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At Least Four Viral Genes Contribute to the Leukemogenicity of Murine Retrovirus MCF 247 in AKR Mice

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Nucleotide sequences encoding gp70, Prp15E, and the U3 region of the long terminal repeat (LTR) distinguish mink cell focus-forming (MCF) retroviruses that can induce leukemia in AKR mice from closely related MCF and ecotropic murine retroviruses that are nonleukemogenic in all inbred mouse strains tested (Lung et al., Cold Spring Harbor Symp. Quant. Biol. 44:1269–1274, 1979; Lung et al., J. Virol. 45:275–290, 1983). We used a set of recombinants constructed in vitro from molecular clones of leukemogenic MCF 247 and nonleukemogenic ecotropic Akv to separate and thereby directly test the role of these genetic elements in disease induction. Leukemogenicity tests of recombinants in AKR mice show that introduction of fragments containing either an MCF LTR or MCF gp70 coding sequences can confer only a very low incidence of disease induction on Akv virus, whereas an MCF type Prp15E alone is completely ineffective. Recombinants with an MCF 247 LTR in combination with MCF Prp15E are moderately oncogenic, whereas those with an MCF 247 LTR plus MCF gp70 coding segment are quite highly leukemogenic. Mice infected with the latter virus show a substantial increase in latent period of disease induction relative to MCF 247; this delay can be reduced when Prp15E, and hence the entire 3′ half of the genome, is from MCF 247. Surprisingly, sequences in the 5′ half of the genome can also contribute to disease induction. We found a good correlation between oncogenicity and recovery of MCF viruses from thymocytes of injected mice, with early recovery and high titers of MCF in the thymus being correlated with high oncogenicity. This correlation held for recombinants with either an MCF or ecotropic type gp70. Together, these results (i) demonstrate that at least four genes contribute to the oncogenicity of MCF viruses in AKR mice and (ii) suggest that recombinants with only some of the necessary MCF type genes induce leukemia because they recombine to generate complete MCF genomes. Although neither Akv nor MCF 247 is leukemogenic in NFS mice, recombinant viruses whose gp70 gene was derived from Akv but whose LTRs were derived from MCF 247 induced a low incidence of leukemia in this mouse strain.

Mink cell focus-forming (MCF) retroviruses are associated with a variety of leukemias and lymphomas and are implicated as the proximal leukemogenic agents in certain inbred strains of mice (18, 20, 39). MCF 247 is the prototype of leukemogenic MCF viruses isolated from high-leukemic AKR mice. It arose by recombination between the nonleukemogenic endogenous ecotropic virus of AKR mice, Akv virus, and sequences from one or more nonecotropic endogenous viruses (5, 14, 20, 38). The goal of this work was to determine which of the genetic elements that distinguish MCF 247 from its ecotropic progenitor, Akv, control the ability of MCF 247 to induce T-cell lymphomas upon injection into AKR mice.

Previous studies from our laboratory (23, 25, 29, 30, 38) and others (3, 4, 11, 34, 35, 42) have identified sequences that distinguish the genomes of oncogenic MCF viruses such as MCF 247 from closely related nononcogenic MCF or ecotropic retroviruses. Nucleotide sequencing in combination with RNase T_1 fingerprints showed that leukemogenic MCF viruses of inbred mice differ from their ecotropic parents in sequences encoding the amino-terminal two-thirds of the viral envelope glycoprotein (gp70) and in the U3 region of the long terminal repeat (LTR). In addition, the sequences of Prp15E, the precursor of virion p15E which anchors gp70 to the virus surface, are recombinant with most of the protein derived from the ecotropic parent, but the carboxy-terminal amino acids are invariably derived from the nonecotropic parent (see Fig. 1) (4, 25, 30, 34). Most (and maybe all) MCF viruses of inbred mice also have limited sequence differences with their ecotropic parents in the 5′ half of their genomes, although neither RNase T_1 fingerprints (29, 30) nor restriction endonuclease maps (3, 35, 36) have revealed 5′-end differences common to all oncogenic MCF isolates. MCF 247 in particular possesses two 5′-end large RNase T_1-resistant oligonucleotides, designated 107 and 103, that are not present in Akv virus and that lie in the *gag* and *pol* genes, respectively (29, 30, 38). Furthermore, nucleotide sequences encoding the carboxy terminus of the MCF 247 *pol* gene (C. A. Holland, unpublished data) are only about 85% homologous with the corresponding sequences of the Akv genome (Fig. 1).

To investigate which genetic elements of MCF viruses contribute to the leukemogenic phenotype, we have studied the oncogenicity of a set of recombinants constructed in vitro between molecular clones of MCF 247 (22) and Akv (28). We find that sequences throughout the 3′ half of the genome must be derived from MCF 247 for a recombinant between MCF 247 and Akv to be as oncogenic as MCF 247 itself. We also find that sequences in the 5′ half of the MCF 247 genome can contribute to oncogenicity.

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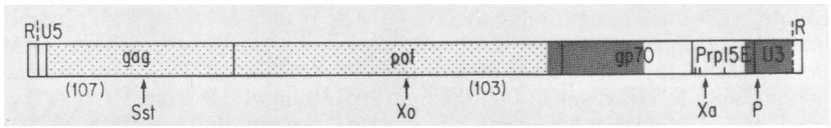

FIG. 1. Diagrammatic representation of the MCF 247 genome indicating which regions are derived from the ecotropic (like Akv [white]) and nonecotropic (different from Akv [grey]) parents. The regions that are (speckled) have not been sequenced and their parental origin has not been determined. Two large MCF-specific T_1 oligonucleotides (107 and 103) not present in Akv lie in this region, but the remainder of the T_1 oligonucleotides are shared with Akv. Conclusions based on DNA sequencing, T_1 oligonucleotide maps, and restriction endonuclease maps (3, 25, 29, 38). Restriction endonuclease sites in DNA clones of MCF 247 and Akv: Sst, *Sst*II; Xo, *Xho*I; Xa, *Xba*I; P, *Pst*I.

