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Envelope Gene and Long Terminal Repeat Determine the Different Biological Properties of Rauscher, Friend, and Moloney Mink Cell Focus-Inducing Viruses

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The nucleotide sequence of the envelope (*env*) gene and the long terminal repeat (LTR) of an infectious clone of Rauscher mink cell focus-inducing (R-MCF) virus has been determined and compared with the published *env* gene and LTR sequences of Friend (F)- and Moloney (M)-MCF viruses. The sequence shows that R-MCF virus, like other MCF viruses, is a recombinant virus. Its *env* gene contains sequences which were acquired from an *env* gene in the mouse genome and which confer on the MCF virus its dualtropic host range. Unlike F-MCF and M-MCF viruses, R-MCF virus will not replicate in NIH 3T3 cells. The deduced amino acid sequence for the gp70 of R-MCF differs from that of F- and M-MCF viruses by 15 amino acids between residues 49 and 138 of gp70. These differences in amino acid sequences may be responsible for the inability of R-MCF virus to replicate in NIH 3T3 cells. The host range of two hybrid viruses constructed in vitro is consistent with this hypothesis. R-MCF virus and Friend murine leukemia virus (F-MLV) show 98% identity in their *env* gene 3′ from the acquired *env* sequences. This contrasts with 82% identity between the *env* gene of R-MCF virus and M-MLV. The LTR of R-MCF shows 98% identity with the LTR of F-MCF as compared to 88% identity with the LTR of M-MCF. This striking similarity between the sequences of R-MCF, F-MCF, and F-MLV is surprising since the Rauscher virus and the Friend virus are thought to have originated independently. The high degree of similarity suggests that Rauscher and Friend viruses have a common origin. In contrast to M-MLV, which induces predominantly a lymphoid disease, R- and F-MCF viruses induce an erythroproliferative disease in NIH Swiss mice. A hybrid R-MCF virus with a genome derived primarily from R-MCF virus and a 3′ end including the U3 region derived from M-MLV induces a lymphoid disease instead of an erythroid disease. This result indicates that it is the U3 region which determines the tissue specificity of the MCF virus-induced disease. It is suggested that the putative viral enhancers in the U3 region play two roles in the process of leukemogenesis: in the Friend and Rauscher disease, the viral enhancers act by increasing the transcription of the MCF *env* gene; in the thymic lymphoma, the enhancers activate mainly the expression of cellular genes.

Rauscher virus (RV) induces, in mice, a disease very similar to that induced by the anemia-inducing Friend virus (FV-A). Both viruses cause an erythroproliferative disease which leads to hepatosplenomegaly accompanied by a severe anemia. The two viruses were isolated independently, RV by Rauscher in 1962 (29) and FV-A by Friend in 1957 (13). Both viruses are in fact mixtures of at least two viruses, Rauscher (R)- or Friend murine leukemia virus (F-MLV), respectively, and a defective virus, the so-called spleen focus-forming virus, R- or F-SFFV, respectively. Both viruses are required for induction of the Rauscher or Friend disease in adult mice. In some virus stocks a mink cell focus-inducing virus, R- or F-MCF, is also present. This virus is replication competent and dualtropic and induces cytopathic foci in mink lung cells.

When injected into newborn NIH mice alone, both R- and F-MLV induce an erythroproliferative disease similar to that induced by RV or FV-A complexes (36, 40). The induction of the disease is correlated with the generation of MCF virus (17, 36, 40). MCF viruses contain *env* gene sequences which are acquired from an endogenous *env* gene and which confer on the MCF virus its dualtropic host range. These endogenous sequences are also present in the *env* gene of SFFV. They are thought to play a role in the induction of disease by both SFFV and MCF viruses. Strong evidence in favor of this conclusion is available. (i) Mutations in the *env* gene of SFFV lead to nonleukemogenic mutants (22, 31). (ii) Experiments with subgenomic fragments of F-SFFV DNA indicate that a fragment containing the *env* gene of F-SFFV is necessary for the induction of disease (21). (iii) Results with hybrid viruses in which different regions of the genome of the nonleukemogenic amphotropic virus 4070 were replaced by homologous regions of F-MCF virus show that, in addition to the long terminal repeat (LTR), the *env* gene of F-MCF virus is required to obtain a pathogenic virus (28).

Most MCF viruses are not leukemogenic by themselves but are leukemogenic as pseudotypes, i.e., as MCF genomes in envelopes provided by helper viruses. Since the titer of MCF virus reached in infected mice is very low (30), the injection of pseudotypes of MCF virus may lead to an increase in the virus titer in the animal to a level required to induce a disease (28, 30). Several MCF viruses have been isolated which do not require an injection as pseudotype to be oncogenic (7, 17, 38). The reason for this difference between the two classes of MCF viruses is unknown. It is possible that the two classes of MCF viruses differ in the rate with which they spread in the animal and that the oncogenic ones are able to infect a sufficient number of cells to cause disease. The existence of MCF viruses which are leukemo-

