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Abelson murine leukemia virus: Molecular cloning of infectious integrated proviral DNA

(recombinant DNA/bacteriophage λ/restriction endonuclease mapping/infectivity)

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ABSTRACT **The integrated proviral genome of Abelson murine leukemia virus (A-MuLV) was cloned in λgt*WES*·λB bacteriophage after *Eco*RI endonuclease digestion and enrichment of proviral sequences by sequential RPC-5 column chromatography and agarose gel electrophoresis. Recombinant DNA clones containing a 7.8-kilobase-pair *Eco*RI insert were shown to have the entire integrated A-MuLV genome with both 5′ and 3′ ends flanked by mink cellular DNA sequences. This DNA fragment was shown to induce focus transformation upon transfection of NIH/3T3 mouse cells. Moreover, focus-forming virus could be rescued from transformed nonproducer cells upon superinfection with a type C helper virus. A polyprotein of molecular weight 120,000 (p120) containing murine leukemia virus *gag* gene determinants was invariably detected by immunoprecipitation analysis of individual transformants induced by the 7.8-kilobase-pair DNA. Molecularly cloned integrated A-MuLV in its infectious form should be of use in elucidating the mechanisms involved in transformation by this virus.**

Abelson murine leukemia virus (A-MuLV) is a replication-defective transforming mouse type C virus isolated from a lymphoma induced in a prednisolone-treated BALB/c mouse that had been infected with Moloney murine leukemia virus (M-MuLV) (1). Unlike other transforming viruses of mammalian origin that generally induce sarcomas, A-MuLV induces B-cell lymphoid leukemia after inoculation into susceptible murine hosts (2). In tissue culture, A-MuLV has been shown to transform both lymphoid cells (3, 4) and fibroblasts (5). By heteroduplex analysis, the A-MuLV genome has been shown to possess sequences homologous to MuLV at its 5′ and 3′ ends with a unique region of about 3.5 kilobase pairs (kbp) in the center of the molecule (6). Thus, this virus, like a number of other replication-defective transforming viruses, appears to represent a genetic recombinant between a helper leukemia virus and cellular DNA.

In an effort to elucidate the mechanism of transformation by A-MuLV, we have attempted to amplify its integrated proviral sequences by taking advantage of recombinant DNA technology to clone a given segment of the mammalian genome (7). We report here the detection, isolation, molecular cloning, and initial characterization of integrated proviral DNA derived from DNA of cells nonproductively transformed by A-MuLV.

MATERIALS AND METHODS

Cells and Viruses. The continuous NIH/3T3 mouse (8) and mink lung (Mv1Lu) cell lines (American Type Culture Collection) were grown respectively in Dulbecco's modified Eagle's medium and RPMI 1640 medium supplemented with 10% calf serum (Colorado Serum, Denver, CO). A-MuLV nonproducer transformants of NIH/3T3 (5) and Mv1Lu (9) were provided by C. Sher (Harvard University) and J. Stephenson (National Cancer Institute). A-MuLV stocks were obtained from tissue culture fluids of NIH/3T3 nonproducer cells superinfected with Rauscher murine leukemia virus (R-MuLV) or M-MuLV.

Enzymes. DNA polymerase of avian myeloblastosis virus was obtained from the Resources and Logistics Program of the National Cancer Institute. Restriction endonucleases were obtained from New England BioLabs or Bethesda Research Laboratories (Rockville, MD), and reaction conditions used were those recommended by the suppliers. Phage T4 ligase was purchased from Bethesda Research Laboratories; T4 polynucleotide kinase and bacterial alkaline phosphatase came from P-L Biochemicals.

Enrichment of A-MuLV Proviral Sequences from Restriction Endonuclease-Cleaved Cellular DNA. High molecular weight DNA was extracted from frozen cell pellets with phenol/*m*-cresol as described by Tiemeier *et al.* (10). DNA was digested with *Eco*RI, extracted with phenol, and precipitated with ethanol. Ten milligrams of *Eco*RI-digested DNA was chromatographed on an RPC-5 column (0.8 × 90 cm) as described (11). The fractionated DNAs were electrophoresed, transferred to nitrocellulose filters according to the method of Southern (12), and hybridized to M-MuLV cDNA. ^{32}P-Labeled M-MuLV cDNA was synthesized by using avian myeloblastosis virus DNA polymerase (reverse transcriptase) (13) and RNA purified from M-MuLV. Fractions that hybridized to the M-MuLV cDNA probe were pooled and applied to a 1% preparative agarose gel and fractionated as described (14). Fractions that hybridized to M-MuLV cDNA were used for molecular cloning.

