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Characterization of Mouse Cellular Deoxyribonucleic Acid Homologous to Abelson Murine Leukemia Virus-Specific Sequences

BEVERLY DALE AND BRAD OZANNE*

Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

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The genome of Abelson murine leukemia virus (A-MuLV) consists of sequences derived from both BALB/c mouse deoxyribonucleic acid and the genome of Moloney murine leukemia virus. Using deoxyribonucleic acid linear intermediates as a source of retroviral deoxyribonucleic acid, we isolated a recombinant plasmid which contained 1.9 kilobases of the 3.5-kilobase mouse-derived sequences found in A-MuLV (A-MuLV-specific sequences). We used this clone, designated pSA-17, as a probe restriction enzyme and Southern blot analyses to examine the arrangement of homologous sequences in BALB/c deoxyribonucleic acid (endogenous Abelson sequences). The endogenous Abelson sequences within the mouse genome were interrupted by noncoding regions, suggesting that a rearrangement of the cell sequences was required to produce the sequence found in the virus. Endogenous Abelson sequences were arranged similarly in mice that were susceptible to A-MuLV tumors and in mice that were resistant to A-MuLV tumors. An examination of three BALB/c plasmacytomas and a BALB/c early B-cell tumor likewise revealed no alteration in the arrangement of the endogenous Abelson sequences. Homology to pSA-17 was also observed in deoxyribonucleic acids prepared from rat, hamster, chicken, and human cells. An isolate of A-MuLV which encoded a 160,000-dalton transforming protein (P160) contained 700 more base pairs of mouse sequences than the standard A-MuLV isolate, which encoded a 120,000-dalton transforming protein (P120).

Abelson murine leukemia virus (A-MuLV) was isolated from a lymphosarcoma that developed in a steroid-treated BALB/c mouse 5 weeks after injection with Moloney murine leukemia virus (M-MuLV) (2). Unlike M-MuLV, A-MuLV is defective for replication and transforms both fibroblasts and lymphoid cells in vitro (34, 37). In mice, A-MuLV demonstrates a unique tissue specificity that produces a fatal nonthymic leukemia within 3 to 6 weeks after inoculation (1). Although the primary target cell type of this virus has not been defined, characterization of A-MuLV tumors of in vitro-transformed lymphoid cells suggests that the target cell type may be a precursor in the B-lymphocyte series (8, 24-26, 41-43).

Studies of other defective acutely transforming retroviruses have shown that each of these viruses is a recombinant between a nondefective nontransforming helper virus and normal genomic deoxyribonucleic acid (DNA) from the animal of isolation (6, 12, 38, 39). The oncogenic potentials of these viruses are believed to result from the acquisition and subsequent expression of host-specific sequences. An analysis of the standard A-MuLV isolate from ANN.1 cells (37) indicated that this isolate also exhibits a recombinant nature (3, 40). In A-MuLV ribonucleic acid (RNA), 1,320 bases at the 5' end and 730 bases at the 3' end are homologous to M-MuLV RNA, whereas the intervening 3,500 bases (designated A-MuLV-specific sequences) are not derived from the parent virus (40). Nucleic acid hybridization studies (3, 15) have shown that the A-MuLV-specific sequences in the viral genome are related to normal mouse genomic DNA (designated endogenous Abelson sequences).

The major translation product of the A-MuLV genome is a 120,000-dalton protein (designated P120). Analyses of P120 molecules produced in vivo and in vitro have identified peptides derived from both the M-MuLV-related sequences and the A-MuLV-specific sequences of the recombinant virus (27, 29, 51). Genetic evidence suggests that P120 is responsible for A-MuLV-induced cellular transformation and that the transformation process involves a protein kinase activity associated with this molecule (28, 52). A relationship between P120 and a 150,000-dalton nor-

mal cell protein (NCP 150) has been found in experiments in which antiserum raised against A-MuLV tumors recognizes NCP 150 on certain normal mouse lymphoid tissues (53). These data, together with the evidence that the A-MuLV genome contains mouse-derived sequences, suggest that endogenous Abelson sequences in mice (presumably those from which the virus arose) code for a protein normally expressed on some lymphoid tissues. The expression of the related A-MuLV-specific sequences as a viral polyprotein results in cellular transformation and may designate the in vivo target for such a transformation event.

Using recombinant DNA technology, we isolated a portion of the A-MuLV genome that lacked M-MuLV sequences. This clone was used as a probe in nucleic acid hybridization studies to determine the arrangement of endogenous Abelson sequences in several strains of mice and the distribution of these sequences in other species. We found that, although endogenous Abelson sequences are arranged similarly in all of the mouse strains and cell types which we examined, the sequence arrangement in the virus is different from that in the mouse genome. In addition, Abelson-related sequences are present in other species. We describe a map of normal mouse cellular sequences that are homologous to the A-MuLV probe.

MATERIALS AND METHODS

Cells and viruses. ANN.1.5 cells (a clone of the A-MuLV-transformed NIH-3T3 cell line ANN.1 [37]) were obtained from J. Toy, Imperial Cancer Research Fund, London, England. The NRK-9 and NIH-3T3 cell lines were obtained from N. Teich, Imperial Cancer Research Fund. The hamster B-cell lymphoma GD36 (11) was obtained from P. Fultz, University of Texas Health Science Center at Dallas. Cells of the BALB/c tumor line BCL_1 (44) were obtained from S. Strober, Stanford University, Stanford, Calif., via E. Vitetta and K. Krolick, University of Texas Health Science Center at Dallas. The BALB/c plasmacytomas NS-1, MPC-11, and SP2/0 were obtained from S. Robertson, University of Texas Health Science Center at Dallas. SVRT is a simian virus 40-transformed human fibroblast cell line isolated by B.O. B6T1 (p5Cl2) is an A-MuLV-induced bone marrow tumor cell line (33) from C57BL/6 mice obtained from R. Risser, McArdle Laboratory, Madison, Wis. All fibroblast cell lines were maintained in Dulbecco modified Eagle medium containing 10% calf serum; B6T1 tumor cells were grown in RPMI 1640 medium containing 20% fetal calf serum and 5×10^{-5} M β-mercaptoethanol, and GD36 cells were grown in RPMI 1640 medium containing 5% fetal calf serum. An A-MuLV(M-MuLV) stock was prepared from ANN-NB1, a clone of ANN.1 cells infected with M-MuLV. The titer of this virus stock was 10^7 plaque-forming units of M-MuLV per ml, as determined by the XC assay (19), and 10^6 focus-forming units of A-MuLV per ml, as determined by the focus assay (36) on Rat-1 cells. An A-MuLV (gibbon ape leukemia virus [GaLV]) stock was prepared from the GibCl6 cell line, a rat embryo fibroblast cell line that produces GaLV and A-MuLV and was obtained from R. Risser.

