JOURNAL OF VIROLOGY, June 1984, p. 813–821
0022-538X/84/070813-09$02.00/0

Vol. 50, No. 3

Characterization of the *env* Gene and Long Terminal Repeat of Molecularly Cloned Friend Mink Cell Focus-Inducing Virus DNA

AKIO ADACHI,[1] KOJI SAKAI,[1] NAOMI KITAMURA,[2] SHIGETADA NAKANISHI,[2] OHTSURA NIWA,[3] MUTSUSHI MATSUYAMA,[4] AND AKINORI ISHIMOTO[1]*

Department of Biophysics, Institute for Virus Research,[1] and Institute for Immunology[2] and Department of Experimental Radiology,[3] Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, and Aichi Cancer Center, Research Institute Nagoya 464,[4] Japan

Received 8 September 1983/Accepted 12 January 1984

The highly oncogenic erythroleukemia-inducing Friend mink cell focus-inducing (MCF) virus was molecularly cloned in phage λgtWES.λB, and the DNA sequences of the *env* gene and the long terminal repeat were determined. The nucleotide sequences of Friend MCF virus and Friend spleen focus-forming virus were quite homologous, supporting the hypothesis that Friend spleen focus-forming virus might be generated via Friend MCF virus from an ecotropic Friend virus mainly by some deletions. Despite their different pathogenicity, the nucleotide sequences of the *env* gene of Friend MCF virus and Moloney MCF virus were quite homologous, suggesting that the putative parent sequence for the generation of both MCF viruses and the recombinational mechanism for their generation might be the same. We compare the amino acid sequences in lymphoid leukemia-inducing ecotropic Moloney virus and Moloney MCF virus, and erythroblastic leukemia-inducing ecotropic Friend virus, Friend-MCF virus, and Friend spleen focus-forming virus. The Friend MCF virus long terminal repeat was found to be 550 base pairs long. This contained two copies of the 39-base-pair tandem repeat, whereas the spleen focus-forming virus genome contained a single copy of the same sequence.

Friend leukemia virus (12) is an acute leukemia virus which consists of at least three components; replication-competent ecotropic Friend virus, replication-defective spleen focus-forming virus (SFFV) (2), and replication-competent dualtropic mink cell focus-inducing (MCF) virus (21, 33, 41). The replication-defective SFFV has been regarded as an erythroleukemia-inducing factor (2). However, the replication-competent ecotropic virus, which had been regarded as a helper virus for SFFV, has been found to be an erythroleukemia-inducing factor when inoculated into newborn NFS mice (21, 42). The third component of Friend leukemia virus, Friend MCF virus, has also been reported as a potent erythroleukemia-inducing factor in newborn NFS mice (21), although a strain of Friend MCF was reported to be nononcogenic per se (33). Except during the latent period, the erythroleukemia induced by the Friend virus complex in NFS mice is pathologically similar to the leukemias induced by Friend MCF virus alone or ecotropic Friend virus alone (21).

The mechanism of erythroleukemogenesis induced by Friend virus in mice is unknown. However, there is experimental evidence that SFFV encodes two proteins; a *gag* gene-related protein, p45 (3), and an *env* gene-related protein, gp52 (4, 10, 35, 45), which has been shown to be a recombinant between sequences of the ecotropic virus and sequences related to the envelope gene of xenotropic virus (34). Recombinant DNA experiments with molecular cloned SFFV have suggested that the *env* gene-related sequences that encode the gp52 protein may be required for erythroleukemia induction (23).

The derivation of Friend MCF virus recombinant *env* gene sequences from ecotropic Friend virus and xenotropic virus-like sequences (11) opened the possibility that the recombinant sequences in Friend MCF virus are also responsible for the induction of erythroleukemia and, thus, that the ecotropic Friend virus causes erythroid leukemia by its ability to induce the formation of Friend MCF virus in which the recombinant sequences are constructed, even if ecotropic virus per se has no oncogenic *env* gene sequences (21).

To elucidate the recombinational sequences responsible for erythroleukemogenesis by Friend SFFV and Friend MCF virus, the highly oncogenic Friend MCF virus isolated from a leukemic NFS mouse inoculated neonatally with ecotropic Friend virus (21) was molecularly cloned, and the DNA sequences of the *env* gene were determined and compared with those of the ecotropic Friend virus and Friend SFFV. Attention has been focused on the role of the long terminal repeat (LTR) as a promoter since leukemia virus without oncogenes may induce leukemia by transcriptional activation of a particular cell gene (17). Thus, in the present study we also determined the DNA sequences of the LTR.

MATERIALS AND METHODS

Cells and virus. Mink lung cell line ATCC CCL-64 cells (18), SC-1 cells (15), and mink S+L− cells derived from CCL-64 (31) were grown and maintained in heated 5% fetal calf serum in Dulbecco modified Eagle minimal essential medium with penicillin (100 U/ml) and streptomycin (100 μg/ml). A highly oncogenic Friend MCF virus, designated MCF-FrNx, was isolated from an erythroleukemic NFS mouse that had been neonatally inoculated with NB-tropic, ecotropic FVA Friend virus (21). The MCF virus was propagated in mink lung cell (18).

Restriction enzymes and digestion. Restriction enzymes were purchased from Takara Shuzo Co. Ltd. (Kyoto, Japan) and Bethesda Research Laboratories (Rockville, Md.). DNA was digested with 2 U of enzyme per μg of DNA under the buffer conditions specified by the manufacturer. For more than one cleavage, a restricted DNA sample was ethanol precipitated, and the pellet was washed in 70% ethanol, air

* Corresponding author.

