

# Genetic Analysis of *Gv1*, a Gene Controlling Transcription of Endogenous Murine Polytopic Proviruses

PETER L. OLIVER AND JONATHAN P. STOYE\*

Division of Virology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom

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***Gv1* is a genetic locus that coordinately regulates the expression of multiple murine leukemia virus-related endogenous proviral sequences. A quantitative nuclease protection assay for typing *Gv1* inheritance has been developed. Use of this assay demonstrates that *Gv1* controls transcription of polytopic but not of modified polytopic endogenous proviruses. A combination of genetic techniques were used to map *Gv1*; these analyses demonstrate that *Gv1* lies approximately 37 centimorgans from the centromeric end of mouse chromosome 13.**

Throughout evolution, the genomes of higher eukaryotes have been colonized by retroviruses and retrovirus-like sequences (2). The best-studied elements are the murine leukemia virus (MLV)-related viruses of mice. Inbred strains of mice contain multiple copies of endogenous MLVs integrated into the genome as proviruses, where they behave as any other Mendelian gene (11). Four classes of endogenous MLV-related proviruses have been isolated, and they are classified according to the structures of their *env* genes (34). Infectious ecotropic viruses can infect murine cells only, xenotropic viruses can infect nonmurine but not murine cells, whereas the polytopic and modified polytopic classes can infect cells of either type (2). The host mechanisms controlling endogenous retroviral gene expression, however, remain unclear, although they are likely to be complex and involve interactions between host cell factors and long terminal repeats (LTRs).

The 129 mouse is unusual among the common inbred strains in that it does not produce infectious virions from endogenous MLV sequences. This mouse strain has therefore been used to model antigen accumulation resulting from the expression of endogenous retroviruses without the confounding effects due to transcription of novel proviruses resulting from in vivo virus replication. The  $G_{IX}$  antigen was the first demonstration of a retroviral gene product in 129 mice. A cytotoxicity assay was established, in which antiserum prepared in inbred rats against a syngeneic leukemia induced by wild-type MLV lysed the thymocytes of some strains of mice such as 129 (designated  $G_{IX}+$ ) and not others ( $G_{IX}-$ ) (12, 31). It was later discovered that  $G_{IX}$  was in fact a type-specific antigenic determinant on gp70, the major glycoprotein component of the MLV envelope (24, 37, 38). Genetic data obtained from crosses between the prototype  $G_{IX}+$  strain 129 and C57BL/6 ( $G_{IX}-$ ) mice revealed that two unlinked chromosomal genes, *Gv1* and *Gv2*, are required for the expression of the  $G_{IX}$  antigen on normal lymphoid cells. The *Gv2* locus controls the  $G_{IX}$  phenotype in a dominant fashion, while *Gv1* is semidominant, with heterozygote mice ( $Gv1^{+/-}$ ) demonstrating a 50% reduction in  $G_{IX}$  expression compared to homozygotes ( $Gv1^{+/+}$ ) (31). Attempts have been made to genetically map *Gv1*, yet these studies have yielded contradictory results (30). *Gv2* has been mapped to chromosome 7, although the chromosome position is based on

the results of only one cross in which *Gv1* was also segregating (32). By serial backcrossing to C57BL/6, an inbred congenic (129  $G_{IX}-$ ) strain was established that only differed from its partner (129  $G_{IX}+$ ) at the *Gv1* locus (29). Unfortunately, this strain is no longer available.

Studies of the congenic strain revealed that the  $G_{IX}-$  phenotype was not simply due to a complete absence of viral antigens, since low levels of both gp70 and the major core protein p30 were detected (17). It was also shown that gp70 molecules found at different tissue sites of 129  $G_{IX}+$  mice, absent or reduced in the congenic strain, have distinct tryptic peptide maps (8), suggesting that multiple proviral genes are being affected by *Gv1*. To examine the extent of this transcriptional control, Moloney MLV DNA was used to probe RNA purified from various tissues from the congenic strain and 129  $G_{IX}+$  mice. It was concluded that a negative allele at the *Gv1* locus correlated with reduced steady-state levels of retroviral RNA and that the two strains differ in the expression of the same complement of retroviral structural genes (18). Northern blotting experiments also showed that the expression of endogenous MLV structural genes appeared to be regulated in a tissue-specific manner (18). Nuclease protection with an LTR-specific probe confirmed that certain MLV transcripts were under-represented in  $G_{IX}-$  tissues and that at least two LTR sequences were under control of *Gv1* based on the pattern of protected products. It was therefore proposed that *Gv1* encodes a *trans*-activating factor that coordinately regulates the expression of multiple proviral loci (19).

Despite this work, the mechanism by which *Gv1* controls proviral expression and the role of *Gv2* remain unclear. To investigate these questions and with the ultimate goal of positionally cloning *Gv1*, we have carried out a genetic analysis of *Gv1*. A reliable nuclease protection assay for the gene has been established, allowing a clearer definition of the targets for *Gv1* action, an assessment of the relative roles of *Gv1* and *Gv2* in controlling proviral transcription, and the genetic mapping of *Gv1*.

## MATERIALS AND METHODS

**Mice.**  $F_1$  and backcross mice were bred from stocks of 129/SvEv, BALB/cJ, and C57BL/6J animals maintained in the Biological Services division of the National Institute for Medical Research. The 129 mice are  $Gv1^+ Gv2^+$ ; the C57BL/6 mice are  $Gv1^- Gv2^-$ , and the BALB/c mice are  $Gv1^- Gv2^+$  (30, 31).

**Nucleic acid purification.** Genomic DNA was prepared from fresh or snap-frozen spleen tissue as previously described (1). Total RNA was prepared from fresh or snap-frozen thymus samples as previously described (3).

\* Corresponding author. Mailing address: Division of Virology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom. Phone: 44-208/959-3666, ext. 2140. Fax: 44-208/906-4477. E-mail: jstoye@nimr.mrc.ac.uk.