MATERIALS AND METHODS

Viruses. Virus stocks were prepared from MCT or SC-1 cells (21) which were transfected with cloned viral DNAs as described previously (19).

Mice and tumor induction. Newborn (<2 days old) AKR/J mice obtained from Jackson Laboratories or AKR/N mice or NFS mice supplied by the Small Animal Production Section of the National Institutes of Health were inoculated by intrathymic injection of 0.02 ml of undiluted tissue culture-grown virus prepared as described previously (22). This corresponds to $10^{3.3}$ to $10^{5.1}$ focus-forming units (FFU) or PFU for MCF, Akv, or recombinant viruses; although in the majority of cases, mice received about $10^{3.5}$ to $10^{3.8}$ PFU or FFU. In any case, no significant correlation between the input virus titers and leukemogenicity was seen in these studies. Mice were checked weekly for evidence of lymphoma and killed by ether or CO_2 anesthesia when disease was far advanced. Only those with grossly enlarged lymphoid organs were regarded as leukemic; histological examination of representative cases confirmed the presence of lymphoid neoplasms. In AKR mice, lymphoma development before 6 months of age was considered an accelerated response. NFS mice were observed for 12 months after inoculation. In both strains the overwhelming majority of tumors were lymphoblastic lymphomas of thymic origin.

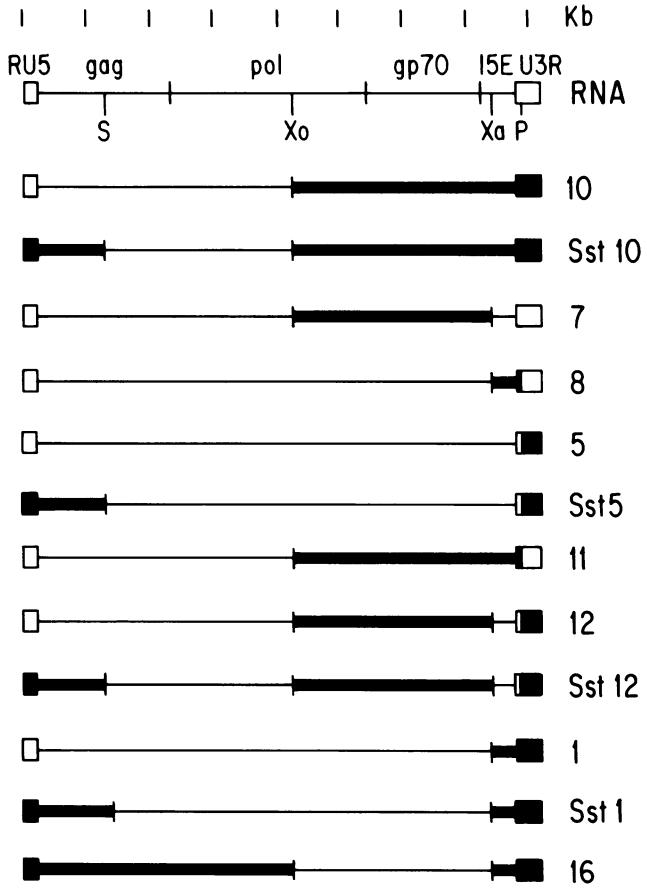

FIG. 2. RNA genomes of recombinants. Restriction sites in DNA clones of MCF 247 and Akv that were used to generate recombinants (P, *Pst*I; S, *Sst*II; Xo, *Xho*I; Xa, *Xba*I) are shown relative to the viral RNA genome on the top line. Each line below represents the predicted structure of the viral RNA produced after transfection of the DNA clones shown in Fig. 2 of the accompanying paper (22). Sequences are derived from Akv (lines) or from MCF 247 (solid areas). These diagrams are based on RNase T_1 fingerprints shown in Fig. 4 of the accompanying paper (22).

Thymocyte antigen and virus assays. Single-cell suspensions of thymocytes were prepared as previously described (24). Tests for amplification of MuLV cell surface antigens were carried out by immunofluorescence assays (40); tests for expression of MCF viruses were performed by infectious center assays of mitomycin C-treated thymocytes on mink lung cells (ATCC CCL 64) and SC-1 mouse cells as previously described (8).

RESULTS

Structure of the recombinants. The RNA viral genomes of the set of recombinants used to determine which viral genes contribute to the leukemogenic phenotype of MCF 247 are shown in Fig. 2. Four restriction endonuclease sites shared by Akv and MCF 247 clones (3, 4, 23, 36) were used to separate fragments that encode gp70, the majority of Prp15E and the LTR as well as the other genetic elements shown in Fig. 1 and 2 and in Fig. 3 of the accompanying paper (22). We will refer to the 3.2-kilobase (kb) *Xho*I-to-*Xba*I fragment as the gp70-containing fragment even though it contains the carboxy terminus of the *pol* gene which, as noted above, differs between MCF 247 and Akv. Likewise, we have used the 0.5-kb *Xba*I-to-*Pst*I and the 2.4-kb *Pst*I-to-*Hin*dIII fragment to exchange the Prp15E coding sequences and LTR sequences, respectively, realizing that these fragments contain other sequences as well. *Sst*II was used to generate derivatives of recombinants 1, 5, 10, and 12 (Fig. 2). It has been noted previously and is demonstrated in the accompanying paper (22) that recombinants with two different LTRs yielded viruses whose U3 regions were derived from the 3′ LTR as shown in Fig. 2.

