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Genetics

Germ line integration of Moloney leukemia virus: Identification of the chromosomal integration site

(genetic transmission/structural gene/segregation analysis/somatic cell hybrids/backcross animals)

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ABSTRACT The chromosomal integration site of the structural gene of Moloney murine leukemia virus (M-MuLV) in the genome of BALB/Mo mice was mapped genetically. These mice transmit the exogenous M-MuLV as an endogenous virus at a single Mendelian locus. Two independent experimental approaches were used: (*i*) Non-virus-producing fibroblasts prepared from homozygous BALB/Mo embryos were fused to Chinese hamster Wg3-h-o cells. In an analysis of 30 independent mouse–Chinese hamster cell hybrid clones, the segregation of the viral genome measured by molecular hybridization and enzymes assigned to 16 different mouse chromosomes were compared. We found a highly concordant segregation of M-MuLV sequences and the mouse enzyme triosephosphate isomerase (TPI, EC 5.3.1.1), whose gene has been assigned to chromosome 6. A further karyotype analysis of 9 clones, in which the chromosomes were identified cytochemically, supported this result. (*ii*) The segregation of the viral genome was studied in backcrosses of BALB/Mo with ABP/J mice. In the backcross ABP/J × (ABP/J × BALB/Mo) a linkage of the M-MuLV genome to the morphological marker *wa-1* on mouse chromosome 6 was found. This confirmed the conclusion that the M-MuLV genome is integrated in mouse chromosome 6. These experiments define the genetic locus *Mov-1*, denoting the genetically transmitted structural gene of M-MuLV in BALB/Mo mice.

Endogenous retroviruses are genetically transmitted as part of the normal genetic complement of a wide variety of animal species. Although the role of these viruses in normal development or natural oncogenesis of their respective hosts is far from being understood, they provide excellent model systems for studying the regulation of eukaryotic gene expression. Exogenous retroviruses, in contrast, are not part of the normal genetic information of an animal but are transmitted horizontally (for reviews, see refs. 1 and 2).

In the mouse a number of rather well-characterized endogenous and exogenous leukemia viruses are known that cause leukemic transformation of different target cells with a high degree of specificity. Several murine genetic factors seem to control the expression of the endogenous murine leukemia viruses (MuLVs) and the target cell specificity of the exogenous MuLVs. Such factors can be structural genes for cell surface receptors (3), factors involved in the integration of proviral DNA into the host genome, including factors determining the specificity of this event, or regulatory genes controlling the expression of the integrated proviral DNA (4). Several genes involved in the replication or regulation of MuLV have been assigned to individual chromosomes: *Akv-1*, the proviral genome of an ecotropic endogenous mouse virus, has been assigned to chromosome 7 (5, 6). Xentropic leukemia virus-inducing loci in two mouse strains have recently been mapped on chromosome 1 (7). Other genes exert their effect on viral replication and oncogenesis; these include *Fv-1* on chromosomes 4 (8), *Rec-1* on chromosome 5 and *Ram-1* on chromosome 8 (9), *Fv-2* on chromosome 9 (10), and *Gv-1* and *Rgv-1* on chromosome 17 (11, 12). A locus controlling the replication of a primate virus has been defined genetically (13). As part of our efforts to elucidate the factors controlling virus-induced leukemic transformation in mice, we describe experiments that have led to the identification of the integration site of the structural gene of the Moloney strain of murine leukemia virus (M-MuLV) in BALB/Mo mice.

This strain of mice has been derived from a mouse embryo that was infected at the four- to eight-cell preimplantation stage (14). The exogenous M-MuLV is genetically transmitted in these animals as an endogenous virus at a single Mendelian locus. A subline of mice has been obtained that is homozygous at the M-MuLV locus (15). Fibroblasts from homozygous BALB/Mo mice fused with Chinese hamster cells were used to assign the chromosomal integration site of M-MuLV in BALB/Mo mice by segregation analysis in somatic cell hybrids. This assignment was confirmed by a classical Mendelian genetic analysis of BALB/Mo backcross animals.

