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Clonal analysis of early and late stages of erythroleukemia induced by molecular clones of integrated spleen focus-forming virus

(retrovirus/recombinant DNA/DNA transfer/erythropoietin independence/leukemic colony-forming cells)

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ABSTRACT **The integrated proviral DNA of the polycythemia-inducing isolate of Friend spleen focus-forming virus ($SFFV_P$) has been identified in rat cell clones nonproductively infected with this replication-defective erythroleukemia virus and cloned in phage λ vectors. These λ$SFFV_P$ recombinants, λ$SFFV_P$502 and λ$SFFV_P$542, contain endonuclease *Eco*RI inserts of size 7.4 and 8.2 kilobases, respectively, and include full copies of the $SFFV_P$ genome, along with host flanking sequences. Infectivity of the cloned $SFFV_P$ genomes was tested by a two-step DNA transfer procedure involving transfection of the cloned DNA into 3T3 mouse fibroblasts or cotransfer of the cloned DNA into thymidine kinase-deficient 3T3 cells together with the cloned thymidine kinase gene of herpes simplex virus, followed by rescue of the transferred DNA by superinfection with a helper virus. Inoculation of the rescued virus into adult mice resulted in the appearance of spleen foci, rapid splenomegaly, and polycythemia. Early after infection, spleen cell populations contained large numbers of cells capable of forming small erythroid colonies *in vitro* (CFU-E) in the absence of erythropoietin. Late after infection, these mice contained cells capable of forming macroscopic colonies (CFU-FV) *in vitro*. These data indicate that molecular clones of $SFFV_P$, in conjunction with a helper virus, induce the appearance of hemopoietic colony-forming cells characteristic of both the early and late stages of Friend leukemia.**

Friend leukemia virus (1) is a retrovirus that induces rapid splenomegaly in adult mice. The original isolate (FV-A; ref. 1) induces anemia, whereas a later isolate (FV-P), derived from stocks of FV-A (2), induces polycythemia. At least two distinguishable defective viruses have been isolated from preparations of Friend virus. Like other rapidly transforming leukemia and sarcoma viruses, both FV-P and FV-A are complexes of two viruses: a defective spleen focus-forming virus ($SFFV_P$ and $SFFV_A$, respectively) and a replication-competent virus [Friend murine leukemia virus (F-MuLV)] that can serve as a helper virus for SFFV (3–5). Although inoculation of F-MuLV into newborn mice of certain inbred strains results in splenomegaly, anemia, and the proliferation of erythroid cells (6, 7), analysis of the diseases induced by different pseudotypes of $SFFV_A$ and $SFFV_P$ has suggested that these defective viruses, rather than their helper viruses, are responsible for differences in the erythroid cell populations observed in FV-A- and FV-P-infected mice (4, 8, 9). Erythroid colony-forming cells (CFU-E) detected in susceptible mice early after infection with different pseudotypes of $SFFV_P$ are independent of the hormone erythropoietin (Epo) (4, 8–10), whereas erythroid progenitor cells in $SFFV_A$-infected mice still require Epo to proliferate (4).

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Both $SFFV_P$ and $SFFV_A$ can induce rapid changes in erythroid progenitor cell populations, but cells capable of autonomous and extensive proliferation *in vitro* or tumorigenic growth *in vivo* can be detected only much later after infection. These cells can be quantitated by their ability to form macroscopic colonies *in vitro* (11, 12) or spleen colonies *in vivo* in genetically anemic Sl/Sl^d recipient mice (13).

The molecular determinants responsible for these differing effects of $SFFV_A$ and $SFFV_P$ on erythroid cells early after infection have not been defined. In addition, it is not known whether different viral functions are necessary to induce the appearance of tumorigenic colony-forming cells late after infection with FV-A or FV-P (13). To approach these questions, we have begun to analyze the organization of the $SFFV_A$ and $SFFV_P$ genomes, using recombinant DNA techniques. In this report, we describe the cloning in phage λ of infectious molecules of integrated $SFFV_P$ proviral DNA from rat cells nonproductively infected with this virus.

MATERIALS AND METHODS

Cells and Viruses. Rat cells nonproductively infected with Friend $SFFV_P$ have been described (4, 14). The $SFFV_P$ nonproducer clones NP501 and NP502 were isolated from NRK cells (14); the $SFFV_P$ nonproducer clone P5 and the $SFFV_A$ nonproducer clone A1 were isolated from RAT-1 cells (4).