We have previously described three independent molecular clones of MCF 247 (22). Because minor biochemical and biological differences between the clones had been noted, all three were used to construct the entire set of recombinants shown in Fig. 2. Two independent isolates of each recombinant were cloned and transfected into SC-1 or MCT cells. The tests to assure purity of the cloned recombinants and the viruses produced after their transfection have been described previously (22).

Leukemogenicity of recombinant viruses in AKR mice. Virus stocks prepared from cells transfected with the cloned

viral DNAs were injected into AKR mice to determine their leukemogenicity. The number of independent molecular clones of each recombinant that were tested and the number of mice injected are shown in Table 1. The results of all the tests are shown in Fig. 3 and 4. Since there were no significant differences in disease induction between groups of animals injected with independent isolates of the same recombinant, data for each recombinant were pooled. Also, *Sst* recombinants and the recombinants from which they were derived (Fig. 3) gave indistinguishable results, so data from animals injected with these pairs of viruses are plotted together in Fig. 3 and 4. Since AKR mice begin to develop thymic lymphomas spontaneously after 6 months of age, all tests were terminated by 180 days.

Virus derived from the three molecular clones of MCF 247 all induced thymic lymphomas in AKR mice with a time course and incidence essentially like that of MCF 247 virus itself (Fig. 3A). Recombinants designated 10 and *Sst* 10, in which the entire 3′ half of the genome is derived from MCF were as leukemogenic as MCF 247 (Fig. 3A and 4). The 3′ half of MCF 247 was divided into three fragments and used to construct recombinants that contain just a gp70 coding segment, just a segment carrying Prp15E, or just the LTR of MCF 247, or any two of these elements. Recombinants with just the gp70 or LTR coding fragment from MCF 247 (recombinants 5 and 7, Fig. 3C) were weakly leukemogenic with less than one third of the animals becoming diseased after a fairly long latent period. Recombinant 8 with just Prp15E coding sequences from MCF 247 was not leukemogenic at all (Fig. 3C). When recombinants carried two of the three elements, gp70, Prp15E, or the LTR from MCF 247 (recombinants 1, 11, and 12, Fig. 3B and 4), the incidence of leukemia increased substantially when one of the two elements was the MCF 247 LTR (recombinants 1 and 12). Recombinant 12 with a gp70 and LTR from MCF 247 induced almost as high an incidence of disease as MCF 247, although with an increased latent period.

The results obtained with recombinant 16 (Fig. 3B and 4) containing most of the genome of MCF 247 except for gp70 were surprising. This virus differs from recombinant 1 (Prp15E and LTR from MCF 247) only in having the 5′ half of its genome derived from MCF 247 (Fig. 2). Compared with recombinant 1, a significantly larger percentage of animals injected with recombinant 16 got leukemia (Fig. 3B). Thus, sequences in the 5′ half of the genome can contribute to leukemogenicity in conjunction with appropriate 3′-end elements.

Amplification of MuLV antigens and MCF virus expression in thymocytes. Amplification of murine leukemia virus (MuLV)-related antigens on the surface of thymic lymphocytes occurs in AKR mice at 5 to 6 months of age and appears in major part to represent enhanced expression of *env* gene product related to dual tropic recombinant MuLVs (17, 24, 32). Antigen amplification is correlated with the emergence of MCF viruses and inoculation of AKR mice with oncogenic MCF isolates results in the early expression in thymus of large amounts of cell surface antigen and high levels of MCF virus, and in development of thymic lymphomas within about 3 months (6, 20, 32). In contrast, in mice inoculated with Akv, no early antigen amplification is detected, and occurrence of leukemia is not accelerated. Mice infected with nonlymphomagenic MCF viruses follow a similar course, and in most instances, little or no replication of input virus in the thymus can be detected (6, 32).

Thymocytes of AKR mice injected with our set of recombinants were examined for amplified expression of cell surface antigen and production of infectious MCF virus at two time periods after inoculation. Tests positive at 4 to 7 weeks reflect either replication of input virus (for recombinants with an MCF gp70) or rapid generation of new MCF viruses (for recombinants with an Akv gp70 and possibly also for recombinants with an MCF type gp70 but lacking other MCF genes needed for replication in thymocytes). Tests at 10 weeks or later were included to detect less

TABLE 1. Leukemogenicity of recombinant viruses in AKR mice

Virus or recombinant	Genome[a]				No. of clones tested (fingerprinted)[b]	No. of mice injected	Incidence (%)[c]	Mean latent period ± variance	Leukemia[e]	
	gag-pol	gp70	p15E	LTR					Early	Late
MCF 247 clones	+	+	+	+	3 (3)	33	91	103 ± 23	++	
10		+	+	+	2 (1)	8	88	96 ± 16	++	
Sst 10	+	+	+	+	2 (2)	25	96	96 ± 16	++	
16	+		+	+	4 (1)	51	82	134 ± 27	+	++
12		+		+	3 (1)	14	79	129 ± 25	+	++
Sst 12	+	+		+	1 (1)	18	94	125 ± 23	+	++
1			+	+	7 (2)	75	65	148 ± 27	±	++
Sst 1	+		+	+	2 (1)	16	68	139 ± 32	±	++
11		+	+		3 (2)	25	36	160 ± 24	−	+
5				+	4 (3)	25	20	151.6 ± 10	−	±
Sst 5	+			+	2 (1)	18	28	151 ± 21	−	±
7		+			3 (1)	22	32	170 ± 8	−	±
8			+		3 (1)	32	0		−	−

[a] +, Genetic element was derived from MCF 247.

