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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

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We used T_1 oligonucleotide maps, in conjunction with available nucleotide sequences of appropriate C-type viruses, to identify regions of the viral genome that distinguish two biological classes of mink cell focus-forming (MCF) viruses described previously by Cloyd et al. (J. Exp. Med. 151:542–522, 1980). We found that leukemogenic MCF viruses from thymus differed from non-leukemogenic MCFs isolated from nonthymic neoplasms in nucleotide sequences encoding Prp15E and the U3 portion of the long terminal repeat (LTR). The thymic isolates possessed recombinant Prp15E genes, with the 5′ to mid portion derived from their ecotropic parents and the extreme 3′ portion invariably derived from their nonecotropic parents. These viruses probably derived the entire U3 portion of their LTRs from their nonecotropic parents. The nonthymic MCFs appeared to inherit their entire Prp15E coding region from their nonecotropic parents. We failed to detect consistent differences in gp70-coding sequences between the two groups of MCFs, but this may simply reflect limitations of the data. The studies presented here, in conjunction with studies from a number of labs indicating a role for MCF gp70 in leukemogenesis, indicate that three genetic elements, gp70, p15E, and the U3 portion of the LTR, may all play a role in determining the leukemogenic phenotype of type C viruses of high-leukemic inbred mice.

High-leukemic inbred strains of mice inherit DNA copies of ecotropic type C viruses, and expression of these loci results in a lifelong viremia (21, 32). Despite compelling evidence that this viremia ultimately results in leukemia (32), a number of findings have suggested that the inherited ecotropic viruses themselves are not leukemogenic but rather give rise, by genetic recombination, to novel type C viruses which are the proximal leukemogenic agents of inbred mice (14, 16, 18, 26, 34). One group of recombinant viruses which may serve as inducers of leukemia are the mink cell focus-forming (MCF) viruses described by Hartley et al. (14) and by Fischinger et al. (10). MCF viruses apparently arise during the lifetime of high-ecotropic-virus mice by recombination between endogenous ecotropic and nonecotropic viruses and invariably appear in leukemias or at the sites of their imminent appearance (14). They possess novel gp70s and, as a result, a novel host range, being able to infect mouse cells like their ecotropic progenitors and mink cells like their putative xenotropic progenitors (3, 6, 8, 9, 31).

To test the hypothesis that MCF viruses are the proximal leukemogenic agents of inbred mice, Cloyd et al. (4, 33) determined the oncogenicity of a set of cloned MCF viruses obtained from preleukemic or leukemic mice of high-leukemic strains. Surprisingly, these viruses fell into two biological groups depending on their mouse strain of origin and the correlated property of whether that strain develops thymomas or leukemias localized grossly in the spleen, these being chiefly tumors of cell lineages other than T cells (Rowe, unpublished results). MCFs isolated from thymus (class I MCFs) accelerated leukemia when injected into AKR mice, whereas isolates from nonthymic tumors (class II MCFs) did not. One MCF virus whose tissue of origin was uncertain had properties intermediate between those of the two classes.

Previously we reported a preliminary analysis that indicated that the genomes of the two

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biologically defined classes of MCF viruses described by Cloyd et al. (4) could be distinguished by the methods of RNase T_1 fingerprinting and oligonucleotide mapping (23). Consistent oligonucleotide differences near the 3′ end of the viral genomes were predictive of the origin from spleen versus thymus of an MCF virus. We were interested in extending these studies by analyzing MCF viruses isolated from additional inbred strains. Furthermore, an important question unanswered by the previous studies was, precisely which of the genetic elements known to reside in the 3′ third of the viral genome consistently differs between biologically distinct MCF viruses and between MCF viruses and their nonleukemogenic ecotropic progenitors? Recently, it has been possible to prepare an improved physical T_1 oligonucleotide map of Akv virus, the prototype ecotropic parent of MCF viruses of inbred mice. Kelly et al. (20) determined the nucleotide sequence of the Prp15E and long terminal repeat (LTR)-coding regions of a molecular DNA clone of an MCF virus. In addition, Van Beveren et al. (38), Herr and Corbin (see reference 19), and Lenz et al. (20) have sequenced, respectively, the LTR-, Prp15E-, and gp70-coding regions of Akv virus. These studies have allowed us to precisely localize T_1 oligonucleotides that distinguish the biological classes of MCF viruses. We find that these oligonucleotides comprise sequences coding for Prp15E and the U3 portion of the LTR.

TABLE 1. Biological properties of MCF isolates whose genomes were analyzed

MCF class	MCF isolate	Mouse strain of origin	Isolated from thymus	Ecotropic parent(s)	Thymotropism[a]	Plating efficiency on SC-1 vs NFS embryo cells	Accelerates AKR leukemia
I	AKR-247	AKR	Yes	Akv-1 or v-2	+	Sc-1 > NFS	Yes
	AKR-13	AKR	Yes	Akv-1 or v-2	+	Sc-1 > NFS	Yes
	AKR-L3 (Leuk-3)[b]	AKR	Yes	Akv-1 or v-2	+	Sc-1 > NFS	Yes
	AKR-L4 (1375-1)	AKR	Yes	Akv-1 or v-2	+	Sc-1 > NFS	Yes
	AKR-L5 (1375-2)	AKR	Yes	Akv-1 or v-2	+	Sc-1 > NFS	Yes
	C58-L1b	C58	Yes	C58v-1, v-2, or v-3	+	Sc-1 > NFS	Yes
Ia	*Akv-1*-C311	NFS.*Akv-1*	Yes	Akv-1	?	Sc-1 > NFS	No
II	*Akv-2*-C34 (V2-34)	NFS.*Akv-2*	No	Akv-2	−	NFS > Sc-1	No
	Akv-2-C78 (CM74)	NFS.*Akv-2*	No	Akv-2	−	NFS > Sc-1	No
	Akv-2-C25 (MB25)	NFS.*Akv-2*	No	Akv-2	−	NFS > Sc-1	No
	Akv-2-C26-2 (CM66)	NFS.*Akv-2*	No	Akv-2	−	NFS > Sc-1	No
	Akv-1-C44-3 (CM78)	NFS.*Akv-1*	No	Akv-1	−	NFS > Sc-1	No
	Akv-1-C93 (CM181)	NFS.*Akv-1*	No	Akv-1	−	NFS > Sc-1	No
	Akv-1-C44-1 (M116)	NFS.*Akv-1*	No	Akv-1	−	NFS > Sc-1	NT[c]
	Akv-1-C44-2 (M111A)	NFS.*Akv-1*	No	Akv-1	−	NFS > Sc-1	No
	C58v-1-C77 (CM156)	NFS.*C58v-1*	No	C58v-1	−	NFS > Sc-1	No
	C58v-2-C45 (C139)	NFS.*C58v-2*	No	C58v-2	−	NFS > Sc-1	No
	Fgv-1-C647 (C207)	NFS. *Fgv-1*	No	Fgv-1	−	NFS > Sc-1	No
Intermediate	*Akv-1*-C36 (V1-36)	NFS.*Akv-1*	Uncertain	Akv-1	Intermediate	Intermediate	Conditional

[a] Ability to replicate in AKR thymus after injection.
[b] Previous designations of isolates are given in parentheses (22).
[c] NT, Not tested.

MATERIALS AND METHODS

Viruses. The origins of the majority of strains studied here are described in Cloyd et al. (4). Three additional strains from NFS mice congenic for *Akv-1* were studied. These were strains *Akv-1*-C311, from normal thymus of a 7-month-old mouse, and *Akv-1*-C44-1 and *Akv-1*-C44-2, from splenic lymphomas in 18-month-old littermates. Strain *Akv-1*-C311 did not accelerate AKR lymphoma, but in the one mouse tested at 2 months after inoculation, the virus had grown to high titer. It resembled other MCFs of thymic origin in having lower plating efficiency (8- to 10-fold) on NFS embryo cells than on Sc-1 cells. Strains *Akv-1*-C44-1 and -C44-2 plated with equal efficiency on the two cell types, as is characteristic of other MCFs of nonthymic origin. Strain *Akv-1*-C44-2 was non-lymphomagenic in AKR; strain *Akv-1*-C44-1 was not tested. All viruses were cloned by two cycles of limiting dilution purification in mink lung cell cultures.

RNase T_1 fingerprints and identification of large T_1 oligonucleotides by secondary digestion. The preparation of uniformly ^{32}P-labeled MCF viral RNA, its digestion with RNase T_1, and separation of the resulting T_1 oligonucleotides by two-dimensional gel electrophoresis were performed as described previously (7). The large T_1 oligonucleotides were eluted from the gels and analyzed further by digestion with pancreatic RNase as described previously (7). All viruses were analyzed at least twice, as were all T_1 oligonucleotides analyzed in each viral fingerprint. In some cases, it was difficult to obtain sufficient amounts of radioactively labeled viral RNA to allow accurate quantitation of the secondary digestion products of individual T_1 oligonucleotides. Thus, these products were frequently analyzed qualitatively and quantitated visually. We believe that visual analysis of the secondary digestion products, combined with the electrophoretic mobility of the T_1 oligonucleotides in the fingerprinting gels, probably provides as accurate an identification as obtained by quantitation of the radioactivity present in each secondary digestion product.

