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Nucleic Acid Sequence and Oncogenic Properties of the HZ2 Feline Sarcoma Virus v-*abl* Insert

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Hardy-Zuckerman 2 feline sarcoma virus (HZ2-FeSV), isolated from a multicentric feline fibrosarcoma is a replication-defective acute transforming feline retrovirus which originated by transduction of feline c-*abl* sequences with feline leukemia virus (FeLV) and is known to encode a 110-kilodalton *gag-abl* fusion protein with tyrosine-specific protein kinase activity (P. Besmer, W. D. Hardy, E. E. Zuckerman, P. J. Bergold, L. Lederman, and H. W. Snyder, Nature (London) 303:825–828, 1983). The nucleotide sequence of the *abl* segment in the HZ2-FeSV genome was determined and compared with the murine and human v-*abl* and c-*abl* sequences. The predicted transforming protein consists of 344 amino acids (aa) of FeLV *gag* origin, 439 aa of *abl* origin, and at least 200 aa of FeLV *pol* origin (p110$^{gag\text{-}abl\text{-}pol}$). The 1,317-base-pair HZ2-FeSV v-*abl* segment (fv-*abl*) corresponds to 5′ *abl* sequences which include the region known to specify the protein kinase domain. The 5′ 189 base pairs of fv-*abl* correspond to 5′ c-*abl* sequences not contained in Abelson murine leukemia virus (MuLV) v-*abl*. The mouse c-*abl* exon which contains these segments was identified, and its nucleotide sequence was determined. Comparison of the predicted amino acid sequence of fv-*abl* with those of Abelson MuLV v-*abl* and c-*abl* revealed five aa differences. The 5′ junction between FeLV and *abl* was found to involve a preferred region in FeLV *gag* p30 (P. Besmer, J. E. Murphy, P. C. George, F. H. Qiu, P. J. Bergold, L. Lederman, H. W. Snyder, D. Brodeur, E. E. Zuckerman, and W. D. Hardy, Nature (London) 320:415–421, 1986). A six-base homology exists at the recombination site between the parental FeLV and the c-*abl* sequences. The 3′ junction between fv-*abl* and FeLV *pol* predicts an in-frame fusion of fv-*abl* and FeLV *pol*. A transformed cell line containing a truncated *gag-abl-pol* protein, p85, that lacks most of the FeLV *pol* sequences was obtained by transfection of NIH 3T3 mouse cells. This result implies that the *pol* sequences of the p110$^{gag\text{-}abl\text{-}pol}$ protein are dispensable for fibroblast transformation. To assess whether the fv-*abl* segment specifies the unique biological properties of HZ2-FeSV, we constructed a Moloney MuLV-based version of HZ2-FeSV, Mo-MuLV(fv-*abl*), in which the fv-*abl* sequences were contained in a genetic context similar to that in HZ2-FeSV. When injected into neonatal NSF/N mice, MoMuLV(fv-*abl*) amphotropic pseudotype virus induced lymphosarcoma of B-cell origin with kinetics similar to those of the disease caused by Abelson MuLV variants which, like HZ2-FeSV, lack 3′ c-*abl* sequences. These results suggest that the fv-*abl* segment does not specify an altered oncogenic potential.

Hardy-Zuckerman 2 feline sarcoma virus (HZ2-FeSV) is a replication-defective acute transforming feline retrovirus which was isolated from a feline leukemia virus (FeLV)-associated feline multicentric fibrosarcoma (9, 11). HZ2-FeSV was generated by transduction of feline c-*abl* sequences with FeLV. The genome of HZ2-FeSV is 6.8 kilobases (kb) and has the organization 5′ *gag-abl-pol-env* 3′. The *abl* sequences in HZ2-FeSV, a segment of 1.4 kb, are known to be homologous with the sequences which specify the protein kinase domain of *abl* (P. J. Bergold, J. Y. J. Wang, W. D. Hardy, V. Littau, E. Johnson, and P. Besmer, Virology, in press). Cells infected by the virus express a 6.3-kb genome RNA and a 2.3-kb subgenomic RNA which represents a deleted *env* mRNA, as well as a 110-kilodalton *gag-abl* polyprotein with associated tyrosine-specific protein kinase activity. In vivo, HZ2-FeSV is thought to be involved in sarcomagenesis, and in vitro the virus transforms fibroblast cell lines (11; Bergold et al., in press).

Abelson murine leukemia virus (Ab-MuLV) originated by transduction of murine c-*abl* sequences by Moloney MuLV (Mo-MuLV) (1, 2, 23). The genetic structure of Ab-MuLV is 5′ *gag-abl-env* 3′. The Ab-MuLV *gag-abl* transforming protein, like the HZ2-FeSV *gag-abl* protein and other related transforming proteins such as pp60^{src} of Rous sarcoma virus, exhibits tyrosine-specific protein kinase activity (31, 63–65). Ab-MuLV was isolated from a nonthymic lymphosarcoma, and in vivo the virus induces lymphoma preferentially of a pre-B-cell phenotype (43, 44). T-cell lymphoma, mastocytoma, plasmacytoma, and myelomonocytic leukemias, however, are induced by Ab-MuLV as well (43, 44, 60). In vitro, Ab-MuLV transforms pre-B lymphocytes, mast cells, and fibroblasts (38, 44, 45). Ab-MuLV also transforms certain interleukin 3-dependent cells to interleukin 3 independence, and furthermore it can stimulate erythroid precursor cells to proliferate and differentiate (16, 36, 38, 56). Genetic analysis of Ab-MuLV v-*abl* (mv-*abl*) has indicated that the protein kinase activity is necessary for transformation of hematopoietic cells and fibroblasts (40, 64); in con-

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trast, 3′ *abl* sequences are dispensable for transformation of fibroblasts; however, their loss modulates in vivo and in vitro transformation potentials for lymphoid cells (40, 44).

c-*abl* is also known to be involved in the 9,22 Philadelphia translocation, which is associated with human chronic myelogenous leukemia. As a result of the 9,22 translocation, the chromosome 22 *bcr* gene is fused to 5′ c-*abl* sequences, and a hybrid protein product with an associated tyrosine protein kinase activity is found in cells carrying this translocation (8, 27, 26, 50).

HZ2-FeSV and Ab-MuLV have different tumor origins, and the two viruses display different specificities for in vivo transformation targets. To investigate the molecular basis of the differing biological properties of the two viruses, we undertook a detailed structural analysis of the HZ2-FeSV genome. The molecular cloning and analysis of the integrated HZ2-FeSV provirus is described elsewhere (Bergold et al., in press). Herein we report the determination of the primary nucleic acid sequence of the fv-*abl* segment. We found that all the HZ2-FeSV-specific sequences derive from feline c-*abl*, including 5′ *abl* sequences which are not contained in mv-*abl*. The nucleic acid sequence analysis also indicated that the HZ2-FeSV transforming protein contains *gag* and *abl*, as well as *pol*, sequences. To investigate whether the fv-*abl* segment specifies the unique biological properties of HZ2-FeSV, we constructed an Mo-MuLV-based version of HZ2-FeSV. The results of in vivo studies in mice suggested that fv-*abl* by itself does not determine an altered oncogenic specificity.

MATERIALS AND METHODS

Cells and viruses. HZ2-FeSV-infected CCL64 mink cells (11), ANN-1 cells (Ab-MuLV P120 nonproducer cells) (48), NIH 3T3 cells (28), and NIH 3T3 transformants, as well as amphotropic murine leukemia virus (292 amph-MuLV [41])-producing NIH 3T3 cells were grown in Dulbecco modified Eagle medium supplemented with 10% calf serum. Transformation assays were done as described previously, with NIH 3T3 cells as indicators, and amph-MuLV titers were determined by using FG10 S^+L^- indicator cells (6, 10). Transfection of NIH 3T3 cells was as described previously (12, 25). Various amounts of DNA (1 to 100 ng) were added to 30 μg of sheared NIH 3T3 carrier DNA for transfection. Foci of transformed cells were observed after 10 to 14 days. For analysis, foci of transformed cells were picked with glass cylinders and grown to mass culture.

Cloned DNAs. Molecular cloning of the integrated HZ2-FeSV provirus from a clonally derived HZ2-FeSV-infected mink cell line is described elsewhere (Bergold et al., in press). The plasmid pHZ2 contains the 12-kb integrated HZ2-FeSV provirus in the vector pBR322. pHZ3.8 contains the 3.8-kb *Hin*dIII fragment, 1.7 to 5.5 kb, of the HZ2-FeSV genome, which includes the fv-*abl* segment in pBR322. p*Pst*0.6 is a 5′ murine c-*abl* fragment which contains the exon with the 5′ recombination site of Ab-MuLV (57). pE3.7 is the murine 5′ 3.7-kb *Eco*RI c-*abl* fragment which contains the upstream exon and was generously provided by J. Wang (57). The J_{11} probe, which is specific for the immunoglobulin heavy-chain J_3 and J_4 regions, was obtained from J. Stavnezer (32).