Preparation of DNA linear intermediates. Monolayers of NRK-9 cells were grown to subconfluency in roller bottles. Infection of these monolayers with A-MuLV(GaLV) or A-MuLV(M-MuLV) was achieved by cocultivating the NRK-9 cells with GibCl6 or ANN-NB1 cells at a ratio of 10:1. At 24 h after infection, the cells were washed twice in phosphate-buffered saline, and low-molecular-weight DNA was extracted as described by Hirt (18). The linear DNA molecules in the Hirt extract were visualized by agarose gel electrophoresis, blotting onto nitrocellulose paper, and hybridization to a ^{32}P-labeled nick-translated clone of M-MuLV in pBR322, which was obtained from E. Gilboa. An estimated 20-fold purification of an A-MuLV(GaLV) linear molecule preparation was achieved by centrifuging the preparation through a 10 to 30% sucrose gradient in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (pH 7.8)–1 mM ethylenediaminetetraacetate–10 mM NaCl for 3 h at 100,000 × *g* in a Sorvall 865B vertical rotor. Fractions were collected, and a sample of each fraction was assayed for linear viral DNA as described above. Fractions positive for A-MuLV linear DNA by hybridization were pooled, and the DNA was precipitated.

Cloning of DNA into phage and plasmid vectors. DNA arms from the modified λWES-λB vector WESB-Sst were obtained from J. Stringer, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. The arms were prepared by the procedure of Maniatis et al. (21), using *Sst*I (an isoschizomer of *Sac*I). DNA from the sucrose gradient-enriched A-MuLV linear molecule preparation was cleaved with *Sac*I under conditions designed to generate partial digests (1 U of enzyme per μg of DNA at 37°C for 10 min). The digested λ arms and linear molecule preparation were ligated (7), and in vitro packaging of recombinant phages into λ particles was accomplished by the method of Blattner et al. (7). Phage were plated onto an approved *Escherichia coli* host. Screening for phage containing the desired insertions was performed by the method of Benton and Davis (4), using M-MuLV [^{32}P]DNA as a probe. Positive plaques were picked, purified, and analyzed by restriction enzyme digestion and gel electrophoresis of the hybrid λ DNA. Appropriate clones were selected for subcloning into pSA801, a derivative of pBR322 containing an *Sst*I site derived from Charon 4A in the normal *Bam*HI site (a gift of E. Stavnezer, Sloan Kettering Memorial Institute, New York, N.Y.). DNA from hybrid phage was prepared by the method of Blattner et al. (7). The A-MuLV sequences in the purified recombinant phage DNA preparation were excised witb *Sac*I and purified by elution into a dialysis bag from an 0.8% agarose gel. The eluted 2.7-kilobase (kb) A-MuLV fragment was extracted with phenol and chloroform and then precipitated with 0.2 M NaCl and ethanol. Purified pSA801 DNA was cut with *Hin*dIII and *Sac*I, mixed with the 2.7-kb *Sac*I fragment (which was also digested

with *Hin*dIII), and ligated at 14°C overnight. *E. coli* DH-1 was transformed to ampicillin resistance with recombinant plasmids from the ligated mixture. Mini-DNA preparations were made by the method of Meagher et al. (22). Large plasmid DNA preparations were made by cesium chloride-ethidium bromide centrifugation of cleared lysates of the host bacterium carrying the desired recombinant plasmid after chloramphenicol amplification.

All recombinant DNA procedures were carried out in accordance with the National Institutes of Health Guidelines for Recombinant DNA Research.

Preparation of high-molecular-weight cellular DNA, restriction enzyme digestion, gel electrophoresis, transfer of DNA fragments and blot hybridization. High-molecular-weight cellular DNAs were prepared from the livers of adult mice belonging to the following strains: AKR, BALB/c, C57BL/6, C3H-He, CBA, DDS-10, DBA/2, A-Str, and Q (obtained from the Imperial Cancer Research Fund animal unit). B6T1 (p5Cl2) DNA was prepared from tumor cells in culture. Other DNA preparations were from established cell lines. DNA prepared from SPA-FAS chicken embryo fibroblasts was obtained from E. Humphries, University of Texas Health Science Center at Dallas. To purify high-molecular-weight DNAs from cell lines and cultured tumor cells, we used the method of Steffen et al. (46), with a slight modification. To purify DNAs from mouse livers, nuclei were prepared by the method of Steffen and Weinberg (47). The nuclei were pelleted, washed, suspended in 10 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.0)–10 mM NaCl–10 mM ethylenediaminetetraacetate, and lysed with 0.5% sodium dodecyl sulfate, and DNA was extracted as described above for the cell lines.

Restriction enzymes were obtained from New England Biolabs, and enzyme digestions were performed as recommended by the supplier. Digests were electrophoresed on 0.8 or 1.0% horizontal agarose gels at 1 V/cm for 16 to 18 h in 36 mM tris(hydroxymethyl)aminomethane base–30 mM NaH_2PO_4–1 mM ethylenediaminetetraacetate (pH 7.9). Agarose gels were treated with 0.25 M HCl for 15 min, denatured in 0.2 N NaOH–0.6 M NaCl for 1 h, and neutralized in 0.5 M tris(hydroxymethyl)aminomethane hydrochloride (pH 8.0)–0.6 M NaCl for 1 h. The DNA fragments were transferred to nitrocellulose sheets by the method of Southern (45) with 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The sheets were rinsed in 2× SSC and dried at 80°C under a vacuum for 2 h. Hybridization to a nick-translated ^{32}P-labeled probe (specific activity $\sim 10^8$ cpm/μg) was performed as described by Grodzicker and Klessig (17). Sheets were exposed to Fuji Rx X-ray film with intensifier screens for 1 to 7 days at −70°C.

