JOURNAL OF VIROLOGY, Oct. 1986, p. 242–250
0022-538X/86/100242-09$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 60, No. 1

# Molecular Analysis and Pathogenesis of the Feline Aplastic Anemia Retrovirus, Feline Leukemia Virus C-SARMA

NORBERT RIEDEL,[1] EDWARD A. HOOVER,[2] PETER W. GASPER,[2] MARGERY O. NICOLSON,[3] AND JAMES I. MULLINS[1]*

*Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115[1]; Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523[2]; and Applied Molecular Genetics, Newbury Park, California 91320[3]*

Received 7 April 1986/Accepted 30 June 1986

**We describe the molecular cloning of an anemogenic feline leukemia virus (FeLV), FeLV-C-Sarma, from the productively infected human rhabdomyosarcoma cell line RD(FeLV-C-S). Molecularly cloned FeLV-C-S proviral DNA yielded infectious virus (mcFeLV-C-S) after transfection of mammalian cells, and virus interference studies using transfection-derived virus demonstrated that our clone encodes FeLV belonging to the C subgroup. mcFeLV-C-S did not induce viremia in eight 8-week-old outbred specific-pathogen-free (SPF) cats. It did, however, induce viremia and a rapid, fatal aplastic anemia due to profound suppression of erythroid stem cell growth in 9 of 10 inoculated newborn, SPF cats within 3 to 8 weeks (21 to 58 days) postinoculation. Thus, the genome of mcFeLV-C-S encodes the determinants responsible for the genetically dominant induction of irreversible erythroid aplasia in outbred cats. A potential clue to the pathogenic determinants of this virus comes from previous work indicating that all FeLV isolates belonging to the C subgroup, an envelope-gene-determined property, and only those belonging to the C subgroup, are potent, consistent inducers of aplastic anemia in cats. To approach the molecular mechanism underlying the induction of this disease, we first determined the nucleotide sequence of the envelope genes and 3′ long terminal repeat of FeLV-C-S and compared it with that of FeLV-B-Gardner-Arnstein (mcFeLV-B-GA), a subgroup-B feline leukemia virus that consistently induces a different disease, myelodysplastic anemia, in neonatal SPF cats. Our analysis revealed that the p15E genes and long terminal repeats of the two FeLV strains are highly homologous, whereas there are major differences in the gp70 proteins, including five regions of significant amino acid differences and apparent sequence substitution. Some of these changes are also reflected in predicted glycosylation sites; the gp70 protein of FeLV-B-GA has 11 potential glycosylation sites, only 8 of which are present in FeLV-C-S.**

Feline leukemia viruses (FeLVs) are a group of horizontally transmitted type C retroviruses capable of induction of neoplastic and degenerative diseases of the feline lymphoreticular system (16, 28, 31, 44). The FeLV family consists of three known subgroups, FeLV-A, FeLV-B, and FeLV-C, defined by virus interference and neutralization (60). Distribution of the different subgroups within feline populations differs markedly. All field isolates of FeLV contain FeLV-A either alone or in a mixture of subgroups (60), whereas FeLV-C has been isolated rarely in nature, and only in animals with severe degenerative disease (23, 31, 48, 60).

Feline aplastic anemia (AA) is a naturally occurring disease known for over a decade to be caused by infection with certain isolates of FeLV (26, 37). Feline AA is characterized by erythroid aplasia manifested as severe nonregenerative anemia, by lymphopenia, and in later stages by granulocytopenia, leukopenia, and myelosclerosis (26, 29). Only a few naturally occurring isolates of FeLV, all of which belong to the C subgroup, are known to be capable of consistent induction of AA (26, 48). The anemogenic capacity of FeLV isolates, therefore, is considered to be specific for subgroup C viruses (30, 48). The pathology of feline AA is directly analogous to that of human AA (12, 32). Since no other virus is known to induce this type of anemia (30, 48), FeLV-C viruses present a unique model for the study of this disease.

FeLV subgroups differ in their host range of infection in vitro. FeLV-A isolates are usually restricted to feline cells, FeLV-B and FeLV-C isolates also replicate well in mink, canine, and human cells, whereas only FeLV-C replicates in guinea pig cells (23, 31, 58). Cell receptor recognition by the gp70 envelope gene product is thought to be one component determining the host range of the virus (20, 33, 61). In avian retroviruses, host range variation correlates with variable regions in the major envelope glycoprotein (13). In murine retroviruses, differences between highly leukemogenic viruses and their more benign relatives cluster within the envelope gene and the long terminal repeat (LTR) (8, 14, 20, 56). The murine mink cell focus-forming (MCF) virus gp70 gene is thought to be derived by recombination between endogenous xenotropic and ecotropic parental sequences, resulting in a virus with a broader host range (4, 10, 14, 56). Comparison of the FeLV-B-Gardner-Arnstein (FeLV-B-GA) and MCF envelope gene sequences reveals regions of specific homology, which led to the hypothesis that FeLV-B-GA might be derived by a mechanism similar to that of MCF viruses, i.e., by somatic recombination between FeLV-A and endogenous FeLV sequences present in the feline genome (15, 63). A somatic recombination origin for FeLV-C was suggested earlier by Russell and Jarrett (58), who found FeLV-C arising in a cat persistently infected with FeLV-A. Biologically cloned FeLV-C virus induces fatal AA in newborn kittens, but by itself it is incapable of inducing viremia or disease in weanling or adult cats (30). In contrast, dual infection of weanling kittens with the nonanemogenic FeLV-A and biologically cloned FeLV-C-Sarma (FeLV-C-S) results in FeLV-C viremia and fatal AA

* Corresponding author.

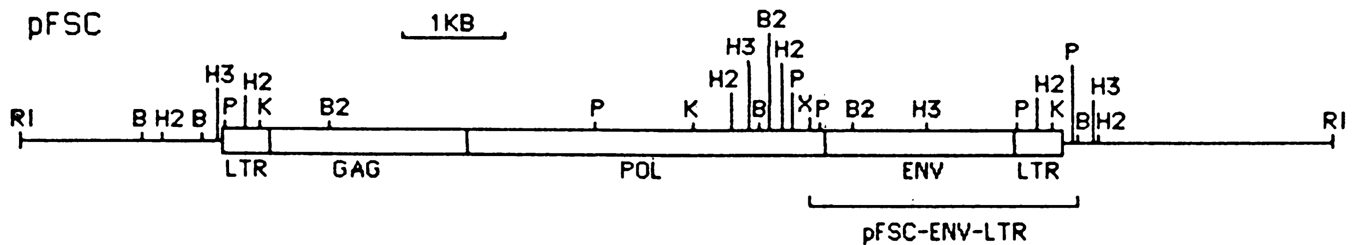


FIG. 1. Restriction map of pFSC. The 8.7-kilobase proviral DNA is contained within a 13-kilobase *Eco*RI fragment. The *env*-LTR region was subcloned as a 2.9-kilobase *Xho*I-*Bam*HI fragment into the *Sal*I-*Bam*HI sites of M13mp18 (pFSC-ENV-LTR). P, *Pst*I; H2, *Hin*cII; K, *Kpn*I; H3, *Hin*dIII; B, *Bam*HI; X, *Xho*I; B2, *Bgl*II; RI, *Eco*RI.

