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Genome Structure of Abelson Murine Leukemia Virus Variants: Proviruses in Fibroblasts and Lymphoid Cells

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We have prepared full-length DNA clones of the Abelson murine leukemia virus (A-MuLV) genome. A specific probe homologous to the central portion of the A-MuLV genome was prepared by nick translation of a subcloned restriction fraction from the cloned DNA. The probe was used to examine the genome structure of several A-MuLV variants. The conclusions are: (i) three viruses coding for Abelson-specific proteins of molecular weight 120,000, 100,000, and 90,000 had genomes indistinguishable in size, suggesting that the shorter proteins are the result of early translational termination; (ii) compared with the genome encoding the 120,000-dalton (120K) protein, a genome coding for a 160K protein was 0.8 kilobase larger in the A-MuLV-specific region; and (iii) a genome coding for a 92K protein had a 700-base pair deletion internal to the coding region. This mutant was transformation defective: its 92K protein lacked the protein kinase activity normally associated with the A-MuLV protein, and cells containing the virus were not morphologically transformed. In addition, we determined the number of A-MuLV proviruses in each of several transformed fibroblast and lymphoid cells prepared by infection in vitro. These experiments show that a single copy of the A-MuLV provirus is sufficient to transform both types of cells and that nonproducer cells generally have only one integrated provirus.

Abelson murine leukemia virus (A-MuLV) is a replication-defective, transforming retrovirus (2). It was derived by passage of the replication-competent Moloney MuLV (M-MuLV) in mice (1) and transforms cells of the B-lymphocyte lineage both in vivo and in vitro (12). In addition, A-MuLV can transform fibroblastic continuous cell lines in vitro (15). The RNA genome of A-MuLV is a hybrid molecule containing portions of the parental M-MuLV at its ends and a large central region, termed *abl*, apparently derived from a single-copy gene in normal mouse DNA (4). This genome is only known to code for a single polypeptide consisting of a portion of the *gag* polyprotein of M-MuLV joined to polypeptide encoded by the *abl* sequence. This protein has a molecular weight of 120,000 in cells containing the prototype strain of A-MuLV, a strain that is thus termed A-MuLV(P120) (8, 25). The protein can be phosphorylated in an in vitro reaction in which the γ-phosphate of ATP is transferred to tyrosine residues on the P120 molecule itself (22). In this regard the protein resembles the smaller pp60^{src} protein encoded by the Rous sarcoma virus genome (6), and this similarity between these viruses suggests that they might transform cells by a common mechanism. Indeed, several other transforming retroviruses, such as feline sarcoma virus (3, 20) and Fujinami virus (H. Hanafusa, personal communication), also encode transforming proteins associated with tyrosine kinase activity.

The A-MuLV P120 protein contains approximately 90,000 daltons of protein encoded by the *abl* sequence. The intact cellular protein, which is apparently the product of the *abl*-related sequences in cellular DNA, is a polypeptide of 150,000 molecular weight termed NCP150 (24); thus, only 60% of the putative original gene product is contained in the A-MuLV(P120) protein. Several variants of A-MuLV have been isolated which produce proteins of sizes different from that of the P120 molecule (13, 14). Using a DNA probe specific for *abl*, we analyzed the structure of the proviruses in cells infected with these variants. In all cases examined, the differences seemed to lie in the *abl* region of the viral

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genome. These studies define the regions of the viral protein needed for protein kinase activity and for cellular transformation. In addition, we determined the number of proviruses contained in a variety of cell lines transformed in vitro and demonstrated that a single provirus is sufficient for transformation.

MATERIALS AND METHODS

Cells and virus. A-MuLV-transformed NIH/3T3 fibroblast cell lines ANN-1, A2, F1-3, Tx-15, AN-P100, AN-P90-25, and AN-P90-33 were described previously (14, 16, 23). The A-MuLV-induced lymphoid line ABPC-22 (7) was used as a source of virus to derive the fibroblast line AN-P160-54. The other lymphoid lines, 18–48 and 2M3, were as described elsewhere (25). Fibroblast lines were grown in Dulbecco modified Eagle medium containing 10% calf serum; lymphoid lines were grown in RPMI 1640 medium containing 10% fetal calf serum and 50 μM β-mercaptoethanol. M-MuLV was prepared from C1-3A, a clonally infected NIH/3T3 producer cell line.