RESULTS

The MCF viruses whose biological properties were studied by Cloyd et al. (4, 33) were isolated from a variety of inbred mouse strains which inherit different ecotropic viral loci. The individual MCF isolates, their biological properties, their tissue and mouse strain of origin, and the ecotropic viral loci present in these mouse strains are listed in Table 1. Three additional isolates obtained more recently by Hartley (unpublished results) are also included in Table 1

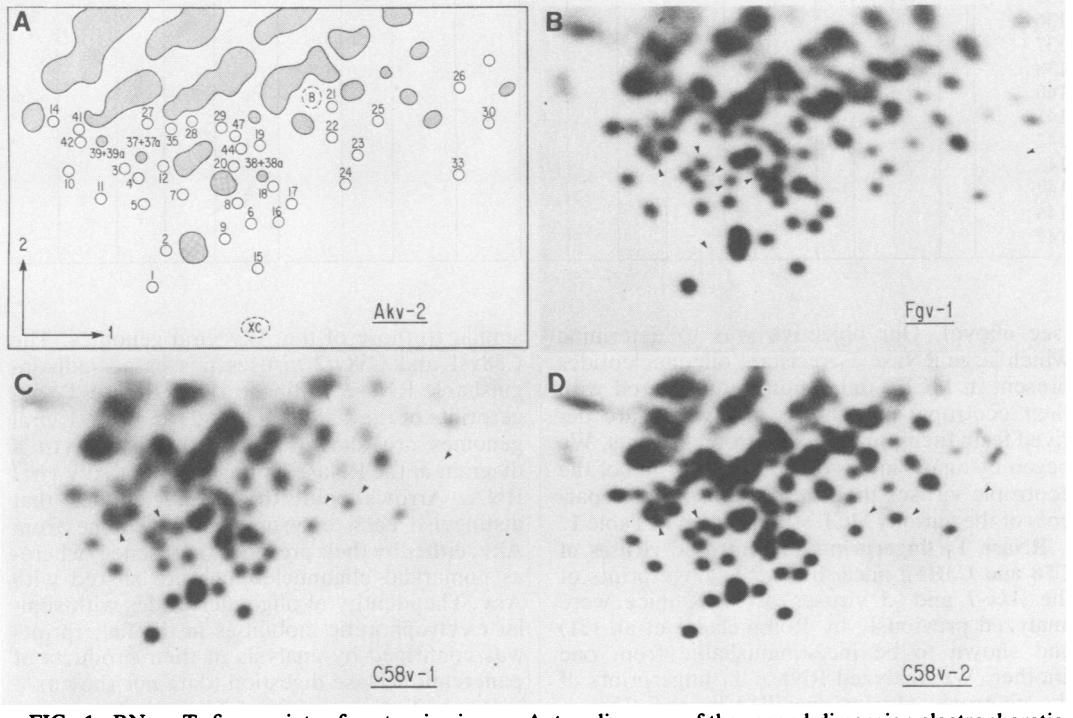

FIG. 1. RNase T_1 fingerprints of ecotropic viruses. Autoradiograms of the second-dimension electrophoretic gels of ^{32}P-labeled, RNase T_1-resistant oligonucleotides of ecotropic viruses of (B) an NFS.*Fgv-1* congenic mouse, (C) an NFS.*C58v-1* congenic mouse, and (D) an NFS.*C58v-2* congenic mouse. Arrows indicate oligonucleotide differences relative to the *Akv-2* diagram. (A) Diagram of RNase T_1 fingerprint of Akv, the ecotropic virus of an NFS.*Akv-1* or NFS.*Akv-2* congenic mouse (30). The arrows indicate the direction of migration in the first and second dimensions of the gel electrophoresis. XC and B, Positions of dye markers xylene cyanol FF and bromphenol blue, respectively.

TABLE 2. Presence of MCF-specific T_1 oligonucleotides in 19 MCF isolates

Oligonucleotide	Presence of MCF-specific T_1 oligonucleotides in MCF isolates:																		
	13	247	L3	L4	L5	L1b	C311	C34	C78	C25	C26-2	C44-3	C93	C44-1	C44-2	C77	C45	C647	C36
101	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?
102		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+
103		+	+							+						+	+		
104	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
106	+	+	+	+	+	(+)										(+)	(+)	(+)	
107	+	+	+																
107a	+		+	+	+														
108	+	+	+	+	+	+													
110								+				+		+	+		+		
111					+	+		+	+	+	+	+	+	+		+		?	+
112								+				+		+					
113					+	+		+	+	+	+	+	+	+		+	+	+	+
114							+	+	+	+	+	+	+	+		+	+	+	
116	+																		
119	+		+	+															
121	+														+				
122	+					+													
123	+		+	+		+									+				
123a	+		+	+			+								+				
125	?				+	+		+	+	+	+	+	+	+	?	+	?	?	?
128			+			+						+							
129											+								
130			+			+													
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135													+						
136															+				
137															+				
138															+		?		
140															+				
141										+	+	+		+		+		+	
142	?														+				
143															+				
144															+				
146																	+	+	
147																	+		

(see above). Our objective was to determine which large RNase T_1-resistant oligonucleotides present in MCF viral genomes are shared with their ecotropic progenitors and which are derived from their putative xenotropic parents. We began by analyzing RNase T_1 fingerprints of the ecotropic viruses that are the presumptive parents of the various MCF strains listed in Table 1.

RNase T_1 fingerprints of ecotropic viruses of C58 and C3HFg mice. RNase T_1 fingerprints of the *Akv-1* and *-2* viruses of AKR mice were analyzed previously by Rommelaere et al. (31) and shown to be indistinguishable from one another. We analyzed RNase T_1 fingerprints of the ecotropic viruses specified by the *C58v-1*, *C58v-2*, and *Fgv-1* loci of C58 and C3HFg mice. These viruses were obtained from NFS mice congenic for these individual ecotropic viral loci. The fingerprints of the genomes of all these viruses were very similar to one another and similar to those of the Akv viral genomes. The C58v-1 and C58v-2 viruses possessed indistinguishable RNase T_1 fingerprints. RNase T_1 fingerprints of the C58v-1, C58v-2, and Fgv-1 viral genomes are shown in Fig. 1 adjacent to a diagram of the RNase T_1 fingerprint of Akv viral RNA. Arrows point to oligonucleotides that distinguish each ecotropic viral genome from Akv, either by their presence or absence, whereas unmarked oligonucleotides are shared with Akv. The identity of oligonucleotides with similar electrophoretic mobilities in the fingerprints was confirmed by analysis of their products of pancreatic RNase digestion (data not shown).

RNase T_1 fingerprints of MCF viral genomes. A detailed analysis of RNase T_1 fingerprints of the genomes of four of the MCF viruses listed in Table 2, MCF 247, MCF 13, MCF *Akv-2*-C34 (V2-34), and MCF *Akv-1*-C36 (V1-36), was described previously by Rommelaere et al. (31).

