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Class II Polytropic Murine Leukemia Viruses (MuLVs) of AKR/J Mice: Possible Role in the Generation of Class I Oncogenic Polytropic MuLVs

LEONARD H. EVANS* AND FRANK G. MALIK

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840

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We examined the frequency of occurrence of polytropic murine leukemia viruses (MuLVs) in the spleens and thymuses of preleukemic AKR/J mice from 1 week to 6 months of age and analyzed the genomic RNAs of several polytropic isolates by RNase T_1 oligonucleotide fingerprinting. Polytropic MuLVs were first detected in the spleens of 3-week-old mice and preceded the appearance of polytropic MuLVs in the thymus by over 1 month. At 4 months of age and older, nearly all mice expressed polytropic MuLVs in both organs. In contrast to previous studies which have identified class I polytropic MuLVs in AKR/J mice, fingerprint analysis of polytropic MuLVs from both young (3- to 4-week-old) and older (5- to 6-month-old) preleukemic mice indicated that a large proportion of viruses at both ages were class II polytropic MuLVs. All polytropic viruses (five isolates) analyzed from 3- to 4-week-old mice were recovered from spleen cells and were class II polytropic MuLVs. In older preleukemic mice, five of seven isolates were class II polytropic MuLVs and two were class I polytropic viruses. Class I and class II polytropic MuLVs were recovered from both the spleens and thymuses of older preleukemic mice. A detailed comparison of the class I and class II polytropic MuLVs from 5- to 6-month-old mice revealed that the nonecotropic gp70 sequences of most of the class I and class II MuLVs were identical, consistent with a common origin for these sequences. In contrast, the nonecotropic p15E sequences of class I MuLVs were clearly derived from different endogenous sequences than the nonecotropic p15E sequences of the class II MuLVs. The in vitro host ranges of class I and class II polytropic viruses were clearly distinguishable. Examination of the in vitro host range of several isolates suggested that the predominant polytropic viruses initially identified in the thymus (2 to 3 months of age) were class II polytropic viruses. The order of appearance of the class I and class II polytropic MuLVs and the identity of the gp70 oligonucleotides of these MuLVs suggested a model for the stepwise generation of class I polytropic MuLVs involving a class II polytropic MuLV intermediate.

Polytropic murine leukemia viruses (MuLVs) arise by recombination of ecotropic MuLVs with endogenous retroviral sequences of mice and are implicated in the etiology of ecotropic MuLV-induced leukemias in several murine systems (6, 9, 14, 16, 18, 28, 31, 32, 37, 38, 43–46). The parental ecotropic viruses are infectious only for murine cells; however, the recombinant polytropic MuLVs exhibit an altered in vitro host range characterized by the ability to infect both murine cells and certain heterologous cell lines (15, 18). Many polytropic viruses induce cytopathic effects (CPE) on mink cells and are thus frequently termed mink cell focus-inducing viruses (18). Polytropic viruses were initially detected as contaminants of ecotropic virus stocks (15) and were soon afterwards detected in AKR mice which harbor endogenous ecotropic MuLVs and exhibit a high incidence of spontaneous leukemia (18).

The structures of the RNA genomes of polytropic MuLVs from AKR mice have been extensively characterized (2, 3, 22, 26, 29, 30, 36, 42). The genomes of these recombinant viruses exhibit closely related structural features with regard to the regions of the genome in which ecotropic sequences have been substituted with nonecotropic sequences, and the sequence homology of the substituted sequences. Polytropic MuLV isolates from AKR mice exhibit two substitutions of ecotropic MuLV sequences with nonecotropic sequences (29, 30). One substitution encompasses the 5′ sequences of the *env* gene and sometimes includes most of the gp70-coding region. A second substitution includes 3′ sequences of the p15E-coding region and extends in the 3′ direction to encompass the U_3 region of the long terminal repeat (LTR). The 5′ region of the p15E-coding sequences of these polytropic MuLVs is derived from the ecotropic MuLV parent. Viruses exhibiting the above-described structure have been termed class I polytropic viruses (29, 30) and are frequently oncogenic (9, 32, 37). Since no endogenous retroviral species has been identified which contains both the 5′ gp70 and the 3′ p15E/U_3 nonecotropic sequences found in class I polytropic MuLVs, it has been postulated that the two nonecotropic substitutions are derived from different endogenous sequences by a stepwise mechanism of recombination (35, 42). In this model the 3′ p15E/U_3 substitution is derived by recombination of the ecotropic MuLV with an endogenous xenotropic MuLV, while the 5′ gp70 sequences are derived by recombination with another endogenous retroviral species. In support of this hypothesis, Thomas and Coffin (42) have identified intermediate recombinant MuLVs in the thymuses of preleukemic mice which contain the nonecotropic 3′ p15E/U_3 sequence but not the 5′ gp70 sequences of the class I polytropic MuLVs. Recombination of this intermediate with an endogenous retroviral species containing the nonecotropic 5′ gp70 sequences would yield a class I polytropic virus. Endogenous xenotropic viruses which could contribute the 3′ p15E/U_3 sequences have been

* Corresponding author.

identified (21, 35, 42); however, the source of the 5′ gp70 sequences is unknown.