[b] Independent clones were constructed with different molecular clones of MCF 247. Parentheses indicate the number of isolates that were analyzed by RNase T_1 fingerprinting.

[c] Percentage of animals injected with virus which were diagnosed at autopsy as having lymphoblastic lymphoma.

[d] Mean latent period, $\underline{X} = \Sigma X_i/N$, where X_i is the number of days from injection until death and N is the number of animals with advanced disease; variance, $\delta = \sqrt{\Sigma(X_i - \bar{x})^2}/\sqrt{N-1}$

[e] Early leukemia occurred in less than 120 days; late leukemia occurred at 120 to 180 days. Leukemogenic potential for early or late leukemia: ++, high; ± intermediate; −, negative.

efficient replication and/or late generation of MCF viruses. All animals were grossly normal at the time of sacrifice. In general, there was remarkably good agreement between time (early or late) and degree of both antigen amplification and recovery of MCF virus, and the rate of lymphoma development (Table 2). Thus, highly leukemogenic recombinant 10, like parental MCF 247 virus, rapidly induced a high level of antigen amplification and MCF virus, whereas the poorly or nonleukemogenic recombinants (5 and 8), like Akv, induced neither antigen amplification nor generation of MCF virus. Recombinants with an MCF type gp70 that showed intermediate (recombinant 12) or low (recombinants 11 and 7) oncogenicity replicated less well in thymocytes than did recombinant 10, inducing only moderate antigen amplification and achieving high levels of virus replication less consistently and at later times. The moderately leukemogenic recombinant 16 with an ecotropic gp70 induced significant early antigen amplification and new MCF virus production in a few mice and high levels were detected in most animals at the later time points. In contrast, recombinant 1 gave a low frequency of early display of antigens and MCF virus correlating with the lower frequency and longer latency of disease.

These results suggest that genes that contribute to oncogenicity facilitate the replication of MCF viruses in the thymus or contribute to the rapid appearance of MCF viruses that replicate well in the thymus.

Leukemogenicity of recombinants in NFS mice. MCF 247 does not cause leukemia when injected into ecotropic MuLV negative NFS mice, and only rarely are tumors induced in low virus inbred mouse strains (8). Studies have shown that endogenous ecotropic virus expression is needed to help

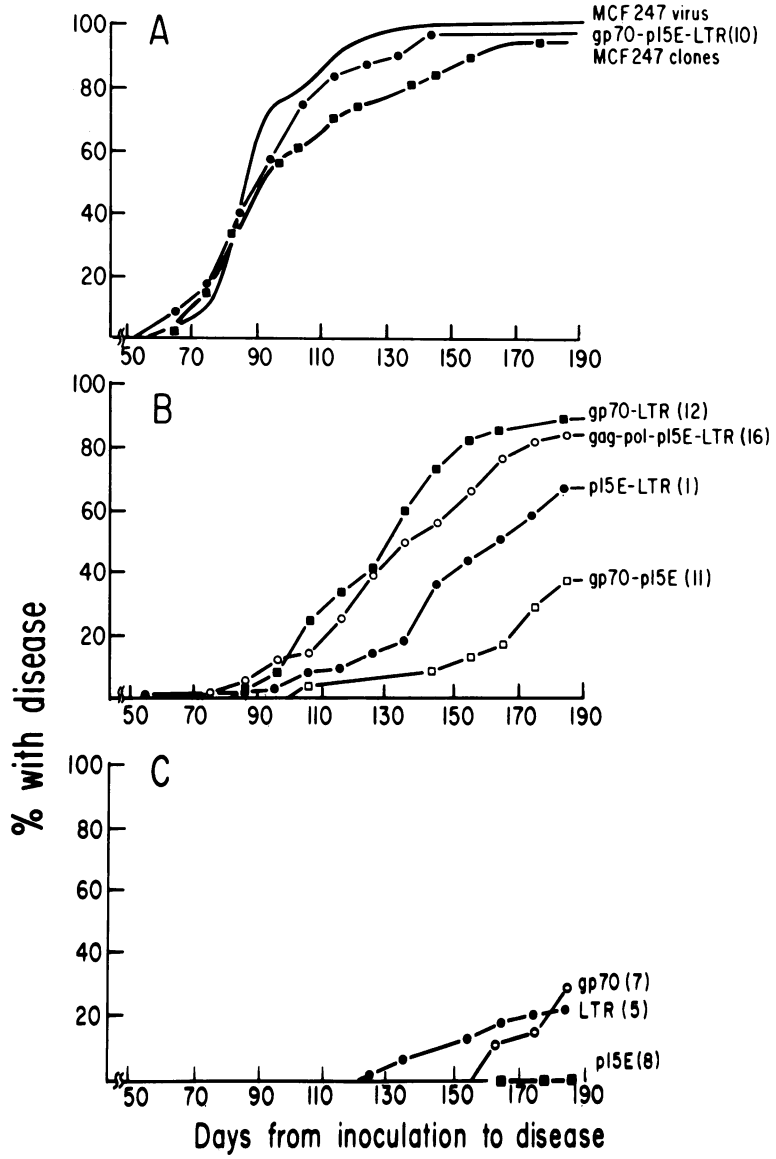

FIG. 3. Induction of T-cell lymphomas in AKR mice (A) by MCF 247 virus (—), recombinant 10 (●), and MCF 247 virus obtained by transfection of molecular clones of MCF 247 (■); (B) by recombinants containing two or more genes from MCF 247, recombinant 12 (■), recombinant 16 (○), recombinant 1 (●), or recombinant 11 (□); (C) by recombinants containing one gene from MCF 247, recombinant 7 (○), recombinant 5 (●), or recombinant 8 (■). Genes derived from MCF 247 in each recombinant are indicated next to the disease induction curves; the recombinant number is in parentheses.

