Molecular Analysis of the Envelope Gene and Long Terminal Repeat of Friend Mink Cell Focus-Inducing Virus: Implications for the Functions of These Sequences

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We sequenced the envelope (env) gene and 3' long terminal repeat of a Friend mink cell focus-inducing virus (F-MCFV). We also sequenced the gp70 coding regions for two cDNA clones of another F-MCFV. The deduced amino acid sequence of the *env* gene products of both F-MCFVs were compared to the corresponding sequences of other MCFVs and of ecotropic viruses. The *env* polypeptides of the different viruses showed long stretches of homology in the carboxy-terminal half of gp70 and in $p15^{env}$ ("constant region"). The amino-terminal half of gp70 was very similar in all MCFVs, but showed extensive variations relative to the ecotropic viruses ("differential region"). This differential region in all MCFVs is of endogeneous origin. We show evidence that this region carries determinants for ecotropic or polytropic host range. No indication could be found that the *env* gene products determine the histological type of disease caused by particular MCFVs. When the long terminal repeats of F-MCFV and Friend murine leukemia virus were compared with those of other viruses causing either lymphatic leukemia or erythroleukemia, several nucleotides were localized which might determine the histological type of disease caused by these viruses.

Mink cell focus-inducing viruses (MCFVs) are polytropic viruses, able to induce cytopathic foci when grown on mink lung fibroblasts (16). The association between a variety of leukemias and MCFVs is becoming increasingly apparent. MCFVs have been implicated in the generation of thymic lymphomas in AKR mice and in the leukemias induced by Moloney murine leukemia virus (M-MuLV), Rauscher MuLV, and Friend MuLV (F-MuLV) (7, 12, 50). Antigenic and tryptic peptide analysis of viral gene products, RNase T_1 oligonucleotide mapping, and heteroduplex, restriction enzyme, and sequence analyses reveal that MCFVs are recombinants between ecotropic MuLVs and endogenous xenotropic virus-like sequences (7, 12, 50).

F-MuLV is an ecotropic, helper-independent, type C retrovirus which induces erythroleukemia at a high rate in newborn mice and lymphatic leukemia with a long latency in adult mice (53). A subgenomic DNA fragment of F-MuLV containing the 3' portion of the polymerase (*pol*) gene, the *env* gene, a single long terminal repeat (LTR), and the 5' portion of the *gag* gene carries the pathogenic functions of F-MuLV (33). A 2.4-kilobase-pair DNA fragment ranging from the 3' portion of the *pol* gene to the 3' portion of the *env* gene contains sufficient information to convert a nonpathogenic MuLV into a leukemia-inducing virus (34). However, these sequences are not sufficient to impart the high disease incidence and brief latency period of F-MuLV to other MuLVs. These properties must be specified by other sequences in the F-MuLV genome.

The onset of erythroleukemia in mice infected with F-MuLV is invariably associated with the generation of Friend MCFV (F-MCFV) (40, 42, 54). F-MuLV replicates to high titers in both resistant strains of mice and in strains that are susceptible to F-MuLV-induced disease; however, F-MCFV is only generated in susceptible strains of mice (40, 41). F-

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MCFV itself induces the same type of erythroproliferative disease as F-MuLV. Resistance to this disease correlates with the endogenous expression of a xenotropic-like gp70related protein that may interfere with the replication or spread of F-MCFV. These results suggest that F-MCFV is a crucial intermediate in the induction of disease by F-MuLV.

Restriction enzyme analysis of a molecularly cloned F-MCFV (clone pFM54B) indicates that a DNA fragment of F-MuLV containing most of the *env* gene has been replaced by endogenous *env*-like sequences (31). To better understand recombined *env* genes, we sequenced this region of the F-MCFV genome. In addition, we sequenced two cDNA clones of the *env* gene of an F-MCFV which we observed in an established Friend virus-infected cell line (45). To identify the sequences which may specify the histological type of leukemia caused by F-MuLV and F-MCFV, the LTR regions of these viruses were also sequenced. These sequences were compared with the LTR sequences of other lymphatic leukemia- and erythroleukemia-inducing murine retroviruses.

MATERIALS AND METHODS

Virus and plasmid. F-MCFV is a helper-independent, polytropic retrovirus which was isolated from a leukemic NIH Swiss mouse after inoculation with a biologically cloned F-MuLV isolate, F-MuLV 201. F-MCFV DNA was molecularly cloned in pBR322 as described (31). One of the resulting clones, pFM54B, which represents a genomic clone of F-MCFV containing a single LTR, was used for sequencing. F-MuLV clone 57 is a helper-independent, ecotropic, highly leukemogenic virus which induces erythroleukemia after inoculation into newborn NIH Swiss mice in the absence of spleen focus-forming virus (SFFV) (32, 33). F-MuLV 57 DNA (pF-MuLV57) was molecularly cloned from viral DNA isolated from fibroblasts infected with F-MuLV clone 201. pF-MuLV57 is a genomic clone which carries a single LTR (33). pF-MuLV57 was used for all F-MuLV sequence analyses.

Cloning of viral mRNA. Polyadenylate-containing RNA from the Eveline cell line, a Friend virus-infected cell line from an STU mouse (45), was prepared as described by Chirgwin et al. (5). Double stranded cDNA was synthesized and tailed with 3'-dCMP and ligated to vector DNA by the method of Land et al. (21). pBR322 and pBR327 (47) cut with *PstI* and tailed with 3'-dGMP were used as vectors for constructing recombinant plasmids. Tetracycline-resistant (ampicillin-sensitive) transformants were screened by colony hybridization (14). A nick-translated DNA fragment of pF-MuLV57 (*HindIII-KpnI* fragment of 3,300 base pairs [bp]) (20) was used to probe for plasmids containing F-MuLV *env* gene sequences. Two clones (pFM1 and pFM2) were obtained which hybridized to the F-MuLV probe.

Cloning of pFM2 in m13mp8 and dideoxy sequencing. The PstI double-stranded cDNA insert of pFM2 was reinserted into the PstI site of m13mp8 replicative-form DNA (J. Messing, Methods Enzymol., in press), and recombinants were transfected into *Escherichia coli* JM103 (27). Bacteria from white plaques were grown in small cultures and screened for m13mp8 replicative-form DNA containing inserts by using a miniscreen procedure (2). Single-stranded phage DNA was isolated from the cultures and sequenced according to the dideoxy nucleotide chain-terminating procedure developed by Sanger et al. (43), using a primer consisting of 15 nucleotides (Bethesda Research Laboratories, Gaithersburg, Md.).

Restriction enzyme analysis. Preparation of plasmid DNA for restriction enzyme analysis and sequencing was performed as previously described (20). Restriction endonucleases were obtained from Bethesda Research Laboratories, Gaithersburg, Md., Boehringer Mannheim, Mannheim, West Germany, and New England Biolabs, Beverly, Mass., and were used according to the specifications of the manufacturer. Restriction enzyme analysis of DNA was performed as described (20).

DNA sequencing by base-specific chemical cleavages. Sequence analysis of the envelope genes of plasmids pFM54B and pFM1 and of the LTR regions of pFM54B and pF-MuLV57 was performed by base-specific chemical cleavages (26). Restriction enzyme fragments were terminally labeled with $[\gamma$ -³²P]ATP (Amersham Radiochemical Centre, Amersham, England) and T4 polynucleotide kinase (Bethesda Research Laboratories). A+G, G, A>C, C>T, and C+T reactions were used for base-specific modifications.

RESULTS AND DISCUSSION

Sequence determination of the env gene and joining regions of F-MCFV. Comparison of the restriction enzyme maps of pFM54B (31) and pF-MuLV57 (20, 31) indicated that the env gene and LTR of pFM54B were located within a region ranging from the single SphI site to the HindIII site in the gag gene. A detailed restriction enzyme analysis of this region was performed (Fig. 1C). Nucleotide sequences of the 3' terminus of the *pol* gene, the *env* gene, and the LTR of pFM54B were determined by using the method of Maxam and Gilbert (26). The sequencing strategy is shown in Fig. 1C. The env-specific PstI insert of pFM1 (about 1,400 bp) was mapped by digestion with restriction endonucleases (Fig. 1B). Comparison of the restriction enzyme maps of pFM1 (Fig. 1B) and pFM54B (Fig. 1C) suggested that pFM1 contained the region coding for gp70 of F-MCFV. The PstI insert of pFM1 was sequenced according to the method of Maxam and Gilbert (26). The sequencing strategy is shown in Fig. 1B. The *env*-specific *PstI* insert of pFM2 (about 400 bp) was recloned into the *PstI* site of m13mp8 replicativeform DNA. Both strands of the insert were sequenced by using the method of Sanger et al. (43). Comparison of the overlapping sequences of pFM2 and pFM1 revealed that both clones were identical in this region (see below). Thus, we assumed that pFM1 and pFM2 are derived from identical F-MCFV genomes. In addition to the sequence coding for the amino terminus of gp70, pFM2 contains the region coding for the peptide leader of the *env* polyprotein. A restriction enzyme map of the *PstI* insert of pFM2 is shown in Fig. 1A. The nucleotide sequences of the envelope genes and joining regions of pFM54B and pFM1/pFM2 are shown in Fig. 2.