Preparation of Cellular and Viral DNA. Cellular DNA for cloning was isolated from cultured cells as described (15). Unintegrated proviral DNA was isolated by the method of Hirt (16) from mouse NIH-3T3 cells infected 18 hr previously with an FV-P preparation containing $SFFV_P$ in excess compared to F-MuLV (14).

Restriction Enzymes and Gel Electrophoresis of DNA. Restriction endonucleases were purchased from Bethesda Research Laboratories (Rockville, MD), New England BioLabs, and Boehringer Mannheim. DNA samples were digested with these enzymes under conditions suggested by the suppliers. Analytical and preparative gels were run in 0.7–1.2% agarose in TA buffer (50 mM Tris/50 mM sodium acetate/18 mM NaCl/2 mM EDTA, pH 8.2). Detection of specific DNA sequences was by the method of Southern (17), using ^{32}P-labeled cDNA.

Preparation of ^{32}P-Labeled FV-P cDNA. DNA complementary to FV-P complex containing a 10-fold excess of $SFFV_P$ over

Abbreviations: FV-P and FV-A, polycythemia and anemia-inducing isolates of Friend leukemia virus, respectively; SFFV, spleen focus-forming virus; F-MuLV, Friend murine leukemia virus; Mo-MSV, Moloney murine sarcoma virus; kb, kilobase(s); Epo, erythropoietin; CFU-FV, colony-forming unit, Friend virus; CFU-E, colony-forming unit, erythroid; LTR, long terminal repeat; TK, thymidine kinase; HAT, hypoxanthine/aminopterin/thymidine.

F-MuLV was synthesized as described (14). Selected ^{32}P-labeled cDNA, enriched for the 3′ end of the viral genome, was prepared by the use of $(dT)_{10}$ as primer (18).

Molecular Cloning. Recombinant DNA was handled in accordance with Medical Research Council of Canada guidelines.

Rat *Eco*RI DNA fragments enriched for $SFFV_P$ sequences (1 μg) were ligated with phage T4 DNA ligase (New England BioLabs) to equal molar amounts of the left and right arms of either λgt*WES*·λB (19) or λ Charon 4A (20) and packaged *in vitro* into phage particles as described (21). Phage were plaqued on *Escherichia coli* K-12 DP50*supF*, and plaques containing recombinant phage were located by the plaque filter hybridization technique (22). Between 1 and 4×10^5 plaques were screened with FV-P ^{32}P-labeled cDNA. Plaques giving positive hybridization were picked and subjected to additional cycles of purification until more than 90% of the phage reacted positively with the FV-P cDNA probe.

Restriction Enzyme Analysis of the Cloned $SFFV_P$ DNA Segments. Recombinant λ-$SFFV_P$ DNA, or the *Eco*RI-digested $SFFV_P$ DNA insert purified by agarose gel electrophoresis, was mapped by double or triple digestions with various restriction endonucleases. Cloned fragments were also analyzed by partial digestion of DNA fragments end-labeled with [γ-^{32}P]ATP by polynucleotide kinase, as described by Smith and Birnstiel (23).

DNA Sequence Analysis. The methods outlined by Maxam and Gilbert (24) were followed. The *Sst* I/*Bgl* II restriction fragment that includes a portion of the 5′ long terminal repeat (LTR) (see Fig. 2) was first 5′-end-labeled with [γ-^{32}P]ATP (2000–5000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and phage T4 polynucleotide kinase then cleaved with *Kpn* I to separate the labeled ends.

Eukaryotic Cell Transformation. Two separate procedures were used to transfer cloned $SFFV_P$ DNA into mouse cells. In the mass transfer method, 5×10^5 mouse NIH-3T3 cells seeded in 100-mm Petri dishes were transfected with 1.5–3.0 μg of recombinant phage DNA as described (25) with modifications (26). The carrier DNA was derived from thymidine kinase-positive (TK^+) mouse cells. Twenty-four hr later, the cells were infected with F-MuLV at a multiplicity of infection of 0.01 plaque-forming unit per cell. The culture medium was harvested 3 days later, filtered, and used to infect DBA/2J mice. In the cotransfer method, 200 ng of the plasmid pxI (27), carrying the *tk* gene of herpes simplex type I virus, was mixed with 0.4–1.5 μg of recombinant phage DNA and transfected, as above, into TK^- Swiss 3T3 cells (28). TK^+ transformants were selected in hypoxanthine/aminopterin/thymidine (HAT) medium, and 2 weeks later colonies were picked. Individual colonies were picked, grown in HAT medium, seeded at 1×10^5 cells per 60-mm Petri dish, and infected with F-MuLV. The medium was harvested and used to infect DBA/2J mice as described above.