(30). The molecular mechanism for the induction of irreversible erythroid aplasia leading to fatal AA, therefore, lies in the molecular analysis of the FeLV-C genome.

Here we describe the molecular cloning of an FeLV-C provirus, demonstrate that it encodes a potent anemogenic virus of predictable, acute pathology, present the nucleotide sequences of its envelope gene and LTR, and compare them with the envelope gene and LTR sequences of FeLV-B-GA, a potent inducer of a distinctly different disease.

## MATERIALS AND METHODS

**Molecular cloning, transfection, and DNA sequence analysis.** The source of FeLV-C virus for these experiments was the biologically cloned isolate of Sarma, FeLV-C-S (60), introduced by infection into human rhabdomyosarcoma cells (38). DNA from rhabdomyosarcoma cells infected with FeLV-C-S was digested with *Eco*RI, an enzyme which does not cleave the FeLV-C-S genome (data not shown), and fractionated on a sucrose gradient (43). Fractions containing 8- to 25-kilobase (kb) DNA were identified by gel electrophoresis and subsequently ligated into the bacteriophage vector Charon 4A (43). The resulting phage library was screened with $^{32}$P-nick-translated pFGB, a probe containing an entire FeLV-B-GA provirus (15). Several clones containing two LTRs were identified by hybridization with a probe specific for exogenous LTR sequences (7) and used to transfect AH927 feline (W. Nelson-Rees), D-17c-1 canine (J. Riggs), rhabdomyosarcoma human (38), and GP104 guinea pig fibroblasts (ATCC 158). Cells were tested for production of reverse transcriptase 4 weeks after transfection (45).

The restriction map of a representative, reverse-transcriptase-positive, and biologically active (see Fig. 1) clone (λFSC5) was determined after subcloning of the entire *Eco*RI fragment into the plasmid vector pK125 (D. Goldberg, personal communication). A *Xho*I-*Bam*HI fragment containing the entire envelope and LTR was subcloned into the *Sal*I-*Bam*HI sites of M13mp18 (46). Deletion clones were then derived by using the double-strand exonuclease BAL 31 (51) and were sequenced by using the dideoxynucleotide chain termination method (59).

**Virus interference test.** Subgroup determination with virus derived from feline fibroblasts transfected with plasmid clone pFSC was performed by O. Jarrett according to protocols described previously (30, 31, 57). In brief, FeLV-producing feline embryo fibroblasts were used as initiators and were challenged by FeLV pseudotypes of murine sarcoma virus (MSV), MSV (FeLV), of subgroups A, B, or C together with a concentration of homologous helper FeLV sufficient to give about 500 foci in a 5-cm plate of uninfected feline embryo fibroblasts. Cells infected with FeLV are resistant to challenge with MSV(FeLV) of the same subgroup, and no foci are observed (30, 31, 57).

**Animal inoculation studies.** Ten newborn specific-pathogen-free cats from a breeding colony at Colorado State University were inoculated intraperitoneally with either $5 \times 10^4$ or $1 \times 10^6$ infectious focus-forming units (clone 81 assay [18]) of FeLV-C-S prepared as cell culture fluid from productively transfected D17-cl cells. One kitten from each litter served as an uninoculated control. At biweekly intervals postinoculation, blood and bone marrow were collected from each cat and methanol-fixed films were prepared for the detection of FeLV viral structural antigen p27 in leukocytes, platelets, and marrow cells by immunofluorescence by using a procedure modified (27) from the original technique of Hardy et al. (24). Complete automated hemograms and cell size analysis were also performed at each sampling interval to assess the presence of anemia or other hematologic abnormality (29). In one litter of cats, clonogenic methylcellulose colony-forming assays for erythroid and granulocyte-macrophage progenitor cells in bone marrow were performed at three intervals postinoculation by procedures described by Abkowitz et al. (1) and modified by Gasper (19) in FeLV-infected cats. All cats were euthanized and necropsied when signs of severe, nonregenerative anemia were apparent. Eight 8-week-old specific-pathogen-free cats were inoculated with $2 \times 10^6$ focus-forming units in a similar fashion, but none developed viremia or anemia.

**Southern blot analysis.** Isolation of DNA from feline tissues and Southern blot analysis were performed as described previously (42). DNAs derived from transfected cell lines and from feline tissues were separated on 1% agarose gels after digestion with *Kpn*I or *Kpn*I plus *Bgl*II, transferred to nitrocellulose, and hybridized with a probe specific for exogenous FeLV sequences (exU3 [42, 44; J. I. Mullins et al., manuscript in preparation]). This analysis allows identification of FeLV-C-S-specific internal virus bands and was performed to determine whether the virus injected into animals had undergone gross viral gene rearrangements within the animal.

## RESULTS

**Molecular cloning, transfection, and analysis of in vitro host range of infection and subgroup.** Proviruses from FeLV-C-S-infected human rhabdomyosarcoma cells were cloned in the bacteriophage vector Charon 4A (43), and eucaryotic insert DNA was transferred to the plasmid vector pK125. A restriction enzyme site map of plasmid clone pFSC, containing an intact provirus, is shown in Fig. 1. Cloned pFSC DNA as well as a molecular clone of FeLV-B-GA (43) was used to transfect cell lines derived from feline, canine, human, and guinea pig cells. Cell culture supernatants were monitored after 4 weeks for reverse transcriptase activity, indicating the production of infectious virus. pFSC produced virus

TABLE 1. Pathogenicity of FeLV-C-S in neonatal specific-pathogen-free cats

| Cat no. | Age at inoculation (days) | Virus dose (FFU)[a] | No. of viremic cats/ no. inoculated | Onset of viremia (days) | Survival (days) | Terminal hematocrit | Terminal BFU-e (% control)[b] | Disease |
|---|---|---|---|---|---|---|---|---|
| 1180 | | None | | Control | 58 | 28 | 100 | None |
| 1175 | 3 | $5 \times 10^4$[a] | 4/5 | 23 | 58 | 5 | 5 | AA |
| 1176 | 3 | $5 \times 10^4$ | | 23 | 58 | 14 | 5 | AA |
| 1177 | 3 | $5 \times 10^4$ | 4/5 | 23 | 58 | 7 | 0 | AA |
| 1179 | 3 | $5 \times 10^4$ | 4/5 | None | 58 | 23 | ND | None |
| 1181 | 3 | $5 \times 10^4$ | 4/5 | 23 | 58 | 10 | ND | AA |
| 617-5 | | None | | Control | 27 | 16 | 100 | None |
| 617-2 | 1 | $1 \times 10^6$ | 3/3 | 19 | 27 | 16 | 43 | AA |
| 617-3 | 1 | $1 \times 10^6$ | 3/3 | 19 | 20 | 10 | 13 | AA |
| 617-4 | 1 | $1 \times 10^6$ | 3/3 | 19 | 27 | 10 | 0 | AA |
| 797-1 | | None | | Control | 21 | 17 | ND | None |
| 797-2 | 1 | $1 \times 10^6$ | 2/2 | 14 | 21 | 11 | ND | AA |
| 797-3 | 1 | $1 \times 10^6$ | 2/2 | 14 | 21 | 10 | ND | AA |

[a] FFU, Focus-forming units (clone 81 assay of Fischinger et al. [18]).
[b] Produced by bone marrow cells in a clonogenic methylcellulose culture assay system. ND, Not done.

capable of growth in all cell lines tested, whereas molecular-clone-derived FeLV-B-GA did not grow in guinea pig cells (data not shown). These results are consistent with previous studies using biologically cloned virus (58). Interference studies conducted with virus derived from transfected feline fibroblasts demonstrated that clone pFSC encodes a subgroup C FeLV (data not shown).