**Nuclease protection.** Constructs containing proviral sequences from the polytropic and modified polytropic classes of MLV were subcloned from clones pMX40 (35) and pMX33 (34) for the synthesis of riboprobes. LTR sequences were amplified from these clones by using the primers PLO4 (5'-CGCAATTAACCTCACTAAAGGG-3') and PLO5 (5'-GCGAATTCATTGGCAGACAC-3'). The PCR products were digested with *Pst*I and *Eco*RI and subcloned into the pGEM 3Zi(+) vector. A 424-bp polytropic-specific sequence was amplified from the *env* region of pMX40 by using the primers PLO33 (5'-GGACTAAGACTGTACCGATC-3') and PLO34 (5'-CTTCGGACAGGGTCAGCTTG-3'). The amplified products were cloned into the pCRII-TOPO vector (Invitrogen) as described by the manufacturer. Riboprobes were synthesized by *in vitro* transcription in a reaction containing 250 ng of linearized template; 1× Transcription Optimized Buffer (Promega); 0.5 mM ATP, CTP, and GTP; 25 to 125 μM UTP; 10 mM dithiothreitol; 50 μCi of [ $\alpha$ -<sup>32</sup>P]UTP (specific activity, 800 Ci/mmol); 10 U of rNasin RNase inhibitor (Promega); and 20 U of T7 RNA polymerase (Promega). Riboprobes were gel purified on denaturing polyacrylamide gels, and nuclease protection was carried out according to the RPA II method (Ambion). The  $\beta$ -actin control plasmid supplied was also used for the synthesis of riboprobes. Supplementation of the *in vitro* transcription reaction with up to 500 μM UTP allowed riboprobes with lower specific activities to be made for reactions in combination with the *env* probe to avoid interference of the  $\beta$ -actin-protected fragment with smaller bands of interest. Typically, 25 μg total RNA was coprecipitated with  $1 \times 10^5$  to  $3 \times 10^5$  cpm of labelled MLV probe plus the  $\beta$ -actin riboprobe as an internal control. Hybridization and RNaseA-T1 digestion was carried out as described by the manufacturer. Protected products were resolved on 5% Sequagel denaturing polyacrylamide gels (National Diagnostics), dried onto filter paper, and analyzed by autoradiography or by using a phosphorimager.

Probe-specific signals were quantified by integrating pixel intensities over defined signal volumes by using ImageQuant software (Molecular Dynamics). Relative intensities were expressed as ratios [(probe signal - background)/( $\beta$ -actin signal - background)] to correct for variation in RNA content, loading, or coprecipitation during the nuclease protection procedure.

**Mapping by microsatellite amplification from pooled DNA samples.** Genome scanning was carried out by using the DNA pooling strategy described by Taylor et al. (36). DNA from individual backcross mice was prepared as described above, and each sample was diluted to 25 ng/μl. Pools were made by mixing DNA from 50 animals typed by nuclease protection. PCR amplification of simple sequence repeats was carried out essentially as previously described (36), although the reaction volume was reduced to 15 μl and the optimal MgCl<sub>2</sub> concentrations were determined empirically where necessary. Eighty-five microsatellite primer pairs, which span 97% of the genome, were purchased from Research Genetics, Inc. PCR products were run on 3% NuSeive (Flowgen) 1% agarose gels stained with ethidium bromide. For those products that could not be resolved on conventional gels, 10 pmol of one primer was end labelled with [ $\gamma$ -<sup>32</sup>P]ATP by using T4 polynucleotide kinase (New England Biolabs), and 0.1 pmol was added per reaction to the standard PCR mix. PCR products were resolved on 5% denaturing polyacrylamide gels.

**REVEAL-PCR.** Repeat element-viral element amplified locus PCR (REVEAL-PCR) was carried out essentially as previously described (16). First, 10 pmol of an intracisternal A-type particle (IAP)-specific oligonucleotide (JS139 or JS140) was end labelled with [ $\gamma$ -<sup>32</sup>P]ATP by using T4 polynucleotide kinase and combined in a PCR reaction with one of three B1 or B2 short interspersed element (SINE) primers (JS134, JS135, or JS136). Labelled PCR products were resolved on 5% denaturing polyacrylamide gels which were dried onto filter paper and exposed to X-ray film overnight.

REVEAL products of interest (REVEAL-1 and REVEAL-2) were excised from dried polyacrylamide gels and eluted in RPA II probe elution buffer (Ambion) overnight at 37°C. The products were reamplified with the same IAP-SINE repeat primer pair used previously without the addition of an end-labelled oligonucleotide, and the PCR products were cloned into the pCRII-TOPO vector as described by the manufacturer (Invitrogen). Inserts were sequenced by standard methods on the 377 automated sequencer (Perkin-Elmer) for internal primer design.

**Radiation hybrid mapping.** PCR analysis of the T31 mouse radiation hybrid panel (Research Genetics) was carried out by using the REVEAL-1-specific primers JS139 (5'-GTTTACCACCTTAGAACACAG-3') and PLO16 (5'-GGAAATAGTAAGTCACACC-3'). First, 20 ng of all 100 hybrid clone DNA samples were amplified in 15 μl of PCR reactions containing 1× PCR Buffer II (Perkin-Elmer), 2.5 mM MgCl<sub>2</sub>, 0.2 mM concentrations of each deoxynucleoside triphosphate (dNTP), 2 pmol of primers, and 0.5 U of AmpliTaq DNA polymerase (Perkin-Elmer). PCR conditions were identical to the REVEAL-PCR protocol, and all reactions were carried out in triplicate. Based on the analysis of products on 2% agarose gels, the hybrid clones were scored according to the presence or absence of a mouse-specific band. The screening results were processed by the software Map Manger QT (20) at The Jackson Laboratory.

