (16, 44), NFS xenotropic (26), or BL/Ka(B) (27) MuLVs, might be tentatively correlated with the thymotropicity and leukemogenicity. In the present note, we describe the nucleotide sequence of the LTR, as it appears in a biologically active molecular clone that we prepared from the unintegrated, linear RadLV/VL$_3$(T+ L+) proviral genome.

BL/RL$_{12}$-NP is a non-virus-producing cell line derived from a radiation-induced C57BL/Ka thymic lymphoma. It is permissive for the replication of the T+ L+ component of the RadLV/VL$_3$ complex (31). A 20-h coculture of BL/VL$_3$ and BL/RL$_{12}$-NP cells was processed for extrachromosomal DNA extraction by the urea-hydroxyapatite method described by Shoyab and Sen (41). The resulting DNA preparation was submitted to a 5 to 20% sucrose gradient velocity sedimentation, and samples of the collected fractions were checked for the presence of virus-specific DNA by the standard procedure of Southern (42) by using a ^{32}P-labeled cDNA transcript from a RadLV-related viral RNA genome (23). The fractions shown to contain an 8.9-kilobase-pair (kbp) proviral DNA molecule were treated with *Pst*I and yielded an 8.3-kbp proviral DNA fragment which was submitted to electrophoresis in a preparative agarose slab gel (42), electroelution (47), and chromatography on a Whatman DE52 column. It was then ligated to a *Pst*I-digested, bacterial alkaline phosphatase-treated pBR322 plasmid (19). The reaction product was used to transform *Escherichia coli* C600 cells (8), and the tetracycline-resistant colonies, containing a recombinant plasmid, were selected and screened by colony hybridization (3). Positive colonies were amplified, processed for rapid alkaline extraction of plasmid DNA (4), and checked for the presence of an 8.3-kbp insert. One of the clones obtained, termed pMOL502, was used in the subsequent experiment.

To test for its biological activity, the *Pst*I insert was ligated with T4 DNA ligase and calcium precipitated from a 30-μg/ml solution (17). Carrier-free DNA samples (3 μg) were used to transfect cultures containing 2×10^5 SC-1 cells at the time of plating (22). As soon as 1 week after transfection, the 10 SC-1 cultures that had been treated released type C particles, as attested to by the incorporation of 0.2 pmol of [^{3}H]deoxyribosylthymine monophosphate in a poly(rA.dT$_{10}$)-primed standard reverse transcriptase reaction. Three weeks after transfection, the clarified medium from one of the cultures (incorporating 6.5 pmol of [^{3}H]deoxyribosylthymine monophosphate) was assayed for thymotropicity as described by Declève et al. (11): clarified culture medium was supplemented with 4 μg of Polybrene (Aldrich Chemical Co.) per ml and inoculated as 50-μl samples intrathymically

* Corresponding author.