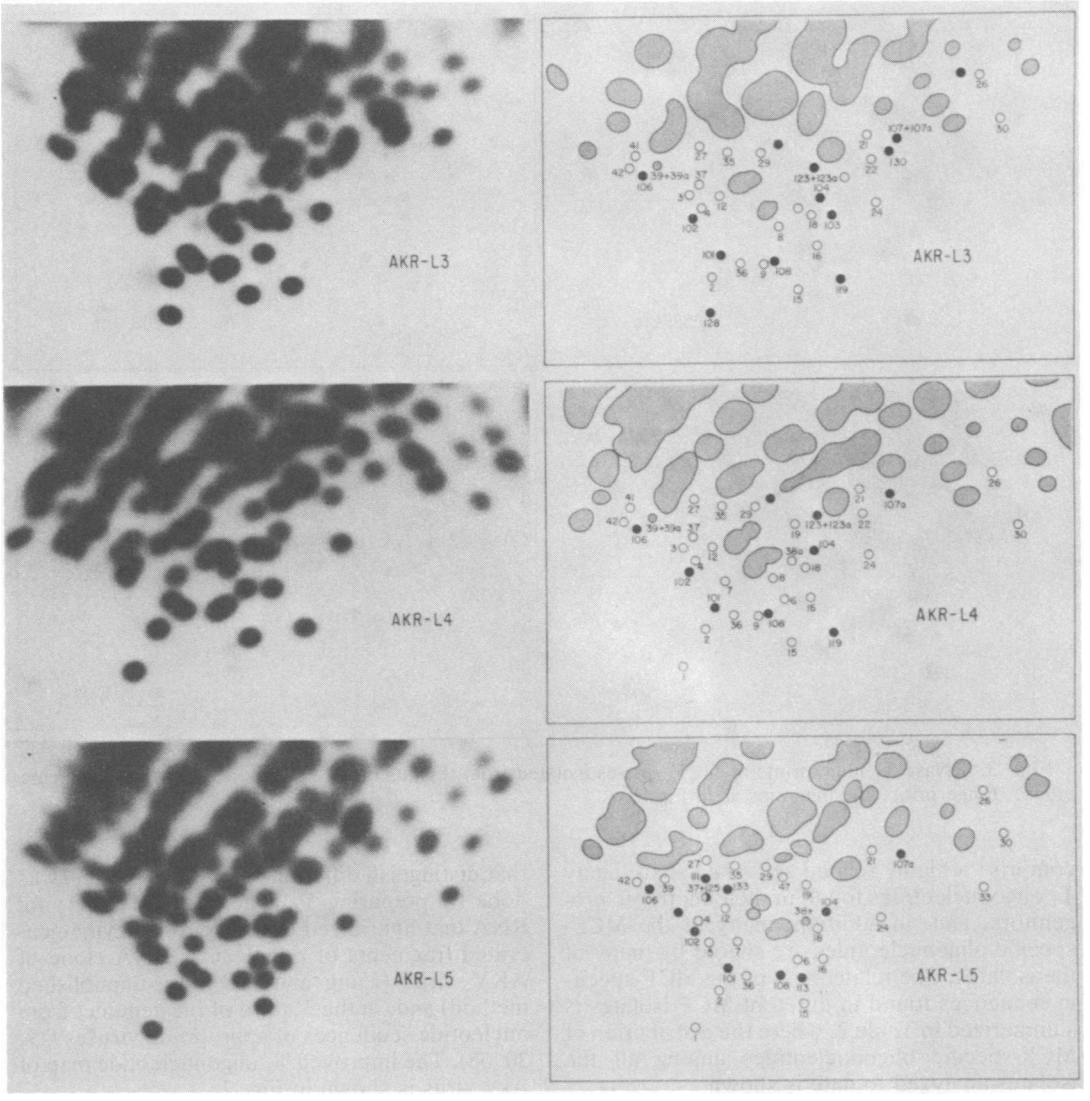

FIG. 2. RNase T_1 fingerprints of MCF viruses isolated from AKR mice. Autoradiograms and diagrams as described in the legend to Fig. 1. Symbols: ○, single T_1 oligonucleotides shared with the ecotropic parent(s) Akv-1 or Akv-2; ●, MCF-specific T_1 oligonucleotides; shaded areas, two or more comigrating T_1 oligonucleotides.

Lung et al. (23) presented a preliminary analysis of the fingerprints of the genomes of the remaining isolates from AKR and from NFS mice congenic for the *Akv-1 and Akv-2* viral loci of AKR (except for the novel isolate *Akv-1*-C311). The complete analysis of the fingerprints of these viruses and the remaining MCF viruses listed in Table 1 is presented here.

RNase T_1 fingerprints of MCF viruses not previously published are shown in Fig. 2 through 6 along with diagrams of the fingerprints. Inspection of these fingerprints reveals that, like the previously analyzed MCFs, all these isolates share large T_1 oligonucleotides with their ecotropic progenitors, all are lacking some ecotropic oligonucleotides, and all possess a set of large T_1 oligonucleotides not present in the ecotropic viruses. Oligonucleotides shared by different viruses are assigned the same numbers; those shared with ecotropic viruses are designated 1, 2, etc., whereas those found only in MCF viruses are designated 101, 102, etc. The identity of oligonucleotides is based on their electrophoretic mobility and on the apparent identity of their products of pancreatic RNase digestion.

A comparison of the T_1 fingerprints of all the MCF viruses listed in Table 1 reveals that even though each fingerprint is unique, the viruses

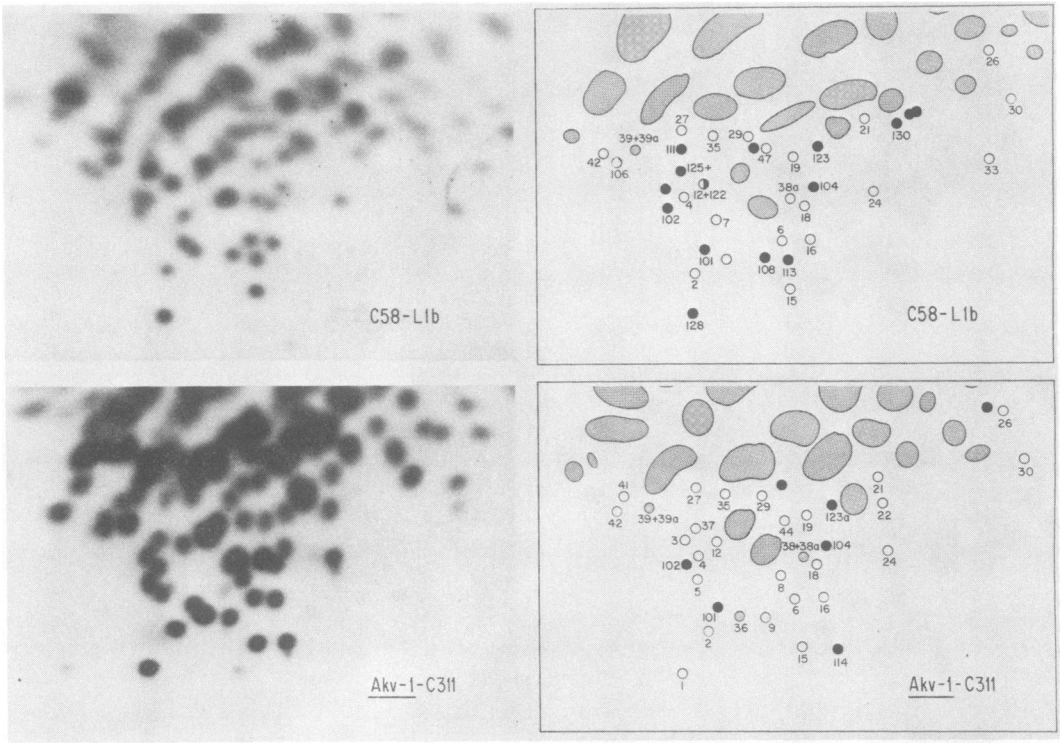

FIG. 3. RNase T_1 fingerprints of MCF viruses isolated from thymus of a C58 and an NFS.*Akv-1* congenic mouse. Fingerprints and diagrams as in Fig. 2.

comprise a highly related set. They share many T_1 oligonucleotides found in their ecotropic progenitors, and, in addition, many of the MCF-specific oligonucleotides are shared by many of the isolates. The relatedness of the MCF-specific sequences found in different MCF isolates is summarized in Table 2, where the distribution of MCF-specific oligonucleotides among all the isolates analyzed to date is shown.

T_1 oligonucleotide maps. Previous studies have shown that large T_1 oligonucleotides shared by different type C viruses occupy the same relative positions in T_1 oligonucleotide maps of the respective viruses (31). Thus, one can use previously determined oligonucleotide maps of Akv and MCF viruses to construct T_1 oligonucleotide maps for the majority of oligonucleotides appearing in fingerprints of the ecotropic and MCF isolates studied here (31). The previously determined oligonucleotide maps of Akv and MCF viruses (31) were constructed by the classical method of Wang et al. (41) and Coffin and Billeter (5) and revealed positions of the large T_1 oligonucleotides relative to each other and to the 3′ end of the viral RNA. We were interested in obtaining more accurate physical locations for the T_1 oligonucleotides along the genome to identify more precisely those genetic elements that distinguish different MCF viruses. This was done by preparing T_1 fingerprints of Akv viral RNA that hybridized to restriction enzyme-generated fragments of a molecular DNA clone of AKV virus (Lung and Hopkins, unpublished method) and, in the 3′ third of the genome, from nucleotide sequences of appropriate viruses (19, 20, 38). The improved T_1 oligonucleotide map of Akv virus is shown in Fig. 7.

(i) Positions of T_1 oligonucleotides shared by ecotropic and MCF viruses. We used the improved T_1 oligonucleotide maps shown in Fig. 7 to deduce the position in MCF genomes of T_1 oligonucleotides shared by MCF viruses and their ecotropic parents. The results of this analysis are shown in Fig. 8 and 9. It is apparent that the genomes of the two biological classes of MCF viruses differ consistently in the following ways. (i) All of the class I (thymic) MCF viruses share some T_1 oligonucleotides, and all share oligonucleotide 18 with their ecotropic parents in the p15E-coding region (Fig. 9). Class II (spleen) MCFs have replaced this entire region with T_1 oligonucleotides derived from their non-ecotropic parent. (ii) Class I MCFs lose the large T_1 oligonucleotide 14 (Fig. 7 through 9) that marks the U3 portion of the LTR of their ecotropic progenitors, whereas the class II MCFs