DNA structure analysis. DNA from tissue culture lines was prepared after lysis of the cells suspended in TE buffer (10 mM Tris-chloride, pH 7.5–1 mM EDTA) at 2×10^6 cells/ml by addition of an equal volume of sodium dodecyl sulfate protease buffer (1% sodium dodecyl sulfate, 50 mM Tris-chloride, pH 7.5, 100 mM NaCl, 10 mM EDTA, and 100 μg of proteinase K per ml). The lysate was digested for 2 h at 37°C with gentle mixing and then gently extracted two to three times with phenol and twice with $CHCl_3$. The DNA was then precipitated by the addition at room temperature of 3 volumes of ethanol. The precipitate was immediately removed and transferred to TE buffer to give a final concentration of 200 to 400 μg/ml. DNA to be prepared from liver was made from purified nuclei. Fresh tissue was minced finely and homogenized in a tight-fitting Dounce homogenizer in buffer (10 mM Tris-chloride, pH 7.5–10 mM NaCl–1.5 mM $MgCl_2$). The nuclei were collected by centrifugation, and DNA was prepared from them as described above for cells.

The DNA (10 μg) for electrophoretic analysis was incubated in buffer (10 mM Tris-chloride, pH 7.5–50 mM NaCl–6 mM $MgCl_2$–0.5 mM dithiothreitol) with 5 U of the appropriate restriction endonuclease (New England Biolabs) for 2 h at 37°C; an additional 2 U was added, and digestion was continued for an additional 2 h. The DNA was then applied to a 0.6% agarose (Sigma Chemical Co.) gel in TBE buffer (90 mM Tris-hydroxide–90 mM boric acid–9 mM EDTA) containing ethidium bromide (1 μg/ml), and electrophoresis was carried out at 2 V/cm for 48 h. The gel was photographed with transillumination by UV light to ensure that approximately equal quantities of DNA were run in each lane. The DNA was denatured and transferred to nitrocellulose (Millipore Corp.) as described (18).

Hybridization to these nitrocellulose sheets was performed by using dextran sulfate as published (21). The sheets were dried and exposed to XR-5 film at −70°C with du Pont Lightning Plus screens. Nick translations of DNA (9) were carried out by using two α-^{32}P-labeled deoxyribonucleoside triphosphates (New England Nuclear Corp.).

Analysis of ^{35}S-labeled proteins by immunoprecipitation and electrophoresis on polyacrylamide gels was as previously described (25).

RESULTS

A-MuLV strains. Several variants of A-MuLV have been detected since the original A-MuLV-induced tumor was first isolated. Most of these variants, arising spontaneously upon growth of virus-containing cells or upon passage of virus from A-MuLV producer cells to uninfected cells, were detected by virtue of alterations in the size of the A-MuLV-specific protein (14). A list of the variants known to date, the size of the protein detected, and their origin is given in Table 1.

Genome size of A-MuLV variants. To analyze the viral genome in various cell lines, the DNA provirus was examined. DNA was isolated from nonproducer cell lines clonally infected with each of the known variants of A-MuLV and was cleaved with either of two enzymes (*Xba*I and *Kpn*I) known to cut only in the long terminal repeat sequences which bracket the integrated provirus (see reference 4 for a complete restriction map of the A-MuLV genome). Thus, a proviral DNA fragment was produced free of adjacent cellular DNA, the length of which should thus be characteristic of the length of the provirus. The cleaved DNA was size fractionated

TABLE 1. *Properties of A-MuLV strains and cell lines*

Virus strain	Cell line	Reference	Protein produced	Genome size (kb)
A-MuLV(P160)	AN-P160-54	12	160K	6.3
	298-18			
	ABPC-22	6		
A-MuLV(P120)	ANN-1	13	120K	5.5
	2M3	10		
	SWR/4	23		
A-MuLV(P100)	AN-P100	12	100K	5.5
A-MuLV(P90) + deletion	AN-P90-25	12	90K	5.5 + 4.0
A-MuLV(P90)	AN-P90-33		90K	5.5
	AN-P90-35			
	234-9			
A-MuLV(P90A) + A-MuLV(P92*td*)	Late-passage A2/M	14	90K + 92K	5.5 + 4.8
	Tx-15	21		
A-MuLV(P90A)	AN-P90A-5		90K	5.5
	AN-P90A-21			
A-MuLV(P92*td*)	F1-3	21	92K	4.8
	F1-6	21		
A-MuLV(P90B) + deletion	Late-passage 18–48	10	90K	5.5 + 4.6
A-MuLV(P90B)	AN-P90B-3		90K	5.5
	AN-P90B-4			
	AN-P90B-5			

by agarose gel electrophoresis, and the viral sequences were detected by hybridization with an A-MuLV-specific DNA probe cloned into pBR322. This probe, described previously (4), consists of a 2.5-kilobase (kb) segment of the A-MuLV genome which lacks any homology to M-MuLV RNA. Any cellular DNA fragments containing *abl*-related sequences would also be detected by this probe.