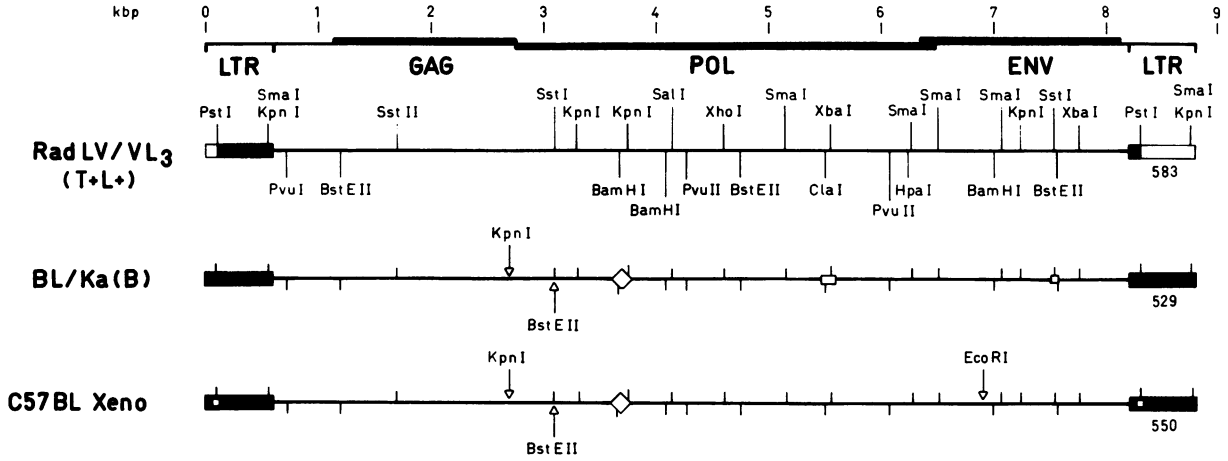


FIG. 1. Restriction enzyme analysis of the RadLV/VL$_3$(T+ L+) proviral genome. The boxes represent the LTRs, the length of which is indicated in bp under the 3′ terminus. The blank areas delineate the sequences not present in the cloned insert. The restriction maps of BL/Ka(B) and BL/Ka(X) are from Grymes et al. (18). Symbols indicate the discrepancy between the relative positioning of the neighboring restriction sites of RadLV and BL/Ka(B) (◇) and missing (□) or additional (⍏) restriction sites.

into 30-day-old female C57BL/Ka mice. Three weeks after inoculation, 10 mice were sacrificed. Thymus cell suspensions were processed for indirect immunofluorescence staining to detect RadLV-related antigens as described by Declève et al. (9, 11). The rat anti-MuLV serum was obtained from W/Fu rats which had been injected intramuscularly for 14 consecutive weeks with 1×10^6 to 10×10^6 W/Fu(SL) cells (9). Fluorescein-conjugated goat anti-rat immunoglobulin (Nordic) was used at a 1:20 dilution in phosphate-buffered saline. In parallel, 30-day-old mice (C57BL/Ka) and 15-day-old rats (R/Cnb) were inoculated intrathymically with the supernatant from SC-1 cells which had become virus producers after transfection. The animals were maintained until they died or showed evidence of severe illness. They were autopsied to check for the presence of a thymic lymphoma.

The 10 mice which were inoculated to test for virus replication in the thymus (11) displayed the presence of RadLV-related antigens in ca. 90% of the thymus cells. Six months after the inoculation, 7 of the 16 rats and 6 of the 10 mice inoculated to test for leukemogenicity had developed a thymic lymphoma. The conclusion that the *Pst*I insert of pMOL502 could only represent the genome of RadLV/VL$_3$(T+ L+) was further confirmed by its restriction map (Fig. 1). It was indeed indistinguishable from that of either the extrachromosomal provirus isolated from the 20-h cocultures of BL/VL$_3$ and BL/RL$_{12}$-NP cells or the integrated proviral species acquired after thymic lymphoma development in RadLV/VL$_3$-inoculated rats (data not shown). Manteuil-Brutlag et al. (34) reported that RadLV/VL$_3$ contains a defective virus characterized by a shorter (6.5-kbp) genome. Its presence was, however, dependent on culture conditions. It was not observed in our RadLV/VL$_3$ cultures, was found in only one of the RadLV stocks investigated by Rassart et al. (37), and is recognized as not being primarily responsible for oncogenesis (18, 37).