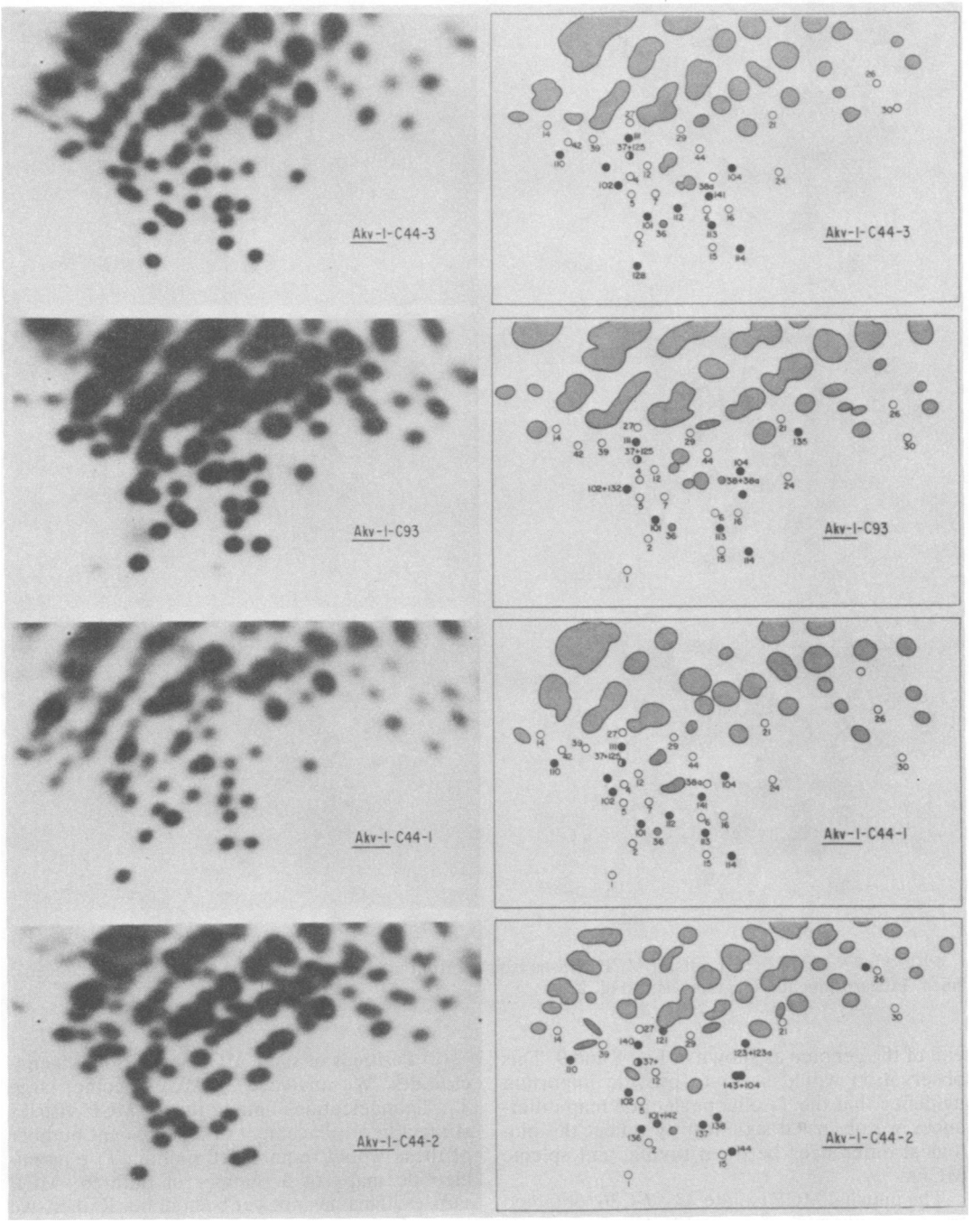

FIG. 4. RNase T_1 fingerprints of MCF viruses isolated from nonthymic neoplasms of NFS.*Akv-1* congenic mice. Fingerprints and diagrams as in Fig. 2.

share this sequence with their ecotropic parents.

Class I MCFs can share T_1 oligonucleotides encoding the C-terminal portion of gp70 with either their ecotropic or nonecotropic parent, whereas class II MCF viruses invariably derive this region from their nonecotropic parents.

A most interesting finding is that MCF *Akv-1*-C311, the MCF virus isolated from the thymus of an NFS.*Akv-1* congenic mouse, a high-virus strain that only infrequently develops thymic lymphomas, resembles other thymic class I MCFs in its T_1 oligonucleotide pattern at the 3′

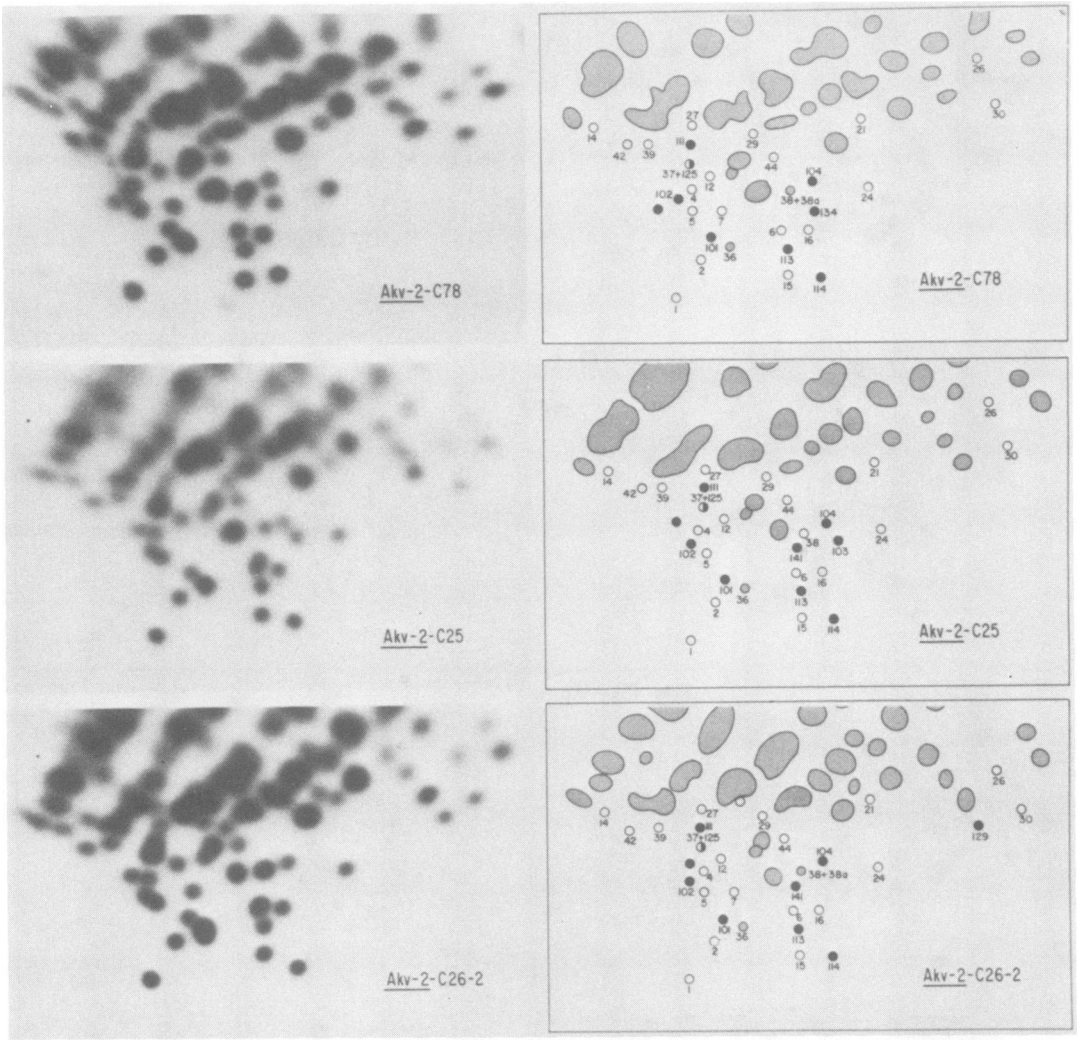

FIG. 5. RNase T_1 fingerprints of MCF viruses isolated from nonthymic neoplasms of NFS.*Akv-2* congenic mice. Fingerprints and diagrams as in Fig. 2.

end of the genome as shown in Fig. 8 and 9. This observation would seem to provide important evidence that the T_1 oligonucleotide map differences we observed significantly reflect the biological differences between thymic and splenic MCFs.

The unusual MCF isolate *Akv-1*-C36, obtained from a leukemia of an NFS.*Akv-1* congenic mouse, whose primary tissue of localization was unclear (Table 1), has biological properties intermediate between those of class I and II MCFs. As noted previously (23), the genome of this virus resembles class I MCFs in having T_1 oligonucleotides encoding p15E shared with the ecotropic parent, but it resembles class II MCFs in possessing the ecotropic T_1 oligonucleotide (14) located in the U3 portion of the LTR.