Infectivity and Erythroid Colony Assays. Filtered cell culture media (0.5 ml), harvested as described above from the 3T3 cultures, was injected intravenously into female DBA/2J mice (The Jackson Laboratories). The induction of spleen foci (3), splenomegaly, and polycythemia was then monitored in these mice. CFU-E were detected (4) and CFU-FV was assayed (11) as described.

RESULTS

Identification of Rat Cell DNA Fragments Containing the Integrated Friend $SFFV_P$ Genome. In preliminary experiments, a restriction map of the unintegrated linear $SFFV_P$ genome was determined by using viral DNA obtained from Hirt extracts of FV-P-infected NIH-3T3 cells. Digestion of this DNA with various restriction enzymes, followed by Southern transfer and hybridization with ^{32}P-labeled FV-P cDNA, indicated that $SFFV_P$ linear proviral DNA was not cleaved by *Eco*RI, *Hin*dIII, *Sal* I, *Xba* II, and *Xho* I.

We next examined the sites of integration of the SFFV genome in rat cell clones nonproductively infected with SFFV. High molecular weight DNA was extracted from three independent rat cell clones nonproductively infected with $SFFV_P$—clones 501, 502, and P5—and one rat cell clone nonproductively infected with $SFFV_A$—clone A1. This DNA was then separated on an agarose gel, and the SFFV sequences were analyzed by Southern transfer and hybridization to ^{32}P-labeled FV-P cDNA. *Eco*RI DNA fragments from the rat SFFV nonproducer cell clones 501, 502, P5, and A1 all contained one or more fragments that hybridized to FV-P cDNA. A single band of size 8.4 kilobases (kb) was observed in clone 501, two bands (7.4 and 14 kb) were detected in clone 502, three bands (8.5 11.5, and 12.5 kb) were observed in clone P5, and a single band of size 6.8 kb was observed in the $SFFV_A$ nonproducer cell clone A1 (Fig. 1).

Cloning of the Integrated $SFFV_P$ Provirus. The *Eco*RI fragments containing $SFFV_P$ sequences were enriched by agarose gel electrophoresis and electroeluted. One microgram of *Eco*RI-digested clone 502 DNA, of size 6–8 kb, and 1 μg of *Eco*RI-digested P5 DNA, of size 8–20 kb, was ligated to the left and right arms of λgt*WES*·λB and λ Charon 4A, respectively. The ligated DNA was packaged *in vitro* and approximately 10^5 recombinant phages were screened with ^{32}P-labeled FV-P cDNA. One positive plaque from 502 DNA and five plaques from clone P5 DNA were identified. The recombinant phage from 502 DNA in λgt*WES*·λB was termed λ$SFFV_P$502 and the clone derived from clone P5 in λ Charon 4A was designated λ$SFFV_P$542. Digestion of λ$SFFV_P$502 with *Eco*RI resulted in four DNA bands; two fragments of size 23 and 14 kb corresponding to the left and right arms of λgt*WES*·λB, and two insert fragments of size 7.4 and 5.8 kb. Southern gel analysis revealed that only the 7.4-kb *Eco*RI fragment contained $SFFV_P$ sequences; this fragment was purified and subcloned in λgt*WES*·λB. Digestion of the recombinant clone λ$SFFV_P$542 with *Eco*RI yielded four fragments, including the λ Charon 4A arms and two inserts with lengths of 8.2 and 6.2 kb. Southern gel analysis indicated that only the 8.2-kb fragment contained SFFV sequences. On the basis of double digestions with various endonucleases, as well as partial digestions as described by Smith and Birnstiel (23), the restriction maps shown in Fig. 2 were obtained. Both $SFFV_P$ clones contained restriction en-