DNA from all cell lines infected with the different A-MuLV strains was found to contain new A-MuLV-related fragments as well as those fragments present in uninfected NIH/3T3 cells (Fig. 1). Lines infected with A-MuLV(P120) and A-MuLV(P100) yielded a new viral DNA fragment 5.5 kb in length when the DNA was cleaved with *Xba*I (Fig. 1A). An identical new band was produced by cleavage with *Kpn*I (data not shown). The genomes encoding the P120 and P100 proteins could thus be unambiguously identified with the 5.5-kb DNA.

The cloned cell line (AN-P90-25) producing P90 contained two new fragments: one 5.5 kb and one 4.0 kb (Fig. 1A). The genome encoding the P90 protein could be either of these. To identify the genome of the A-MuLV(P90) strain, virus was rescued from line AN-P90-25 by superinfection with M-MuLV helper and used to clonally infect NIH/3T3 cells. New nonproducer transformed cell lines were isolated from this infection, and DNA from them was analyzed as before (Fig. 1B). These lines, all producing P90 protein, now contained only a 5.5-kb genome. Thus, like the A-MuLV(P120) and A-MuLV(P100) strains, the A-MuLV(P90) strain was represented by a 5.5-kb *Xba*I fragment. Further analyses using another restriction endonuclease, *Sac*I (data not shown), showed that two internal DNA fragments were also indistinguishable in all three strains. Thus, no gross change was detected in the genome size or organization that could be responsible for the altered size of the protein encoded by these strains. We have never observed passage of the 4.0-kb

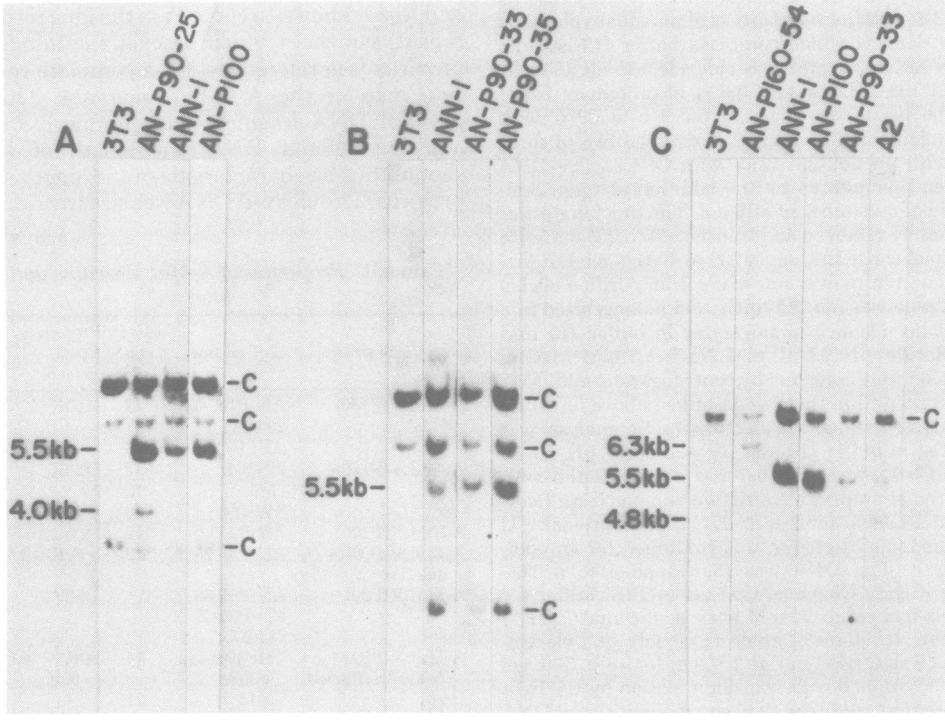

FIG. 1. *Analysis of proviral DNAs contained in various A-MuLV-infected cells. (A) DNAs isolated from the indicated cell lines were cleaved with XbaI to release most of the linear provirus (from the site in left long terminal repeat to the site in the right long terminal repeat) from the flanking sequences. The DNAs were displayed on a 0.6% agarose gel, blotted, and hybridized with an A-MuLV-specific probe (plasmid pAB3Sub3; 4). Those fragments present in uninfected cell DNA are marked "C"; the sizes of proviral fragments, as determined from DNA markers of known size, are shown. (B) DNAs from the indicated cell lines were cleaved with XbaI and analyzed as in (A). (C) DNAs from the indicated cell lines were cleaved with KpnI and analyzed as in (A).*

DNA fragment present in AN-P90-25 to other cells; preliminary mapping (data not shown) suggests that this is a defective provirus of A-MuLV not bracketed by two complete long terminal repeats.