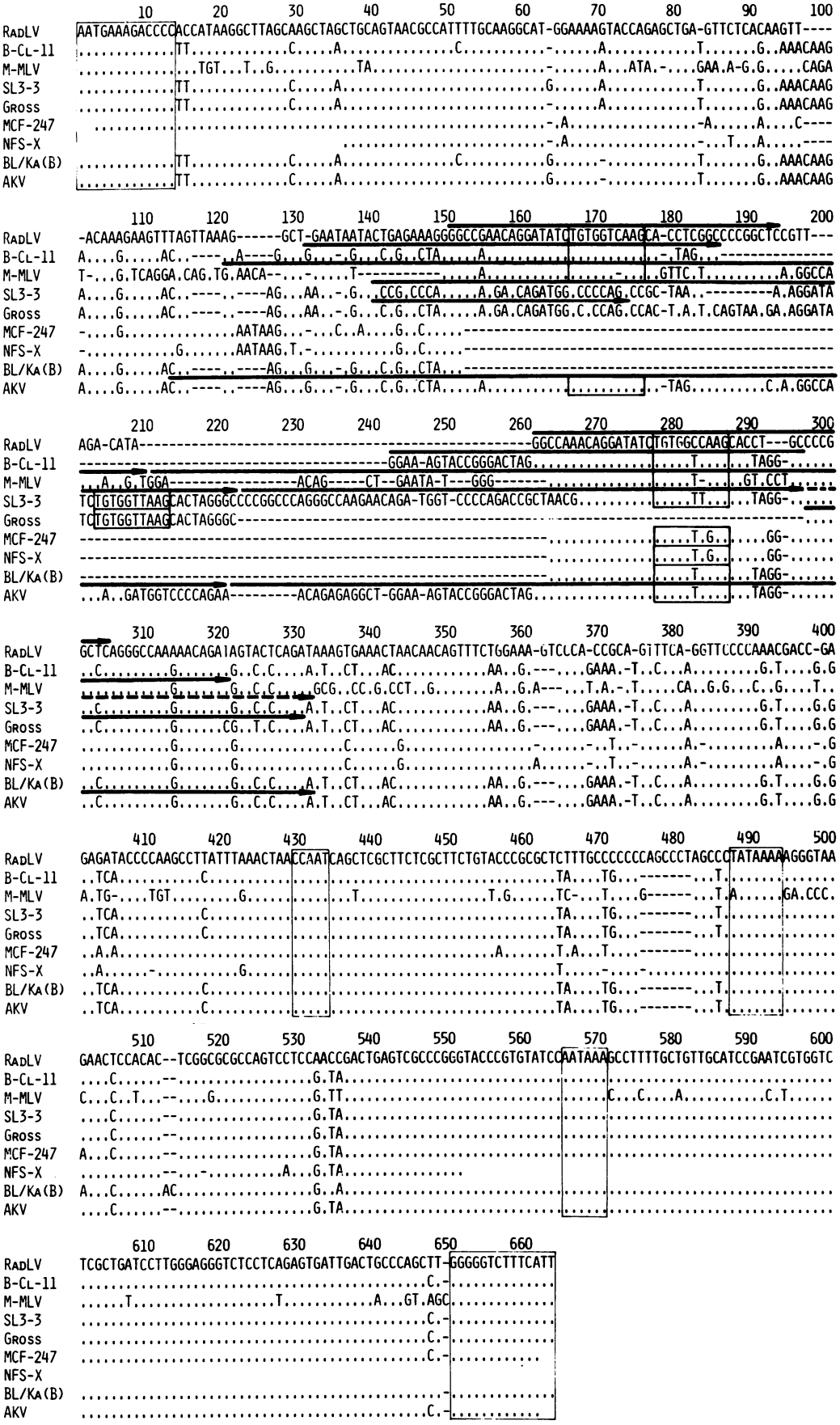
Our results clearly demonstrate that a full-length genome is sufficient to induce leukemia. Because of its extreme thymotropism (32), one might be astonished by the fact that RadLV/VL$_3$(T+ L+) was capable of transfecting SC-1 cells. However, SC-1 cells could be used by the same group to propagate RadLV (18), and other RadLV isolates replicated efficiently on these cells (37).

The restriction map of RadLV/VL$_3$(T+ L+) shows one discrepancy with that of a provirus cloned by Grymes et al. (18) in lambda phage DNA (the relative position of two neighboring *Bam*HI and *Kpn*I sites at coordinate 3.7 kbp), due perhaps to some inaccuracy in mapping two closely neighboring sites. The molecular clones of RadLV genomes described elsewhere (37) displayed the same relative positions of these sites as did RadLV/VL$_3$(T+ L+). Grymes et al. (18) interpreted their failure to demonstrate biological activity as the possible result of small lesions in the cloned DNA rather than of permutation of the clone with respect to unintegrated linear DNA.

A comparison between the restriction maps of RadLV/VL$_3$(T+ L+) and BL/Ka(B) reveals some distinct features. The first is the absence in RadLV/VL$_3$(T+ L+) of two restriction sites (*Kpn*I and *Bst*EII) located close to one another around the *gag-pol* gene junction. The second is the absence in BL/Ka(B) of two neighboring sites (*Cla*I and *Xba*I) in the *pol* gene. The third difference resides in the *env* gene, where BL/Ka(B) lacks a recognition site for *Sst*I. Finally, a fourth dissimilarity is the greater length of the LTR of RadLV/VL$_3$(T+ L+) (583 kbp instead of 529 kbp).