(ii) Positions of some MCF-specific T_1 oligonucleotides. We analyzed 37 MCF-specific, large T_1 oligonucleotides among the 19 MCF viruses studied here. Placement of a significant number of these would require determining T_1 oligonucleotide maps of a number of different MCF viruses. This has not yet been done. Rather, we have used the T_1 oligonucleotide maps determined previously by Rommelaere et al. (31) for two MCF viruses (MCF 247 and MCF *Akv-2*-C34) to determine the approximate location and the presence or absence of 12 MCF-specific T_1 oligonucleotides among the different isolates studied. It should be noted that the region of MCF 247 whose nucleotide sequence has been determined (the region encoding the C-terminal end of gp70, Prp15E, and the LTR) contained

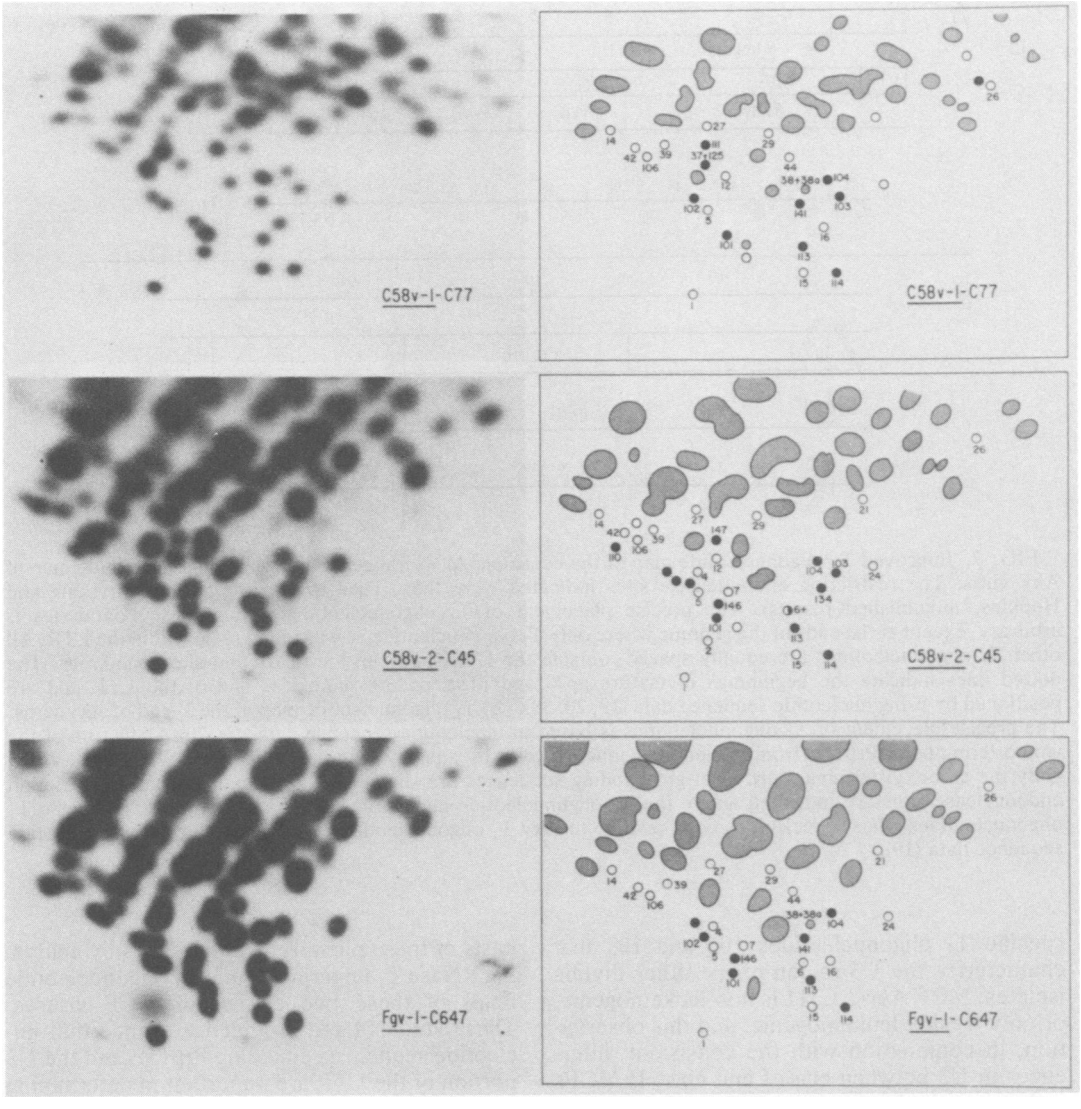

FIG. 6. RNase T_1 fingerprints of MCF viruses isolated from nonthymic neoplasms of NFS.*C58v-1*, NFS.*C58v-2*, or NFS.*Fgv-1* congenic mice. Fingerprints and diagrams as in Fig. 2.

only three of the MCF-specific T_1 oligonucleotides we had analyzed (19). The placement of these three important oligonucleotides is shown in Fig. 7B. The presence or absence of the nine crudely mapped and the three precisely localized MCF-specific T_1 oligonucleotides for all the MCFs studied is shown in Fig. 10. It is apparent from Fig. 10 that the nonecotropic parents of MCFs are a highly related set of viruses. Interestingly, the two classes of MCF viruses, with the exception of the unusual class I isolate MCF *Akv-1*-C311 (see below), can be distinguished by T_1 oligonucleotide 108, located in the U3 portion of the LTR. Note also that all MCFs (with the possible exception of MCF *Akv-1*-C36) possess the MCF-specific T_1 oligonucleotide designated 101 that lies at the C-terminal end of Prp15E-coding sequences (20). By analogy with studies of Moloney murine leukemia virus, it seems likely that this T_1 oligonucleotide encodes a region of Prp15E that is cleaved off during the maturation of virus particles to yield mature p15E and a small peptide (12, 17, 40).

Interestingly, the unusual non-leukemogenic, thymic MCF isolate *Akv-1*-C311 differs from other class I MCFs in its LTR. Although, as shown above, it has lost the ecotropic LTR oligonucleotide 14, it has not acquired the MCF-

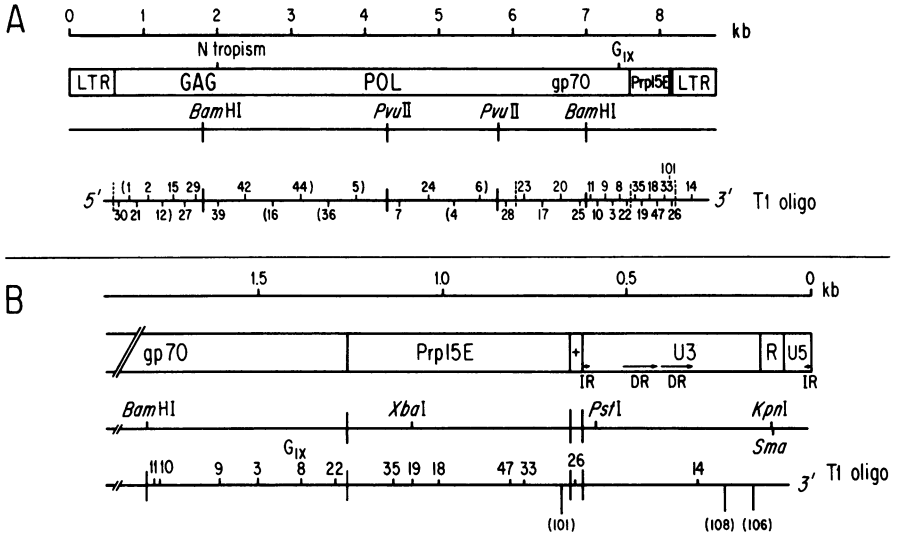

FIG. 7. Improved T_1 oligonucleotide map of the ecotropic Akv viral genome. (A) T_1 oligonucleotide map of Akv virus. The restriction endonuclease sites indicated were those used to construct the map (Lung and Hopkins, unpublished results). The precise placement of T_1 oligonucleotides within these boundaries is arbitrary, except at the ends of the genome where only T_1 oligonucleotide 14 was found to lie within the LTR. All other T_1 oligonucleotides are equally spaced, outside the LTR, between the restriction site boundaries. The dotted lines indicate the beginnings of mature gp70 and p15E coding sequences and of the LTR and are positioned by using nucleotide sequence data (19, 20, 38). (B) T_1 oligonucleotide map of the 3' end of Akv virus. The precise placement of T_1 oligonucleotides is based on nucleotide sequence data (19, 20). Definition of the amino terminus of Prp15E from nucleotide sequences was by analogy with data for Moloney leukemia virus. Only the carboxyl terminal portion of gp70-coding sequences are shown. Several commonly used restriction endonuclease sites are indicated above the T_1 oligonucleotide maps. 101, 106, and 108 are MCF-specific T_1 oligonucleotides whose precise positions relative to Akv T_1 oligonucleotides were determined from nucleotide sequence data (19).

specific T_1 oligonucleotides 106 and 108 that characterize the U3 region of the other thymic isolates. MCF *Akv-1*-C311 is non-leukemogenic, or only weakly leukemogenic, and this observation, in conjunction with the consistent differences in U3 between class I and class II MCFs or between leukemogenic MCFs and their non-leukemogenic ecotropic progenitors, might be taken as further evidence that the precise nucleotide sequence of the U3 region of the LTR is important in determining the leukemogenic phenotype of MCF viruses.

DISCUSSION

MCF viruses isolated from thymomas or from thymus of inbred mice that normally develop thymomas, are generally thymotropic, plate preferentially on SC-1 versus NFS embryo cells, and induce accelerated leukemia in AKR mice (4, 33). MCF viruses isolated from nonthymic leukemias do not replicate efficiently in the thymus, plate preferentially on NFS embryo versus SC-1 cells, and generally fail to induce leukemia when injected into young mice of a variety of strains. We investigated the genetic basis of these phenotypic differences by analyzing RNase T_1 fingerprints and T_1 oligonucleotide maps of these two classes of MCF viruses. These studies have provided evidence that nucleotide sequences encoding Prp15E and the U3 portion of the LTR are important in determining the phenotypes of MCF viruses. The importance of gp70 was already apparent, of course, since it defines and led to the discovery of MCF viruses (10, 14).