Cleavage with the enzyme *Kpn*I demonstrated that cell lines producing the P160 A-MuLV-specific protein had a viral genome approximately 6.3 kb long (Fig. 1C) or 800 base pairs (bp) larger than the A-MuLV(P120) 5.5-kb genome. The fragment was also produced by cleavage with *Xba*I, but comigrated with a fragment from the cellular gene. The viral fragment could be detected only by the increase in intensity of labeling relative to that of the uninfected NIH/3T3 cell DNA (see Fig. 3).

Analysis of A2 cell DNA and virus. The nonproducer cell line A2 was derived by low-multiplicity infection of NIH/3T3 cells with a mixed virus stock containing A-MuLV and M-MuLV helper (16). The cell line at that time produced A-MuLV-specific proteins of two sizes (25): the normal P120 protein and a second protein of approximately 92,000 molecular weight which we now term P92. During passage, however, production of the larger protein ceased, and only A-MuLV-specific polypeptides approximately 90,000 daltons in size could be detected. This cell line, after the change in expression had occurred, was used as a source of virus to prepare molecular clones of the A-MuLV genome (4). These experiments resulted in the isolation of two sets of clones: clones containing a full-length genome and shorter clones containing a 700-bp deletion in the *abl* region. It seemed likely that the A2 cell line contained two proviruses.

This explanation was confirmed by direct analysis of DNA from an early passage of A2 cells. Digestion with *Xba*I showed the presence of two fragments (Fig. 1C). One was 5.5 kb, like those proviral fragments in cells producing P120, P100, or P90; the other was 4.8 kb. An identical pattern was found in a late-passage strain of A2 cells that had been superinfected with M-MuLV (A2/M cells); in this strain the change of P120 expression to P90 expression had occurred. We conclude that two proviral genomes were present in A2 cells and that the alteration that occurred during the switch from P120 to P90 production was not detectable as a size change in the A-MuLV DNA.

Isolation of new virus strains producing P90 and P92, a defective transforming protein. To separate the two viruses in A2 cells, the virus rescued from the cells was used to infect NIH/3T3 cells (multiplicity of infection of 0.3 focus-forming units/cell), and the cells were cloned in microtiter dishes immediately after infection. After 10 days, the cloned cell lines were scored for the presence of helper virus and for the transformed morphology characteristic of Abelson-infected NIH/3T3 cells.

Twenty nonproducer clones with a grossly transformed morphology were examined for the size of their A-MuLV proviral DNA. All clones contained the larger A-MuLV viral genome (Fig. 2A shows 11 of these). Many, but not all, of these clones also contained the smaller viral genome; those clones with the small provirus were indistinguishable in morphology from clones not carrying the small provirus. We conclude that the large genome was sufficient for effecting morphological transformation of these cells and that the small viral genome present in A2 cells was not essential for the process. All clones tested produced an A-MuLV-specific protein of approximately 90,000 daltons, confirming that the larger genome in A2 cells encoded this protein. We term this protein P90A to distinguish it from another 90,000-molecular-weight variant isolated independently (see Table 1). The protein size and the genome size of these independently arising A-MuLV strains were indistinguishable.

From the cell lines infected with A2-derived virus, 18 morphologically normal clones were screened for the presence of A-MuLV-specific protein by immunoprecipitation. As expected, most were apparently uninfected; two cell lines, however, were detected which produced a 92,000-molecular-weight A-MuLV-specific protein. Analysis of the DNA of these cell lines revealed that only the smaller viral genome was present. An example of one of the lines, F1-3, is shown in Fig. 2B; one of the morphologically transformed clones containing both proviruses, isolated from the same infection, Tx-15, is shown for comparison. Thus, the deleted genome was defective for morphological transformation of NIH/3T3 cells; we therefore term this viral strain A-MuLV(P92*td*). Further analysis of the activity of this protein indicated that the protein kinase activity associated with other A-MuLV proteins was lacking in this protein (23). Circular DNA from the A-MuLV(P92*td*) strain has been molecularly cloned, and the 700-base deletion has been localized to the 5′ half of the *abl* region (4; see Fig. 6).

