The Endogenous Ecotropic Murine Retroviruses *Emv-16* and *Emv-17* Are Both Capable of Producing New Proviral Insertions in the Mouse Genome

CSILLA SZABO, YOUNG KI KIM, AND WILLIE H. MARK*

Section of Genetics and Development, Cornell University, Ithaca, New York 14853-2703

Received 8 April 1993/Accepted 16 June 1993

New germ line proviral insertions are acquired at a high frequency by the progeny of SWR/J-RF/J hybrid female mice that carry the endogenous ecotropic murine leukemia proviruses *Emv-16* and *Emv-17*. The tight linkage of these RF/J strain proviral loci has prevented genetic segregation of the retroviral genomes. Hence, it is not known whether both of these proviruses are capable of giving rise to new proviral insertions. We have molecularly cloned *Emv-16* and *Emv-17* and have characterized them *in vitro* and *in vivo*. Restriction enzyme analysis of the recombinant clones revealed that the proviral genomes are very similar to each other and closely resemble the wild-type AKR virus. A comparison of the flanking cellular DNA suggests that the *Emv-16* and *Emv-17* loci did not arise by simple duplication of a viral insertion site within the RF/J genome but most likely are independent integration events. Both proviruses produce infectious virus when transfected into NIH 3T3 cells, indicating that they are nondefective retroviruses. Exogenous infection of SWR/J mice with either *Emv-16* or *Emv-17* leads to viremia in the host animals, and in both cases, progeny of viremic females acquire new proviral insertions. The ability of these retroviruses to generate novel retroviral integration sites in the mouse genome provides a simple method for inducing insertional mutations in mice.

The RF/J mouse strain carries three endogenous ecotropic murine leukemia viruses (MuLVs), *Emv-1*, *Emv-16*, and *Emv-17* (9, 15). *Emv-1* is a defective provirus located on chromosome 5 (15), whereas the *Emv-16* and *Emv-17* proviral loci are tightly linked on chromosome 1 (3). Although virus spread is restricted in RF/J mice by both genetic and nongenetic factors (14, 16, 19), *Emv-16* and *Emv-17* induce viremia when transmitted as a single dominant locus to permissive hosts, such as the SWR/J strain (8, 15).

Offspring of viremic RF/J-SWR/J hybrid female mice carrying *Emv-16* and *Emv-17* acquire new proviral insertions in their germ line at a high frequency (8). Since provirus acquisition is observed only in offspring of viremic females, it has been postulated that exogenous infection of oocytes followed by proviral integration during early embryonic development is the most likely mechanism for producing these germ line insertional events (2, 8, 12, 24). Evidence supporting this theory was obtained from studies in which proviral insertions were observed in the offspring of SWR/J females that had been inoculated as neonates with virus derived from RF/J-SWR/J hybrid mouse tissues (12, 18). Viral RNA expression studies of RF/J-SWR/J hybrid females (12, 17) and ovarian transplantation studies (12) further indicated that the oocyte can be the target of viral infection.

Although *Emv-16* and *Emv-17* are readily transmitted to a permissive host by breeding, the tight linkage of these proviruses (within 0.11 ± 0.11 centimorgans) has prevented their genetic segregation through backcrossing (2, 3, 8, 24). The lack of readily detectable differences between the viral genomic DNAs, such as restriction fragment length polymorphisms, also hinders the distinction of *Emv-16* from *Emv-17*. Thus, it has not been previously possible to determine whether each of these viruses is capable of giving rise to the spontaneous germ line proviral insertions observed in

progeny of viremic SWR/J-RF/J hybrid females. In fact, it was not clear whether both *Emv-16* and *Emv-17* are biologically active.

To address these questions, we isolated the *Emv-16* and *Emv-17* proviral loci from the RF/J mouse genome as individual recombinant DNA clones. Two EMBL4 phage libraries containing size-selected *EcoRI*-digested RF/J spleen genomic DNA were screened (23) with the ecotropic virus-specific pAKV-4 probe (a gift from Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) (7). The *Emv-16* provirus was isolated as a 13.5-kb genomic DNA fragment, while the *Emv-1* and *Emv-17* proviruses were each cloned as 18.6-kb fragments (3, 15; data not shown).