Molecular Cloning and Identification of A-MuLV Proviral Recombinant Phage. The EK2 certified vector λgt*WES*·λB and host *Escherichia coli* DP50*supF* and *E. coli* strain LE392 were generously provided by J. G. Seidman and L. Enquist (National Institutes of Health). Purification of λgt*WES*·λB arms was performed after digestion of DNA with *Eco*RI as described by Maniatis *et al.* (15) and used for ligation and packaging. Partially purified proviral DNA containing *Eco*RI fragments and vector DNA were adjusted to 30 and 100 μg/ml, respectively, and treated with T4 DNA ligase at 200 units/ml for 18 hr at 4°C. The resultant recombinant DNA was packaged *in vitro* into phage particles (16) and plated onto *E. coli* K-12 DP50*supF*. Plaques containing recombinant phage were located by the plaque filter hybridization technique (17), replated at limiting dilution, and plaque purified until the number of recombinant phage in the population was greater than 95%. All cloning experiments were carried out under P2/EK2 containment conditions (*Federal Register*, Dec. 22, 1978, Part VII).

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Abbreviations: MuLV, murine leukemia virus; A-MuLV, M-MuLV, and R-MuLV, Abelson, Moloney, and Rauscher MuLVs; kbp, kilobase pair(s); LTR, large terminal repeat.

Restriction Endonuclease Mapping. Recombinant phage DNA was prepared as described (18). DNA inserts containing proviral sequences were separated from λgt*WES*·λB arms by *Eco*RI digestion and preparative agarose gel electrophoresis. Restriction endonuclease mapping of insert was carried out by both double digestion analysis and the partial digestion method of Smith and Birnstiel (19).

Transfection with Molecularly Cloned A-MuLV Proviral DNA. Transfections were carried out by the calcium precipitation method of Graham and van der Eb (20) as described (21). NIH/3T3 cells were used as recipients in the transfection assay. Foci were scored at 2–3 weeks. For analysis of virus rescue, individual foci induced at limiting dilution were picked by the cloning cylinder technique and grown up to mass culture, and supernatants were analyzed for A-MuLV focus formation on NIH/3T3 cells after superinfection with M-MuLV as described (22).

Immunoprecipitation Assays. *Antisera.* Goat anti-MuLV p15, p12, and p30 sera were obtained from the Resources and Logistics Program of the National Cancer Institutes. *Cell labeling and immunoprecipitation.* Exponentially growing cultures were labeled with [^{35}S]methionine (560 Ci/mmol) at 0.05 mCi/ml (1 Ci = 3.7×10^{10} becquerels) for 3 hr in Dulbecco's modified Eagle's medium lacking unlabeled methionine. Radiolabeled cells were typed and immunoprecipitated as described (23). Immunoprecipitates were analyzed by linear 6–12% polyacrylamide gels as reported (23).

RESULTS

Enrichment of Cellular DNA Fragments Containing Integrated A-MuLV. DNA isolated from A-MuLV-transformed Mv1Lu cells was digested with restriction endonuclease *Eco*RI. This enzyme had first been shown not to inactivate transforming activity of A-MuLV-transformed Mv1Lu cellular DNA or to cleave within the unintegrated linear form of the A-MuLV genome (data not shown). In order to enrich for proviral DNA-containing fragments, we first fractionated the DNA by RPC-5 column chromatography. When individual fractions were analyzed by analytical agarose gel electrophoresis followed by transfer to nitrocellulose membrane filters and hybridization to M-MuLV cDNA, three regions of hybridization were observed (Fig. 1). Hybridizable bands were estimated as 7.8, 10.2, and 11 kbp in size by using λ DNA cut with *Hin*dIII as molecular weight markers on an analytical agarose gel. Under the same conditions, DNA obtained from control Mv1Lu cells failed to show any detectable hybridizable bands. To further purify and resolve the three bands, those fractions that hybridized to M-MuLV cDNA were pooled and subjected to high resolution preparative gel electrophoresis. Aliquots of each fraction were again electrophoresed in a 1% analytical agarose gel, transferred to nitrocellulose filters, and hybridized to M-MuLV cDNA. As shown in Fig. 1, for the 7.8-kbp fragment, considerable enrichment of DNA was obtained by this approach. Similar purification was achieved for 10.2- and 11-kbp fragments (data not shown).