RESULTS

Molecular cloning of A-MuLV-specific sequences. The sources of retroviral DNA useful for molecular cloning into procaryotic vectors include both the linear and the circular intermediates formed during retrovirus infection before integration into the host genome. We used A-MuLV linear molecules, which were consistently present in larger quantities than circular intermediates in Hirt extracts prepared from A-MuLV-infected NRK-9 cells (data not shown). Because of the homologous sequences shared by A-MuLV and M-MuLV (Fig. 1), we used a clone of M-MuLV in pBR322 as a probe for detecting A-MuLV molecules during the cloning procedures. Linear molecules were prepared by using an A-MuLV stock with a GaLV pseudotype. The GaLV genome contains limited homology to M-MuLV (5), and thus our use of the GaLV pseudotype lessened the possibility of selecting clones of the helper virus.

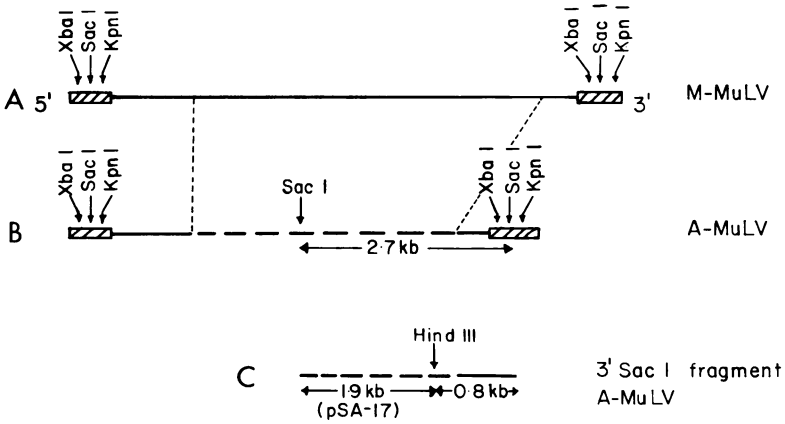

FIG. 1. *Schematic representations of retrovirus molecules involved in the cloning of A-MuLV-specific sequences. (A) M-MuLV linear DNA molecule with restriction enzyme sites in the long terminal repeat (cross-hatched bars) (15). (B) A-MuLV linear DNA molecule showing sequences homologous to M-MuLV (solid lines) and specific to A-MuLV (dashed lines). The long terminal repeat restriction enzyme sites were predicted from homology to M-MuLV. (C) The 3' 2.7-kb SacI fragment cloned into the λ vector WESB-Sst. Cleavage of the fragment with HindIII generated the 1.9-kb A-MuLV-specific fragment contained in the plasmid clone pSA-17.*

A feature of the retroviral linear intermediate is a 600-base pair long terminal repeat found at both ends of the linear DNA molecule (14). Because heteroduplex mapping of A-MuLV RNA with the M-MuLV genome revealed absolute homology at the 5′ and 3′ ends (40), we assumed that the linear DNA intermediate generated by A-MuLV had the same restriction enzyme sites in the long terminal repeat as described previously for M-MuLV (Fig. 1). Enzymes which cut in the M-MuLV long terminal repeat include *Xba*I, *Kpn*I, and *Sac*I (14). Because of the availability of the useful λ vector WESB-Sst with *Sac*I insertion sites, *Sac*I was chosen for the initial cloning procedures. The A-MuLV linear molecule also possesses an internal *Sac*I site (Dale and Ozanne, unpublished data). Therefore, partial digestion with *Sac*I generates a subgenomic A-MuLV molecule lacking 400 and 200 bases at the 5′ and 3′ ends, respectively, as well as two *Sac*I fragments of similar size (2.8 and 2.7 kb). Any one of these fragments represents a suitable A-MuLV-specific probe after removal of the remaining M-MuLV-specific sequences.

The A-MuLV(GaLV) linear molecules were partially digested with *Sac*I, ligated to arms prepared from the λ vector WESB-Sst, packaged, and plated onto the approved host bacterium. Six positive clones were picked, one of which contained a 2.7-kb insertion. A comparison of the restriction enzyme pattern of this fragment with the patterns of A-MuLV linear molecules (data not shown) revealed it to be the 3′ *Sac*I fragment of A-MuLV DNA (Fig. 1). Cleavage of this fragment with *Hin*dIII produced a 1.9-kb fragment and an 0.8-kb fragment, which were visualized by ethidium bromide. When these fragments were hybridized to M-MuLV [^{32}P]RNA, only the 0.8-kb fragment was recognized. The A-MuLV specificity of the 1.9-kb fragment is illustrated in Fig. 2. The positive λ clone was digested either with *Sac*I alone (Fig. 2, lanes a and c) or with *Sac*I and *Hin*dIII (lanes b and d). M-MuLV 70S [^{32}P]RNA hybridized to the 2.7-kb *Sac*I fragment (Fig. 2, lane a) and to the 0.8-kb *Sac*I-*Hin*dIII fragment (lane b), but not to the 1.9-kb *Sac*I-*Hin*dIII fragment. Hybridization of the same filter to A-MuLV(M-MuLV) 70S [^{32}P]RNA (ratio of A-MuLV molecules to M-MuLV molecules, 1:10) visualized the 1.9-kb fragment also. This indicated that the 1.9-kb fragment possessed specificity for A-MuLV but not M-MuLV. This 1.9-kb A-MuLV-specific fragment (Fig. 1) was subcloned into pSA801, a pBR322 derivative with a *Sac*I site substituted at the *Bam*HI site in the tetracycline resistance gene. This clone was designated pSA-17. Additional evidence that the insertion in this clone

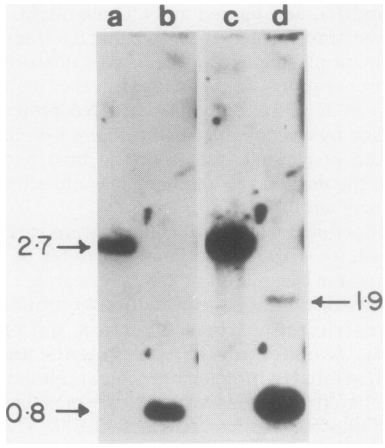

FIG. 2. *Specificity of A-MuLV [^{32}P]RNA for the 1.9-kb A-MuLV-specific DNA fragment. DNA was prepared from A-MuLV–λ hybrids containing the 2.7-kb A-MuLV 3′ SacI fragment and was digested with either SacI (lanes a and c) or HindIII and SacI (lanes b and d). Digests were electrophoresed, blotted onto nitrocellulose, and hybridized to either M-MuLV 70S [^{32}P]RNA (lanes a and b) or A-MuLV(M-MuLV) 70S [^{32}P]RNA (lanes c and d).*

was indeed derived from the non-M-MuLV sequences of A-MuLV was that pSA-17 [^{32}P]DNA recognized A-MuLV but not M-MuLV linear molecules in a mixed preparation (data not shown).