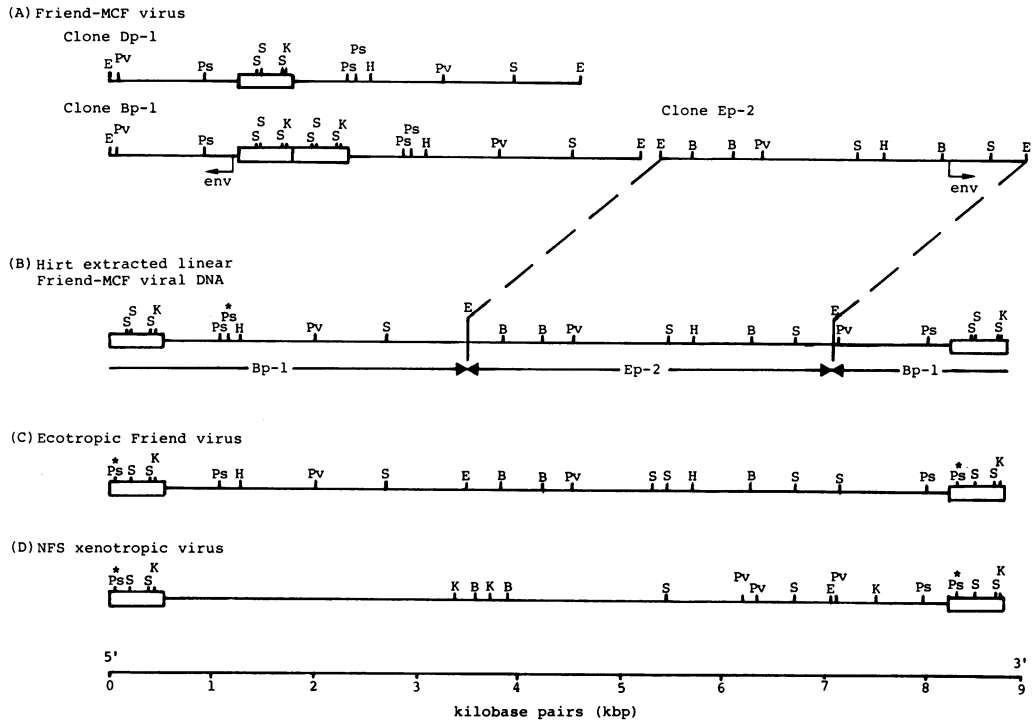

FIG. 1. Restriction endonuclease cleavage maps of Friend MCF virus Bp-1, Dp-1, and Ep-2 clones (A). Hirt-extracted linear Friend MCF viral DNA (B), putative parental ecotropic Friend virus DNA genome (C) (7, 22), and the NFS xenotropic virus DNA genome (D) (7) are shown for comparison. The asterisk in B marks a *Pst*I site that does not exist in either C or D. The asterisks in C and D mark two *Pst*I sites that do not exist in B. Abbreviations: B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I; Ps, *Pst*I; Pv, *Pvu*II; S, *Sma*I; E, *Eco*RI.

dried, and suspended in the reaction buffer of the second enzyme. Digested DNA was analyzed by electrophoresis at 30 V on 0.7% agarose horizontal slab gels. The standard size marker mixture consisted of DNA fragments ranging from 23.7 to 0.10 kilobases and was prepared by the product of *Hin*dIII- or *Eco*RI-cleaved lambda DNA.

Isolation of viral DNA for molecular cloning. Subconfluent mink lung cells in four 10-cm plate dishes were infected with the virus at a multiplicity of infection of 5 in the presence of polybrene (39) to get unintegrated viral DNA for molecular cloning. Unintegrated viral DNA was prepared from the Hirt supernatant (19) of infected mink lung cells 24 h after

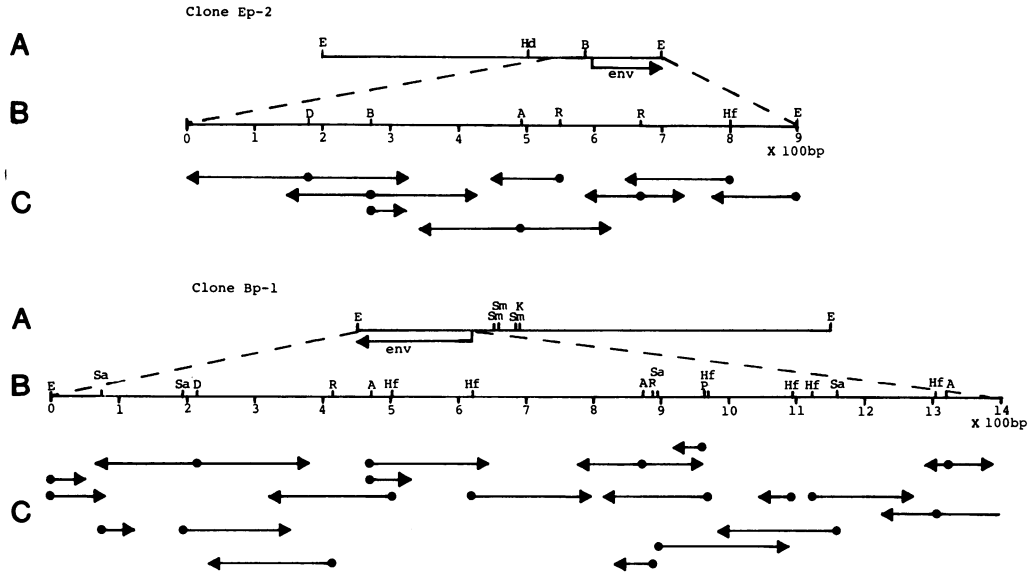

FIG. 2. Sequencing strategy for the *env* gene of Friend MCF virus. (A) Schematic representation of the Friend-MCF virus DNA clones Bp-1 and Ep-2. (B) Cleavage sites in the *Eco*RI-*Sma* fragment (Bp-1) and *Hin*dIII-*Eco*RI fragment (Ep-2) of the cutting enzymes used for sequencing. (C) Sequencing strategy. The closed circles on the lines represent 5′ ends labeled with [λ-^{32}P]ATP. Abbreviations: A, *Alu*I; B, *Bam*HI; D, *Dde*I; E, *Eco*RI; K, *Kpn*I; R, *Rsa*I; P, *Pst*I; Hd, *Hin*dIII; Hf, *Hin*fI; Sa, *Sau*3AI; Sm, *Sma*I.