**In vivo pathogenesis of FeLV-C-S.** Cell-free supernatants of canine cells productively transfected with FeLV-C-S were injected into neonatal (≤3-day-old) or 8-week-old cats. None of the 8-week-old animals developed viremia, whereas nine of ten neonatal cats developed persistent viremia by 3 weeks postinoculation and rapidly progressive nonregenerative anemia, which was terminal between 3 and 8 weeks postinoculation (Table 1). In marrow clonogenic assays, these cats demonstrated a precipitous decline in erythroid progenitor colony-forming cells (burst-forming units, erythroid, BFU-e) after the onset of viremia and before the onset of anemia, which persisted throughout the disease course. Anemia was defined as a decline in the hematocrit. For one litter, serial assays of bone marrow BFU-e and CFU (erythroid and granulocyte-macrophage) progenitor cells were performed (Fig. 2). Marked decreases in BFU-e and erythroid CFU were evident in each inoculated animal relative to an uninoculated littermate control (Fig. 2). A parallel decrease in marrow erythroid progenitor cells was evident in stained films and in counts of total marrow nucleated cells (not shown). In contrast, no significant decrease in granulocyte-macrophage CFU was apparent at these same intervals (Fig. 2).

Restriction enzyme analysis of exogenous viral DNA present in terminal-stage tissues of animals with AA revealed a low level of proviral DNA (up to one copy per cell in the bone marrow and spleen) with no evidence of gross viral gene rearrangement or clonal expansion of FeLV-infected cells (data not shown).

These results demonstrate that molecularly cloned FeLV-C-S is a potent, reproducible inducer of erythroid-cell-specific pathogenesis comparable in potency to biologically passaged isolates of FeLV-C (5, 19, 26, 30, 48, 64).

In contrast, inoculation of neonatal cats with molecular-clone-derived FeLV-B-GA virus specifically and reproduc-

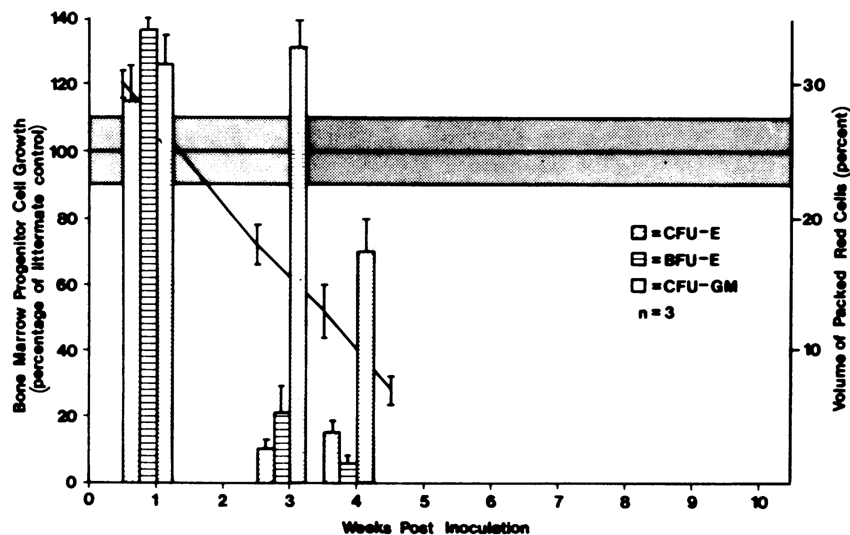


FIG. 2. Serial clonogenic assays of bone marrow progenitor growth in cats inoculated with molecular-clone-derived FeLV-C-S.

ibly induces a distinctly different disease, myelodysplastic anemia (E. A. Hoover et al., manuscript in preparation) in animals derived from the same population of specific-pathogen-free cats. Thus, the specific pathogenic outcome of infection is a genetically dominant effect attributable to the genetic differences between these two FeLV isolates. Elucidation of these differences requires nucleotide sequence analysis and comparison of their genomes.

**Nucleotide sequence analysis and comparison with FeLV-B-GA.** The FeLV-C-S sequence (Fig. 3) comprises 2,520 nucleotides from the *Pst*I site immediately 5′ to the envelope gene to the end of the 3′ LTR. The nucleotide sequence of the envelope genes and the beginning of the 3′ LTR of an independent isolate of FeLV-C-S was recently determined by Luciw et al. (36a). The two sequences are identical in gp70 but differ by two changes (A to C at position 1536 and A to G at position 1886) in p15E and by an insertion of a C at position 1988 of the sequence shown in Fig. 3. A comparison of the FeLV-C-S LTR with the LTR of FeLV-B-GA is shown in Fig. 3B (15, 47, 66). The FeLV-B-GA LTR sequence used for comparative studies was taken from Elder and Mullins (15). The FeLV-B-GA LTR sequence published by Nunberg et al. (47) is identical, and the LTR sequence determined by Wuensch et al. (66) differs by a single A to T change at position 2287.

**The envelope polypeptides of FeLV-C-S and FeLV-B-GA.** The amino acid sequences of the envelope proteins of FeLV have not been determined. However, we assume that they are derived by a mechanism analogous to that in other retroviruses, i.e., by translation of a spliced mRNA with coding sequences derived from the 3′ end of the viral genome (39). A splice acceptor consensus sequence is located approximately 200 base pairs 5′ to the start point of the deduced amino acid sequence shown in Fig. 4A (D. Doggett, A. L. Drake, M. E. Rowe, V. Stallard, V. Hirsch, J. C. Neil, J. H. Elder, and J. I. Mullins, submitted for publication). Elder and Mullins (15) assigned the tentative location for the N terminus of the envelope polyprotein of FeLV-B-GA by aligning the large open reading frame present in their sequence with the coding sequences of murine leukemia virus envelope genes (15, 49). The first start codon of the FeLV-C-S envelope gene was positioned in an analogous manner (Fig. 3A, position 1). There are, however, two methionine codons positioned two triplets apart in the region of the presumed start codon, and we do not know which one determines the beginning of the gp70 precursor. The first methionine residue is followed by 16 hydrophilic amino acids (positions 1 to 16) and a hydrophobic region spanning 16 amino acids (positions 17 to 32), a pattern characteristic for signal peptides of membrane proteins (3). We believe this region encodes the leader sequence of the envelope precursor.

