Role of a Membrane Glycoprotein in Friend Virus Erythroleukemia: Nucleotide Sequences of Nonleukemogenic Mutant and Spontaneous Revertant Viruses

JING-PO LI, RICHARD K. BESTWICK, CURTIS MACHIDA, AND DAVID KABAT*

Department of Biochemistry, School of Medicine, Oregon Health Sciences University, Portland, Oregon 97201

Received 5 August 1985/Accepted 8 October 1985

We previously isolated spontaneous *env* gene mutants of Friend spleen focus-forming virus that are nonleukemogenic in adult mice but form leukemogenic revertants in newborns; we found that the revertants contain secondary *env* mutations. To identify sites in the encoded membrane glycoprotein that are important for its pathogenic function, we molecularly cloned and partially sequenced the *env* genes of two mutant viruses (clone 63 and clone 4) and one revertant (clone 4^{REV}). Clone 63 contained three noncontiguous point mutations that caused nonconservative amino acid substitutions of Gly-119→Arg-119, Cys-180→Tyr-180, and Gly-203→Arg-203 in the xenotropic-related domain of the *env* glycoprotein. These substitutions were presumably responsible for the altered electrophoretic and pathogenic properties of the mutant glycoprotein. The presence of these and several other G-A nucleotide substitutions at different sites in one spontaneous mutant provided striking evidence that error-rich proviruses can form during retroviral replication. Clone 4 contained a point mutation that generated a premature termination codon at amino acid residue 304 (Gln-304→Ochre-304). This termination codon was located immediately after the proposed xenotropic-ecotropic recombination site and eliminated the ecotropic-related domain, including the putative membrane anchor of the glycoprotein. Clone 4^{REV} was a true revertant derived from clone 4 in which the premature termination codon had back-mutated to re-form the wild-type sequence. These results confirm an essential role for the *env* gene in Friend spleen focus-forming virus pathogenesis and suggest that the encoded membrane glycoprotein contains different domains that contribute to its pathogenic function.

Friend spleen focus-forming virus (F-SFFV) is a defective murine type C retrovirus that, in the presence of a helper virus, can cause erythroleukemia in adult mice (9). The F-SFFV *env* gene encodes a membrane glycoprotein with an apparent M_r of 55,000 (gp55) that is structurally and immunologically related to the envelope glycoproteins of dual-tropic recombinant murine leukemia viruses (7, 24); there is now substantial evidence that this glycoprotein is essential for pathogenesis (16-21, 26; for a review, see reference 25). We previously reported the isolation and characterization of several spontaneous, stable, nonleukemogenic and weakly leukemogenic SFFV mutants with mutations in nonoverlapping regions of their *env* genes (20, 21, 26). When these mutants were injected into newborn mice, revertants formed that were fully leukemogenic in mice of all ages (19). To analyze the specific structural features that contribute to gp55 function, we molecularly cloned two nonpathogenic F-SFFV *env* mutants and one revertant. Partial DNA sequence analysis indicated that the two mutants have alterations in different regions of their *env* genes. A single-nucleotide back mutation in the revertant virus restored its pathogenic function.

MATERIALS AND METHODS

Cells and viruses. All F-SFFV mutant viruses were derived from the normal rat kidney clone 1 nonproducer cell line (31, 32) as described previously (19, 26). NIH 3T3 cells infected with these viruses were maintained in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum and antibiotics.

Hirt extraction and molecular cloning of F-SFFV mutant

and revertant proviral DNAs. Preparation of unintegrated proviral DNA and its cloning into a λ bacteriophage vector have been described previously (3). Briefly, the unintegrated proviral DNAs were extracted from virus-infected NIH 3T3 cell cultures by the procedure of Hirt (11), and the circular forms were cloned into the vector λ Charon 28 (Bethesda Research Laboratories, Gaithersburg, Md.) through a unique *Hind*III site. A *Hind*III-*Kpn*I fragment containing the *env* gene and part of the long terminal repeat (LTR) was then isolated from the recombinant λ DNA for restriction endonuclease analysis and subcloning into an M13 bacteriophage for sequencing analysis.

Restriction endonuclease analysis. Hirt supernatant or recombinant λ DNA was digested with restriction endonucleases as recommended by the supplier (New England BioLabs Inc., Beverly, Mass.). Digested DNA was fractionated on agarose gels and transferred to nitrocellulose paper by the method of Southern (29). After transfer, the blot was hybridized with a ³²P-labeled SFFV-specific probe made of a 620-base-pair *Bam*HI-*Eco*RI *env* gene fragment from molecularly cloned F-SFFV (18). The hybridization procedure has also been described previously (3).