MATERIALS AND METHODS

Somatic Cell Hybrids. Cells were maintained in Dulbecco's modification of Eagle's medium supplemented with 10% fetal bovine serum. Culture medium for selection and maintenance of hybrid cells was supplemented with 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine (HAT selection medium) and 1 mM ouabain (16). Non-virus-producing BALB/Mo fibroblasts were prepared from homozygous BALB/Mo embryos at day 15 of gestation. Eight embryos were removed from the uterus sterilely and dipped extensively in ether to inactivate contaminating maternal virus. Fibroblast cultures were prepared and divided after one passage: one aliquot of each culture was frozen and stored at −80°C, whereas the second aliquot was passaged further in the presence of Polybrene to facilitate the spread of possibly contaminating virus. After several passages the cultures were tested for virus production by the XC plaque assay. Five of the eight cultures were found to be virus-negative, whereas three produced virus. The frozen aliquots of two of the virus-negative fibroblast preparations were then used to establish the mouse–Chinese hamster cell hybrids for the segregation analysis.

Mixtures of mouse embryo fibroblasts (6th passage) and a 5-fold excess of Wg3-h-o cells (derivative of Wg3-h cells, deficient in hypoxanthine phosphoribosyltransferase and resistant

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Abbreviations: MuLV, murine leukemia virus; M-MuLV, Moloney murine leukemia virus; TPI, triosephosphate isomerase.

to 1 mM ouabain) were fused by using polyethylene glycol as described (16, 17). All isolated mouse–Chinese hamster hybrid clones were free of mycoplasma contamination (18).

Molecular Hybridization. The *in vitro* synthesis of M-MuLV cDNA, its selection against uninfected BALB/c DNA to remove all sequences that cross-hybridize with endogenous viruses, and the quantitation of M-MuLV-specific sequences in mouse DNA using the selected cDNA in a cDNA excess hybridization reaction were done essentially as described (15, 19). DNA from mouse tissues or tissue culture cells was extracted by the method of Kirby (20), purified by CsCl/ethidium bromide equilibrium centrifugation, and boiled in alkali as described (14, 15).

Chromosome Identification Techniques. The Giemsa/trypsin banding technique was used to identify Chinese hamster and mouse chromosomes. In addition, mouse chromosomes were identified by the use of the fluorescent benzimidazole dye Hoechst 33 258 (21).

Enzyme Determinations. The hamster and mouse forms of enzymes were separated by starch gel or polyacrylamide gel electrophoresis as described (22). The following enzyme assays were carried out: acid phosphatase (ACP-1, EC 3.1.3.2) (23), adenylate kinase (AK-1 and AK-2, EC 2.7.4.3) (24), adenine phosphoribosyltransferase (APRT, EC 2.4.2.7), dipeptidase-1 and dipeptidase-2 (DIP-1, DIP-2, EC 3.4.11.-), esterase-10 (ES-10, EC 3.1.1.1), mannosephosphate isomerase (MPI, EC 5.3.1.8), phosphoglucomutase-1 and phosphoglucomutase-2 (PGM-1, PGM-2, EC 2.7.5.1), tripeptidase-1 (TRIP-1, EC 3.4.11.-), glucosephosphate isomerase (GPI, EC 5.3.1.9) (22), glutamate oxalacetate transaminase-1 (GOT-1, EC 2.6.1.1) (25), glyoxalase-1 (GLO-1, EC 4.4.1.5) (26), galactokinase (GalK, EC 2.7.1.6) (27), hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8) (28), and triosephosphate isomerase (TPI, EC 5.3.1.1) (29).

RESULTS

Assignment of M-MuLV Proviral Sequences by Somatic Cell Hybridization. Fibroblasts from BALB/Mo embryos were isolated and fused with established Chinese hamster cells. Mouse fibroblasts *in vitro* are susceptible to infection by M-MuLV, and infected fibroblasts usually harbor multiple copies of the viral genome (Table 1 and ref. 30), most likely integrated at different sites (L. Bacheler and H. Fan, personal communication). Amplification and reintegration in either mouse or hamster chromosomes of the viral genome during preparation of the fibroblasts or as a result of the cell fusion event would have made impossible an identification of the original integration site. Therefore it was of great importance to use non-virus-producing BALB/Mo fibroblasts for the cell fusion.