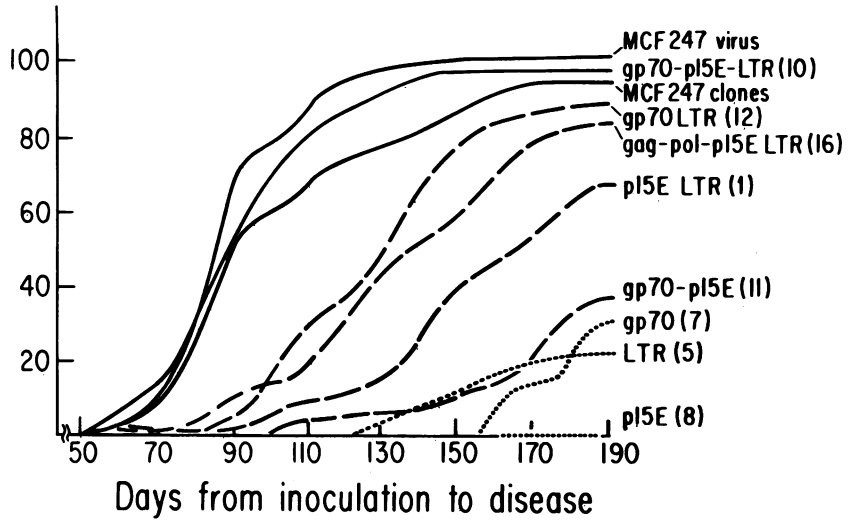

FIG. 4. Composite figure showing induction of T-cell lymphomas in AKR mice by all the viruses tested in this study. The figure is a redrawing of Fig. 3A, B, and C.

MCF viruses replicate efficiently and thus to cause lymphoma (7, 8). We were interested to determine that, if freed from the MCF gp70, other MCF genetic elements could confer oncogenicity on the Akv genome in conjunction with an Akv coded gp70. Recombinants 1, 5, 8, and 16, whose gp70s are derived from ecotropic Akv, but whose LTR (recombinant 5), Prp15E (recombinant 8), Prp15E and LTR (recombinant 1), or Prp15E, LTR, and 5' half of the genome (recombinant 16) are derived from MCF 247 were injected into newborn NFS mice. The majority of mice were monitored for disease for 1 year. The results of the study are shown in Table 3. Both recombinants 1, 5, and 16 induced a number of T cell lymphomas in NFS mice (incidence of ca. 30%). Recombinant 8 containing Prp15E from MCF 247 failed to induce T-cell leukemias; however only five mice were injected with this virus. The significance of a case of B-cell lymphoma in one mouse each injected with recombinants 1 and 8 is unknown. These results show that the MCF 247 LTR contributes to leukemogenicity in NFS mice, but the numbers of mice studied are too small to allow determination of the importance of other genetic elements.

DISCUSSION

Although previous studies have shown that the genomes of leukemogenic MCF viruses of inbred mice differ from those of their nonleukemogenic ecotropic parents in se-

TABLE 2. Antigen amplification and MCF virus expression in thymocytes of preleukemic AKR mice inoculated with recombinant viruses

Virus	Origin of gp70	Time after injection in (wk)	No. mice tested (no. of clones)	Thymocytes					
				Cell surface antigen[a]			MCF Virus recovery[b]		
				0–tr	+–++	≥+++	0	$\leq 10^{2.7}$	$\geq 10^{3}$
MCF 247	MCF	5	2	0	0	2	0	0	2
Akv 623	*Eco*	10–11	6	6	0	0	6	0	0
Recombinants									
10	MCF	4–5	5 (2)	0	0	5	0	0	5
		11	2 (1)	0	0	2	0	0	2
16	*Eco*	6	6 (2)	4	2	0	3	3	0
		10–11	7 (3)	1	2	4	1	3	3
12	MCF	6–7	6 (3)	6	0	0	3	2	1
		10	2 (1)	1	1	0	0	1	1
1	*Eco*	4–7	19 (7)	18	1	0	16	2	0
		10	4 (2)	1	2	1	0	2	2
11	MCF	4	2 (1)	2	0	0	0	2	0
		12	3 (1)	0	2	1	0	0	3
5	*Eco*	5	5 (2)	5	0	0	4	0	0
		10–18	5 (3)	4	0	0	5	0	0
7	MCF	5	4 (2)	4	0	0	2	2	0
		10–11	4 (2)	3	1	0	1	3	0
8	*Eco*	8–11	6 (2)	6	0	0	6	0	0

[a] Surface fluorescence was detected after exposure of cells to fluorescein-conjugated goat serum against Tween-ether-disrupted Moloney MuLV and was scored by estimating the percentage of positive cells and intensity of staining. 0–tr, <5%, dull; +, 5–20%, moderate; ++, 20–50%, moderate to bright; ≥+++, >50%, bright.

[b] Log_{10} MCF virus-producing thymus cells per 10^7.

TABLE 3. Induction of leukemia in NFS mice

Virus	Incidence (%) at[a]:	
	6 mo	12 mo
Recombinants		
1	6/41 (15)	13/41 (32)[b]
16	5/22 (23)	6/22 (27)
8	0/6	1/5[b]
5	4/14 (29)[c]	
MCF 247	0/13	0/13

[a] Incidence was the number of mice (found dead or killed) with lymphoma/number of mice inoculated.