**Microsatellite mapping.** Markers were chosen based on polymorphism between the 129 and the C57BL/6 or BALB/c strains where data were available. The sequence of primers for microsatellites in the region of interest were obtained from The Jackson Laboratory Mouse Genome Informatics World Wide Web site (23). DNA samples (50 ng) were amplified in 15-μl PCR reactions containing 1× PCR buffer I (Perkin-Elmer), 0.2 mM concentrations of each

dNTP, 2 pmol each primer, and 0.2 U of AmpliTaq DNA polymerase. The initial denaturation step was 2 min at 94°C, followed by 45 cycles of denaturation for 20 s at 94°C, annealing for 30 s at 55°C, and extension for 30 s at 72°C before a final 10-min extension at 72°C. PCR products were resolved on conventional or polyacrylamide gels as described above. Linkage and quantitative trait locus (QTL) data were analyzed by using the Map Manager QT program (20).

## RESULTS

**Characterization of *Gv1* controlled transcription.** To allow studies of the inheritance of *Gv1*, we set out to devise a reliable nuclease protection assay for quantifying proviral expression. U3 probes were designed to allow the independent analysis of both the polytropic and modified polytropic classes of endogenous MLV that are present in multiple copies in all inbred strains of mice. Previous studies (34) have shown that the LTRs of modified polytropic proviruses contain two inserts, of 5 and 42 nucleotides, compared to polytropic proviruses in the U3 region (Fig. 1A). Probes to this region will therefore yield clearly distinguishable full-length protected fragments in nuclease protection experiments. Total thymus RNA from 129, C57BL/6, BALB/c, and F<sub>1</sub> (129 × C57BL/6 and 129 × BALB/c) mice were hybridized to the polytropic and modified polytropic LTR probes by using  $\beta$ -actin as an internal control. Quantitative studies with the polytropic probe revealed that the full-length protected product of 272 nucleotides was clearly reduced in the *Gv1*<sup>-</sup> strains compared to 129 (Fig. 1B, compare lane 4 to lanes 5 and 6). Expression of this LTR sequence was consistently lower in the C57BL/6 strain than in the BALB/c strain, and this was reflected in the F<sub>1</sub> mice, which showed an intermediate level of proviral expression compared to the 129 (*Gv1*<sup>+</sup>) animals. These levels of expression are consistent with the semidominant nature of *Gv1* inheritance. The full-length protected fragment, with the modified polytropic probe, did not differ in intensity between the *Gv1*<sup>+</sup> and *Gv1*<sup>-</sup> strains (Fig. 1B, lanes 9 to 13), implying that the *Gv1* locus does not affect the expression of the modified polytropic class of endogenous MLV.

To further investigate the nature of *Gv1* transcriptional control, nuclease protection experiments were carried out with an *env* probe designed to allow simultaneous measurement of both polytropic and modified polytropic proviral expression. These two classes of provirus can be distinguished by a 27-nucleotide deletion in modified polytropic viruses compared to polytropic viruses (34). By using a polytropic probe, polytropic transcripts will therefore protect a fragment of 424 nucleotides, whereas modified polytropic sequences will protect two fragments of 212 and 185 nucleotides (Fig. 2A). Figure 2B shows the result of such an experiment to examine *env* gene expression in thymus. *Gv1*<sup>-</sup> strains express less polytropic *env* mRNA than 129 as judged by the intensity of the 424-nucleotide full-length protected fragment. The patterns of expression observed are comparable to the results obtained when using the polytropic LTR probe. By contrast, the intensities of the 212- and 185-nucleotide protected fragments do not vary between the different strains. These results confirm that expression of the polytropic but not of the modified polytropic class of endogenous MLV is regulated by *Gv1*.

**Genetic mapping of *Gv1*.** C57BL/6 and BALB/c backcrosses were set up to follow the segregation of *Gv1*. Nuclease protection data was collected from 230 animals from the backcross populations by using the polytropic LTR riboprobe (Table 1). In each set of reactions, the corrected intensity of the full-length protected product was compared to a reaction by using RNA from the appropriate F<sub>1</sub> mouse. If the band intensity was over 75% of the F<sub>1</sub> value, the mouse was classified as heterozygous at the *Gv1* locus, whereas *Gv1*<sup>-</sup> animals were those that