Bacterial cells, plasmids, and viruses. Growth of bacteria, plasmid preparations, and transformations were done as described previously (14). The M13 phages mp10, mp18, and mp19 were grown in *E. coli* JM103 and JM107 (66).

DNA sequence analysis. Nucleic acid sequences were determined by the chain termination method of Sanger et al. (47) after subcloning of fragments into the phage M13 vectors mp10, mp18, and mp19 (66).

Analysis of cellular DNAs. High-molecular-weight DNAs from tissue culture cell lines were prepared as described previously (14). For preparation of DNAs from murine tumors, spleen, and kidney, tissue was Dounce homogenized in TNE (10 mM Tris, 100 mM NaCl, 10 mM EDTA, pH 8.0), and nuclei were collected by low-speed centrifugation. DNA then was prepared from nuclei by the proteinase K-sodium dodecyl sulfate method as described previously (14). For gel analysis, 15 μg of high-molecular-weight DNA was digested with the appropriate restriction enzyme, fractionated in 1% agarose, transferred to Zetabind nylon membranes, and hybridized with ^{32}P-labeled probes as described previously (14). When rehybridizing Southern blots, we first washed the blots in 0.4 M NaOH at 42°C (30 min) and then neutralized them in 0.2 M Tris–0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (pH 7.5)–0.5% sodium dodecyl sulfate at 42°C (30 min) and 0.1× SSC at 25°C. Hybridization then was carried out as usual.

Protein analysis. Extracts of cells labeled with [3H]leucine were immunoprecipitated and analyzed by sodium dodecyl sulfate-polyacrylamide gel-electrophoresis as described previously (51). Rabbit anti-FeLV p27 antiserum was obtained from W. D. Hardy (Sloan-Kettering Institute).

Construction of Mo-MuLV(fv-*abl*). An Mo-MuLV mutant, pMo-MuLV(Xo) (see Fig. 5), which was obtained by introduction of an *Xho* linker at the *Hpa*I site at 7.6 kb of the Mo-MuLV map and deletion of the sequences between the *Xho*I site at 1.6 kb and the new *Xho*I site at 7.6 kb, was generously provided by S. Brigham and S. P. Goff, Columbia University (unpublished data). pMo-MuLV(Xo) was digested with the restriction enzyme *Xho*I, the protruding ends were filled in with T4 DNA polymerase in the presence of deoxynucleoside triphosphates, and the resulting product was dephosphorylated with calf intestinal phosphatase. The 3.8-kb *Hin*dIII fragment, 1.7 to 5.5 kb of the HZ2-FeSV map, which includes *gag* p30, fv-*abl*, *pol*, and *env* sequences, was obtained by digestion with *Hin*dIII, and the protruding ends were filled in by T4 DNA polymerase. The modified 3.8-kb *Hin*dIII fragment then was inserted into the modified pMo-MuLV(Xo) by blunt-end ligation and used to transform E. coli HB101. Plasmid DNAs obtained from colonies were then screened for the presence of the 3.8 kb *Hin*dIII fragment in the appropriate orientation (the *Hin*dIII sites were regenerated as part of the reaction scheme).

Animal inoculations and histopathology. NFS/N mice were obtained from a colony maintained at the Sloan-Kettering Institute (P. V. O'Donnell). Neonatal mice (less than 48 h old) were inoculated intraperitoneally with 100 μl of titered cell-free virus stock with 30-gauge needles. Mice were checked daily for unusual breathing patterns, hindleg paralysis, and enlargement of spleen or lymph nodes. If animals were ill, they were sacrificed with CO_2 and autopsied. All organs were examined, and portions were prepared for histology. Tumor tissue was frozen at −70°C, and DNA was prepared as described above.

RESULTS

HZ2-FeSV encodes a protein with *gag*, *abl*, and *pol* sequences. The nucleotide sequence of the fv-*abl* segment was determined by the dideoxy chain termination method of Sanger et al. after subcloning of restriction fragments into the M13 vectors mp10, mp11, mp18, and mp19 (47, 66). The nucleotide sequence was determined on both strands, and

overlaps were obtained for all restriction sites. The nucleotide sequence of HZ2-FeSV from the *Hin*dIII site at 1.65 kb of the HZ2-FeSV restriction map to an *Alu*I site at 3.75 kb, 24 nucleotides 5′ of the *Kpn*I site (Fig. 1), is shown in Fig. 2. One open reading frame extends all the way from nucleotide −155.

To identify the 5′ recombination sites between FeLV and fv-*abl*, the sequence was compared with the known FeLV *gag* genome. Likewise, the approximate 3′ recombination site between fv-*abl* and FeLV *pol* was established by comparing the HZ2-FeSV sequence with mv-*abl* and Mo-MuLV *pol* (42, 49). The 3′ recombination site in FeLV *pol* corresponds to Mo-MuLV sequences 534 nucleotides from the reverse transcriptase-endonuclease boundary in the *pol* gene (Fig. 2; 18).

On the basis of the known FeLV *gag* nucleic acid sequence and the observation that the 5′ 1.9 kb of the HZ2-FeSV genome is colinear with the Gardner-Arnstein FeLV (GA-FeLV) genome, the *gag*-derived segment in the HZ2-FeSV *gag-abl* protein is predicted to contain 344 amino acids with sequences of p15, p10, and 148 amino acids of p30 (30). The fv-*abl* segment consists of 1,317 nucleotides and specifies 439 amino acids. At the 3′ end, the fv-*abl* open reading frame extends into the correct FeLV *pol* reading frame for at least 618 nucleotides. This presumption was established by comparison of the HZ2-FeSV *pol*, the known GA-FeLV *pol*, and the Mo-MuLV *pol* sequences (Fig. 3C).The known size of 110 kilodaltons for the HZ2-FeSV *gag-abl* protein predicts a site for termination of translation which is not too far beyond the HZ2-FeSV *pol* sequences we had determined.

By Southern blot analysis, the fv-*abl* insert has previously been shown to contain 5′ *abl* sequences (Bergold et al., in press). Comparison of the fv-*abl* sequence with mv-*abl* and c-*abl* indicated homology with 5′ c-*abl* sequences which are not contained in mv-*abl* (189 nucleotides) and with 5′ sequences of mv-*abl* which include the sequences of the tyrosine kinase domain (1,127 nucleotides). The 5′ 189-nucleotide segment of fv-*abl* derives, on the one hand, from the exon which contains the 5′ mv-*abl* recombination site and, on the other hand, from another c-*abl* exon which is approximately 400 nucleotides upstream (Fig. 3A and B) (57). We identified the upstream exon sequences by hybridization of blots containing mouse 5′ c-*abl* DNA digested with the restriction enzyme *Pst*I with an *Hin*dIII-*Alu*I probe derived from nucleotides −156 to 111 (Fig. 1). The nucleotide sequence of the 0.28-kb *Pst*I fragment which contained these fv-*abl*-related sequences was determined and is shown in Fig. 3B. The 3′ end of the *abl* homology in this sequence is bounded by a splice donor.

The fv-*abl* and mouse c-*abl* and v-*abl* nucleic acid sequences are contiguous, and only five differences exist among the amino acid sequences which are specified by them. This result indicates that fv-*abl* originated from the feline *abl* locus and demonstrates that feline and mouse *abl* are highly conserved genes.

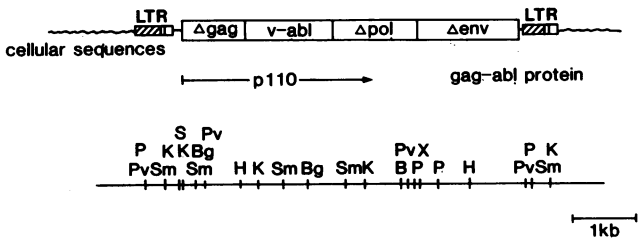


FIG. 1. Genetic structure and restriction map of the integrated HZ2-FeSV provirus. Abbreviations for restriction enzymes: B, *Bam*HI; Bg, BglII; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I; Sm, *Sma*I; X, *Xho*I.

Comparison of the protein sequences of the transmembrane receptor kinases with those of the *src-abl* family has enabled identification of a common enzymatic domain, TPK (tyrosine protein kinase), which includes a nucleotide binding site (20, 33, 39, 58, 67). With fv-*abl*, the protein kinase domain includes nucleotides 540 to 1305. The 3′ fv-*abl* boundary in HZ2-FeSV is 4 amino acids downstream of the 3′ boundary of the protein kinase domain. The 3′ fv-*abl* boundary, therefore, very accurately delineates the 3′ end of the protein kinase domain of fv-*abl*.