Nucleotide sequence analysis. The *Hind*III-*Kpn*I restriction fragments encompassing the *env* gene and part of the LTR were isolated from the recombinant λ DNA by double digestion with the appropriate enzymes and then electroeluted from agarose gel as described by Maniatis et al. (22). The DNA fragments were further cleaved with one or a combination of *Hin*PI, *Sau*3A, *Alu*I, *Sma*I, and *Eco*RI restriction endonucleases, and the resulting subfragments were ligated into the appropriate cloning sites of the M13 vectors MP8 and MP9. Sequencing was performed by the Sanger dideoxynucleotide method (27), using the protocol of

* Corresponding author.

FIG. 1. (A) Southern blot analysis. Lanes: 1, lambda-HindIII marker; 2 and 3, undigested Hirt supernatant DNA from clone 63- and clone 4-infected cells, respectively; 4 and 5, BamHI digestions of Hirt supernatant DNA from clone 63- and clone 4-infected cells, respectively. 6 and 7, EcoRI digestions of Hirt supernatant DNA from clone 63- and clone 4-infected cells, respectively. Previously we noted a fragment of about 600 base pairs that could hybridize to the *env* gene-specific probe from a Hirt extract of cells infected with clone 63 after the Hirt DNA was digested with a mixture of BamHI and EcoRI restriction endonucleases (26). However, this fragment could not be found when Hirt DNA preparations containing less contaminating genomic DNA were used. It is likely that the 600-base-pair fragment observed previously was derived from an F-SFFV-like endogenous sequence that is present in the genomic DNA. (B) Restriction maps of the linear and the two circular forms of un-integrated proviral DNA for wild-type Lilly-Steeves strain F-SFFV (33).

Messing (23). The sequencing primer was either the 15-mer or 17-mer (New England Biolabs). The Klenow fragment of *Escherichia coli* DNA polymerase I was obtained from New England Nuclear Corp., Boston, Mass.

Compilation and sequence analysis were performed on an Apple II+ microcomputer with the programs of Larson and Messing (14) and Fristensky et al. (10).

RESULTS AND DISCUSSION

Southern blot and restriction endonuclease analyses of proviral DNA from Hirt supernatants. We reported previously that the clone 4 and clone 63 F-SFFV mutants encode *env*-related glycoproteins with apparent M_r s of 40,000 (gp40) and 58,000 (gp58), respectively (26). However, the sizes of their proviral DNAs and of their genomic and subgenomic RNAs were indistinguishable from those of wild-type F-SFFV (26).

To study these mutants further, we analyzed un-integrated proviral DNAs from the Hirt supernatants of infected NIH 3T3 fibroblasts. A restriction endonuclease DNA blot analysis of the proviral DNAs with an SFFV-specific probe containing the BamHI-EcoRI region of the *env* gene is shown in Fig. 1. After digestion with BamHI, three fragments of clone 4 proviral DNA hybridized to the probe (Fig. 1A, lane 5). These fragments were about 4.7, 4.2, and 1.9 Kilobases (kb) long and were derived from the two circular forms with either one or two LTRs and from the linear form of proviral DNA, respectively, as expected from the restriction map of wild-type F-SFFV (17).

Clone 63 lost the BamHI site that occurs slightly 5' to the *env* gene (Fig. 1A). Accordingly, the proviral DNA formed three BamHI fragments with sizes of about 6.2, 5.7, and 3.4 kb. In addition, clone 63 lacked an EcoRI site within its *env*

gene, because three EcoRI fragments of about 6.2, 5.7, and 3.1 kb hybridized to the probe (lane 6), although only a single 1.8-kb EcoRI fragment from clone 4 and wild-type proviral DNAs hybridized to the probe (lane 7). Similar studies of clone 4^{REV} indicated that its proviral DNA was indistinguishable in size and restriction map from that of clone 4 and wild-type F-SFFVs.