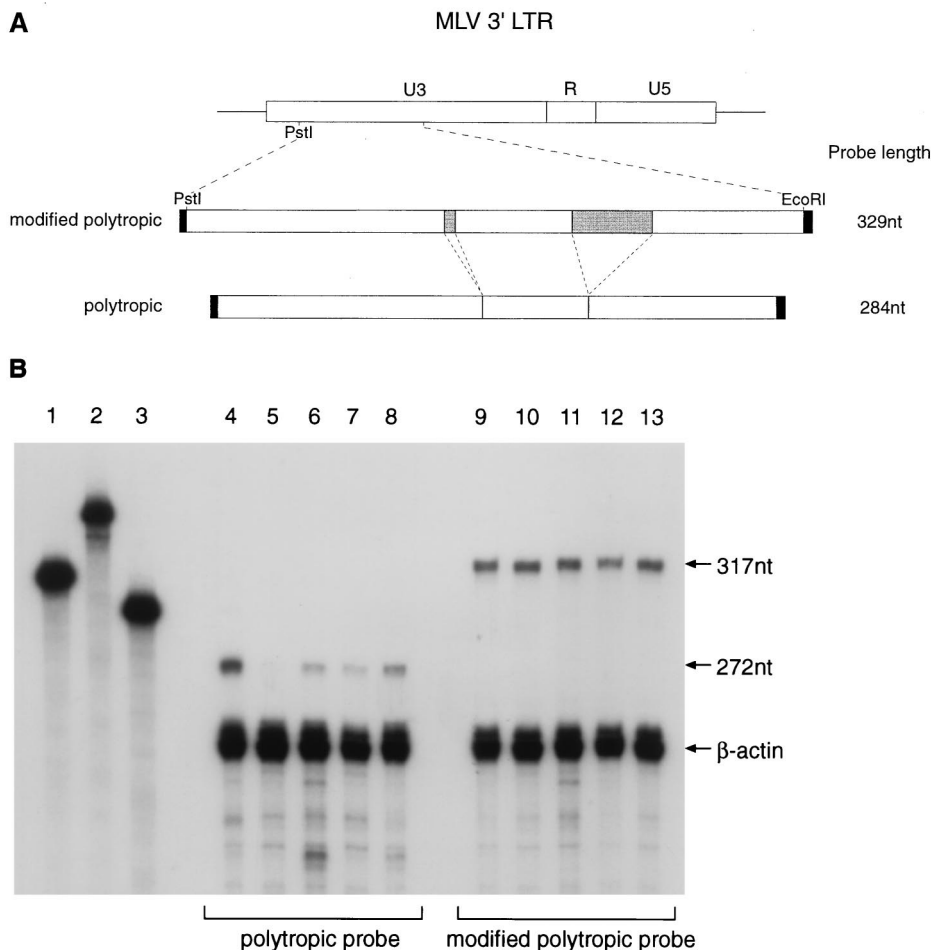


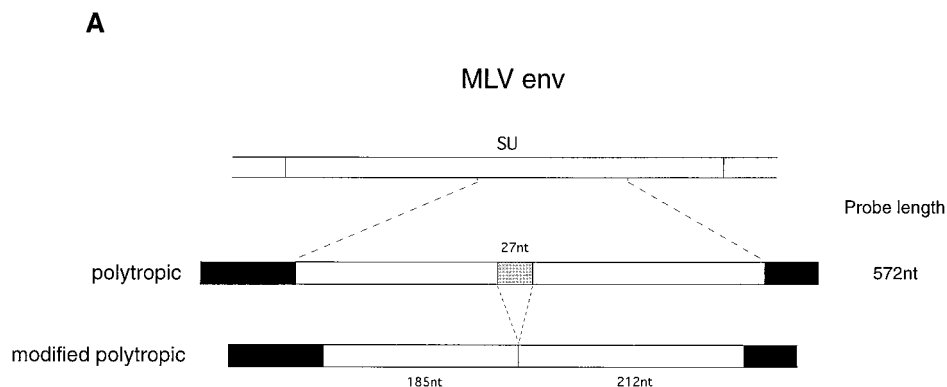
FIG. 1. Quantitative nuclease protection demonstrates differential expression of the polytropic class of endogenous MLV LTR sequences in *Gv1*<sup>-</sup> strains of mice. (A) The MLV LTR region cloned for the synthesis of riboprobes. Shaded areas indicate two regions, of 5 and 42 bp, of the modified polytropic sequence absent in the polytropic clone. (B) Quantitative nuclease protection of thymus total RNA with an LTR probe and a  $\beta$ -actin internal control probe. Lanes 4 to 8, protected products with polytropic probe; lanes 9 to 13, modified polytropic probe. The strains of mice analyzed plus the relative intensities of the full-length protected products, corrected for  $\beta$ -actin are as follows: lane 4, 129, 0.118; lane 5, C57BL/6, 0.006; lane 6, BALB/c 0.032; lane 7, 129  $\times$  C57BL/6, 0.030; lane 8, 129  $\times$  BALB/c, 0.064; lane 9, 129, 0.108; lane 10, C57BL/6, 0.128; lane 11, BALB/c, 0.112; lane 12, 129  $\times$  C57BL/6, 0.109; and lane 13, 129  $\times$  BALB/c, 0.121. The undigested  $\beta$ -actin, modified polytropic, and polytropic probes (lanes 1, 2, and 3, respectively) are longer than the full-length protected products due to retention of plasmid sequence from the *in vitro* transcription reaction (regions shaded black in panel A).

demonstrated intensity values of <55%. An example from the C57BL/6 backcross is shown in Fig. 3. The nuclease protection data from both backcross populations show a bimodal distribution (Fig. 4). Of the 230 animals typed for *Gv1* by using the assay, 111 (48%) carry the 129 allele, which is a finding consistent with the segregation of a single gene. These results also indicate that *Gv2* does not play a role in regulating proviral expression since essentially equivalent distributions were seen whether (129  $\times$  C57BL/6) or not (129  $\times$  BALB/c) the gene was segregating in the cross.

To map *Gv1* in the backcross populations, a genome scan with 85 microsatellite markers was carried out based on the method of Taylor et al. (36). The phenotypic pooling method relies on the detection of markers in a segregating population, grouped together according to their phenotype at a target locus. Linked markers are identified by a change in the relative intensity of microsatellite PCR products from each DNA pool. Linkage can be identified with a 20-centimorgan (cM) distance from each marker typed. Linkage to *Gv1* could be excluded from 74% of the genome from the data obtained from 41 of the

primer pairs (Fig. 5). The remaining microsatellites could not be typed due to failure of one or both of the alleles to amplify or lack of polymorphism between the strains (data not shown). It was therefore decided to attempt REVEAL-PCR (16) as an alternative mapping method.

This multilocus mapping approach examines the inheritance of IAP elements and their proximity to SINEs in a PCR-based assay (Fig. 6A). To find a marker linked to *Gv1*, REVEAL products from 23 C57BL/6 backcross mice typed as heterozygous at the *Gv1* locus were generated with all six combinations of IAP and SINE repeat primers (16). PCR products of 129 origin that were present in most of the backcross DNA samples were considered as possible mapping candidates. Of approximately 30 REVEAL products derived from the JS140/134 primer pair, one band (REVEAL-2) was present in 20 of the 23 animals, indicating linkage (Fig. 6B). Closer linkage was demonstrated by a product amplified by using the JS139/136 primer pair, REVEAL-1, with only one backcross mouse showing the absence of the band at approximately 360 bp. Both REVEAL products were excised from polyacrylamide gels,