Homology has been noted previously for the N-terminal sequences of v-*abl* and v-*src* (nucleotides 190 to 540 of fv-*abl*) (7, 57). Homology between *src* and *abl* also exists in the 5′ sequences of fv-*abl* which are not present in mv-*abl*. The N termini of *abl* and the *src* family, which includes the oncogenes *yes* and *fgr*, then have distinctive common structural features. The functional significance of this relationship, however, is currently not known.

A major difference between the *abl* and *src* genes is their C-terminal domains. The *abl* C-terminal domain contains 845 amino acids; in contrast, the c-*src* C terminus consists only of 24 amino acids (17, 19, 58). In HZ2-FeSV, the entire C-terminal domain of c-*abl* (843 amino acids) is missing. Instead, fv-*abl* is fused to 3′ *pol* sequences, and an open reading frame in the *pol* gene is sustained for more than 600 nucleotides. Evidence suggesting that the p110 transforming protein contains *pol* sequences was obtained in the following way. Analysis of the p110 *gag-abl* transforming protein in cells obtained by transfection of NIH 3T3 cells with pHZ2-FeSV DNA and subsequent cloning of foci of transformed cells, revealed one transformant (PB8) which expressed a 90-kilodalton *gag-abl* protein much rather than the wild-type p110 protein (Fig. 4A). The PB8 cells which contained the mutant p90 protein displayed morphological transformation indistinguishable from that of wild-type transformants. To investigate the basis of the alteration in the *gag-abl* protein, we determined by blot hybridization whether proviral sequences were altered in these cells (Fig. 4B). In the DNA from wild-type HZ2-FeSV, PB8 and nontransformed control cells, the v-*abl* probe detected restriction fragments that derive from c-*abl*; in HZ2-FeSV-transformed cells, in addition, virus-specific fragments of 1.9 and 1.3 kb (*Sma*I) and 2.2 kb (*Kpn*I) were seen (Fig. 4B, lanes 6 and 9). In contrast, in PB8 cell DNA, virus-specific fragments of 1.9 and 0.9 kb (*Sma*I) and 3.8 kb (*Kpn*I) were found (Fig. 4B, lanes 7 and 10). This result indicated that, in the HZ2-FeSV provirus, 3′ proviral sequences are altered. A new *Sma*I site was found approximately 50 to 100 nucleotides 3′ of the *abl-pol* boundary and, where investigated, restriction sites of the HZ2-FeSV provirus 3′ of this new site were found to be altered (data not shown), suggesting that the entire 3′ end of the provirus was missing. In agreement with this interpretation, no focus-forming virus was obtained upon rescue from the PB8 cells with amph-MuLV. Taken together, our analyses suggested that the missing sequences in the mutant p90 protein are of *pol* origin and are not essential for transformation of fibroblasts.

Construction of Mo-MuLV(fv-*abl*). HZ2-FeSV was originally obtained from a cat with multicentric fibrosarcoma and is thought to be involved in sarcomagenesis (11). It is, however, not known why the feline *abl* virus is involved in sarcomagenesis much rather than leukemogenesis. Several

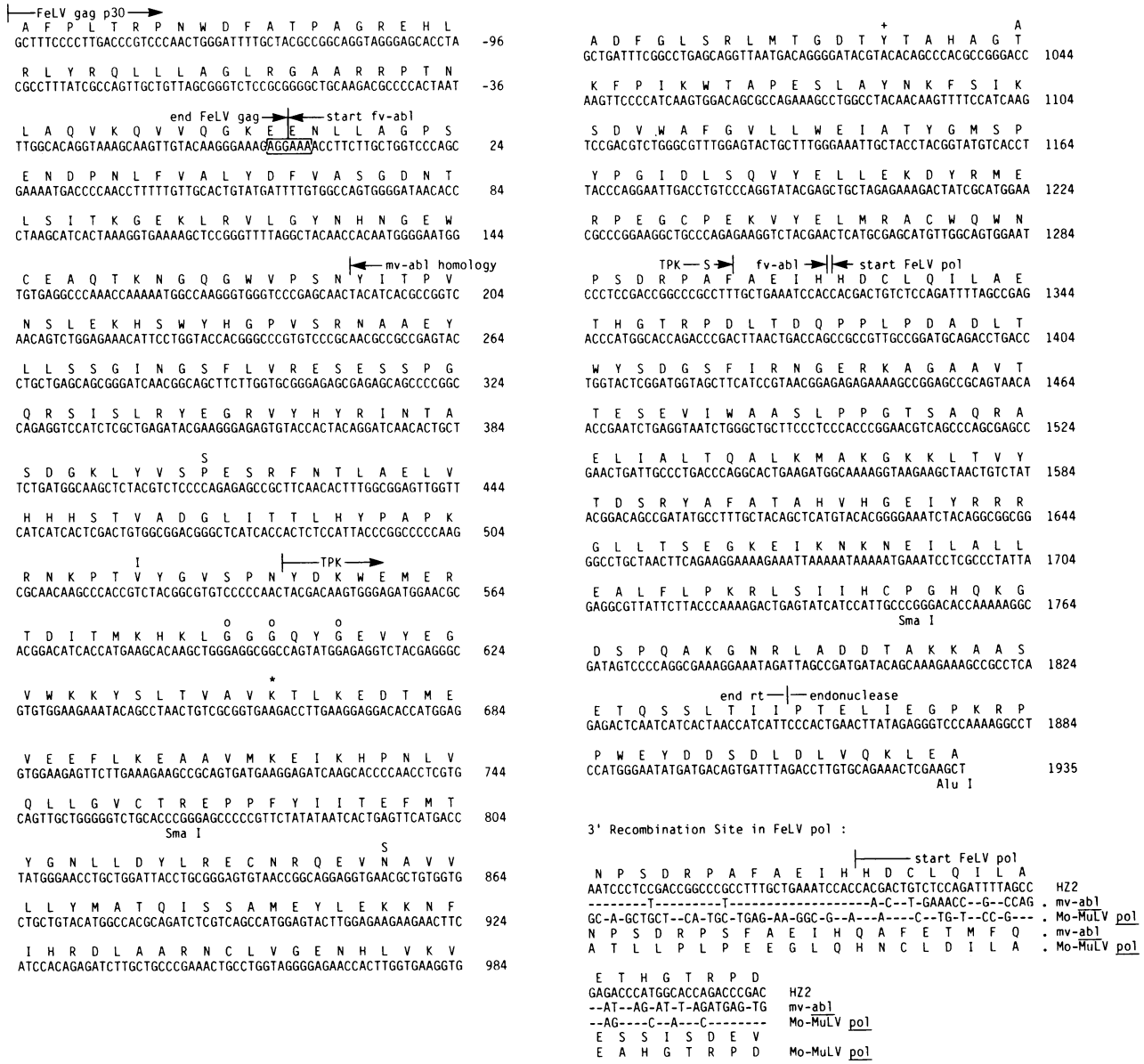
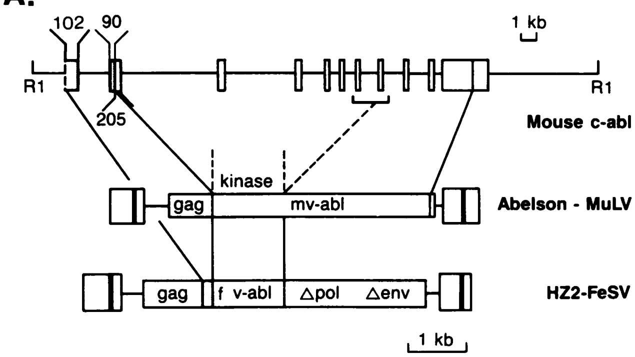


FIG. 2. Nucleotide sequence and predicted amino acid sequence of fv-*abl*. The complete nucleotide sequence of the fv-*abl* region, 1.8 to 3.9 kb of the HZ2-FeSV genome, was determined by the dideoxynucleotide chain termination method of Sanger et al. after subcloning of fragments into the M13 vectors mp10, mp18, and mp19 (47, 66). The predicted amino acid sequence of the only long open reading frame is shown above with single-letter amino acid notations. The numbers at the end of each line refer to nucleotides, with position +1 defining the beginning of fv-*abl*. The nucleotide sequence was determined on both strands, and all restriction site were crossed. The 5′ recombination site between *gag* and fv-*abl* was determined by comparison of the *gag-abl* sequence with that of the known FeLV *gag* sequences and the sequence of the upstream mouse c-*abl* exon (Fig. 3B). To determine the 3′ recombination site between fv-*abl* and FeLV *pol*, we compared the *gag-abl-pol* sequence with mv-*abl* and Mo-MuLV *pol*. Symbols: *, The lysine residue whose position aligns with a lysine in the nucleotide-binding site of protein kinases (67); +, the tyrosine residue whose position aligns with Tyr-416 of pp60$^{v\text{-}src}$.