It was proposed that cleavage of the envelope precursor into gp70 and p15E molecules is mediated by a trypsin-like protease recognizing a doublet of two basic amino acids (50), and two arginine residues are positioned immediately preceding the presumed p15E N terminus of FeLV-C-S (positions 441 and 442).

A comparison between deduced FeLV-C-S and FeLV-B-GA envelope proteins is shown in Fig. 4. The envelope gene of FeLV-C-S has an open reading frame of 1,917 nucleotides, beginning with the leader sequence (Fig. 3A, nucleotide 1). The total coding capacity is 639 amino acids, of which 33 constitute the N-terminal signal peptide (nucleotides 1 to 99), 409 constitute gp70 (nucleotides 100 to 1,326), and 197 constitute p15E (nucleotides 1,327 to 1,917). The gp70 of FeLV-C-S is 23 amino acids shorter than that of FeLV-B-GA. Most major differences between the gp70s of FeLV-C-S and FeLV-B-GA lie in the N-terminal half of the sequence and can be summarized as follows. Five blocks of apparent substitution, characterized by significant amino acid differences and in four cases by deletions or insertions or both, were identified (Fig. 4A, boxed areas). The first block comprises 32 amino acids in FeLV-C-S and 25 amino acids in FeLV-B-GA. Depending on the alignment chosen, approximately 21 of 25 amino acids differ, of which 15 represent nonconservative changes. Both regions are hydrophilic; 10 of 32 amino acids are charged in FeLV-C-S and 6 of 25 are charged in FeLV-B-GA, and approximately 50% of all amino acids in both sequences have uncharged polar side chains. The second block spans 9 amino acids in FeLV-C-S (positions 127 to 135) and 4 amino acids in FeLV-B-GA. The FeLV-C-S sequence contains two prolines, whereas there is no proline in FeLV-B-GA; the importance of this observation is that introduction of prolines interrupts what might otherwise be a α-helical structure, creating a rigid kink or bend (55). The third block has a length of 12 amino acids in FeLV-C-S (beginning at position 170) which are distinct in sequence from the 36 amino acid segment in FeLV-B-GA. The FeLV-C-S block is strongly hydrophilic with 4 charged and 8 uncharged polar amino acids, whereas the sequence in FeLV-B-GA contains 6 charged, 23 uncharged polar, and 7 hydrophobic amino acids. The FeLV-C-S block contains no prolines, whereas the FeLV-B-GA block has one. The fourth block begins at amino acid 252 in FeLV-C-S and comprises 26 amino acids in FeLV-C-S and 37 amino acids in FeLV-B-GA. Depending on the alignment chosen, at least 11 of 26 amino acids differ, including seven nonconservative changes. The sequence in FeLV-B-GA contains five prolines compared with two in FeLV-C-S. In FeLV-B-GA, the first 16 amino acids constitute a hydrophilic region (4 charged amino acids), whereas the next 16 amino acids form a hydrophobic region (10 hydrophobic amino acids). In constrast, the FeLV-C-S sequence is weakly hydrophilic overall (4 positively charged, 18 polar, and 8 hydrophobic amino acids).

An additional cluster of nonconservative amino acid changes not involving deletions or insertions occurs within the N-terminal half of these proteins (beginning at position 155 of FeLV-C-S). Here, three amino acid changes, two nonconservative, occur over a span of five amino acids.

The C-terminal regions of gp70 show a high degree of homology between the two viruses examined, with the exception of a stretch of 10 amino acids (Fig. 4A, fifth boxed area, positions 371 to 380 in FeLV-C-S). Both sequences are hydrophilic, however; four positively charged lysine residues appear in FeLV-C-S, whereas no lysines and a single negatively charged glutamic acid residue occur in the FeLV-B-GA sequence.

The gp70 molecules of FeLV-B-GA and FeLV-C-S share eight potential glycosylation signals (Asn-X-Thr/Ser) (41) (Fig. 4A, overlined). However, three additional glycosylation sites were found in FeLV-B-GA, each within a block of evident sequence substitution (Fig. 4A, double underlined).

As mentioned previously, the gp70 of FeLV-B-GA was shown to share three regions of significant homology with murine MCF viruses, regions which are not shared with murine ecotropic virus gp70s (15). Three of these four regions (Fig. 4A, underlined) are also conserved in FeLV-C-S.

The p15E proteins of FeLV-C-S and FeLV-B-GA show 95% homology at the DNA level and 97.5% homology at the