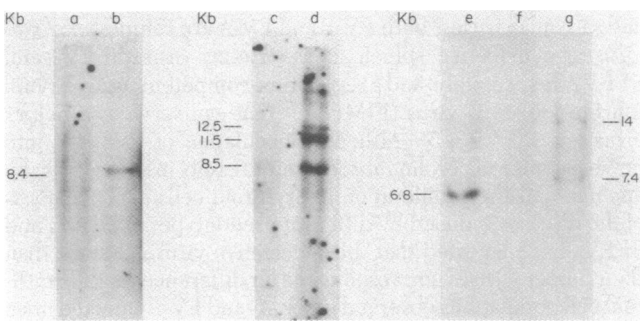

FIG. 1. Detection of $SFFV_P$ and $SFFV_A$ proviral DNA sequences. DNA from RAT-1 and NRK cells nonproductively infected with either $SFFV_P$ or $SFFV_A$ was digested with *Eco*RI and electrophoretically separated on 0.8% agarose gels. The DNA was transferred to nitrocellulose filters and hybridized to ^{32}P-labeled FV-P cDNA. Restriction enzyme fragments of phage λ served as molecular weight markers. Lane a, uninfected NRK DNA; lane b, NRK-$SFFV_P$501; lane c, uninfected RAT-1 DNA; lane d, RAT-1-$SFFV_P$P5; lane e, RAT-1-$SFFV_A$A1; lane f, uninfected NRK DNA; lane g, NRK-$SFFV_P$502.

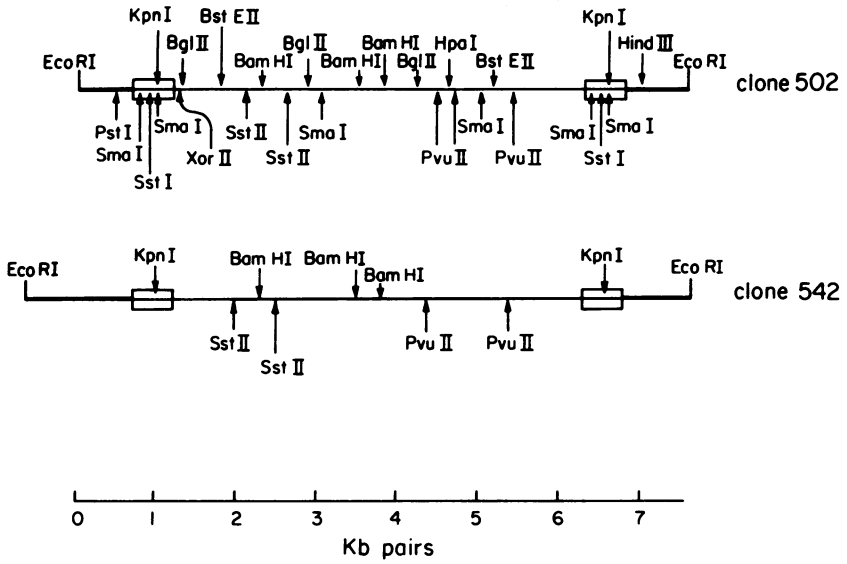

FIG. 2. Restriction enzyme maps of λSFFV$_P$502 and λSFFV$_P$542. The map was derived by cleavage of recombinant phage DNAs with various restriction enzymes followed by agarose gel electrophoresis. Neither DNA was cut by *Xba* I, *Xho* I, or *Sal* I. Orientation of the SFFV$_P$ genome was obtained by using a 3′-specific cDNA probe (Fig. 3) and is shown 5′ to 3′ reading from left to right. Adjacent rat cellular sequences are indicated by thick lines, and the LTRs are represented by the boxes.

zyme sites for *Kpn* I, *Sst* I, and *Sma* I, located approximately 5.8 kb apart at opposite ends of the viral genome. The presence of these repeated restriction enzyme sites are suggestive of direct repeated sequences or LTRs at the termini of the integrated SFFV$_P$ genome. Comparison of the restriction enzyme maps of SFFV$_P$502 and SFFV$_P$542 indicate that they are very similar; however, the size of the cellular flanking sequences adjacent to the integrated provirus in these two clones are different.