possibilities exist for the differing oncogenic properties of HZ2-FeSV and Ab-MuLV: the fv-*abl* segment, the HZ2-FeSV long terminal repeat sequences, and the FeLV helper virus are possible determinants of the oncogenic properties of HZ2-FeSV. We attempted to investigate whether the structural differences between fv-*abl*, including the 3′ *pol* segment, and mv-*abl* determine the altered biological properties of HZ2-FeSV. Ab-MuLV originated by transduction of murine c-*abl* with Mo-MuLV (1, 2, 23). An Mo-MuLV containing the chicken v-*src* gene has recently been constructed and found to be sarcomagenic in mice (21). Mo-MuLV, therefore, appeared to be a good vector to investigate whether the fv-*abl* segment determines sarcomagenicity in mice. An Mo-MuLV-based virus was constructed in which the fv-*abl* sequences were contained in a similar genetic context as in HZ2-FeSV. The scheme for construction of Mo-MuLV(fv-*abl*) is shown in Fig. 5. The MuLV *gag* p30 sequences and FeLV p30 sequences are spliced such that a four-amino-acid duplication is generated. The transforming protein in this virus consists of 315 amino acids of MuLV *gag*, 52 amino acids of FeLV p30, 439 amino acids of fv-*abl*, and more than 200 amino acids of the FeLV *pol*

A.



B.

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 L  Q  R  P  V  A  S  D  F  E  P  Q  G  L  S  E  A  A  R  W    60
CTGCAGAGGCCAGTGGCATCTGACTTTGAGCCCCAGGGTCTCAGCGAAGCAGCTCGATGG   c-abl
Pst I
      |<-start fv-abl homology
 N  S  K  E  N  L  L  A  G  P  S  E  N  D  P  N  L  F  V  A   120
AACTCCAAGGAAAACCTTCTTGCTGGGCCCAGTGAAAATGACCCCAACCTTTTTGTGGCA   c-abl
GGGAAAG------------------T-----C-----------------------T---   HZ2

                                     exon boundary ->|
 L  Y  D  F  V  A  S  G  D  N  T  L  S  I  T  K               180
CTCTATGATTTTGTGGCCAGTGGAGATAACACTCTCAGCATCACTAAAGGTAAAGGAGAG   c-abl
--G-------------------G--------C--A---------------G--AAGCTC   HZ2
                                                              240
ATGGCAAGCAAAGGGGGGGGGTGGTGGCTCTAGGTGGGCCAGCCCTTTGCTCCCAAGACC   c-abl
CG--TTTTAGGCTACAACCACAAT                                      HZ2
                                      277
TTTATAATTTGAGAGCAGAAATGCCCTGCAG        c-abl
                         Pst I
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C.

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                                                           49
HZ2 pol  HDCLQILAETHGTRPDLTDQPPLPDADLTWYSDGSFIRNG ERKAGAAVTT
Mo pol   -N--D----A----------- -----H---T---SLLQEGQ---------

                                                          100
HZ2 pol  ESEVIWAASLPPGTSAQRAELIALTQALKMAKGKKLTVYTDSRYAFATAH
Mo pol   -T-----KA--A------------------E----N------------

                                                          150
HZ2 pol  VHGEIYRRRGLLTSEGKEIKNKNEILALLEALFLPKRLSIIHCPGHQKGD
Mo pol   I--------------------D------K-------------------H
GA pol                                                ------

                       rt --+-- endonuclease       198
HZ2 pol  SPQAKGNRLADDTAKKAA SETQSSLTIIPTELIEGPKRPPWEYDDSDL
Mo pol   -AE-R---M--QA-R---IT--PDTS-LL I-NSSPYTSEHFH-TVT-IK
GA pol   ------------------ T--H----VL----------L--------