Molecular cloning. The strategy used to clone the mutant and revertant proviral DNA was described earlier (3, 18). The circular form of proviral DNA in the Hirt supernatant was purified by agarose gel electrophoresis and was then linearized by HindIII digestion before ligation with λ Charon 28 HindIII arms that had been pretreated with calf intestinal alkaline phosphatase. After screening about 2×10^4 to 5×10^4 plaques with an SFFV *env*-specific probe as described above, a few to several hundred positive plaques were obtained from each virus. In each case, one recombinant λ -F-SFFV clone was isolated and analyzed by restriction mapping. They all contained a viral insert of about 5.7 kb (with one copy of the LTR), and the restriction patterns were identical to those obtained for the corresponding Hirt extracts (data not shown). A HindIII-KpnI fragment containing the *env* gene was then isolated from each recombinant clone in sufficient amount for DNA sequence analysis.

DNA sequence analysis. The wild-type F-SFFV that we used for our original mutant isolations (26) was a Lilly-Steeves polycythemic strain that had been biologically cloned into normal rat kidney cells and then molecularly cloned by Linemeyer et al. (18) and sequenced by Wolff et al. (33). Consequently, we have in all cases compared our sequences with the sequence obtained by these investigators. Because the clone 4 and clone 63 F-SFFVs contain mutations that affect the carboxyl-terminal domain and amino-terminal gp55 domain, respectively (26), we focused our sequence studies on the corresponding regions of their

FIG. 2. DNA sequence analysis. Top, Schematic representation of the wild-type F-SFFV *env* gene (33) and its flanking regions. The *env* gene coding region starts as nucleotide 229 and ends at nucleotide 1455. X-E, Proposed xenotropic-ecotropic recombination site. (A) Regions of clone 4 (C.4) *env* gene that have been sequenced. Arrows indicate point mutations found in this clone; their locations are indicated by nucleotide numbers relative to the wild-type *env* gene. The nucleotide substitutions of these mutations are indicated in parentheses. (B and C) Regions that have been sequenced for clone 63 (C.63) and clone 4^{REV} (C.4 REV), respectively.

env genes. The results of partial DNA sequence analysis of the cloned *env* genes of the mutant and revertant viruses are shown in Fig. 2. All the mutations found in the *env* genes of these viruses were point mutations as determined by comparison with the published sequence (33) of the wild-type

Lilly-Steeves strain of F-SFFV. A summary of these point mutations compared with various wild-type sequences, including that of the Lilly-Steeves strain (referred to as S1), is contained in Table 1; a comparison of the amino acid sequences of these *env* genes is shown in Table 2.

It is noteworthy that in sequencing these mutant and revertant F-SFFVs, we obtained information for the entire *env* gene, and many regions were sequenced twice (Fig. 2). In considering the implications of this sequence information,

TABLE 1. Comparison of nucleotide substitutions with wild-type F-SFFV sequences

Nucleotide no. ^a	<i>env</i> gene sequence ^b						
	Clone 4	Clone 63	Clone 4 ^{REV}	S1	Si	Sax	Sa
125	NA	G*	NA	A	A	A	NA
165	G	A*	NA	G	G	G	NA
292	G	G	NA	A	G	G	A
295	C	C	NA	T	C	C	C
471	NA	A	NA	G	A	A	A
529	NA	A	NA	G	G	G	A
583	G	A*	NA	G	G	G	G
767	G	A*	NA	G	G	G	G
784	G	A	NA	G	G	A	G
835	G	A*	NA	G	G	G	G
945	C	C	NA	T	T	T	C
948	T	T	NA	C	T	T	T
986	T	T	NA	C	T	T	T
1138	T*	C	C	C	C	C	C
1368	C	C	NA	T	C	C	C
1488	T	T	T	C	T	T	NA

^a According to Wolff et al. (33).

^b F-SFFV strains: S1, Lilly and Steeves (15, 33); Si, Ikawa et al. (1, 12); Sax, Axelrod and Steeves (2, 4); Sa, anemia-inducing strain (13, 34). *, Unique nucleotide substitutions. NA, Data not available.

TABLE 2. Comparison of amino acid substitutions in sequences of F-SFFV mutants with various wild-type sequences

Nucleotide no.	Amino acid residue no. (from amino terminus)	Amino acid residue ^a						
		Clone 63	Clone 4	S1	Si	Sax	Sa	Sr
292	21	Val	Val	Ile	Val	Val	Ile	Ile
471	81	Ile		Met	Ile	Ile	Ile	Ile
529	101	Thr		Ala	Ala	Ala	Thr	Ala
583	119	Arg*	Gly	Gly	Gly	Gly	Gly	Gly
767	180	Try*	Cys	Cys	Cys	Cys	Cys	Cys
784	186	Lys	Glu	Glu	Glu	Lys	Glu	Glu
835	203	Arg*	Gly	Gly	Gly	Gly	Gly	Gly
986	253	Leu	Leu	Pro	Leu	Leu	Leu	Leu
1138	304	Gln	Ochre* (TAA)	Gln	Gln	Gln	Gln	Gln