Genomic differences between biologically distinct MCFs. (i) p15E. Class I MCFs have recombinant Prp15E genes; class II MCFs inherit Prp15E from their nonecotropic parents.

Class I MCF viruses isolated from thymus share some T_1 oligonucleotides with their ecotropic parents in sequences that encode p15E, whereas the class II, nonthymic isolates possess only nonecotropic T_1 oligonucleotides in this region. Although different thymic MCF isolates share different numbers of T_1 oligonucleotides encoding p15E with their ecotropic parents, one ecotropic T_1 oligonucleotide, 18 (Fig. 7 through 9), was present in all of the isolates. Oligonucleotide 18 lies between nucleotides 226 and 246 in

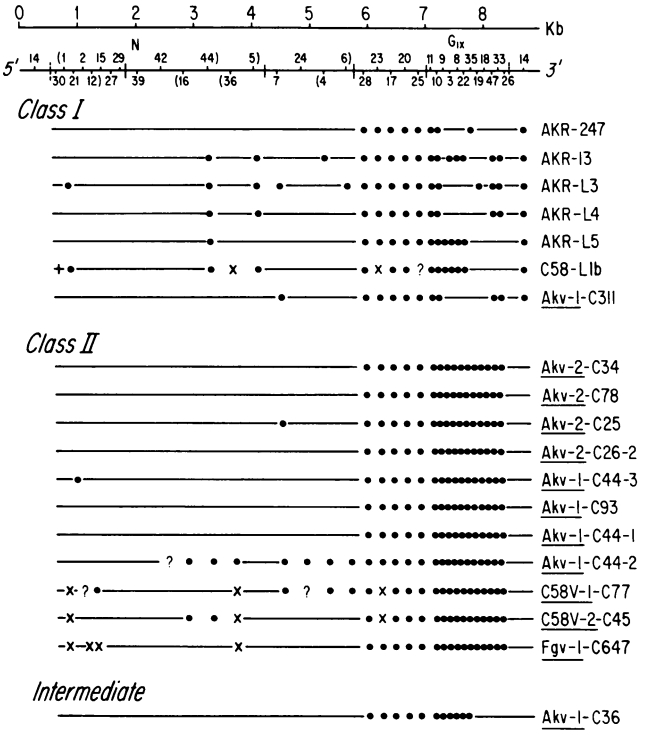

FIG. 8. Ecotropic T_1 oligonucleotides shared with MCF viruses. The improved T_1 oligonucleotide map of the Akv viral genome (from Fig. 7A) is shown at the top. Each line below the map represents the genome of an MCF virus. Symbols: ———, T_1 oligonucleotides shared by the ecotropic and MCF viruses; ●, ecotropic T_1 oligonucleotides that are absent in the MCF T_1 fingerprints; +, corresponding Akv T_1 oligonucleotide is not present in the presumptive ecotropic parent of the MCF, but is present in the MCF virus and presumably was derived from the nonecotropic parent; ×, corresponding Akv T_1 oligonucleotide is not present in the ecotropic parent of the MCF and so is not expected to appear in the MCF; ?, presence of the corresponding Akv oligonucleotide in the MCF virus is uncertain, usually because its position in the second dimension fingerprinting gel is now occupied by one or more MCF-specific T_1 oligonucleotides.

the 603-nucleotide open reading frame of Prp15E (19). Because we have not yet identified the nonecotropic parent(s) of MCFs, our analysis alone could not reveal whether oligonucleotide 18 was inherited from the ecotropic or nonecotropic parent. However, W. Herr (personal communication) has found that sequences in this region of p15E are ecotropic specific in a hybridization assay. Furthermore, Chattopadhyay et al. (2) found that an *Xba*I restriction enzyme site that Kelly et al. (19) located 58 nucleotides to the 5′ side of oligonucleotide 18, and within p15E coding sequences, is ecotropic specific and present in class I MCFs. Thus, we conclude that the oligonucleotide 18 present in thymic class I MCFs was derived from the ecotropic parents. This conclusion, in conjunction with the presence of ecotropic type-specific T_1 oligonucleotides in the 5′ end of MCF genomes, the presence of nonecotropic sequences encoding portions of gp70, the C-terminal end of Prp15E, and the U3 region, means that class I MCFs arise by a minimum of three crossover events (at least two events surrounding gp70 coding sequences and one in the middle or toward the 3′ end of the Prp15E-coding sequences).

Although the 5′ to midportion of the p15E gene is inherited from the ecotropic parent in class I MCFs, these viruses invariably derive sequences encoding the C-terminal end of Prp15E from their nonecotropic parent. This region of the genome is monitored by the MCF-specific T_1 oligonucleotide designated 101, which is present in all the class I and class II MCF viruses analyzed here. DNA sequencing of a DNA clone of MCF 247 and the corresponding region of Akv virus revealed that oligonucleotide 101 signals a region of alteration in MCF 247 relative to its ecotropic parent that extends out to the 3′ end of the genome (19). In MCF 247, the introduction of non-Akv sequences in this region results in changes in amino acids near the C terminus of Prp15E (19, 20). Akv virus possesses the sequence lys-thr-ile-glu-asp-cys-lys-

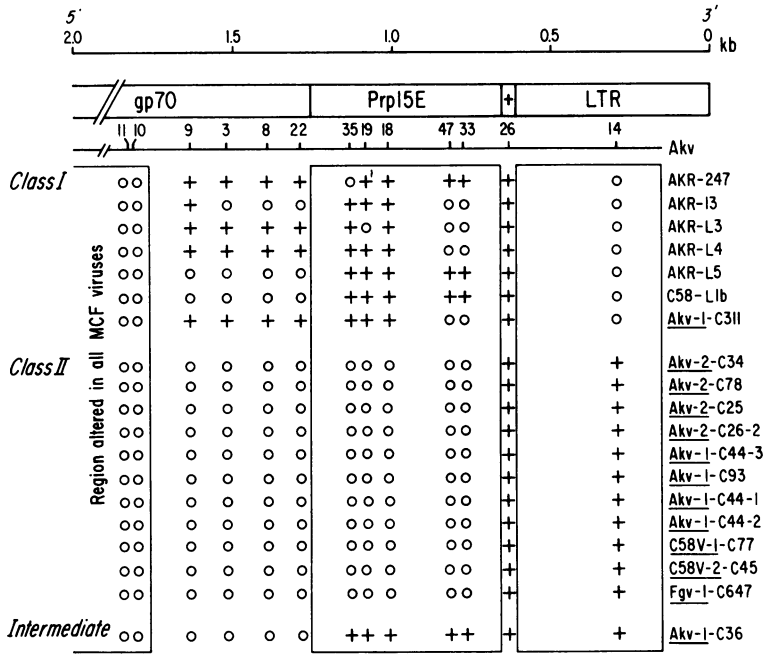

FIG. 9. Ecotropic T_1 oligonucleotides from the 3' end shared with MCF viruses. The 13 3'-terminal T_1 oligonucleotides of the Akv genome (Fig. 7B) are shown and their presence (+) or absence (O) in each MCF virus is indicated.

ser-arg-glu, MCF 247 has lys-ser-ile-asp-pro-glu-glu-val-glu-ser-arg-glu. These amino acids may be cleaved off during virus maturation to yield mature p15E (12, 17, 40). Why the nucleotide or amino acid sequences in this region might be critical to the phenotype or to the construction of viable MCF viruses is unknown. It should be noted that although all class I and II MCF viruses share T_1 oligonucleotide 101 at the 3' end of *env*, it is of course possible that the precise nucleotide (and amino acid) sequences surrounding this oligonucleotide differ for the two sets of viruses.

Our observations would seem to confirm the importance of nucleotide sequences of p15E in determining the biological properties of class I versus class II MCFs, but they do not allow us to conclude which (if any) of the recognized phenotypes of class I MCF viruses depend on the presence of ecotropic sequences in the amino terminal half of the p15E gene. However, analysis of a set of weakly leukemogenic viruses isolated from AKR mice by O'Donnell et al. (27) implies that the presence of ecotropic sequences encoding the middle of p15E in class I MCFs is genetically linked to the highly leukemogenic phenotype (Lung, O'Donnell, and Hopkins, unpublished results).

Oligonucleotide 26 lies in the putative origin of +DNA strand synthesis, between Prp15E and the LTR. This region of the genome is highly conserved among murine leukemia viruses, and so, not surprisingly, oligonucleotide 26 is found in ecotropic as well as class I and II MCFs. In class I MCFs this entire region is almost certainly derived from the nonecotropic parent.

(ii) U3. It is probable that all of the class I (thymic) MCFs derive the entire U3 portion of their LTRs from their putative xenotropic parents, a result confirmed for MCF 247, whose LTR has been sequenced (19). All class I MCFs lack ecotropic T_1 oligonucleotide 14 (Fig. 8 and 9), and, with the exception of the unusual thymic isolate MCF *Akv-1*-C311, all possess oligonucleotides 106 and 108 in U3 (Fig. 10). Class II MCFs share the ecotropic T_1 oligonucleotide marker 14, present in U3. Thus our analysis is consistent with the possibility that these viruses derive their U3 region entirely from their ecotropic progenitors, although we cannot exclude the possibility that we simply fail to detect recombination events in the U3 region of class II MCFs.