              206
HZ2 pol  DLVQKLEA
Mo pol   --T --G-
GA pol   --------
```

FIG. 3. Sequence comparisons. (A) Schematic comparison of the HZ2-FeSV genome with that of Ab-MuLV and the mouse c-*abl* locus. The shaded area indicates the upstream *abl* sequences unique to fv-*abl*. (B) Nucleotide sequence of the upstream mouse c-*abl* exon, which contains the 5′ sequences of fv-*abl*. The predicted amino acid sequence is shown above the nucleotide sequence in single-letter notations. The extent of homology with fv-*abl*, as well as the 3′ exon boundary, is indicated. (C) Comparison of HZ2-FeSV *pol*, Mo-MuLV *pol*, and GA-FeLV *pol* amino acid sequences. The HZ2-FeSV *pol* amino acid sequence corresponding to nucleotides 1317 to 1935 (Fig. 2) is shown in the top line. The corresponding Mo-MuLV *pol* sequence, nucleotides 4074 to 4691, and the known GA-FeLV *pol* sequences are shown below (13, 49, 58; unpublished data). Amino acid identities are indicated by a dash, and gaps are indicated by empty spaces.

origin. Foci of transformed cells characteristic of Ab-MuLV and HZ2-FeSV were obtained upon transfection of NIH 3T3 cells with Mo-MuLV(fv-*abl*) DNA, and Mo-MuLV(fv-*abl*)(amph-MuLV) pseudotype virus stocks with titers of 1.3 $\times 10^5$ focus-forming units per ml were generated by infection of NIH 3T3 transformants with amph-MuLV.

Oncogenic potential of Mo-MuLV(fv-*abl*) in mice. We investigated the oncogenic properties of Mo-MuLV(fv-*abl*) in mice. Most studies with Ab-MuLV in the past were done with Mo-MuLV as a helper virus. Mo-MuLV is a potent leukemogenic agent with an average latency of 90 days. Amph-MuLV is a good helper virus for Ab-MuLV-induced lymphoma as well (Table 1; N. Rosenberg, personal communication). In contrast to Mo-MuLV, by itself, amph-MuLV induces neoplasms only rarely and after a long latency period (more than 6 months) (Table 1; 22, 41). To avoid the complication of helper virus disease, we used amph-MuLV as a helper virus much rather than Mo-MuLV. Neonatal (1 to 2 days old) NFS/N mice were inoculated with 1×10^3 to 3×10^3 focus-forming units of Mo-MuLV(fv-*abl*) (amph-MuLV) pseudotype virus stock. The results of this experiment are summarized in Table 1. Mo-MuLV(fv-*abl*)-infected animals began to die 29 days postinfection. The gross pathology and histopathology indicated lymphoblastoid lymphosarcoma as the cause of death. The peripheral lymph nodes were enlarged and filled with tumor cells; the spleens were enlarged and infiltrated with tumor cells; in some animals, large masses of tumor tissue surrounded the lumbar vertebrae, and the thymuses were normal. After 120 days, 50% of the animals had died (Table 1).

To establish a role for Mo-MuLV(fv-*abl*) in tumorigenesis in these animals, we analyzed the Mo-MuLV(fv-*abl*) proviruses in tumor DNA by blot analysis (Fig. 6). Southern blots containing DNAs from kidney (lanes 1 and 4), spleen (lanes 2 and 5), and tumor (lanes 3 and 6) digested with the restriction enzymes *Hin*dIII were hybridized with a ^{32}P-labeled 5′ mouse c-*abl* probe (Fig. 6A). This probe detected c-*abl* fragments found in both normal and tumor tissues and a 3.8-kb v-*abl* fragment of the Mo-MuL(fv-*abl*) genome that was found in tissues containing tumor cells. In both animals, the proviral fragment was found in DNAs from tumor tissue and spleen, and in animal 2 a faint v-*abl* band was seen in kidney DNA as well. These data demonstrated that the recombinant Mo-MuLV(fv-*abl*) virus is involved in the formation of these tumors, and they also indicated tumor involvement of the spleen and metastasis to other organs.

The Mo-MuLV(fv-*abl*) genome does not contain sites for the restriction enzyme *Eco*RI and, as a consequence, *Eco*RI restriction fragments which contain proviral integrations are detected only if they represent a fraction of at least 5 to 10% of the total; that is, the tumors are clonal in nature. To investigate the possibility that the tumors contained clonal proviral integrations, we hybridized a blot containing the DNAs, shown in Fig. 6A, digested with *Eco*RI, with the 5′ c-*abl* probe (Fig. 6B). Prominent c-*abl* bands of 6.0 and 2.3 kb were seen in all DNA samples, as well as in tumor-specific fragments. In tumor 2, at least four distinct fragments were seen, the most abundant one representing 20 to 25% of the total. The tumor-infiltrated spleen of the same animal contained fragments that were also found in the main tumor but in different proportions. These results indicated that the Mo-MuLV(fv-*abl*)-induced tumors are oligoclonal with regard to proviral integrations.

Ab-MuLV-induced tumors are mostly lymphoid in origin, corresponding predominantly to cells of early stages of B-cell differentiation (4, 5, 43, 44). Rearrangements of the

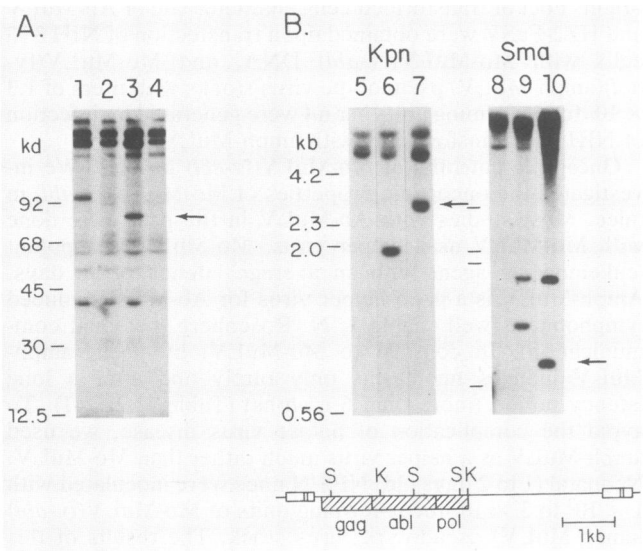

FIG. 4. Identification and characterization of an HZ2-FeSV variant (NIH PB8 cells) missing the *pol* sequences. The HZ2-FeSV provirus and the *gag-abl* protein product in transformants derived by transfection of NIH 3T3 cells with pHZ2-FeSV DNA were characterized as follows. (A) Extracts of cells labeled with [^{3}H]leucine were immunoprecipitated and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lanes: 1 and 2, NIH PB7 cells; 3 and 4, NIH PB8 cells; 1 and 3, rabbit anti-FeLV p27 serum; 2 and 4, normal rabbit serum. Molecular sizes are indicated on the left in kilodaltons (kd). Southern blots containing DNAs from NIH 3T3 cells (lanes 5 and 8), NIH PB7 cells (lanes 6 and 9), and NIH PB8 cells (lanes 7 and 10) digested with the restriction enzymes *Kpn*I and *Sma*I, lanes 1 to 3 and 4 to 6, respectively, were hybridized with a ^{32}P-labeled *abl* hybridization probe (pAB103Hc; unpublished data). Migration of *Hin*dIII lambda DNA is indicated in kilobase pairs. The arrows indicate the altered protein product and altered restriction fragments in NIH PB8 cells. A schematic of the HZ2-FeSV genome is shown below panel B. The shaded box indicates the *gag-abl-pol* coding region. The restriction sites for the enzymes *Sma*I (S) and *Kpn*I (K) which are relevant to the analysis are indicated.

immunoglobulin genes are distinctive markers for stages in B-cell development. In the earliest described stage, the D_H and J_H regions of the immunoglobulin heavy-chain gene are rearranged without involvement of the variable region V_H (5). In mature B cells, the immunoglobulin light-chain gene is rearranged as well. To investigate whether Mo-MuLV(fv-*abl*)-induced tumors display characteristics of the B-cell lineage similar to those of Ab-MuLV-induced tumors, we determined whether the immunoglobulin heavy-chain J_H segment is rearranged in tumor DNAs. A Southern blot containing the DNAs shown in Fig. 6A digested with the restriction enzyme *Eco*RI was therefore hybridized with a J_H probe (Fig. 6C). In normal DNAs, the 6.0-kb germ line J_H fragment was seen. In DNAs obtained from tumor tissue, however, the germ line J_H fragment was lost. Instead, new restriction fragments containing rearranged J sequences were observed. These results suggested that Mo-MuLV(fv-*abl*)-induced tumors, similar to Ab-MuLV-induced tumors, displayed characteristics of the B-cell lineage. In agreement with this result, no rearrangement of the T-cell receptor gamma gene was seen (data not shown). Taken together, these findings indicated that the Mo-MuLV construct containing the fv-*abl* segment [Mo-MuLV(fv-*abl*)] induces tumors in mice which are indistinguishable from Ab-MuLV-induced tumors. This result then implies that the fv-*abl* segment does not determine sarcomagenicity in mice.