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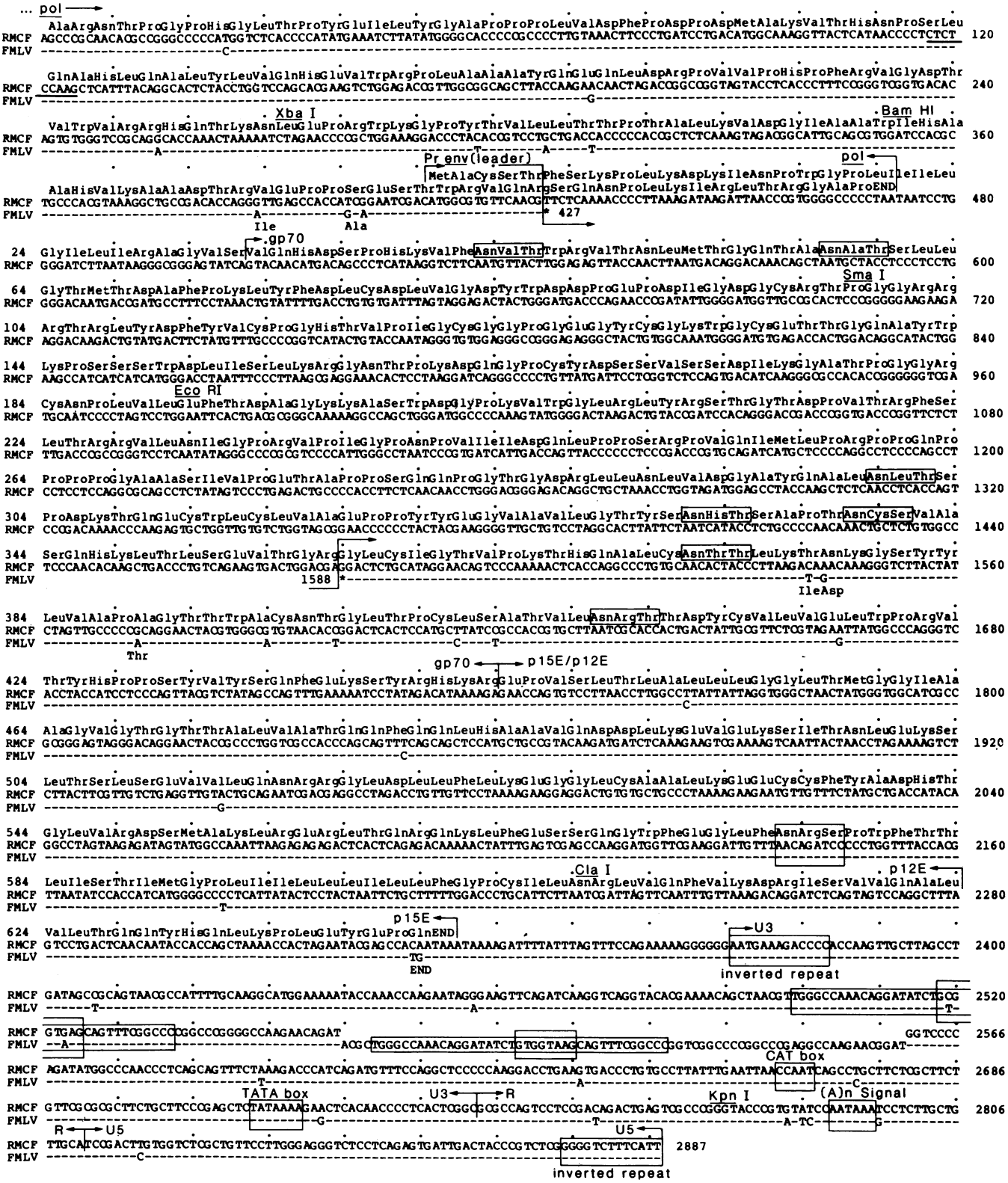

FIG. 1. Nucleotide sequence of the *env* gene and of the LTR of R-MCF26 DNA. The deduced amino acid sequence is given above the nucleotide sequence. Differing nucleotides and amino acids of F-MLV57 DNA (18, 27) are shown below the nucleotide sequence. Bold line, Splice acceptor site; zigzag arrows, beginning and end of acquired *env* sequences; open boxes in *env* gene, potential glycosylation sites; unlabeled open boxes in U3 region, direct repeats with core sequences.

genic by themselves confirms the conclusion that MCF viruses are oncogenic.

Recently, the nucleotide sequences of the *env* gene and the LTR of several cloned F-MCF viruses have been published (1, 19). In this paper we compare the published *env* and LTR sequences of F-MCF viruses with the analogous sequences of an infectious molecular clone of R-MCF virus (clone pR-MCF26) recently isolated by us. This comparison reveals a striking similarity between the *env* and LTR sequences of F- and R-MCF virus.