The enriched proviral DNA fragments were ligated to the arms of λgt*WES*·λB DNA and packaged *in vitro* (16), and the resultant phage particles were cloned in *E. coli* K-12 DP50*sup*F. The sequential procedures used for enriching A-MuLV-related DNA fragments enabled us to identify 1 out of every 6000 plaques by hybridization with M-MuLV cDNA. Twenty clones were picked from agar plates, amplified in DP50*sup*F, and retested by hybridization with M-MuLV cDNA.

Restriction Enzyme Analysis of Recombinant A-MuLV Clones. A-MuLV is known to contain M-MuLV sequences on both 5′ and 3′ ends (6). Thus, we compared the restriction digestion pattern obtained with each fragment with that of M-MuLV cloned at the *Hin*dIII site from the circular form of the unintegrated DNA molecule (unpublished data). The restriction map shown in Fig. 2 closely corresponds to that previously reported for the M-MuLV genome by Gilboa *et al.* (24). The large terminal repeat (LTR) of M-MuLV contains a series of restriction sites that includes *Sma* I, *Sac* I, *Xba* I, *Kpn* I, and *Ava* I. This constellation of sites was found in two regions of the 7.8-kbp DNA insert, establishing the existence of two M-MuLV LTRs within this fragment as well as their localization. These findings were confirmed by partial sequence analysis (data not shown). The 10.2- and 11.0-kbp fragments lacked this restriction pattern, and were not analyzed in further detail in the present studies.

The orientation of viral sequences within the 7.8-kbp fragment was deduced from analysis of restriction sites known to