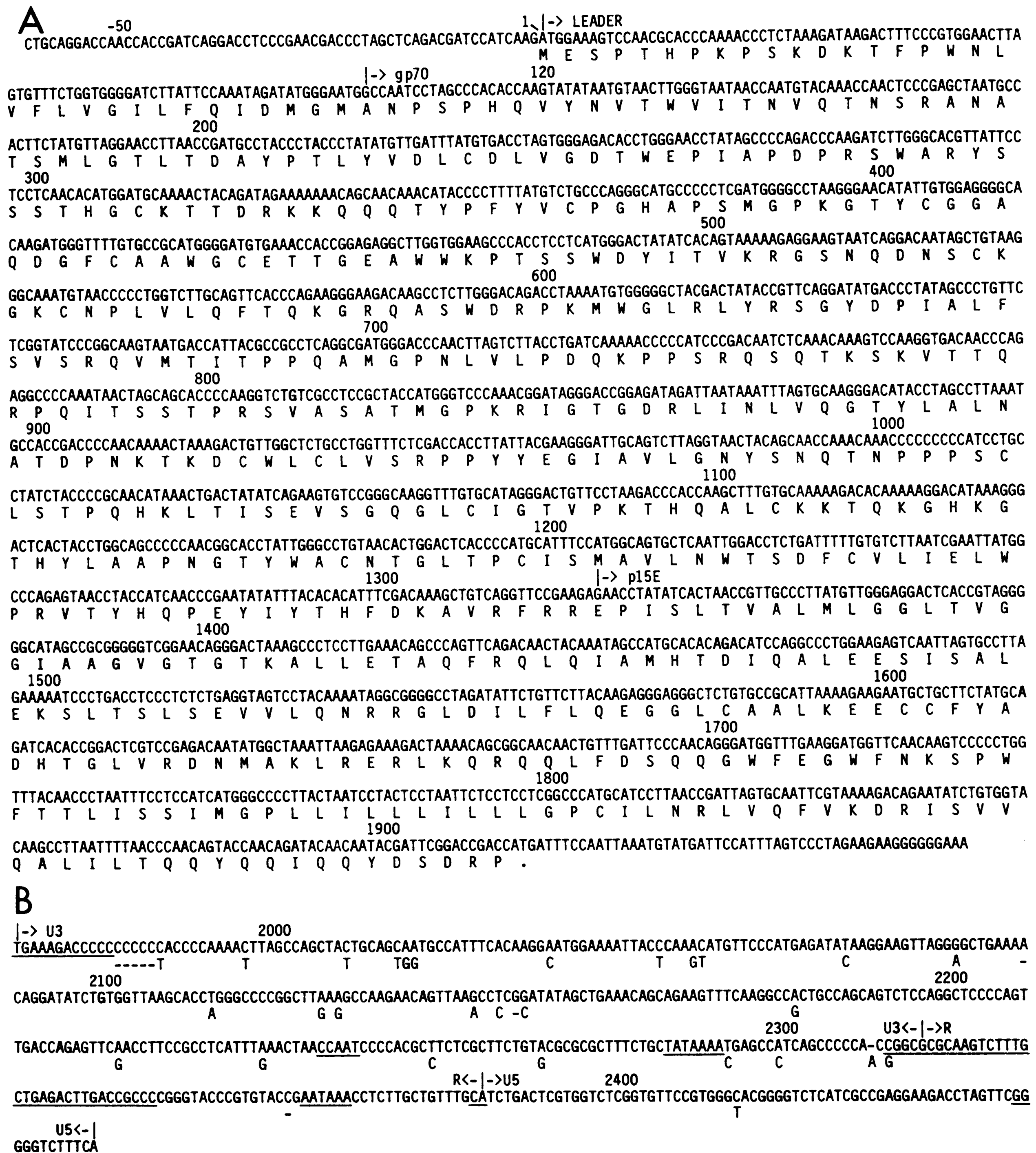

FIG. 3. Nucleotide and deduced amino acid sequence of the envelope gene (A) and the 3′ LTR (B) of FeLV-C-S. (A) The sequence begins at the *Pst*I site immediately 5′ to the envelope gene (position −61). Position 1 indicates the beginning of the envelope gene leader sequence, and the mature gp70 begins at position 100. The beginning of the p15E gene is indicated at position 1,327. (B) The LTR sequence of FeLV-C-S and a comparison with the LTR sequence of FeLV-B-GA. The beginnings of the U3, R, and U5 regions are indicated. The palindromic sequence (see text), regulatory and signal sequences such as the inverted repeats and the CCAAT (21) and Goldberg-Hogness (TATAAAA) (11, 17, 53) boxes, the presumed polyadenylation recognition signal (AATAAA) (54), and the polyadenylation addition site (CA) (54) are underlined. Nucleotides of FeLV-B-GA not shared with FeLV-C-S are indicated below the FeLV-C-S sequence. Dashes are introduced to maintain maximum alignment.

```
A
                                                             51                                 86
FeLV-C-S   MESPTHPKPSKDKTFPWNLVFLVGILFQIDMGMANPSPHQVYNVTWVITNVQTNSRANATSMLGTLTDAYPTLYVDLCDLVGDTWEPIAPDPRSWARYSSSTHGCK 106
FeLV-B-GA                LS           T  I               T   LV GTK             F  M F    II N  N SDQE FPGYGCDQPMRRW- 105

                               127                         155            170
FeLV-C-S   TTDRKKQQQTYPFYVCPGHAPSMGPKGTYCGGAQDGFCAAWGCETTGEAWWKPTSSWDYITVKRGSNQDNS-CKGK-----------------------CNPLVLQ 188
FeLV-B-GA  ------  RNT         ---NR Q--   P      V        TY R           K VT GIYQ S GGWCGPCYDKAVHSSTTGASEGGR    I   200

                                                                          252
FeLV-C-S   FTQKGRQASWDRPKMWGLRLYRSGYDPIALFSVSRQVMTITPPQAMGPNLVLPDQKPPSRQSQTKSKVTTQRPQ-------ITS--------STPRSVASATMGPK 279
FeLV-B-GA         T   G  S                                                IE R  PHHS GNGGTPG  LVNASIAPL  VTP   ----   302

                                                                                                      371
FeLV-C-S   RIGTGDRLINLVQGTYLALNATDPNKTKDCWLCLVSRPPYYEGIAVLGNYSNQTNPPPSCLSTPQHKLTISEVSGQGLCIGTVPKTHQALQKKTQKGHKGTHYLAA 385
FeLV-B-GA                           R                   I                I                            NE  Q  T A      408

FeLV-C-S   PNGTYWACNTGLTPCISMAVLNWTSDFCVLIELWPRVTYHQPEYIYTHFDKAVRFRR 442
FeLV-B-GA                                              V    A  A     465

B
                                         31        41
FeLV-C-S   EPISLTVALMLGGLTVGGIAAGVGTGTKALLETAQFRQLQIAMHTDIQALEESISALEKSLTSLSEVVLQNRRGLDILFLQEGGLCAALKEECCFYADHTGLVRDN 106
FeLV-B-GA                                I         M

                                                              158                             190 194
FeLV-C-S   MAKLRERLKQRQQLFDSQQGWFEGWFNKSPWFTTLISSIMGPLLILLLILLLGPCILNRLVQFVKDRISVVQALILTQQYQQIQQYDSDRP 197
FeLV-B-GA                                                     F                               K   P
```

FIG. 4. Deduced amino acid sequence comparison of the proposed *env* polypeptides (gp70 [A] and p15E [B] proteins) of FeLV-C-S and FeLV-B-GA. Gaps introduced to allow maximum alignment of the two sequences are indicated by dashed lines. Underlined sequences show regions of high homology between FeLV-B-GA and murine MCF virus gp70, as described earlier (15). Boxed areas represent blocks of sequence substitution, and numbers above the sequence indicate the beginnings of regions discussed in the text. Common potential glycosylation sites are overlined; those unique for FeLV-B-GA are double underlined.

amino acid level. Only two of five amino acid changes are nonconservative; glutamine to lysine at position 190 and serine to proline at position 194. Processing of the murine p15E is accompanied by removal of a 16-amino-acid C-terminal sequence called R, recognized in Moloney murine leukemia virus by Green et al. (20). The analogous region would span 17 amino acids in both FeLV proteins. Thus, if FeLV proteins are similarly processed, only 15 amino acids of the C terminus are expected to face the cytoplasmic side of the plasma and virus membrane. Both nonconservative amino acid changes between deduced FeLV-C-S and FeLV-B-GA p15E proteins are found within the C-terminal R region and are presumably not present in the mature protein.

**Structure of the 3′ LTR.** Retroviral LTRs contain regulatory sequences typical for eucaryotic genes (17). These include the CCAAT box (21) and the Goldberg-Hogness box (11, 17, 53) in the U3 region of the LTR, which play roles in the promotion of transcription. The U3 regions also contain enhancerlike sequences (36), and the FeSV-B-GA LTR was previously shown to have promoter and enhancer activity (9). Several regions of the FeLV-B-GA and FeLV-C-S LTR show strong homology to a 72-base-pair repeat in MSV, the latter of which was shown to enhance transcription of papovavirus early genes when substituted for the 72-base-pair repeat of simian virus 40 (34).