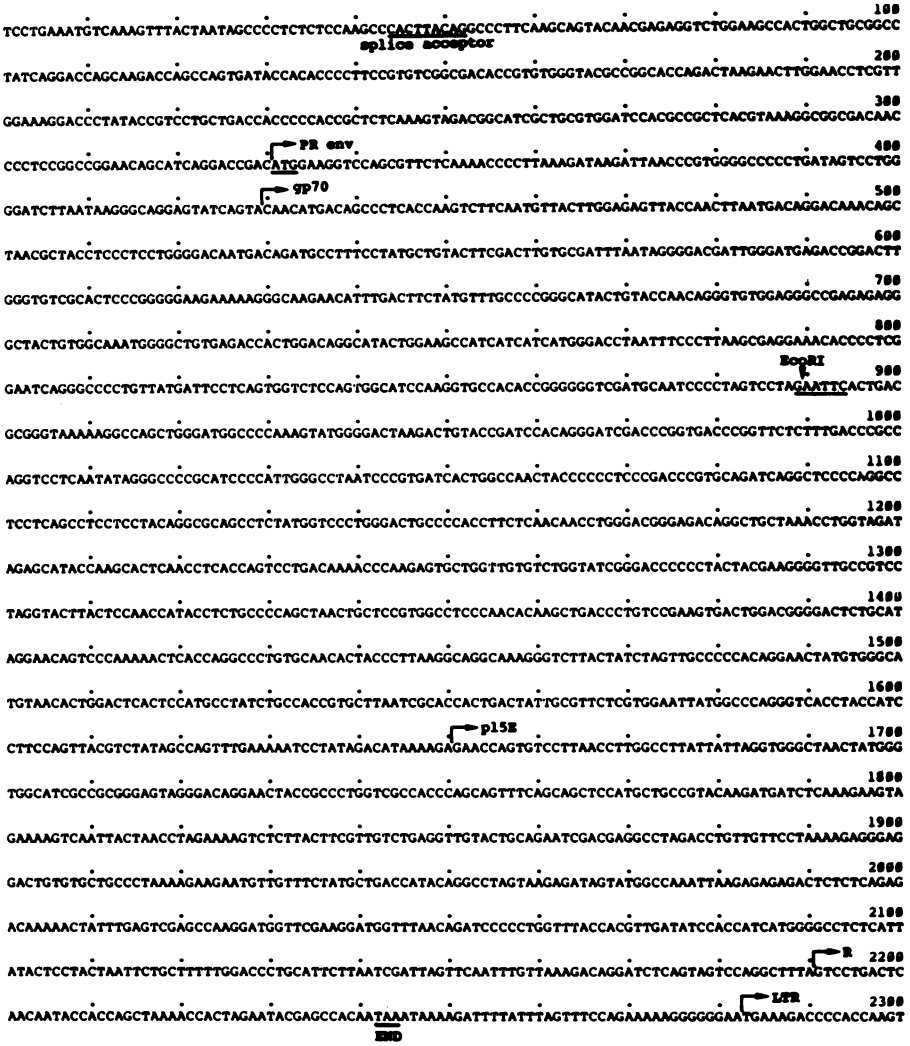

FIG. 3. Nucleotide sequence of the *env* gene region of Friend MCF virus.

infection. Supercoiled viral DNA was separated from 0.8% low-melting-point agarose (Bethesda Research Laboratories) gels under the conditions specified by the manufacturer from the part where the existence of specific DNA was shown by Southern blots.

Hybridization and DNA probe. Agarose gel electrophoresis and DNA transfer onto a nitrocellulose membrane were performed by the technique of Southern (38). Virus-specific DNA fragments transferred to nitrocellulose membranes were detected by hybridization with ^{32}P-labeled Moloney murine leukemia virus (MuLV) DNA cloned into pBR322 kindly provided by R. A. Weinberg, Massachusetts Institute of Technology, Cambridge, Mass. (20). The probe DNA was labeled by nick translation (26) and had specific activities of 2×10^7 to 40×10^7 cpm/μg. Hybridization was performed as previously described (14).

Molecular cloning of Friend MCF viral DNA. *Eco*RI-digested, supercoiled DNA was mixed with λgtWES.λB vector arms, ligated with T4 DNA ligase, packaged in vitro into infectious phage particles, and plated on *Escherichia coli* DP50 *supF* as previously described (14). Recombinant phage plaques were screened with ^{32}P-labeled Moloney MuLV DNA. The MuLV-reactive DNA inserts from the lambda clones were released by *Eco*RI cleavage and ligated to *Eco*RI-digested pBR322 DNA by incubation with T4 DNA ligase. *E. coli* K-12 strain HB101 cells were transformed by the ligation mixture, and the recombinant plasmids containing MuLV-reactive DNA inserts were identified as previously described (7).

DNA sequence. The DNA sequence was determined by the chemical methods of Maxam and Gilbert (27).

Transfection. The viral inserts were excised from pBR322 recombinants by cleavage with *Eco*RI and ligated with T4 DNA ligase. Transfections were performed on SC-1 cells (15) with ligated viral DNA by a modification (43) of the original calcium phosphate precipitation method (13). Viral production in the supernatant of SC-1 cells was measured by focus assay in the mink S+L− cells (31) and by the foci in mink lung cells (16).

Animals. The NFS mouse strain is an inbred strain from NIH Swiss mice originally supplied by the animal production section of the National Institutes of Health. A continuous single line was maintained in our laboratory by sibling mating. A 0.2-ml sample of the recovered MCF virus (10^4