DISCUSSION

HZ2-FeSV contains 5′ *abl* sequences. Two different retroviral transductions of c-*abl* are known: Ab-MuLV and HZ2-FeSV (11, 23). In human chronic myelogenous leukemia, c-*abl* is translocated to chromosome 22 with the result of a gene fusion (26, 27, 50). In all three events, the N terminus of the c-*abl* protein is replaced: in the acute transforming retroviruses by *gag* sequences and in human chronic myelogenous leukemia by the N terminus of the *bcr* gene product. In human chronic myelogenous leukemia, the *bcr-abl* protein fusions are obtained by RNA splicing of *bcr* to the common 5′ c-*abl* exon (7). In HZ2-FeSV, the 5′ recombination site between FeLV *gag* and c-*abl* is 72 nucleotides 3′ of the splice acceptor of the common exon, and in A6-MuLV, the recombination site in c-*abl* is in the following exon 263 nucleotides 3′ of the splice acceptor. Since all three c-*abl* activations involve replacement of N-terminal c-*abl* sequences, it is reasonable to assume that this characteristic is a determining factor in the oncogenic activation of c-*abl*.

The fv-*abl* segment is colinear with the known murine v-*abl* and human c-*abl* sequences, and only five amino acid differences exist between them. It is difficult to know whether these changes represent species differences or whether they are differences between the feline c-*abl* and v-*abl* sequences and thus possibly contribute to oncogene activation.

Distal to the kinase domain in c-*abl*, there is a c-terminal segment of 843 amino acids. This protein segment is present in the *bcr-abl* protein as well as in the p160 *gag-abl* protein of the P160 strain of Ab-MuLV. In HZ2-FeSV, these sequences are missing; instead, fv-*abl* is fused to 3′ *pol* sequences, and an open reading frame in the *pol* gene is sustained for more than 600 nucleotides. Genetic variants exist of the P160 strain of Ab-MuLV in which C-terminal sequences are missing as well. The loss of C-terminal sequences then does not constitute a common denominator of *abl* activation.

Mo-MuLV(fv-*abl*) induces lymphosarcoma in mice. To assess whether the differences between the v-*abl* segments (the extra 5′ sequences, the amino acid changes, and the substitution of 3′ c-*abl* sequences with FeLV *pol* sequences) determine the oncogenic properties of the virus, we constructed a murine version of HZ2-FeSV, Mo-MuLV(fv-*abl*). Like Ab-MuLV(amph-MuLV), Mo-MuLV(fv-*abl*)(amph-MuLV) induces lymphosarcoma of B-cell origin. The tumors, however, occur with prolonged latency, and tumor incidence is less than 50%. These properties resemble those of Ab-MuLV variants which, like v-*abl* lack most of the C-terminal c-*abl* sequences (44, 46; N. Rosenberg, personal communication). These results suggest that the unique structural features of fv-*abl* (vis-à-vis mv-*abl*) do not affect the neoplastic spectrum associated with mv-*abl*. However, it cannot be ruled out that these structural features play a role in feline fibrosarcoma induction.

The tumors were found to be oligoclonal, suggesting that fv-*abl*-induced leukemogenesis is a multistage process and that the virus is the probable initiating agent. This result is in agreement with experiments indicating a progression of the transformed phenotype upon infection of bone marrow cells with Ab-MuLV in vitro (59, 60, 61), as well as with the finding of rearranged c-*myc* sequences and v-*abl* sequences

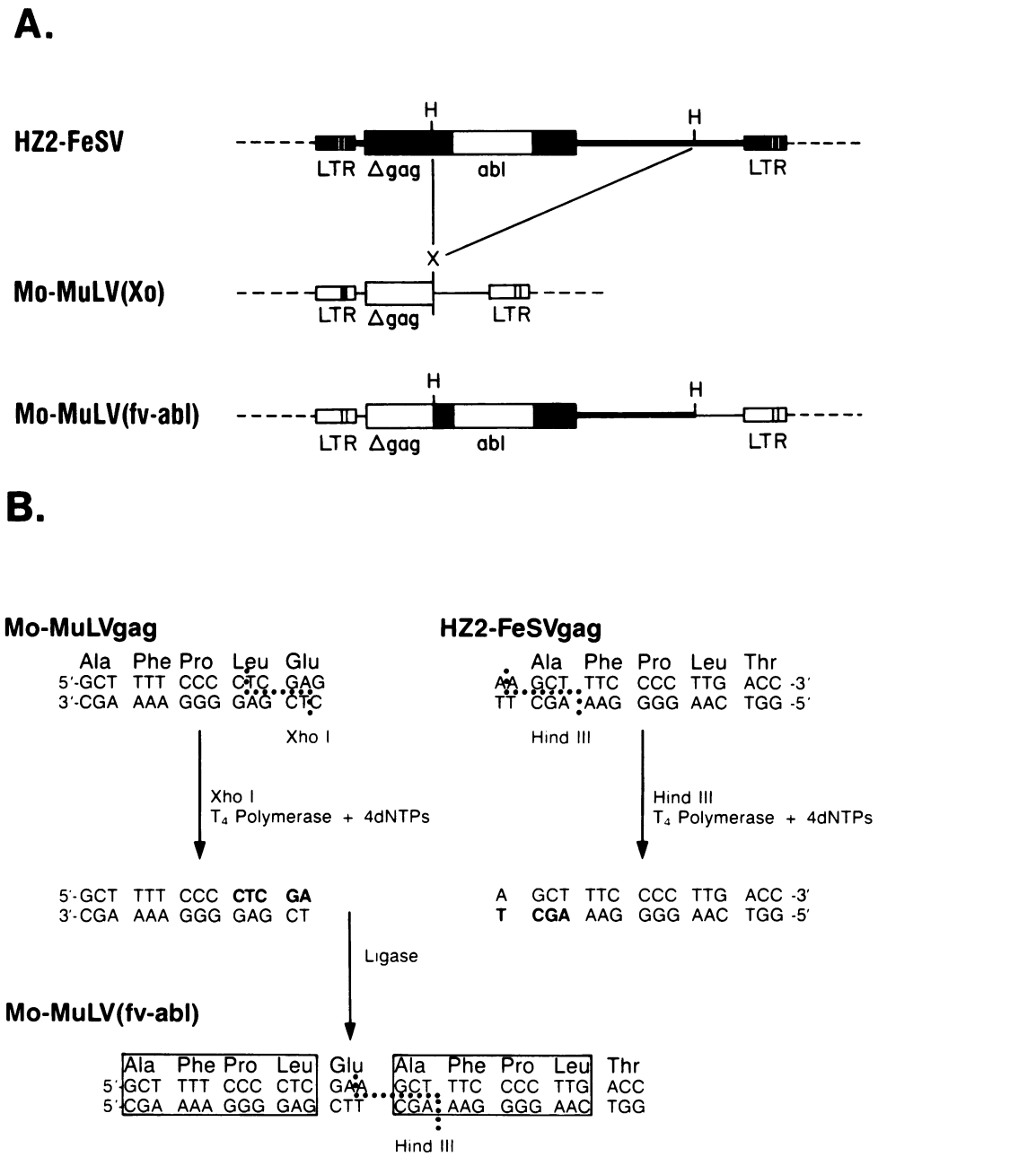

FIG. 5. Construction of Mo-MuLV(fv-*abl*). (A) Schematic representation of the construction. Only features relevant to the construction are shown. Integrated HZ2-FeSV provirus: FeLV-derived portions are solid. The *gag-abl-pol* coding region is indicated by a large box, and long terminal repeat (LTR) sequences are indicated by small boxes. Integrated Mo-MuLV(Xo) provirus: Mo-MuLV-derived sequences are indicated by open boxes. Integrated Mo-MuLV(fv-*abl*) provirus: Mo-MuLV-derived sequences are shown as open boxes, and HZ2-FeSV-derived sequences are shown as solid boxes. The following restriction sites relevant for the construction are shown: H, *Hin*dIII; X, *Xho*I. (B) Nucleic acid sequences of MoMuLV *gag*, HZ2-FeSV *gag*, and Mo-MuLV(fv-*abl*) *gag* around the *Xho*I and *Hin*dIII sites, respectively, are shown. The schematic of the enzymatic steps is indicated. Nucleotides filled in by T4 DNA polymerase are shown in boldface. The duplicated sequence segment of Mo-MuLV(fv-*abl*) is shown by open boxes.

in plasmacytomas induced with pristane in conjunction with Ab-MuLV, similarly indicating multiple events in Ab-MuLV-induced tumorigenesis (34, 37). The oligoclonal nature of the tumors is in contrast with our results obtained with fv-*abl*-associated fibrosarcoma, in which the tumors were found to be polyclonal (Bergold et al., in press).

5′ recombination site in a preferred region of recombination in FeLV p30. The 5′ junction between the FeLV and *abl* sequences in HZ2-FeSV was found at nucleotide 1034 of the FeLV *gag* gene. Four acute transforming feline retroviruses, namely, GA-FeSV, HZ4-FeSV, M-FeLV, and HZ2-FeSV, have *gag-onc* junctions that are clustered within 24 base pairs defining a preferred region of recombination. The significance of the recombination site cluster and the unique sequence motifs found in this region of FeLV p30, as well as in the vicinity of other retrovirus-*onc* junctions, have been

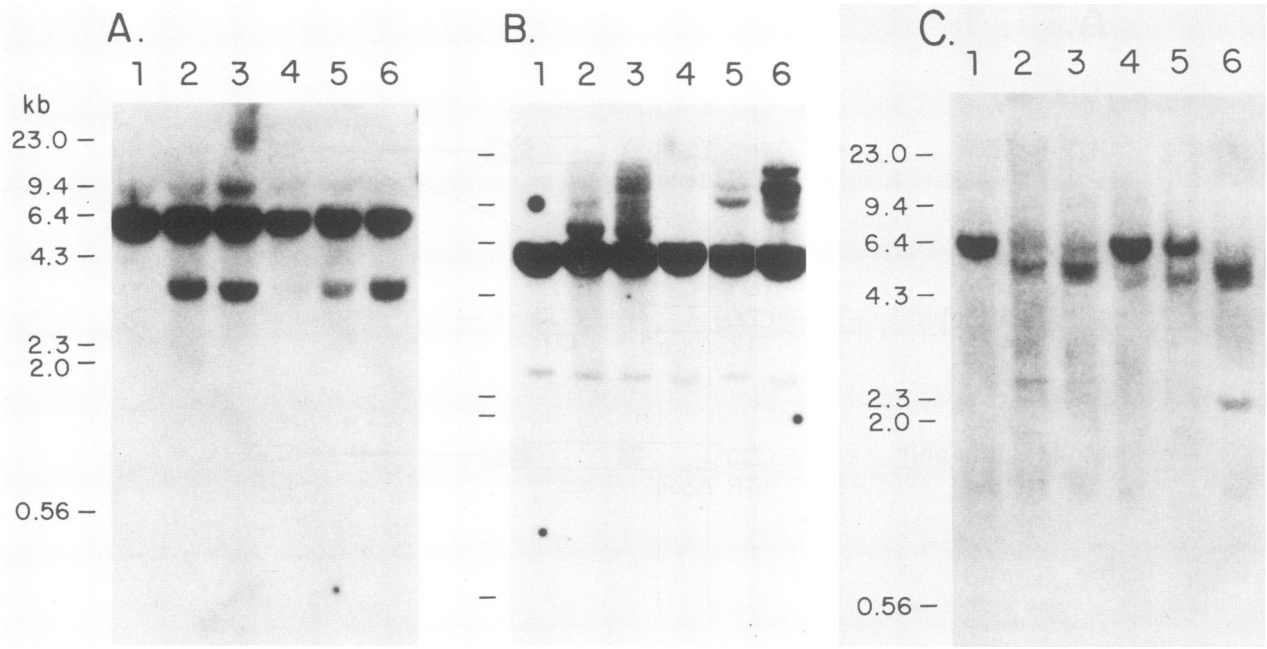

FIG. 6. Analysis of DNAs obtained from Mo-MuLV(fv-*abl*)-induced NFS mouse tumors. Characterization of proviral insertions: Southern blots containing DNAs digested with the restriction enzymes *Hin*dIII (panel A) and *Eco*RI (panel B) from kidney (lanes 1 and 4), enlarged spleen (lanes 2 and 5), and tumor tissues (lanes 3 and 6) from two NFS/N mice with lymphosarcoma (lanes 1 to 3 and 4 to 6) obtained with Mo-MuLV(fv-*abl*)(amph-MuLV) virus were hybridized with a ^{32}P-labeled 5′ c-*abl* probe. (C) Characterization of immunoglobulin heavy-chain J_H sequences in NFS/N mouse tumor DNAs. A Southern blot containing the same DNAs as in panel A, digested with the restriction enzyme *Eco*RI, was hybridized with a ^{32}P-labeled J_{11} probe. Migration of *Hin*dIII lambda DNA is indicated in kilobases.