Sequence determination of the LTR of F-MuLV and of a region 5' to the *env* gene. Restriction enzyme maps of the 3'-terminal portion of the *pol* gene, the *env* gene, the LTR of F-MuLV, and the nucleotide sequence of the *env* gene of F-MuLV have been published before (20, 24, 31, 32). We determined the sequence of the 3' terminus of the *pol* gene and the sequence of the LTR of pF-MuLV57. The restriction enzyme maps and the sequencing strategies for these regions are shown in Fig. 1D.

3'-Terminal portion of the *pol* gene of F-MCFV (pFM54B) and F-MuLV. Figure 3 shows the nucleotide sequences of the 3'-terminal regions of the *pol* genes and the 5'-terminal regions of the *env* genes of F-MCFV (pFM54B) and F-MuLV. The *pol* gene and *env* gene overlap by 58 nucleotides (19 1/3 codons) in both viral genomes. Within the overlapping region of *pol* and *env* genes, there are 13 differences in the nucleotide sequences of F-MCFV and F-MuLV. These differences lead to 12 amino acid differences in the frame coding for the *env* polypeptide but to only 3 differences in the frame coding for the DNA polymerase. Thus, the carboxyterminal region of the polymerase is conserved to a higher degree than the amino terminus of the *env* polypeptide.

LTRs of F-MCFV and F-MuLV. Retroviral LTRs appear to contain sequences that control synthesis of progeny viral RNA and viral mRNA, reverse transcription of viral RNA by DNA polymerase, and integration of viral DNA into cellular DNA and contribute to the pathogenic potential of retroviruses (8, 33, 51, 52). In addition, U₃ sequences can influence the tissue tropism exhibited by some murine leukemia viruses (4, 9). Presumably, these sequences contain tissue-specific transcriptional enhancers which control the level of viral gene expression in different target cells. LTRs of several species of retroviruses differ in size and nucleotide sequence (6, 10, 18, 48, 49, 56). Despite these differences, certain nucleotide sequences which are believed to be important for the functions mentioned above are present in the LTRs of different virus species (8, 51, 52).

We sequenced the LTRs of F-MCFV (pFM54B) and F-MuLV (pF-MuLV57) (Fig. 2 and 4). By comparison with known sequences, we localized the regions U_3 -R- U_5 , where U_3 and U_5 designate unique sequences originating from the 3' and 5' ends, respectively, of viral RNA and R designates a sequence of viral RNA that is redundant at both ends. The nucleotide sequences of the LTRs of F-MCFV and F-MuLV were very similar to the sequences obtained for other murine (3, 6, 22, 46) and feline (11, 15; M. Wünsch, A. S. Schulz, W. Koch, R. Friedrich, and G. Hunsmann, EMBO J., in press) retroviruses and contained the putative control elements generally present in retroviral LTRs (8, 52). The LTR of F-MCFV consists of 514 bp. The LTR of F-MuLV contains 591 bp. The U_3 regions of both LTRs start with 13 nucleotides

which are found as an inverted repeat at the 3' termini of the U_5 regions. This inverted repeat is also contained in the LTRs of several other murine (3, 6, 11, 15, 22, 46) and feline (11, 15) retroviruses. The U_3 of F-MuLV contains a stretch of 65 nucleotide pairs which is repeated in tandem. As suggested for similar direct repeat sequences in the U_3 of other retroviruses, this region presumably has an enhancer function for effective expression of the proviral DNA (8, 52). F-MCFV contains no direct repeat. The 3' portion of the direct repeat of F-MuLV has an insert of nine nucleotide pairs which is part of a small, nearly perfect direct repeat. A CAT and a TATA box, which presumably are necessary

for the initiation of transcription (8, 52), are located in the U_3 of F-MCFV and F-MuLV. A possible Z-DNA segment which is believed to be involved in regulation of transcription (30) is also present in the U_3 region of F-MCFV and F-MuLV. Signals which are hypothesized to be involved in polyadenylation of viral RNA (AATAAA, 22 bp before the start of U_5 , and CA, at the very end of R) are found in F-MCFV and F-MuLV.

Sequences in the LTR as possible determinants of the histological type of leukemia. When we aligned the LTRs of F-MCFV and F-MuLV with the LTRs of the lymphatic-inducing viruses Moloney MCFV (M-MCFV) and M-MuLV



FIG. 1. Physical mapping and use of restriction enzyme fragments for sequence analyses. Closed circles represent oligonucleotide primers used for polymerization reactions (dideoxy nucleotide sequencing). Open circles represent 5' ends labeled with $[\gamma^{-32}P]ATP$ (sequencing with base-specific chemical cleavages). Arrows indicate fragments from which unambiguous sequences were obtained. (A) Restriction endonuclease cleavage sites of the *env*-specific *PstI* insert of clone pFM2. (B) Restriction endonuclease cleavage sites of the *env*-specific *PstI* insert of clone pFM2. (C) Restriction endonuclease cleavage sites to 74-bp deletion in the LTR of F-MCFV with respect to F-MuLV. (D) Restriction endonuclease cleavage sites of the *pol* gene and the LTR of F-MULV within the *SphI-Hind*III fragment. The numbers in and near the LTRs of F-MCFV (C) and F-MuLV (D) indicate functional regions as follows: 1 and 8, inverted repeats at the ends of the LTRs; 2 and 3, direct repeats in the U₃ of F-MuLV; 4, CAT box; 5, putative Z-DNA segment; 6, TATA box; 7, polyadenylation signal; 9, primer binding site for minus-strand synthesis; 10, splic donor site for generation of subgenomic 21S mRNA. Abbreviations: CHO, potential glycosylation site; A, AccI; Ba, Smal; Sp, SphI; SI, SstI; SII, SstI; T, Tth1111; X, XbaI.