Orientation of the SFFV$_P$ Genome. To determine the orientation of the cloned SFFV proviral DNA, a ^{32}P-labeled cDNA probe enriched for the 3′ end of the viral RNA was hybridized to DNA fragments generated by various restriction endonucleases. Because the ends of the integrated provirus are repeated, a 3′-specific cDNA probe will hybridize to DNA fragments from either the 3′ or the 5′ end. Therefore, the 7.4-kb *Eco*RI insert of clone SFFV$_P$502 was first digested with *Kpn* I and *Sst* I to remove the LTRs (see Fig. 2), and DNA was then further digested with *Bgl* II. As shown in Fig. 3, the 2.5-kb fragment generated by *Bgl* II–*Sst* I–*Kpn* I digestion hybridized to the 3′-specific cDNA probe, indicating that this fragment is derived from the 3′ end of the viral genome. As expected, small fragments of size 0.7–0.8 kb containing the LTRs, between the *Kpn* I/*Sst* I sites and the cellular *Eco*RI site, also hybridized to this probe (Fig. 3).

To confirm this orientation, the nucleotide sequence of the *Sst* I/*Bam*HI DNA fragment at the 5′ end of the viral genome was determined by the method of Maxam and Gilbert (24). Fig. 4 summarizes a partial sequence analysis of 70 nucleotides within this fragment. These sequences were also compared to the corresponding sequences from the U5 region of the LTR of Moloney murine sarcoma virus (Mo-MSV) (29). As can be seen in Fig. 4, there is a high degree of homology between the 3′ ends of the U5 region of Mo-MSV and Friend SFFV$_P$ LTRs. In addition, the sequence -T-G-G-G-G-G-C-T-C-G-T-C-C-G-G-G-A-T-, immediately 3′ to the end of the LTR, is complementary to the 3′ end of proline tRNA (30). Because the tRNA binding site is a structural feature of the 5′ end of retrovirus genomes, the orientation of the cloned SFFV$_P$ inserts is confirmed.

Infectivity of Cloned SFFV$_P$ DNA. To determine whether the SFFV$_P$ inserts had biological activity, 3T3 fibroblasts were transfected with the recombinant clones 502 and 542. Because SFFV$_P$ is replication defective and does not transform fibroblasts, a two-step procedure to assay for the biological activity of this virus was employed. Two methods were used. In the mass transfer method, cloned SFFV$_P$ DNA was transferred into NIH-3T3 cells by the calcium phosphate procedure, followed by superinfection of these cells with the replication-competent helper virus F-MuLV. Filtered supernatant fluids were then injected into adult DBA/2J mice. As shown in Table 1, the viruses produced after transfection and rescue with F-MuLV all induced rapid splenomegaly and an increased hematocrit in adult mice, whereas virus rescued from mock-transfected 3T3 cells did not induce any changes in spleen weight in DBA/2J mice.

The transfection protocol utilized above clearly indicated that the SFFV$_P$ recombinant clones had biological activity. However, this procedure did not allow the isolation of fibroblast cell

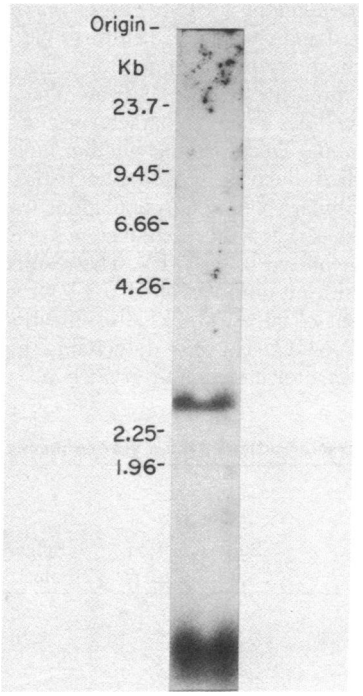

FIG. 3. Orientation of SFFV$_P$502 DNA. The 7.4-kb *Eco*RI insert from λSFFV$_P$502 was digested with *Kpn* I and *Sst* I, followed by *Bgl* II. The DNA fragments were then separated electrophoretically on a 1.2% agarose gel. Southern blot analysis was then carried out with a ^{32}P-labeled cDNA probe specific for the 3′ end of the viral genome, as described in the text.