Interestingly, the unusual MCF virus isolated from the thymus of an NFS.*Akv-1* congenic mouse, MCF *Akv-1*-C311, differs from the other thymic MCFs in lacking oligonucleotides 106 and 108. Biologically, this isolate differs from the other class I MCFs in being non-leukemogenic or only weakly so. Thus, the T_1 oligonucleotide pattern in the U3 region of MCF *Akv-1*-

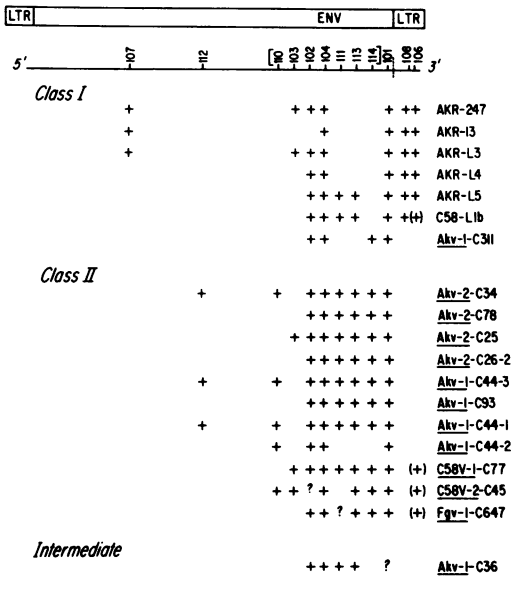

FIG. 10. Presence of some MCF-specific T_1 oligonucleotides in T_1 fingerprints of all MCF viruses analyzed. Only T_1 oligonucleotides mapped by Rommelaere et al. (31) are shown, except for oligonucleotide 108 in U3, whose placement was determined from nucleotide sequencing of a DNA clone of MCF 247 (19). The DNA sequences also revealed the precise placement of T_1 oligonucleotides 101 and 106 (Fig. 7B). The placement of all other T_1 oligonucleotides is arbitrary and limited only by their placement relative to Akv T_1 oligonucleotides. Endogenous ecotropic viruses of C58 and C3H/Figge possess oligonucleotide 106 in U3 (+). Thus, this oligonucleotide can only be considered MCF specific when the ecotropic parent of an MCF lacks 106, as is the case with Akv-1 or -2 viruses. Since all class I MCFs lose the U3 Akv oligonucleotide 14 and usually acquire oligonucleotides 106 and 108 in U3, it seems likely that 106 and the entire U3 region are acquired from the nonecotropic parent in MCF C58-L1b and MCF *Akv-1*-C311. Class II MCFs, all of which possess the ecotropic oligonucleotide 14 in U3, also possess 106 in U3 if they arise from ecotropic viruses possessing 106. In these cases, it seems likely that 106 (and possibly the entire U3 region) was acquired from the ecotropic parent.

C311, in conjunction with the consistent differences in U3 seen between class I and class II MCFs and between leukemogenic MCFs and their non-leukemogenic progenitors, supports the notion that particular nucleotide sequences in U3, characterized by the presence of both oligonucleotides 106 and 108 and by the absence of oligonucleotide 14, are correlated with the leukemogenic phenotype among MCFs of inbred mice. That nucleotide sequences of U3 are important in leukemogenesis by type C viruses has been deduced from genetic studies of avian leukosis viruses (29, 36), where these sequences may affect transcriptional activation of the cellular *myc* gene (15, 25, 28).

gp70. Our analysis failed to detect consistent differences in gp70-coding sequences between the two biological classes of MCF viruses. However, it is entirely possible that this simply reflects limitations of the data. Futhermore, we have never been able to identify ecotropic T_1 oligonucleotides that might be correlated with the mouse-tropic phenotype of dual tropic MCF viruses. This might reflect the fact that T_1 oligonucleotides represent only 5 to 10% of the genome. For example, Coffin and his collaborators found that dual-tropic avian type C viruses obtained by recombination of subgroup B or D and E viruses possessed only one of the recognized parental subgroup E T_1 oligonucleotides located in the gp85-coding sequences (37). Alternatively, the gp70s of some MCFs may be inherited in toto from as yet unidentified endogenous viruses with dual tropic host range, a possibility suggested by several investigators (2; A. Rein, submitted for publication).

Our observations concerning sequence patterns in the sequences coding Prp15E, the C-terminal portion of gp70, and the LTR of class I versus class II MCFs are summarized in Fig. 11.

Further observations concerning the nonecotropic parents of MCF viruses. Examination of MCF genomic T_1 fingerprints reveals that the nonecotropic, putatively xenotropic, parents of MCFs are a highly related set of viral genomes (Table 2 and Fig. 10). The data are not sufficient to allow us to determine how many different xenotropic genomes can participate in the formation of MCFs within a given inbred strain, although one can say with confidence that the MCF isolate *Akv-1*-C44-2 had a different nonecotropic parent(s) than the other isolates from NFS.*Akv-1* congenic mice.

Several years ago, J. Rommelaere and N. Hopkins analyzed RNase T_1 fingerprints of xenotropic viruses isolated from several inbred mouse strains in an attempt to identify the nonecotropic parents of MCF viruses (unpublished results). These studies revealed that certain xenotropic viruses possess many, and in some cases almost all, of the nonecotropic T_1 oligonucleotides present in MCF viruses and thus are clearly highly related to the missing MCF parents. However, the xenotropic viruses usually lack at least one or more MCF-specific T_1 oligonucleotides, suggesting that the xenotropic viruses readily obtained from inbred mice may not be those involved in MCF formation. Others have reached similar conclusions (8, 11).

The oligonucleotide data indicate that MCF virus formation involves multiple recombinational events; these events might occur sequen-

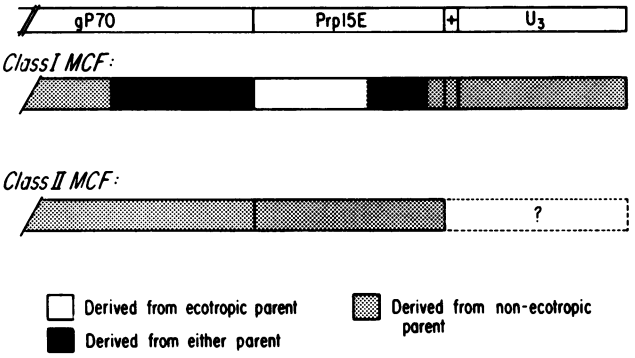

FIG. 11. Diagramatic representation of the parental origin of 3'-end sequences in class I versus class II MCFs. Class I MCFs can inherit sequences encoding the 3' end of gp70 from either their ecotropic or nonecotropic parent, whereas class II viruses lack all of the ecotropic T_1 oligonucleotides in this region. Class I MCFs possess recombinant Prp15E genes with the 5' half or more completely or almost completely shared with the ecotropic parent, whereas the 3' half or at least the extreme 3' end of the gene is always of nonecotropic origin. Class II MCFs possess only nonecotropic T_1 oligonucleotides in the Prp15E region. In class I MCFs, the nonecotropic sequences commencing towards the 3' end of Prp15E extend through U3. Our data are consistent with the possibility that class II MCFs inherit U3 from their ecotropic parents, but we have too little T_1 oligonucleotide data for U3s of nonecotropic viruses to be confident of this conclusion.

tially, and C. Y. Thomas and J. M. Coffin (personal communication) have recently obtained evidence that this is indeed the case. Thus it might be expected that MCF viruses may have two or more nonecotropic parents, and one might not expect to find a single endogenous type C virus possessing all of the MCF-specific T_1 oligonucleotides.

Conclusions. The original suggestion of Hartley et al. (14) that MCF viruses might be the proximal leukemogens of high-leukemic mice has received support from the ability of certain MCFs to induce leukemia (4), from the inevitable appearance of MCFs in leukemias induced by a variety of ecotropic viruses (14, 35), and from the recent finding that although AKR and other high-virus mouse strain leukemias possess multiple reinsertions of type C viruses these reinsertions are seldom ecotropic proviral genomes, but rather appear to be MCF proviruses (39; W. Herr, personal communication; F. Yoshimura, personal communication). Although numerous studies have emphasized the importance of gp70 in leukemogenesis by MCF viruses (6, 14, 24; J. N. Ihle, J. C. Lee, L. Enjuanes, L. Cicurel, I. Horak, and L. Pepersack, *in Cold Spring Harbor Conferences on Cell Proliferation: Viruses in Naturally Occurring Cancer*, in press), the biochemical analysis reported here and elsewhere (1, 23; Lung, O'Donnell, and Hopkins, unpublished results) implies that the viral p15E and U3 must also be considered as determinants of this phenotype. Given the complexity of in vivo leukemogenesis, one can imagine any number of ways in which these (three) viral genetic elements might affect the outcome of an MCF infection. In particular, they might affect the ability of a virus to replicate efficiently in appropriate cell types or affect the ability of the host to respond immunologically to the virus or to infected cells, two interactions known to affect the susceptibility of mice to type C viral leukemogenesis (21; T. Cloyd, J. W. Hartley, and W. P. Rowe, unpublished results). Recently, the stunning finding that nondefective avian type C viruses may induce leukemias by transcriptional activation of a particular cell gene (15, 29, 36) has focused attention on the role of viruses as essentially mutagenic agents in tumorigenesis. Thus, gp70, p15E, and the LTR might play a role in helping MCF viruses to effectively locate and replicate in their target cells and possibly to integrate preferentially into the chromosome. Alternatively, Scolnick and his collaborators (22) have obtained evidence that the oncogene of the erythroleukemia-inducing spleen focus-forming virus component of Friend virus is an MCF-type glycoprotein, gp52, a result analogous to the original proposal of Elder et al. (6) that certain MCF gp70s might stimulate uncontrolled proliferation of T cells. Recently, C. A. Holland, using molecular cloning techniques, has succeeded in constructing recombinant viruses that possess just one or two of the three genetic elements, gp70, Prp15E, or the LTR, from a leukemogenic MCF virus and the remainder of the viral genome from non-leukemogenic Akv virus (C. A. Holland and N. Hopkins, unpublished studies). Analysis of the biological properties of these viruses may allow one to determine the contribution of each genetic element to leukemogenesis by MCF viruses.

