JOURNAL OF VIROLOGY, July 1983, p. 24–32
0022-538X/83/070024-09$02.00/0

Vol. 47, No. 1

The High Leukemogenic Potential of Gross Passage A Murine Leukemia Virus Maps in the Region of the Genome Corresponding to the Long Terminal Repeat and to the 3′ End of *env*

LUC DESGROSEILLERS,[1] RICHARD VILLEMUR,[1] AND PAUL JOLICOEUR[1,2]*

Institut de Recherches Cliniques de Montréal, Montreal, Quebec H2W 1R7, Canada,[1] and Département de Microbiologie et d'Immunologie, Université de Montréal, Montreal, Quebec, Canada[2]

Received 4 January 1983/Accepted 28 March 1983

The Gross passage A murine leukemia virus (MuLV) is a highly leukemogenic, ecotropic fibrotropic retrovirus. Its genome is similar to that of other nonleukemogenic ecotropic fibrotropic MuLVs but differs at the 3′ end and in the long terminal repeat. To determine whether these modifications were related to its leukemogenic potential, we constructed a viral DNA recombinant in vitro with cloned infectious DNA from this highly leukemogenic Gross passage A MuLV and from a weakly leukemogenic endogenous BALB/c B-tropic MuLV. Infectious viruses, recovered after microinjection of murine cells with recombinant DNA, were injected into newborn mice. We show here that the Gross passage A 1.35-kilobase-pair *Kpn*I fragment (harboring part of gp70, all of p15E, and the long terminal repeat) is sufficient to confer a high leukemogenic potential to this recombinant.

Several different oncogenes have been identified in defective retroviruses (1). These usually induce malignant diseases with a short latency. The nondefective replication-competent retroviruses usually induce neoplasms after a long latency, and no oncogenes have yet been identified in their genomes. The Gross passage A murine leukemia virus (MuLV) is one of these retroviruses. It is a highly leukemogenic viral preparation which appears to contain a mixture of nondefective ecotropic, xenotropic, and recombinant dualtropic MuLVs (8). One viral isolate, propagated in vitro in NIH cells, has retained its leukemogenic potential (3). This leukemogenic MuLV was ecotropic, N-tropic, fibrotropic, and XC^+. We have recently cloned the genome of this ecotropic, N-tropic MuLV in procaryotic vectors and have shown that the virus recovered after transfection of this infectious DNA is leukemogenic (38). By fingerprint analysis of its RNA genome (2) and by restriction endonuclease mapping of its viral DNA genome (38), major differences were observed within the 3′ end of the genome, as compared with its putative parent, the nonleukemogenic AKR MuLV, suggesting that this region might be involved in the leukemogenic potential of this virus. Similar modifications have been detected in several MuLVs isolated from lymphoid cell lines established from spontaneous AKR thymoma (24, 25). Also, the nondefective murine mink cell focus-inducing (MCF) viruses, which are thought to be involved in the leukemogenic process (5, 8, 13, 22), have been shown to have acquired xenotropic sequences in the *env* gene through recombination (7, 31, 33). Similarly, the *env* gene coding for the gp52 protein of spleen focus-forming virus has been shown to be required for the induction of erythroid cell proliferation (17). In the avian system, studies with in vivo-derived recombinants between oncogenic and nononcogenic nondefective avian leukosis viruses have shown that the U3 long terminal repeat (LTR) determines their oncogenic potential (30, 37).

These studies directly or indirectly suggest that the *env* gene or the LTR or both confer the leukemogenic potential to these viruses. To identify unambiguously the Gross A MuLV sequences responsible for leukemogenicity, we constructed DNA recombinants in vitro with parental DNA from this highly leukemogenic Gross A MuLV and the weakly leukemogenic BALB/c B-tropic MuLV. Infectious viruses were recovered from cells microinjected with recombinant DNA, and their leukemogenic potentials were tested in mice. We present here our results obtained with one recombinant DNA. We show that the Gross A 1.35-kilobase-pair (kbp) *Kpn*I fragment (harboring the 3′ end of *env* and the LTR) is sufficient to confer a high leukemogenic potential to this recombinant.

MATERIALS AND METHODS

Cells and viruses. The origin of the Fv-1$^-$ SC-1 cells has been given elsewhere (12). The infectious viruses recovered after transfection of pGD-17 cloned DNA from Gross passage A MuLV (38) and of λB16 cloned DNA (26) from BALB/c B-tropic (B-Cl-11) MuLV (15) have been described previously. pGD-17 MuLV was propagated in NIH/3T3 cells. B-Cl-11 and λB16 MuLVs were propagated in BALB/3T3 cells, and p5GD-1 MuLV was propagated in SC-1 cells. Cells were grown in Dulbecco modified Eagle medium supplemented with 10% calf serum (GIBCO Laboratories, Grand Island, N.Y.).