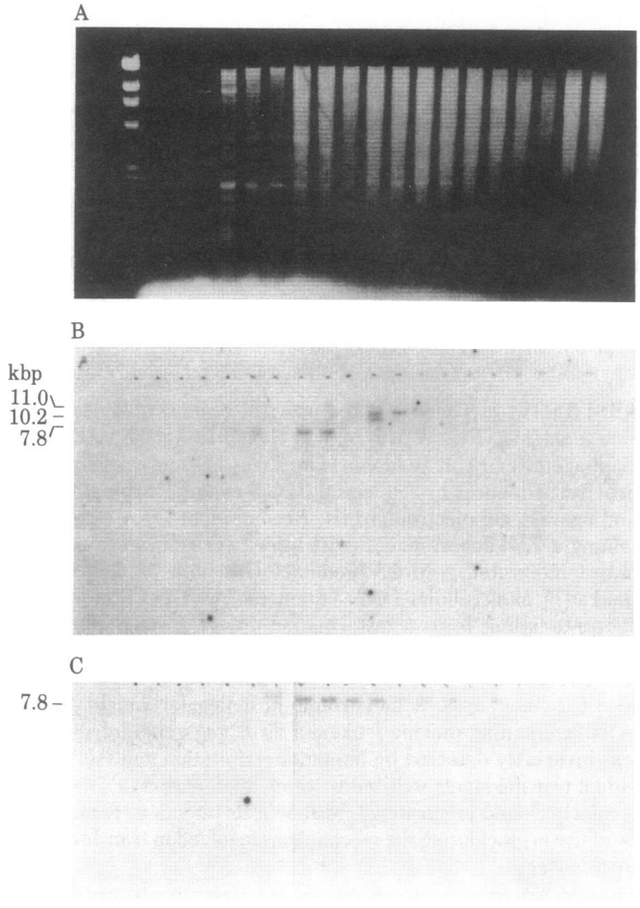

FIG. 1. Detection and enrichment of integrated A-MuLV proviral DNA sequences. DNA from A-MuLV-transformed mink cells was digested with *Eco*RI and fractionated by RPC-5 chromatography. Individual fractions were analyzed on 0.7% agarose gels. After electrophoresis the DNA was transferred to a nitrocellulose filter and hybridized to ^{32}P-labeled cDNA of M-MuLV. Fractions that hybridized to the radioactive probe were pooled and further fractionated by preparative electrophoresis. Small samples of individual fractions were again analyzed on 0.7% agarose gels, transferred to nitrocellulose filters, and hybridized again to M-MuLV cDNA probe. (*A*) Ethidium bromide-stained gel of RPC-5 column fractions. The left lane is molecular weight markers. (*B*) Autoradiogram of RPC-5-fractionated DNA of *A* transferred to nitrocellulose filter and hybridized to [^{32}P]cDNA of M-MuLV. (*C*) Autoradiogram of preparative gel electrophoresis fractions. RPC-5 fractions 7 and 8 (containing the 7.8-kbp hybridizable fragment) were pooled and applied to a preparative gel. Individual fractions were analyzed on a 0.7% agarose gel, transferred to nitrocellulose filters, and hybridized to [^{32}P]cDNA of M-MuLV.

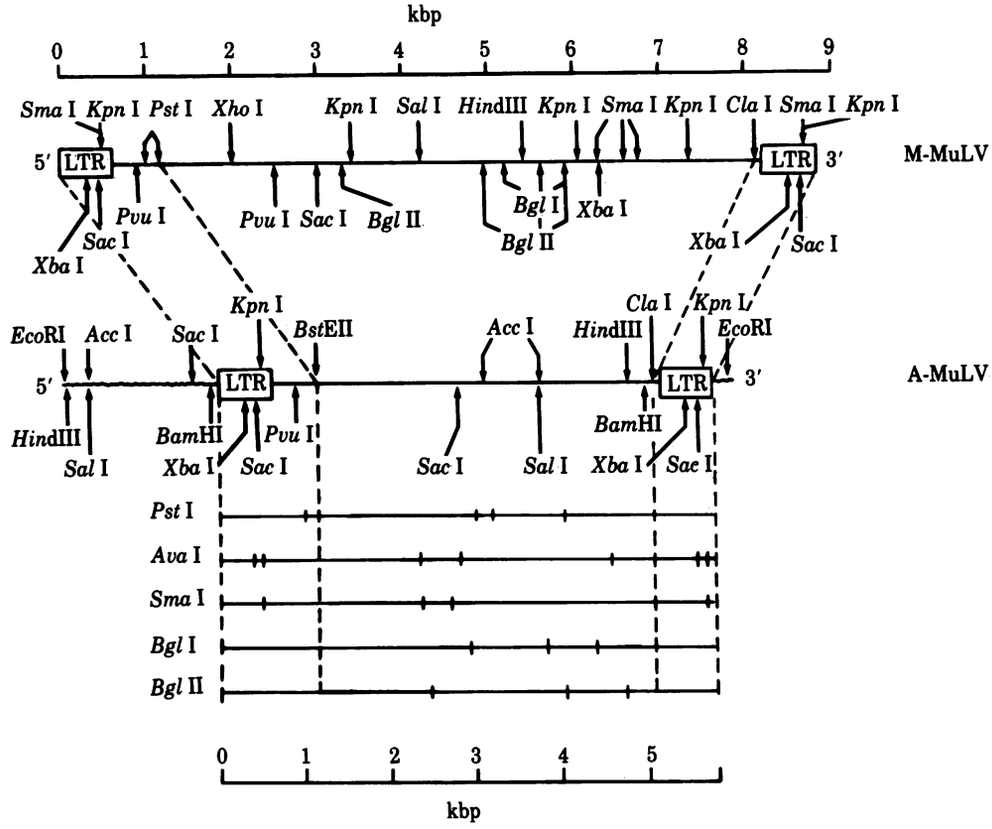

FIG. 2. Restriction endonuclease map of A-MuLV proviral DNA derived from λAM-1 containing a 7.8-kbp *Eco*RI insert. Flanking host cellular DNA sequences are represented by wavy lines, and the large terminal repeats (LTRs) are indicated as rectangles. For comparison, the restriction map of M-MuLV (unpublished data; ref. 24) is also included.

occur exclusively toward the 5′ end of the M-MuLV genome. Thus, a *Pvu* I site, which occurs 0.9 kbp from the 5′ terminus, and two *Pst* I sites, localized 1.0 and 1.15 kbp from the 5′ terminus, were found in an identical configuration within the 7.8-kbp fragment, 2.7, 2.8, and 2.95 kbp, respectively, from one end. These results oriented that end as containing the 5′ terminus of the viral genome (Fig. 2). From the location of the two LTRs, it was determined that proviral DNA was flanked by 1.8- and a 0.2-kbp of mink cellular sequences at the 5′ end and 3′ ends, respectively (Fig. 2).

