JOURNAL OF VIROLOGY, Nov. 1981, p. 431-439
0022-538X/81/110431-09$02.00/0

Vol. 40, No. 2

Structural Organization and Biological Acitivity of Molecular Clones of the Integrated Genome of a BALB/c Mouse Sarcoma Virus

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Received 30 April 1981/Accepted 16 June 1981

BALB/c mouse sarcoma virus (BALB-MSV) is a spontaneously occurring transforming retrovirus of mouse origin. The integrated form of the viral genome was cloned from the DNA of a BALB-MSV-transformed nonproducer NRK cell line in the Charon 9 strain of bacteriophage λ. In transfection assays, the 19-kilobase-pair (kbp) recombinant DNA clone transformed NIH/3T3 mouse cells with an efficiency of 3×10^4 focus-forming units per pmol. Such transformants possessed typical BALB-MSV morphology and released BALB-MSV after helper virus superinfection. A 6.8-kbp DNA segment within the 19-kbp DNA possessed restriction enzyme sites identical to those of the linear BALB-MSV genome. Long terminal repeats of approximately 0.6 kbp were localized at either end of the viral genome by the presence of a repeated constellation of restriction sites and by hybridization of segments containing these sites with nick-translated Moloney murine leukemia virus long terminal repeat DNA. A continuous segment of at least 0.6 and no more than 0.9 kbp of helper virus-unrelated sequences was localized toward the 3′ end of the viral genome in relation to viral RNA. A probe composed of these sequences detected six *Eco*RI-generated DNA bands in normal mouse cell DNA as well as a smaller number of bands in rat and human DNAs. These studies demonstrate that BALB-MSV, like previously characterized avian and mammalian transforming retroviruses, arose by recombination of a type C helper virus with a well-conserved cellular gene.

Transforming retroviruses of mouse origin have been isolated from naturally occurring tumors and from tumors that arose after passage of leukemia viruses in vivo (3, 13, 26, 28). Moloney murine sarcoma virus (M-MSV) and Abelson murine leukemia virus (A-MuLV) have been the most intensively studied of these viruses. The genome of each has been shown to be composed of some information of Moloney murine leukemia virus (M-MuLV). Each also contains a distinct set of sequences which show homology with cellular sequences present at low copy numbers within the normal mouse genome (14, 17, 41, 42). Recent evidence indicates that the cell-derived sequences of M-MSV and A-MuLV are essential for their respective transforming functions (4, 8, 27, 33, 35). Such studies imply that these viruses arose by recombination between a type C helper virus and cellular genes with transforming potential.

Our laboratory has characterized the biological properties of a sarcoma virus, BALB-MSV (2), which was initially isolated from a BALB/c mouse hemangiosarcoma (28). Like other mammalian transforming retroviruses, BALB-MSV is replication defective. The fact that BALB-MSV stocks contain an excess of helper virus has made it difficult to perform detailed biochemical analyses of the BALB-MSV genome. The recent application of recombinant DNA techniques to the study of retroviruses has made it possible to isolate and amplify viral genetic sequences for analyses of their structures and functions. This communication describes the molecular cloning of the integrated form of BALB-MSV and the characterization of its viral genomic structure.

MATERIALS AND METHODS

Cells and viruses. Mouse NIH/3T3 (22) and NRK (11) continuous cell lines were utilized. The isolation of a clonal BALB-MSV nonproducer transformant of NRK cells, BALB-MSV NRK Cl 18, has been described (2). Clonal strains of M-MuLV (25), AKR-MuLV (15), mouse amphotropic virus (16), and simian sarcoma-associated virus (1) were also utilized.

DNA transfection. DNA transfection was performed by the calcium phosphate precipitation method of Graham and van der Eb (18) as previously described (33). Recipient NIH/3T3 cells were used in

the transfection assay. Foci were scored after 2 to 3 weeks. To test for the presence of rescuable sarcoma virus, individual foci induced at a limiting DNA dilution were picked by the cloning cylinder technique, grown up to mass culture, and superinfected with M-MuLV. Tissue culture fluids from productively infected cultures were assayed 2 to 3 weeks later for focus-forming virus on NIH/3T3 cells, as previously described (33).

Enzymes. Avian myeloblastosis virus DNA polymerase was obtained from the Biological Carcinogenesis Branch, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. Restriction endonucleases and T4 ligase were purchased from New England Biolabs and Bethesda Research Laboratories. Reaction conditions were those suggested by the suppliers. Bacterial alkaline phosphatase and T4 polynucleotide kinase were purchased from P. L. Biochemicals.