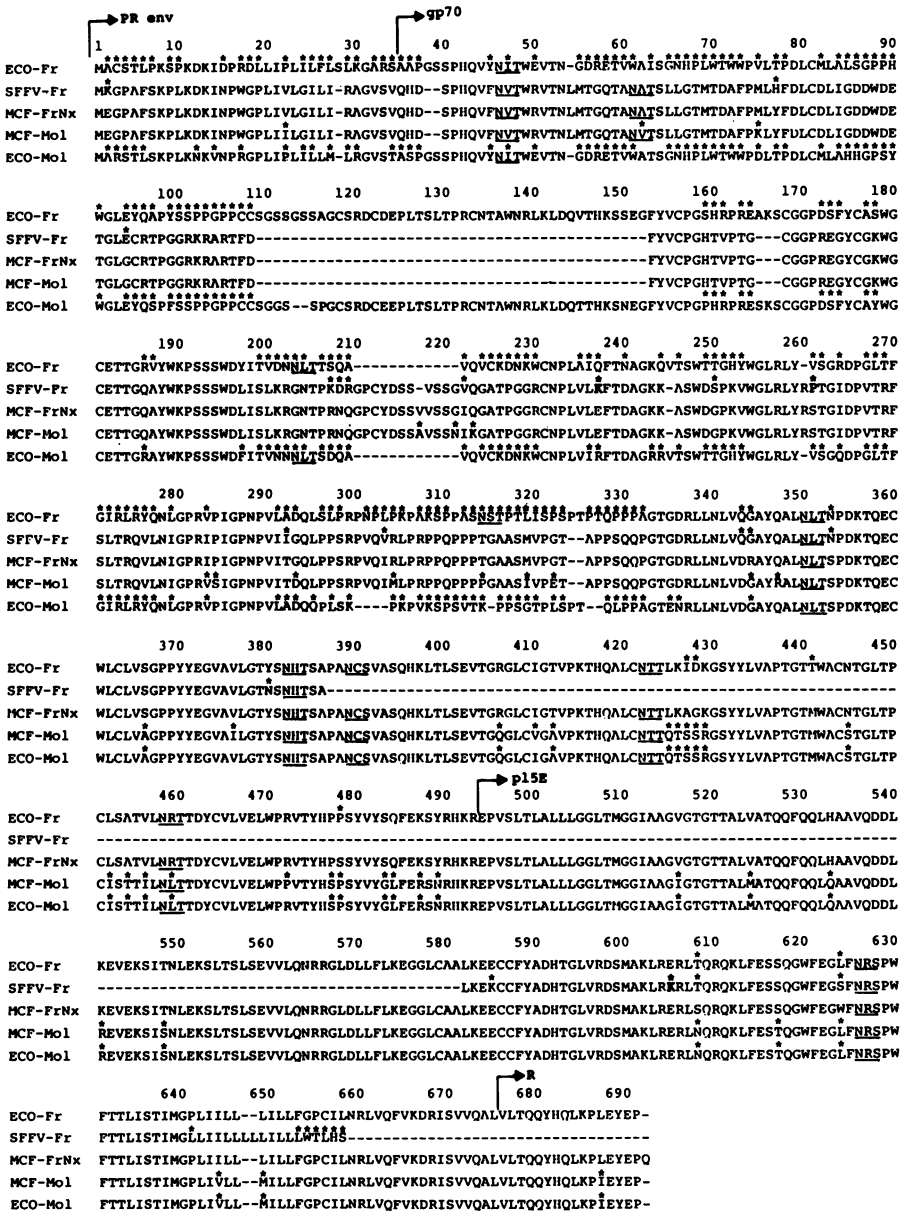

FIG. 4. Deduced amino acid sequence of the Friend MCF virus *env* gene. In addition to Friend MCF virus, sequences of ecotropic Friend virus (22), ecotropic Moloney virus (37), and Moloney MCF virus (5) *env* genes and the Friend SFFV (9) *env*-related sequence are shown for comparison. Amino acids different from those of Friend MCF virus are indicated by asterisks. Potential glycosylation sites are underlined. Abbreviations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

S+L− mink cell focus-forming units per mouse) harvested from SC-1 cells transfected with the DNA was inoculated intraperitoneally into newborn NFS mice.

RESULTS

Molecular cloning of Friend MCF virus. To analyze the structure of Friend MCF viral DNA and to determine the appropriate enzyme for molecular cloning, in vivo circular unintegrated viral DNA was digested with *Eco*RI, *Hin*dIII, and *Bam*HI. After digestion, samples were run on 0.7% agarose gels, transferred to nitrocellulose filters, and hybridized to ^{32}P-labeled Moloney virus DNA. Although the ecotropic Friend virus and the xenotropic virus from NFS mice had one *Eco*RI site at a different place (Fig. 1) (7, 22), both *Eco*RI and *Hin*dIII cleaved the Friend MCF genome twice, indicating the presence of an additional *Eco*RI site compared with the ecotropic Friend virus or the xenotropic virus. *Bam*HI cleaved the genome three times. For molecular cloning, the closed circular forms of unintegrated, supercoiled viral DNA were cleaved with *Eco*RI and ligated to *Eco*RI-cleaved phage λgtWES.λB DNA. Packaging of this ligated DNA generated about 5,000 plaques. Recombinant phages containing permuted viral inserts were identified by hybridization with ^{32}P-labeled Moloney MuLV and ^{32}P-labeled LTR-specific probes (*Sma*II-*Pvu*II, 290-base-pair [bp] fragment in LTR) from Moloney MuLV. Four Friend

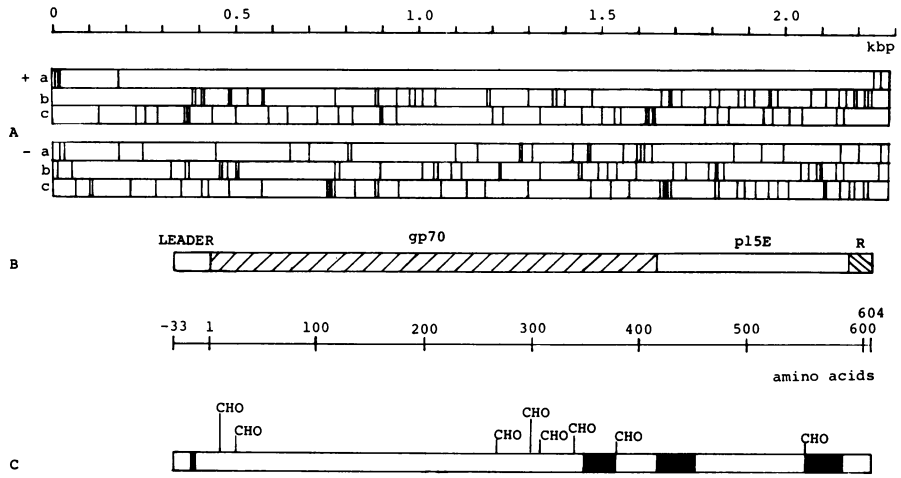

FIG. 5. Friend MCF virus characterization of the *env* gene and its products. (A) Distribution of termination codons in the coding (+) and noncoding (−) strands in all reading frames (a, b, and c). (B) Representation of the *env* polypeptide within the boundaries of A. (C) Potential glycosylation sites (CHO) and large, uncharged, hydrophobic regions (closed boxes) in the *env* polypeptide. This figure was arranged like the figure for ecotropic Friend virus (22) for easy comparison.

MCF virus recombinants, designated C1-B and C1-C (5.1-kilobase-pair [kbp] insert), C1-D (4.6 kbp), and C1-E (3.3 kbp) was obtained. The LTR-specific probe obtained from Moloney MuLV was hybridized with C1-B (two LTR copies), C1-C (two LTR copies), and C1-D (one LTR copy), but not with C1-E. None of recombinants, C1-B, C1-C, and C1-D, was hybridized with the ^{32}P-labeled virus-specific insert of C1-E. For further analysis, the C1-B, C1-C, C1-D, and C1-E viral DNA inserts were subcloned into pBR322 at the *Eco*RI site and designated Bp-1, Cp-1, Dp-1, and Ep-2, respectively. Each cloned fragment was subjected to restriction endonuclease mapping (Fig. 1). Comparison of the restriction site and the location of the LTR with published data for ecotropic Friend virus and xenotropic virus from NFS mice (7, 22) allowed us to compose the full sequence of Friend MCF virus by the rearrangement of C1-B and C1-E (Fig. 1), since Friend MCF virus is considered to be a recombinant between ecotropic Friend virus and xenotropic virus-like sequence in NFS mice. For comparison, restriction endonuclease mapping of ecotropic Friend virus (7) and xenotropic MuLV from NFS mice (7) is also shown in Fig. 1.