In contrast to the erythroproliferative disease induced by RV and Friend virus, Moloney (M)-MLV and M-MCF viruses cause a lymphoid disease most often characterized by a thymic lymphoma. Chatis et al. (5, 6) have recently constructed hybrid F-MLVs which induce a lymphoid disease instead of an erythroid disease. The genomes of these hybrid F-MLVs were primarily derived from F-MLV but contained sequences of M-MLV which included the U3 region at their 3′ end. Conversely, the introduction of the U3 region of F-MLV into the M-MLV genome led to a hybrid virus which caused an erythroproliferative disease. These results show the important role that the U3 region plays in determining the tissue specificity of the disease. The induction of disease by both F-MLV and M-MLV is correlated with the generation of MCF virus. MCF virus generated in lymphoid cells by M-MLV may differ from MCF virus generated in erythroid cells by R- or F-MLV. Therefore, it seemed of interest to test whether R-MCF virus generated in erythroid cells was able to induce a disease in lymphoid cells with a U3 region of M-MLV. The biological properties of two hybrid viruses in which a portion of the *env* gene or sequences encompassing the U3 region of R-MCF virus were substituted by sequences of M-MCF or M-MLV are described here.

MATERIALS AND METHODS

Cells and viruses. The source of NIH 3T3 and mink lung cells (line Mv 1Lu [NBL-7]) has been described previously (38). Biologically cloned R-MCF-1 (38) was used as a source for the molecular cloning. R-MCF-1 is derived from an RV complex that had been passaged in vivo for over 10 years (25).

Molecular cloning of R-MCF-1 virus. Unintegrated circular viral DNA, isolated by the Hirt method (16) from mink lung cells infected with R-MCF-1 virus, was linearized by digestion with restriction enzyme *Eco*RI and ligated to *Eco*RI-cleaved Charon 30 phage DNA. Plaques obtained from the packaged ligation mixture were screened with an M-MCF *env* probe and amplified as described by Blattner et al. (3). The R-MCF-1 insert was subcloned in the *Eco*RI site of pBR322 by standard techniques. The resulting recombinant plasmid, designated pR-MCF26, contains an infectious permuted R-MCF viral DNA insert.

Cloned viral DNAs. Plasmid pM-MCF$_1$-1 contains a hybrid Moloney mink cell focus-forming provirus (4). It was generated in vitro by ligating the cloned 5′ half of BALB/M-MLV DNA (pMLV$_1$-1) to the cloned 3′ half of BALB/M-MCF DNA (pM-MCF$_1$-16). Plasmid pLTR3 contains the 3′ M-MLV LTR and 140 nucleotides upstream of the LTR. It was kindly given to us by Rolf Muller and has been described by Muller and Muller (24). Both plasmid DNAs were used to construct recombinant viral genomes between R-MCF and M-MCF or M-MLV. Plasmid pRV-1 DNA contains an R-MLV provirus subcloned in the *Eco*RI site of pBR322 (15). It was generously provided by E. P. Reddy.

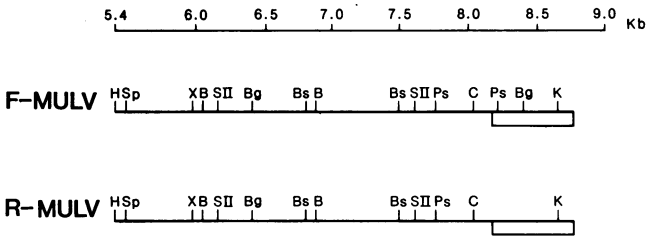

FIG. 2. Restriction map of F-MLV DNA, as deduced from the published nucleotide sequence of F-MLV57 (18), and revised restriction map of R-MLV, clone RV-1 (15). B, *Bam*HI; Bg, *Bgl*I; Bs, *Bst*EII; C, *Cla*I; H, *Hin*dIII; K, *Kpn*I; Ps, *Pst*I; SII, *Sst*II; Sp, *Sph*I; X, *Xba*I.

Cloned recombinant viral DNAs. Plasmid pRM-1 contains a recombinant R-MCF DNA in which an *Eco*RI-*Cla*I fragment (extending from nucleotide 980 to nucleotide 2232 in Fig. 1) was removed from the *env* gene and replaced by the corresponding fragment of M-MCF DNA (see Fig. 4). Plasmid pRM-4 contains a recombinant R-MCF DNA in which the *Cla*I-*Kpn*I fragment at the 3′ end of the viral genome (extending from nucleotide 2233 to nucleotide 2779) was substituted by the corresponding fragment of M-MLV DNA (see Fig. 4). This fragment, which contains all of the U3 region, extends by 99 nucleotides into the carboxy terminus of the *env* gene and by 36 nucleotides into the R region of the LTR.

Transfection of viral DNAs. Transfection of DNA on mink lung cells was carried out by Stow and Wilkie's modification (35) of the calcium phosphate coprecipitation technique of Graham and van der Eb (14). The assay for reverse transcriptase activity was done as described previously (38).

Nucleotide sequence analysis. All DNA sequence analyses were performed by the partial chemical degradation procedure of Maxam and Gilbert (23). An outline of the techniques used has been described previously (37).

RESULTS

Biological properties and molecular cloning of R-MCF-1 virus. R-MCF-1 virus (38) was isolated from the serum of leukemic SJL/J mice infected with an RV stock which had been passaged in vivo for over 10 years (25). R-MCF-1 virus induces an erythroproliferative disease when injected into newborn NIH Swiss mice in the absence of helper virus (38). This property distinguishes it from helper-requiring R-MCF viruses isolated from splenic tumors induced by the injection of R-MLV (unpublished data). Another property of R-MCF-1 virus not shared by most MCF viruses is its inability to replicate in NIH 3T3 cells.