Isolation of unintegrated BALB-MSV DNA. BALB-MSV NRK Cl 18 nonproducer rat cells were infected with simian sarcoma-associated virus, and the resulting pseudotype virus was used to infect NRK cells. After 18 h, unintegrated viral DNA was prepared by Hirt extraction and CsCl-ethidium bromide (EtBr) centrifugation (8, 20). BALB-MSV proviral DNA was detected by hybridization of its mouse helper virus-related sequences to an AKR-MuLV cDNA probe (32).

Molecular cloning. High-molecular-weight DNA was isolated from BALB-MSV NRK Cl 18 cells by the method of Gross-Bellard et al. (19). Enrichment for DNA fragments containing BALB-MSV sequences was effected by centrifugation of 0.4 mg of *Eco*RI-cleaved DNA through a 5 to 35% linear sucrose density gradient in 0.1 M NaCl–0.1% sodium dodecyl sulfate–0.001 M EDTA–0.01 M Tris, pH 7.5, at 21,000 rpm for 14 h at 20°C in a Beckman SW27 rotor. Fractions (1 ml) were collected, and 80 μl of each was analyzed by agarose gel electrophoresis, Southern blotting, and hybridization to an AKR-MuLV cDNA probe. Fractions containing virus-related sequences were pooled and ligated to *Eco*RI-cleaved Charon 9 DNA (7, 9). Recombinant DNA was packaged into phage particles by the method of Hohn (21). Screening for BALB-MSV-containing phage clones was performed by standard techniques (5). Charon 9 was provided by F. R. Blattner, in vitro packaging cells BHB2688 and BHB2690 were a gift from B. Hohn, and phage host LE392 was obtained from J. Seidman. The phage were propagated and the DNA was purified as previously described (12).

Subcloning of recombinant DNAs into plasmid vectors was performed by using pBR322 or pBR325 (obtained from Bethesda Research Laboratories). Transformation experiments were performed according to the procedure of Taketo (40). Colonies were screened by using appropriate ^{32}P, nick-translated probes (31). Plasmid DNAs were isolated by CsCl-EtBr centrifugation of cleared lysates (24).

Restriction endonuclease mapping. Unintegrated BALB-MSV DNA was analyzed by performing single and double digests with restriction enzymes. Cloned DNA inserts were separated from vector DNA by preparative gel electrophoresis and then isolated by electroelution (23). Double-digestion analysis and the partial-digestion method of Smith and Birnstiel (37) were used for restriction endonuclease mapping of recombinant DNA clones. Virus-related sequences were identified by transferring DNA to nitrocellulose by the Southern technique followed by hybridization to nick-translated cloned DNA probes or to cDNA as previously described (8, 38). Blots were then washed at 62°C in 0.1× SSC (1× SSC = 0.15 M NaCl plus 0.015 M sodium citrate) and autoradiographed with Kodak XR5 film.

RESULTS

Analysis of unintegrated BALB-MSV DNA. The cloning of the integrated BALB-MSV genome required knowledge of restriction enzymes which did not cleave within the proviral DNA. Linear BALB-MSV unintegrated DNA, isolated from the Hirt supernatant of newly infected cells, was used for this analysis. Figure 1 shows the results of restriction endonuclease digestion of the 6.8-kilobase-pair (kbp) linear BALB-MSV DNA. Restriction enzymes which did not cleave the viral DNA included *Eco*RI, *Xho*I, and *Sal*I (Fig. 1, lanes 2, 4, and 5, respectively). *Hin*dIII cut the linear molecule twice (Fig. 1, lane 3), whereas *Xba*I cleaved once, giving bands of 5.8 and 1.0 kbp (Fig. 1, lane 6). *Pst*I reduced the size of the genome to 6.2 kbp (lane 7). The fact that we did not detect a 0.6-kbp band suggested that *Pst*I cut the molecule more than once.

When *Eco*RI-cleaved, high-molecular-weight DNAs from BALB-MSV NRK Cl 18 and uninfected NRK cells were compared by agarose gel electrophoresis and Southern blotting analysis with an AKR-MuLV cDNA probe, we observed a unique 19-kbp DNA band in the nonproducer cells. To confirm that this band represented the integrated BALB-MSV proviral DNA, we isolated it by preparative gel electrophoresis and cleaved it with *Pst*I. Analysis of the products by the Southern technique revealed a 6.2-kbp DNA fragment characteristic of *Pst*I-cleaved BALB-MSV DNA (data not shown). This was taken as further evidence that the 19-kbp band contained the integrated BALB-MSV genome.