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	Ασαι Αστστασοστέςος λολοκικό και και ματά το τραγιατικό το τραγιάτα τη	90
	AGTAGACGGĊATCGCTGCGŤGGATCCACGĊCGCTCACGTÁNAAGCGGCGACAACCCCTCCGGCCGGAACAGCATCAGCACCACATGAA Hetgiu	180
-30	T T λ GGTCCAGCGTTCTCAAAACĊCCTTAAAGAŤAAGATTAACĊCGTGGGGGCĊCCTGATAGTCCTGGGGATCŤTAATAAGGGĊAGGAGTATCÅ GlyProAlaPheSerLysProLeuLysAspLysIleAsnProTrpGlyProLeuIleValLeuGlyIleLeuIleArgAlaGlyValSer CysSerThr	270
7D	A GTACCACATĠACAGCCCTCÁTCAGGTCTTĊ <u>AATGTTACT</u> ŤGGAGAGTTAĊCAACTTAATĠACAGGACAAÁCAGCT <u>AATĠĊTACC</u> TCCCTĊ ValProHisspserProHisGlnValPheAsnValThrTrpArgValThrAsnLeuMetThrGlyGlnThrAlaAsnAlaThrSerLeu Gln	360
31	T CTGGGGACAÀTGACCGATGĊCTTTCCCAAÀCTGTACTTTĠACTTGTGCGÀTTTAATAGGĠGACGACTGGĠATGAGACTGĠACTCGGGTGŤ LeuGlyThrMetThrAspAlaPheProLysLeuTyrPheAspLeuCysAspLeuIleGlyAspAspTrpAspGluThrGlyLeuGlyCys end pFN2	450
61	GCACTCCCCGGGGGAAGAAAAAGGGCAAGAACATTGACTTCTATGTTTGCCCCGGGCATACTGTACCAACAGGGTGTGGAGGCCCGAGA ArgThrProGlyGlyArgLyBArgAlaArgThrPheAspPheTyrValCyBProGlyHisthrValProThrGlyCyBGlyGlyProArg Ala	540
91	GAGGGGTACTGTGGGCAAAATGGGGGCTGTGAGACCACTGGAČAGGCATACTGGAAGCCATCATCATCATGGGACCTAATTTCCCTTAAGCGA GluGlyTyrCysGlyLysTrpGlyCysGluThrThrGlyGlnAlaTyrTrpLysProSerSerSerTrpAspLeuIleSerLeuLysArg Leu	630
121	GGANACACCĊCTCĠGAATCÅGGGCCCCTGŤTATGATTCCŤCAGCGGTCŤĊAGGGGCATĊĈAGGG ^C GGCCÅCACCGGGGGĠŤCGATGCAĂŤ GlyAsnThrProArgAsnGlnGlyProCysTyrAspSerSerAlaValSerSerGlyIleGlnGlyAlaThrProGlyGlyArgCysAsn Gln Asp Lys	720
151	CCCCTAGTCĊTĂGAATTCAĊTGACGCGGGŤAAAAAGGCCÁGCTGGGATGĠČCCCAAAGTÁTGGGGACTAÁGACTGTACCĠATCCACAGGĂ ProleuValleuGluPheThrAspAlaGlyLysLysAlaSerTrpAspGlyProLysValTrpGlyLeuArgLeuTyrArgSerThrGly	810
181	ACCGACCCGĠTGACCCGGTṫCTCTTTGACĊCGCCĂGGTCĊTCAATATAGĠGCCCCGCĂTĊCCATTGGGĊCTAATCCCĠŤGATCA ¹ TGAĊ ThrAspProValThrArgPheSerLeuThrArgGinValLeuAsnIleGlyProArgIleProIleGlyProAsnProValIleThrAsp Arg	900
211	CAGTTACCCCCCCCGACCCGGGCAGATCATGCTCCCCCAGGCCTCCCCTACAGGCGCAGCCTCTATA GInLeuProProSerArgProValGInIleMetLeuProArgProProGInProSerProThrGlyAlaAlaSerIle Pro Pro ValProGluThr	978
237	GCCCCACCTTCTCAA CAACCTGGGACGGGAGACAGACTGCTAAACCTGGTAGATGGAGCCTACCAAGCACTC <u>AACCTCACC</u> AGTCCTGAC GInProGlyThrGlyAspArgLeuLeuAsnLeuValAspGlyAlaTyrGlnAlaLeuAsnLeuThrBerProAsp AlaProProSerGln	1053
262	ANANCCCANĠAGTGCTGGTŤGTGTCTGGTŇŤCGGÂCCCĊCCTACTACGÅAGGGGTTGCĊGTCCTAGGTŇCCTAČTC <u>AÁCCATACC</u> TCŤ LysThrGlnGluCysTrpLeuCysLeuValSerGlyProProTyrTyrGluGlyValAlaValLeuGlyThrTyrSerAsnHisThrSer AlaGlu	1143
292	GCCCCAGCT <u>ÅACTGCTCC</u> GTGGCCTCCCAÅCACAAGCTGÅCCCGGÅCGGÅCTGGGÅCÅGGGÅCTCCGGÅGÅGGÅGTČCCCAÅÅ AlaProAla[AsnCysSerValAlaSerGlnHisLysLeuThrLeuSerGluValThrGlyGlnGlyLeuCysValGlyAlaValProLys Arg 11e Thr	1233
322	T C C C T TT G A A A T A T. A T. C A. A T. C G. G T. ACCCATCAGGCCCTGTGT <u>ATACCCACCCAGAATACAAGC</u> GACGGCTCTACTATCTGGCTGCCCGCCGGGACCATTTGGGCTTGCGAAC ThrHisGlnAlaLeuCysAsnThrThrCin <u>AsnThrSer</u> AspGlySerTyrTyrLeuAlaAlaProAlaGlyThrIleTrAlaCysAsn LeuLys AsnLysValVal	1323
352	$ \begin{array}{cccccc} C & A & A & T & C & C & C & G & T & T & G & T & C & T & C & A & A & T & A & C & G & C & A & A & A & C & G & C & A & A & A & A & A & A & A & A & A$	1413
382	TCT CA CCA A A TCCTATGCT A C CT TATA A T CACTCCCCTGGTTATGGTTATGGCCAGTTTGAGAGAAAAACCAAATATAAAAGAGAGCGGGTGTCATTAACTCTGGCCCTGGTGTGGGA HisSerProGlyTyrValTyrGlyGlnPheGluArgLysTrLysTrLysArgGlu <u>ProValSerLeuThrLeuAlaLeuLeuLeuGly</u> Pro Ser LysSerTyrArgHis <i>p15env/p12env</i>	1503
412	G Å T C C G GGACTTACTÁTGGGCGGCAŤAGCTGCAGGÁGTAGGGACÁGGAACTACCGĊCCTGGTCGCĆACCAGCAGŤTCCAGCAGCŤCCATGCTGCĆ GlyLeuThrHetGlyGlyIleAlaAlaGlyValGlyThrGlyThrThrAlaLeuValAlaThrGInGInPheGInGInLeuHisAlaAla	1593
442	GTACAAGATĠATCTCAAAGÁAGTCGAAAAĠTCAATTACTÁACCTAGAAAÁĠTCTCTTACŤTCGTTGTCTĠAGGTTGTGCŤGCAGAATCGÁ ValGinAspAspLeuLysGiuValGiuLysSerileThrAsnLeuGiuLysSerLeuThrSerLeuSerGiuValValLeuGinAsnArg	1683
472	CGAGGCCTAĞACCTGTTGTŤCCTAAAAGAÁAGAGGACTGŤGTGCTGCCČŤAAAAGAAGAÁTGTTGTTTĊŤATGCTGACCÁTACAGGCCTÁ ArgGlyLeuAspLeuLeuPheLeuLysGluArgGlyLeuCysAlaAlaLeuLysGluGluCysCysPheTyrAlaAspHisThrGlyLeu	1773
502	GTANGAGATÁGTATGGCCAÁATTANGAGAĠAGACTCACTĊAGAGACAAAÁACTATTTGAĠTCGAGCCAAĠGATGGTTCGÀAGGATGGTTGTŤ ValargaspSerHetalelysleuargGluargleuThrGlnArgGlnLysleuPheGluSerSerGlnGlyTrpPheGluGlyLeuPhe	1863
532	ANCAGATECCCTGGTTTACCACGTTAATATCCACCATCATGGGGCCTCTCATTATACTCTACTACTACTACTACTACTACTACT	1953
562	CTTAATCGAŤTAGTTCAATŤTGTTAAAGAČAGGATCTCAĠŤAGTCCAGGČTTTAGTCCTĠACTCAACAAŤACCACCAGCŤAAAACCACTÁ LeuAsnargleuValGinPheValLysAspargileSerValValGinAlaleuValLeuThrGinGinTyrHisGinLeuLysProLeu	2043
592	end pl2env _> - LTR - UJ GARTAGGAGCATGATAAATAAAAGATTTTATTTAGTTTCCAGAAAAAGGGGGGGAATGAAAGACCCCACCAAGTTGCTTAGCCTGATAGC GlutyrGluPro end pl5env	2133
	CGCAGTAACĠCCATTTTGCĂAGGCATGGAĂAAATACCAAĂCCAAGAATAĠGGAAGTTCAĠATCAAGGGCĠGGTACACGAĂAACAGCTAAċ	2223
	GTTGGGCCAÄACAAGATATĊTGCGGTAAGĊAGTTTCGGCĊCCGGCCCGGĠGCCAAGAACÅGATGGTCCCĊAGATATGGCĊCAACCCTCAĠ	2313
	CAGTTTCTTĂAGACCCATCĂGATGTTTCCĂGGCTCCCCCĂAGGACCTGĂĂATGACCCTGTGCCTTĂTTTĂAATTAACCAĂTCAGCCCGCT	2403
	TCTCGCTTCTGCGCGCGCCTTTTGCTTCCCGAGCTCTATAAAAGAGCTCACAACCCCTCACTCGGCGCGCGC	2493
	GCCCGGGTACCCATGTACCAATAAATCCTCTTGCTGTTGCATCCGACTTGTGGTCTCGCTGTTGGGAGGGTCTCCTCAGAGTGAT	2583
	TGACTACCCGTCTCGGGGGGTCTTTCATTTGGGGGGCTCGTCCGGGATCTGGAGACCCTTGCCCAAGGACCACCGACCCACCGGGAGGT	2673

FIG. 2. Nucleotide sequence and deduced amino acid sequence of the env gene of F-MCFV (pFM54B). Differing nucleotides and amino acids of pFM1 and pFM2 are also shown. Regions of uncharged amino acids are underlined. Potential glycosylation sites are enclosed in boxes. The sequence presented here differs from the preliminary sequence distributed at the Cold Spring Harbor RNA Tumor Virus Meeting in May 1983 at positions 896, 1539, and 1969. Also given is the nucleotide sequence of the LTR of F-MCFV (pFM54B).