R-MCF-1 DNA was molecularly cloned in the *Eco*RI site of pBR322 as described in Materials and Methods. pR-MCF26 contains an infectious, permuted R-MCF-1 viral DNA with a single LTR. pR-MCF26 was used for all sequence analyses.

Sequence analysis of the *env* gene and the 3′ LTR of R-MCF-1 virus. A region extending from the *Hin*dIII site in the polymerase (*pol*) gene to the 3′ end of the LTR was chosen for the sequence analysis of pR-MCF26. The nucleotide sequence was determined according to the method of Maxam and Gilbert (23). Figure 1 shows the nucleotide sequence of the 3′-terminal portion of the *pol* gene, the total *env* gene, and the LTR of pR-MCF26. The deduced amino acid sequence is indicated above the nucleic acid sequence. The nucleotide sequence predicts an *env* gene product of 640

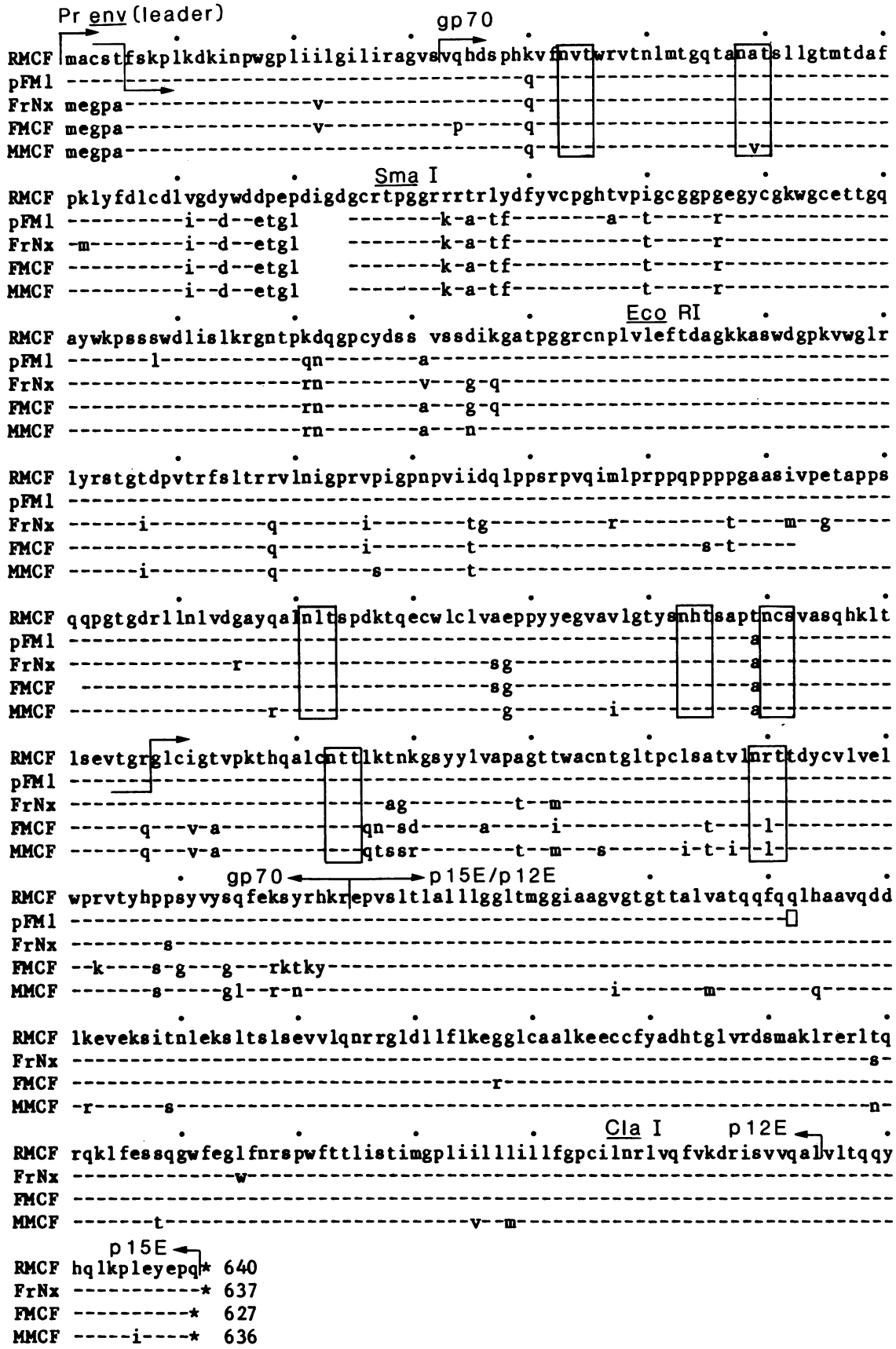

FIG. 3. Deduced amino acid sequences of the *env* genes of R-MCF26, pFM1 (19), MCF-FrNx (1), F-MCF54B (19, 26), and M-MCF-1 (4). Potential glycosylation sites are enclosed in boxes. Zigzag arrows, Beginning and end of acquired *env* sequences of R-MCF-1 and FM1 virus.

amino acids with a leader sequence of 32 amino acids and a gp70 of 411 amino acids.