[b] Includes one B-cell lymphoma of follicular center-cell type, occurring at 11 months.

[c] These were only monitored through 8 months, at which time incidence was unchanged.

quences encoding gp70, Prp15E, the U3 portion of the LTR, and sometimes also in sequences 5′ of *env* (4, 23, 25, 29, 30, 38), it remained to be shown whether each of these genetic elements contributes directly to the leukemogenicity of MCF viruses. The present study indicates that they do and clearly demonstrates that no single gene or any subset of fragments we used contains the determinants of the full oncogenic potential of MCF 247. While an MCF type LTR or gp70 coding segment alone confers only very low oncogenicity on Akv virus, recombinants with an MCF 247 LTR in combination with either the MCF gp70 segment or Prp15E are quite highly leukemogenic in AKR mice. However, the entire 3′ half of the genome must be derived from MCF 247 for a virus to induce the high incidence of lymphoma with a latent period indistinguishable from MCF 247 itself (Fig. 2). In addition, the 5′ half of the MCF 247 viral genome can enhance oncogenicity in combination with an MCF type Prp15E and LTR.

In AKR mice inoculated with the recombinant viruses, the time and degree of amplification of MuLV antigens was generally concordant with expression of MCF virus, and both measures were correlated with the incidence and latency of lymphoma. The patterns obtained suggest that when recombinants with just one or two genetic elements derived from MCF 247 induce leukemia in AKR mice, they do so by recombining with endogenous retrovirus sequences to generate a complete MCF-type genome. Thus, recombinants with more of their genome derived from MCF 247 would be more leukemogenic because they would need to acquire fewer genetic elements to achieve full oncogenic potential. For example, recombinant 10, containing the entire 3′ half of the MCF 247 genome, is itself most likely the proximal leukemogen which led to rapid and high frequency lymphoma, whereas in mice inoculated with the other less active recombinants, new oncogenic MCF viruses were generated with different degrees of efficiency. It will be noted that recombinants 1, 7, and 11, although of low oncogenicity by the assay used in this study, did induce in the majority of recipients both antigen amplification and moderate- to high-titer replication of MCF virus by 10 to 12 weeks. It is possible that an acceleration of lymphomagenesis compared with that in uninoculated controls, would have been seen if the mice had been observed for more than 180 days. Other studies have indicated that although replication of virus in cells of the thymus is required for leukemogenesis, not all mice in which virus production is demonstrable develop thymic lymphomas (8, 32).

Although it is clear from these studies that several genetic elements of the oncogenic MCF viral genome contribute to leukemogenesis, the specific roles of the different MCF viral genes are still in large part undefined.

LTR. Given recent findings that MCF proviruses integrate adjacent to protooncogenes c-*myc* (9; Y. Li, C. A. Holland, J. W. Hartley, and N. Hopkins, Proc. Natl. Acad. Sci. U.S.A., in press) and *pim-1* (10) in some spontaneous and MCF-induced AKR thymic lymphomas, it seems that retroviral genes must, in part, function to direct the MCF genome toward this end. Recent studies (22) showing that the MCF 247 LTR encodes an in vitro host range property that is correlated with the thymotropism of class I MCFs, and that other retroviral LTRs encode disease specificity and tissue tropism, probably within their transcriptional enhancers (1, 2, 12, 13), suggests a role for the MCF LTR in leukemogenesis. The ability of MCF 247 to replicate well in the thymus and to activate protooncogenes efficiently in T cells probably resides in part within the LTR. Certain MCF viral enhancer elements may have a specific functional association with a particular target cell population, perhaps immature lymphocytes of the thymic cortex (6).

gp70. Because the gp70 coding fragment we used to construct recombinants between MCF 247 and Akv contains sequences encoding the carboxy-terminal portion of *pol*, it is possible that the determinant of leukemogenicity contained in this fragment is in either the MCF type gp70 or *pol* gene or both. Recently, the murine retrovirus *pol* gene has been shown to encode three proteins, the most carboxy-terminal of which probably functions as an integrase for proviral DNA (27, 41). We noted that the carboxy-terminal portion of *pol*, which was present on the fragment we used to exchange gp70 coding sequences, is only about 85% homologous between Akv and MCF 247. Conceivably, the MCF and Akv integrase proteins could have different specificities and efficiencies and might also function more efficiently in conjunction with their respective LTRs. (It must be emphasized again that we do not know from these studies whether the carboxy-terminal segment of *pol* contributes to leukemogenicity.) Extensive data already support a role for MCF gp70 in leukemogenesis (16). Whether, as has been suggested, this protein is specifically mitogenic for mouse thymocytes (31) and thereby contributes to oncogenesis remains unknown. Other possibilities are that MCF envelope proteins facilitate infection of thymic target cells, presumably at a postattachment replicative step (6), or that an MCF type gp70 can, by utilization of different cell surface receptors, bypass the interference resulting from ecotropic virus infection in high-virus mice such as AKR and thereby facilitate virus spread in the thymus and replication in target cells. An MCF 247-type gp70 thus might indirectly increase viral enhancer activity or cellular oncogene activation, or might directly alter the sensitivity of target cells to mitogenic stimuli.