Molecular cloning of integrated BALB-MSV provirus. The 19-kbp DNA fragment containing BALB-MSV DNA was enriched approximately 20-fold by sucrose density gradient centrifugation and was ligated to *Eco*RI-cleaved Charon 9 λ DNA. This vector can accept DNA fragments up to 22 kbp in length (9). Approximately 5×10^5 in vitro-packaged phage were screened by hybridization with an AKR-MuLV cDNA probe. The one positive plaque obtained was purified by plating at high dilution. The DNA from this clone, designated λ9EB-18, was

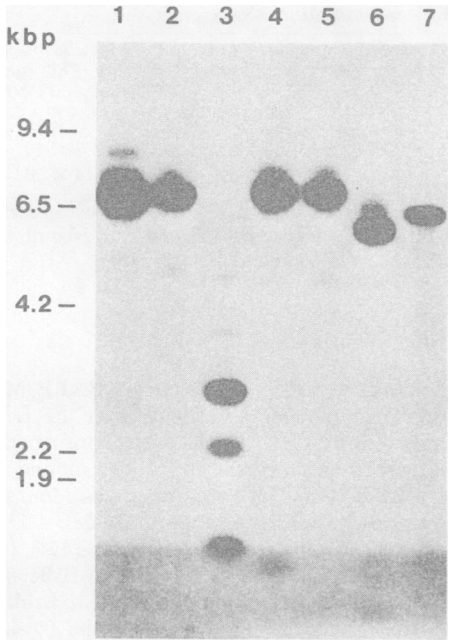

FIG. 1. *Restriction enzyme analysis of BALB-MSV linear DNA. Low-molecular-weight DNA isolated by Hirt extraction of NRK cells acutely infected with the simian sarcoma-associated virus pseudotype of BALB-MSV was fractionated by CsCl-EtBr gradient centrifugation. Fractions containing linear DNA were pooled and digested with various restriction enzymes, electrophoresed through 1.0% agarose, and transferred to nitrocellulose. BALB-MSV DNA was detected by hybridization with ^{32}P-AKR-MuLV cDNA as described in the text. Untreated BALB-MSV linear DNA is shown in lane 1; also shown is BALB-MSV linear DNA after cleavage with EcoRI (lane 2), HindIII (lane 3), XhoI (lane 4), SalI (lane 5), XbaI (lane 6), and PstI (lane 7). Fragments generated by cleavage of λ DNA with HindIII served as molecular weight standards.*

cleaved with *Eco*RI or *Pst*I and subjected to Southern blotting analysis. Respective 19- and 6.2-kbp inserts were observed after hybridization with AKR-MuLV cDNA. This pattern was consistent with results from our preliminary analysis of linear BALB-MSV DNA.

Recombinant phage λ9EB-18 DNA was used to construct subclones of the BALB-MSV insert. These included plasmid clone pE-8, a pBR325 subclone of the entire 19-kbp *Eco*RI insert of λ9EB-18 (Fig. 2, lanes 2 and 4), and plasmid clone pP-7, a pBR325 subclone of the 6.2-kbp *Pst*I fragment of BALB-MSV (Fig. 2, lanes 1 and 3).

Transforming activity of recombinant DNA clones. To determine whether the 19-kbp recombinant DNA clone contained the entire BALB-MSV provirus, we examined the biological activity of this DNA by transfection analysis. NIH/3T3 cells were exposed to various amounts of DNA, and cultures were observed for the appearance of transformed foci. Within 2 to 3 weeks, foci characteristic of parental BALB-MSV were readily observed. The transforming activity of λ9EB-18 DNA was 3×10^4 focus-forming units per pmol of viral DNA insert (Table 1). The specific transforming activity of 19-kbp DNA subcloned in pBR322 was approximately fourfold lower. When individual transformed foci were grown up to mass culture and superinfected with M-MuLV or mouse amphotropic virus, sarcoma virus capable of inducing transformed foci characteristic of BALB-MSV was rescued. These results indicated that the 19-kbp recombinant DNA clone contained information required for BALB-MSV transforming and rescue functions.