Like other MCF viruses, R-MCF virus contains sequences in its *env* gene which are acquired from an *env* gene endogenous to the mouse. To determine the junction points between the acquired and parental *env* sequences in R-MCF virus, the nucleotide sequence of R-MCF DNA was compared with the published *env* sequence of F-MLV DNA (18). This was necessary since no sequence data for R-MLV, the putative parent of R-MCF virus, were available. However, this comparison is appropriate, since the restriction maps of the 3′ halves of F-MLV and R-MLV DNA were found to be identical except for the presence of two additional restriction sites in the LTR of F-MLV DNA (Fig. 2). Comparison of the sequence of R-MCF virus with the sequence of F-MLV suggests that the acquired *env* sequences begin 15 base pairs (bp) into the *env* gene of R-MCF virus, at nucleotide 427, and end near nucleotide 1479 of R-MCF DNA (or nucleotide 1588 of F-MLV DNA in Fig. 1).

The LTR of R-MCF virus consists of 517 bp in contrast to the 591 bp of F-MLV. As often in MCF viruses, the U3 region of R-MCF virus contains sequences homologous to the 5′ half but misses sequences homologous to the 3′ half of the direct repeat.

In vitro host range of two hybrid R-MCF viruses. A property of R-MCF virus not shared by F- and M-MCF viruses is its inability to replicate in NIH 3T3 cells. Between residues 49 and 138, the deduced amino acid sequence of the gp70 of R-MCF virus differs at 15 positions from the deduced gp70 sequences of F- and M-MCF viruses (Fig. 3). These differences may be responsible for the inability of R-MCF virus to replicate in NIH 3T3 cells. The host range of two hybrid viruses is consistent with this hypothesis: both hybrid viruses were constructed to test their in vivo pathogenicity. RM-1 virus is a hybrid R-MCF virus in which nucleotides 980 to 2232 of the *env* gene of R-MCF virus were replaced by the corresponding region of M-MCF virus (Fig. 4). This region codes for two-thirds of gp70, residues 158 to 411, and for all except the C-terminal 16 residues of p12E. RM-4 virus is a hybrid R-MCF virus in which the 3′ end of the viral genome was replaced by the corresponding region of M-MLV (Fig. 4). The exchanged region which contains all of the U3 region extends by 99 nucleotides into the carboxy terminus of the *env* gene and by 36 nucleotides into the R region of the LTR. RM-1 and RM-4 viruses do not replicate in NIH 3T3 cells (Table 1). Both hybrid viruses contain the *env* sequences by which R-MCF differs from F- and M-MCF viruses.

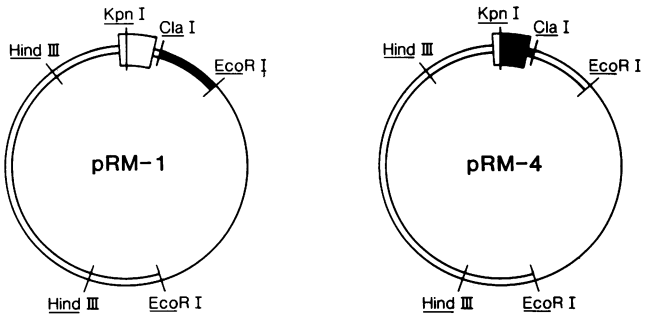

FIG. 4. Schematic representation of two recombinant plasmids. In pRM-1, an *Eco*RI-*Cla*I fragment of R-MCF DNA has been replaced by the analogous fragment of M-MCF DNA. This region codes for two-thirds of gp70, residues 158 to 411, and for all, except the C-terminal 16 residues, of p12E. In pRM-4, a *Cla*I-*Kpn*I fragment of R-MCF DNA has been replaced by the analogous fragment of M-MLV DNA. This fragment includes all of the U3 region and extends by 99 nucleotides into the carboxy terminus of the *env* gene and by 36 nucleotides into the R region of the LTR. Boxes, LTR; single line, pBR322; double line, R-MCF; thick line, M-MCF; closed box, M-MLV.

TABLE 1. In vitro host range of hybrid viruses[a]

Virus	Reverse transcriptase activity (pmol/ml) in[b]:	
	NIH 3T3 cells	Mink lung cells
R-MCF[c]	<1	66.7
RM-1[d]	<1	60.0
RM-4[e]	<1	70.3
M-MCF[f]	62.5	60.2

[a] The host range of each virus was determined by a reverse transcriptase assay. Cultures of both NIH 3T3 and mink lung cells were infected with filtered 24-h harvests of mink lung cells producing MCF or hybrid virus. The infected cultures were transferred at 10^5 cells per 5-cm dish when the cells had grown to confluence. Three weeks after the original infection, reverse transcriptase assays were made on 24-h-old culture media as described previously (38). The values represent the averages of four plates.

[b] [^{3}H]dGTP (picomoles) incorporated in 1 h per 1.0 ml of medium (1 pmol = 1,500 to 2,000 cpm).