Prp15E. According to nucleotide sequence data, the Prp15E proteins of MCF 247 and Akv differ primarily in their carboxy-termini and share 192 of 201 amino acids. It is striking that the presence of the fragment carrying an MCF-type Prp15E can substantially shorten the latent period of disease induction (recombinants 10 versus 12; Figure 3A and B and Fig. 4), and also increase the incidence of disease (recombinants 1 versus 5; Fig. 3B and C and Fig. 4) although only in combination with an MCF LTR. Other groups have reported similar effects of Prp15E on latent period of retroviral induced lymphomogenesis (13, 26, 33). Our analysis of MCF expression in thymocytes of injected AKR mice suggests that the Prp15E coding fragment of MCF is needed for efficient replication of MCF viruses in thymocytes (compare recombinants 10 and 12 in Table 2).

pol and gag. Because recombinant 16 (Fig. 2), whose Prp15E, LTR, and 5′ half are derived from MCF 247, was more leukemogenic than recombinant 1 with just Prp15E and the LTR of MCF 247, sequences in *gag* or *pol* (5′ of *Xho*I) must contribute to oncogenicity. Since *Sst*II recombinants (see Fig. 2) were not more leukemogenic than their progenitors, the sequences that contribute to leukemogenicity may lie between *Sst*II and *Xho*I, a *gag-pol* coding region. To determine which gene or genes in this region might be important will require further study. It is interesting that Robinson et al. (37) have identified a determinant of osteopetrosis in this region of the genome of certain nondefective avian leukosis viruses and have also suggested that accumulation of unintegrated proviral DNA plays a role in the development of this disease (H. Robinson, personal communication). Interestingly, unintegrated MCF proviral DNA has also been observed in AKR thymuses. Also AKR thymic lymphomas contain multiple insertions of MCF proviruses (Li et al, in press). Conceivably the *pol* genes (reverse transcriptase or integrase) may affect proviral DNA accumulation which in turn could play a role in disease induction.

Cancer has long been recognized as a multistep phenomenon. In the AKR mouse, among these steps is the generation of MCF viruses, which after acquiring a unique combination of genes, rapidly induce T cell lymphomas (42). The mechanisms of accelerated lymphomagenesis are gradually being clarified through identification of viral genome segments affecting induction of leukemias and lymphomas and analysis of their function.

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LITERATURE CITED

1. **Chatis, P. A., C. A. Holland, J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1983. Role for the 3′ end of the genome in determining disease specificity of Friend and Moloney murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **80**:4408–4411.
2. **Chatis, P. A., C. A. Holland, J. E. Silver, T. N. Fredrickson, N. Hopkins, and J. W. Hartley.** 1984. A 3′ end fragment encompassing the transcriptional enhancers of nondefective Friend virus confers erythroleukemogenicity on Moloney leukemia virus. J. Virol. **52**:248–254.
3. **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.
4. **Chattopadhyay, S. K., M. R. Lander, S. Gupta, E. Rands, and D. R. Lowy.** 1981. Origin of mink cytopathic focus-forming (MCF) viruses: comparison with ecotropic and xenotropic murine leukemia virus genomes. Virology **113**:465–483.
5. **Chattopadhyay, S. K., M. R. Lander, E. Rands, and D. R. Lowry.** 1980. Structure of endogenous murine leukemia virus DNA in mouse genomes. Proc. Natl. Acad. Sci. U.S.A. **77**:5774–5778.
6. **Cloyd, M. W.** 1983. Characterization of target cells for MCF viruses in AKR mice. Cell **32**:217–225.
7. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1980. Lymphomagenicity of recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **151**:542–552.
8. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1981. Genetic study of lymphoma induction by AKR mink cell focus-inducing murine leukemia virus in AKR×NFS crosses. J. Exp. Med. **154**:450.
9. **Corcoran, L. M., J. M. Adams, A. R. Dunn, and S. Cory.** 1984. Murine T lymphomas in which the cellular *myc* oncogene has been activated by retroviral insertion. Cell **37**:113–122.
10. **Cuypers, H. T., G. Selten, W. Quint, M. Zijestra, E. R. Maandag, W. Boelens, P. van Wezenbeeck, C. Melief, and A. Berns.** 1984. Murine leukemia virus inducted T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. Cell **37**:141–150.
11. **Datta, S. K., C. Y. Thomas, J. A. Nicklas, and J. M. Coffin.** 1983. Thymic epithelial genotype influences the production of recombinant leukemogenic retroviruses in mice. J. Virol. **47**:33–45.
12. **DesGroseillers, L., E. Rassart, and P. Jolicoeur.** 1983. Thymotropism of murine leukemia virus is conferred by its long terminal repeat. Proc. Natl. Acad. Sci. U.S.A. **80**:4203–4207.
13. **DesGroseillers, L., R. Villemur, and P. Jolicoeur.** 1983. The high leukemogenic potential of Gross passage A murine leukemia virus maps in the region of the genome corresponding to the long terminal repeat and to the 3′ end of env. J. Virol. **47**:24–32.
14. **Elder, J., F. Jensen, R. 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. U.S.A. **74**:4676–4680.
15. **Even, J., S. J. Anderson, A. Hampe, F. Galibert, D. Lowy, G. Khoury, and C. J. Sherr.** 1983. Mutant feline sarcoma proviruses containing the viral oncogen (v-*fes*) and either feline or murine control elements. J. Virol. **45**:1004–1016.
16. **Famulari, N. G.** 1982. Murine leukemia viruses with recombinant env genes: a discussion of their role in leukemogenesis. Curr. Top. Microbiol. Immunol. **103**:75–108.
17. **Famulari, N. G., and D. Cieplensby.** 1983. A time-course study of MuLV *env* expression in the AKR thymus: quantitative and qualitative analysis of ecotropic and recombinant virus gene products. Virology **132**:282–291.
18. **Fischinger, P. J., S. Nomura, and D. P. Bolognesi.** 1975. A novel murine oncornavirus with dual eco- and xenotropic properties. Proc. Natl. Acad. Sci. U.S.A. **72**:5150–5155.
19. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
20. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. U.S.A. **74**:789–792.
21. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lacks host-range restrictions for murine leukemia viruses. Virology **65**:128–134.
22. **Holland, C. A., J. Wozney, P. A. Chatis, N. Hopkins, and J. W. Hartley.** 1984. Construction of recombinants between molecular clones of murine retrovirus MCF 247 and Akv: determinant of an in vitro host range property that maps in the long terminal repeat. J. Virol. **53**:152–157.
23. **Holland, C. A., J. Wozney, and N. Hopkins.** 1983. The nucleotide sequence of the gp70 gene of murine retrovirus, MCF 247. J. Virol. **47**:413–420.
24. **Kawashima, K., H. Ikeda, J. W. Hartley, E. Stockert, W. P. Rowe, and L. J. Old.** 1976. Changes in expression of murine leukemia virus antigens and production of xenotropic virus in the late preleukemic period in AKR mice. Proc. Natl. Acad. Sci. U.S.A. **73**:4680.
25. **Kelly, M., C. A. Holland, M. L. Lung, S. K. Chattopadhyay, D. R. Lowy, and N. Hopkins.** 1983. Nucleotide sequence of the 3′ end of MCF 247 murine leukemia virus. J. Virol. **45**:291–298.
26. **Lenz, J., D. Celander, R. L. Crowther, R. Patarca, D. W. Perkins, and W. A. Haseltine.** 1984. Determination of the leukemogenicity of a murine retrovirus by sequences within the long