(Fig. 4) we found a high degree of homology between the LTR sequences. However, some nucleotide exchanges as well as insertions and deletions were observed. Most differences were in the U₃ regions. M-MuLV has a perfect major tandem direct repeat flanked by small direct repeats, whereas F-MuLV has a tandem direct repeat which contains a few mismatches and a 9-bp insertion/deletion. Neither F-MCFV nor M-MCFV showed a large direct repeat. Since M-MCFV

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1	F-NCFV	ccc	A CC	GCT	тст	, , ,	GTA	GYC	GGC	ATC	GCT	GCG	TGG	ATC	cic	GCC	GCT	CAC	GT7		GCG	GCGAC	ллссс	• ctccg	GCCGGÅ
		Pro	Thr	Ala	Leu	Lys	Val	λsp	Gly	Ile	λla	λla	Trp	Ile	Gln	Ala	λla	His	sVal	Lys	λla	λlaTh	rThrP	roProl	laGly
2	F-NulV	*	*	*	¥	¥	¥	*	*	*	¥	*	*	¥	*	*	*	*	*	*	*	*	λspT	hrarg	IleGlu
		•••	•••	•••	•••	•••	•••	•••	•••	т	X	•••	•••	•••	•••	т	c			G	т	c	G A . A	.CAG.I	ATT.λG
1	λCλGCλTC <i>env pol</i> ThrλlaSe	λGGA (ype) rGly	CCG ptic Pro	SλCλ de M oThr	TGG etG Trp	λλG luĢ Lys	GTC lyP Val	CAG roA Gln	CGT laP Arg	TCT heS Ser	CAA erL Gln	• AAC AST	CCC ProL Pro	TTA euL Leu	.ys) iLys	SATA Spl SIle	λGλ ysI λrg	TT) lei Lei	AACC AsnF uThr	CGT ProT Arg	GGG rpG Gly	GCCCC lyPro AlaPr	CTAAT Leull o <i>end</i>	• AGTCCI eValL(<i>poly</i> :	rgggg λ euGly merase
2	ProProAl	aGlu <i>Y pej</i>	Ser D <i>tic</i> T	te te	* * λ	Arg laC CGT	* ysS	* erT	* hrL	* .euP	* ro	* 5	* SerP	* ro C	*	*	* *	*)	* Asp	* *	¥ rgλ	ThrSe spLeu	r <i>end</i> * *	' <i>poly</i> Pro H	<i>merase</i> * Ile

FIG. 3. Nucleotide sequence of the 3' ends of the *pol* genes and the 5' ends of the *env* genes of F-MCFV (pFM54B) and F-MuLV. The amino acids of the frames for polymerase and *env* polypeptide are also given. Dots indicate identical nucleotides and asterisks denote identical amino acids in F-MuLV when compared to F-MCFV.

is highly leukemogenic, the presence of a large direct repeat in U_3 apparently is not necessary for the leukemogenic potential of MCFVs. When we compared polytropic viruses F-MCFV and M-MCFV with the ecotropic viruses F-MuLV and M-MuLV, we did not observe a single nucleotide which was specific for either polytropic or ecotropic viruses. Thus, we assume that the sequences responsible for the expanded host tropism of MCFVs are not located in the LTR but in another part of the genome (see below).

We compared the LTRs of the Friend viruses with those of Moloney viruses. We found certain positions (e.g., the region between nucleotides 2093 and 2115 or between nucleotides 2188 and 2192 of F-MCFV) (Fig. 4) which are different between these two groups of viruses. Recent experiments in other laboratories have suggested that the LTR plays a crucial role in determining the type of disease caused by different leukemia viruses (4, 9). The differences in the LTRs described above may be the molecular basis for these properties. Friend SFFV (F-SFFV) is an erythroleukemiainducing virus. The LTR of F-SFFV (6) is nearly identical to the LTR of F-MCFV (comparison not shown). Therefore, the LTR sequence differences between F-MCFV and the Moloney viruses could be determinants of erythroid versus lymphoid virally induced leukemia. However, when the LTR sequence of AKR MCF247 (a virus which accelerates T-cell leukemia in AKR mice) (19) was also examined (comparison not shown), only some of these nucleotide differences remained. These sequences are indicated by an arrow in Fig. 4.

Comparison of the *env* genes of pFM54B and pFM1/pFM2. Figure 2 shows the composition of the nucleotide sequences obtained for the F-MCFV clones pFM54B, pFM1, and pFM2. The sequence of pFM54B has one large open reading frame consisting of 2,028 nucleotides. This frame starts at nucleotide 28 and terminates at position 2055 with two stop condons. The amino acid sequences for this reading frame starting at the first ATG codon are also shown in Fig. 2.

The sequence coding for the peptide leader of the *env* polyprotein in pFM54B and pFM2 consists of 32 codons. There are seven nucleotide differences in this region of these MCFV clones, five of which give rise to a change in the corresponding amino acid. The open reading frame which encodes gp70 encompasses 1,197 nucleotides (399 codons) in pFM54B and 1,224 nucleotides (408 codons) in pFM1/pFM2. In the sequence coding for the proline-rich region (PR) of

gp70 (see below), 27 nucleotides (nine codons) are deleted in pFM54B at position 978. In the gp70's of pFM54B and pFM1, 103 (8.6%) nucleotide differences exist leading to 33 (8.3%) amino acid changes (including the deletion of 27 nucleotides). Nineteen of these nucleotide changes (11 amino acid changes) are situated in the *env* sequence from the initiation codon up to the end of the PR (differential region, see below), whereas 84 nucleotide changes (22 amino acid changes) occur in gp70 downstream of the PR.

The 27 nucleotides in the PR of pFM1 which are deleted from pFM54B are present in other polytropic viruses (3, 25; C. Holland, personal communication) and in F-SFFV (1, 6, 58). Interestingly, this deletion in pFM54B gives a portion of the PRs which are different in F-MCFV and F-MuLV (positions 943 to 963 in pFM54B in Fig. 5A) the exact same length between stretches which are homologous in the two *env* genes. In vitro recombinants of F-MuLV containing the *env* gene of pFM54B yielded infectious but nonpathogenic virus (unpublished data). It is possible that this deletion is responsible for the nonpathogenicity of this clone. Experiments are in progress to test the importance of these deleted sequences in causing disease.

Comparison of the *env* genes and *env* polypeptides of F-MCFV and F-MuLV. Figures 5A and B show the 3' termini of the *pol* and *env* genes of F-MCFV (pFM54B) and F-MuLV (pF-MuLV57). Also shown are the amino acid sequences of the *env* polypeptides. Alignment was performed to obtain maximal homology of the two nucleotide sequences.

Comparison of the nucleotide sequences of F-MCFV (pFM54B) and F-MuLV show identical sequences in the *pol* gene of both viruses up to nucleotide 36. The two viral sequences then vary up to nucleotide 1509 in F-MCFV. We therefore conclude that the nucleotides between positions 36 and 1650 in F-MuLV have been replaced by endogenous sequences, thereby forming F-MCFV (clone pFM54B). The substitution begins in the 3' portion of the *pol* gene about 120 nucleotides upstream from the initiator ATG of the *env* gene and ends at or near nucleotide 1510 (in F-MCFV) within the sequence coding for p15^{env}. The substituted sequence in F-MCFV (pFM54B) is 141 nucleotides shorter than the corresponding F-MuLV sequence. The two *env* genes share the same initiation and termination codons and are translated in the same reading frame.