The restriction map of the Friend MCF genome was more similar to the map of ecotropic Friend virus than to that of xenotropic virus from NFS mice (7). However, Friend MCF virus had two *Eco*RI sites, one of which was located at the same site as in the xenotropic virus. The location of the two *Eco*RI sites in the Friend MCF genome suggests the possibility that MCF-FrNX is a recombinant between ecotropic Friend virus and endogenous xenotropic virus-like sequence in NFS mice. The Friend MCF virus lost the *Pst*I site in LTRs that existed in the putative parental ecotropic Friend virus.

Infectivity of clone Bp-1 and Ep-2 Friend MCF viral DNA. The infectivity of the recombinant viral DNAs was tested by the calcium phosphate transfection procedure (43). Viral inserts in Bp-1 and Ep-2 were excised by cleavage with *Eco*RI. Viral DNAs from Bp-1 (0.75 μg) and Ep-2 (0.25 μg) were ligated with T4 DNA ligase. Ethidium bromide staining of the gel showed that most of the DNAs were converted to circular or linear dimers and several other unknown forms of higher-molecular-weight DNA. After confirmation of the ligation by agarose gel electrophoresis, religated DNA (0.1 to 0.2 μg per plate) was transfected onto 6-cm plates containing SC-1 cells and tested for its ability to generate infectious virus. Two days after transfection, the SC-1 cells were passaged and cultured with mink lung cells (18). Viral production was observed in the supernatant of the Sc-1 cells 10 days after transfection. The virus recovered after this transfection had the same biological characteristics as the parental MuLV MCF-FrNx; it was a dualtropic NB-tropic MCF virus. Inoculation of this virus into newborn NFS mice induced leukemia in 100% of animals within 3 months. The gross and microscopic leukemic abnormalities developed by the virus were identical to those developed by the original parent viral stock of MCF-FrNx.

DNA sequence of Friend MCF virus envelope gene. Comparison of the restriction site with ecotropic Friend virus suggested the location of the *env* gene in the cloned Friend MCF viral DNA to be shown in Fig. 1 and 2. The sequencing strategy and the additional restriction enzyme cleavage sites used are shown in Fig. 2. The nucleotide sequence is presented in Fig. 3. The deduced amino acid sequences for

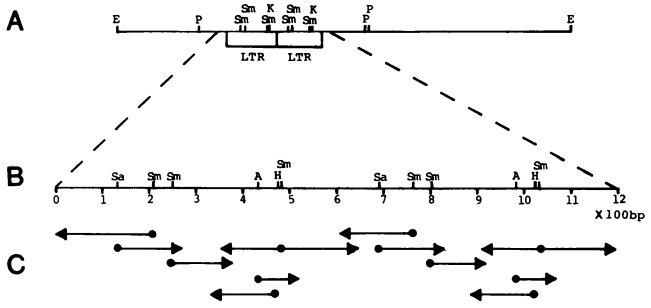

FIG. 6. Sequencing strategy for LTR of Friend MCF virus. (A) Schematic representation of the Friend MCF virus DNA clone Bp-1. (B) Cleavage sites in the *Pst*I-*Pst*I fragment (Bp-1). (C) Sequencing strategy. The closed circles on the lines represent 5′ ends labeled with [^{32}P]ATP. Abbreviations: A, *Alu*I; E, *Eco*RI; H, *Hin*fI; K, *Kpn*I; P, *Pst*I; Sa, *Sau*3AI; Sm, *Sma*I.