An LTR sequence comparison between FeLV-C-S and FeLV-B-GA is shown in Fig. 3B. The 12-base inverted repeats found at the ends of the LTRs are identical, whereas the inverted repeat of FeLV-C-S is immediately followed by five cytosine residues not found in FeLV-B-GA. The FeLV-C-S LTR encompasses the following 489 nucleotides (482 for FeLV-B-GA): 346 nucleotides in U3 (340 for FeLV-B-GA, over which the homology is 92.5%); 68 nucleotides in the R region (67 for FeLV-B-GA, with no base substitutions); and 75 nucleotides in both U5 regions, in which there is 98.7% homology. A palindromic sequence within the R region (Fig. 3B, underlined) was proposed to function as a termination sequence of transcription due to the formation of a hairpinlike structure (2, 15, 25); 35 of 36 bases in this region are conserved between the two LTRs.

## DISCUSSION

We described molecular cloning of FeLV-C-S and demonstrated that it alone is sufficient to induce rapid, fatal AA in neonatal kittens associated with ablation of bone marrow BFU-e. Since subgroup C FeLVs are the only known inducers of nonregenerative AA, studies with this prototype virus should permit us to examine mechanisms and pathways of stem cell suppression. The relevance of retroviruses as agents of aplasia is amplified by the increasing body of evidence indicating that naturally occurring human retroviruses are responsible for both cytosuppressive and cytoproliferative hemolymphatic human diseases (35, 40, 52, 65). Analogy to the pathogenic spectrum of feline retroviruses suggests that human hemolymphotropic viruses may be implicated in marrow aplasias of humans.

A comparison of the gp70, p15E, and LTR sequences of FeLV-C-S and FeLV-B-GA revealed substantial sequence differences only in the gp70 regions, including five blocks of apparent substitution. DNA sequence analysis revealed that the unique, FeLV-B-specific gp70 sequences originate by recombination between a subgroup A-like virus and a member of the major family of endogenous FeLV provirus-like sequences (J. I. Mullins et al., manuscript in preparation). The deduced amino acid sequences in four of these blocks differ in length, pattern of charged and uncharged polar side chains, and number of proline residues and in general display a high number of nonconservative amino acid changes. The sequences of FeLV-C-S and FeLV-B-GA in each block were found to be hydrophilic, suggesting that they are likely antigens by being exposed to the protein surface (55). The gp70 of FeLV-B-GA is 23 amino acids longer than that of FeLV-C-S and has three additional glycosylation signals. The differences noted probably play a role in generating the distinct subgroup-specific structural domains of these proteins.

In nature, FeLV-B and FeLV-C are found only in mixtures with FeLV-A, which is thought to function as an in vivo helper virus, and under experimental conditions only, can grow in weanling and adult cats that are already viremic for FeLV-A (30). The supportive effect of FeLV-A on subgroups B and C growth might be attributed to an immunosuppressive effect of FeLV-A on its host, thus allowing FeLV-B and FeLV-C to escape the host immune system (30), or to the fact that many more cells in the cat may be susceptible to FeLV-A compared with FeLV-B and FeLV-C, thus allowing rapid spread and growth of subgroups B and C in weanling and adult cats by phenotypic mixing (30). The gp70 protein might therefore play an important role in target-cell specificity for pathogenesis. However, growth of FeLV-C-S in kittens is not restricted to cells of the hemopoietic system (26), indicating that additional factors, coded for by the FeLV-C genome, may play a crucial role in the rapid depletion of early erythroid progenitor cells.

Comparison of the p15E proteins and LTRs of FeLV subgroups B and C revealed a high degree of homology. The few changes in the putative enhancerlike regions of the FeLV-B-GA and FeLV-C-S LTRs are mostly transition mutations, which may not be sufficient to account for the described differences in pathogenesis, but the importance of single nucleotide changes to the function of this region has not been adequately explored. The five cytosines positioned immediately downstream of the inverted repeat at the 5′ end of the FeLV-C-S U3 region are not unique to this strain of FeLV; four cytosines are present in the same position of the FeLV-B-Snyder-Theilen LTR (22). Previous studies of murine viruses reported that substitution of the LTR of a leukemogenic virus (SL3-3) into a nonleukemogenic virus (Akv) results in the formation of a potent leukemogenic virus in mice (8). However, the role of other regions of the viral genome during induction of leukemia by SL3-3 viruses was not examined, and recent data (C. Y. Thomas, personal communication) show that induction of leukemia by the SL3-3 virus is associated with the formation of envelope gene recombinant murine leukemia viruses.

Additional regulatory processes on the level of provirus integration, the requirement of a certain cellular environment for activity, and possibly provirus-encoded *trans*-acting transcription activators (6, 62) could contribute to host range, tissue tropism, and viral pathogenesis. We are therefore constructing recombinant viruses between a minimally pathogenic FeLV-A (J. Overbaugh, E. A. Hoover, and J. I. Mullins, unpublished data) and FeLV-C-S to investigate the role of individual viral genes in pathogenic specificity.

## ACKNOWLEDGMENTS

We thank O. Jarrett for performing the virus interference test, M. Krempin for expert technical assistance, and N. Davidson, in whose laboratory initial phases of this research were conducted. We also thank Dan Faulkner and Temple Smith for assistance in computer analysis of DNA sequences.

This work was supported by grants to J.I.M. from the National Science Foundation (PCM8216351) and by Public Health Service grant CA32563 to E.A.H. and J.I.M. from the National Institutes of Health. N.R. is a fellow of the Deutsche Forschungsgemeinschaft, and J.I.M. was a scholar of the American Cancer Society and is currently recipient of a research career development award from the National Institutes of Health.