Within the region coding for $p15^{env}$, the nucleotide se-

- 11.7

1	P-MCPV GAGCCAT GATAAAAAAAAAAAAAAAAAAAAAAATTTAATTT	2107
2	P-MuLV	2248
3	<i>M-MCFV</i>	2396
1	<i>N-MULV</i>	7846
	Glupro end p15env inverted repeat AA AA	
		2196
2		2170
3		2486
1	C TTA A GATGG	7936
-	Ā	
1	GCTAACGTTGGGCCAAACAAGATATCTGCGGTAAGCAGTTTCGGCCCCGGGCCCAAGAACAGAT	2264
2	AC GC TGGGCCA	2417
3		2555
•		8026
1	GGTCCCCAGATATGGCCCAACCCTCAG	2291
2	AACAGGATATCTGTGGTAAGCAGTTTCGGCCCGGTCGGCCCCGAGGCCAAGAACGGAT	2507
3	GCTG	2582
1	AACAGGATATCTGTGGTAAGCAGTT CCTGCCCCGGCTCAGGGCCAAGAACAGAT	8107
	· · · · · · · · · · · · · · · · · · ·	
1	слаттстталалсссатслаататтсслаастсссссалаалссталаталссстататталатталатсласссаст	2381
2	······································	2597
3		2672
4	······································	819/
1		2468
2		2687
3		2762
4		8287
	Z DNA TATA box	/23
	· · · · · · · · · · · · · · · · · · ·	
1	GTCGCCCGGGTACCCGTGTATCCAATAAATCCTCTTGCTGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCAGAGT	2558
2	······································	2///
3	······································	2852
•		113
1	GATTGACTACCCGTCT CGGGGGTCTTTCATTGGGGGGCTCGTCCGGGATCTGGAGACCCTTGCCCAAGGACCACCGACCACCACCGGG	2647
2		2866
3	λG	2882
4		203
	inverted repeat primer binding site	
	· · · · · · · · · · · · · · · · · · ·	
1	AGGTAAGUTGGUUAGUAATIGATUTGTUTGTUU ATTGTUUGTG	2693
2	Icitigatigatifategeccg	2955
		293
•	splice donor	275

FIG. 4. Nucleotide sequences of the LTRs and adjacent regions of F-MCFV (pFM54B), F-MuLV, M-MCFV (3), and M-MuLV (46). The sequences were aligned to obtain maximal homology. Arrows denote some of the differences between lymphatic leukemia- and erythroleukemia-inducing viruses (see text). Dots indicate identical nucleotides as in F-MCFV. Gaps have been introduced to allow optimal alignment. Regions of functional importance are enclosed in boxes. Direct repeats are underlined. Position numbers for M-MCFV and M-MuLV are taken from references 3 and 46, respectively.

quences of F-MCFV (pFM54B) and F-MuLV are identical from nucleotide 1510 to nucleotide 2112 (5' terminal portion of the U₃ region in the LTR) with one exception: a change at nucleotide 1693 in the region encoding $p15^{env}$ in F-MCFV leads to an arginine, whereas F-MCFV carries a glycine at the corresponding position. In all MCFVs studied so far, the borders of the substituted regions can be recognized by a change from complete homology (outside the recombination site) to sequence variations (within the recombination sites). Since the change at position 1693 in F-MCFV is located far downstream (180 nucleotides) from the proposed recombination site of F-MCFV and F-MuLV (see below), we suggest that this difference is due to a point mutation which occurred after the generation of F-MCFV.

The substitution in pFM1/pFM2 is considerably shorter

than that in pFM54B. It begins within the sequence coding for the peptide leader at or near nucleotide 166 in F-MuLV (Fig. 5A) and ends near position 1211. There are few nucleotide differences between pFM1/pFM2 and F-MuLV clone 57 outside the substituted region (e.g., positions 173, 175, 1152, 1161, 1191, and 1227; Fig. 2). Since pFM1/pFM2 are derived from a Friend virus-infected cell line of an STU mouse (44) and not from F-MuLV clone 201 as are pFM54B and pF-MuLV57, we consider these differences to represent point mutations present in the progenitor MCFV of pFM1/pFM2.

The *env* gene product of F-MCFV (pFM54B), as deduced from the nucleotide sequence, is 636 amino acids long (including the deleted sequence in the PR; Table 1). This is 39 amino acids shorter than the proposed *env* polypeptide

		78
	<u>Friend-Nuly</u>	78
	CATCGCTGCGTGGATCCACGCCGCTCACGTAAAAGCGGCGACAACCCCTCCGGCCGG	168
	T	165
-27 -30	TTCTCANANACCCCTTANAGATANGATTANCCCGTGGGGC CCCCTGATAGTCTGGGGATCTTANTANGGGAGGAGTATCA PheSerLysProLeuLysAspLysIleAsnProTrpGly ProLeuIleValLeuGlyIleLeuIleArgAlaGlyValSer LeuPro * SerPro * * * Asp * ArgAspLeuLeuIle * * LeuPheLeuSer * LysGly * ArgSerAla C.CTC	249 255
		gp70 F-Nul.V
1 2	GTACCACATGACAGCCCTCATCAGGTCTTCAATGTTACTTGGAGAGATTACCAACTAATGACAGGACAAACAGCTAATGCTACCTCCCTC	339
		402
31 31	CTGGGGACAATGACCGATGCCTTTCCCAAACTGTACTTGACTTGGCGATTTAATAGGGGAC LeuGlyThrHetThrAsplaPheProLysLeuTyrPheAspleuCysAspleuIleGlyAsp AsnHisProLeuTrpThrTrpTrp * Val * ThrPro * * * * Het * AlaLeuSerGlyProProHisTrpGlyLeuGluTyr	402
		417
61	AspTrpAspGluThr AspTrpAspGluThr GlnAlaProTyrSerSerProProGlyProProCysCysSerGlySerSerGlySerSerAlaGlyCysSerAr t vs * * * Pro	•••
•••	CAGGCCCCCTATTCCTCGCCCCCGGGGCCCCCTTGTTGCTCAGGGAGCAGCGGGAGCAGTGCAGGCTGTTCCAGAC.CC.C	522
57 91	GGACTCGGGTGTCGCACTCCCGGGGGAAGAAAAAGGGCAAGAACATTTGAC GlyLeuGlyCysArgThrProGlyGlyArgLysArgAlaArgThrPheAsp LeuThrSerLeuThrProArgCysAsnThrAlaTrpAsn * LeuLysLeuAspGlnValThrHisLysSerSerGluGly * * *	477
	TTGAC.TCCCTCAC.CGGT.CAAC.CTGCCT.AACCTTAAGCTAGACCCAGGTAACTCATAAATCAAGTGAGGGATC	612
77 121	Teregradue Construction CysProlyHisthValProThrGly CysGlyGlyProArgGluGlyTyrCysGlyLysGlyCysGluThrThrGly ***** SerHiskrg * ArgGluAlaLysSer[****] AspSerPhe * * AlaSer[*********	228
		702
104	CAGGCATACTGGAAGCCATCATCATCATGGGACCTAATTTCCCTTAAGCGAGGAAACACCCCTCGGAATCAGGGCCCCTGTTATGATTCC GInalaTyrTrpLysProSerSerSerTrpAspLeuIleSerLeuLysArgGlyAsnThrProArgAsnGlnGlyProCysTyrAspSer JargVall * * * * * * * * * * * * * * * * * *	648
	AGA.T	768
134	TCAGCGGTCTCCAGTGGCATCCAGGGTGCCACACCGGGGGGTCGATGCAATCCCCTAGTCCTAGAATTCACTGACGCGGGTAAAAAGGCC SerAlaValSerSerGlyIleGlnGlyAlaThrProGlyGlyArgCysAsnProLeuValLeuGluPheThrAspAlaGlyLysLysAla Gln * * GlnValCysLysAspAsnLysTrp * * * AlaIleGln * * Asn * * * GlnVal	730
	CAG., TCAGGTATAAG.CAA.AAGTGGT.G.CTA.CC.G., TAAC.GCT.	846
164	AGCTGGGATGGCCCCAAAGTATGGGGACTAAGACTGTACCGATCCACAGGAACCGACCCGGTGACCCGGTTCTTTTGACCCGCCAG SetTrpAspGlyProLysValTrpGlyLeuArgLeuTyrArgSetThrGlyThrAspProValThrArgPheSerLeuThrArgGln Thr * ThrThrGlyHistry * * * * * * * * * * * * * * * * * * GlyLeuThr * GlyLloarg	825
	ACCTCAACAACTGGAC.CTATTC.TT.GTCTGCGGG.CTTACTGGGA.CCGA.T.AGA	933
193 228	GTCCTCAATATAGGGCCCCGCATCCCCATTGGGCCTAATCCCGTGATCACTGACCAGTTACCCCCCCC	915
	TAT. AACATGGG.AAGCCC.GG.AAC.TT.G.T.C.GTAAT.CCA end differential region	1017
223	CCCAGGCCTCCTCAG ProArgProProGln ProSerProThrGlyAlaAlaSerIleGlnProGly	966
256	* Lys * AlaLysSerProProAlaSerAsnSerThrProThrLeuIleSer * * * * ProThrGlnProProProAla *AAG.CATCTCCCCCCGCCTCTAATTCGACTCCCACATTGATTTCCGCTCC.A.TCAGC.CCCG.C.G.AA	1107
	ACGGGAGACAGACTGCTAAAACCTGGTAGAATGGAGCCTACCAAGCACTCAACCTCACCAGTCCTGACAAAAACCCAAGAGTGCTGGTTGTGT	1056
240 286	ThrGlyAspArgLeuLeuAsnLeuValAspGlyAlaTyrGlnAlaLeuAsnLeuThrSerProAspLysThrGlnGluCysTrpLeuCys * * * * * * * * * * Gln * * * * * * * * * * Asn * * * * * * * * * * * * * * * * * * *	•
	GT. λT λC. G	1197