Genome structure of proviruses in lymphoid cells. To examine the genome structure of the A-MuLV variants in transformed lymphoid cells, DNA was isolated from a variety of cloned cell lines prepared by infection of bone marrow cells in vitro. The DNAs were cleaved with *Xba*I and *Kpn*I as before to release a proviral DNA fragment whose size was characteristic of the viral strain. The DNA was separated by electrophoresis and hybridized with the *abl*

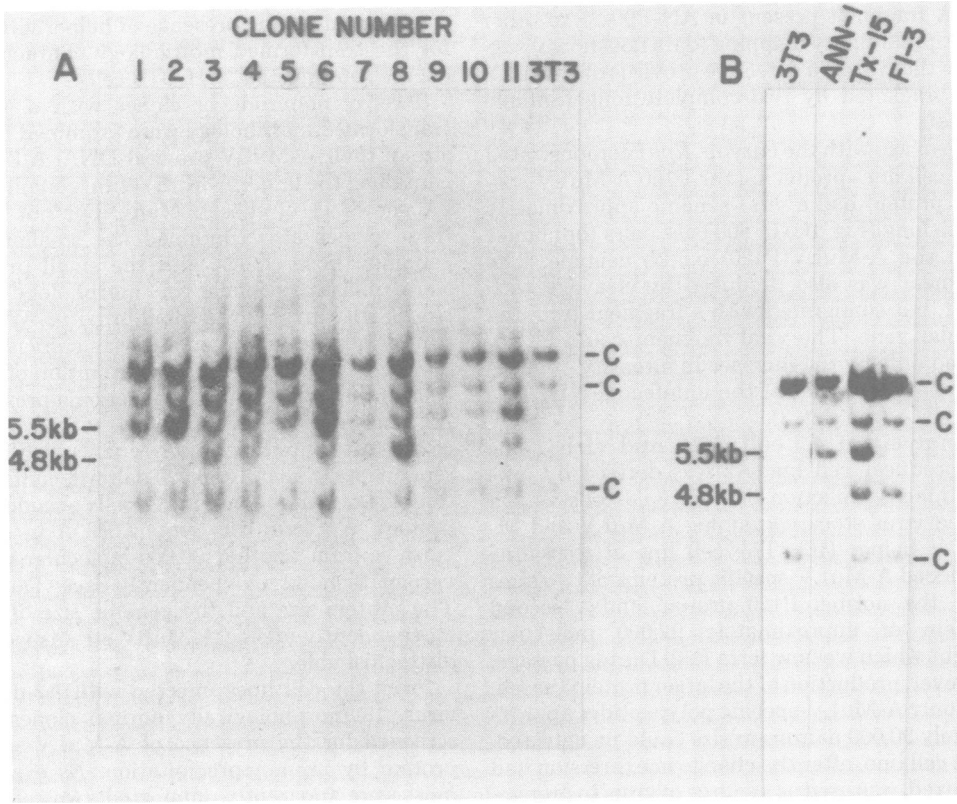

FIG. 2. *Identification of the two genomes cloned from A2 cells. (A) The DNAs were isolated from morphologically transformed cell lines clonally infected with virus rescued from A2 cells. DNA from each cell line was cleaved with XbaI, displayed on an agarose gel, blotted, and hybridized as in Fig. 1. The DNA fragments present in uninfected cells are marked "C"; the sizes of the two A-MuLV proviruses are shown. (B) DNAs from the indicated cell lines were cleaved with XbaI and processed as in (A).*

probe. In all cases (Fig. 3A), the genome size was identical to that of the virus used to infect the cells.

The intensity of hybridization to the proviral DNA, in comparison with the hybridization to the endogenous cellular DNA fragments, suggested that these cells contained only a single provirus. To determine the number of proviruses more accurately, the DNAs were cleaved with enzymes which do not cut within the provirus; thus, the size of the provirus-containing DNA fragment produced should depend on the arrangement of restriction endonuclease cleavage sites in the cell DNA flanking the provirus. Previous work with other retroviruses has shown that they integrate into so many different sites that cutting outside the proviral DNA produces distinguishable fragment sizes from independent integration events (19). All of the A-MuLV-transformed lymphoid cells lines examined had only a single provirus-containing fragment after cleavage with *Eco*RI (Fig. 3B). Similar results were obtained after cutting the DNA with *Xho*I (data not shown). We conclude that the virus strains producing P90, P120, or P160 are all capable of transforming lymphoid cells without alteration in genome structure and by integration of only a single provirus.