Construction of the DNA recombinant. The infectious viral DNA genomes λB16 (26) and λGD17 (38) from BALB/c B-tropic and Gross passage A MuLV, respectively, were described previously. They were subcloned into pBR322 at the *Hin*dIII site and designated pB16-5 and pGD-17, respectively. pB16-5 DNA (10 μg) was partially digested with *Kpn*I (New England Biolabs), and four bands in the range of 10 to 13 kbp were separated on a 1% agarose gel and isolated by electroelution (28). Their structures were characterized by complete digestion with *Kpn*I. Band 2 (11.2 kbp), which was shown to be deleted of its 0.6-kbp LTR and 3'-terminal 1.35-kbp *Kpn*I fragments, was used for ligation. pGD-17 DNA (20 μg) was cleaved with *Kpn*I, and the 1.35-kbp fragment was purified on a 1.4% agarose gel. This fragment was ligated to band 2 (11.2 kbp) of pB16-5 with 20 U of T_4 DNA ligase (New England Biolabs) at 12°C for 16 h in 0.01 ml of 50 mM Tris-hydrochloride (pH 7.5)–10 mM $MgCl_2$–20 mM dithiothreitol–1 mM ATP (ligase buffer) and was used to transform (19) *Escherichia coli* JF1161 (35). Colonies that hybridized with the ^{32}P-labeled (29) 1.35-kbp *Kpn*I fragment were isolated by the method of Grunstein and Hogness (11) and grown. Positive clones were characterized with *Pst*I, *Xba*I, and *Bam*HI to ascertain their correct recombinant structure.

Microinjection procedure. The viral insert of p5GD-1 DNA (2 μg) was excised from pBR322 by digestion with *Hin*dIII (Boehringer Mannheim), phenol and ether extracted, and ligated with T_4 DNA ligase (50 U) in 0.5 ml of the ligase buffer (28). The ligated recombinant viral genome was phenol extracted and ethanol precipitated. DNA was suspended (200 μg/ml) in 1% KCl and microinjected into 500 to 1,000 SC-1 cells as described previously (28). Cells (10^5) were seeded in 60-mm petri dishes 24 h before microinjection. Infectious viruses were recovered in the culture supernatant 10 days later.

DNA sequencing. pGD-17 DNA (250 μg) was digested with *Pst*I and *Bgl*I, and the short 580-bp fragment, separated by 6% polyacrylamide gel electrophoresis, was eluted (20) and further cleaved with *Hin*fI, *Dde*I, *Alu*I, or *Sau*3A. The resulting fragments (0.5 μg) were treated with alkaline phosphatase (Boehringer Mannheim) and labeled at their 5' ends with 600 to 900 μCi of [γ-^{32}P]ATP (New England Nuclear Corp.; 3,000 Ci/mmol) and 20 U of polynucleotide kinase (Bethesda Research Lab) as previously described (20). The same fragments (0.5 μg) were also labeled by filling out the 3' ends with one α-^{32}P-labeled deoxynucleotide triphosphate (New England Nuclear Corp.; 3,000 Ci/mmol) and the Klenow fragment of *Pol*I (9). This labeling reaction was performed in 20 μl of a solution containing 50 mM potassium phosphate (pH 7.4), 5 mM $MgCl_2$, 10 mM dithiothreitol, 50 to 100 μCi of one α-^{32}P-labeled deoxynucleotide triphosphate, and 0.5 U of the Klenow fragment for 20 min at 20°C. End-labeled DNA fragments were cleaved with appropriate restriction endonucleases or were strand separated and then isolated by polyacrylamide gel electrophoresis as previously described (20). The nucleotide sequence was determined by the procedure of Maxam and Gilbert (20).

RESULTS

Characteristics of parental MuLVs used in this study. The main biological characteristics of the parental MuLVs used in this study are summarized in Table 1. Gross A MuLV preparations have been reported to be highly leukemogenic in a variety of mouse strains, inducing leukemia after a relatively short latency (3 to 6 months) (3, 10, 38). We reported that 5×10^4 to 5×10^5 PFU of the endogenous B-tropic BALB/c MuLV (B-Cl-11) has a low leukemogenic potential, inducing leukemia only after a mean latent period of 11 months (15). The molecular characterization of the DNA genomes of these two parental MuLVs has been carried out. A detailed restriction endonuclease map is available for both genomes (26, 27, 38), and the infectious viral DNA genomes of Gross A and B-Cl-11 MuLV have been cloned in procaryotic vectors and

TABLE 1. Biological characteristics of the parental MuLVs used for in vitro constructions

Parental MuLV	Origin	Host range	Fv-1 tropism determinant	XC plaques	Leukemogenicity	Latency (mo)
BALB/c B-tropic	Normal spleen	Ecotropic Fibrotropic Thymotropic	B-tropic	XC$^+$	Weakly leukemogenic[b]	11
Gross passage A	AKR thymoma (serial passages)[a]	Ecotropic Fibrotropic Thymotropic	N-tropic	XC$^+$	Highly leukemogenic[c]	3–6

[a] Reference 10.
[b] Reference 15.
[c] References 3 and 10.

designated, respectively, pGD-17 (38) and λB16 (26) (or pB16-5 after subcloning into pBR322). The virus recovered after transfection with pGD-17 DNA has been shown to be highly leukemogenic (38). The virus recovered after transfection of λB16 DNA appeared weakly leukemogenic, being unable to induce thymomas by 9 months after its intrathymic inoculation into newborn SIM.R mice (Table 2). Intraperitoneal injection of the same virus gives rise to a thymoma in one of eight mice injected after 9 months (unpublished data). Therefore, these two parental MuLVs appear to have a sufficiently distinct leukemogenic potential to use it as a biological marker of each parental genome.