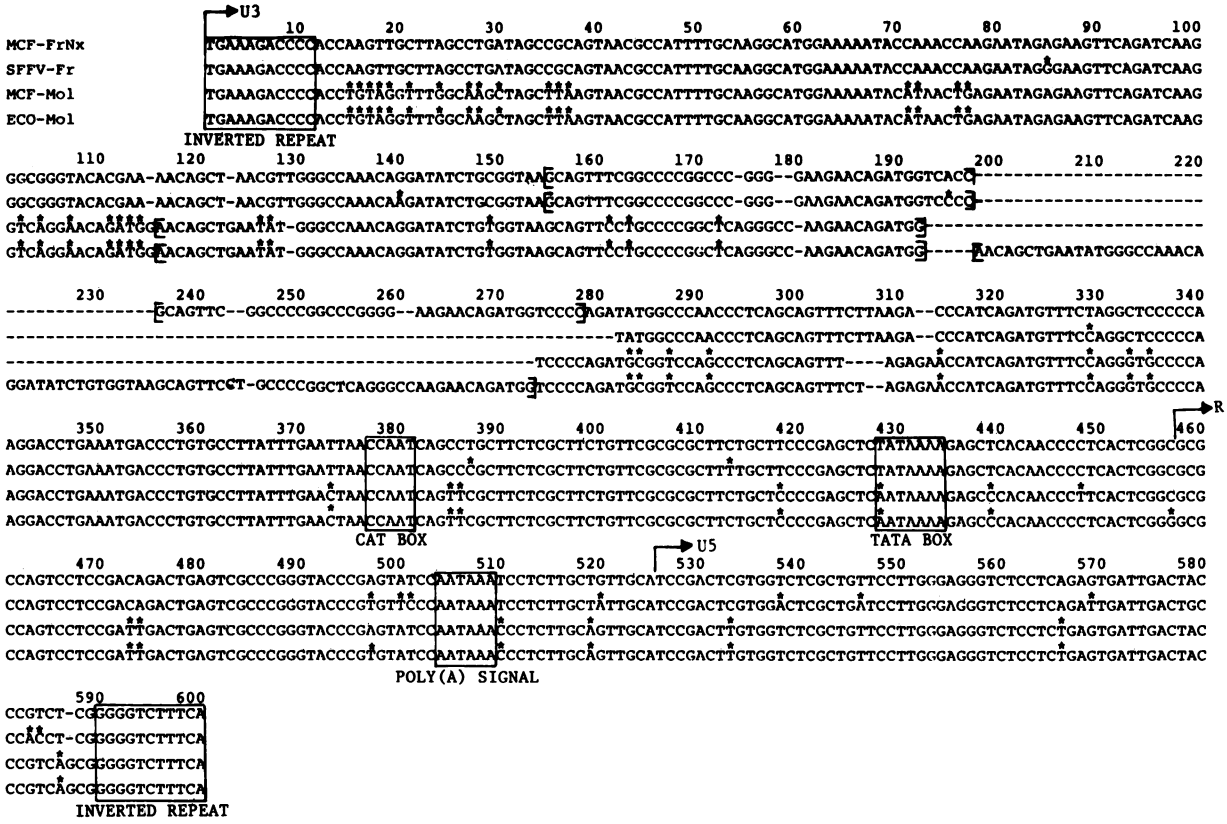

FIG. 7. Nucleotide sequence of the Friend MCF virus LTR. Friend SFFV (8), Moloney-MCF virus (5), and ecotropic Moloney virus (37) are shown for comparison. Nucleotides different from those of MCF-FrNx are indicated by asterisks. The major structural features of this region are indicated. The internal tandem repeat sequences are enclosed in brackets.

this reading frame are shown in Fig. 4. The sequence had one large open frame consisting of 1,911 nucleotides (Fig. 5). The mRNA for the *env* gene products is spliced from a precursor RNA. The splice acceptor sequence (36) was located 287 nucleotides upstream from the peptide leader (Fig. 3). The rough location of the sequence coding for the envelope polyprotein and the border of gp70 and p15E within the *env* gene was determined by comparing the nucleotide sequence with previously published data for ecotropic Friend virus (22) and Moloney MCF virus (5). In this frame, a methionine codon is 99 nucleotides upstream from the proposed NH_2 terminal of gp70, a glutamine residue (Fig. 4). It is possible that the carboxyl and NH_2-terminal amino acids of Friend MCF gp70 and p15E are actually located a few amino acids to the left or right, since neither the carboxyl nor the NH_2 terminal of the *env* polypeptide of Friend MCF virus has been determined yet.

The carbohydrates are linked to proteins via the side chains of asparagine residues in Asn-X-Thr or Asn-X-Ser sequences (28, 32). Seven glycosylation sites were found in the amino acid sequence of Friend MCF gp70 (Fig. 4 and 5). It has been reported that glycosylation sites within the *env* gene are highly conserved in the different MuLVs (22). Six of the seven glycosylation sites were at the same sites as in ecotropic Friend virus. The other was not found in ecotropic Friend virus, but was found in Friend SFFV and Moloney MCF virus. Whether all of the seven potential sites in the Friend MCF gp70 are actually glycosylated is not known at present.

Comparison of the nucleotide and amino acid sequences of Friend MCF virus with the sequences of ecotropic Friend virus (22), Friend SFFV (9), and Moloney MCF virus (5) revealed some distinctive features. (i) Friend MCF virus seemed to demonstrate substitution with some endogenous nonecotropic virus-like (or xenotropic virus-like) sequences. Nucleotide sequence analysis of the *env* gene of Friend MCF virus DNA showed that the substitutions were in the NH_2-terminal portion of the envelope protein. The substitution began in the carboxyl terminous of the *pol* gene and ended at about amino acid no. 322. Assuming identical processing sites in ecotropic Friend virus (22) and Friend MCF virus, the leader region, gp70, p15E, and R consisted of 33, 407, 180, and 17 amino acids, respectively. (ii) The nucleotide sequences of the *env* gene of Friend MCF and the *env*-related sequence of Friend SFFV were quite homologous. Comparison showed a 585-bp deletion in this region in Friend SFFV. (iii) The nucleotide sequence of the Friend MCF virus *env* gene is 114 nucleotides shorter than the corresponding ecotropic Friend nucleotide sequence. Ecotropic Friend virus, Friend MCF virus, Friend SFFV, and Moloney MCF virus share the same initiation codon and are translated in the same reading frame. The carboxyl termini of gp70 and p15E of Friend MCF and ecotropic Friend virus were identical. (iv) The amino acid sequences of Friend MCF virus, Moloney MCF virus, and Friend SFFV were very similar (95% homology). (v) It has been reported that the most variable and proline-rich site among the *env* gene of Akv, ecotropic Friend virus, and ecotropic Moloney virus is

located between amino acids no. 233 and 283 (22). However, the amino acid sequence in this region (no. 281 to no. 332 in Fig. 4) of Friend MCF virus and Friend SFFV (9) showed strong (96%) homology, and the sequence of Friend MCF virus and Moloney MCF virus showed moderate (86%) homology. This difference in homology among Friend MCF virus, Friend SFFV, and Moloney MCF virus seemed to reflect the difference in their pathogenicity. However, the amino acid sequence homologies in this region between our Friend MCF virus and two other strains of Friend SFFV which were recently reported were not so strong as that shown in the Fig. 4; 74% homology (44) and 84% homology (1). Incidentally, the amino acid sequence in this region of Friend MCF and ecotropic Friend virus or ecotropic Moloney virus showed very low (30%) homology.

DNA sequence of Friend MCF virus LTR. To elucidate the possible role of the LTR on erythroleukemia induction by Friend MCF virus, we studied the nucleotide sequence of the Friend MCF virus LTR, which may play a critical role in the promoter insertion mechanism of oncogenesis (17), and compared it with the nucleotide sequence in ecotropic Moloney virus, Friend SFFV, and Moloney MCF virus.

Figure 6 shows the strategy used to sequence the various restriction fragments, and Fig. 7 illustrates the nucleotide sequence obtained. For comparison, the previously reported LTR nucleotide sequences of Moloney MCF virus (5), ecotropic Moloney virs (37), and Friend SFFV (8) are also shown in Fig. 7. The Friend MCF virus LTR was found to be 550 bp long. This includes an 11-bp-long inverted repeat at the termini of the LTR, CAT box, TATA box, and polyadenylate addition signals (AATAAA). Apart from these highly conserved sequences, considerable nucleotide heterogeneity (14%) between Friend MCF virus and Moloney MCF virus was observed in the U3 region, whereas only 2% nucleotide dissimilarity was noted between Friend MCF virus and Friend SFFV. Nucleotide sequences of the R and U5 region were 95% homologous between Friend MCF virus and Moloney MCF virus. The major difference between the LTRs of Friend MCF virus and Friend SFFV was in the 39-bp tandem repeat: the Friend SFFV had only one copy of it. In Moloney MCF virus, the tandem repeat that was present in ecotropic Moloney virus was also missing.