**FIG. 2.** Quantitative nuclease protection demonstrates differential expression of the polytropic class of endogenous MLV *env* sequences in *Gv1*<sup>-/-</sup> strains of mice. (A) The region of MLV *env* cloned for the synthesis of riboprobes. The shaded area indicates a 27-bp region absent in the modified polytropic clone. Solid black regions indicate plasmid sequence retained in the probes from the in vitro transcription reaction. (B) Quantitative nuclease protection of thymus total RNA with a polytropic *env* probe and a  $\beta$ -actin internal control probe. The strains of mice analyzed and the relative intensities of the full-length protected products, corrected for  $\beta$ -actin are as follows: lane 1, 129, 0.214; lane 2, C57BL/6, 0.013; lane 3, BALB/c, 0.032; lane 4, 129  $\times$  C57BL/6, 0.096; and lane 5, 129  $\times$  BALB/c, 0.123. The relative intensities of the 212- and 185-nucleotide modified polytropic-specific fragments are as follows (respectively): lane 1, 0.086 and 0.110; lane 2, 0.080 and 0.114; lane 3, 0.075 and 0.108; lane 4, 0.091 and 0.122; and lane 5, 0.093 and 0.127. No  $\beta$ -actin probe was added to the 129  $\times$  C57BL/6 RNA sample in lane 6.

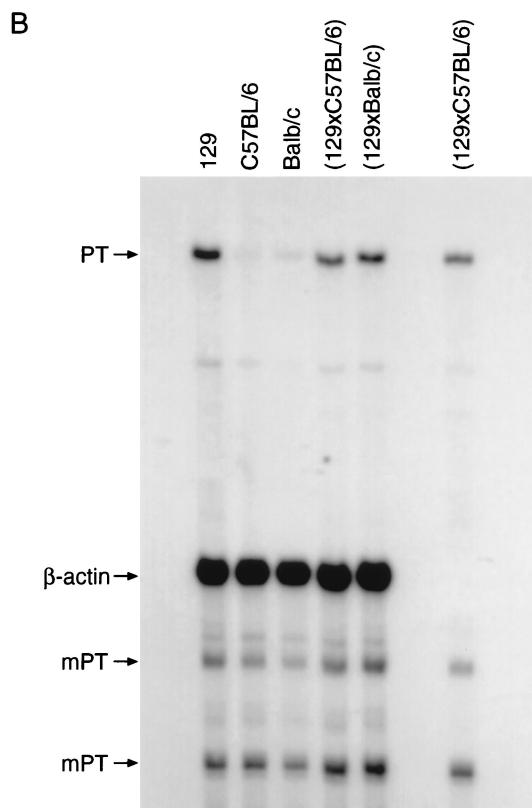
reamplified, cloned, and sequenced. Internal primers to REVEAL-1 were designed, allowing animals to be screened for the presence of this marker by PCR on conventional gels (Fig. 6C). Linkage was confirmed when REVEAL-1 was amplified from the remaining 93 C57BL/6 backcross progeny, with only seven further animals showing apparent (see below) recombination between the marker and *Gv1*. Linkage was also established to the REVEAL-1 marker in the BALB/c backcross (15 apparent recombinants in 100 animals tested), confirming that the same locus was responsible for the proviral expression phenotype observed.

To map *Gv1*, it was first necessary to identify the chromosomal position of the REVEAL-1 marker. For this purpose, we used the technique of whole genome radiation hybrid mapping. The T31 mouse whole-genome panel comprises 100 hybrid cell lines derived by fusing irradiated 129 embryonic stem cells with cells from a hamster fibroblast line (22). Different hybrids retain different fragments of mouse chromosomes, and these have been extensively characterized by microsatellite mapping (13). Scoring REVEAL-1 on the T31 radiation hybrid panel showed that it mapped to mouse chromosome 13. The marker, now termed *D13Nimr1*, was placed between the mic-

**TABLE 1.** Nuclease protection data inferring *Gv1* genotype from two backcross populations

<i>Gv1</i> genotype	C57BL/6 backcross	BALB/c backcross	Total
<i>Gv1</i> <sup>-/-</sup>	66	50	116
<i>Gv1</i> <sup>+/-</sup>	57	54	111
Unknown <sup>a</sup>	1	2	3
Total no. of animals	124	106	230

<sup>a</sup> Values from these animals lay exactly at the cutoff point between *Gv1*<sup>-/-</sup> and *Gv1*<sup>+/-</sup>.



rosatellites *D13Mit157* and *D13Mit66* with LOD scores of 11.8 and 9.0, respectively (data not shown). This region of chromosome 13 is part of the fraction of the mouse genome which could not be excluded by the pooling method (Fig. 5).

To characterize the map position of *Gv1*, microsatellites flanking *D13Nimr1* were tested on the C57BL/6 backcross DNA pools. Of the 32 chromosome 13 markers analyzed that have been mapped to between 32 and 49 cM, only 7 appeared to be polymorphic between the 129 and C57BL/6 strains. Linkage to *Gv1* was confirmed by using this method, and all 116 backcross mice were consequently screened with the same seven markers. The haplotypes of the animals are shown in Fig. 7A.

Eight mice in the C57BL/6 backcross appeared to be recombinant between *Gv1* and both the closest flanking markers, *D13Mit13* and *D13Mit39*. These double recombinants in a very



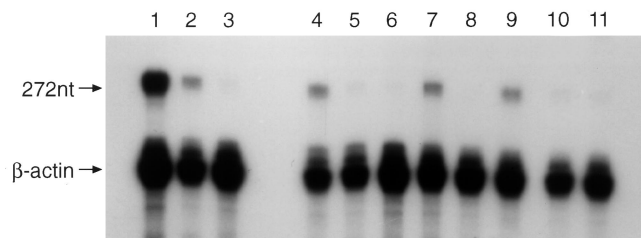


FIG. 3. Nuclease protection of C57BL/6 backcross mice indicates *Gv1* genotype. Thymus total RNA from the mouse strains indicated was analyzed by quantitative nuclease protection by using the polytropic LTR riboprobe and a  $\beta$ -actin internal control probe. Relative intensity of the 272-nucleotide fragments corrected for  $\beta$ -actin as follows: lane 1, 0.225; lane 2, 0.065; lane 3, 0.009; lane 4, 0.076; lane 6, 0.016; lane 6, 0.005; lane 7, 0.056; lane 8, 0.009; lane 9, 0.066; lane 10, 0.018; and lane 11, 0.015. Mice were classified as *Gv1*<sup>+/-</sup> (lanes 4, 7, and 9) by similarity (>75%) of the band intensity to RNA from the F<sub>1</sub> animal (lane 2) or as *Gv1*<sup>-/-</sup> (lanes 5, 6, 7, 10, and 11) if the value obtained was under 55% of the F<sub>1</sub> protected product intensity.