[c] R-MCF, Molecular clone R-MCF26.

[d] RM-1, R-MCF/M-MCF *env* (*Eco*RI-*Cla*I).

[e] RM-4, R-MCF/M-MCF LTR (*Cla*I-*Kpn*I).

[f] M-MCF, Molecular clone M-MCF-1.

In vivo pathogenicity of two hybrid R-MCF viruses. RM-1 and RM-4 viruses were tested for their in vivo pathogenicity by inoculation into newborn NIH Swiss mice. Within 6 months, 56% of the mice inoculated with RM-1 virus developed an erythroproliferative disease similar to that induced by R-MCF virus; the mice had enlarged livers and spleens infiltrated with erythroblasts, large numbers of erythroid precursor cells in the peripheral blood, and a severe anemia. Thymus and lymph nodes were of normal size. No significant difference in the pathogenicity of R-MCF and RM-1 viruses was observed (Table 2). This result shows that the substitution of two-thirds of the *env* gene sequences of R-MCF virus by the corresponding *env* gene sequences of M-MCF virus does not affect the ability of the virus to infect and transform cells of the erythroid lineage in vivo.

The in vivo pathogenicity of RM-4 virus is shown in Table 3. Of 40 animals injected, none developed an erythroid disease. Three animals had a thymic lymphoma; two of the three animals had, in addition, enlarged lymph nodes and a slightly enlarged spleen. A fourth animal had enlarged lymph nodes and a slightly enlarged spleen. (Due to the early stage of the disease and the high background of normal T and B cells in the spleen, the lineage of the blast cells in the spleen could not be ascertained.) The low incidence (10%) of diseased animals after inoculation of RM-4 virus is in sharp contrast with the incidence of >50% after inoculation of R-MCF or RM-1 virus (Table 2). It is of interest that a similar low incidence of thymic lymphoma (3 of 35) was previously observed after inoculation of M-MCF virus into newborn NIH mice (39). The induction of thymic lymphoma in 3 of 40 mice inoculated with RM-4 virus suggests that the U3 region derived from M-MLV is responsible for the change in the disease specificity.

The thymotropism of RM-4 virus is also suggested by the following experiment. Virus produced by thymocytes of mice 7 weeks after virus inoculation was quantified by a reverse transcriptase assay. The thymocytes were cocultured with mink lung cells for five days, after which reverse

TABLE 2. Pathogenicity of RM-1 virus[a]

Virus	Disease incidence		Latency (wk)		Spleen (g)		Liver (g)		Hematocrit (%)	
	Erythroid	Lymphoid	Avg	Range	Avg	Range	Avg	Range	Avg	Range
R-MCF[b]	18/34	0/34	15.3	10–24	1.3	0.7–1.8	2.5	1.2–3.4	14.4	9–21
RM-1[c]	20/36	0/36	20.2	13–26	1.2	0.5–2.0	3.4	1.6–5.7	15.4	7–28
Control	0/37	0/37			0.1	0.08–0.13	1.2	1.1–1.4	48.0	45–52

[a] NIH Swiss mice were inoculated intraperitoneally 24 to 48 h after birth with 0.1 ml of filtered or centrifuged 24-h harvests from virus-producing mink lung cells. The mice were sacrificed at an advanced stage of disease. Latency of the disease, weight of spleen and liver, and hematocrits of the diseased animals are shown.

[b] R-MCF, Molecular clone 26.

[c] RM-1, R-MCF/M-MCF *env* (*Eco*RI-*Cla*I).

transcriptase activity was assayed in the medium. The observed reverse transcriptase activity is a function of the number of infected thymocytes plated, the virus yield per cell, and the spread of the virus in the mink lung cells. The reverse transcriptase activity in thymocyte cultures from mice inoculated with RM-4 virus (69.4 ± 30.1 pmol/ml) was approximately 10 times higher than that obtained from R-MCF-1-inoculated mice (5.5 ± 4.1 pmol/ml). This result is consistent with the conclusion drawn from the in vivo pathogenicity tests.

DISCUSSION

Sequence analysis of the *env* gene of R-MCF-1 virus. The nucleotide sequence of the *env* gene and the LTR of a molecular clone of R-MCF-1 virus has been determined. The sequence predicts an *env* gene product of 640 amino acids with a leader sequence of 32 amino acids and a gp70 of 411 amino acids. Like other MCF viruses, R-MCF virus has in its *env* gene acquired sequences derived from an *env* gene in the mouse genome. The putative junction points between the acquired and parental sequences (nucleotides 427 and 1479 in Fig. 1) are very similar, if not identical, to those of the F-MCF clone FM1, isolated from a Friend virus-infected cell line (32) and sequenced by Koch et al. (19). Downstream from the acquired sequences, the *env* sequence of R-MCF DNA differs by only 14 (out of 891) bp from the *env* sequence of F-MLV (Fig. 1). Except for two nucleotide differences which lead to a change in the terminal amino acid, none of the remaining 12 nucleotide differences give rise to a change in the protein sequence. It is of interest that 10 of the differing nucleotides of R-MCF DNA are identical to the corresponding nucleotides in FM1 DNA. The four remaining nucleotides fall outside of the available sequence of FM1 virus. In summary, 5′ and 3′ from the acquired *env* sequences, the *env* genes of R-MCF-1 and FM1 viruses have a sequence identity of 99% and the *env* genes of R-MCF-1 and F-MLV have a sequence identity of 98%. A similar comparison (not shown) of the *env* gene sequence of R-MCF-1 virus and M-MLV (33) shows an identity of only 82%.