Construction of viral DNA recombinant p5GD-1. To map the region responsible for the higher leukemogenic potential of Gross A MuLV, we constructed recombinants in vitro between these two parental DNA genomes: the N-tropic, thymotropic, highly leukemogenic Gross A MuLV pGD-17 DNA and the B-tropic, thymotropic, weakly leukemogenic B-Cl-11 MuLV pB16-5 DNA. These constructions were facilitated by the fact that they share several identical restriction endonuclease sites (26, 27, 38). One recombinant (p5GD-1) was constructed by inserting the shorter 1.35-kbp *Kpn*I fragment from pGD-17 DNA into a partially *Kpn*I-digested pB16-5 DNA genome (Fig. 1). This short pGD-17 DNA fragment, which essentially covers the LTR, all of p15E, and the carboxy terminus of gp70, harbors significant modifications as compared with the identical region of the AKR MuLV, its putative parent (2, 38). The short Gross A 1.35-kbp *Kpn*I fragment was ligated to the complementary fragment of pB16-5 (linked to pBR322), and the hybrid molecule was cloned into *E. coli*. Several clones were picked and studied by using restriction endonucleases. One clone (p5GD-1) which appeared to have the desired structure was studied further. This cloned recombinant DNA molecule was studied by using restriction endonucleases to ascertain the origin of each fragment and prove its recombinant structure. The absence of the *Xba*I site (Fig. 2, lanes g through k) and the presence of two *Pst*I sites in the LTR, thus generating a 140-bp fragment (Fig. 2, lanes o through q), indicated that the short 1.35-kbp *Kpn*I fragment was derived from the parental pGD-17 DNA. The presence of a larger 1.2-kbp *Bam*HI-*Hin*dIII fragment (1.2 versus 1.15 kbp; Fig. 2, lanes d through f) and of an additional *Pvu*II site in the *env* region, generating a 1.25-kbp fragment (Fig. 2, lanes a through c), indicated that each of the two other possible *Kpn*I fragments (7.3 and 3.9 kbp) was derived from the parental pB16-5 DNA.

Recovery of infectious MuLV from recombinant p5GD-1 DNA. To recover infectious viruses, the 8.2-kbp viral insert of p5GD-1 DNA was first excised with *Hin*dIII and ligated with T_4 DNA ligase to obtain nonpermuted circular viral genomes or linear concatemers. Previous experiments have indeed indicated that these two steps are necessary to achieve infectivity (26, 38). These DNA molecules (200 μg/ml) were then microinjected into SC-1 cells. Infectious viruses were recovered in the culture supernatant 10 days later and titers were determined on NIH/3T3 and BALB/3T3 cells to determine their Fv-1 tropism. p5GD-1 MuLV was B-tropic (data not shown), suggesting that the *gag* region was derived from parental B-tropic pB16-5 DNA. Indeed, we have recently shown (L. DesGroseillers and P. Jolicoeur, manuscript in preparation) that the B-tropic host range of pB16-5 MuLV maps in the *gag* *Bam*H1-*Hin*dIII fragment. This fragment would be included on the 7.3-kbp *Kpn*I fragment made of pBR322 sequences fused with viral sequences (Fig. 1).

Leukemogenicity of p5GD-1 MuLV. To determine the leukemogenic potential of p5GD-1 recombinant MuLV, it was injected, along with

TABLE 2. Biological characterization of p5GD-1 recombinant MuLV

Virus injected[a]	XC (10^4 PFU/ml)[b]	Fv-1 tropism determinant[b]	Mouse strain	No. of mice with thymomas/no. of mice inoculated[c]	Latency (mo)
p5GD-1	2	B-tropic	SIM.R	9/17	6–7
B-C1-11	6	B-tropic	SIM.R	0/5	9
λB16	5	B-tropic	SIM.R	0/8	7
pGD-17[d]	1	N-tropic	SIM.S	5/15	7–8

[a] Recovery of infectious MuLV from λB16 DNA (the viral DNA which was subcloned into pBR322 and designated pB16-5) and from pGD-17 DNA has been described previously (26, 38). Viruses were filtered (0.45-μm membrane filter, type HAWP; Millipore Corp.) before intrathymic (0.05-ml) injection of newborn mice.

[b] Titration was carried out by the XC assay (32) by using NIH/3T3 or BALB/3T3 cells.

[c] Mice were considered positive when the thymus showed an asymmetric enlargement of more than twofold as compared with thymuses from uninoculated mice. In 50% of the mice with thymoma, the spleen was also enlarged two- to fourfold.

[d] Reported from reference 38.