Recognition of virus-specific and endogenous Abelson sequences by pSA-17. The specificity of pSA-17 for both viral and endogenous Abelson sequences was determined by analyzing restriction enzyme digests of DNAs prepared from both uninfected and A-MuLV-infected cells. Samples of DNA were cut with either *Eco*RI or *Xba*I, fractionated by gel electrophoresis, blotted onto nitrocellulose, and hybridized to pSA-17 [^{32}P]DNA (Fig. 3). We had previously determined that the enzyme *Eco*RI did not cut within the A-MuLV linear DNA molecule (data not shown). Therefore, hybridization to *Eco*RI-digested DNA from A-MuLV-transformed cells should have generated a band representing an integrated provirus which was larger than the 6.1 kb of the linear A-MuLV DNA molecule. In addition, if the endogenous Abelson sequences were arranged as they appeared in the virus, another fragment larger than 3.5 kb (the size of the mouse-derived sequences in the A-MuLV genome) representing the endogenous locus should have been detected. The single band appearing in *Eco*RI digests of uninfected mouse DNA at 28 kb (Fig. 3, lanes a, b, and e) represented the endogenous Abelson sequences (15). Additional bands were observed in A-MuLV-transformed fibroblasts (Fig. 3, lane d)

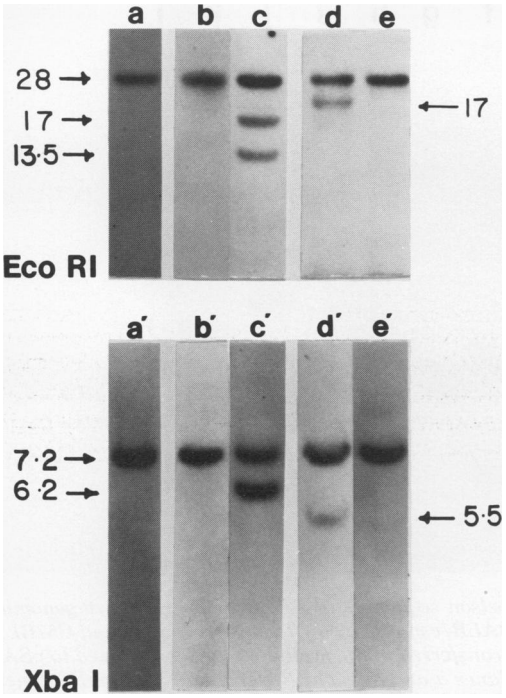

FIG. 3. *Visualization of A-MuLV-specific and endogenous Abelson sequences by the A-MuLV-specific probe pSA-17. Cellular DNAs were prepared from uninfected (lanes a, b, e, a', b', and e') and A-MuLV-infected (lanes c, d, c', and d') mouse cells, digested with EcoRI or XbaI, electrophoresed, transferred to nitrocellulose, and hybridized to pSA-17 [^{32}P]DNA. The DNAs used were BALB/c liver (lanes a and a'), C57BL/6 liver (lanes b and b'), B6T1 (p5Cl2) (lanes c and c'), ANN.1.5 (lanes d and d'), and NIH-3T3 (lanes e and e').*

and A-MuLV-transformed bone marrow cells (lane c) at 17 kb (lane d) and at 17 and 13.5 kb (lane c). That these additional bands represented integrated proviral DNA was determined by digesting the same DNAs with *Xba*I, which cuts within the long terminal repeat of the virus and removes a total of 600 bases from the viral linear DNA (Fig. 1). *Xba*I digestion of uninfected cell DNA (Fig. 3, lanes a', b', and e') generated a single band of 7.2 kb (the endogenous Abelson sequences), whereas digestion of A-MuLV-transformed cell DNA (Fig. 3, lanes c' and d') resulted in two bands, the 7.2-kb band of endogenous Abelson sequences and an A-MuLV-specific band. The virus-specific band in ANN.1.5 cells (Fig. 3, lane d') was at 5.5 kb, the expected size after removal of 600 base pairs from a standard 6.1-kb A-MuLV integrated provirus. Only one virus-specific band (larger than the provirus in ANN.1.5 cells) was found in B6T1 DNA, as would be expected if both integrated proviruses observed in the *Eco*RI digestion were cleaved within their long terminal repeat sequences, thereby removing the flanking cellular DNA. The size variation shown by the A-MuLV proviruses in ANN.1.5 and B6T1 cells is discussed below.

Organization of Abelson-specific sequences in BALB/c mice. Moloney murine sarcoma virus is similar to A-MuLV in that it has acquired host cell sequences which apparently render the virus tumorigenic (12). The fragment of cellular DNA from which Moloney murine sarcoma virus presumably arose has been isolated, and heteroduplex mapping of this cell fragment with the cloned virus has revealed that the two sets of sequences are colinear (23). Our results and those of others (15) indicate that a different relationship exists between the endogenous Abelson sequences in BALB/c DNA and the A-MuLV-specific sequences present in pSA-17. When high-molecular-weight DNA prepared from BALB/c mouse livers was digested with *Eco*RI (an enzyme that does not cut A-MuLV-specific sequences) and hybridized to pSA-17 [^{32}P]DNA, all of the homologous cellular sequences were contained within a single 28-kb fragment (Fig. 3, lane a). However, when BALB/c DNA was digested with other restriction enzymes that did not cut the A-MuLV-specific sequences in pSA-17 (*Xba*I, *Kpn*I, *Bam*HI, *Hin*dIII, and *Sac*I), more bands were generated than would have been predicted if the endogenous Abelson sequences were present as a single-copy gene colinear with A-MuLV-specific sequences (Fig. 4, lanes a through f). With each enzyme one band of intense hybridization and one or more less intense bands were present. For example, in the *Sac*I digest of BALB/c DNA (Fig. 4, lane e), homology to pSA-17 was observed at 2.35 kb and faintly at 5.7 kb. Although a double digestion with *Sac*I and *Hin*dIII generated a band at 1.9 kb (the same size as the *Sac*I-*Hin*dIII A-MuLV-specific insertion in pSA-17), faint hybridization was also observed at 4.5 kb (Fig. 4, lane f). The following two explanations were possible: (i) A-MuLV-specific sequences were intact in BALB/c DNA, and there were additional sequences of lesser homology contained within the single *Eco*RI fragment, which generated the fainter bands in other enzyme digests; or (ii) A-MuLV-specific sequences were dispersed in the BALB/c genome, and the viral sequences were the result of some type of rearrangement of endogenous Abelson sequences. Our data suggested that the latter explanation was correct. By comparing the patterns generated by the restriction enzymes that cut within the A-MuLV-specific pSA-17 (data not shown), we were able to ascertain that the 1.9-kb *Sac*I-*Hin*dIII fragment in the BALB/c