A recent report from this laboratory described the occurrence of polytropic MuLVs in spleens of very young (3-week-old) AKR/J mice (11). Analyses of one of the polytropic isolates from a young mouse revealed a structure typical of a class II polytropic virus. Class II polytropic viruses are characterized by a single substitution of nonecotropic sequences which encompasses the 5′ gp70 sequences and frequently includes the entire *env* gene (29, 30). The LTR of class II polytropic viruses is of ecotropic origin. Polytropic viruses had not been previously identified in AKR mice younger than 5 to 6 months of age (2, 18, 37, 38, 39, 47), and class II polytropic MuLVs had previously only been identified in other mouse strains (29, 30, 41). A class II polytropic virus could potentially participate as the donor of the gp70 sequences in a stepwise mechanism of class I polytropic MuLV generation similar to that proposed by Thomas and Coffin (42). However, it was unclear from our previous analyses if the structure or the occurrence of class II polytropic MuLVs in AKR/J mice was consistent with a role as an intermediate in the generation of class I polytropic MuLVs. In this study we examined the occurrence of polytropic MuLVs in the spleens and thymuses of mice throughout the preleukemic period (1 week to 6 months). We identified class II as well as class I viruses in both the spleens and thymuses of late preleukemic (5- to 6-month-old) mice and determined that oligonucleotides of some class I and class II viruses were identical in their 5′ gp70-coding sequences but differed in their 3′ p15E sequences. Furthermore, we determined that all our isolates from the spleens of young mice were class II polytropic viruses and that the predominant polytropic viruses initially identified in thymuses of mice between 2 and 3 months of age were also very likely class II polytropic MuLVs. Our results suggest that gp70 sequences of class I and class II polytropic MuLVs from AKR/J mice have a common origin and that class II polytropic MuLVs are intermediates in the generation of oncogenic class I polytropic MuLVs.

MATERIALS AND METHODS

Mice, assays, cells, and virus isolation. AKR/J mice were obtained from Jackson Laboratory (Bar Harbor, Maine) and maintained as breeding stocks at the Rocky Mountain Laboratories. Mice were sacrificed at various ages, and their spleen and thymus cells were assayed as infectious centers (IC) by a focal immunofluorescence assay (FIA) on live cells with a monoclonal antibody specifically reactive with polytropic viruses, as described previously (40). IC assays, as well as assays of cell-free viruses, were performed with SC-1 cells (mouse) (17), *Mus dunni* cells (27), and mink lung fibroblasts (19). Viruses were isolated by picking individual foci of fluorescent cells from the initial IC assays at limiting dilutions of spleen or thymus cells. The infected cells were propagated, and viruses harvested from the growth media were subsequently cloned by multiple (greater than 3) endpoint dilutions by the FIA.

Propagation of virus isolates and RNase T_1 oligonucleotide fingerprinting. The viruses were propagated on *M. dunni* cells and metabolically labeled with ^{32}P as described previously (12). The procedures for fingerprinting, mapping, and secondary analysis of T_1 oligonucleotides after digestion with RNase A have been described previously in detail (12). The oligonucleotide maps of each virus isolate were deduced from fingerprints of seven to nine different size classes of poly(A)-containing fragments of virion RNA.

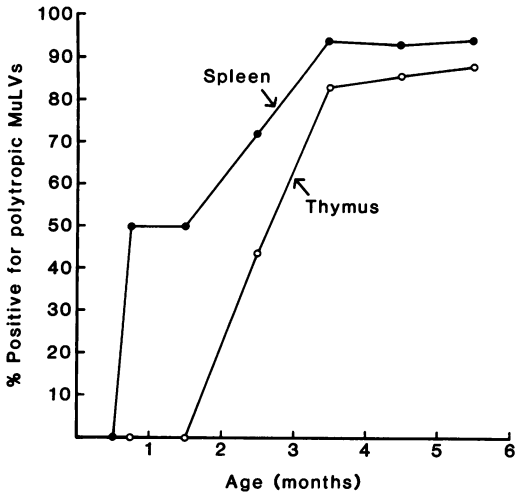


FIG. 1. Frequency of detection of polytropic viruses in preleukemic AKR/J mice of various ages. The spleen and thymus cells of preleukemic mice from 1 week to 6 months of age were assayed as ICs by the FIA with antibody Hy 7 on *M. dunni*, SC-1, and mink cells. Spleen or thymus cells from mice which scored with Hy 7 on any of the cell lines were considered positive. The percentage of mice which expressed polytropic viruses in the spleen or thymus was calculated for different intervals of age. The number of mice tested in each age interval was: 1 to 3 weeks, 12 mice; 3 to 4 weeks, 12 mice; 1 to 2 months, 14 mice; 2 to 3 months, 18 mice; 3 to 4 months, 18 mice; 4 to 5 months, 14 mice; and 5 to 6 months, 16 mice. The youngest age in which polytropic MuLVs were detected in the spleen was 21 days and in the thymus was 59 days.