short genetic interval are indicative of mis-typing in the assay used to genotype the backcross animals (26). The proviral expression levels of these eight mice, based on the nuclease protection data, lie towards the center of the bimodal distribution, between 60 and 80% of the heterozygote figure. This was also the case for the 15 apparent recombinants from the BALB/c cross. This suggests that errors in the quantification method are large enough to account for the incorrect genotyping of a small percentage of animals. Assuming this to be the case, *Gv1* cosegregates with both *D13Nimr1* and *D13Mit39*, placing the gene at 37 cM from the centromere (Fig. 7B). To confirm linkage to this region of chromosome 13, the nuclease protection and backcross mapping data from all 116 mice of

the C57BL/6 backcross, including the 8 animals which we believe were mis-typed, were combined and analyzed as a QTL (Fig. 7C). The highest LOD score was obtained around the chromosomal position of *D13Nimr1* and *D13Mit39*, suggesting strong linkage between a locus at approximately 37 cM on mouse chromosome 13 and the proviral expression levels observed in the backcross population, thereby confirming the map location derived from the haplotype data.

DISCUSSION

Despite intensive studies, we know little about the factors controlling expression of endogenous retroviruses (2). Elucidation of the mode of action of *Gv1* might be expected to shed light on these processes. The genetic mapping of *Gv1* reported here represents the first step towards the eventual cloning and characterization of the gene.

Throughout this study we have assumed that the gene we have mapped to chromosome 13 does indeed correspond to the locus controlling G<sub>IX</sub> antigen expression that was originally described. Of particular concern was the possibility that we might have been following merely the inheritance of a single provirus rather than the *trans*-acting *Gv1*. Unfortunately, the congenic G<sub>IX</sub><sup>-</sup> strain is no longer available, so direct tests for the presence of chromosome 13 markers of C57BL/6 origin in the congenic strain are not possible. Nevertheless, our assumption of identity does not seem unreasonable since the properties of our gene appear concordant with those of *Gv1*.

G<sub>IX</sub> was described as a thymus antigen expressed by 129 mice but not by BALB/c or C57BL/6 mice (31). Inheritance was semidominant. We have monitored the thymic expression of polytropic MLV sequences in crosses between 129 (positive) and BALB/c or C57BL/6 (negative), and the transcription lev-

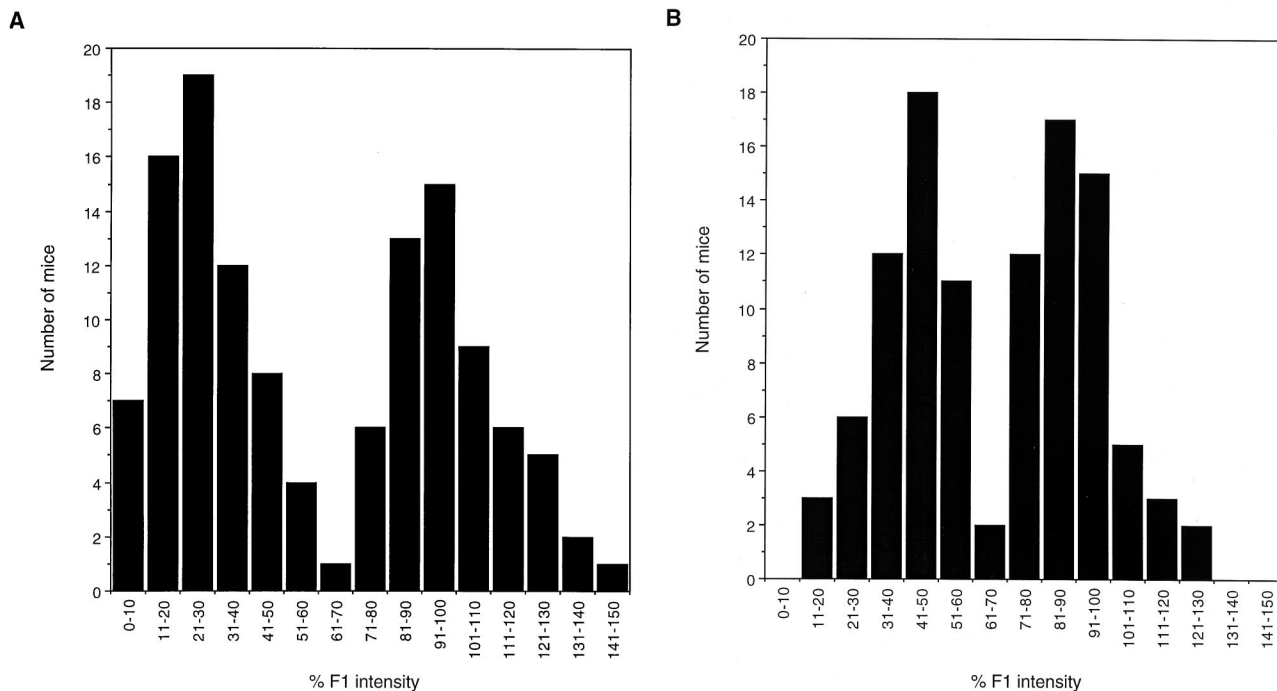


FIG. 4. A single locus is responsible for the proviral expression levels observed in two backcross populations. A distribution of the quantitative nuclease protection data with the polytropic LTR riboprobe from the two backcross populations indicated is shown. Data are shown as a percentage of the relative intensity of the full-length protected product compared to RNA from F<sub>1</sub> mice. The bimodal distribution of the data suggests that a single locus is controlling the proviral expression levels observed.