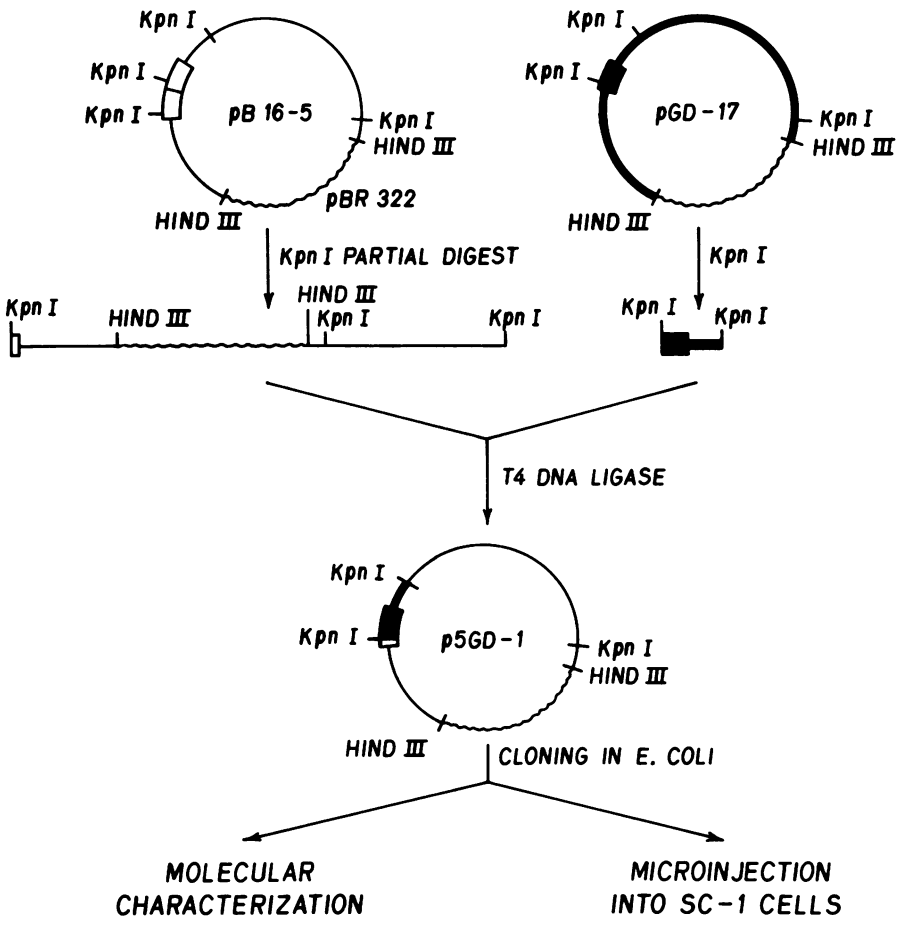

FIG. 1. Construction of the p5GD-1 MuLV recombinant. The 11.2-kbp *Kpn*I fragment obtained from a partial digest of pB16-5 DNA was ligated with the complementary 1.35-kbp *Kpn*I fragment from pGD-17 DNA to generate a complete permutated hybrid viral genome which was amplified in *E. coli*. One clone (p5GD-1) that had the correct recombinant structure was studied further. Symbols: Open and black boxes, LTR; line, pB16-5; thick line, pGD-17; wavy line, pBR322.

appropriate control MuLVs, into the thymus of newborn Fv-1^n (SIM.S) or FV-1^b (SIM.R) permissive mice. SIM.S and SIM.R mice, which are congenic for the Fv-1 locus (39), were used to minimize genetic differences between strains in their susceptibility to leukemia. Mice were observed weekly for signs of disease, and all were sacrificed after 6 to 9 months. A significant percentage of the mice injected with recombinant p5GD-1 MuLV had thymic leukemia after a 6- to 7-month latent period (Table 2). None of the animals injected with parental λB16 or B-Cl-11 MuLV was leukemic after 7 or 9 months, whereas parental Gross A pGD-17 MuLV induced thymomas in 5 of 15 animals injected after the same period. The latent period for the appearance of the disease was longer than usual in this experiment, presumably because of the low titers of our virus stocks. These results indicated that the short Gross A 1.35-kbp *Kpn*I fragment was sufficient to confer a high leukemogenic potential to this recombinant.

Sequence analysis of the 3' end of *env*. Because the 1.35-kbp *Kpn*I fragment seemed to be significant for the higher leukemogenic potential of gross A MuLV, it was of interest to determine which types of modifications could be detected on this segment, as compared with the nonleukemogenic AKR virus, its putative parent. Our previous restriction analysis identified only two changes (the absence of the *Xba*I site and the presence of an additional *Pst*I site) on this fragment (38). We have already reported, however, the sequence of the pGD-17 Gross A LTR and showed that it has point mutations and a 36-bp insertion in the U3 99-bp direct repeat, as compared with its putative parent, the AKR MuLV genome (38). To determine whether