FIG. 5

chain of F-MuLV (675 amino acids). Most of the differences in the *env* polypeptides of F-MCFV and F-MuLV are located in the leader peptide and in gp70, whereas $p15^{env}$ is highly conserved. The proposed signal peptides of the *env* precursor polypeptides of F-MCFV and F-MuLV consist of 32 and 34 amino acids, respectively. The nucleotide and amino acid sequences of the F-MCFV leader peptide are very different from those of the parental F-MuLV. However, in this portion of the *env* polypeptide of both viruses, a stretch of uncharged, mostly apolar amino acids is found. The conservation of this hydrophobic region points to the important function of the signal peptide in the excretion of the env polypeptide through cell membranes.

The signal peptide cleavage site of the F-MuLV precursor polypeptide has been determined (23), whereas this site is not known for F-MCFV. In Rauscher MCFV, the mature gp70 polypeptide chain starts with NH₂-Val-Gln-His-Asp (44). We therefore assume that the mature gp70 of F-MCFV

	B and differential region	-
		966
	Gin ProserProThrGivalaAlaSerIleGinProGiv	
	LysserProProlaserlanserThrProThrLeuIleSer * * * * ProThrGinProProProl *	
	Priend-Muly A., TCTCCCCCCCCCCTTANTICGACTCCCACATTGATTGC., G., C., TCC.A., TCAGC.CCCG.C.G.A., A	1107
240 286	ACGGGAGACAGACTGCTAAACCTGGTAGATGGAGCCTACCAAGCACTCAACCTCACCAGTCCTGACAAAAACCCAAGAGTGGTGGTTGTGT ThrGlyAspArgLeuLeuAsnLeuValAspGlyAlaTyrGlnAlaLeuAsnLeuThrSerProAspLysThrGlnGluCysTrpLeuCys * * * * * * * * * Gln * * * * * * * * * Asn * * * * * * * * * *	1056
		119/
270	CTGGTATCGGGACCCCCCTACTACGAAGGGGTTGCCGTCCTAGGTACCTACTCCAACCATACCTCTGCCCCAGCTAACTGCTCCGTGGCC LeuValSerGlyProProTyrTyrGluGlyValAlaValLeuGlyThrTyrSerAsnHisThrSerAlaProAlaAsnCysSerValAla	1146
010		1287
300 346	TCCCAACAAGCTGACCCTGTCCGAAGTAACCGGACAGGGACTCTGCGTAGGAGCAGTTCCCAAAACCCATCAGGCCCTGTGTAATACC SerGlnHisLysLeuThrLeuSerGluValThrGlyGlnGlyLeuCysValGlyAlaValProLysThrHisGlnAlaLeuCysAsnThr * * * * * * * * * * * * * * * * * * *	1236
	······································	13//
330 376	ACCCAGAATACAAGCGACGGGTCCTACTATCTGGCTGCTCCCGGCGGGACCATTTGGGCTTGCAACACTGGGCTCACTCCCTGCCTATCT ThrGlnAsnThrSerAspGlySerTyrTyrLeuAlaAlaProAlaGlyThrIleTrpAlaCysAsnThrGlyLeuThrProCysLeuSer * LeuLysIleAspLys * * * * Val * * Thr * * Thr * * * * * * * * * * * *	1326
		1467
360	ACTACTGTACTCAACCTCACCGATTACTGTGTGTGTGTGAGCTCTGGCCAAAGGTGACCTACCACTCCCCTGGTTATGTTTATGGC ThrThrValLeuAsnLeuThrThrAspTyrCysValLeuValGluLeuTrpProLysValThrTyrHisSerProGlyTyrValTyrGly	1416
406	λla * * * * λrg * * * * * * * * * * * * * * λrg * * * * Pro * Ser * * * Ser	
	G.CC.,GTT.,GTC.,TC.,AT.AC.GCTC.T.,CACA	1557
390	CAGTTTGAGAGAAAAACCAAATATAAAAGAGAGCCGGTGTCATTAACTCTGGCCCTGCTGTTGGGAGGACTTACTATGGGCGGCATAGCT GInPheGluArgLysThrLysTyrLysArgGluProValSerLeuThrLeuAlaLeuLeuGlyGlyLeuThrMetGlyGlyIleAla	1506
436		1647
		104/
	GCAGGAGTAGGGACAGGAACTACCGCCCCCGGTCGCCACCAGCAGCTCCAGCAGCTCCATGCCGCGTACAAGATGATCTCAAAGAAGTC	1596
420	AlaGiyValGiyThrGiyThrThrAlaLeuValAlaThrGinGinPheGinGinLeuHisAlaXlaValGinAspAspLeuLysGiuVal	
466		
		1737
450	GANANGTCANTTACTANCCTAGANANGTCTCTTACTTCGTTGTCTGAGGTTGTGCTGCAGAATCGACGAGGCCTAGACCTGTTGTTCCTA	1686
496		
		1827
480 526	AAAGAAAGAGACTGTGTGCTGCCCTAAAAGAAGAAGAATGTTGTTGTTGTAGCCGATACAGGCCTAGTAAGAGATAGTATGGCCAAATTA LysGluArgGlyLeuCysAlaAlaLeuLysGluGluCysCysPheTyrAlaAspHisThrGlyLeuValArgAspSerMetAlaLysLeu * * Gly * * * * * * * * * * * * * * * * * * *	1776
	6	1917
510 556	AGAGAGAGACTCACTCAGAGACAAAAACTATTTGAGTCGAGCCAAGGATGGTTCGAAGGATTGTTAACAGATCCCCCTGGTTTACCACG ArgGluArgLeuThrGlnArgGlnLysLeuPheGluSerSerGlnGlyTrpPheGluGlyLeuPheAsnArgSerProTrpPheThrThr * * * * * * * * * * * * * * * * * * *	1866
		2007
540 586	TTAATATCCACCATCATGGGGGCCTCTCATTATACTCCTCATTCTAATTCTGCTTTTTGGACCCTGCATTCTTAATCGATTAGTTCAATTGTT LeuileSerThrileHetGlyProLeuileIleLeuLeuLeuIleLeuLeuPheGlyProCysileLeuAsnArgLeuValGinPheVal * * * * * * * * * * * * * * * * * * *	1956
	and plann — and plann —	2097
570 616	<pre></pre>	2046
		2187
	GATTTTATTTAGTTTCCAGAAAAAGGGGGGAATGAAAGACCCCACCAAGTTGCTTAGCCTGATAGCCGCAGTAACGCCATTTTGCAAGGC	2136 2277

FIG. 5. Nucleotide sequences and deduced amino acid sequences of the *env* genes of F-MCFV (pFM54B) and F-MuLV (pF-MuLV57) (20). The two nucleotide sequences were aligned to obtain maximal homology. Asterisks indicate identical amino acids in F-MuLV with respect to F-MCFV. Gaps have been introduced to allow optimal alignment. (A) 5' portions of the *env* genes and their products of F-MCFV and F-MuLV representing the differential region (see text). Stretches of identity (at least four identical amino acids) between the two *env* polypeptides are enclosed in boxes. (B) 3' portions of the *env* genes and carboxy-terminal portion of the *env* polypeptides of F-MCFV and F-MuLV representing the constant region (see text). Stretches of divergence (at least five different amino acids) between the two *env* polypeptides are enclosed in boxes.

begins with the related sequence NH₂-Val-Pro-His-Asp. The carboxy terminus of gp70 of F-MCFV has been located by analogy to F-MuLV (20).