At the present time, we cannot yet make a correlation between the thymotropic or leukemogenic properties of RadLV/VL$_3$(T+ L+) and specific features of its proviral genome. Biological and biochemical studies on mink cell

FIG. 2. Nucleotide sequence of the RadLV/VL$_3$(T+ L+) LTR compared with that of the lymphomagenic BALB/c B-tropic MuLV (B-Cl-11) (14), Moloney MuLV (40), SL3-3 MuLV (30), Gross passage A MuLV (45), and MCF 247 MuLV (25), of the xenotropic NFS-X MuLV (26), and of the ecotropic BL/Ka(B) (27) and Akv (44) MuLVs. The sequences are arranged relative to each other with respect to their homologies. The nucleotide positions are numbered accordingly. Boxes delineate the terminal inverted repeats, the core sequences in the direct repeats, the promoter signals, and the polyadenylation signal. Thick arrows above the sequences indicate the direct repeats; the dashed arrow delineates a third repeat in SL3-3.


RADLV
B-CL-11
M-MLV
SL3-3
GROSS
MCF-247
NFS-X
BL/KA(B)
AKV

focus-forming (MCF) viruses have emphasized differences in the membrane glycoprotein structural gene when they were compared with ecotropic viruses (15, 20). Similarly, mutations in this gene caused a loss of leukemogenicity in case of Friend spleen focus-forming virus (39). Comparing MCF viruses which differ in oncogenicity, Lung et al. (33) showed that the U3 portion of the LTR and the regions coding for gp70 and p15E could all play a role in determining leukemogenicity. However, RadLV/VL_3 is devoid of MCF activity (10) and the nucleotide sequence of its *env* gene is very similar to that of Akv MuLV (J. Merregaert, J. M. Nuyten, and M. Janowski, submitted for publication). Therefore, tentative comparisons should remain subject to caution. On the other hand, studies on avian leukosis viruses have established that the U3 region of the LTR determines their growth in rat fibroblasts and their oncogenic potential (38). Similarly, the same region might be responsible for the disease specificity of Moloney MuLV (6), the thymotropicity of a BALB/c MuLV (14), and leukemogenicity of SL3-3 MuLV (5, 30).

We determined the nucleotide sequence of the LTR of the pMOL502 insert according to the method of Maxam and Gilbert (35) and compared it with the already known LTR sequence of the lymphomagenic BALB/c B-tropic MuLV (B-Cl-11) (14), Moloney MuLV (40), SL3-3 MuLV (30), Gross passage A MuLV (45), and MCF 247 MuLV (25), of the xenotropic NFS-X MuLV (26), and of the ecotropic nonleukemogenic BL/Ka(B) (27) and Akv (44) MuLVs (Fig. 2). All the LTRs contain, in the same order and approximately at the same distance from each other, the consensus CCAAT and TATAAAAA promoter sequences and the AATAAA polyadenylation signal. The LTRs for which the entire nucleotide sequence are available display identical, 13-base-pair (bp) inverted repeats at their extremities.

Sequence homologies were calculated in the domain where the sequence of NFS-X LTR is available (positions 36 to 551), not taking into account the region where a direct repeat lacks in some viruses (positions 152 to 263). The nucleotide sequence of RadLV/VL_3(T+ L+) LTR resembles more that of the xenotropic NFS (91%) MuLV than that of the other retroviruses (80, 70, 78, 72, 88, 79, and 79%, respectively, for the retroviruses listed above). Furthermore, RadLV/VL_3(T+ L+) compares with NFS-X in that it displays a unique 6-bp insertion upstream of the TATA box, as was found also in the case of the xenotropic NZB-X MuLV (36). These observations suggest that the recombinational event leading to the generation of RadLV (2) involved an LTR from an endogenous xenotropic retrovirus of the C57BL/Ka mouse.