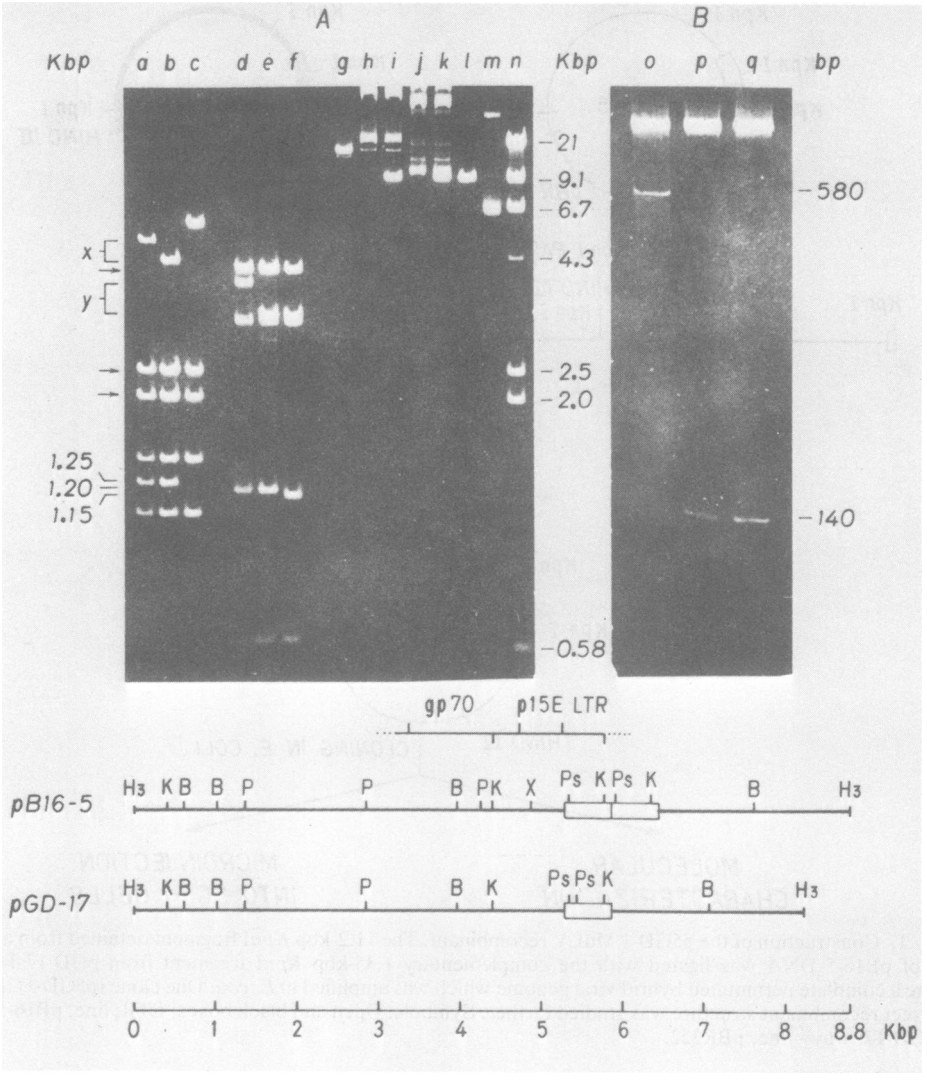

FIG. 2. Molecular characterization of p5GD-1 DNA. Parental pB16-5, pGD-17, and recombinant p5GD-1 DNAs (2 μg each) were digested with restriction endonucleases, and DNA fragments were separated on 1.4% agarose (A) or 6% polyacrylamide (B) gels, as previously described (20, 27). Bands were stained with ethidium bromide and revealed by UV illumination (26). (A) pB16-5 (lanes a, d, and g), p5GD-1 (lanes b, e, h, and i), and pGD-17 (lanes c, f, j, and k) DNAs cleaved with *Hin*dIII-*Pvu*II (lanes a, b, and c), *Hin*dIII-*Bam*HI (lanes d, e, and f), and *Xba*I (lanes g through k). Plasmid pPy2B1A (containing one *Xba*I site) was added into the *Xba*I reaction tubes (lanes i and k) to monitor complete digestion in this assay. *Xba*I-digested (lane l) and undigested (lane m) pPy2B1A DNA are also shown. *Hin*dIII-digested λ DNA (lane n). (B) *Pst*I digestions of pB16-5 (lane o), p5GD-1 (lane p), and pGD-17 (lane q) DNAs. The larger and the smaller x and y fragments contain two copies and one copy of LTR, respectively. The arrows indicate pBR322 fragments. B, *Bam*HI; H3, *Hin*dIII; K, *Kpn*I; P, *Pvu*II; Ps, *Pst*I.

these modifications on the LTR could account by themselves for its high leukemogenic potential or whether additional modifications could be detected in the other part of the 1.35-kbp *Kpn*I pGD-17 DNA fragment, we sequenced the region adjacent to the LTR by the Maxam and Gilbert technique (20) (Fig. 3). As shown, eight point mutations could be detected in this region, as compared with the AKR MuLV sequence (16), and six of them (at positions 41, 85, 403, 423, 486, and 495) generated codons for different amino acids, thus mutating the p15E protein. The two other mutations (positions 78 and 221) did not affect the amino acids coded. By comparison with the corresponding sequence of the leukemogenic Moloney MuLV (34), the point