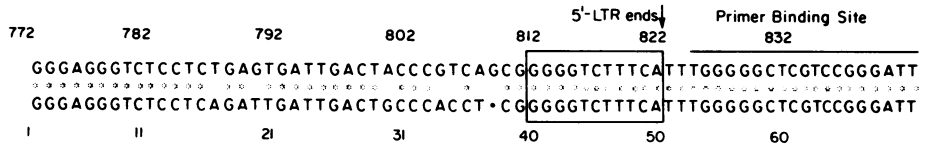

FIG. 4. Comparison of the nucleotide sequence of part of the 5' LTR of Mo-MSV (29) and the corresponding region of SFFV$_P$502. The top strand is the Mo-MSV sequence [numbering the same as that used by Dhar *et al.* (29)], and the bottom strand is the SFFV$_P$502 sequence (arbitrary numbering). Asterisks between the sequences denote identity of the bases. The region corresponding to the 11-base-pair inverted repeat at the terminus of the LTR is boxed.

clones nonproductively transfected with molecularly cloned SFFV$_P$ DNA. Therefore, a second cotransfer method was used. This procedure makes use of the observation by Wigler *et al.* (31) that selection of stable DNA transformants also selects for a subpopulation of cells competent for uptake of unlinked DNA. To select for such cell clones, we cotransferred a recombinant plasmid containing the herpes virus *tk* gene along with a 100-fold excess of λSFFV$_P$502 DNA into TK$^-$ 3T3 cells, selecting for cells with a TK$^+$ phenotype in HAT medium. Cell clones capable of growing in HAT medium were isolated and superinfected with F-MuLV, and the filtered culture fluids were injected into DBA/2J mice to assay for SFFV$_P$. The data in Table 2 indicate that four out of five of the TK$^+$ 3T3 clones contained a rescuable SFFV genome capable of inducing spleen foci. Thus, this protocol allows for the isolation of stable transformants transfected with molecularly cloned SFFV$_P$ DNA.

Effect of Cloned SFFV$_P$ DNA on Hemopoietic Colony-Forming Cells. To determine whether the molecular clones of SFFV$_P$ could also induce alterations in hemopoietic colony-forming cells, spleen cells from mice infected with SFFV$_P$ rescued by the mass transfection procedure were analyzed for Epo-independent CFU-E. The results shown in Table 1 indicate that virus harvested from 3T3 cells transfected with SFFV$_P$ DNA and rescued with F-MuLV induced a large increase in the number of Epo-independent CFU-E. Virus harvested from the mock-transfected and rescued 3T3 cultures did not induce any alterations in these erythroid progenitor cells.

The results presented above indicate that cloned SFFV$_P$ DNA induces cellular changes characteristic of the early phase of Friend leukemia. To determine whether molecular clones of SFFV$_P$ could also induce changes characteristic of the late phase of Friend leukemia, spleen cell populations from mice inoculated 4–6 weeks earlier with rescued clones of SFFV$_P$ were assayed for their content of CFU-FV. The results in Table 1 indicate that significant numbers of CFU-FV were present in the enlarged spleens of mice 5 weeks after infection with rescued SFFV$_P$ DNA. No CFU-FV were detected in these mice within the first 3 weeks after infection or in mice inoculated with culture medium from the mock-transfected 3T3 cultures rescued with F-MuLV.

DISCUSSION

In this communication, we have reported the isolation of molecular clones containing the integrated proviral DNA from the polycythemia-inducing strain of Friend spleen focus-forming virus (SFFV$_P$). These clones were isolated from a library of *Eco*RI-generated DNA fragments propagated in phage λ from two rat cell clones nonproductively infected with SFFV$_P$. Both SFFV$_P$ clones contain regions, located 5.8 kb apart, that, at the level of restriction enzyme sites, appear to be direct terminal repeats. Similar to the LTRs of Mo-MSV (29), the LTRs of Friend SFFV$_P$ contain restriction enzyme sites for *Kpn* I and *Sst* I and are approximately 0.5 kb in size (unpublished data). Nucleotide sequence analysis, as well as DNA transfer studies, have also indicated that the LTR of SFFV$_P$ includes a functional promoter region that can activate the expression of downstream coding sequences (unpublished data).

The cloned SFFV$_P$ genomes, rescued after gene transfer from either TK$^+$ transformants or from uncloned populations of 3T3 cells, were infectious as assayed by their ability to induce rapid splenomegaly and polycythemia in adult mice. Clonal analysis of the spleen cell populations from these mice indicated that the cloned SFFV$_P$ genome induced the appearance of both Epo-independent erythroid colony-forming cells (CFU-E) and tumorigenic colony-forming cells (CFU-FV). Thus, these molecular clones encode the genetic information necessary for the induction of both the early and late stages of the erythroleukemia associated with SFFV$_P$.