discussed elsewhere (13). In procaryotes, short sequence homologies are known to facilitate formation of large chromosomal deletions (3). Short sequence homologies between the parental strands at retrovirus-*onc* recombination sites have been found in some acute transforming retroviruses (Ab-MuLV, HZ5-FeSV, simian sarcoma virus, FBJ murine sarcoma virus, 3611 murine sarcoma virus, reticuloendotheliosis virus strain T, and CMII avian leukosis virus) but not in others (12, 15, 29, 54, 55, 57, 62). These homologies, therefore, may facilitate recombination, but they are not a prerequisite. With HZ2-FeSV, a six-base homology was observed at the 5′ recombination site between the parental FeLV p30 and the mouse c-*abl* sequences. Since the murine and human 5′ *abl* sequences are highly conserved at the nucleotide level, we assume that this homology applies to the feline c-*abl* sequences as well. The c-*onc* sequences corresponding to the 5′ recombination sites in HZ4-FeSV, GA-FeSV, and Mo-FeLV are not known, and the question of homology at the recombination sites in these viruses cannot be determined at this time. Thus, the significance of sequence homologies at recombination sites which involve the preferred FeLV p30 region cannot be assessed yet. The formation of acute transforming retroviruses is thought to involve five steps: (i) upstream integration of an FeLV provirus, (ii) a deletion at the DNA level in which FeLV *gag* sequences become fused with c-*onc* coding sequences, (iii) transcription of a hybrid RNA composed of 5′ retrovirus and *onc* sequences and RNA splicing, (iv) packaging of the hybrid RNA into a retrovirus particle, and (v) recombination of 3′ retroviral sequences with the chimeric RNA in a subsequent cycle of infection (24, 52, 58). In a variant scheme, RNA splicing has been proposed as a mechanism for the formation of 5′ retrovirus-*onc* junctions (62). Unless functional constraints for expression of the transforming protein are a determinant for the recombination event, our present and previous observations suggest that formation of 5′ *gag-onc* junctions of acute transforming retroviruses are facilitated by recombinogenic sequences, as well as by short sequence homologies at the recombination site.

TABLE 1. Induction of lymphosarcoma by Mo-MuLV(fv-abl) (amph-MuLV)[a]

Virus stock	Lymphosarcoma frequency	Mean (range) latency period (days)[b]
Mo-MuLV(fv-abl) (amph-MuLV)	14/28	54 (29–116)
Ab-MuLV(amph-MuLV)	21/21	33 (21–50)
amph-MuLV	0/15	NA[c]

[a] Neonatal NFS/N mice were injected intraperitoneally with 1×10^3 to 3×10^3 focus-forming units of the indicated virus.
[b] Scored at 120 days postinjection.
[c] NA, Not applicable.

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LITERATURE CITED

1. **Abelson, H. T., and L. S. Rabstein.** 1970. Influence of prednisolone on Moloney leukemia virus in Balb/c mice. Cancer Res. **30**:2208–2212.
2. **Abelson, H. T., and L. S. Rabstein.** 1970. Lymphosarcoma virus: virus-induced thymic independent disease in mice. Cancer Res. **30**:2213–2222.
3. **Albertini, A. M., M. Hofer, M. P. Calos, and J. H. Miller.** 1982. On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions. Cell **29**:319–328.
4. **Alt, F., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore.** 1981. Organization and reorganization of immunoglobulin genes in A-MuLV transformed cells: rearrangement of heavy but not light chain genes. Cell **27**:381–390.
5. **Alt, F. W., G. D. Yancopoulos, T. K. Blackwell, C. Wood, E. Thomas, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore.** 1985. Ordered rearrangement of immunoglobulin heavy chain variable region segments. EMBO J. **3**:1209–1219.
6. **Bassin, R. H., N. Tuttle, and P. J. Fischinger.** 1971. Rapid cell culture assay technique for murine leukemia viruses. Nature (London) **269**:56–58.
7. **Ben-Neriah, Y., A. Bernards, M. Paskind, G. Q. Daley, and D. Baltimore.** 1986. Alternative 5′ exons in c-*abl* mRNA. Cell **44**: 577–586.
8. **Ben-Neriah, Y., G. Q. Daley, A.-M. Mes-Masson, O. N. Witte, and D. Baltimore.** 1986. The chronic myelogenous leukemia specific P210 protein is the product of the *bcr-abl* hybrid gene. Science **233**:212–214.
9. **Besmer, P.** 1983. Acute transforming feline retroviruses. Curr. Top. Microbiol. Immunol. **107**:1–28.
10. **Besmer, P., and D. Baltimore.** 1977. Mechanism of restriction of ecotropic and xenotropic murine leukemia viruses and formation of pseudotypes between the two viruses. J. Virol. **21**:965–973.
11. **Besmer, P., W. D. Hardy, E. E. Zuckerman, P. J. Bergold, L. Lederman, and H. W. Snyder.** 1983. The Hardy-Zuckerman 2-FeSV, a new feline retrovirus with oncogene homology to Abelson-MuLV. Nature (London) **303**:825–828.
12. **Besmer, P., E. Lader, P. C. George, P. J. Bergold, F. H. Qiu, E. E. Zuckerman, and W. D. Hardy.** 1986. A new acute transforming feline retrovirus with *fms* homology specifies a C-terminally truncated version of the c-*fms* protein that is different from the SM-feline sarcoma virus v-*fms* protein. J. Virol. **60**:194–203.
13. **Besmer, P., J. E. Murphy, P. C. George, F. H. Qiu, P. J. Bergold, L. Lederman, H. W. Snyder, D. Brodeur, E. E. Zuckerman, and W. D. Hardy.** 1986. A new acute transforming feline retrovirus and relationship of its oncogene *kit* with the protein kinase gene family. Nature (London) **320**:415–421.
14. **Besmer, P., H. W. Snyder, J. E. Murphy, W. D. Hardy, Jr., and A. Parodi.** 1983. The Parodi-Irgens feline sarcoma virus and simian sarcoma virus have homologous oncogenes, but in different contexts of the viral genomes. J. Virol. **46**:606–613.
15. **Bonner, T. I., S. B. Kerby, P. Sutrave, M. A. Gunnell, G. Mark, and U. R. Rapp.** 1985. Structure and biological activity of human homologs of the *raf/mil* oncogene. Mol. Cell. Biol. **5**: 1400–1407.
16. **Cook, W. D., D. Metcalf, N. A. Nicola, A. W. Burgess, and F. Walker.** 1985. Malignant transformation of a growth factor dependent myeloid cell line by Abelson virus without evidence of an autocrine mechanism. Cell **41**:677–683.
17. **Cooper, J. A., K. L. Gould, C. A. Cartwright, and T. Hunter.** 1986. Tyr^{527} is phosphorylated in $pp60^{c\text{-}src}$: implications for regulation. Science **231**:1431–1434.
18. **Copeland, T. D., G. F. Gerard, C. W. Hixson, and S. Oroszlan.** 1985. Amino- and carboxy-terminal sequence of Moloney murine leukemia virus reverse transcriptase. Virology **143**:676–679.
19. **Courtneidge, S. A.** 1985. Activation of the $pp60^{c\text{-}src}$ kinase by middle T antigen binding and dephosphorylation. EMBO J. **4**:1471–1477.
20. **Feng, D. F., M. S. Johnson, and R. F. Doolittle.** 1985. Aligning amino acid sequences: comparison of commonly used methods. J. Mol. Evol. **21**:112–125.
21. **Feuerman, M. H., B. R. Davis, P. K. Pattengale, and H. Fan.** 1985. Generation of a recombinant Moloney murine leukemia virus carrying the v-*src* gene of avian sarcoma virus: transformation in vitro and pathogenesis in vivo. J. Virol. **54**:804–816.
22. **Gardner, M. B.** 1978. Type C viruses of wild mice: characterization and natural history of amphotropic, ecotropic, and xenotropic MuLV. Curr. Top. Microbiol. Immunol. **79**:215–259.
23. **Goff, S. P., E. Gilboa, O. N. Witte, and D. Baltimore.** 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: studies with cloned viral DNA. Cell **22**:777–785.
24. **Goldfarb, M. P., and R. A. Weinberg.** 1981. Generation of novel, biologically active Harvey sarcoma virus via apparent illegitimate recombination. J. Virol. **38**:136–150.
25. **Graham, E. L., and A. J. Van der Eb.** 1973. Transformation of rat cells with human adenovirus 5. Virology **52**:456–467.
26. **Heisterkamp, N., K. Stam, J. Groffen, A. deKlein, and G. Grosveld.** 1985. Structural organization of the *bcr* gene and its role in the Ph′ translocation. Nature (London) **315**:758–761.
27. **Heisterkamp, N., J. R. Stephenson, J. Groffen, P. F. Hanson, A. deKlein, C. R. Bartram, and G. Grosveld.** 1983. Localization of the c-*abl* oncogene adjacent to a translocation breakpoint in chronic myelogenous leukemia. Nature (London) **306**:239–242.