^a For abbreviations, see Table 1, footnote b. Sr, Rauscher SFFV. *, Unique amino acid alterations.

we assumed that sequences that occur in one or more of the published wild-type sequences must be compatible with gp55 pathogenic function. By this criterion, there was only one relevant mutation in the clone 4 mutant; it occurred at nucleotide 1138 and caused premature termination at amino acid 304 (Table 2). This premature termination codon occurred immediately after the proposed xenotropic-ecotropic recombination site on the *env* gene, and therefore it eliminated the ecotropic-related glycoprotein domain, including the putative hydrophobic membrane anchor that occurs near the carboxyl terminus. This result correlates well with previous evidence that the mutant synthesizes a truncated *env* glycoprotein with an intact N-terminal domain that can be immunoprecipitated with an anti-dual-tropic virus antiserum but lacks a C-terminal domain (26). The apparent size (M_r , 40,000) of the *env* glycoprotein encoded by this mutant also corresponded to the placement of this premature termination codon. Consequently, the ecotropic-related domain of the *env* glycoprotein must be important for the maintenance of full pathogenicity.

As described above, clone 4^{REV} was a pathogenic revertant of the clone 4 mutant. Clone 4^{REV} contained the wild-type sequence at nucleotide 1138, indicating that the premature termination codon in clone 4 was repaired by a back T→C transition to regenerate the wild-type sequence (Table 1). Since clone 4^{REV} is fully active (19), these results support the conclusion that a single point mutation at nucleotide 1138 in clone 4 is the cause for the loss of F-SFFV pathogenicity.

We previously concluded that clone 4^{REV} contains an intragenic suppressor mutation in its *env* gene and that it is not a true revertant (19). That conclusion was based on a slight difference in the electrophoretic mobility of the *env* glycoproteins encoded by the wild-type and clone 4^{REV} (apparent M_r , 56,000) F-SFFVs. Conceivably, that difference is caused by one of the pathologically neutral differences between the clone 4 and S1 sequences indicated in Table 2. Our sequence results suggest that the pathologically relevant mutation in clone 4 has reverted in clone 4^{REV}. Clone 4, clone 63, and clone 4^{REV} have identical sequences in their LTRs (Jing-Po Li, R. K. Bestwick, and D. Kabat, submitted for publication).

When the same criteria were applied to the sequence data from the clone 63 mutant, three potentially relevant mutations were observed at nucleotides 583, 767, and 835, which caused nonconservative amino acid substitutions of Gly-119→Arg-119, Cys-180→Tyr-180, and Gly-203→Arg-203, respectively (Table 2). Presumably, these substitutions would affect protein folding and possibly disulfide bond formation and could account for the slightly reduced electrophoretic mobility of the encoded glycoprotein compared with that of gp55 (26). Amino acid substitutions in the *ras* protein alter its electrophoretic mobility in polyacrylamide gels containing sodium dodecyl sulfate (6, 8, 28, 30). These changes in gp55 probably also cause the reduced reactivity of the glycoprotein with an antiserum that is specific for dual-tropic murine leukemia viruses (26) and account for its lack of pathogenic activity. It is surprising that this single spontaneous mutant contained mutations that caused at least three noncontiguous and nonconservative amino acid substitutions. Additional mutations at nucleotides 529 and 784 caused amino acid substitutions that were presumably pathogenically neutral because they occurred in a wild-type sequence. In addition, clone 63 contained apparent mutations at nucleotides 125 and 165 in the adjacent *pol* gene. Strikingly, these were all G-A substitutions (Table 1). This plethora of muta-

tions implies that there may be error-rich retroviral reverse transcripts. Conceivably, these might be formed by a mutant or improperly folded reverse transcriptase or by a transcriptase that lacks a regulatory subunit.

The G→A mutations in clone 63 at nucleotides 165 and 784 would destroy the *Bam*HI and *Eco*RI recognition sequences, respectively. This result explains the changes in the restriction endonuclease digestion patterns of the proviral DNA from the Hirt supernatant (Fig. 1). The fact that the unintegrated DNA from the Hirt supernatant contained these noncontiguous mutations establishes that they were present in the original clone 63 mutant and that they were not formed during this molecular cloning study.