Table 1. Quantitation of M-MuLV-specific sequences in mouse DNA by cDNA excess hybridization

Source of DNA	Fraction hybridized	X	Copies/haploid mouse genome
Heterozygous BALB/Mo embryo	0.093	0.102	0.5
Homozygous BALB/Mo embryo	0.163	0.195	1.0
Non-virus-producing BALB/Mo fibroblasts*	0.193	0.240	1.2
Virus-producing BALB/Mo fibroblasts*	0.464	0.866	4.4

Hybridization reactions using 15 μg of mouse DNA were carried out and the fraction X of M-MuLV-specific sequences in the various cell DNAs as compared to the input cDNA was computed from the equation $X/(1 + X)$ = fraction hybridized (19).

* The fibroblasts used in these experiments were transformed by simian virus 40 and cloned to obtain permanent cell lines. This had no effect on the number of M-MuLV genome copies per diploid cell genome equivalent (data not shown).

The mouse–Chinese hamster cell hybrids segregated mouse chromosomes as indicated by chromosome analyses (see Tables 3 and 4). The mapping of the viral integration site was achieved by correlating the segregation of individual mouse chromosomes with the segregation of the viral genome in 30 independent clones. Unequivocal evidence for the presence or absence of the viral structural gene was obtained by quantitating the viral sequences in cell hybrid clones by molecular hybridization. Our cDNA excess hybridization assay using an M-MuLV-specific cDNA probe has been described in detail elsewhere (15, 19). This cDNA does not cross-hybridize with endogenous murine viruses, and the assay allows us to determine the number of M-MuLV genome copies in a given DNA by direct computation or by comparison with standard mouse DNAs with known contents of viral sequences. Table 1 shows that, as expected, DNA isolated from heterozygous BALB/Mo embryos had 0.5 M-MuLV genome copies and DNA from homozygous embryos had 1.0 M-MuLV genome copy per haploid mouse genome equivalent (15). A non-virus-producing BALB/Mo fibroblast line also showed one M-MuLV genome copy per haploid mouse genome (Table 1). Another fibroblast line that did express the viral genome (as determined by the XC plaque assay) showed amplification of the M-MuLV sequences to approximately four viral genome copies per haploid mouse genome (Table 1). Amplification of M-MuLV-specific DNA was found reproducibly also in diploid BALB/Mo fibroblasts producing infectious virus after superinfection with M-MuLV *in vitro* (data not shown).

When the 30 mouse–Chinese hamster hybrid clones used for the segregation analysis were analyzed for M-MuLV-specific sequences, no evidence for amplification of the viral genome in any of the clones was found (Table 2). Similarly, each clone was tested for the presence of infectious virus by the XC plaque assay, and all clones were found to be XC-negative (data not shown). This showed that there was no activation and reintegration of the viral genome as a result of the cell fusion event (31).

The results of the segregation analysis are summarized in Tables 2 and 3. It was found that the M-MuLV sequences segregated highly concordantly (in 93%—i.e., in 28 of the 30 clones) with mouse TPI, whose gene has recently been assigned to mouse chromosome 6 (29). In only two clones (= 7%) was the segregation discordant: one clone (2W14) was negative for expression of mouse TPI but had retained the viral sequences, and one clone (2W18) expressed mouse TPI but showed no hybridization to M-MuLV cDNA.

The low discordance rate of the segregation of the viral genome and mouse TPI suggested that the M-MuLV integration site is located on chromosome 6. However, for mouse chromosomes 3, 13, 15, and 16 no enzyme tests were available. Therefore, nine clones were selected for a further karyotype analysis in which the chromosomes were identified by Giemsa/trypsin banding and fluorescent staining. This allowed us to determine the discordance rates for the four chromosomes that had not been tested in the isozyme analysis and to analyze the chromosome content of the two clones in which the segregation of M-MuLV sequences and TPI had been found to be discordant. The result of this analysis is shown in Table 4: again, the lowest discordance was found between segregation of M-MuLV sequences and mouse chromosome 6 (only one exception). The discordances for chromosomes 3, 13, 15, and 16 were higher, excluding these chromosomes as integration sites.

Table 2. Quantitation of M-MuLV-specific sequences in mouse–Chinese hamster cell hybrids by molecular hybridization and correlation to mouse TPI activity

Hybrid clone	M-MuLV copies per hybrid cell*	Mouse TPI activity
2W2	1.6	+
2W3	1.0	+
2W5	1.4	+
2W6	1.2	+
2W8	0	−
2W14	1.3	−
2W18	0	+
6W4	0	−
6W5	0	−
Homozygous BALB/Mo	2.0	
Heterozygous BALB/Mo	1.0	

Thirty hybrid clones were assayed for M-MuLV-specific sequences by molecular hybridization and mouse TPI enzyme activity. The data for nine representative clones are shown in this table.