We also examined the 6.2-kbp *Pst*I DNA subclone (pP-7) by transfection analysis. The transforming activity of *Pst*I-cleaved plasmid DNA was 4×10^3 FFU of viral DNA per pmol, a level similar to that of the pBR322 subclone of 19-kbp

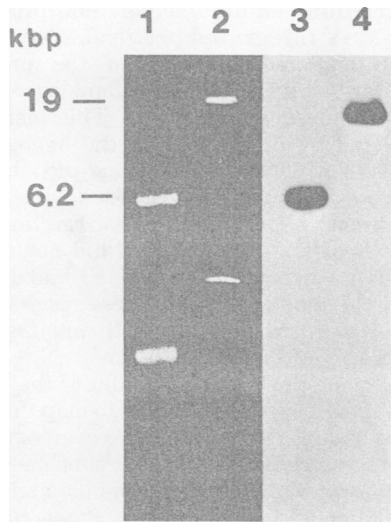

FIG. 2. *Characterization of molecularly cloned BALB-MSV DNA. The integrated form of BALB-MSV DNA cloned in Charon 9 was subcloned in pBR325 by using either PstI (clone pP-7) or EcoRI (clone pE-8). These generated, respectively, 6.2-kbp (lanes 1 and 3) and 19-kbp (lanes 2 and 4) inserts. The resulting recombinant plasmid DNAs were cleaved with the appropriate restriction enzymes, electrophoresed on a 0.7% agarose gel, stained with EtBr (lanes 1 and 2, respectively), and analyzed with ^{32}P-AKR-MuLV cDNA by Southern blotting (lanes 3 and 4, respectively). For aiding in the visualization of the pP-7 insert, plasmid DNAs were digested with SalI. This enzyme cleaves the 5.8-kbp vector but not the BALB-MSV inserts.*

TABLE 1. *Biological activity of BALB-MSV recombinant DNA clones*

Recombinant DNA	Designation	Foci/plate with indicated amt (μg/plate) of DNA[a]				Specific infectivity (FFU[c]/pmol of viral DNA)
		5.0	0.5	0.05[b]	0.005[b]	
19-kbp *Eco*RI λ	λ9EB-18	TMC[d]	>100	15, 60	3, 8	3×10^4
19-kbp *Eco*RI pBR325	pE-8	TMC	>100	15, 40	1, 2	7×10^3
6.2-kbp *Pst*I pBR325	pP-7	TMC	>100	20, 35	2, 4	4×10^3

[a] DNA transfection was performed as described in the text, utilizing salmon sperm DNA (50 μg) as the carrier. Before transfection analysis, recombinant clones λ9EB-18 and pE-8 were cleaved with *Eco*RI, and recombinant clone pP-7 was cleaved with *Pst*I. Focus formation on NIH/3T3 cells was scored at 14 to 21 days.

[b] Results of duplicate samples.

[c] FFU, Focus-forming units.

[d] TMC, Too many to count.

DNA (Table 1). Similar results were obtained when uncleaved recombinant plasmid pP-7 DNA was used. Thus, the 6.2-kbp *Pst*I DNA subclone contained, at a minimum, the region of the viral genome required for transformation. Focus-forming virus could be rescued from cells transformed by either *Pst*I-cleaved or uncleaved pP-7 DNA. In each case, the titer of MSV released was around 100-fold lower than that observed with transformants containing the 19-kbp DNA clone.

Restriction endonuclease mapping of BALB-MSV integrated proviral DNA. A restriction endonuclease map of the proviral BALB-MSV genome, including flanking cellular regions, is presented in Fig. 3. This map was constructed by first analyzing the biologically active 6.2-kbp DNA of *Pst*I clone pP-7 by appropriate single and double digests to determine the positions of restriction sites. Enzymes including *Eco*RI, *Xho*I, and *Sal*I did not cleave pP-7 DNA, whereas *Pvu*I, *Hpa*I, *Sst*I, and *Xba*I cleaved the molecule once. These results were identical to those obtained with unintegrated linear BALB-MSV DNA (Fig. 1).

Since clone pP-7 lacked 0.6 kbp of the linear viral genome, it was not possible to map the sites near the ends of the molecule. To overcome this difficulty, we generated *Hin*dIII subclones that encompassed both the viral genomic and adjacent cellular sequences (Fig. 3). Three *Hin*dIII subgenomic clones, pH-2g, pH-4a, and pH-9c, were oriented to the *Pst*I clone pP-7 on the basis of single and double digests with various restriction enzymes (Fig. 3). Utilizing pH-2g and pH-9c subclones, we could extend the map of viral DNA past the *Pst*I sites and into the adjacent cellular sequences.