Why do our clone 4 and clone 63 mutant viruses have common sequence differences that distinguish them from the virus sequenced by Wolff et al. (33)? We confirmed the sequence differences of our mutants by isolating different sets of M13 recombinant clones and sequencing in the opposite orientation. These common differences are difficult to understand because our wild-type virus was derived from the same normal rat kidney clone 1 nonproducer cell line that was the source of the virus analyzed by Wolff et al. One possible explanation is that there may be errors at these positions in the published sequence (33). This sequence suggests that there is a cytosine at nucleotide 986 (Table 1) that would predict a *Bgl*II restriction site at this position in the *env* gene (33). However, we did not find this *Bgl*II site in the recombinant plasmid 4-1a3 that we obtained from D. Linemeyer (18) and that was used by Wolff et al. in their sequence analysis (33). Similarly, our DNA sequence data predict a *Sau*3AI restriction site at nucleotide 1488, and this restriction site is present in the 4-1a3 plasmid (data not shown). In addition, we sequenced an M13 clone derived from this plasmid that contained a region of the *env* gene between nucleotides 813 and 1017, and we found a cytosine at nucleotide 945 and a thymine at nucleotides 948 and 986 (data not shown), the same result obtained for both our mutants at these positions (Table 1). Although overall the sequence data for our mutant clones indicate that they have a closer relationship to the F-SFFV S1 wild-type sequence (33) than to the other F-SFFV *env* gene sequences, the F-SFFV S1 sequence differs from all the others at several positions (e.g., nucleotides 295, 471, 948, 986, 1368, and 1488) (Table 1). A second explanation is that there could be several pathogenic F-SFFVs in normal rat kidney clone 1 cells or that F-SFFV sequences can evolve rapidly without loss of pathogenesis. The fact that the clone 63 mutant contained several noncontiguous mutations, and other results (5), is consistent with the possibility that F-SFFVs can evolve rapidly.

The mechanism of F-SFFV pathogenesis is not fully understood. Our results strongly support the conclusion that the membrane glycoprotein encoded by the F-SFFV *env* gene is essential for the erythroblast proliferation that characterizes the first stage of leukemogenesis. Our results also confirm earlier evidence that pathogenesis can be reduced by mutations that alter different domains of the gp55 membrane glycoprotein. In addition, our results imply that spontaneous retroviral mutants may frequently be caused by C-T and G-A transitions and that occasional reverse transcripts may contain a relatively large number of noncontiguous specific transitions. This latter observation is important because it suggests that mutations may form not only by random low-frequency errors but also by error-prone polymerases that cause multiple specific mutations within single nucleic acids.

ACKNOWLEDGMENTS

We thank Jennifer Au and Bruce Boswell for excellent assistance.

This research was supported by Public Health Service grant CA25810 from the National Institutes of Health. R.K.B. is the recipient of a Special Fellowship from the Leukemia Society of America.