* The numbers of viral genome copies per cell were calculated from the hybridization data as described before (refs. 14, 19; compare Table 1) on the basis of an average DNA content of the hybrid cells of 2.7 times that of the diploid mouse genome equivalent. This figure was determined as follows: In metaphase preparations of five representative clones the chromosomes were differentiated as described under *Materials and Methods*. The lengths of the individual chromosomes were measured, and the total length of all mouse and hamster chromosomes was used to calculate the relative amount of mouse and hamster DNA and the total DNA content of the cells, assuming a direct correlation of chromosome length and DNA content.

Chromosome 15, which showed a rather low discordance in this analysis (only two exceptions), was clearly excluded by the sexual genetic analysis described in the next section. In neither of the two discordant clones (2W14 and 2W18, Table 2) could an intact mouse chromosome 6 be detected, although one of them was positive in the hybridization assay, whereas the other one expressed mouse TPI. The most likely reason for these discrepancies is a fragmentation of mouse chromosome 6 and an independent translocation of the fragments. In conclusion, the segregation analysis in the mouse–Chinese hamster somatic cell hybrids suggested strongly that the structural gene of M-MuLV in BALB/Mo mice is integrated in chromosome 6.

Assignment of the M-MuLV Integration Site by Sexual Genetics. As a second, independent, attempt to map the chromosomal integration site of M-MuLV, a segregation analysis by classical Mendelian genetics was performed. In a first experiment C57BL/6J × (C57BL/6J × BALB/Mo) backcross animals were analyzed and no linkage of the M-MuLV genome to markers on mouse chromosomes 2, 4, 7, 8, or 9 was found (32). Because the segregation analysis in mouse–Chinese hamster somatic cell hybrids suggested mouse chromosome 6 as the possible integration site for M-MuLV (see above), we used the linkage test strain ABP/J for further backcross analysis. ABP/J mice carry the morphological marker *wa-1* (waved) on chromosome 6 and the coat color marker *bt* (belted) on chromosome 15 (33) (genotype −, *bt, wa-1*). The (ABP/J × BALB/Mo)F_1 animals are viremic (15) and show straight whiskers and no belt, because both morphological markers are recessive (F_1 genotype Mo^+, +, +/−, *bt, wa-1*). F_1 males were backcrossed to ABP/J females and the ABP/J × (ABP/J × BALB/Mo) backcross animals were analyzed for the presence of M-MuLV in serum and spleen extracts and for the expression of the two recessive markers *wa-1* and *bt*.

For the following reason only paternal transmission was studied in this analysis: BALB/Mo females transmit the virus genetically as well as congenitally via the milk. Therefore 100% of the offspring of BALB/Mo females are viremic (15). In contrast, BALB/Mo males transmit the virus only genetically. Control experiments in which the presence or absence of the M-MuLV genome in backcross animals was determined by molecular hybridization analysis of their liver DNAs have shown that viremia is a reliable indicator for paternal transmission of the viral genome.

Table 3. Correlation between mouse enzymes and M-MuLV sequences in Chinese hamster–mouse hybrid clones

		Number of hybrid clones					
			Enzyme retention/M-MuLV sequences present				
Enzyme	Mouse chromosome	Total	+/+	−/−	+/−	−/+	% discordance
DIP-1	1	30	11	10	3	6	30
AK-1	2	30	7	11	2	10	40
PGM-2	4	30	7	7	6	10	53
PGM-1	5	30	6	9	4	11	50
TPI	6	30	16	12	1	1	7
GPI	7	24	13	6	3	2	21
APRT	8	30	12	9	4	5	30
MPI	9	30	7	11	2	10	40
TRIP-1	10	30	12	10	3	5	27
GalK	11	30	3	12	1	14	50
ACP-1	12	26	7	6	4	9	50
ES-10	14	30	13	8	5	4	30
GLO-1	17	30	12	8	5	5	33
DIP-2	18	30	5	13	0	12	40
GOT-1*	19	30	0	13	0	17	57
HPRT	X	30	17	0	13	0	43

Thirty hybrid clones were tested for the presence of mouse enzymes and M-MuLV sequences. See *Enzyme Determinations* for full names of enzymes. The columns labeled +/+, −/−, +/−, and −/+ indicate the number of clones in which the enzyme and viral sequences were present (+) or absent (−).