In an effort to determine the respective lengths of M-MuLV sequences present at the 5′ and 3′ termini of the integrated proviral genome, we utilized a variety of enzymes known to cleave M-MuLV in these regions. To the right of the *Pst* I sites, *Xho* I, *Sal* I, and *Bgl* II sites present within M-MuLV DNA were not detected. Moreover, new restriction sites, including those of *Bgl* II, *Sma* I, and *Pst* I, were observed (Fig. 2). Thus, restriction analysis helped to define the extent of M-MuLV sequences at the 5′ end of the A-MuLV genome as between 1.15 and 1.3 kbp from the 5′ end. At the 3′ terminus of the integrated proviral genome, there was no matching of restriction sites to the left of the *Cla* I site, which is located 100 bases from the 3′ LTR. Furthermore, new sites, including those of *Hin*dIII, *Bam*HI, and *Bgl* II, were found within 0.2 kbp of the LTR. These findings argued that only a limited stretch of about 100–200 base pairs of helper viral sequences at the 3′ terminus was present within the A-MuLV proviral genome.

Biologic Activity of the Molecularly Cloned Integrated Form of A-MuLV. In order to further examine the nature of the DNA clones obtained, we compared their biologic activities in a transfection assay utilizing NIH/3T3 cells. As shown in Table 1, the 7.8-kbp DNA clone (λAM-1) was infectious after *Eco*RI digestion and purification and also as the uncut form containing the λ arms. Moreover, in each case focus formation was one-hit, indicating the capacity of a single DNA molecule to induce transformation. The morphology of foci induced by the 7.8-kbp DNA fragment was indistinguishable from that induced by A-MuLV.

A property of replication-defective transforming oncoviruses is the ability to be rescued by a replication-competent type C

Table 1. Biologic activity of cloned 7.8-kbp *Eco*RI DNA fragment containing A-MuLV proviral genome

Recombinant λAM-1	Foci per plate from indicated amounts of added DNA (μg per plate) 3.0	0.3	0.03	0.003	Infectivity, focus-forming units/μg	Transformants with rescuable A-MuLV,* no. rescuable/no. tested
Uncut	>100	30, 45	3, 8	0, 0	$10^{2.2}$	8/8
*Eco*RI-digested	>100	30, 50	4, 12	0, 1	$10^{2.5}$	7/7
Purified insert	—	TMTC	80, 100	9, 15	$10^{3.5}$	5/5

TMTC, too many to count.

* Individual transformed foci were isolated at limiting dilution, grown up to mass culture, and superinfected with M-MuLV. At 2 weeks, tissue culture fluids were assayed for rescued A-MuLV on NIH/3T3 cells.