To verify the identities of the cloned proviruses, recombinant phage clones were characterized by using the restriction enzyme *PvuII*, which cuts twice in ecotropic MuLV proviral sequences (20). The pAKV-4 probe specifically detects the 3' virus-cell DNA junction fragment, which is unique for each proviral integration site. Three junction fragments of 6.6, 4.7, and 4.2 kb were observed on Southern blots of *EcoRI*- and *PvuII*-digested cloned DNAs, as shown in Fig. 1A, which correspond to the integration sites of *Emv-17*, *Emv-16*, and *Emv-1*, respectively (8, 9, 15). The *Emv-16* junction fragment is approximately 0.2 kb smaller than that observed in *PvuII* digests of genomic RF/J DNA, since the *EcoRI* site used for cloning is located between the 3' viral long terminal repeat and the *PvuII* site in the flanking cellular DNA (data not shown).

The restriction maps of the *Emv-16* and *Emv-17* clones schematically diagrammed in Fig. 1B show the distinct cleavage patterns of the cellular sequences flanking these proviral loci. The observed differences indicate that the close linkage of these endogenous retroviral genomes is not due to a simple tandem duplication of a chromosomal region containing a single proviral insertion (3, 8) but is likely to have resulted from independent integration events.

* Corresponding author. Electronic mail address: WM2X@vax5.CIT.CORNELL.EDU.

FIG. 1. Molecular analysis of the *Emv-16* and *Emv-17* proviral loci. (A) Southern blot of EMBL-4 lambda phage clones containing RF/J genomic DNA digested with *PvuII* and *EcoRI* and hybridized with the pAKV-4 probe. RF/J genomic DNA digested with *PvuII* is shown to indicate the alignment of the clones with the endogenous junction fragments. The junction fragments of 6.6, 4.7, and 4.2 kb represent the insertion sites of *Emv-17*, *Emv-16*, and *Emv-1*, respectively. Recombinant clones 1-1 to 1-4 contain *Emv-1*, clones 17-1 to 17-3 contain *Emv-17*, and clones 16-1 to 16-3 contain *Emv-16*. Molecular size markers of 9.9, 6.6, 4.7, 4.4, and 4.2 kb are indicated. (B) Restriction maps of the recombinant clones containing *Emv-16* and *Emv-17*. Solid bars, the viral genome; open regions, the viral long terminal repeats; hatched bars, flanking cellular DNA. The map of AKR-623 is shown for comparison. The location of the pAKV-4 probe within the viral genome is indicated. B, *BamHI*; H, *HindIII*; K, *KpnI*; P, *PstI*; RI, *EcoRI*; S, *SalI*; V, *PvuII*; X, *XbaI*.

Further restriction analysis of the *Emv-16* and *Emv-17* proviral genomes revealed extensive similarity between these viruses and AKR-623 (a gift from W. Herr [6, 13]), a cloned derivative of the *Akv-1* endogenous ecotropic MuLV (data not shown). No restriction site polymorphisms were observed between *Emv-16* and *Emv-17* among the 49 enzymes tested, a sample of 1,496 nucleotides, or approximately 16.7% of the retroviral genome.

Only seven enzymes detected differences between AKR-623 and the *Emv-16* and *Emv-17* viruses. One of these differences is a *HindIII* site present in the genomes of *Emv-16* and *Emv-17*, as previously shown by Spence et al. (24), which is not found in AKR-623. This site, common to ecotropic MuLVs (4), is approximately 2.9 kb from the 5' end of the proviral genome (Fig. 1B). The enzymes *SacII*, *BssHII*, *SpeI*, *KpnI*, *DraI*, and *HhaI* identified additional polymorphisms that can be attributed to a 100-bp duplication of the viral transcriptional enhancer located in the U3 region of the AKR-623 long terminal repeat (20, 26). These results demonstrate that *Emv-16* and *Emv-17* are highly homologous and support the view that the closely related AKR-type endogenous ecotropic MuLVs in inbred mouse strains arose from a common ancestral origin (5, 11, 25) via germ line reinfection (21).

To determine the capacities of the individual proviruses to express replication-competent virus, 5 μ g of the cloned viral DNAs was transfected into NIH 3T3 cells by the method of

calcium phosphate precipitation (1, 27). Cell-free virus stocks were obtained from the culture supernatant of the transfected cells and monitored for the presence of infectious virus on NIH 3T3 cells by the XC plaque assay (22).