Long terminal repeats (LTRs) have been shown to be a common structural feature of the proviral DNAs of avian and mammalian retroviruses (6). *Kpn*I cleaved viral DNA in identical locations with respect to the two *Pst*I sites (Fig. 3). It seemed likely that these two respective 0.4-kbp domains resided within the BALB-MSV LTRs. The presence and location of the LTRs of the integrated BALB-MSV was confirmed by using, as a nick-translated probe, cloned M-MuLV LTR. This DNA fragment was generated by *Kpn*I digestion of a clone of M-MSV DNA containing tandemly arranged LTRs (41). Southern blots of the pH-2g and pH-9c subclones, but not the pH-4a subclone, hybridized to the LTR probe. Furthermore, the 0.4-kbp, *Kpn*I-to-*Pst*I fragment derived from either pH-2g or pH-9c DNA hybridized to this probe.

LTRs of previously studied mouse type C viruses have been shown to be 0.5 to 0.6 kbp (10, 30). When we compared the 6.2-kbp distance between either set of terminal *Kpn*I or *Pst*I sites with the 6.8-kbp length of the linear-integrated BALB-MSV proviral DNA, we reasoned that the length of the BALB-MSV LTR must be approximately 0.6 kbp. *Hin*dIII and *Pvu*I produced asymmetrical cuts at the left side of the integrated proviral DNA, helping further to approximate the positions of the viral LTRs (Fig. 3).

Orientation of integrated BALB-MSV DNA in relation to viral genomic RNA. The gene order of the type C retrovirus is known to be 5′-*gag-pol-env*-3′ (6, 43). It is also known that BALB-MSV codes for the expression of MuLV *gag* gene products (2). Thus, we used a nick-translated probe containing M-MuLV *gag* gene sequences in an effort to orient the BALB-MSV genome in relation to viral RNA. This approach was previously shown to be useful in orienting other retroviral genomes (32). A M-MSV subclone spanning *Xho*I to *Sal*I sites and containing M-MuLV *gag* gene sequences as well as the 5′ portion of the *pol* gene (E. P. Reddy, E. Canaani, S. R. Tronick, and S. A. Aaronson, unpublished data) was hybridized to the three *Hin*dIII subclones. *Hin*dIII subclones pH-2g and pH-4a hybridized to this probe, whereas pH-9C did not (Fig. 4). When the same fragments were hybridized with a nick-translated probe of the entire

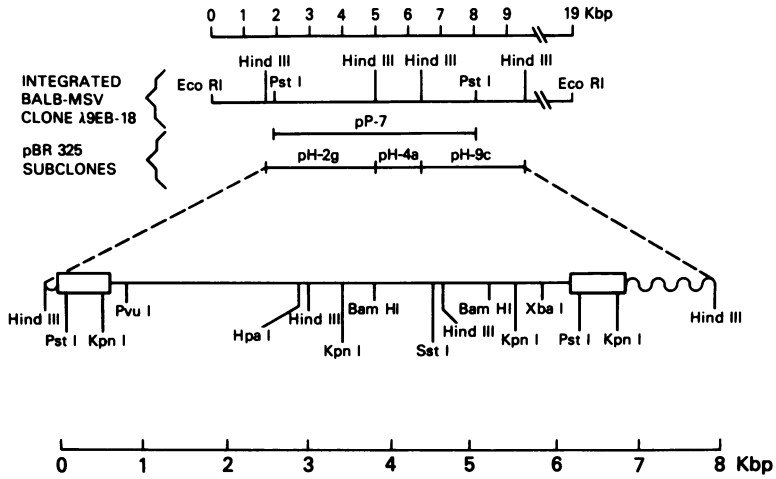

FIG. 3. *Restriction map of the 19-kbp clone containing integrated BALB-MSV. Subcloned DNA fragments used in restriction mapping are as indicated. Open rectangles represent the LTRs, and cellular sequences flanking the viral genome are indicated by wavy lines.*