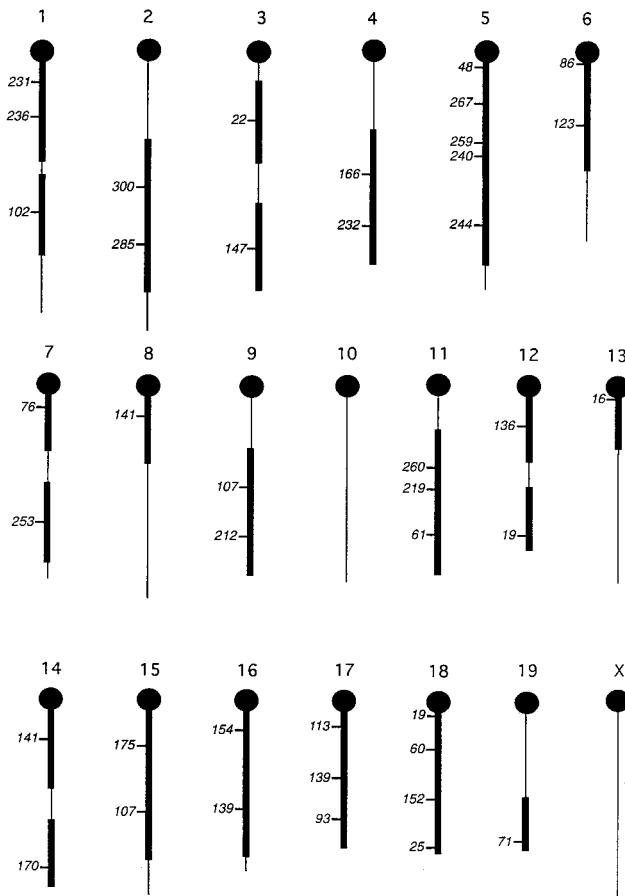


FIG. 5. Linkage of *Gv1* is excluded from 74% of the mouse genome. Chromosomal locations of the 41 microsatellite markers successfully amplified and analysed by the DNA pooling method are shown. The Mit number (6) of each marker is shown, and the centromeric ends of the chromosomes are indicated by black circles. Linkage to *Gv1* has been excluded from the regions represented by thickened lines, which correspond to 74% of the mouse genome.

els are consistent with the inheritance of a single, semidominant gene. The protected products that appeared absent in the thymus RNA from congenic 129 mice in the experiments of Levy et al. (19) are in fact consistent with the use of a modified polytropic probe for detecting polytropic LTR sequences. We note that one polytropic provirus, *Pmv41* (10), from DBA mice, has been shown to lie within the interval containing the gene we have mapped. However, this provirus is not present in 129 mice, and no other polytropic proviruses have been mapped to this region of chromosome 13 (9). Hybridization studies with the polytropic-specific oligonucleotide probe JS5 (35) have confirmed the absence of *Pmv41* in 129/SvEv mice (33). Analysis of a small panel of backcross mice revealed no linkage between 129 proviruses and polytropic virus expression (33), a result consistent with the proposition that *Gv1* encodes a *trans*-acting gene product.

The observation that polytropic but not modified polytropic sequences are responsive to *Gv1* are consistent with the predicted  $G_{IX}$  phenotypes of these classes of MLV sequence. The appearance of the  $G_{IX}$  antigen is correlated with the absence of a glycosylation signal in the *env* gene of ecotropic MLV (7); sequence data (34) from the equivalent region of polytropic and modified polytropic MLV reveals that the former, but not the latter, class of sequence also lacks a glycosylation signal.

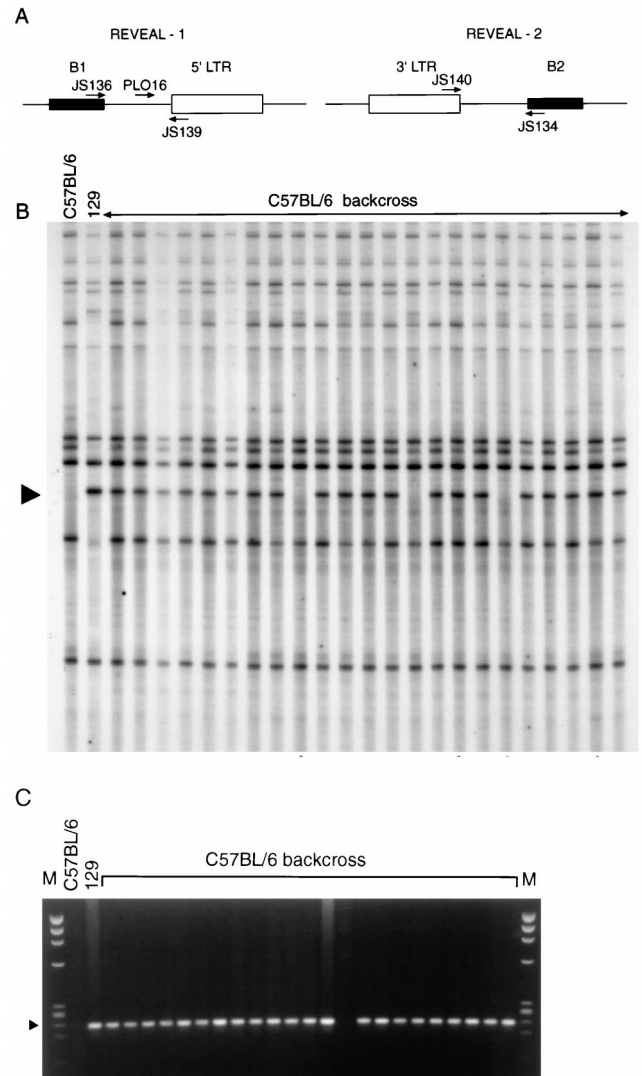
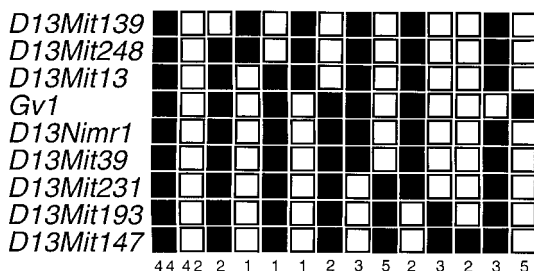