DISCUSSION

The Friend leukemia virus complex is a unique oncogenic retrovirus whose oncogenic mechanism may be different from that of other acute leukemia viruses and sarcoma viruses, since no oncogenic proteins coded for by a non-virus-related gene derived from a cellular gene have been detected in the infected cells. Another unique property of the Friend virus complex is that it includes at least three components that can induce erythroleukemia independently. However, the mechanism(s) of erythroleukemogenesis by each of these components remains unknown. The ability of Friend SFFV to induce erythroleukemia was shown by inoculating mice with Friend SFFV that had been rescued by Moloney MuLV (40) or amphotropic virus (24), which does not induce erythroleukemia by itself. Linemeyer et al. showed that the gp52 protein encoded by SFFV is required for the virus-induced proliferation of erythroid precursor cells (23).

NFS mice neonatally inoculated with ecotropic Friend virus developed erythroid leukemia (21, 41). However, erythroleukemia induction by ecotropic Friend virus may be ascribable to its ability to induce the formation of Friend MCF virus, since all of the mice inoculated with ecotropic Friend virus manifested the rapid emergence of the MCF-type virus (21). The observation made by T_1 RNase-resistant fingerprint analysis (11), that Friend MCF virus has an *env* gene sequence homologous to the *env*-related sequence of Friend SFFV, supports this hypothesis. In this study, we examined the nucleotide sequence of the *env* gene and the LTR of the highly oncogenic Friend MCF virus to elucidate its mechanism of erythroleukemogenesis and compared our findings with those reported for Friend SFFV (8, 9) and ecotropic Friend virus (22).

Analysis of the *env* and LTR nucleotide sequences suggested that Friend MCF virus is also a recombinant in which almost the same regions of the *env* gene are substituted by the same endogenous nonecotropic virus-like sequences as in Moloney MCF virus. Despite the observed close homology of the nucleotide and amino acid sequences in Friend MCF and Moloney MCF viruses, the pathogenicity of these two viruses is different. Moloney MCF virus does not induce erythroid leukemia; rather, it induces thymic lymphoid leukemia. The identification of the nucleotide sequence that codes for this difference in pathogenicity represents a challenge. However, we could not identify the nucleotide sequence that is responsible for erythroid leukemia induction when the *env* gene nucleotide sequences of Friend MCF virus, ecotropic Friend virus, Friend SFFV, Moloney MCF virus, and ecotropic Moloney viruses were compared. Studies by Scolnick and his collaborators have shown that the 3′ portion of the genome (gp70, p15E, LTR) plays a determinant role in erythroid leukemia induction (23, 25, 29). Oliff and Ruschetti showed that the 2.4-kbp fragment of the Friend MuLV genome contains the sequences responsible for Friend MuLV-induced erythroid leukemia (30). However, Chatis et al. found that a recombinant virus whose genome was derived primarily from Friend MuLV, but which had 621 nucleotides of Moloney MuLV information at its 3′ end, did not induce erythroid leukemia, but induced thymic lymphomas, inspite of the presence of Oliff's 2.4-kbp fragment in the Chatis et al. recombinant (6). These data show that both the *env* gene and the LTR of ecotropic Friend virus might play a role in leukemia induction. These data also suggest that it is very difficult to identify the sequence responsible for the erythroid leukemia induction and to determine whether erythroid leukemia induction by the ecotropic Friend virus is ascribable to the appearance of MCF virus. Recently, we have constructed a recombinant whose genome is derived primarily from Friend MCF virus, but which has the same 621 nucleotides of Moloney MuLV as the Chatis et al. recombinant, which might show the possible role of the Friend MCF virus on the erythroleukemia induction. The characteristics of the Friend MCF virus and the Moloney virus LTRs are under investigation.

ACKNOWLEDGMENT

This work was partly supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

LITERATURE CITED

1. **Amanuma, H., A. Katori, M. Obata, N. Sagata, and Y. Ikawa.** 1983. Complete nucleotide sequence of the gene for the specific glycoprotein (gp55) of Friend spleen focus-forming virus. Proc. Natl. Acad. Sci. U.S.A. **80**:3913–3917.
2. **Axelrad, A. A., and R. A. Steeves.** 1964. Assay for Friend leukemia virus: rapid quantitative method based on enumeration of macroscopic spleen foci in mice. Virology **24**:513–518.
3. **Barbacid M., D. H. Troxler, E. M. Scolnick, and S. A. Aaronson.**