Emv-1 is a known defective provirus (15) and as expected produced no XC plaques (<1 PFU/ml of supernatant) on infected NIH 3T3 cells. In contrast, both *Emv-16* ($2.5 \times 10^6 \pm 0.4 \times 10^6$ PFU/ml) and *Emv-17* ($4.3 \times 10^6 \pm 0.1 \times 10^6$ PFU/ml) are capable of producing infectious virus at levels comparable to that of AKR-623 ($5.9 \times 10^5 \pm 0.6 \times 10^5$ PFU/ml), which was used as a positive control. Our results differ from those described by Lock et al. (12), who observed a higher level of virus production by AKR-623 possibly because of its enhancer duplication (26). To assess the promoter strength of the individual viruses, clonal cell lines carrying a single copy of each proviral genome would need to be established.

Since *Emv-16* and *Emv-17* are both replication competent, we wished to evaluate the abilities of the two viruses to produce new proviral insertions in progeny of viremic female mice. For this purpose, 1- to 2-day-old SWR/J neonates were inoculated by subcutaneous injection with 0.05 ml of supernatant virus stocks of either *Emv-16*, *Emv-17*, or AKR-623. Successful infections were verified by monitoring inoculated animals for viremia at 6 to 8 weeks of age. Approximately 1.0 cm of tail tissue was homogenized in the presence of culture media, and the virus titers of cell extracts were determined

FIG. 2. Identification of new proviral insertions in progeny of SWR/J females infected with *Emv-16*, *Emv-17*, or AKR-623. Southern blot of tail DNA (approximately 10 µg per lane) obtained from progeny mice that was digested with *PvuII* and probed with pAKV-4. Each junction fragment represents a unique proviral integration site in the SWR/J genome. Lanes: 1 to 4, AKR-623 mice; 5, 6, and 9 to 14, *Emv-16* mice; 7 and 8, *Emv-17* mice. S, SWR/J genomic DNA; R, RF/J genomic DNA; M, DNA size marker. Molecular size markers of 23.1, 6.6, 4.7, 4.4, and 4.2 kb are indicated.

as before by the XC plaque assay (22). Females that expressed virus (>5 PFU/cm of tail tissue) were then bred with wild-type SWR/J males. For comparison, females that tested negative for virus expression from each set of inoculations were included in this study. In both cases, the progeny obtained were examined for the presence of new proviral insertions by Southern blot analysis.

Representative Southern blots of *PvuII*-digested genomic tail DNAs from progeny mice carrying new proviral insertions are shown in Fig. 2. Each new provirus is detected as a novel virus-cell junction fragment by hybridization with the pAKV-4 probe. These results clearly show that *Emv-16* (Fig. 2, lanes 5, 6, and 9 to 14) and *Emv-17* (Fig. 2, lanes 7 and 8) are both capable of giving rise to new proviral insertions in progeny of females expressing these viruses. New proviral insertions were observed only in the progeny of viremic females (Table 1).

Table 1 summarizes the data from the Southern blot experiments. Ten of the 242 (4.1%) progeny derived from *Emv-16*-infected viremic females acquired a total of 16 new proviral insertions, an average of 0.07 provirus per animal. Eight new proviral loci were identified among six of the 352 progeny (1.7%) obtained from *Emv-17*-infected mothers, a frequency of 0.02 provirus per animal. These numbers are lower than those reported by Panthier et al. (18), who observed a frequency 0.15 provirus per animal in similar experiments. We believe this difference is due to the mode of

virus inoculation; subcutaneous injection of virus may be a less efficient means of achieving successful infection of host animals than intraperitoneal injection, the method used by Panthier et al. (18). The low frequency of provirus acquisition (0.03 provirus per animal) obtained by Lock et al. (12) from subcutaneously inoculated neonates supports this view. Genetic transmission of the *Emv-16* and *Emv-17* loci to permissive hosts seems to give the highest frequency of proviral insertion, approximately 0.1 to 0.3 provirus per animal (2, 8, 12, 24; our unpublished results).