The amino acid sequences of F-MCFV and F-MuLV are very different in the amino-terminal portion of gp70 up to position 238 (in F-MCFV) (Fig. 5A). We call the *env* gene sequence up to position 963 in F-MCFV the differential region, since only a few stretches of identity between F-MCFV and F-MuLV can be detected (boxes in Fig. 5A).

Starting at amino acid 239 in F-MCFV, the carboxyterminal regions of gp70 in F-MCFV and F-MuLV are very similar (Fig. 5B). In this constant region, only two stretches with significant differences (boxes in Fig. 5B) are present.

The *env* precursor polypeptides of F-MCFV, F-MuLV (20), and other mammalian retroviruses (3, 11, 22, 46; Wünsch et al., in press) contain stretches of uncharged, mostly nonpolar amino acids located in homologous regions. The apolar regions of F-MCFV are underlined in Fig. 2. Some of these regions could allow polypeptides to penetrate through or integrate in cellular and viral membranes according to the model proposed by Lenz et al. (22).

Possible role of the PR of gp70. The gp70's of all MCFVs and MuLVs examined to date and the gp52 of F-SFFV contain a region strongly enriched in proline residues (PR) at the carboxy terminus of the differential region. In Fig. 6, the PRs of two F-MCFVs, M-MCFV (3), F-SFFV (6, 58), F-MuLV (20), M-MuLV (46), and Akv (22) are compared. In these regions the relative amount of proline residues varies between 31 and 36% depending on the virus. MCFVs and F-SFFV have similar amino acid sequences in their PRs, whereas the ecotropic MuLVs have different sequences compared with the MCFVs and F-SFFV. The PRs of all ecotropic MuLVs include a stretch of about 35 amino acids where the gp70's of these viruses are remarkably different with respect to each other (20) (hypervariable region of ecotropic gp70's; see below). As discussed previously (20), the different amino acid sequences of the PRs of the gp70's of ecotropic MuLVs could allow these viruses to interact with specific receptors, thus defining the host range of these viruses.

The PR of clone pMo-MCF-16 (3) of the lymphatic leukemia-inducing M-MCFV and the PR of clone pFM1 of the erythroleukemia-inducing F-MCFV are nearly identical. Therefore, we can exclude the possibility that this region is involved in determining the histological type of disease caused by MCFVs. Rather, the PR might be responsible for the polytropic host range of the MCFVs. It could carry the sequences utilized by xenotropic and polytropic viruses for

 TABLE 1. Calculated molecular weights of proposed F-MCFV

 env polypeptides^a

ol wt of the opolypeptide
69,155
3,396
43,944 ^b
21,834
19,867

 a Clone pFM54B was taken for calculation; however, the sequence of 27 nucleotides which is deleted in the PR of this clone was added before calculation.

^b Since some amino acids might be cleaved off the carboxy terminus of gp70, the actual number might be slightly smaller.

Comparison of the amino acid sequences of env polypeptides of different viruses. Figure 7 shows a comparison of the amino acid sequences of the env polypeptides of F-MCFV (pFM54B, pFM1, and pFM2), M-MCFV (3), F-SFFV (6, 58) and F-MuLV (20). The sequences of the env polypeptides of the MCFVs and F-SFFV have a high degree of similarity. As described above, the sequence of the F-MuLV env polypeptide ranging from the leader peptide to the carboxy terminus of the PR is dissimilar with respect to the MCFV and F-SFFV sequence (differential region; Fig. 5A). F-MuLV and these other viruses have very similar polypeptide sequences from the carboxy terminus of the PR region up to the end of p15^{env} (constant region; Fig. 5B). At the carboxy terminus, the $p15^{env}$ -related sequence of F-SFFV gp52 is 33 amino acids shorter than the $p15^{env}$ of F-MCFV, M-MCFV, and F-MuLV. The carboxy-terminal six amino acids of gp52 are different with respect to the p15^{env} of the other viruses, which have a common sequence in this region.

Localization of possible glycosylation sites. Asn-X-Thr/Ser sequences are known to be possible sites for glycosylation of retroviral polypeptides (13, 29). Glycosylation sites within the env gene products are highly conserved within different host range classes of mammalian retroviruses (3, 11, 20, 22, 46; Wünsch et al., in press). This argues for a strong selective pressure in favor of their conservation. The gp70's of F-MCFV, M-MCFV, and F-MuLV carry six potential glycosylation sites in homologous positions (boxes in Fig. 7). The three amino-terminal sites are also contained in gp52 of F-SFFV, whereas the three carboxy-terminal sites are deleted in F-SFFV. F-MCFV has one additional possible glycosylation site (at amino acid 26) shared by F-MCFV and F-SFFV and one site (at amino acid 332) not found in M-MCFV or F-SFFV. F-MuLV has two additional sites (at amino acids 168 and 266) not found in these other viruses. Whether all of the potential sites in the gp70's and gp52 are glycosylated is unknown at present. The sequence Asn-Arg-Ser in p15^{env} of the env polypeptides of murine retroviruses is not glycosylated (28, 37, 57).

General structure of the *env* gene. Comparison of murine retrovirus *env* genes and their products shows that they are composed of clearly distinguishable regions (Fig. 5A, 5B, and 7). The sequence between the amino terminus of the leader peptide and the end of the PR is highly conserved within the polytropic group of viruses and within the ecotropic viruses but very different between polytropic and ecotropic viruses (differential region). The remainder of the *env* polypeptide is composed of a region which is very similar between polytropic and ecotropic viruses (constant region). A comprehensive view of the elements of the *env* polypeptide is given in Fig. 8.

A few sections of the differential region are homologous between the ecotropic and polytropic viruses (boxes in Fig. 5A, dotted areas in Fig. 8). The constant regions contain two blocks with variable character (boxes in Fig. 5B, white areas in Fig. 8).

As described above, the PR is located at the border of the differential and constant regions. In the *env* polypeptides of all ecotropic mouse viruses studied so far, the PR is the only larger segment with considerable variability. The total *env* polypeptide seems, therefore, to consist of the following elements: differential region (without PR), PR, and constant region. The structure of the *env* polypeptide is reminiscent of the structure of the immunoglobulins which consist of sepa-



FIG. 6. PRs in the gp70's of F-MCFV (clones pFM1 and pFM54B), M-MCFV (3), F-MuLV (20), M-MuLV (46) and Akv (22) and in the gp52 of F-SFFV (6, 58). The standard one-letter amino acid code is used. Dots indicate identical amino acids with respect to F-MCFV clone pFM1. Proline residues are underlined. Gaps have been introduced to allow optimal alignment. The numbering of the amino acids of the individual sequences refers to their positions in gp70 (or gp52).

rate genetic elements: variable region, PR (hinge), and constant region (17, 55).

It has been suggested recently (Wünsch et al., in press) that the constant portions of gp70 and $p15^{env}$ of mammalian retroviruses are two separate genetic elements which together with the N-terminus of the differential region can be traced to a common short primordial *env*-related gene. We have proposed that the present day *env* gene has been generated by at least two tandem duplications of this primordial *env*-related genetic element and by additional recombinational exchanges and mutations.

Possible functions of the elements of the *env* gene and its products. Comparison of the putative points of recombination which have led to the generation of MCFVs shows that the differential region is the part of the *env* gene in all MCFVs which is replaced by endogenous sequences. In some viruses, e.g., clone pFM1/pFM2 of F-MCFV, little more than the differential region has been substituted. We therefore assume that the differential region is the part of the *env* gene which determines the ecotropic or polytropic host range of the virus. As described above, the PR may contain determinants for this function.

Various parts of the constant region have been replaced by endogenous sequences in the *env* genes of the MCFVs analyzed so far. These endogenous sequences are very similar to their ecotropic partners: pFM1 carries only three and pMo-MCF₁-16 carries only four varying amino acids in this part of the constant region. Thus, we can assume that the constant regions of ecotropic and polytropic viruses carry domains for those functions which are common to all viruses, e.g., determinants for infectivity and structural properties necessary for interaction with cellular membranes.