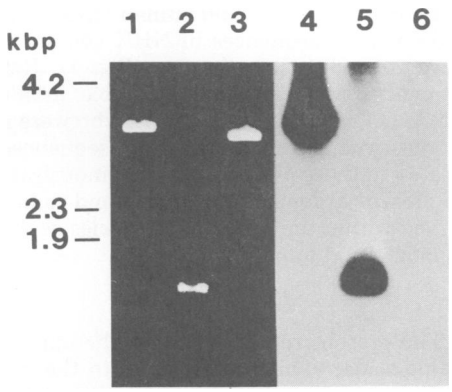

FIG. 4. *Orientation of cloned BALB-MSV DNA in relation to viral genomic RNA. Inserts derived from the three nonoverlapping HindIII subclones of BALB-MSV DNA (see Fig. 3) were subjected to agarose gel electrophoresis and Southern blotting. The Southern blots were hybridized with a cloned DNA probe specific for the 5′ region (XhoI to SalI) of M-MuLV. Clone pH-2g is shown in lanes 1 and 4; clone pH-4a is shown in lanes 2 and 5; and clone pH-9c is shown in lanes 3 and 6. EtBr-stained DNAs are displayed in lanes 1, 2, and 3, and autoradiograms of Southern blots are shown in lanes 4, 5, and 6.*

M-MuLV genome, all fragments were hybridized (data not shown). These results localized MuLV *gag* gene sequences to the left half of the physical map of integrated BALB-MSV, thus orienting the left end as 5′ in relation to BALB-MSV genomic RNA.

Localization of BALB-MSV nucleotide sequences unrelated to MuLV. We next attempted to identify and localize sequences not related to MuLV within the BALB-MSV genome. A variety of fragments representing the entire BALB-MSV genome were produced by digestion of the DNAs of subclones pH-2g, pH-4a, and pH-9C with a battery of restriction enzymes. These fragments were tested for ability to hybridize with nick-translated M-MuLV DNA. All tested restriction fragments of subclones pH-2g and pH-4a hybridized to the M-MuLV DNA probe. With pH-9c, a fragment bounded by *Hind*III and *Bam*HI sites (4.5 to 5.2 kbp on the BALB-MSV map shown in Fig. 3) was the only segment that did not hybridize with this probe (Fig. 5). Since *Sst*I-*Bam*HI (4.4 to 5.2 kbp on the map) and *Bam*HI-*Kpn*I (5.2 to 5.4 kbp on the map) fragments hybridized, the helper virus-unrelated region of BALB MSV must be at least 0.6 and no more than 0.9 kbp in length. The insert of a subgenomic clone (designated clone pHB-1) of the *Hind*III to the *Bam*HI fragment of clone pH-9C was nick translated and was hybridized with M-MuLV DNA; hybridization was not detected (Fig. 6). The close genetic homology among MuLV strains is well established. However, to rule out the possibility that the region defined by the pHB-1 probe was a helper-related sequence present in the B-tropic helper virus but not in M-MuLV DNA, we hybridized 35S B-tropic BALB helper virus RNA to nick-translated pHB-1 DNA after Northern blotting. No detectable hybridization was observed under stringent or relaxed annealing and washing conditions, even after very long exposures. These results confirm the lack of homology between MuLV's and the *Hind*III-*Bam*HI region of BALB-MSV.

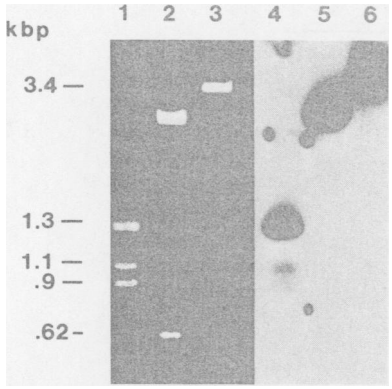

FIG. 5. *Demonstration of helper virus-unrelated sequences within the BALB-MSV genome. For localizing the region of the BALB-MSV genome unrelated to helper viral DNA, the insert of the subgenomic clone pH-9c (lanes 3 and 6) and its KpnI (lanes 1 and 4) and BamHI (lanes 2 and 5) digests were electrophoresed on agarose gels and stained with EtBr (lanes 1, 2, and 3). DNA was then transferred to a nitrocellulose filter, hybridized to ^{32}P-labeled M-MuLV DNA, and autoradiographed (lanes 4, 5, and 6).*