Acquisition of proviral insertions was also observed in the progeny of viremic AKR-623-infected females (Fig. 2, lanes 1 to 4) (Table 1). Fifteen new proviruses were detected in 7 of the 309 (2.3%) progeny analyzed, giving an average frequency of 0.05 provirus per animal. This result confirms the previous findings of Lock et al. (12), who reported the ability of the AKR-623 virus to cause new proviral insertions in the germ line of progeny mice.

In several instances, multiple proviral insertions were identified in a single animal (Fig. 2, lanes 2, 3, 8, 10, 12, and 14), as previously seen in the offspring of RF/J-SWR/J hybrid mice (2, 8, 12, 24). Southern blot analysis of *SacI*-digested tail DNA permitted us to conclude that the proviral loci detected by *PvuII* digestion represent the maximum number of integration sites in each animal, since *SacI* releases a junction fragment distinct from that generated by *PvuII* (data not shown).

TABLE 1. Frequency of provirus acquisition in progeny of SWR/J female mice infected with *Emv-16*, *Emv-17*, or AKR-623

Class of mouse according to virus used for infection	No. of progeny	No. of progeny with new proviruses	Frequency of progeny with new proviruses (θ)	No. of new proviruses	Avg no. of proviruses/animal
<i>Emv-16</i>	242	10	0.041 ^a	16	0.07
<i>Emv-17</i>	352	6	0.017 ^a	8	0.02
AKR-623	309	7	0.023 ^a	15	0.05
Nonviremic ^b	114	0	<0.009 ^c	0	<0.009 ^c

^a Analysis of Poisson-distributed variables [$f(k) = e^{-\theta}\theta^k/k!$] indicates significant differences among all three groups ($P < 0.05$) (see reference 10).

^b Females in this group were inoculated with either *Emv-16*, *Emv-17*, or AKR-623 and tested negative for virus expression by the XC plaque assay.

^c Limit of detection.

TABLE 2. Virus expression in ovaries of SWR/J female mice and frequency of provirus acquisition in their progeny

Virus type used for infection and mouse no.	Virus titer (PFU [10^3]/ovary)	No. of progeny analyzed	No. of progeny with new proviruses (%)	No. of new proviruses	Avg. no. of proviruses/animal
<i>Emv-16</i>					
103	1.98	41	0	0	0
106	0.38	32	0	0	0
108	ND ^a	24	1 (4.2)	1	0.04
110	0.18	43	3 (7.0)	3	0.07
<i>Emv-17</i>					
43	0.10	22	2 (9.1)	2	0.09
66	0.11	25	0	0	0
AKR-623					
4	6.0	21	0	0	0
9	5.4	16	1 (6.3)	4	0.25
13	0.50	13	0	0	0
14	0.40	26	1 (3.8)	1	0.04
15	0.07	18	3 (16.7)	8	0.44
20	13.8	14	0	0	0
31	0.98	15	0	0	0

^a ND, not detected (less than 10 PFU per ovary).

One of the newly acquired *Emv-16*-derived proviral loci was found to contain a restriction fragment polymorphism which can be attributed to a 1.0-kb deletion within the viral genome (data not shown). None of the other new proviral insertions were distinguishable from the parental viruses by restriction enzyme analysis. The mutation identified by this polymorphism probably arose during virus replication in the maternal tissues, since multiple rounds of virus reinfection can occur in the somata of RF/J-SWR/J hybrid animals prior to infection of the germ line (24).

Since proviral acquisition has been suggested to occur by exogenous infection of the oocyte (8, 12), we were interested in determining whether the level of virus expression in the ovaries influences the frequency of new insertions in progeny mice. Cell extracts derived from the ovaries of viremic females were therefore monitored for virus production by the XC assay, and Table 2 shows the results of this analysis.

First, it can be seen that the amount of virus detected in the ovary can vary as much as 100-fold (compare mouse 15 and mouse 20 in Table 2). Second, there appears to be no obvious correlation between the amount of virus present in ovarian tissues and the frequency of provirus acquisition in the offspring. Some females show high levels of virus expression (mouse 4 and mouse 20), yet their progeny had no new proviral insertions; conversely, animals (mouse 15 and mouse 108) which expressed virus at low levels or even below the limit of detection nonetheless produced offspring containing new proviral insertions.