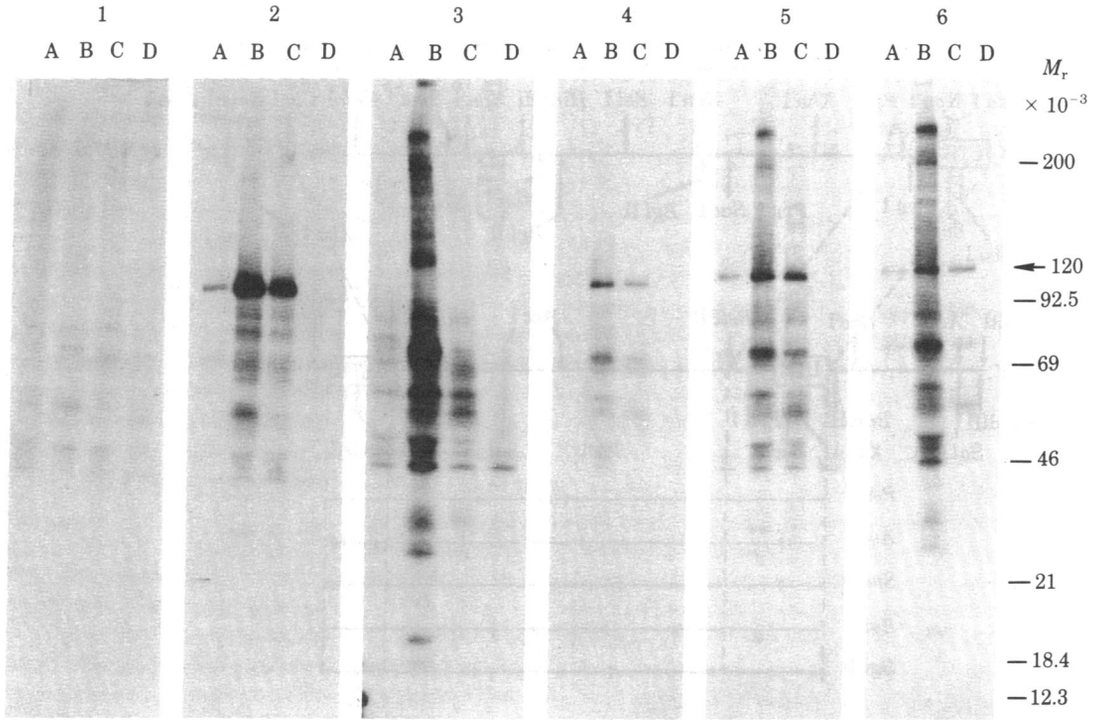

FIG. 3. Electrophoretic analysis of immunoprecipitates of labeled cell extracts after precipitation with antiviral antisera. Cells were incubated for 3 hr with [^{35}S]methionine and the cytoplasmic extracts were treated with: lanes A, goat anti-MuLV (BALB virus 2); lanes B, goat anti-disrupted M-MuLV; lanes C, goat anti-purified M-MuLV p15; and lanes D, normal goat serum. The immune complexes were precipitated by the addition of staphylococcal protein A-Sepharose, washed, dissolved in 2% sodium dodecyl sulfate containing 0.1 M dithiothreitol at 90°C, and subjected to electrophoretic analysis on 6–12% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. The gels were prepared as described (23). ^{14}C-Labeled proteins were used as molecular weight markers. Gel 1, uninfected mink lung cells; gel 2, mink lung cells nonproductively infected with A-MuLV; gel 3, control NIH/3T3 cells; gels 4, 5, and 6, NIH/3T3 cells transformed by molecularly cloned 7.8-kbp cloned DNA fragments. The three cell lines represent cells grown from three different foci.

helper virus. To examine whether transformants induced by the 7.8-kbp DNA fragment contained rescuable A-MuLV, we isolated individual foci induced at limiting DNA dilution. None of the transformants released detectable virus as measured by reverse transcriptase assay of 50-fold concentrates of tissue culture fluids. However, after superinfection with either M-MuLV or R-MuLV, high-titered focus-forming activity ranging from $10^{4.0}$ to $10^{5.0}$ focus-forming units/ml was released from all tested transformants (Table 1).

A-MuLV Expression by Cloned DNA Transformants. A-MuLV has been shown to code for expression of a M_r 120,000 protein (p120), which contains MuLV *gag* gene products p15, p12, and a small portion of p30, as well as a region unrelated to known MuLV proteins (2). The latter is presumed to be encoded by the cell-derived insertion sequence of A-MuLV (2). We examined transformants induced by the 7.8-kbp cloned DNA fragment for expression of MuLV-related gene products by immunoprecipitation analysis. As shown in Fig. 3, sodium dodecyl sulfate/polyacrylamide gel electrophoretic analysis of [^{35}S]methionine-labeled cell extracts of an A-MuLV nonproducer mink cell line revealed a M_r 120,000 protein (p120) precipitable with anti-MuLV serum prepared against disrupted MuLV or purified MuLV p15. This protein was not precipitated with normal goat serum, nor was it detected in uninfected mink cells by anti-MuLV antisera. Transformants induced by molecularly cloned 7.8-kbp DNA were invariably found to contain p120 precipitable by the same antiserum, whereas the control NIH/3T3 cells did not exhibit the synthesis of such a protein (Fig. 3). These results established that p120 expression is associated with transformation by cloned integrated A-MuLV DNA.