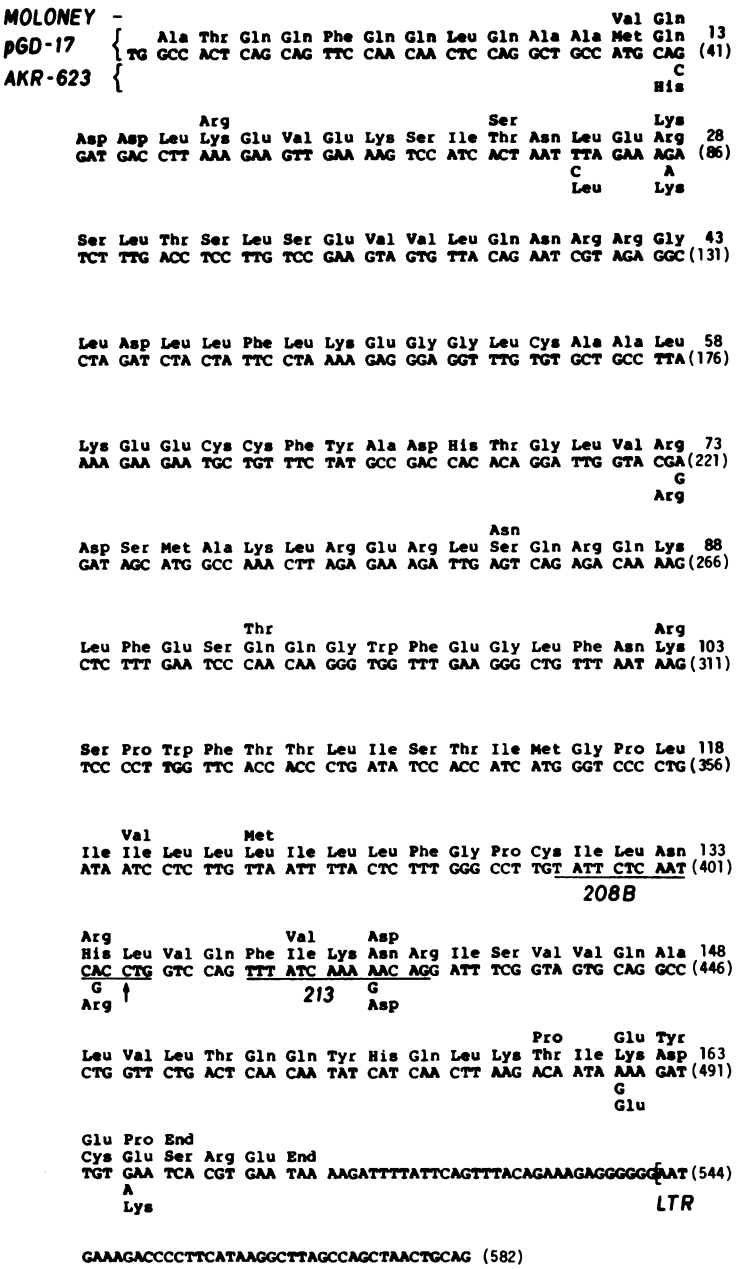

FIG. 3. Nucleotide sequence of the 3′ end of Gross passage A pGD-17 DNA. Base changes and amino acid differences of the same region of the putative parental nonleukemogenic AKR MuLV (clone 623) previously sequenced (16) are presented for comparison. Amino acid differences with p15E of leukemogenic Moloney MuLV (34) are also shown. All amino acids of p15E from AKR and Moloney MuLVs and all bases of AKR MuLV which are not indicated are identical to those of Gross passage A MuLV (pGD-17). The C at position 405 (arrow) was previously sequenced in T_1 oligonucleotide 208B as an A (2). This band was lost on the sequencing gel, suggesting the presence of a methyl group (20). G was present at this position on the opposite strand. The position of T_1 oligonucleotides 208B and 213, previously sequenced (2), is shown.

mutation at position 41 is the most likely to be biologically significant, with both the Gross A and Moloney p15E being similar at this site (*gln*) and different from the AKR MuLV (*his*). At four other positions (85, 403, 423, and 486), the amino acids differ between AKR MuLV and Gross A MuLV, but they are identical between AKR MuLV and Moloney MuLV, suggesting they might not be involved in leukemogenicity.

DISCUSSION

Using molecular cloning techniques, we have shown that the sequences of Gross passage A MuLV located within the 3′ end 1.35-kbp *Kpn*I fragment confers a high leukemogenic potential to this virus. The mutated p15E protein or the rearrangement in the U3 LTR (38) or both could account for this high leukemogenic potential. U5, R, and the right part of U3 LTR cannot be responsible for this effect, since the pGD-17 LTR sequence (38) and the pB16-5 LTR sequence that we have recently obtained (L. Des-Groseillers, E. Rassart, and P. Jolicoeur, Proc. Natl. Acad. Sci. U.S.A., in press), are identical in U5, R, and part of U3. A sequence of about 345 bp, coding for the carboxy terminus of gp70, could also harbor this leukemogenic potential. At least four amino acid mutations (corresponding to T_1 oligonucleotides 207, 211, and 205) have previously been described for this portion of Gross A gp70 as compared with the AKR MuLV gp70 (2, 16).

Previous studies have reported a strong correlation between the leukemogenicity of several SL ecotropic AKR MuLVs and the presence of the T_1 oligonucleotide 36B in U3 LTR (2, 24, 25). This oligonucleotide was always associated with p15E oligonucleotide 208B in leukemogenic viral isolates (24), suggesting a role for this protein in the leukemogenic process. The correlation between leukemogenic potential and the presence of ecotropism-specific sequences in p15E in various AKR MCF MuLVs also suggests a role for this protein in leukemogenicity (4). In a recent study, Lung et al. (18) have provided additional information on the importance of p15E ecotropic sequences in determining the leukemogenic potential of AKR MCF MuLVs. The isolation from AKR/J mouse preleukemic thymuses of several ecotropic MuLVs which had recombined within p15E and U3 LTR has also stressed the biological importance of this region (36). Studies with in vivo-derived recombinants between oncogenic and nononcogenic avian leukosis viruses also indicate that U3 LTR determines their oncogenic potential (37), although more recent evidence suggests that another region might contribute to it (30). We do not know yet which of the 3′ end of *env* or U3 LTR sequences is necessary and sufficient to confer the leukemogenic potential. If the U3 LTR alone is sufficient for leukemogenicity, then it could be through an "enhancer" effect on transcription of adjacent cellular oncogenes as shown with avian leukosis virus (14, 21, 23). It could also facilitate viral replication in target thymocytes through its rearranged 99-bp direct repeat (38), as we have recently found for Gross passage A and for another BALB/c MuLV (Des-Groseillers et al., in press). A close correlation between thymotropism and leukemogenicity of MuLV has indeed been reported (5, 6).