28. **Jainchill, J. L., S. A. Aaronson, and G. Todaro.** 1969. Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. J. Virol. **4**:549–553.
29. **Josephs, S. F., R. Dalla Favera, E. P. Gelman, R. C. Gallo, and F. Wong-Stahl.** 1983. 5′ viral and human cellular sequences corresponding to the transforming gene of simian sarcoma virus. Science **219**:503–505.
30. **Laprevotte, I., A. Hampe, C. J. Sherr, and F. Galibert.** 1984. Nucleotide sequence of the *gag* gene and *gag-pol* junction of feline leukemia virus. J. Virol. **50**:884–894.
31. **Lederman, L., M. C. Singhal, P. Besmer, E. E. Zuckerman, W. D. Hardy, and H. W. Snyder.** 1985. Immunological and biochemical characterization of HZ2 feline sarcoma virus and Abelson murine leukemia virus translation products. J. Gen. Virol. **66**:2057–2063.
32. **Marcu, K. B., J. Banerji, N. A. Penncavage, R. Lang, and N. Arnheim.** 1980. 5′ flanking region of immunoglobulin heavy chain constant region genes displays length heterogeneity in germlines of inbred mouse strains. Cell **22**:187–196.
33. **Mark, G. E., and U. P. Rapp.** 1984. Primary structure of v-*raf*: relatedness to the *src* family of oncogenes. Science **224**:285–289.
34. **Mushinski, J. F., M. Potter, S. R. Bauer, and E. P. Reddy.** 1983. DNA rearrangement and altered RNA expression of the c-*myb* oncogene in mouse plasmoid lymphosarcoma. Science **220**: 795–798.
35. **Nowell, P. C.** 1986. Mechanisms of tumor progression. Cancer Res. **46**:2203–2207.
36. **Oliff, A., O. Agranovsky, M. D. McKinney, V. V. V. S. Marty, and R. Bauchwitz.** 1985. Friend murine leukemia virus-immortalized myeloid cells are converted into tumorigenic cell lines by Abelson leukemia viruses. Proc. Natl. Acad. Sci. USA **82**:3306–3310.
37. **Ohno, S., S. Migita, F. Wiener, M. Babonits, G. Klein, J. F. Mushinski, and M. Potter.** 1984. Chromosomal translocations activating *myc* sequences and transduction of v-*abl* are critical events in the rapid induction by pristane and Abelson virus. J. Exp. Med. **159**:1762–1777.
38. **Pierce, J. H., P. P. DiFiore, S. A. Aaronson, M. Potter, J. Pamphrey, A. Scott, and J. N. Ihle.** 1985. Neoplastic transformation of mast cells by Abelson-MuLV: abrogation of Il-3 dependence by a nonautocrine mechanism. Cell **41**:685–693.
39. **Prywes, R., J. G. Foulkes, and D. Baltimore.** 1985. The minimum transforming region of v-*abl* is the segment encoding protein tyrosine kinase. J. Virol. **54**:114–122.
40. **Prywes, R., J. G. Foulkes, N. Rosenberg, and D. Baltimore.** 1983. Sequences of the A-MuLV protein needed for fibroblast and lymphoid cell transformation. Cell **34**:569–579.

41. **Rasheed, S., M. B. Gardner, and E. Chan.** 1976. Amphotropic host range of naturally occurring wild mouse leukemia viruses. J. Virol. **19**:13–18.
42. **Reddy, E. P., M. J. Smith, and Srinivasan.** 1983. Nucleotide sequence of Ableson murine leukemia virus genome: structural similarity of its transforming gene to other *onc* gene products with tyrosine specific kinase activity. Proc. Natl. Acad. Sci. USA **80**:3623–3627.
43. **Risser, R.** 1982. The pathogenesis of Abelson virus lymphomas of the mouse. Biochim. Biophys. Acta **651**:213–244.
44. **Rosenberg, N.** 1982. Abelson murine leukemia virus. Curr. Top. Microbiol. Immunol. **101**:95–126.
45. **Rosenberg, N., and D. Baltimore.** 1976. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. J. Exp. Med. **143**:1453–1463.
46. **Rosenberg, N. E., D. R. Clark, and O. N. Witte.** 1980. Abelson murine leukemia virus mutants deficient in kinase activity and lymphoid cell transformation. J. Virol. **36**:766–774.
47. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
48. **Scherr, D. C., and R. Siegler.** 1975. Direct transformation of 3T3 cells by Abelson murine leukemia virus. Nature (London) **253**: 729–731.
49. **Shinnik, T. M., R. A. Lerner, and J. G. Sutcliff.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293**:543–548.
50. **Shtivelman, E., B. Lifshitz, R. P. Gale, and E. Canaani.** 1985. Fused transcripts of *abl* and *bcr* genes in chronic myelogenous leukemia. Nature (London) **315**:550–554.
51. **Snyder, H. W.** 1982. Biochemical characterization of protein kinase activities associated with transforming gene products of the Snyder-Theilen and Gardner-Arnstein strains of feline sarcoma virus. Virology **117**:165–172.
52. **Swanstrom, R., R. C. Parker, H. E. Varmus, and J. M. Bishop.** 1983. Transduction of a cellular oncogene: the genesis of Rous sarcoma virus. Proc. Natl. Acad. Sci. USA **80**:2519–2523.
53. **Takeya, T., H. Hanafusa.** 1983. Structure and sequence of the cellular gene homologous to the RSV *src* gene and the mechanism for generating the transforming virus. Cell **32**:881–890.
54. **vanBeveren, C., F. van Straaten, T. Curran, R. Muller, and I. M. Verma.** 1983. Analysis of FBJ-MuSV provirus and c-*fos* gene reveals that viral and cellular *fos* gene products have different carboxy termini. Cell **32**:1241–1255.
55. **Walther, N., R. Lurz, T. Patchinsky, H. W. Jansen, and K. Bister.** 1985. Molecular cloning of proviral DNA and structural analysis of the transduced *myc* oncogene of avian oncovirus CMII. J. Virol. **54**:576–585.
56. **Waneck, G. L., L. Keyes, and N. Rosenberg.** 1981. Abelson virus drives the differentiation of Harvey virus-infected erythroid cells. Cell **44**:337–344. **26**:79–89.
57. **Wang, J. Y. J., F. Ledley, S. P. Foff, R. Lee, Y. Groner, and D. Baltimore.** 1984. The mouse c-*abl* locus: molecular cloning and characterization. Cell **36**:349–356.
58. **Weiss, R., N. Teich, H. Varmus, and J. Coffin.** 1982. Molecular biology of tumor viruses, 2nd ed.: RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
59. **Whitlock, C. A., and O. N. Witte.** 1982. Abelson virus-infected cells can exhibit restricted in vitro growth and low oncogenic potential. J. Virol. **40**:577–584.
60. **Whitlock, C. A., and O. N. Witte.** 1985. The complexity of virus-cell interactions in Abelson virus infection of lymphoid and other hematopoietic cells. Adv. Immunol. **37**:74–98.
61. **Whitlock, C. A., S. F. Ziegler, and O. N. Witte.** 1983. Progression of the transformed phenotype in clonal lines of Abelson virus-infected lymphocytes. Mol. Cell. Biol. **3**:596–604.
62. **Wilhelmsen, K. C., K. Eggleton, and H. M. Temin.** 1984. Nucleic acid sequences of the oncogene v-*rel* in reticuloendotheliosis virus strain T and its cellular homolog, the proto-oncogene c-*rel*. J. Virol. **52**:172–182.
63. **Witte, O. N., A. Dasgupta, and D. Baltimore.** 1980. Abelson murine leukemia virus protein is phosphorylated *in vitro* to form phosphotyrosine. Nature (London) **283**:826–831.
64. **Witte, O. N., S. P. Goff, N. Rosenberg, and D. Baltimore.** 1980. A transformation defective mutant of Abelson murine leukemia virus lacks protein kinase activity. Proc. Natl. Acad. Sci. USA **77**:4993–4997.
65. **Witte, O. N., N. Rosenberg, M. Paskind, A. Shields, and D. Baltimore.** 1978. Identification of an Abelson murine leukemia virus encoded protein present in transformed fibroblasts and lymphoid cells. Proc. Natl. Acad. Sci. USA **75**:2488–2492.
66. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
67. **Zoller, M. J., N. C. Nelson, and S. S. Taylor.** 1981. Affinity labeling of cAMP-dependent protein kinase with *p*-fluorosulfonylbenzoyl adenosine. J. Biol. Chem. **256**:10837–10842.