Viral genomes in 18-48 cells. Examination of one transformed lymphoid cell line yielded a more complicated pattern. The virus-producing cell line 18-48 was prepared by infection of bone marrow cells in vitro with the P120 strain of A-MuLV, and it originally made P120 (25). After long-term passage, however, the line began to produce smaller A-MuLV-specific proteins (100,000 to 90,000 molecular weight) as well as the P120. Analysis of the DNA revealed two classes of proviruses: a provirus 5.5 kb in size, identical to that coding for the P120 or P90 proteins, and a second band 4.6 kb in length (Fig. 4). To determine which genome encoded the A-MuLV proteins, culture medium was harvested and used to clonally infect NIH/3T3 fi-

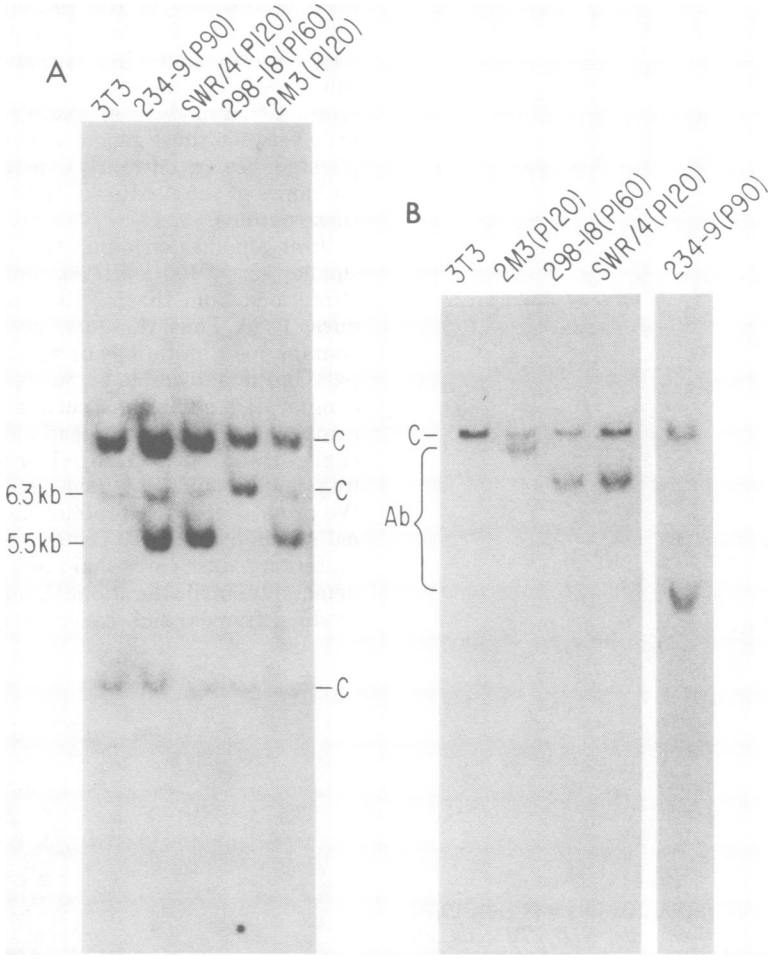

FIG. 3. *Analysis of proviral DNAs in lymphoid cell lines. (A) DNAs from the indicated cell lines were cleaved with XbaI, separated by electrophoresis, and hybridized with the abl-specific probe. The genome sizes are marked. (B) DNAs from the indicated cell lines were cleaved with EcoRI to release a fragment whose size was determined by the cellular sequences flanking the provirus. The DNAs were separated by electrophoresis and analyzed as in Fig. 1.*

broblasts. Six morphologically transformed clones were isolated and examined for the size of the A-MuLV-specific protein and the size of the A-MuLV provirus. All lines produced only a 90,000-molecular-weight protein termed P90B to distinguish it from the other independent proteins of this size (Fig. 5), and all contained only the 5.5-kb provirus (data not shown). We were not able to rescue the 4.6-kb provirus. We conclude that 18–48 originally contained an active P120 provirus 5.5 kb in length. During passage the virus switched to producing P90B without gross genomic changes, much as A2 cells did. Like A2 cells, 18–48 also carries a smaller provirus, but unlike that of A2 cells, this provirus apparently cannot be rescued and may not code for protein.