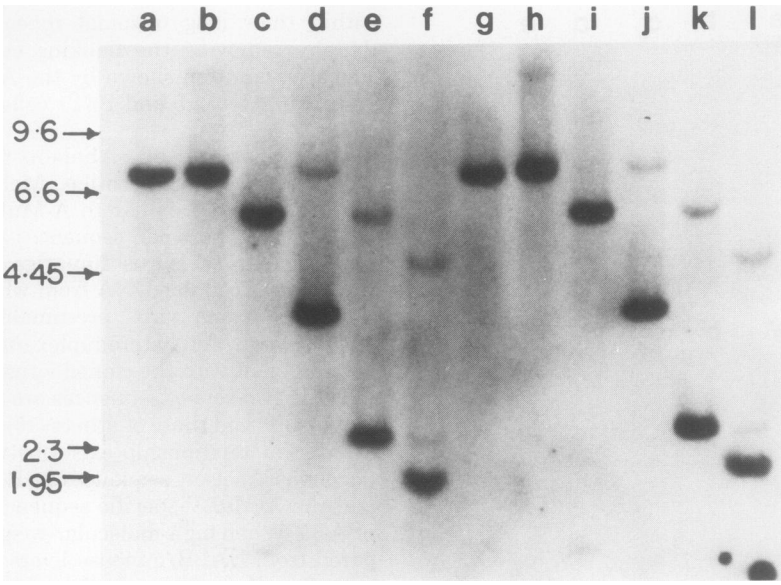

FIG. 4. *Restriction enzyme patterns of endogenous Abelson sequences in BALB/c and C57BL/6 genomic DNAs. Enzyme digests of cellular DNAs prepared from BALB/c mouse livers (lanes a through f) and C57BL/6 mouse livers (lanes g through l) were electrophoresed, transferred to nitrocellulose, and hybridized to pSA-17 [^{32}P]DNA. The restriction enzymes used were XbaI (lanes a and g), KpnI (lanes b and h), BamHI (lanes c and i), HindIII (lanes d and j), SacI (lanes e and k), and HindIII-SacI (lanes f and l). Molecular weights were determined by migration of λ DNA fragments digested with HindIII, as shown on the left. (The faint band appearing at 2.35 kb in the HindIII-SacI double digest [lanes f and l] in this figure was due to incomplete digestion by HindIII.)*

digest (Fig. 4, lane f) was in fact not identical to the 1.9-kb pSA-17 insertion. Although both shared the same 3′ *Hin*dIII site, their 5′ *Sac*I sites were derived from different parts of the normal cell genome. These internal patterns also indicated that the more intense band in each digest in Fig. 4, lanes a through f, represented the bulk of the A-MuLV-specific sequences at the 3′ end of the viral sequences in pSA-17 and that the fainter band represented sequences at the 5′ end of our probe. When the fragments were aligned as a linear sequence, as suggested by the single *Eco*RI 28-kb fragment, the restriction map shown in Fig. 5 was generated. Some of the restriction fragments observed in Fig. 4, lanes c through f, are represented schematically above the proposed map. A major feature of this map is the separation of the 5′ and 3′ sequences of A-MuLV-specific DNA by a noncoding region of at least 4,000 bases in endogenous Abelson DNA. The representation of the smaller intervening sequence at the 5′ end was suggested by double digestions with *Bam*HI and other enzymes. This representation of endogenous Abelson sequences is consistent with the data of Goff et al. (15).

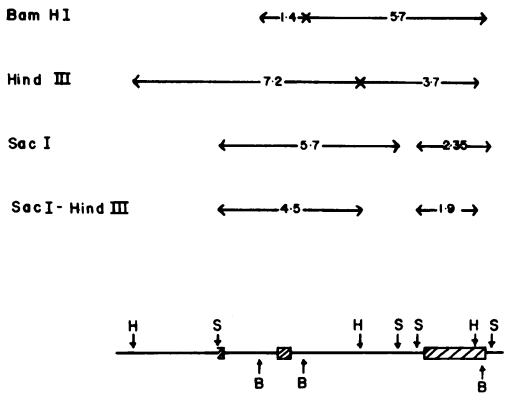

FIG. 5. *Arrangement of the endogenous Abelson sequences recognized by the A-MuLV specific probe pSA-17. The fragments generated by BamHI (B), HindIII (H), SacI (S), and HindIII-Sac I are shown above the proposed restriction enzyme map of the endogenous Abelson sequences. The cellular sequences homologous to the A-MuLV-specific probe are indicated by cross-hatched bars. Solid lines represent nonhomologous cellular sequences.*

Arrangement of endogenous Abelson sequences in lymphoid cells. It is well known that in certain lymphoid cells, particularly B-cells, rearrangements of genomic DNA occur during differentiation and that these rearrangements result in the generation of novel combinations of genes for immunoglobulin not found as such in germ line DNA (49). Certain se-

quences are deleted so that the gene in any particular clone of B-cells contains contiguous sequences of DNA which are not so arranged in other tissues of the same animal. Since a cellular protein antigenically related to P120 (NCP 150) is expressed only on lymphoid cells (53), we examined whether the gene coding for this protein might also have been rearranged during differentiation. One might predict that this alteration would lead to a sequence arrangement in the differentiated cells that was identical to that found in the virus. DNAs were prepared from cells of the BCL_1 tumor (a spontaneous BALB/c tumor with the characteristics of immature B-cells) and from three BALB/c plasmacytomas. These DNAs were digested with various restriction enzymes, and the patterns of hybridization to pSA-17 were compared with the pattern of BALB/c liver DNA. A comparison of the restriction patterns obtained with four different enzymes revealed no obvious differences in the arrangement of endogenous Abelson sequences (data not shown). Figure 6 shows a comparison of *Eco*RI digestions of DNAs from a liver, the BCL_1 tumor, and a plasmacytoma. We also examined endogenous Abelson sequences in thymus DNA and likewise found no rearrangement (data not shown).