Do MCFVs contain disease-specific sequences in their env polypeptide? Since MCFVs induce the same type of disease as their ecotropic progenitor viruses and since MCFVs are believed to be the ultimate cause of these diseases, we tried to identify sequences which are characteristic for particular histological types of leukemia. It has been shown previously (34) that a 2.4-kilobase-pair fragment of F-MuLV encompassing about 700 bp of the *pol* gene and almost the entire *env* gene contains sequences which contribute to erythroleukemia. Since the *pol* gene is not a likely candidate for determining disease specificity (34), we restricted our analysis to the *env* gene (Fig. 7 and Table 2).

We compared all of the available data on amino acid sequences of the *env* polypeptides of murine retroviruses which cause either lymphatic leukemia or erythroleukemia.

Desian	¥2	D.C	Amino acids at the following positions"										
Region	virus	Kelerence	27	139–141	181	200-201	253	282	514	523	550	553	
Differential	F-MCFV clone pFM54B	This paper	Α	GIQ	Т	I P							
	F-MCFV clone pFM1/2	This paper	Α	DIK	Т	VΡ							
	F-SFFV	6,58	Α	GVL	Т	VΡ							
	F-SFFV	1	Α	GVQ	Ι	ΙP							
	M-MCFV	3	v	NIK	Ι	V S							
	MCF247	C. Holland ^b	Α	DIK	I	VP							
Constant	F-MCFV clone pFM54B	This paper					0	v	Т	S	I	L	
	F-MCFV clone pFM1/2	This paper					ò	v					
	F-SFFV	6,58					ò	v	Т	S	I	L	
	F-SFFV	1					Q	v	Т	S	Ι	L	
	M-MCFV	3					Ŕ	Ι	Ν	Т	v	Μ	
	MCF247	C. Holland ^b					Q	v	S	0	Ι	L	
	F-MuLV	20					Q	v	Т	ŝ	I	L	
	M-MuLV	46					ò	v	Ν	Т	v	Μ	
	Akv	22					ò	v	S	0	Ι	L	

TABLE 2. Comparison of amino acids at selected sites of different viruses

^a Positions refer to Fig. 7.

^b Personal communication.

	differential reg	ion		
	- Leader .	• • • • 90 70	(gp52)	•
1 F-NCFV pFN54B	MEGPAFSKPLKDKINPWG	PLIVLGILIRAGVSVPHDSPHQ	VENVTWRVTNLMTGQTANATSLI	LGTMTDAF
2 pFN1/pFN2	.ACST			
3 N-NCFV				
4 F-SFFV	ST	I		E
5 F-MULV	ACSTLP.SPD.RDLI	LT LFLS.KG.RSAA.GS.	Y. L. F. GDRE VW I GI	
	• •			
2		DWDEIGLGCKIFG	SARARIED FIVE	
7		••••••	••••••••	· · · · A · · ·
· · · · · · · · · · · · · · · · · · ·		•••••••••	•••••	• • • • • • • • •
4 · · · · · · · · · · · Π · · · · · · ·		•••••••••••		••••••
5 .V.IP	HWGLETQAPTSSPPGPPCCS	GSSGSSAGCSR.C.PLISLIPRC	NTAWN.LKLDQVTHKSSEG	SHR.R 1
•	• •	• • •	• •	•
7 G CGGPREGYCGKW	GCETTGQAYWKPSSSWDLIS	LKRGNTPRNQGPCYDSSAVSSGIQ	GATPGGRCNPLVLEFTDAGKKA	SWDGPKV 1
2	•••••L•••••	QD.K	· • • • • • • • • • • • • • • • • • • •	1
3		N.K	, 	1
4		<u></u> .KDVL		A 1
5 EAKSDSFAS.		VDNNL.T .QQVCKD	NKWAIQNQV	TTTGHY 2
•	• •	• • •	end differential re	aion —
1 WGLRLYRSTGTDPVTR	FSLTRQVLNIGPRIPIGPNP	VITDQLPPSRPVQIMLPRPPQ	PSPTGAASI	GP 2
2	R		P P VPE	
7	2V	••••••	P P VPC	TAPPSO 2
/	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	т і тр і		TAPPDO 2
5 V D CIT				1AFFF4 2
JVKULI	. GIKLKIQ.LV	.LASLFNFK.AKSFF	ASPIS PILISPIUPP	PA Z
1 GTGDRLLNLVDGAYQA 2	LNLTSPDKTQECWLCLVSGPI 	PYYEGVAVLGTYS <mark>ŇHT</mark> SAPA <mark>NCS</mark> V 	ASQHKLTLSEVTGQGLCVGAVP	KTHQALON 3
			end an70 n15env/	12004
	GTIMACNTGL TPCI STTVI	TTTDYCVI VEI WPKVTYHSPCYVY	GREERKTKYKREPVSI TI ALLI	GGI TMCCT A
			C KCYDU	30CTRI001 4
	м с т т	P C		····· 4
/		•••••••••••••••••••••••••••••••••••••••		••••••
			C	,
<u></u> LKIDKVI	· · · · · · · · · · · · · · · · · · ·	<u>к.</u> к	5KSTKH	••••• 4
•	• •	• • •	• •	• _
7 AAGVGTGTTALVATQQ	FQQLHAAVQDDLKEVEKSITI	NLEKSLTSLSEVVLQNRRGLDLLF	LKERGLCAALKEECCFYADHTG	LVRDŞMAK 5
2	end pFN1			4
3IH	QRS		G	5
6			K	3
5			G	5
			end constant re	gion
•	• •	• •	end plaenv - end pl	Senv -
1 LRERLTOROKI FESSO	GWFEGLENRSPWETTLISTI	MGPLIILL LILLEGPCILNRIV	OF VKDRISVVQAL VI TOOVHOL	KPLEYEP* 5
2	and a sector of a sector of a			
- 7 N T	1 1	V M		1 + 4
σπ		····▼·· ⊓······························	and an 52	
•			ena gpJZ	3
J			· • • • • • • • • • • • • • • • • • • •	* 6

FIG. 7. Comparison of the deduced amino acid sequences of the *env* polypeptides of F-MCFV (clones pFM54B, pFM1, and pFM2), M-MCFV (3), F-MuLV (20), and the *env*-related polypeptide of F-SFFV (6, 58). The standard one-letter amino acid code is used. Sequences are aligned to obtain maximal identity. Comparison of nucleotide sequences helped find proper alignment in some parts. Gaps have been introduced to allow optimal alignment. Dots indicate identical amino acids with respect to F-MCFV (pFM54B). Possible glycosylation sites are enclosed in boxes.

The following differences can be found among the clones listed in Table 2 (differences between the F-MCFV clones pFM1/pFM2 and pFM54B were regarded as not significant and not taken into consideration; positions refer to pFM54B of Fig. 7): positions 27, 139, 141, 181, 201, 253, 282, 292 to 486, 514, 523, 550, 553, and 591. The changes at positions 27, 139, 141, 181, 201, 253, 282, 514, 523, 550, 553, and 591 occur in one or more of the lymphatic leukemia viruses (M-MuLV, M-MCFV, Akv, or AKR MCF247) and in one or more of the

erythroleukemia viruses (F-MuLV, F-MCFV, or SFFV). Since these changes occur in both types of viruses, they cannot specify the type of disease produced by these agents. Changes which lie between positions 292 and 486 fall into the region of the F-MCFV genome which is deleted from the SFFV genome.

These comparisons show that there is no site in the amino acid sequence of the env polypeptide of different MCFVs which clearly determines their disease specificity. We there-



FIG. 8. General structure of *env* polypeptides of MCFVs, MuLVs, and F-SFFV. Differential region, PR, and constant region are explained in the text. Arrows indicate the locations of the putative recombination sites involved in generation of MCFVs. Dotted areas represent stretches of identical amino acids in F-MCFV and F-MuLV. White boxes are stretches of differing amino acids. Also indicated is the major deletion in F-SFFV. The length of the polypeptide is taken from F-MCFV(pFM54B).

fore must assume that the *env* gene of a given MCFV cannot contribute to different histological types of disease and that other sequences in the MCFV genome are responsible for determining what type of disease a particular virus will induce.

We localized several nucleotide positions in the LTRs of different viruses which seem to be characteristic of either lymphatic leukemia- or erythroleukemia-inducing viruses (see above). However, additional biological tests with in vivo- or in vitro-generated recombinant viruses will be necessary to determine the exact role these regions play in determining pathogenicity and disease specificity.

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