DISCUSSION

A remarkable number of active A-MuLV strains has been identified (14) which produce Abelson-specific proteins of different sizes (Table 1). The genome sizes of these viral strains have now been determined and are summarized in Fig. 6. The largest genome, 6.3 kb in length, codes for the largest Abelson protein, P160. This strain is found in cell lines made with virus originating in the laboratories of W. Rowe and M. Potter at the National Institutes of Health (7) and is widely used as a wild-type virus. A 5.5-

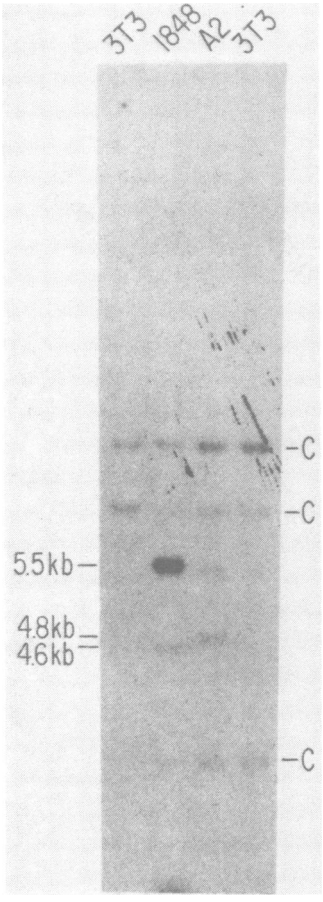

FIG. 4. *Analysis of provirus in 18–48 cells. DNAs from late-passage 18–48 cells, NIH/3T3 cells, and late-passage A2/M cells were cleaved with XbaI and analyzed as before. The sizes of the proviral DNAs are marked.*

kb genome, encoding the P120 protein, is found in the ANN-1 cell line and in most lines transformed by virus rescued from ANN-1. This strain is the wild-type virus from our laboratory. The genealogy of these strains is obscure: it seems likely that the P160 genome was the original isolate which subsequently suffered a deletion of 800 bp to give rise to the shorter P120 genome. We cannot rule out the possibility, however, that the P120 strain was the original virus and gave rise to the larger genome by a duplication or an insertion of 800 bp. There could even have been multiple A-MuLV's in the original isolate. Preliminary mapping (S. Latt, S. P. Goff, and D. Baltimore, unpublished data) places the difference between these strains near the center of the virus, within the portion of the *abl* region which encodes the protein. It should be noted that the difference in the size of the A-MuLV genomes roughly accounts for the difference in the size of the proteins. Thus, it is probable that the extra sequences of the P160 strain are translated in phase and that termination occurs at the same point as in the P120 strain. Other models are possible, and the difference between these genomes may be more complicated. We are currently constructing molecular clones of the A-MuLV(P160) genome to test these notions.

Viral genomes encoding two proteins of still smaller size, P100 and P90, had no detectable alterations from the 5.5-kb parent which produces P120. Thus, these two genomes probably contain point mutations or very small deletions (<50 bp) undetectable by our techniques. More complicated alterations such as inversions or combined deletions/duplications which do not change the size of *Xba*I, *Kpn*I, or *Sac*I restriction fragments would also have escaped detection. We propose that the proteins lack the C-terminal region of the P120 protein; either nonsense mutations cause early chain termination, or undetectably small alterations change translational reading frames and cause termination soon

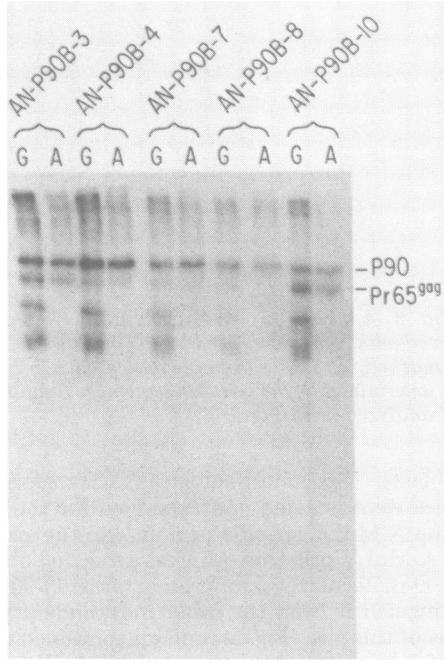

FIG. 5. *Electrophoretic analysis of the A-MuLV-specific proteins produced by clones of NIH/3T3 cells infected with late-passage 18–48 virus. Extracts from each line, labeled with [^{35}S]methionine, were immunoprecipitated with (lanes G) goat anti-MuLV antiserum (20) or (lanes A) mouse anti-AbT sera (1), and the precipitated proteins were separated on a 10% polyacrylamide gel and visualized by fluorography. Each clone contained an A-MuLV-specific protein of 90,000 daltons.*