LITERATURE CITED

- 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. USA* **80**:3913-3917.
- Axelrod, A. A., and R. A. Steeves. 1964. Assay for Friend leukemia virus: a rapid quantitative method based on enumeration of macroscopic spleen foci in mice. *Virology* **24**:513-518.
- Bestwick, R. K., B. A. Boswell, and D. Kabat. 1984. Molecular cloning of biologically active Rauscher spleen focus-forming virus and the sequences of its *env* gene and long terminal repeat. *J. Virol.* **51**:695-705.
- Clark, S. P., and T. 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. USA* **80**:5037-5041.
- Clark, S. P., and T. W. Mak. 1984. Fluidity of a retrovirus genome. *J. Virol.* **50**:759-765.
- Der, C., and G. Cooper. 1983. Altered gene products are associated with activation of cellular *ras*^K genes in human lung and colon carcinomas. *Cell* **32**:201-208.
- 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.
- Fasano, O., T. Aldrich, F. Tamanai, E. Taparowsky, M. Furth, and M. Wigler. 1984. Analysis of the transforming potential of the human H-ras gene by random mutagenesis. *Proc. Natl. Acad. Sci. USA* **81**:4008-4012.
- 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.
- Fristensky, B., J. Lis, and R. Wu. 1982. Portable microcomputer software for nucleotide sequence analysis. *Nucleic Acids Res.* **10**:6451-6463.
- Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. *J. Mol. Biol.* **26**:365-369.
- Ikawa, Y., M. Aida, and Y. Indue. 1976. Isolation and characterization of high and low differentiation-inducible Friend leukemia lines. *Gann* **67**:767-770.
- Kaminchik, J., W. D. Hankins, S. K. Ruscetti, D. L. Linemeyer, and E. M. Scolnick. 1982. Molecular cloning of biologically active proviral DNA of the anemia-inducing strain of spleen focus-forming virus. *J. Virol.* **44**:922-931.
- Larson, R., and J. Messing. 1982. Apple II software for M13 shotgun DNA sequencing. *Nucleic Acids Res.* **10**:39-49.
- Lilly, F., and R. A. Steeves. 1973. B-tropic Friend-virus: a host-range pseudotype of spleen focus-forming virus (SFFV). *Virology* **43**:223-233.
- Linemeyer, D., S. Ruscetti, E. Scolnick, L. Evans, and P. 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. USA* **78**:1401-1405.
- 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.
- Linemeyer, D. L., S. K. Ruscetti, J. G. Menke, and E. M. Scolnick. 1980. Recovery of biologically active spleen focus-forming virus from molecularly cloned spleen focus-forming virus-pBR322 circular DNA by cotransfection with infectious type C retroviral DNA. *J. Virol.* **35**:710-721.
- Machida, C., R. Bestwick, B. Boswell, and D. Kabat. 1985. Role of a membrane glycoprotein in Friend virus-induced erythroleukemia: studies of mutant and revertant viruses. *Virology* **144**:158-172.
- Machida, C. A., R. K. Bestwick, and D. Kabat. 1984. Reduced leukemogenicity caused by mutations in the membrane glycoprotein gene of Rauscher spleen focus-forming virus. *J. Virol.* **49**:394-402.
- Machida, C. A., R. K. Bestwick, and D. Kabat. 1985. A weakly pathogenic Rauscher spleen focus-forming virus mutant that lacks the carboxyl-terminal membrane anchor of its envelope glycoprotein. *J. Virol.* **53**:990-993.
- Maniatis, T., E. Fritsch, and J. Sambrook (ed.). 1982. *Molecular cloning: a laboratory manual*, p. 164-170. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Messing, J. 1983. New M13 vectors for cloning. *Methods Enzymol.* **101**:20-78.
- 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.
- Ruscetti, S., and L. Wolff. 1984. Spleen focus-forming virus: relationship of an altered envelope gene to the development of a rapid erythroleukemia. *Curr. Top. Microbiol. Immunol.* **112**:21-44.
- Ruta, M., R. Bestwick, C. Machida, and D. Kabat. 1983. Loss of leukemogenicity caused by mutations in the membrane glycoprotein structural gene of Friend spleen focus-forming virus. *Proc. Natl. Acad. Sci. USA* **80**:4704-4708.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463-5467.
- Shimizu, K., M. Goldfarb, Y. Suard, M. Perucho, Y. Li, T. Kamata, J. Feramisco, E. Stavnezer, J. Fogh, and M. Wigler. 1983. Three human transforming genes are related to the viral *ras* oncogenes. *Proc. Natl. Acad. Sci. USA* **80**:2112-2116.
- Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503-517.
- Tabin, C., S. Bradley, C. Bargmann, R. Weinberg, A. Papageroge, E. Scolnick, R. Dhar, D. Lowy, and E. Chang. 1982. Mechanism of activation of a human oncogene. *Nature (London)* **300**:143-148.
- Troxler, D., D. Lowy, R. Howk, H. Young, and E. Scolnick. 1977. Friend strain of spleen focus-forming virus is a recombinant between ecotropic type C virus and the *env* gene region of xenotropic type C virus. *Proc. Natl. Acad. Sci. USA* **74**:4671-4675.
- Troxler, D. H., W. P. Parks, W. C. Vass, and E. M. Scolnick. 1977. Isolation of a fibroblast nonproducer cell line containing the Friend strain of the spleen focus-forming virus. *Virology* **76**:602-615.
- Wolff, L., E. Scolnick, and S. Ruscetti. 1983. Envelope gene of the Friend spleen focus-forming virus: deletion and insertions in the 3' gp70/p15E-encoding region have resulted in unique features in the primary structure of its protein product. *Proc. Natl. Acad. Sci. USA* **80**:4718-4722.
- Wolff, L., J. Kaminchik, W. D. Hankins, and S. K. Ruscetti. 1985. Sequence comparisons of the anemia- and polycythemia-inducing strains of Friend spleen focus-forming virus. *J. Virol.* **53**:570-578.