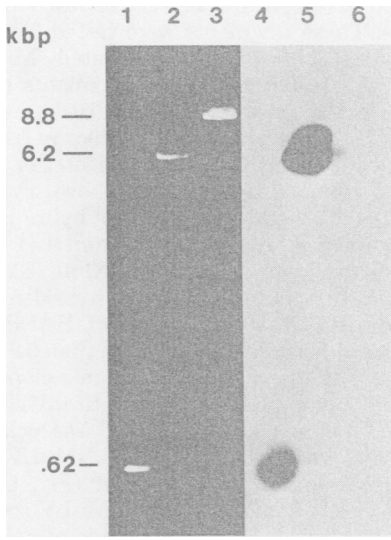

FIG. 6. *Lack of homology of MuLV with BALB-MSV sequences encompassed by the subcloned BamHI-to-HindIII fragment (pHB-1) of HindIII clone pH-9c. Cloned M-MuLV DNA (lanes 3 and 6), the isolated BamHI-to-HindIII insert of pHB-1 (lanes 1 and 4), and the PstI fragment of BALB-MSV (lanes 2 and 5) were electrophoresed and stained with ethidium bromide (lanes 1, 2, and 3). DNA was then transferred to a nitrocellulose filter, hybridized to the ^{32}P-labeled insert of clone pHB-1, and autoradiographed (lanes 4, 5, and 6).*

Homology of BALB-MSV helper virus-unrelated sequences with normal cellular DNA. Helper virus-unrelated sequences of a number of previously studied transforming retroviruses have been shown to be homologous with sequences present at low copy numbers in normal cellular DNA. To examine this possibility for BALB-MSV, we utilized the pHB-1 cloned insert as a hybridization probe in Southern blots of mouse cellular DNA cleaved with *Eco*RI. This probe detected several discrete *Eco*RI DNA fragments in cellular DNAs of NIH/Swiss and BALB/c mouse strains (Fig. 7). In each case, six bands with molecular weights corresponding to DNA sizes of 23, 13.5, 9.1, 7.4, 7.1, and 4.3 kbp were demonstrated. In Fig. 7, the 7.4- and 7.1-kbp bands were not resolved owing to the length of exposure required to demonstrate the less reactive 13.5- and 4.3-kbp bands. As one means of studying the degree of conservation of the cell-related sequences of BALB-MSV, the pHB-1 probe was hybridized to *Eco*RI-cleaved rat and human DNAs. BALB-MSV-related sequences in NRK cellular DNA were 20 and 13 kbp in size (Fig. 7). Related sequences were also demonstrable in human DNA. DNA bands of 9.2 and 7.4 kbp were readily observed. Thus, BALB-MSV sequences not related to those of MuLV were demonstrated to be present at low copy numbers and to be conserved within the genomes of species as diverse as mouse and human.

DISCUSSION

The present report describes the application of molecular cloning techniques to the investigation of the structural organization of BALB-MSV, a naturally occurring transforming retrovirus of mice. The integrated form of the BALB-MSV genome, including cellular flanking sequences, was isolated by cleaving nonproducer-cell DNA with *Eco*RI and cloning the fragments in the lambda phage vector Charon 9. Restriction enzyme mapping revealed a 6.8-kbp region within this 19-kbp cloned DNA fragment which was identical to BALB-MSV viral DNA. The cloned DNA induced transformed foci indistinguishable from those induced by BALB-MSV upon transfection of NIH/3T3 cells. Moreover, transformants released biologically active virus upon helper virus rescue. Thus, cloned DNA retained the known functional characteristics of the parental virus genome.

Several important features of the molecular organization of integrated BALB-MSV provirus were deduced from restriction mapping and molecular hybridization analyses (Fig. 8). LTRs,

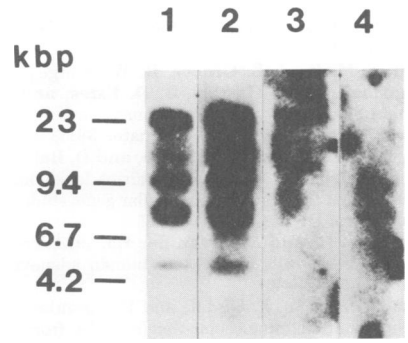

FIG. 7. *Analysis of normal cellular DNA with a probe composed of helper virus-unrelated sequences of BALB-MSV (insert of clone pHB-1). High-molecular-weight cellular DNA was cleaved with EcoRI, electrophoresed on a 0.7% agarose gel, blotted to a nitrocellulose filter, and hybridized with the ^{32}P-labeled insert of clone pHB-1. DNAs included those of NIH/Swiss 3T3 (lane 1) and BALB/c (lane 2) mouse strains, NRK rat cells (lane 3), and human placenta (lane 4).*