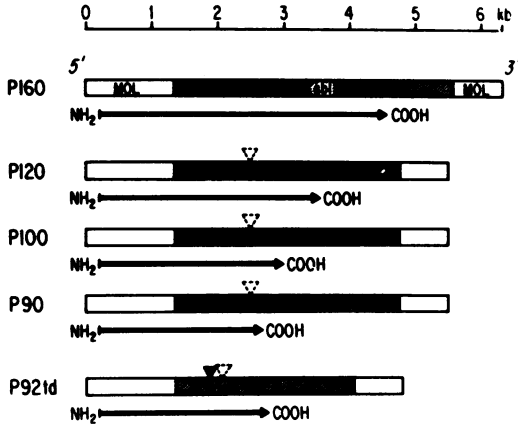

FIG. 6. *Structure of A-MuLV genomes. The open boxes represent M-MuLV-specific regions (MOL), and the shaded regions are the abl sequences for five different A-MuLV strains. The dark line under each represented genome shows the region encoding the A-MuLV polypeptide. The position of the sequence missing in all strains except the A-MuLV(P160) is not known accurately and is designated by a broken triangle. The position of the additional deletion found in the A-MuLV(P92td) strain is marked by a solid triangle.*

thereafter. If this is the correct interpretation, the C-terminal 30,000 daltons of the P120 is unnecessary for the morphological transformation of fibroblasts. It should be noted that it has been shown that the P90 virus, while retaining some of its transforming activity, has a reduced ability to transform lymphoid cells (14) and also has a reduced activity in the in vitro protein kinase activity as compared with the P120 parental strain (13). In spite of these defects, when A-MuLV(P90) does successfully transform lymphoid cells, it accomplishes the feat with only a single integrated provirus (Fig. 3). The mutation which gave rise to the P90 protein apparently can occur with a high frequency because two other independent events yielded an apparently identical variant, the P90A strain found in late-passage A2 cells and the P90B strain found in late-passage 18–48 cells.

Probably the most significant viral strain analyzed here is the transformation-defective virus A-MuLV(P92*td*). NIH/3T3 cell lines carrying this virus do not show the characteristic transformed morphological changes of other A-MuLV-transformed lines (23). The virus is unable to transform lymphoid cells in vivo or in vitro, and the P92 protein produced has no associated protein kinase activity, although this protein can act as an acceptor for phosphate if mixed with a wild-type A-MuLV protein (23). The deletion within this strain suggests that the P120 protein is essential for transformation. The mapping of this 700-bp deletion (4) places the alteration within the region normally encoding the P120 protein; moreover, the size of the deletion accounts for the smaller size of the P92 protein. The deletion therefore probably does not disturb the translational reading frame and allows read-through to a termination codon at the normal position. The region deleted corresponds to a stretch of amino acids 50,000 to 78,000 daltons from the N terminus of the P120. Whereas the C-terminal 30,000 daltons of protein is apparently dispensible for at least some of the P120 function [as shown by the A-MuLV(P90) strains], this internal region cannot be removed without complete loss of function.

It is worth noting the surprising plasticity of the A-MuLV protein and its remarkable ability to retain at least some function even after the loss of large segments of protein. If the P160 protein is assumed to be the true parental viral protein, then 70,000 daltons—more than half of the 120,000 to 130,000 daltons encoded by the *abl* region—is dispensible. Perhaps virus-induced transformation requires only one of the functions normally performed by this cellular protein. No mutations have yet been found in the *gag*-specific portion of the Abelson protein; the function performed by this region is therefore unknown.

Many of the viral variants isolated were derived after passage of virus stocks to fresh cells (e.g., those in A2 cells). These mutations probably arose upon reverse transcription of viral RNA or at some other early stage of infection (17). Many others arose by spontaneous mutation in integrated proviruses during cell passage (e.g., those viruses coding for the P100 and P90 proteins). The appearance of these variants is unexpected since mutations arising in proviruses depend on errors occurring during cell passage probably during cell-directed DNA replication; this should be a very infrequent event. These mutants may have been exposed inadvertently to a selective pressure that favored the accumulation of rare variants. It is possible, for example, that the presence of full-length P120 protein is deleterious to the growth of fibroblasts in a way that the P90 and P100 proteins are not.

Lymphoid cell lines derived by in vitro infection of bone marrow cells apparently carry proviruses whose size (characteristic of the A-MuLV strain) is identical to that of the proviruses in fibroblast lines. Thus, although the successful transformation of bone marrow cells is less efficient than that of NIH/3T3 cells (10), no gross alteration of viral structure is required to effect the rarer event. In most cases examined, the cells carry a single provirus, proving that a single integration event can suffice to transform

and immortalize the cell. Similar results have been obtained from analyses of the avian sarcoma proviruses (5). Thus, the requirement for specific helper viruses for A-MuLV lymphoid cell transformation (11) cannot be a consequence of the helper catalyzing multiple integrations of the A-MuLV genome.

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