TABLE 3. Pathogenicity of RM-4 virus[a]

Mouse no.	Latency (wk)	Thymic lymphoma (g)	Lymph nodes[b]	Spleen wt (g)	Liver wt (g)	Hematocrit (%)
628-1	12	1.2	−	0.1	0.9	43
630-1	14	0.5	+	0.5	2.5	ND[c]
627-2	16		+	0.4	1.2	35
627-3	26	0.4	+	0.6	4.5	27

[a] Of 40 NIH Swiss mice inoculated 24 to 48 h after birth with RM-4 virus, 4 mice developed a leukemic disease within 26 weeks.

[b] +, Enlarged lymph nodes; −, lymph nodes of normal size.

[c] ND, Not determined.

Comparison of the deduced amino acid sequences of the PrENV proteins of R-, F-, and M-MCF viruses. In Fig. 3 the deduced amino acid sequence of the PrENV protein of R-MCF-1 virus is compared with the published PrENV protein sequences of three F-MCF viruses and one M-MCF virus (1, 4, 19). The results can be summarized as follows. (i) The leader sequences of the PrENV proteins of R-MCF and FM1 virus are identical. They differ by five amino acids from the leader sequences of the three other MCF viruses. (ii) The gp70 of R-MCF virus differs between residues 49 and 138 by 15 amino acids from the gp70s of the other MCF viruses. Results with two hybrid viruses suggest that these differences in amino acid sequence are responsible for the unique inability of R-MCF virus to replicate in NIH 3T3 cells. (iii) 3′ from the acquired *env* gene regions, the PrENV proteins of R-MCF, F-MCF, and FM1 virus show 99% sequence identity. In contrast, the PrENV proteins of R-MCF and M-MCF have a sequence identity of only 89%.

Comparison of the LTRs of Rauscher, Friend, and Moloney viruses. Sequences in the LTR of leukemia viruses play an important role in determining the tissue specificity of virus-induced leukemic disease (5, 6, 11, 12). The sequences thought to determine the tissue tropism are the putative enhancer sequences or direct repeats in the U3 region of the LTR. In Fig. 5, the LTR sequences of viruses which induce an erythroproliferative disease (F-MLV, R-MCF, F-MCF, FrNx-MCF) are compared with the LTR sequences of viruses which induce a lymphoid disease (M-MCF, M-MLV). Most nucleotide differences between the two groups of viruses are observed in the U3 region. In R-MCF, F-MCF, and M-MCF, sequences corresponding to the 3′ half of the direct repeat are missing, whereas short stretches of these sequences are present in FrNx-MCF. In the 5′ half of the direct repeat, R-MCF virus shows 98% identity with F-MCF virus and 81% identity with M-MCF virus. Comparing the total LTR sequences, the identity between R-MCF and F-MCF equals 98%; that between R-MCF and M-MCF equals 88%.

Relatedness of Rauscher and Friend viruses. The nucleotide sequences of the *env* gene and the LTR of R- and F-MCF viruses show an unusual degree of homology for viruses of independent origin. A similar high degree of homology was recently observed by Bestwick et al. between the *env* gene and LTR sequences of R-SFFV and the polycythemia-inducing F-SFFV (2). The similarity between the two sequences is especially striking in the *env* gene: both *env* genes have junctions between the acquired and parental sequences at the same amino acid, both contain an identical deletion of 518 bp, and both have a single base pair insertion which leads to a premature termination at the same amino acid of