## LITERATURE CITED

1. **Abkowitz, J. L., R. L. Ott, J. M. Nakamura, L. Steinmann, P. J. Fialkow, and J. W. Adamson.** 1985. Feline glucose-6-phosphatase dehydrogenase cellular mosaicism: application to the study of retrovirus-induced pure red cell aplasia. J. Clin. Invest. **75**:133–140.
2. **Benz, E. W., R. M. Wydro, B. Nadal-Ginard, and D. Dina.** 1980. Moloney murine sarcoma proviral DNA is a transcriptional unit. Nature (London) **288**:665–669.
3. **Blobel, G., and B. D. Dobberstein.** 1975. Transfer of proteins across membranes. J. Cell Biol. **67**:835–885.
4. **Bosselman, R. A., F. Van Straaten, C. Van 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.
5. **Boyce, J. T., E. A. Hoover, G. J. Kociba, and R. G. Olsen.** 1981. Feline leukemia virus-induced erythroid aplasia: in vitro hemopoietic culture studies. Exp. Hematol. (NY) **9**:990–1001.
6. **Broome, S., and W. Gilbert.** 1985. Rous sarcoma virus encodes a transcriptional activator. Cell **40**:537–546.
7. **Casey, J. W., A. Roach, J. I. Mullins, K. B. Burck, M. O. Nicolson, M. B. Gardner, and N. Davidson.** 1981. The U3 portion of the feline leukemia virus identifies horizontally acquired proviruses in leukemic cats. Proc. Natl. Acad. Sci. USA **77**:7778–7782.
8. **Celander, D., and W. A. Haseltine.** 1984. Tissue-specific transcription preference as a determinant of cell tropism and leukemogenic potential of murine retroviruses. Nature (London) **312**:159–162.
9. **Chang, E. H., M. E. Furth, E. M. Scolnick, and D. R. Lowy.** 1982. Tumorigenic transformation of mammalian cells induced by a normal human gene homologus to the oncogene of Harvey murine sarcoma virus. Nature (London) **297**:479–483.
10. **Chattopadhyay, S. K., M. W. Cloyd, D. L. Linemeyer, M. R. Lander, E. Rands, and D. R. Lowy.** 1982. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. Nature (London) **295**:25–31.
11. **Corden, J., B. Wasylyk, A. Buchwalder, P. Sassone-Corsi, C. Kedinger, and P. Chambon.** 1980. Promoter sequences of eukaryotic protein-coding genes. Science **209**:1406–1414.
12. **Doney, K. C., B. Torok-Storb, S. Dahlberg, C. D. Buckner, P. Martin, J. A. Hanse, E. D. Thomas, and R. Storb.** 1984. Immunosuppressive therapy of severe aplastic anemia. Prog. Clin. Biol. Res. **148**:259–270.
13. **Dorner, A. J., J. P. Stoye, and J. M. Coffin.** 1985. Molecular basis of host range variation in avian retroviruses. J. Virol. **53**:32–39.
14. **Elder, J. H., J. W. Gautsch, F. C. Jensen, R. A. Lerner, J. W. Hartley, and W. P. Rowe.** 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (*env*) gene recombinants. Proc. Natl. Acad. Sci. USA **74**:4676–4680.
15. **Elder, J. H., and J. I. Mullins.** 1983. Nucleotide sequence of the envelope gene of Gardner-Arnstein feline leukemia virus B reveals unique sequence homologies with a murine mink cell focus-forming virus. J. Virol. **46**:871–880.
16. **Essex, M.** 1975. Horizontally and vertically transmitted oncornavirus of cats. Adv. Cancer Res. **21**:175–248.
17. **Estratiasis, A., J. W. Posakony, T. Maniatis, R. M. Lawn, C. O'Connell, R. A. Spitz, J. K. DeRiel, B. G. Forget, S. M. Weissman, J. L. Slightom, A. E. Blechl, O. Smithies, F. E. Baralle, C. C. Shoulders, and N. J. Proudfoot.** 1980. The structure and evolution of the human β-globin gene family. Cell **21**:653–668.
18. **Fischinger, P. J., C. S. Blevins, and S. Nomura.** 1974. Simple, quantitative assay for both xenotropic murine and ecotropic feline leukemia viruses. J. Virol. **14**:177–179.
19. **Gasper, P. W., and E. A. Hoover.** 1983. Feline leukemia virus strain specific ablatement of erythroid burst forming units (BFU-3). Leuk. Rev. Internat. **1**:71–72.
20. **Green, N., T. Shinnick, O. Witte, A. Ponticelli, J. G. Sutcliffe, and R. A. Lerner.** 1981. Sequence-specific antibodies show that maturation of Moloney leukemia virus envelope polyprotein involves removal of a COOH-terminal peptide. Proc. Natl. Acad. Sci. USA **78**:6023–6027.
21. **Grosschedl, H., and M. L. Birnstiel.** 1980. Identification of regulatory sequences in the prelude sequences of an H2A

histone gene by the study of specific deletion mutants in vivo. Proc. Natl. Acad. Sci. USA **77**:1432–1436.

22. **Hampe, A., M. Gobet, J. Even, C. J. Sherr, and F. Galibert.** 1983. Nucleotide sequences of feline sarcoma virus long terminal repeats and 5′ leaders show extensive homology to those of other mammalian retroviruses. J. Virol. **45**:466–472.
23. **Hardy, W. D., Jr., P. W. Hess, E. G. MacEwen, A. K. McClelland, E. E. Zuckerman, M. Essex, S. M. Cotter, and O. Jarrett.** 1976. Biology of feline leukemia virus in the natural environment. Cancer Res. **36**:582–588.
24. **Hardy, W. D., Jr., Y. Hirshaut, and P. W. Hess.** 1973. Detection of feline leukemia virus and other mammalian oncornaviruses by immunofluorescence, p. 778–799. *In* R. M. Dutcher, and L. Chieco-Bianchi (ed.), Unifying concepts of leukemia: proceedings. S. Karger, New York.
25. **Honigman, A., and A. Panet.** 1983. A unique sequence in murine leukemia virus long terminal repeat functions as a termination signal for transcription in *Escherichia coli*. J. Virol. **45**:456–461.
26. **Hoover, E. A., G. J. Kociba, W. D. Hardy, Jr., and D. S. Yohn.** 1974. Erythroid hypoplasia in cats inoculated with feline leukemia virus. J. Natl. Cancer Inst. **53**:1271–1276.
27. **Hoover, E. A., L. E. Mathes, J. L. Rojko, J. P. Schaller, and R. G. Olsen.** 1978. Modifications of procedures for demonstration of feline leukemia virus group specific antigen by immunofluorescence. Am. J. Vet. Res. **39**:1877–1880.
28. **Hoover, E. A., R. G. Olsen, W. D. Hardy, Jr., and J. P. Schaller.** 1977. Horizontal transmission of feline leukemia virus under experimental conditions. J. Natl. Cancer Inst. **58**:443–444.
29. **Hoover, E. A., J. L. Rojko, and R. G. Olsen.** 1980. Pathogenesis of feline leukemia virus infection, p. 32–51. *In* R. G. Olsen (ed.), Feline leukemia. CRC Press, Inc., Boca Raton, Fla.
30. **Jarrett, O., M. C. Golder, S. Toth, D. E. Onions, and M. F. Stewart.** 1984. Interaction between feline leukemia virus subgroups in the pathogenesis of erythroid hypoplasia. Int. J. Cancer **34**:283–288.
31. **Jarrett, O., W. D. Hardy, M. C. Golden, and D. Hay.** 1978. The frequency of occurrence of feline leukemia virus subgroups in cats. Int. J. Cancer **21**:334–337.
32. **Kelleher, J. F., N. L. C. Luban, P. P. Mortimer, and T. Kamimura.** 1983. Human serum parvovirus: a specific cause of aplastic crisis in children with hereditary spherocytosis. J. Pediatr. **102**:720.
33. **Lenz, J., R. Crowther, A. Straceski, and W. Haseltine.** 1982. Nucleotide sequence of the Akv *env* gene. J. Virol. **42**:519–529.
34. **Levinson, B., G. Khoury, G. Van De Woude, and P. Gruss.** 1982. Activation of SV40 genome by 72-base-pair tandem repeats of Moloney sarcoma virus. Nature (London) **295**:568–572.
35. **Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Oshiro.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science **225**:840–842.
36. **Luciw, P. W., J. M. Bishop, H. E. Varmus, and M. R. Capecchi.** 1983. Location and function of retroviral and SV40 sequences that enhance biochemical transformation after microinjection of DNA. Cell **33**:705–716.