DISCUSSION

A-MuLV is the only known mammalian transforming oncovirus with a specificity for transformation of lymphoid cells. Thus, this virus system offers a unique opportunity to study the effects of a transforming virus on a differentiating mammalian cell population. The advent of molecular cloning techniques has recently made it possible to amplify the unintegrated and integrated proviruses of RNA tumor viruses and to more readily investigate the structure and functions of their transforming genes at a molecular level (18, 25, 26). In the present report, we have utilized these techniques to purify and amplify the integrated A-MuLV provirus. A 7.8-kbp DNA *Eco*RI restriction fragment cloned from A-MuLV-transformed mink cells was shown to code for a M_r 120,000 protein containing MuLV *gag* gene determinants and presumed to be the A-MuLV transforming gene product (27, 28). Moreover, the cloned DNA was found to possess the ability to transform cells in tissue culture.

The strategy for enrichment of proviral sequences by sequential RPC-5 and agarose gel electrophoresis was similar to that previously utilized for cloning of mouse globin and immunoglobulin genes (7, 10, 11) as well as the integrated Moloney murine sarcoma virus genome (25). The demonstration that approximately 1 out of 6000 plaques contained A-MuLV DNA indicates an enrichment of proviral sequences by a factor of 200-fold over their unique sequence representation within the cellular DNA of the A-MuLV transformant from which these sequences were cloned. Both 10.2- and 11.0-kbp DNA fragments, which were also detected by hybridization with MuLV cDNA in this transformant, were molecularly cloned as well. However, these molecules lacked restriction sites that identi-

fied A-MuLV within them and also failed to demonstrate biologic activity by transfection analysis. Thus, the origin of the 10.2- and 11.0-kbp DNA fragments remains to be determined.

Restriction endonuclease mapping of the biologically active 7.8-kbp DNA fragment revealed several structural features of the cloned integrated A-MuLV provirus. Identification of a constellation of restriction sites, known to exist within the LTR of M-MuLV (24), established the presence of and localization of these structures within this DNA fragment. By comparison of the restriction maps of the 7.8-kbp DNA fragment containing integrated A-MuLV genome with that of M-MuLV, we established that the 5.8-kbp A-MuLV genome contained at least 1.15 kbp of M-MuLV sequences at its 5′ end and 0.75 kbp at its 3′ end. Moreover, approximately 3.6 kbp in the center of the A-MuLV provirus were completely dissimilar from MuLV by restriction mapping. These findings argue strongly that the central portion of the A-MuLV genome contains its cellular insertion sequence, an observation consistent with that obtained from heteroduplex analysis of A-MuLV RNA and M-MuLV cDNA (6). The remaining sequences adjacent to the A-MuLV genome within the 7.8-kbp DNA fragment must represent flanking mink cellular information cloned along with the integrated provirus.

The transforming activity of the integrated A-MuLV genome in transfection studies was comparable to that reported for integrated DNA of murine sarcoma virus (29). Of note, infectious A-MuLV was rescued at high efficiency by superinfection with helper leukemia virus from each DNA transformant analyzed. In contrast, we have found that transformants induced by a permuted form of the unintegrated murine sarcoma virus genome are most frequently not virus rescuable (unpublished observations). This molecule contains an intact transforming region, but its LTRs are located in tandem in the middle of the molecule. Thus, structural integrity of the linear provirus with LTRs at its termini may be necessary for efficient virus rescue.

A-MuLV induces lymphosarcomas *in vivo* but transforms fibroblasts as well as lymphoid cells *in vitro* (2). There is accumulating evidence that p120, its putative transforming gene product, contains a protein kinase activity that specifically phosphorylates tyrosine residues (30). Normal thymocytes have been shown to express a protein that is immunologically cross-reactive with the A-MuLV p120 and to exhibit protein kinase activity as well (2). It is not yet known whether the specificity for transformation of different target cells by A-MuLV is mediated by the same or different virus-coded gene products. The cloning of A-MuLV proviral DNA in its infectious form makes it feasible to utilize site-specific mutagenesis (31) in an effort to generate conditional lethal virus mutants. Such studies may help to establish the important functional domains of the A-MuLV transforming gene product(s) and to determine whether fibroblastic and lymphoid cell transforming functions reside in the same or different virus-coded genes.