Arrangement of endogenous Abelson sequences in mouse strains other than BALB/c. Not all mouse strains are equally susceptible to Abelson disease. In fact, most mice are resistant as adults; the exceptions to this are the strain of A-MuLV origin, BALB/c, and closely related strains (32). If the expression or antigenicity of the presumed gene product of the endogenous Abelson sequences (NCP 150) affects susceptibility to Abelson disease, susceptible and resistant strains might exhibit sequence heterogeneity, which could be detected as variations in the restriction enzyme pattern of the endogenous Abelson locus. In the experiment shown in Fig. 4, enzyme digests of BALB/c (lanes a through f) and C57BL/6 (lanes g through l) DNAs were selected for a comparison of endogenous Abelson sequences since BALB/c is the mouse strain of origin and is susceptible to Abelson disease and C57BL/6 has been regarded as the prototype resistant mouse strain. A comparison of these two strains showed that the patterns of distribution of endogenous Abelson sequences were apparently identical. Nine other mouse strains were examined and showed the same pattern. An example of the uniformity of the mouse patterns is shown in the *Sac*I digests in Fig. 7, lanes a through i. Fine mapping of the sequences with other restriction enzymes or the use of a probe representing the 5′ end of the A-MuLV-specific sequences may yet reveal differences between BALB/c mice and other mouse strains.

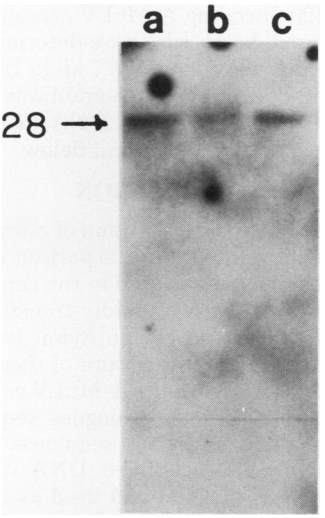

FIG. 6. *Endogenous Abelson sequences in BALB/c DNAs from livers and lymphoid tumors. DNAs prepared from BALB/c livers (lane a), BCL_1 tumor cells (lane b), and cells of MPC 11, a BALB/c plasmacytoma (lane c), were cut with EcoRI, electrophoresed, transferred to nitrocellulose, and hybridized to pSA-17 [^{32}P]DNA.*

Conservation of Abelson-specific sequences in other species. In other acutely transforming retrovirus systems, it has been shown that the cell-specific transforming sequences are conserved in vertebrate species (6, 13, 48). To determine whether the BALB/c-derived sequences of A-MuLV are similarly conserved, we digested DNAs prepared from rat, hamster, human, and chicken cells with various enzymes and hybridized them to pSA-17 [^{32}P]-DNA (Fig. 7, lanes j through m, and Fig. 8). Conservation of Abelson-specific sequences was evident in each species examined. The intensities of hybridization to the various DNAs suggested a higher sequence homology in the closely related rodent species than in the distantly related human and chicken cells.

Structural relationship between A-MuLV genomes encoding P120 and P160 transforming proteins. It is now clear that although the standard A-MuLV isolate from ANN.1 cells encodes a 120,000-dalton transforming protein (P120) (D. J. Grunwald, B. Dale, P. Jelen, B. Sugden, B. Ozanne, and R. Risser, manuscript submitted), other A-MuLV isolates encode transforming proteins that are related antigenically but vary in molecular weight. The A-MuLV-specific polyprotein observed in B6T1 cells was a 160,000-dalton protein (P160) rather than the standard P120 molecule described in

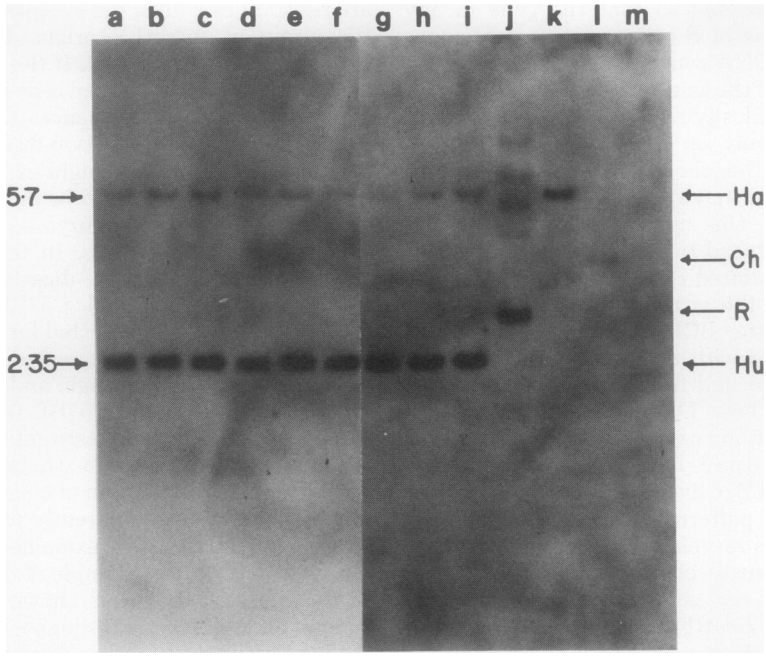

FIG. 7. *SacI patterns of endogenous Abelson sequences in DNAs from mice and other species. DNAs were prepared from the following nine strains of mice: lane a, BALB/c; lane b, C57BL/6; lane c, C3H-He; lane d, AKR; lane e, DBA/2; lane f, Q; lane g, DDS-10; lane h, CBA; and lane i, A-Str. DNAs were also prepared from NRK-9 rat cells (lane j), GD36 hamster cells (lane k), SPAFAS chicken cells (lane l), and SVRT human cells (lane m). These DNAs were digested with SacI, electrophoresed, transferred to nitrocellulose, and hybridized to pSA-17 [^{32}P]DNA. In the mouse strains a major band of homology was found at 2.35 kb and a minor band was found at 5.7 kb. Major bands were also found in rat DNA (lane j) (R) and hamster DNA (lane k) (Ha). Faint areas of hybridization were found in chicken DNA (lane l) (Ch) and human DNA (lane m) (Hu).*