The LTR of RadLV/LV_3(T+ L+) contains two copies of a 43-bp sequence. Similar structures are found in the other lymphomagenic viruses, except in the case of MCF 247. They result from deletions and rearrangements, as compared with their allelic stretches of the 99-bp repeats AKR MuLV. In the case of RadLV/VL_3(T+ L+), they are separated by 11 nucleotides and differ by two point mutations, one being located in the core sequence TGTGG$^{T}_{C}$CAAG, analogs of which are found in the enhancer elements (28, 29, 38) constituted by the direct repeats in viruses as diverse as simian virus 40, human BK virus, polyoma virus, or retroviruses. Such elements can act at a distance from the initiation site of RNA synthesis independently from their orientation and from their position relative to the gene they control. Those from Moloney MuLV, Abelson MuLV, and SL3-3 MuLV display such properties (29, 43, 46). Moreover, they are likely to be responsible for the leukemogenicity of SL3-3 (5, 30) and for the thymotropicity of Gross passage A (13) and B-Cl-11 MuLV (14). The only relevant difference between the latter and its nonthymotropic counterpart BALB/c N-tropic MuLV (N-Cl-35) is in the deletions and rearrangements leading to the appearance of the tandem repeats. It is therefore tempting to make a parallel with the compared features of RadLV/VL_3(T+ L+) and BL/Ka(B), which is comparable to N-Cl-35 in that it does not display direct repeats. The LTRs of MCF 247 and of the xenotropic NFS and NZB (36) MuLVs do not contain such repeats either. That of MCF 247 confers only moderate oncogenicity when it substitutes for the corresponding region in Akv (21). Possibly of interest would be the possibility that a xenotropic-specific LTR might become responsible for thymotropic and leukemogenic behavior through the acquisition of such repeats.

Whether the thymotropicity and leukemogenicity of RadLV/VL_3(T+ L+) actually reside in its LTR (being determined by direct repeats acting as enhancer signals) or in other parts of its genome [possibly those which differ from the BL/Ka(B) genome] will hopefully be answered by the results of the in vitro recombination experiments which are presently under way.

We thank Jean-Marie Nuyten and Elisabeth Franssen for their excellent technical assistance and R. R. O'Neill for the communication of his yet unpublished results.

This work was performed with the financial participation of the European Atomic Energy Commission (contract BIO-D-379-81-B), the Belgian Fonds de la Recherche Scientifique Médicale, and the European Late Effects Project Group.

LITERATURE CITED

1. **Benade, L. E., and J. N. Ihle.** 1980. Different serotypes of B-tropic murine leukemia viruses and association with endogenous ecotropic viral loci. Virology **106**:374–386.
2. **Benade, L. E., J. N. Ihle, and A. Declève.** 1978. Serological characterization of B-tropic viruses of C57BL mice: possible origin by recombination of endogenous N-tropic and xenotropic viruses. Proc. Natl. Acad. Sci. U.S.A. **75**:4553–4557.
3. **Benton, W. D., and R. W. Davies.** 1977. Screening recombinant clones by hybridization to single plaques in situ. Science **196**:180–181.
4. **Birnboïm, H. C., and J. Doly.** 1977. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
5. **Celander, D., and W. A. Haseltine.** 1984. Tissue-specific transcription preference as a determinant of cell tropism and leukemogenic potential of murine retroviruses. 1984. Nature (London) **312**:159–162.
6. **Chatis, P. A., C. A. Holland, J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1983. Role for the 3′ end of the genome determine disease specificity of Friend and Moloney murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **80**:4408–4411.
7. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1980. Lymphomagenicity of recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **151**:542–552.
8. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
9. **Declève, A., M. Lieberman, J. N. Ihle, and H. S. Kaplan.** 1976. Biological and serological characterization of radiation leukemia virus. Proc. Natl. Acad. Sci. U.S.A. **73**:4675–4679.
10. **Declève, A., M. Lieberman, J. N. Ihle, P. N. Rosenthal, M. L. Lung, and H. S. Kaplan.** 1978. Physicochemical, biological and serological properties of a leukemogenic virus isolated from cultured RadLV-induced lymphomas of C57BL/Ka mice. Virology **90**:23–25.