Note Added in Proof. Goff *et al.* (32) have recently reported the cloning of permuted forms of the unintegrated A-MuLV genome.

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1. Abelson, H. T. & Rabstein, L. S. (1970) *Cancer Res.* **30,** 2208–2212.
2. Rosenberg, N. & Baltimore, D. (1980) in *Viral Oncology,* ed. Klein, G. (Raven, New York), pp. 187–203.
3. Sklar, M. D., White, B. J. & Rowe, W. P. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4077–4081.
4. Rosenberg, N., Baltimore, D. & Scher, C. D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1932–1936.
5. Scher, C. D. & Siegler, R. (1975) *Nature (London)* **253,** 729–731.
6. Shields, A., Goff, S., Paskind, M., Otto, G. & Baltimore, D. (1979) *Cell* **18,** 955–962.
7. Seidman, J. G., Leder, A., Edgell, M. H., Polsky, F., Tilghman, S. M., Tiemeier, D. C. & Leder, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3881–3885.
8. Jainchill, J. C., Aaronson, S. A. & Todaro, G. J. (1969) *J. Virol.* **4,** 549–553.
9. Sacks, T. L., Hershey, E. J. & Stephenson, J. R. (1979) *Virology* **97,** 231–240.
10. Tiemeier, D. C., Tilghman, S. M. & Leder, P. (1977) *Gene* **2,** 173–191.
11. Tilghman, S. M., Tiemeier, D. C., Polsky, F., Edgell, M. H., Seidman, J. G., Leder, A., Enquist, L. W., Norman, B. & Leder, P. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4406–4410.
12. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
13. Taylor, J. M., Illmensee, R. & Summers, J. (1976) *Biochim. Biophys. Acta* **442,** 324–330.
14. Polsky, F., Edgell, M. H., Seidman, J. G. & Leder, P. (1978) *Anal, Biochem.* **87,** 397–410.
15. Maniatis, T., Hardison, R. C., Lacy, E., Laver, U., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell* **15,** 687–701.
16. Sternberg, N., Tiemeier, D. C. & Enquist, L. (1977) *Gene* **1,** 255–280.
17. Benton, D. & Davis, R. W. (1977) *Science* **196,** 180–182.
18. Tronick, S. R., Robbins, K. C., Canaani, E., Devare, S. G., Andersen, P. R. & Aaronson, S. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6314–6318.
19. Smith, H. O. & Birnstiel, M. L. (1976) *Nucleic Acids Res.* **3,** 2387–2398.
20. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52,** 456–461.
21. Canaani, E., Robbins, K. C. & Aaronson, S. A. (1979) *Nature (London)* **282,** 378–383.
22. Aaronson, S. A. & Rowe, W. P. (1970) *Virology* **42,** 9–19.
23. Barbacid, M., Lauver, A. V. & Devare, S. G. (1980) *J. Virol.* **33,** 196–207.
24. Gilboa, E., Goff, S., Shields, A., Yoshimura, F., Mitra, S. & Baltimore, D. (1979) *Cell* **16,** 863–874.
25. Vande Woude, G. F., Oskarsson, M., Enquist, L. W., Nomura, S., Sullivan, M. & Fischinger, P. J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4464–4468.
26. O'Rear, J. J., Mizutani, S., Hoffman, G., Fiandt, M. & Temin, H. M. (1980) *Cell* **20,** 423–430.
27. Witte, O. N., Rosenberg, N., Paskind, M., Shields, A. & Baltimore, D. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2488–2492.
28. Reynolds, R. K., Van de Ven, W. J. M. & Stephenson, J. R. (1978) *J. Virol.* **28,** 665–670.
29. Blair, D. G., McClements, W. L., Oskarsson, M., Fischinger, P. J. & Vande Woude, G. F. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3504–3508.
30. Witte, O. N., Dasgupta, A. & Baltimore, D. (1980) *Nature (London)* **283,** 826–831.
31. Peden, K. W. C., Pipas, J. M., Pearson-White, S. & Nathans, D. (1980) *Science* **209,** 1392–1396.
32. Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. (1980) *Cell* **22,** 777–785.