* All 30 hybrid clones were found to be negative for expression of mouse glutamate oxalacetate transaminase-1; however, chromosome 19 was found to be present in two of nine hybrid clones analyzed by trypsin/Giemsa banding and fluorescent staining (see Table 4).

Table 4. Correlation between mouse chromosomes and M-MuLV sequences in Chinese hamster–mouse hybrid clones

Mouse chromosome	Number of hybrid clones: Total	Chromosome retention/M-MuLV sequences present: +/+	−/−	+/−	−/+	% discordance
1	9	3	3	1	2	33
2	9	3	4	0	2	22
3	9	3	2	2	2	44
4	9	2	1	3	3	67
5	9	1	2	2	4	67
6	9	4	4	0	1	11
7	9	3	3	1	2	33
8	9	4	3	1	1	22
9	9	1	4	0	4	44
10	9	3	4	0	2	22
11	9	1	4	0	4	44
12	9	2	3	1	3	44
13	9	3	3	1	2	33
14	9	2	3	1	3	44
15	9	3	4	0	2	22
16	9	2	4	0	3	33
17	9	2	2	2	3	56
18	9	0	4	0	5	56
19	9	2	4	0	3	33
X	9	5	0	4	0	44

Nine hybrid clones were tested by Giemsa banding and fluorescent staining for the presence of mouse chromosomes and by molecular hybridization for M-MuLV sequences. The columns labeled +/+, −/−, +/−, and −/+ indicate the number of clones in which the chromosome and viral sequences were present (+) or absent (−). Between 17 and 51 metaphases per clone were karyotyped. Clones were scored as negative when none of the examined cells contained a given chromosome, and as positive when at least 15% of the cells contained that chromosome.

The result of the analysis of more than 200 ABP/J × (ABP/J × BALB/Mo) backcross animals is given in Table 5. When the segregation of the M-MuLV genome and *wa-1* marker was analyzed, 69% of the BC_1 animals had the parental genotype and 31% were recombinants. Thus the segregation of the M-MuLV and *wa-1* loci is linked, confirming the conclusion that the M-MuLV genome is integrated on mouse chormosome 6. As controls, the segregation of the M-MuLV and *bt* loci as well as the *wa-1* and *bt* loci was analyzed, and these markers were found to segregate independently: the frequences of recombinant genotypes were 47% and 46%, respectively (Table 5). This result clearly excludes chromosome 15 as the integration site.

DISCUSSION

In the experiments described here we have used mouse–Chinese hamster somatic cell hydrids as well as a classical Mendelian genetic analysis to map the chromosomal integration site of the structural gene of M-MuLV in BALB/Mo mice. The somatic cell hybridization experiments were made possible by the availablility of BALB/Mo fibroblasts that did not express the endogenous M-MuLV genome. As outlined above, this was an essential prerequisite, because virus expression leading to subsequent reintegration of viral sequences would have made impossible the identification of the original integration site. The data presented in Table 1 show an amplification of virus-specific sequences in M-MuLV-producing BALB/Mo fibroblasts as compared to nonproducing fibroblasts. Thus virus gene expression is accompanied by virus gene amplification in nontarget fibroblasts *in vitro*. This is in accordance with the situation *in vivo*, where it has been shown that virus expression is associated with viral gene amplification in target cells during the preleukemic and leukemic phases of AKR mice (19) or BALB/Mo mice (34). Our observations are not in agreement with the findings of Chattopadhyay *et al.* (35), who failed to detect AKR virus gene amplification in virus-producing as compared to non-virus-producing AKR fibroblasts.