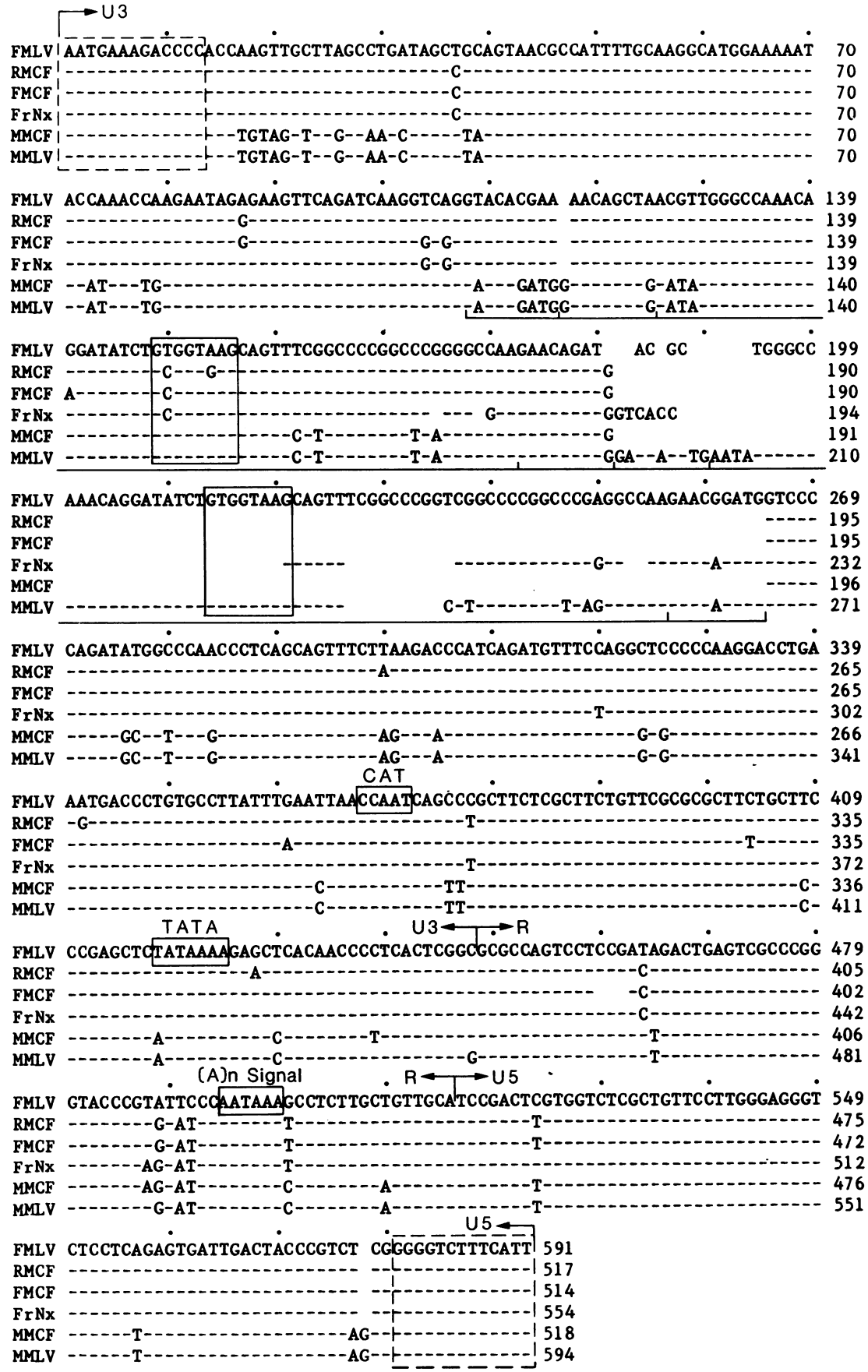

FIG. 5. Nucleotide sequences of the LTRs of F-MLV57 (18), R-MCF26, F-MCF54B (19), MCF-FrNx (1), M-MCF-1 (4), and M-MLV-1 (33). The region of direct repeats is underlined. The inverted repeats are enclosed in a dashed outline; the core sequences are enclosed in a solid outline.

the p15E coding sequence. This finding together with the results of this study strongly suggest that the Rauscher and Friend virus complexes have a common origin. The few differences between the two complexes and their components can be readily explained by the long history of independent passage both in vivo and in vitro.

Lymphoma induction by a hybrid R-MCF virus with a U3 region from M-MLV. We have shown above that a hybrid R-MCF virus, in which the 3′ end of the viral genome comprising the U3 region was replaced by the corresponding region of M-MLV, induces a lymphoid instead of an erythroid disease. This result is similar to that of Chatis et al. (5) in which the analogous fragment of F-MLV was replaced by a fragment of M-MLV. The result also shows that the envelope glycoprotein of R-MCF virus, a virus generated in cells of the erythroid lineage, is able to adsorb to both erythroid and lymphoid precursor cells. This was previously suggested by the observation that in two mice (of 105 mice injected) thymomas have developed after inoculation of R-MCF virus (40). A similar situation exists for MCF viruses with a B-cell or T-cell tropism. Virus-binding studies by Cloyd show that these MCF viruses attach equally well to B and T cells (8).

The role of the putative enhancer sequences in the U3 region may be different in the induction of an erythroid disease from the induction of a lymphoid disease. The Rauscher and Friend diseases represent predominantly an early stage in the process of leukemogenesis. The requirement for a recombinant *env* gene product in the induction of Friend disease (21, 28) suggests that it causes the initial proliferation of the hematopoietic cells. Therefore, the role of the R- or F-MCF virus enhancers would be to increase the transcription of the MCF envelope gene in target cells of the erythroid lineage. The lymphoid disease, on the other hand, represents in most cases a late stage in the leukemogenic process. Although in the early (preleukemic) stage of the disease, the enhancers of M-MLV or M-MCF virus may also act by increasing the expression of the MCF *env* gene in lymphoid precursor cells, at a later stage an activation of c-*myc* (9, 20, 34), Pim-1 (10), or other cellular genes by the viral enhancer sequences may be crucial for the development of lymphomas.

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ADDENDUM IN PROOF

After this manuscript was submitted for publication, Ishimoto et al. (Virology **41**:30–42, 1985) described a hybrid F-MCF virus in which the 3′ end of the viral genome, comprising the U3 region, was substituted with the corresponding sequences from M-MLV. This hybrid F-MCF virus induced thymic lymphomas in 10% of the inoculated mice.

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