terminal repeat. Nature (London) **308**:467–470.

27. **Levin, J. G., S. C. Hu, A. Rein, L. I. Messer, and B. I. Gerwin.** 1984. Murine leukemia virus mutant with a frameshift in the reverse transcriptase coding region: implications for *pol* gene structure. J. Virol. **51**:470–478.
28. **Lowy, D. R., E. Rands, S. K. Chattopadhyay, C. F. Ganon, and G. L. Hager.** 1980. Molecular cloning of infectious integrated murine leukemia virus DNA from infected mouse cells. Proc. Natl. Acad. Sci. U.S.A. **77**:614–618.
29. **Lung, M. L., J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1983. Large RNase T_1-resistant oligonucleotides encoding p15E and the U3 region of the long terminal repeat distinguish two biological classes of mink cell focus-forming type C viruses of inbred mice. J. Virol. **45**:275–290.
30. **Lung, M. L., C. Hering, J. W. Hartley, W. P. Rowe, and N. Hopkins.** 1979. Analysis of the genomes of mink cell focus-inducing murine type C viruses: a progress report. Cold Spring Harbor Symp. Quant. Biol. **44**:1269–1274.
31. **McGrath, M. S., and I. L. Weissman.** 1979. AKR leukemogenesis: identification and biological significance of thymic lymphoma receptors for AKR retroviruses. Cell **17**:65–75.
32. **O'Donnell, P. V., E. Stockert, Y. Obata, and L. J. Old.** 1981. Leukemogenic properties of AKR dualtropic (MCF) viruses: amplification of murine leukemia virus-related antigens on thymocytes and acceleration of leukemia development in AKR mice. Virology **112**:548–563.
33. **Oliff, A., K. Signorelli, and L. Collins.** 1984. The envelope gene and long terminal repeat sequences contribute to the pathogenic phenotype of helper-independent Friend viruses. J. Virol. **5**:788–794.
34. **Quint, W., W. Boelens, P. van Wezenbeek, T. Cuypers, E. Robanus Maandag, G. Selten, and A. Berns.** 1984. Generation of AKR-mink cell focus forming viruses: a conserved single copy xenotropic-like provirus provides recombinant long terminal repeat sequences. J. Virol. **50**:432–438.
35. **Quint, W., W. Quax, H. von de Putten, and A. Berns.** 1981. Characterization of AKR murine leukemia virus sequences in AKR mouse substrains and structure of integrated recombinant genomes in tumor tissues. J. Virol. **39**:1–10.
36. **Rands, E., D. R. Lowy, M. R. Lander, and S. K. Chattopadhyay.** 1981. Restriction endonuclease mapping of ecotropic murine leukemia viral DNAs: size and heterogeneity of the long terminal repeat. Virology **108**:445–452.
37. **Robinson, H. L., B. M. Blais, P. N. Tsichlis, and J. M. Coffin.** 1982. At least two regions of the viral genome determine the oncogenic potential of avian leukosis viruses. Proc. Natl. Acad. Sci. U.S.A. **79**:1225–1229.
38. **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. U.S.A. **75**:495–499.
39. **Rowe, W. P., M. W. Cloyd, and J. W. Hartley.** 1979. Status of the association of mink cell focus forming viruses with leukemogenesis. Cold Spring Harbor Symp. Quant. Biol. **44**:1265–1268.
40. **Rowe, W. P., and J. W. Hartley.** 1983. Genes affecting mink cell focus-inducing (MCF) murine leukemia virus infection and spontaneous lymphoma in AKR F1 hybrids. J. Exp. Med. **158**:353–364.
41. **Schwartzberg, P., J. Colicelli, and S. P. Goff.** 1984. Construction and analysis of deletion mutations in the *pol* gene of Moloney murine leukemia virus: a new viral function required for productive infection. Cell **37**:1043–1052.
42. **Thomas, C. Y., and J. M. Coffin.** 1982. Genetic alterations of RNA leukemia viruses associated with the development of spontaneous thymic leukemia in AKR/J mice. J. Virol. **43**: 416–426.