Table 5. Correlation between morphological markers and M-MuLV expression in ABP/J × (ABP/J × BALB/Mo) backcross animals

Genotype		Number of animals		%
Mo^+/−	+/*wa-1*	74	33.0	68.8 parental
−/−	*wa-1*/*wa-1*	80	35.7	
Mo^+/−	*wa-1*/*wa-1*	37	16.5	31.2 recombinant
−/−	+/*wa-1*	33	14.7	
Mo^+/−	+/*bt*	52	23.7	53.4 parental
−/−	*bt*/*bt*	65	29.7	
Mo^+/−	*bt*/*bt*	56	25.6	46.6 recombinant
−/−	+/*bt*	46	21.0	
wa-1/*wa-1*	*bt*/*bt*	54	24.0	54.2 parental
+/*wa-1*	+/*bt*	68	30.2	
wa-1/*wa-1*	+/*bt*	54	24.0	45.8 recombinant
+/*wa-1*	*bt*/*bt*	49	21.8	

ABP/J females (genotype −, *wa-1*, *bt*) were mated with (ABP/J × BALB/Mo)F_1 males (genotype Mo^+, +, +/−, *wa-1*, *bt*) and the progeny were scored for waved whiskers, white belt, and expression of M-MuLV.

We wish to emphasize here that we employed an M-MuLV-specific quantitative molecular hybridization assay to determine the presence or absence of the M-MuLV genome in the individual hybrid clones. Thus our analysis directly assigned the structural M-MuLV gene without being dependent on expression or activation of the viral genome. These latter parameters are not necessarily indicative of the presence or absence of the structural viral gene because they might be influenced by complex interactions between regulatory host genes and structural viral genes (4).

The segregation of M-MuLV sequences was concordant in 28 of 30 mouse–Chinese hamster cell hybrid clones analyzed with the mouse enzyme TPI (Tables 2 and 3) which has recently been assigned to mouse chromosome 6 (29). With all other mouse chromosomes the discordance of segregation was higher, suggesting strongly that the M-MuLV structural gene in BALB/Mo mice is integrated in mouse chromosome 6. This conclusion was supported by the karyotype analysis using cytochemical methods (Table 4). Moreover, an identical result was obtained by the analysis of ABP/J × (ABP/J × BALB/Mo) backcrosses, which showed that the M-MuLV integration site is on mouse chromosome 6 at a distance of 31 recombination units from the *wa-1* marker (Table 5). We propose to call this locus *Mov-1*, denoting the genetically transmitted structural gene of M-MuLV in BALB/Mo mice.

BALB/Mo mice develop a specific, thymus-derived leukemia similar to the disease observed in AKR mice. In both cases virus expression is restricted to the target organs and a somatic amplification of virus-specific sequences is observed during leukemogenesis (14, 15, 19, 34). Moreover, a trisomy of mouse chromosome 15 is frequently observed in lymphomas induced by AKR virus as well as by M-MuLV (36, 37). In contrast, other MuLV show a different target cell specificity: e.g., the Abelson

strain of MuLV causes a B cell lymphosarcoma (38) and the Friend leukemia virus complex transforms erythroid precursor cells (39). It has been suggested that different but specific integration sites on the host genome are the molecular basis for the target cell specificity of the various MuLVs (40). In the light of this hypothesis it was of interest to learn whether the striking similarities between the diseases induced by the AKR virus and M-MuLV are due to an allelic integration site. The results of the experiments described here so far do not confirm this hypothesis: the *Akv-1* locus, one of the structural genes of the AKR virus, has been assigned to mouse chromosome 7 (5), whereas our work assigns the M-MuLV gene to chromosome 6. Increasing experimental evidence indicates that there are several integration sites for MuLV on the mouse genome: (*i*) three independently segregating AKR-MuLV loci in AKR mice (5, 41); (*ii*) two nonallelic loci for endogenous AKR-type MuLV genomes in the two related mouse strains C3H and C57BL/6 (42); and (*iii*) multiple integration sites for M-MuLV as identified by restriction enzyme analysis of M-MuLV-infected fibroblasts (L. Bacheler and H. Fan, personal communication) as well as of leukemic tissues of BALB/Mo mice (ref. 43; A. Berns and H. v. Putten, personal communication). Because all BALB/Mo mice are progeny of a single animal infected as a preimplantation embryo (14), *Mov-1* represents one of several possible loci for germ line-transmitted MuLV. The mapping of the chromosomal location of additional, unassigned, sites for AKR-type endogenous MuLV genomes will have to show if *Mov-1* and any of these sites are allelic. It will be of special interest to learn whether the different sites are equivalent or whether a hierarchy of integration sites exists with respect to the frequency of integration or their importance for leukemic transformation.

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