11. **Declève, A., M. Lieberman, O. Niwa, and H. S. Kaplan.** 1974. Rapid in vivo assay technique for murine leukemia viruses. Nature (London) **252**:79–81.
12. **Declève, A., C. Sato, M. Lieberman, and H. S. Kaplan.** 1974. Selective thymic localization of murine leukemia virus related antigens in C57BL/Ka mice after inoculation with murine radiation leukemia virus. Proc. Natl. Acad. Sci. U.S.A. **71**: 3124–3128.
13. **DesGroseillers, L., and P. Jolicoeur.** 1984. The tandem direct repeats within the long terminal repeat of murine leukemia viruses are the primary determinant of their leukemogenic potential. J. Virol. **52**:945–952.
14. **DesGroseillers, L., E. Rassart, and P. Jolicoeur.** 1983. Thymotropism of murine leukemia virus is conferred by its long terminal repeat. Proc. Natl. Acad. Sci. U.S.A. **80**:4203–4207.
15. **Elder, J. F., J. W. Gautsch, F. C. Jensen, R. A. Lerner, J. W. Hartley, and W. P. Rowe.** 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (env) gene recombinants. Proc. Natl. Acad. Sci. U.S.A. **74**:4676–4680.
16. **Etzerodt, M., T. Mikkelsen, F. S. Pedersen, N. O. Kjeldgaard, and P. Jorgensen.** 1984. The nucleotide sequence of the Akv murine leukemia virus genome. Virology **204**:196–207.
17. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
18. **Grymes, R. A., M. L. Scott, J. P. Kim, K. E. Fry, and H. S. Kaplan.** 1983. Molecular studies of the Radiation leukemia virus (RadLV) and related retrovirus of C57BL/Ka mice. Prog. Nucleic Acids Res. Mol. Biol. **29**:53–73.
19. **Hager, G. L., E. H. Chang, H. W. Chan, C. S. Garon, M. A. Israel, M. A. Martin, E. M. Scolnick, and D. R. Lowy.** 1979. Molecular cloning of the Harvey sarcoma virus closed circular DNA intermediates: initial structural and biological characterization. J. Virol. **31**:795–809.
20. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. U.S.A. **74**:789–792.
21. **Holland, C. A., J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1985. At least four viral genes contribute to the leukemogenicity of murine retrovirus MCF 247 in AKR mice. J. Virol. **53**:158–165.
22. **Hsu, I. C., and W. K. Yang.** 1977. DNA transfection of ecotropic murine leukemia viruses in mouse cell cultures. Cancer Res. **37**:1709–1714.
23. **Janowski, M., J. Boniver, and J. R. Maisin.** 1982. Common proviral integration sites in C57BL mouse lymphomas induced by radiation leukemia virus and absence of novel virus-related sequences in radiogenic lymphoma DNA. Leukemia Res. **6**:285–287.
24. **Kaplan, H. S.** 1967. On the natural history of the murine leukemias: presidential address. Cancer Res. **27**:1325–1340.
25. **Kelly, M., C. A. Holland, M. L. Lung, S. K. Chattopadhyay, D. R. Lowy, and N. H. Hopkins.** 1983. Nucleotide sequence of the 3′ end of MCF 247 murine leukemia virus. J. Virol. **45**:291–298.
26. **Khan, A. S., and M. A. Martin.** 1983. Endogenous murine leukemia provirus long terminal repeats contain a unique 190 base pair insert. Proc. Natl. Acad. Sci. U.S.A. **80**:2699–2703.
27. **Kim, J. P., H. S. Kaplan, and K. E. Fry.** 1982. Characterization of an infective molecular clone of the B-tropic, ecotropic BL/Ka(B) murine retrovirus genome. J. Virol. **44**:217–225.
28. **Laimins, L. A., P. Gruss, R. Pozzatti, and G. Khoury.** 1984. Characterization of enhancer elements in the long terminal repeat of Moloney murine sarcoma virus. J. Virol. **49**:183–189.
29. **Laimins, L. A., G. Khoury, C. Gorman, B. Howard, and P. Gruss.** 1982. Host-specific activation of transcription by tandem repeat from simian virus 40 and Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **79**:6453–6457.