ANN.1 cells. This larger transforming protein may be explained by the observation that the *Xba*I-cleaved provirus in B6T1 DNA (Fig. 3, lane c') is 700 base pairs larger than the provirus in ANN.1.5 DNA (Fig. 3, lane d'). Goff et al. (personal communication) have also reported a larger genome associated with an A-MuLV isolate encoding P160. A comparison of the *Sac*I digests of the P160-encoding virus in B6T1 cells and the P120-encoding virus in ANN.1.5 cells suggested that the P160-encoding A-MuLV genome possesses an additional *Sac*I site. When B6T1 cells were digested with *Sac*I and hybridized to pSA-17 [^{32}P]DNA, a 700-base fragment was detected in addition to the 2.7-kb *Sac*I fragment observed in digestions of the P120-encoding genome (data not shown). In a similar experiment (Fig. 9), DNAs prepared from B6T1 cells (lane a), ANN.1.5 cells (lane b), and uninfected mouse cells (lane c) were digested with *Kpn*I and *Sac*I. The bands at 3.6 and 2.35 kb in lane c (uninfected mouse cells) represented endogenous Abelson sequences and were also present in lanes a and b. The band at 2.7 kb in both of the A-MuLV-transformed DNAs (lanes a and b) corresponded in size to the 3' *Sac*I fragment of the P120-encoding A-MuLV genome (Fig. 1). An additional band, which we determined to be *Sac*I specific, was found at 0.7 kb in B6T1 DNA only. A possible origin of the additional *Sac*I site and the 700 base pairs of DNA in the P160-encoding genome is discussed below.

DISCUSSION

In A-MuLV, the substitution of normal mouse nucleic acid sequences for a portion of the M-MuLV genome has resulted in the generation of a replication-defective, rapidly transforming virus that causes an acute nonthymic leukemia in mice. We examined the nature of these BALB/c-derived sequences in the A-MuLV genome and their relationship to homologous sequences in mice (endogenous Abelson sequences). A DNA fragment of A-MuLV linear DNA was cloned into an *E. coli* plasmid and used as a probe in Southern blot restriction enzyme analyses of high-molecular-weight cellular DNAs prepared from mouse cells and cells of other species. Our results indicated the following: (i) endogenous Abelson sequences are arranged similarly in all of the mouse strains which we examined; (ii) other species possess nucleic acid sequences ho-

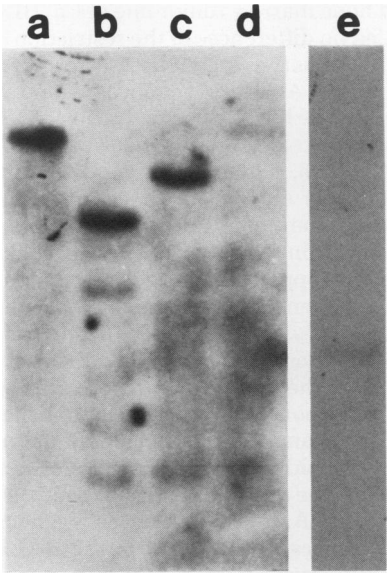

FIG. 8. *Endogenous Abelson sequences in mouse, rat, hamster, chicken, and human DNAs. High-molecular-weight DNAs were prepared from BALB/c livers (lane a), NRK-9 rat cells (lane b), GD36 hamster cells (lane c), SPAFAS chicken cells (lane d), and SVRT human cells (lane e), digested with EcoRI, electrophoresed, blotted onto nitrocellulose, and hybridized to pSA-17 [^{32}P]DNA.*

mologous to A-MuLV-specific sequences; (iii) the BALB/c-derived sequences as they appear in the virus are markedly different from the arrangement of homologous sequences in the mouse genome; and (iv) there is no obvious rearrangement of endogenous Abelson sequences in the DNA of cells representing end-stage differentiation of the B-cell lineage or in thymocytes.

Although no association between the expression of Abelson-related NCP 150 and susceptibility to Abelson disease has been made yet, the unique occurrence of NCP 150 in lymphoid tissue (53) suggests a possible mechanism of tissue specificity, consistent with the "differentiation block" theory proposed by Graf et al. (16). An examination of DNAs prepared from nine strains of mice has revealed no differences in the restriction enzyme patterns of endogenous Abelson-specific sequences in these mice compared with the strain of origin, BALB/c. Therefore, we conclude that the unique susceptibility of BALB/c mice and the relative resistance of most other strains (32) cannot be explained by gross differences in the organization of endogenous Abelson sequences in these mice as detected by our probe. An analysis of the distribution of endogenous Abelson sequences in various mouse strains with a probe specifying the 5′ end of the A-MuLV-specific sequences may detect such differences.

We found that A-MuLV is similar to other defective transforming retroviruses, the genomes of which contain sequences that are related to normal cellular DNA and are conserved in vertebrate species (6, 12, 48). A high degree of homology with the pSA-17 probe was observed in DNA prepared from hamster and rat cells. Less homology was found in chicken and human DNAs, which is consistent with expected evolutionary divergence. It seems likely that proteins related in structure and function to NCP 150 are present in these nonmurine species.

The most interesting result of this study is the disparity between the organization of A-MuLV-specific sequences in the viral genome and the organization of endogenous Abelson sequences in mice. Our probe in this study (pSA-17) contains a 1.9-kb fragment of A-MuLV-specific sequences bounded by a *Sac*I site on the 5′ end

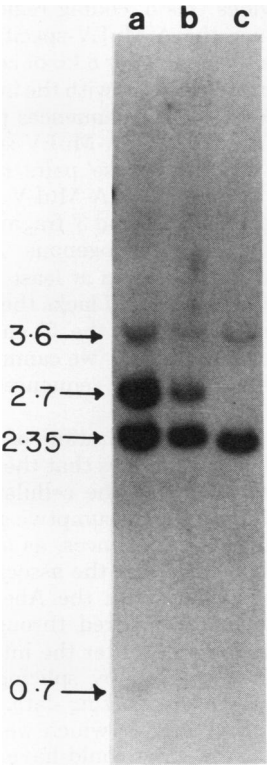

FIG. 9. *Detection of the 700-base pair SacI fragment in the A-MuLV provirus encoding P160. DNAs prepared from B6T1 (p5Cl2) cells (lane a), ANN.1.5 cells (lane b), and C57BL/6 livers (lane c) were digested with KpnI and SacI, electrophoresed, transferred to nitrocellulose, and hybridized to pSA-17 [^{32}P]DNA.*