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LITERATURE CITED

1. **Buchhagen, D. L., F. S. Pedersen, R. L. Crowther, and W. A. Haseltine.** 1980. Most sequence differences between the genomes of the Akv virus and a leukemogenic Gross A virus passaged *in vitro* are located near the 3′ terminus. Proc. Natl. Acad. Sci. U.S.A. **77**:4359–4363.
2. **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.
3. **Chien, Y., I. Verma, T. Shih, E. Scolnick, and N. Davidson.** 1978. Heteroduplex analysis of the sequence relations between the RNAs of mink cell focus-inducing and murine leukemia viruses. J. Virol. **28**:352–360.
4. **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.
5. **Coffin, J. M., and M. A. Billeter.** 1976. A physical map of Rous sarcoma virus genome. J. Mol. Biol. **100**:293–318.
6. **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.
7. **Faller, D. V., and N. Hopkins.** 1977. RNase T1 resistant oligonucleotides of B-tropic murine leukemia virus from BALB/c and five of its NB-tropic derivatives. J. Virol. **23**:188–195.
8. **Faller, D. V., and N. Hopkins.** 1978. T1 oligonucleotide maps of Moloney and HIX murine leukemia viruses. Virology **90**:265–273.
9. **Fischinger, P., A. Frankel, J. Elder, R. Lerner, J. Ihle, and D. Bolognesi.** 1978. Biological, immunological, and biochemical evidence that HIX virus is a recombinant between Moloney leukemia virus and a murine xenotropic C-type virus. Virology **90**:241–254.
10. **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.
11. **Green, N., H. Hiai, J. H. Elder, R. A. Schwartz, K. H. Khiroya, C. Y. Thomas, P. N. Tsichlis, and J. M. Coffin.** 1980. Expression of leukemogenic recombinant viruses associated with a recessive gene in HRS/J mice. J. Exp. Med. **152**:249–264.
12. **Green, N., T. M. Shinnick, O. N. Witte, A. Ponticelli, J. G. Sutcliffe, and R. A. Lerner.** 1981. Sequence-specific antibodies show that maturation of Moloney leukemia virus envelope polyprotein involves removal of a COOH-terminal peptide. Proc. Natl. Acad. Sci. U.S.A. **78**:6023–6027.
13. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology **65**:128–134.
14. **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.
15. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1981. Activation of a cellular *onc* gene by promoter insertion in ALV-induced lymphoid leukosis. Nature (London) **290**:475–480.
16. **Kaplan, H.** 1967. On the natural history of the murine leukemias: presidential address. Cancer Res. **27**:1325–1340.
17. **Karshin, W. L., L. J. Arcement, R. B. Naso, and R. B. Arlinghams.** 1977. Common precursor for Rauscher leukemia virus gp69/71, p15(E) and p12(E). J. Virol. **23**:787–789.
18. **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**:4680–4684.
19. **Kelly, M., C. A. Holland, M. L. Lung, S. K. Chattopadhyay, D. R. Lowy, and N. H. Hopkins.** 1982. Nucleotide sequence of the 3′ end of MCF 247 murine leukemia virus. J. Virol. **45**:291–298.
20. **Lenz, J., R. Crowther, A. Straceski, and W. Haseltine.** 1982. Nucleotide sequence of the Akv *env* gene. J. Virol. **42**:519–529.
21. **Lilly, F., M. L. Duran-Reynals, and W. P. Rowe.** 1975. Correlation of early murine leukemia virus titer and H-2 type with spontaneous leukemia in mice of the BALB/c × AKR cross: a genetic analysis. J. Exp. Med. **141**:882–889.
22. **Linemeyer, D. L., S. K. Ruscetti, E. M. Scolnick, L. H. Evans, and P. H. Duesberg.** 1981. Biological activity of the spleen focus-forming virus is encoded by a molecularly cloned subgenomic fragment of spleen focus-forming virus DNA. Proc. Natl. Acad. Sci. U.S.A. **78**:1401–1405.
23. **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.
24. **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.
25. **Neel, B. G., W. S. Hayward, H. C. Robinson, J. M. Fang, and S. M. Astrin.** 1981. Avian leukosis-induced tumors have common proviral integration sites and synthesize discrete new RNAs: oncogenesis by promoter insertion. Cell **23**:323–334.
26. **Nishizuka, Y., and K. Nakakuki.** 1968. Acceleration of leukemogenesis in AKR mice by grafts, cell suspensions, and cell-free centrifugates of thymuses from preleukemic AKR donors. Int. J. Cancer **3**:203–210.
27. **O'Donnell, R. V., E. Stockert, Y. Obata, A. B. DeLeo, and L. J. Old.** 1979. Murine-leukemia-virus-related cell-surface antigens as serological markers of AKR ecotropic, xenotropic, and dual-tropic viruses. Cold Spring Harbor Symp. Quant. Biol. **44**:1255–1264.
28. **Payne, G. S., S. A. Courtneidge, L. B. Crittenden, A. M. Fadly, J. M. Bishop, and H. E. Varmus.** 1981. Analysis of avian leukosis virus DNA and RNA in bursal tumors: viral gene expression is not required for maintenance of the tumor state. Cell **23**:311–322.
29. **Robinson, H. L., M. N. Pearson, D. W. DeSimone, P. N. Tsichlis, and J. M. Coffin.** 1979. Subgroup-E avian-leukosis-virus-associated disease in chickens. Cold Spring Harbor Symp. Quant. Biol. **44**:1133–1142.
30. **Rommelaere, J., D. V. Faller, and N. Hopkins.** 1977. RNase T_1-resistant oligonucleotides of Akv-1 and Akv-2 type C viruses of AKR mice. J. Virol. **24**:690–694.
31. **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.
32. **Rowe, W. P.** 1977. Leukemia virus genomes in the chromosomal DNA of the mouse. Harvey Lect. Ser. **71**:173–192.
33. **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.

34. **Rowe, W. P., and T. Pincus.** 1972. Quantitative studies of naturally occurring murine leukemia virus infection in AKR mice. J. Exp. Med. **135**:429–436.
35. **Troxler, D. H., E. Yuan, D. Linemeyer, S. Ruscetti, and E. M. Scolnick.** 1978. Helper-independent mink cell focus-inducing strains of Friend murine type-C virus: potential relationship to the origin of replication defective spleen focus-forming virus. J. Exp. Med. **148**:639–653.
36. **Tsichlis, P. N., and J. M. Coffin.** 1979. Role of the C region in relative growth rates of endogenous and exogenous avian oncoviruses. Cold Spring Harbor Symp. Quant. Biol. **44**:1123–1132.
37. **Tsichlis, P. N., K. F. Conklin, and J. M. Coffin.** 1980. Mutant and recombinant avian retroviruses with extended host range. Proc. Natl. Acad. Sci. U.S.A. **77**:536–540.
38. **Van Beveren, C., E. Rands, S. K. Chattopadhyay, D. R. Lowy, and I. M. Verma.** 1982. Long terminal repeat of murine retroviral DNAs: sequence analysis, host-proviral junctions, and preintegration site. J. Virol. **41**:542–556.
39. **van der Putten, H., W. Quint, J. van Raaij, E. R. Maandag, I. M. Verma, and A. Berns.** 1981. M-MLV-induced leukemogenesis: integration and structure of recombinant proviruses in tumors. Cell **24**:729–739.
40. **van de Ven, W. J. M., D. van Zaane, C. Onnekink, and H. P. J. Bloemers.** 1978. Impaired processing of precursor polypeptides of temperature-sensitive mutants of Rauscher murine leukemia virus. J. Virol. **25**:553–561.
41. **Wang, L., D. Galehouse, P. P. Mellon, P. Duesberg, W. S. Mason, and P. K. Vogt.** 1976. Mapping oligonucleotides of Rous sarcoma virus RNA that segregate with polymerase and group-specific antigen markers in recombinants. Proc. Natl. Acad. Sci. U.S.A. **73**:3952–3956.