30. **Lenz, J., D. Celander, R. L. Growther, R. Patarca, D. W. Perkins, and W. A. Haseltine.** 1984. Determination of the leukemogenicity of a murine retrovirus by sequences within the long terminal repeat. Nature (London) **308**:467–472.
31. **Lieberman, M., A. Declève, E. P. Gelman, and H. S. Kaplan.** 1977. Biological and serological characterization of the C-type RNA viruses isolated from the C57BL/Ka strain of mice. II. Induction and propagation of the isolates, p. 231–246. *In* J. F. Duplan (ed.), Radiation-induced leukemogenesis and related viruses. North-Holland Publishing Co., Amsterdam.
32. **Lieberman, M., A. Declève, P. Ricciardi-Castagnoli, J. Boniver, O. J. Finn, and H. S. Kaplan.** 1979. Establishment, characterization, and virus expression of cell lines derived from radiation, and from virus-induced lymphomas of C57BL/Ka mice. Int. J. Cancer **24**:168–177.
33. **Lung, M. L., J. W. Hartley, W. P. Rowe, and N. H. Hopkins.** 1983. Large RNase T_1-resistant oligonucleotides encoding p15E and the U3 region of the long terminal repeat distinguish two biological classes of mink cell focus-forming type C viruses of inbred mice. J. Virol. **45**:275–290.
34. **Manteuil-Brutlag, S., S. Liu, and H. S. Kaplan.** 1980. Radiation leukemia virus contains two distinct viral RNA. Cell **19**:643–652.
35. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
36. **O'Neill, R. R., C. E. Buckler, T. S. Theodore, M. A. Martin, and R. Repaske.** 1985. Envelope and long terminal repeat sequences of a cloned infectious NZB xenotropic murine leukemia virus. J. Virol. **53**:100–106.
37. **Rassart, E., P. Sankar-Mistry, G. Lemay, L. DesGroseillers, and P. Jolicoeur.** 1983. New class of leukemogenic ecotropic recombinant leukemia virus isolated from radiation-induced thymomas of C57BL/6 mice. J. Virol. **45**:565–575.
38. **Robinson, H., B. M. Blais, P. N. Tsichlis, and J. M. Coffin.** 1982. At least two regions of the viral genome determine the oncogenic potential of avian leukosis viruses. Proc. Natl. Acad. Sci. U.S.A. **79**:1225–1229.
39. **Ruta, M., R. Bestwick, C. Machida, and D. Kabat.** 1983. Loss of leukemogenicity caused by mutations in the membrane glycoprotein structural gene of Friend spleen focus-forming virus. Proc. Natl. Acad. Sci. U.S.A. **80**:4704–4708.
40. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293**:543–548.
41. **Shoyab, M., and A. Sen.** 1979. The isolation of extrachromosomal DNA by hydroxyapatite chromatography. Methods Enzymol. **68**:199–206.
42. **Southern, E.** 1979. Gel electrophoresis of restriction fragments. Methods Enzymol. **68**:152–176.
43. **Srinivasan, A., E. P. Reddy, C. Y. Dunn, and S. A. Aaronson.** 1984. Molecular dissection of transcriptional control elements within the long terminal repeat of the retrovirus. Science **223**:286–289.
44. **van Beveren, C., E. Rands, S. L. Chattopadhyay, D. R. Lowy, and I. M. Verma.** 1981. Long terminal repeat of murine retroviral DNAs: sequence analysis, host-proviral junctions, and preintegration site. J. Virol. **41**:542–556.
45. **Villemur, R., E. Rassart, L. DesGroseillers, and P. Jolicoeur.** 1983. Molecular cloning of viral DNA from leukemogenic Gross passage A murine leukemia virus and nucleotide sequence of its long terminal repeat. J. Virol. **45**:539–546.
46. **Wood, T. G., M. L. McGeady, D. G. Blair, and G. F. Vande Woude.** 1983. Long terminal repeat enhancement of v-*mos* transforming activity: identification of essential regions. J. Virol. **46**:726–736.
47. **Yang, R. C. A., J. Lis, and R. Wu.** 1979. Elution of DNA from agarose gels after electrophoresis. Methods Enzymol. **68**: 176–182.