If the leukemogenic potential is conferred by other regions of the genome, either by themselves or in association with U3 LTR, our results would suggest that the 3′ end of *env* is a likely candidate. Other regions in *gag* and *pol* or in *env* might be important, however, as suggested earlier (17, 24, 28), and as found with avian retroviruses (30). If so, the choice of the weakly leukemogenic B-tropic BALB/c MuLV as one of the parents in the construction of p5GD-1 MuLV was fortunate, since these sequences might be present on this parental pB16-5 genome and absent in nonleukemogenic N-tropic AKR or BALB/c MuLV. Other recombinants are being constructed to test this hypothesis.

The mechanism by which this *env*-U3 region contributes to the leukemogenic potential of Gross A MuLV remains unclear. This region might induce transformation directly by its integration adjacent to an oncogene (14, 21, 23) or by a specific activity of p15E in the transformation process. Alternatively, this region might somehow promote recombination with endogenous sequences more efficiently to generate recombinant viruses, possibly MCF MuLVs, which could be the actual leukemogenic agents. Indeed, it has recently been shown that the development of thymic leukemia after injection of Gross passage A MuLV preparations is accompanied by the expression of recombinant MCF-type MuLVs and of ecotropic MuLVs (8). The recombinant MCF MuLVs that were isolated were found to be leukemogenic. The isolated ecotropic MuLVs were also leukemogenic, but MCF-type pr-*env* protein could be detected in the thymomas induced by them, suggesting that the leukemogenic potential of ecotropic MuLVs might be related to their ability to generate recombinant MuLVs. We are currently testing the thymomas generated by p5GD-1 MuLV for the presence of MCF MuLV footprints. The approach described here should help to determine which sequences of the ecotropic Gross passage A MuLV genome are necessary and sufficient to generate recombinant MCF MuLV. This approach should also help to elucidate whether ecotropic Gross passage A MuLV in-

duces leukemia directly or through its ability to generate recombinant MCF MuLVs.

ACKNOWLEDGMENTS

We are grateful to Marylin Smith for helpful comments on the manuscript.

L.D.G. and R.V. are recipients of a fellowship from the Medical Research Council of Canada and the Fonds de la Recherche en Santé du Québec, respectively. This work was supported by grants from the Medical Research Council of Canada and from the National Cancer Institute of Canada to P.J.

ADDENDUM IN PROOF

We constructed another recombinant (designated p7GD-1) with the short 3'-end 1.35-kbp *Kpn*I fragment from pGD-17 DNA and the long complementary 11.2-kbp *Kpn*I fragment from pN20-7 DNA, a cloned infectious viral genome from the nonleukemogenic BALB/c N-tropic MuLV (DesGroseillers et al., in press). The virus recovered from this recombinant DNA was also leukemogenic, suggesting that the p5GD-1 sequences derived from the weakly leukemogenic pB16-5 genome are unlikely to contribute to the leukemogenic potential of this recombinant.