The rat cells nonproductively infected with SFFV$_P$ contained one to three copies of the SFFV$_P$ provirus, integrated at different sites in the rat genome. These results are consistent with the conclusion that SFFV$_P$, like other retroviruses, has the potential to integrate at multiple sites in the host genome. The present studies were carried out with fibroblasts that are not transformed by SFFV$_P$ (4, 14). It will be of interest to determine whether the integration of SFFV$_P$ into the genomic DNA of

Table 1. Characterization of the erythroleukemia induced by molecular clones of SFFV$_P$

DNA source	Superinfection with F-MuLV	Spleen foci	Spleen weight, g	Hematocrit, %	Erythroid colonies, CFU-E/10^5 spleen cells: Without Epo	Erythroid colonies, CFU-E/10^5 spleen cells: With Epo	Tumorigenic colony-forming cells, CFU-FV/5 × 10^5 spleen cells
—	−	0	0.06	53	0	24	0
—	+	0	0.11	45	0	40	0
λSFFV$_P$502	+	—	1.7	70	2900	1300	6.5
λSFFV$_P$502 (*Eco*RI-digested)	+	20	2.57	69	—	—	9.5
λSFFV$_P$502	+	>200	2.81	76	—	—	28
λSFFV$_P$542	+	—	3.28	79	6900	7100	9.5

DNA transfections and superinfections with F-MuLV$_P$ were carried out as described in the text. Spleen foci were counted on day 9 and spleen weight, hematocrits, and CFU-E were determined on day 28. Epo was added at 0.5 unit/ml as indicated. The number of CFU-FV was determined on day 35. A dash indicates not determined.

Table 2. Isolation of $SFFV_P$ nonproducers by cotransfer

Exp.	Transfecting DNA, ng: $SFFV_P$502	Transfecting DNA, ng: pxI(*tk*)	TK^+ transformant clone	Number of spleen foci after rescue with F-MuLV
1	1500	250	1	0
			2	>50
			3	>20
2	400	200	a	50–100
			b	50–100

λ$SFFV_P$502 DNA and plasmid pxI DNA, containing the *tk* gene of herpes simplex virus, were cotransferred into TK^- 3T3 cells and TK^+ transformants were selected. HAT-resistant colonies were picked, grown up in HAT medium, and rescued with F-MuLV. The number of spleen foci was determined 9 days after virus inoculation. A minimum of three mice were used per clone.

CFU-FV is also random or, conversely, whether there are specific sites of integration that are associated with leukemic transformation similar to those recently described for avian leukosis (32, 33) and spleen necrosis virus (34).

Linemeyer *et al.* (35) have reported the molecular cloning of the unintegrated form of $SFFV_P$. These clones also induced splenomegaly and polycythemia in adult mice after a two-step transfer procedure similar to that described here. However, there are a number of differences between the recombinant clones isolated in these studies. First, the clones described in this study are colinear with the viral genome, whereas clones of the unintegrated form of $SFFV_P$ are a circular permutation of the viral genome. Second, the recombinant clones described in this study contain cellular sequences flanking the integrated proviral DNA and hence should be useful in analyzing the integration of this virus into the host genome. Third, there are several differences in the restriction enzyme maps of the two strains of $SFFV_P$ described in these studies. For example, whereas our strain of $SFFV_P$ does not contain recognition sites for *Hin*dIII or *Eco*RI, the strain of $SFFV_P$ used by Linemeyer *et al.* (35) was cleaved twice by *Eco*RI and once by *Hin*dIII.

The results presented in this study suggest that molecular clones of $SFFV_P$, in conjunction with F-MuLV, can induce both the preleukemic and leukemic stages of erythroleukemia. However, these results do not necessarily suggest that the $SFFV_P$ genome encodes genetic information whose continuous expression is necessary for malignant transformation. We have previously suggested (12) that the SFFV genome may act only as an initial carcinogen that stimulates the proliferation of erythroid cells, which then progress to a more malignant phenotype. The isolation of infectious molecular clones of $SFFV_P$, together with the development of colony assays for analyzing the hemopoietic cell populations both early (10) and late (11–13) after virus infection, should help elucidate the molecular and cellular events leading to the leukemic transformation of hemopoietic progenitor cells by Friend leukemia virus.

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