Our results suggest that oocyte infection is a stochastic event, a conclusion that is supported by data from previous studies (2, 8, 12, 18, 24). In particular, Bautch showed that the frequency of proviral insertion in progeny mice varied dramatically among individual females as well as among different litters of the same female (2). We also observed wide variations in provirus acquisition among the inbred SWR/J animals used in the present study (Table 2). Furthermore, progeny which acquire proviral insertions often contain multiple integrants (Table 1; Fig. 2), and the incidence of these new insertions fits a Poisson distribution (10), suggesting that provirus acquisition is a rare but random event.

Statistical analysis with the Mantel limit factors for comparing the means of Poisson-distributed variables (10) suggests that the average frequency of provirus acquisition is significantly different among *Emv-16*, *Emv-17*, and AKR-623 ($P < 0.05$). *Emv-16* yielded the highest average frequency, with 4.1% of progeny containing new insertions, whereas *Emv-17* gave the lowest frequency (1.7%) and AKR-623 exhibited an intermediate frequency (2.3%). These apparent differences may reflect the differential abilities of *Emv-16*, *Emv-17*, and AKR-623 to infect oocytes. Although the similarity of the three viral genomes argues against this possibility, the tropism of these viruses for oocytes has not been experimentally determined.

It is interesting to note that, among the *Srev* lines of mice derived from RF/J-SWR/J hybrid progeny described by Spence et al. (24), those that are competent for virus expression appear to fall into two classes: mice which show a high frequency of provirus acquisition (average frequency, 7.9% of progeny) and mice which exhibit a lower level of acquisition of proviral insertions (average frequency, 3.8%). This observation could be interpreted to suggest that two classes of *Srev* animals exist, those containing *Emv-16* and those containing *Emv-17*, thus reflecting the inherently different capacities of these retroviruses to give rise to new germ line proviral integrations. Unfortunately, the lack of polymorphisms between the two viruses precludes a simple testing of this hypothesis.

In conclusion, the molecular cloning of *Emv-16* and *Emv-17* has permitted us to show unambiguously that these endogenous viruses of the RF/J strain are both infectious and that they are highly homologous to the wild-type AKR endogenous ecotropic MuLV. Studies with mice clearly demonstrated that both *Emv-16* and *Emv-17*, as well as AKR-623, can produce spontaneous proviral insertions in the genomes of offspring from viremic females. The proviral insertions acquired by RF/J-SWR/J hybrid backcross progeny have been shown to be mutagenic (24), indicating that these retroviruses may provide a simple means of generating insertional mutations in mice. We are currently investigating the use of specially designed retroviral transgenes to estab-

lish mouse lines capable of producing retroviral insertions at a high frequency.

We thank Winship Herr for providing the AKV-4 and AKR-623 recombinant DNA clones used in this study. The technical support of Tamar Hughes during the course of these experiments is gratefully acknowledged. We express special thanks to E. C. Kindahl for helpful suggestions in statistical analysis and to D. L. Holzschu, C. R. Parrish, and M. F. Wolfner for critical comments on the manuscript.

Y. K. Kim was supported in part by the Cornell Hughes Scholars Program for Undergraduate Research. C. Szabo was supported by NIH training grant GM07617.