LITERATURE CITED

1. **Bishop, J. M., and H. E. Varmus.** 1982. Functions and origins of retroviral transforming genes, p. 999–1108. *In* R. A. Weiss et al. (ed.), RNA tumor viruses, molecular biology of tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. **Buchhagen, D. L., F. S. Pedersen, R. L. Crowther, and W. A. Haseltine.** 1980. Most sequence differences between the genomes of the AKV virus and a leukemogenic Gross A virus passaged *in vitro* are located near the 3' terminus. Proc. Natl. Acad. Sci. U.S.A. **77**:4359–4363.
3. **Buchhagen, D. L., T. Pincus, O. Stutman, and E. Fleissner.** 1976. Leukemogenic activity of murine type C viruses after long-term passage *in vitro*. Int. J. Cancer **18**:835–842.
4. **Chattopadhyay, S. K., M. W. Cloyd, D. L. Linemeyer, M. R. Lander, E. Rands, and D. R. Lowy.** 1982. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. Nature (London) **295**:25–31.
5. **Cloyd, M. W., J. W. Hartly, and W. P. Rowe.** 1980. Lymphomagenicity of recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **151**:542–552.
6. **Decleve, 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.
7. **Elder, J. H., 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.
8. **Famulari, N. G., C. F. Koehne, and P. V. O'Donnell.** 1982. Leukemogenesis by Gross passage A murine leukemia virus: expression of viruses with recombinant *env* genes in transformed cells. Proc. Natl. Acad. Sci. U.S.A. **79**:3872–3876.
9. **Friedman, T., P. Laporte, and A. Estey.** 1978. Nucleotide sequence studies of polyoma DNA. J. Biol. Chem. **253**:6561–6567.
10. **Gross, L.** 1970. Oncogenic viruses. Pergamon Press, Inc., Elmsford, N.Y.
11. **Grunstein, M., and D. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. U.S.A. **72**:3961–3965.
12. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology **65**:128–134.
13. **Hartley, J. W., N. R. 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.
14. **Hayward, W. S., B. F. Neel, and S. M. Astrin.** 1981. Activation of a cellular *onc* gene by promotor insertion in ALV-induced lymphoid leukosis. Nature (London) **290**:475–480.
15. **Jolicoeur, P., N. Rosenberg, A. Cotellessa, and D. Baltimore.** 1978. Leukemogenicity of clonal isolates of murine leukemia viruses. J. Natl. Cancer Inst. **60**:1473–1476.
16. **Lenz, J., R. Crowther, A. Straceski, and W. Haseltine.** 1982. Nucleotide sequence of the Akv *env* gene. J. Virol. **42**:519–529.
17. **Linemeyer, D. L., J. G. Menke, S. K. Ruscetti, L. H. Evans, and E. M. Scolnick.** 1982. Envelope gene sequences which encode the gp52 protein of spleen focus-forming virus are required for the induction of erythroid cell proliferation. J. Virol. **43**:223–233.
18. **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.
19. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **73**:453–471.
20. **Maxam, A., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
21. **Neel, B. G., W. S. Hayward, H. L. Robinson, J. Fang, and S. M. Astrin.** 1981. Avian leukosis virus-induced tumors have common proviral integration sites and synthesize discrete new RNAs: oncogenesis by promotor insertion. Cell **23**:323–334.
22. **O'Donnell, P. V., E. Stockert, Y. Obata, and L. J. Old.** 1981. Leukemogenic properties of AKR dualtropic (MCF) viruses: amplification of murine leukemia virus related antigens on thymocytes and acceleration of leukemia development in AKR mice. Virology **112**:548–563.
23. **Payne, G. S., J. M. Bishop, and H. E. Varmus.** 1982. Multiple arrangements of viral DNA and an activated host oncogene in bursal lymphomas. Nature (London) **295**:209–214.
24. **Pedersen, F. S., R. L. Crowther, E. F. Hays, R. C. Nowinski, and W. A. Haseltine.** 1982. Structure of retroviral RNAs produced by cell lines derived from spontaneous lymphomas of AKR mice. J. Virol. **41**:18–29.
25. **Pedersen, F. S., R. L. Crowther, D. Y. Tenney, A. M. Reimold, and W. A. Haseltine.** 1981. Novel leukaemogenic retroviruses isolated from cell line derived from spontaneous AKR tumour. Nature (London) **292**:167–170.
26. **Rassart, E., L. DesGroseillers, and P. Jolicoeur.** 1981. Molecular cloning of B- and N-tropic endogenous BALB/c murine leukemia virus circular DNA intermediates: isolation and characterization of infectious recombinant clones. J. Virol. **39**:162–171.
27. **Rassart, E., and P. Jolicoeur.** 1980. Restriction endonuclease mapping of unintegrated viral DNA of B- and N-tropic BALB/c murine leukemia virus. J. Virol. **35**:812–823.
28. **Rassart, E., P. Sankar-Mistry, G. Lemay, L. DesGroseillers, and P. Jolicoeur.** 1983. New class of leukemogenic ecotropic recombinant murine leukemia virus isolated from radiation-induced thymomas of C57BL/6 mice. J. Virol. **45**:565–575.
29. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–257.
30. **Robinson, H. L., 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.
31. **Rommelaere, J., D. V. Faller, and N. Hopkins.** 1978.

Characterization and mapping of RNase T_1-resistant oligonucleotides derived from the genomes of Akv and MCF murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **75**:495–499.

32. **Rowe, W. P., W. E. Pugh, and J. W. Hartley.** 1970. Plaque assay techniques for murine leukemia viruses. Virology **42**:1136–1139.
33. **Shih, T. Y., M. O. Weeks, D. H. Troxler, J. M. Coffin, and E. M. Scolnick.** 1978. Mapping host range-specific oligonucleotides within genomes of the ecotropic and mink cell focus-inducing strains of Moloney murine leukemia virus. J. Virol. **26**:71–83.
34. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukaemia virus. Nature (London) **293**:543–548.
35. **Storms, R. K., E. W. Holowachuck, and J. D. Friesen.** 1981. Genetic complementation of the *Saccharomyces cerevisiae leu2* gene by the *Escherichia coli leuB* gene. Mol. Cell. Biol. **1**:836–842.
36. **Thomas, C. Y., and J. M. Coffin.** 1982. Genetic alterations of RNA leukemia viruses associated with the development of spontaneous thymic leukemia in AKR/J mice. J. Virol. **43**:416–426.
37. **Tsichlis, P. N., and J. M. Coffin.** 1979. Role of the C region in relative growth rates of endogenous and exogenous avian oncoviruses. Cold Spring Harbor Symp. Quant. Biol. **44**:1123–1132.
38. **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.
39. **Ware, L. M., and A. A. Axelrad.** 1972. Inherited resistance to N- and B-tropic murine leukemia viruses *in vitro*: evidence that congenic mouse strains SIM and SIM.R differ at the Fv-1 locus. Virology **50**:339–348.