1978. Analysis of translational products of Friend strains of spleen focus-forming virus. J. Virol. **27**:826–830.
4. **Bilello, J. A., G. Colletta, G. Warnecke, G. Koch, D. Frisby, I. B. Pragnell, and W. Ostertag.** 1980. Analysis of the expression of spleen focus-forming virus (SFFV)-related RNA and gp55, a Friend and Rauscher virus-specific protein. Virology **107**:331–344.
5. **Bosselman, R. A., F. Straaten, C. Beveren, I. M. Verma, and M. Vogt.** 1982. Analysis of the env gene of a molecularly cloned and biologically active Moloney mink cell focus-forming proviral DNA. J. Virol. **44**:19–31.
6. **Chatis, P. A., C. A. Holland, J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1983. Role for the 3′ end of the genome in determining disease specificity of Friend and Moloney murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **80**:4408–4411.
7. **Chattopadhyay, S. K., A I. Oliff, D. L. Linemeyer, M. R. Lander, and D. R. Lowy.** 1981. Genomes of murine leukemia viruses isolated from wild mice. J. Virol. **39**:777–791.
8. **Clark, S. P., and T. W. Mak.** 1982. Nucleotide sequences of the murine retrovirus Friend $SFFV_p$ long repeats: identification of a structure with extensive dyad symmetry 5′ to the TATA box. Nucleic Acids Res. **10**:3315–3330.
9. **Clark, S. P., and T. W. Mak.** 1983. Complete nucleotide sequence of an infectious clone of Friend spleen focus-forming provirus: gp55 is an envelope fusion glycoprotein. Proc. Natl. Acad. Sci. U.S.A. **80**:5037–5041.
10. **Dresler, S., M. Ruta, M. J. Murray, and D. Kabat.** 1979. Glycoprotein encoded by the Friend spleen focus-forming virus. J. Virol. **30**:564–575.
11. **Evans, L., M. Nunn, P. Duesberg, D. Troxler, and E. Scolnick.** 1979. RNAs of defective and nondefective components of Friend anemia and polycythemia virus strains identified and compared. Cold Spring Harbor Symp. Quant. Biol. **44**:823–835.
12. **Friend, C.** 1957. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J. Exp. Med. **105**:307–318.
13. **Graham, R., and A. V. Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
14. **Hager, G., E. H. Chang, H. W. Chan, C. F. Garon, M. A. Israel, M. A. Martin, E. M. Scolnick, and D. R. Lowy.** 1979. Molecular cloning of the Harvey sarcoma virus closed circular DNA intermediates: initial structural and biological characterization. J. Virol. **31**:795–809.
15. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia virus. Virology **56**:128–134.
16. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphoma. Proc. Natl. Acad. Sci. U.S.A. **74**:789–792.
17. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1981. Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature (London) **290**:475–480.
18. **Henderson, I. C., M. M. Lieber, and G. J. Todaro.** 1974. Mink cell line MuILu (CCL64) focus formation and generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. Virology **60**:282–287.
19. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
20. **Hoffmann, J. W., D. Steffen, J. Gusella, C. Tabin, S. Bird, D. Cowing, and R. A. Weinberg.** 1982. DNA methylation affecting the expression of murine leukemia proviruses. J. Virol. **44**:144–157.
21. **Ishimoto, A., A. Adachi, K. Sakai, T. Yorifuji, and S. Tsuruta.** 1981. Rapid emergence of mink cell focus-forming (MCF) virus in various mice infected with NB-tropic Friend virus. Virology **113**:644–655.
22. **Koch, W., G. Hunsmann, and R. Friedruch.** 1983. Nucleotide sequence of the envelope gene of Friend murine leukemia virus. J. Virol. **45**:1–9.
23. **Linemeyer, D. L., J. G. Menke, S. K. Ruscetti, L. H. Evans, and E. M. Scolnick.** 1982. Envelope gene sequences which encode the gp52 protein of spleen focus-forming virus are required for the induction of erythroid cell proliferation. J. Virol. **43**:223–233.
24. **Linemeyer, D. L., D. L. Ruscetti, J. G. Menke, and E. M. Scolnick.** 1980. Recovery of biologically active spleen focus-forming virus-pBR322 circular DNA by cotransfection with infectious type C retroviral DNA. J. Virol. **35**:710–721.
25. **Linemeyer, D. L., S. K. Ruschetti, E. M. Scolnick, L. H. Evans, and P. H. Duesberg.** 1981. Biological activity of the spleen focus-forming virus is encoded by a molecularly cloned subgenomic fragment of spleen focus-forming virus DNA. Proc. Natl. Acad. Sci. U.S.A. **78**:1401–1405.
26. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage λ. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
27. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:560–564.
28. **Montreuil, J.** 1980. Primary structure of glycoprotein glycans. Basis for the molecular biology of glycoproteins. Adv. Carbohydr. Chem. Biochem. **37**:157–223.
29. **Oliff, A., D. Linemeyer, S. Ruschett, R. Lowe, D. Lowy, and E. Scolnick.** 1980. Subgenomic fragments of molecularly cloned Friend murine leukemia virus DNA contains the gene(s) responsible for Friend murine leukemia virus-induced disease. J. Virol. **35**:924–936.
30. **Oliff, A., and S. Ruscetti.** 1983. A 2.4-kilobase-pair fragment of the Friend murine leukemia virus genome contains the sequences responsible for Friend murine leukemia virus-induced erythroleukemia. J. Virol. **46**:718–725.
31. **Peebles, P. T.** 1975. An in vitro focus-induction assay for xenotropic murine leukemia virus, feline leukemia virus C, and the feline-primate viruses RD-114/CCC/M-7. Virology **67**:288–291.
32. **Rosner, M. R., L. S. Grinna, and P. W. Robbins.** 1980. Differences in glycosylation patterns of closely related murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **77**:67–71.
33. **Ruscetti, S., L. Davis, J. Feild, and A. Oliff.** 1981. Friend murine leukemia virus-induced leukemia is associated with the formation of mink cell focus-inducing xenotropic viral envelope genes. J. Exp. Med. **134**:907–920.
34. **Ruscetti, S. K., D. Linemeyer, J. Feild, D. Troxler, and E. M. Scolnick.** 1979. Characterization of a protein found in cells infected with the spleen focus-forming virus that shares immunological cross-reactivity with the gp70 found in mink cell focus-inducing virus particles. J. Virol. **30**:787–798.
35. **Ruscetti, S., D. Troxler, D. Linemeyer, and E. Scolnick.** 1980. Three laboratory strains of spleen focus-forming virus: comparison of their genomes and translational products. J. Virol. **33**:140–151.
36. **Sharp, P.** 1981. Speculations of RNA splicing. Cell **23**:643–646.
37. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293**:543–548.
38. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
39. **Toyoshima, K., and P. K. Vogt.** 1969. Enhancement and inhibition of avian sarcoma viruses by polycations and polyanions. Virology **38**:414–426.
40. **Troxler, D. H., S. K. Ruscetti, D. L. Linemeyer, and E. M. Scolnick.** 1980. Helper-independent and replication-defective erythroblastosis-inducing viruses contained within anemia-inducing Friend virus complex (FV-A). Virology. **102**:28–45.
41. **Troxler, D. H., and E. M. Scolnick.** 1978. Rapid leukemia induced by cloned strain of replicating murine type-C virus: association with induction of xenotropic-related RNA sequences contained in spleen focus-forming virus. Virology **85**:17–27.
42. **Troxler, D. H., E. Yuhan, D. Linemeyer, S. Ruscetti, and E. M. Scolnick.** 1978. Helper independent mink cell focus-inducing strains of Friend murine type-C virus: potential relationship to the origin of replication-defective spleen focus-forming virus. J. Exp. Med. **148**:639–653.

43. **Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin.** 1979. DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. Proc. Natl. Acad. Sci. U.S.A. **76**:1373–1376.
44. **Wolff, L., E. Scolnick, and S. Ruscetti.** 1983. Envelope gene of the Friend spleen focus-forming virus: deletion and insertions in 3'gp70/p15E-encoding region have resulted in unique features in the primary structure of its protein product. Proc. Natl. Acad. Sci. U.S.A. **80**:4718–4722.
45. **Yoshida, M., and H. Yoshikura.** 1980. Analysis of spleen focus-forming virus-specific glycoprotein with a molecular weight of 55,000 (gp55). J. Virol. **33**:587–596.