REFERENCES

1. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.). 1987. Current protocols in molecular biology, vol. 1. John Wiley & Sons, Inc., New York.
2. Bautch, V. L. 1986. Genetic background affects integration frequency of ecotropic proviral sequences into the mouse germ line. *J. Virol.* **60**:693-701.
3. Buchberg, A. M., B. A. Taylor, N. A. Jenkins, and N. G. Copeland. 1986. Chromosomal localization of *Emv-16* and *Emv-17*, two closely linked ecotropic proviruses of RF/J mice. *J. Virol.* **60**:1175-1178.
4. 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.
5. Chattopadhyay, S. K., M. R. Lander, and W. P. Rowe. 1980. Close similarity between endogenous ecotropic virus of *Mus musculus molossinus* and AKR virus. *J. Virol.* **36**:499-505.
6. Herr, W. 1984. Nucleotide sequence of AKV murine leukemia virus. *J. Virol.* **49**:471-478.
7. Herr, W., and W. Gilbert. 1983. Somatically acquired recombinant murine leukemia proviruses in thymic leukemias of AKR/J mice. *J. Virol.* **46**:70-82.
8. Jenkins, N. A., and N. G. Copeland. 1985. High frequency germline acquisition of ecotropic MuLV proviruses in SWR/J-RF/J hybrid mice. *Cell* **43**:811-819.
9. Jenkins, N. A., N. G. Copeland, B. A. Taylor, and B. K. Lee. 1982. Organization, distribution, and stability of endogenous ecotropic murine leukemia virus DNA sequences in chromosomes of *Mus musculus*. *J. Virol.* **43**:26-36.
10. Johnson, N. L., and S. Kotz. 1970. Distributions in statistics: discrete distributions, p. 87-121. John Wiley & Sons, Inc., New York.
11. King, S. R., J. M. Horowitz, and R. Risser. 1987. Nucleotide conservation of endogenous ecotropic murine leukemia proviruses in inbred mice: implications for viral origin and dispersal. *Virology* **157**:543-547.
12. Lock, L. F., E. Keshet, D. J. Gilbert, N. A. Jenkins, and N. G. Copeland. 1988. Studies of the mechanism of spontaneous germline ecotropic provirus acquisition in mice. *EMBO J.* **7**:4169-4177.
13. 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.
14. Mayer, A., F. Duran Struuck, M. L. Duran-Reynals, and F. Lilly. 1980. Maternally transmitted resistance to lymphoma development in mice of reciprocal crosses of the RF/J and AKR/J strains. *Cell* **19**:431-436.
15. McCubrey, J., J. M. Horowitz, and R. Risser. 1982. Structure and expression of endogenous ecotropic murine leukemia viruses in RF/J mice. *J. Exp. Med.* **156**:1461-1474.
16. Melamedoff, M., F. Lilly, and M. L. Duran-Reynals. 1983. Suppression of endogenous murine leukemia virus by maternal resistance factor. *J. Exp. Med.* **158**:506-514.
17. Panthier, J. J., and H. Condamine. 1987. Expression of ecotropic MuLV in ovaries of SWR/J-RF/J hybrid mice. *Ann. Inst. Pasteur/Virol.* **138**:409-422.
18. Panthier, J. J., H. Condamine, and F. Jacob. 1988. Inoculation of newborn SWR/J females with an ecotropic murine leukemia virus can produce transgenic mice. *Proc. Natl. Acad. Sci. USA* **85**:1156-1160.
19. Pincus, T., J. Hartley, and W. P. Rowe. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. *J. Exp. Med.* **133**:1219-1233.
20. Rands, E., D. R. Lowy, M. R. Lander, and S. K. Chattopadhyay. 1981. Restriction endonuclease mapping of ecotropic murine leukemia viral DNAs: size and sequence heterogeneity of the long terminal repeat. *Virology* **108**:445-452.
21. Rowe, W. P., and C. A. Kozak. 1980. Germ-line reinsertions of AKR murine leukemia virus genomes in *Akv-1* congenic mice. *Proc. Natl. Acad. Sci. USA* **77**:4871-4874.
22. Rowe, W. P., W. E. Pugh, and J. W. Hartley. 1970. Plaque assay techniques for murine leukemia viruses. *Virology* **42**:1136-1139.
23. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
24. Spence, S. E., D. J. Gilbert, D. A. Swing, N. G. Copeland, and N. A. Jenkins. 1989. Spontaneous germ line virus infection and retroviral insertional mutagenesis in eighteen transgenic *Srev* lines of mice. *Mol. Cell. Biol.* **9**:177-184.
25. Steffen, D. L., S. Bird, and R. A. Weinberg. 1980. Evidence for the Asiatic origin of endogenous AKR-type murine leukemia proviruses. *J. Virol.* **35**:824-835.
26. Van Beveren, C., E. Rands, S. K. Chattopadhyay, D. R. Lowy, and I. M. Verma. 1982. Long terminal repeat of murine retroviral DNAs: sequence analysis, host-proviral junctions, and preintegration site. *J. Virol.* **41**:542-556.
27. Wigler, M., A. Pellicer, S. Silverstein, and R. Axel. 1978. Biochemical transfer of single-copy eucaryotic genes using total cellular DNA as donor. *Cell* **14**:725-731.