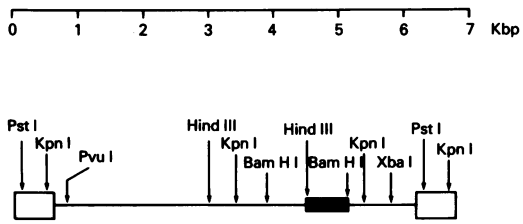

FIG. 8. *Physical map of BALB-MSV DNA. The position of sequences not related to MuLV is indicated by the closed rectangle, and LTRs are denoted by open rectangles.*

approximately 0.6 kbp in length, were defined by the presence of symmetrical *Pst*I and *Kpn*I restriction sites located, respectively, at 1.9 and 2.3 kbp, and at 8.1 and 8.5 kbp within the 19-kbp *Eco*RI DNA clone. Moreover, DNA fragments encompassing these sites hybridized with nick-translated cloned M-MuLV LTR DNA. The viral LTRs contain signals for transcription initiation and termination and, thus, are thought to be essential for efficient viral RNA expression. The *Pst*I subclone, which lacked the distal 20% of the 5′ LTR and 80% of the 3′ LTR, transformed cells efficiently. Moreover, MSV was rescued from transformants containing this DNA, although at a lower titer than that observed with transformants containing the complete BALB-MSV genome. These findings indicate that intact LTRs are probably required for efficient rescue of BALB-MSV.

BALB-MSV is known to code for helper viral gene products indistinguishable from those of B-tropic MuLV present in the initial BALB-MSV isolate (2). In the present study, the restriction map of the helper virus-related portions of BALB-MSV was strikingly similar to that of a B-tropic MuLV (29), providing further evidence that the helper virus sequences of BALB-MSV were derived from those of B-tropic MuLV.

The recombinational events involved in the formation of most transforming retroviruses so far studied appear to include the deletion of helper virus sequences and the incorporation of host cell information. The location of the cellular insertion within different transforming viruses has been found to vary. The size of the BALB-MSV genome, 6.8 kbp, must reflect the loss of helper virus information, since the MuLV genome is around 8.8 kbp in length. In the present studies, we identified a region within BALB-MSV of at least 0.6 kbp which was unrelated to MuLV but was instead homologous with sequences present in normal mouse cellular DNA. This region was localized toward the 3′ terminus of the viral genome in relation to its RNA (Fig. 8). In comparison to previous isolates, the structural organization of BALB-MSV most closely resembles that reported for M-MSV (41, 42) and simian sarcoma virus (32a).

Evidence indicates that independent sarcoma virus isolates of the same species may contain the same or related cell-derived (designated *onc*) sequences (36, 39, 44). Other studies have indicated relatedness among the *onc* sequences of transforming retroviruses isolated from species as diverse as chicken and cat (34). There have been several independent isolates of mouse-transforming viruses. The *onc* sequences of cloned BALB-MSV do not show detectable homology with those of M-MSV (41) and A-MuLV (38a; P. R. Andersen, S. G. Devare, S. R. Tronick, R. W. Ellis, S. A. Aaronson, and E. M. Scolnick, Cell, in press). However, the *onc* sequences of BALB-MSV have recently been shown to be homologous with those of Harvey-MSV, a transforming retrovirus of rat origin (Andersen et al., in press). Thus, transforming retroviruses appear to have been generated by recombination of helper viruses with only a limited number of distinct cellular genes.

The cell-derived sequences of BALB-MSV were detected in several DNA fragments of *Eco*RI-cleaved normal mouse cellular DNA. Such findings suggest that there exists a family of BALB-MSV related genes or that the structure of a single-copy, normal cellular analog is considerably more complicated than that of its viral counterpart. The probe of BALB-MSV cell-derived sequences also detected a smaller number of *Eco*RI-generated fragments of rat

and human DNAs. These findings indicate that sequences related to BALB-MSV have been conserved during the evolution of mammalian species. The role of these sequences in virally induced and naturally occurring neoplasms can now be more readily assessed by using as probes the well-defined cloned segments of the BALB-MSV genome generated in this study.

ACKNOWLEDGMENTS

We thank Eli Canaani for providing the M-MuLV-specific probes and Claire Dunn and Terry Sarantou for excellent technical assistance.

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