and a *Hin*dIII site on the 3′ end (Fig. 1). This constitutes 55% of the total mouse-related DNA found in the P120-encoding A-MuLV genome. We have determined that the A-MuLV-specific sequences present in pSA-17 are not contiguous in endogenous Abelson DNA but are interrupted by intervening sequences in the mouse genome (Fig. 5). Although cleavage of mouse DNA with *Sac*I and *Hin*dIII generates a 1.9-kb fragment after hybridization to pSA-17, mapping with restriction enzymes cutting within the fragment indicated that only about 1.6 kb of the cell band is homologous to our probe. Although the two 1.9-kb fragments (cellular and viral) share the same 3′ *Hin*dIII site, the *Sac*I site at the 5′ end of the endogenous fragment is not the *Sac*I site found in the coding region of A-MuLV DNA. A large intervening sequence of about 4 kb separates the remaining A-MuLV-specific sequences of pSA-17 (the 5′ end). These 5′ sequences generate the fainter band found in the restriction enzyme digests of pSA-17. *Bam*HI digestions suggest that another intervening sequence further subdivides this 5′ coding region (data not shown). Thus, the A-MuLV-specific sequences in pSA-17 are spread over 8 kb of cellular DNA. This pattern is consistent with the interpretation of endogenous Abelson sequences presented by Goff et al. (15). Using an A-MuLV-specific probe containing 400 more base pairs than pSA-17 toward the 5′ end of the A-MuLV genome and subdividing this into 5′ and 3′ fragments, Goff et al. suggested that endogenous Abelson sequences are distributed in at least four distinct coding regions. As pSA-17 lacks the 1.5 kb of A-MuLV which comprises the 5′ end of the A-MuLV-specific sequences, we cannot determine whether other intervening sequences might exist in the cellular gene.

The segmented organization of endogenous Abelson sequences implies that the virus represents a partial copy of the cellular messenger RNA of NCP 150, the presumptive gene product of the endogenous sequences, as suggested by Goff et al. (15). Whether the association of the M-MuLV sequences with the Abelson-specific cellular sequences occurred through an RNA intermediate before or after the intervening sequences were removed by splicing cannot be determined from the existing data. An alternative to the RNA models which we are investigating is that the virus could have arisen via a recombinational event with DNA from a cell in which the endogenous Abelson sequences were rearranged before expression, a phenomenon already demonstrated in lymphoid cells (49). An examination of the arrangements of endogenous Abelson sequences in an immature B-cell tumor line, three plasmacytomas, and an A-MuLV-induced bone marrow tumor line of C57BL/6 mice revealed no differences in the restriction enzyme patterns. Another possible site for rearranged endogenous Abelson sequences would be thymocytes. The highest concentration of NCP 150, the normal cell protein recognized by A-MuLV P120 antisera, is found in thymocytes (53). Also, since T-cells are the target cells for M-MuLV (30), the parent of A-MuLV, perhaps early replication within the target tissue would have provided the opportunity for a recombinational event between M-MuLV and endogenous Abelson sequences. Our analyses indicate that endogenous Abelson sequences are arranged in the thymus as they are in other normal mouse tissues. Still another alternative is that the cell in which rearrangement occurred is not present after lymphoid maturation. Whether the original source of the A-MuLV-specific sequences was DNA or RNA, it is clear that endogenous Abelson sequences as they exist in the BALB/c genome require considerable rearrangement in order to generate the sequences as they appear in the viral genome.

Indeed, it appears that the virus genome is still capable of undergoing variation. It is probable that the original isolate of A-MuLV encodes a protein of 160,000 daltons (P160) and that the P120-encoding virus arose by a deletion (Grunwald et al., manuscript submitted). Still other A-MuLV isolates specify proteins of varying sizes which retain transforming ability and kinase activity (31, 35). We have demonstrated that the genome which encodes P160 is 700 bases larger than the genome which encodes P120 (Fig. 9). Grunwald et al. (manuscript submitted) have shown by tryptic digest analysis that P120 is probably not a simple truncation of P160. This suggests that the added sequences in the P160-encoding virus are toward the 5′ end of the A-MuLV-specific sequences. Digestion of the P160-encoding provirus with *Sac*I generates a fragment of 700 bases in addition to the *Sac*I fragment found in the P120-encoding virus. Digestions with *Hin*dIII and *Sac*I generate a 1.9-kb fragment, which is also found in the P120-encoding virus, as well as the 700-base *Sac*I-specific fragment (data not shown). We propose that the 1.9-kb fragment observed in the P160-encoding virus is not analogous to the *Sac*I-*Hin*dIII fragment from the P120-encoding virus but rather is the *Sac*I-*Hin*dIII fragment seen in digestions of the endogenous Abelson sequences, of which only 1.6 kb is found in our probe (Fig. 5 and 10). This difference would account for 300 bases of the 700-base insertion in the P160-encoding virus, with the remaining sequences derived from somewhere else in the large 4-kb intervening sequences. To generate the P120-

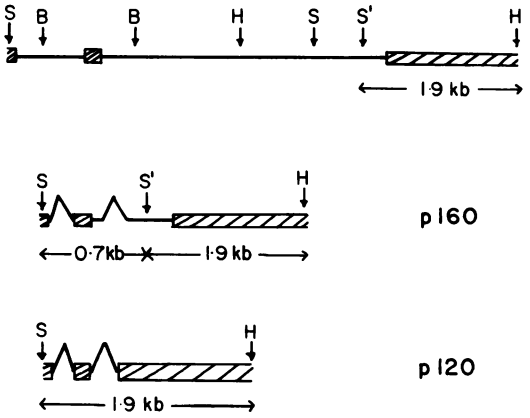

FIG. 10. *Schematic representation of the proposed relationship between the P160-encoding and P120-encoding A-MuLV isolates and the endogenous Abelson sequences. Sequences homologous to pSA-17 are represented by cross-hatched bars. Nonhomologous cellular sequences are represented by solid lines.*

encoding virus, the 700 bases including the P160-specific *Sac*I site (S') would be deleted, perhaps by RNA splicing (Fig. 10). Deletions in the same area of the genome might also account for other clones of A-MuLV that encode proteins which are smaller than P120. The apparently plastic nature of the A-MuLV-encoded fusion protein suggests that only a small portion of NCP 150 is required for transformation when expressed as a viral protein. Further studies on the function and structure of NCP 150 and its gene will be required to determine whether the oncogenic potential of A-MuLV results from inappropriate expression of a normal cell function or whether the fusion protein specified by the virus has acquired novel activities that are not present in NCP 150 alone.

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