36a. **Luciw, P., D. Parker, S. Potter, and R. Najarian.** 1985. Feline leukemia virus (FeLV), strains A/Glasgow-1 and C, *env* genes, p. 1000–1004. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses, appendix B. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

37. **Mackey, L., W. Jarrett, O. Jarrett, and H. Laird.** 1975. Anemia associated with feline leukemia infection in cats. J. Natl. Cancer Inst. **54**:209–217.
38. **McAllister, R. M., J. Melnyk, J. Z. Finkelstein, E. C. Adams, Jr., and M. B. Gardner.** 1969. Cultivation in vitro of cells derived from a human rhabdomyosarcoma. Cancer **24**:520–526.
39. **Mellon, P., and P. M. Duesberg.** 1977. Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3′ half and the 5′ terminus of virion RNA. Nature (London) **270**:631–634.
40. **Montagnier, L., J. C. Cherman, F. Barré-Sinoussi, S. Chamaret, J. Grüest, M. T. Nügeyre, F. Rey, C. Daügüet, C. Axler-Blin, F. Vézinet-Brün, C. Roüzioüx, G. A. Saimot, W. Rozenbaüm, J. C. Gluckman, D. Klatzmann, E. Vilmer, C. Griscelli, C. Foyer-Gazengel, and J. B. Brunet.** 1984. A new human T-lymphotropic retrovirus: characterization and possible role in lymphadenopathy and aquired immune deficiency syndrome, p. 363–379. *In* R. C. Gallo, M. E. Essex, and L. Gross (ed.), Human T cell leukemia-lymphoma viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Montreuil, J.** 1980. Primary structure of glycoprotein glycans: basis for the molecular biology of glycoproteins. Adv. Carbohydr. Chem. Biochem. **37**:157–223.
42. **Mullins, J. I., D. S. Brody, R. C. Binari, Jr., and S. M. Cotter.** 1984. Viral transduction of c-*myc* gene in naturally occurring feline leukemias. Nature (London) **308**:856–858.
43. **Mullins, J. I., J. W. Casey, M. O. Nicolson, K. B. Burck, and N. Davidson.** 1981. Sequence arrangement and biological activity of cloned feline leukemia virus proviruses from a virus-productive human cell line. J. Virol. **38**:688–703
44. **Mullins, J. I., C. Chen, and E. A. Hoover.** 1986. Disease-specific and tissue-specific production of unintegrated feline leukemia virus variant DNA in feline AIDS. Nature (London) **319**:333–336.
45. **Nicolson, M. O., F. Hairi, H. M. Krempin, R. M. McAllister, and R. V. Gilden.** 1976. Infectious proviral DNA in human cells infected with transformation defective type C viruses. Virology **70**:301–312.
46. **Norrander, J., T. Kempe, and J. Messing.** 1983. Improved M13 vectors using oligonucleotide-directed mutagenesis. Gene **26**:101–106.
47. **Nunberg, J. H., M. E. Williams, and M. A. Innis.** 1984. Nucleotide sequences of the envelope genes of two isolates of feline leukemia virus subgroup B. J. Virol. **49**:629–632.
48. **Onions, D., O. Jarrett, N. Testa, F. Frassoni, and S. Toth.** 1982. Selective effect of feline leukemia virus on early erythroid precursors. Nature (London) **296**:156–158.
49. **Oroszlan, S., L. E. Henderson, T. D. Copeland, A. M. Shultz, and E. M. Rabin.** 1980. Processing and structure of murine leukemia virus *gag* and *env* gene encoded polyproteins, p. 219–232. *In* G. Koch and D. Richter (ed.), Biosynthesis, modification and processing of cellular and viral polyproteins. Academic Press, Inc., New York.
50. **Pinter, A., and E. Fleissner.** 1977. The presence of disulfide-linked gp70-p15(E) complexes in AKR murine leukemia virus. Virology **83**:417–422.
51. **Poncz, M., D. Solowiejczyk, M. Ballantine, E. Schwartz, and S. Surrey.** 1982. "Non-random" DNA sequence analysis in bacteriophage M13 by the dideoxy chain-termination method. Proc. Natl. Acad. Sci. USA **79**:4298–4302.
52. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
53. **Proudfoot, N. J.** 1979. Eukaryotic promoters? Nature (London) **279**:376.
54. **Proudfoot, N. J., and G. G. Brownlee.** 1974. Sequence of the 3′ end of the globin mRNA shows homology with immunoglobulin light chain mRNA. Nature (London) **252**:359–362.
55. **Ramachandran, G. N., and V. Sasisekharan.** 1968. Conformation of polypeptides and proteins. Adv. Protein Chem. **23**:283–437.
56. **Rommelaere, J., D. V. Faller, and N. Hopkins.** 1978. Characterization and mapping of RNase T1-resistant oligonucleotides derived from the genomes of Akv and MCF murine leukemia viruses. Proc. Natl. Acad. Sci. USA **75**:495–499.
57. **Russell, P. H., and O. Jarrett.** 1976. An improved assay for feline leukemia virus pseudotypes of murine sarcoma virus. J. Gen. Virol. **31**:139–143.
58. **Russell, P. H., and O. Jarrett.** 1978. The occurence of feline leukemia virus neutralizing antibodies in cats. Int. J. Cancer **22**:351–357.
59. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.

60. **Sarma, P. S., and T. Log.** 1973. Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization tests. Virology **54:**160–169.
61. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293:**543–548.
62. **Sodroski, J. G., C. A. Rosen, and W. A. Haseltine.** 1984. *trans*-acting transcriptional activation of the long terminal repeat of human T lymphotropic viruses in infected cells. Science **225:**381–385.
63. **Soe, L. H., B. G. Devi, J. I. Mullins, and P. Roy-Burman.** 1983. Molecular cloning and characterization of endogenous feline leukemia virus sequences from a cat genomic library. J. Virol. **46:**829–840.
64. **Testa, N. G., D. Onions, O. Jarrett, F. Frassoni, and J. F. Eliason.** 1983. Haemopoietic colony formation (BFU-E, GM-CFC) during the development of pure red cell hypoplasia induced in the cat by feline leukemia virus. Leuk. Res. **7:**103–115.
65. **Wong-Staal, F., and R. C. Gallo.** 1985. The family of human T-lymphotropic leukemia viruses: HTLV-I as the cause of adult T-cell leukemia and HTLV-III as the acquired immunodeficiency syndrome. Blood **65:**235–263.
66. **Wuensch, M., A. S. Schulz, W. Koch, R. Friedrich, and G. Hunsmann.** 1983. Sequence analysis of Gardner-Arnstein feline leukemia virus envelope gene reveals common structural properties of mammalian retroviral envelope genes. EMBO J. **2:**2239–2246.