FIG. 6. Two IAP proviral markers are linked to *Gv1*. (A) Relative positions of the primer pairs used to generate two sets of REVEAL-PCR products. (B) REVEAL-PCR products amplified with JS140 and JS134, in the presence of end-labelled primer JS140, from the genomic DNA samples indicated. The arrow indicates the position of REVEAL-2, which is absent in C57BL/6 mice but was amplified from 129 mice. Of 23 backcross animals predicted to be heterozygous at the *Gv1* locus, 20 show the presence of this band, indicating linkage. (C) Genomic DNA from the same 23 C57BL/6 backcross mice as in panel B was amplified by using primers JS139 and PLO-16 derived from the marker REVEAL-1. Products were run on a 2% agarose gel against the  $\phi x174/HaeIII$  marker (M). The arrow indicates the position of the *D13Nimr1* product at 229 bp. Of 23 backcross animals, 22 show the presence of the band, indicating linkage.

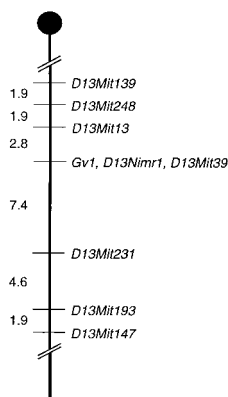
Polytropic MLV (bases 1277 to 1285 [34]) is predicted to encode Asp-Leu-Thr, whereas modified polytropic MLV (bases 1250 to 1258) encodes Asn-Leu-Thr.

The expression of the  $G_{IX}$  antigen is controlled by two genes, *Gv1* and *Gv2*, and it has been hypothesized that both genes play a role in proviral transcription. Although there is a slight difference in the distribution of the nuclease protection data from the C57BL/6 and BALB/c backcrosses, this study suggests otherwise. It has been shown here that only *Gv1* has any transcriptional control activity, as a bimodal distribution was observed in both backcross populations. This suggests that

A



B



C

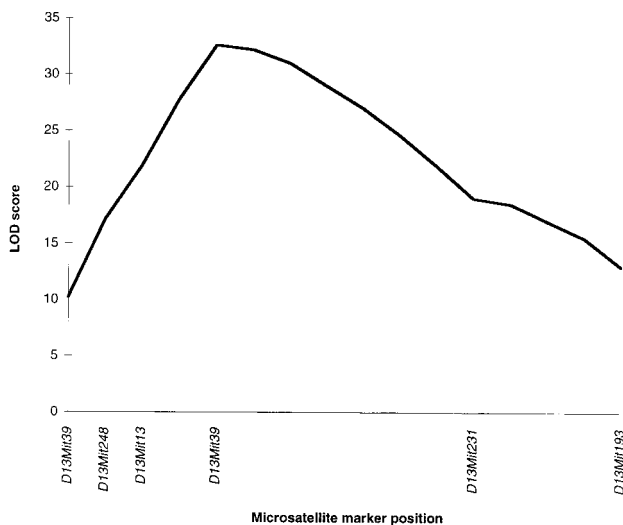


FIG. 7. *Gv1* maps to chromosome 13. (A) Haplotype analysis of the 116 C57BL/6 backcross progeny typed for markers flanking the REVEAL-PCR product, REVEAL-1. Each column represents the allele inherited from the (129 × C57BL/6)<sub>F1</sub> parent. The number of offspring inheriting each allele is indicated at the bottom of each column; shaded boxes indicate the presence of a 129 allele, and the open boxes indicate the presence of a C57BL/6 allele. (B) Partial linkage map of chromosome 13. The relative position of *Gv1* with respect to the linked markers indicated was determined by using the Map Manager QT program, without taking into account the double-recombinant animals. Map distances (in cM) are indicated on the left. (C) QTL analysis of all 116 backcross animals based on proviral expression levels observed.

*Gv2* must act posttranscriptionally and may play a role in MLV surface protein expression.

One of the major functions of the LTR is to regulate the transcription of retroviral genes by providing signals recognized by cellular transcriptional control machinery. Positive and negative regulatory elements have been identified in the LTR, and many transcription factors are known to bind to the U3 region directly. The factor recognition sequences are usually short and may be repeated several times in each LTR, and the signals themselves have also been shown to have different effects in different cell types (4). Since polytropic and modified polytropic MLVs differ in their responsiveness to *Gv1*, U3 sequences polymorphic between the two classes may therefore be a target for such a *trans*-activating factor. Attempts will be made to identify possible binding sites by using a gel mobility assay; a factor present in the 129 mice but not in the C57BL/6 or BALB/c strains that binds to polytropic-specific U3 probes could be a good candidate for the gene product of *Gv1*.

Based on the map position of *Gv1*, we have attempted to identify possible candidate genes within the interval between *D13Mit13* and *D13Mit231* (28). A number of potential DNA binding factors, for example, a number of zinc-finger-related genes, *Zfp68-rs1*, *Zfp71-rs1*, and *Zfp85-rs1* (21), as well as sequences related to the gene encoding the high-mobility-group chromatin protein, Hmg-1 (25), were identified; however, none provides a compelling candidate for *Gv1*. Possibly more interesting are two genes with *trans* effects, *mdac*, modifier of *Dac*, (15) and *Rsl*, regulator of sex-limited protein, *Slp* (14). The latter case is particularly intriguing because a potential target for *Rsl* is the LTR of an ancient endogenous retrovirus which

imposes androgen control over the expression of *Slp* (27). We are currently refining the map position of *Gv1* with the intention of cloning the gene by using the positional or positional candidate approach (5).

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