RESULTS

Incidence of polytropic MuLVs in preleukemic AKR/J mice. It was previously demonstrated that polytropic viruses can be detected in the spleens, but not in the thymuses, of mice less than 1 month of age (11). At 5 to 6 months of age polytropic viruses were found in both organs. It was of interest to determine the lag period between the initial detection of polytropic MuLVs in the spleen and the subsequent appearance of polytropic viruses in the thymus. To accomplish this, AKR/J mice of various ages were sacrificed, and their spleens and thymus cells were assayed as IC on in vitro cell lines by the FIA with antibody Hy 7 (8) (Fig. 1). Hy 7 is unreactive with ecotropic MuLVs but yields intensely fluorescent foci with all polytropic MuLVs we have isolated from AKR/J mice. Although Hy 7 is not as broadly reactive as some of our polytropic-specific antibodies (one of which reacts with virtually all polytropic isolates tested) (4, 5), IC titers of AKR/J spleen and thymus cells with this antibody equaled or exceeded titers obtained with any other polytropic-specific antibody, indicating that Hy 7 reacts with the vast majority of detectable polytropic MuLVs in these mice. The incidence of polytropic MuLVs in the spleen preceded polytropic MuLVs in the thymus by over 1 month (Fig. 1). The youngest mouse in which polytropic MuLVs were detected in the spleen was 21 days of age, whereas thymic polytropic viruses were first observed in a mouse 59 days of age. After the initial detection of polytropic MuLVs in the spleen or thymus, the percentage of mice exhibiting polytropic viruses in these organs abruptly increased, approaching a 50% incidence within 2 weeks. By 3 to 4 months of age a large percentage of mice expressed polytropic viruses in both organs.

TABLE 1. Relative infectivity of in vitro cell lines by polytropic MuLVs from 5 to 6-month-old AKR/J mice

Virus designation[a]	Relative infectivity[b]			Induction of CPE on mink cells
	M. dunni	SC-1	Mink	
M60P-T	12	3	1	+[c]
M73P-S	2	0.3	1	+[c]
M62P-S	1,260	16	1	−
M72P-S	2,000	25	1	−
M75P-S	1,990	31	1	−
M75P-T	830	10	1	−
M79P-T	2,510	32	1	−

[a] Designation of the viruses is as follows. The first three characters identify the mouse from which the isolate was obtained. The fourth character (P) indicates the virus class (polytropic), and the last character (S or T) identifies the organ (spleen or thymus).

[b] Titers on *M. dunni* and SC-1 cells were normalized to the titers on mink cells.

[c] CPE induced by M60P-T were much smaller than those induced by M73P-S.

In vitro properties of polytropic MuLVs from 5- to 6-month-old AKR/J mice. Polytropic MuLVs isolated from young mice (ca. 1 month old) were previously found to be approximately 10-fold more infectious for SC-1 cells than for mink cells and approximately 100-fold more infectious for an *M. dunni* cell line than for SC-1 cells (11). None of the isolates from young mice induced CPE on mink cells. In this study we analyzed in detail seven polytropic MuLVs from 5- to 6-month-old mice. Five of these viruses exhibited an in vitro host range similar to the host range of viruses from young mice (Table 1) and did not induce CPE on mink cells. However, two of the isolates (M60P-T and M73P-S) did induce CPE on mink cells. The CPE-positive viruses exhibited titers on mink cells which were approximately equal to titers on SC-1 cells and were only moderately lower than titers on *M. dunni* cells. Even though polytropic viruses with distinctly different in vitro properties were identified, we were unable to distinguish any of the polytropic viruses we isolated from AKR/J mice on the basis of their reactivities to a panel of hybridoma antibodies (data not shown).

RNase T_1-resistant oligonucleotide analyses of polytropic MuLVs from 5- to 6-month-old mice. Previous analyses of polytropic MuLVs from older preleukemic or leukemic AKR mice have revealed only class I polytropic MuLVs (2, 3, 29, 30, 36, 42). As noted earlier, we recently reported the analysis of one polytropic isolate from a young AKR/J mouse which revealed that it was a class II MuLV, characterized by a single substitution of nonecotropic sequences encompassing the entire *env* gene but not including the LTR (11). Here we fingerprinted and mapped the oligonucleotides of each of the seven isolates from 5- to 6-month-old mice. These analyses were done to determine the occurrence and approximate proportion of class II polytropic MuLVs in older mice, the heterogeneity of class II isolates, and their structural relationship to class I polytropic MuLVs. The fingerprints of the viruses revealed that all the isolates were unique. Although some viruses differed from others by only a few oligonucleotides, all were distinguishable from one another in both the residual ecotropic oligonucleotides and the nonecotropic oligonucleotides contained in their genomes (Fig. 2 to 4). Oligonucleotide maps of the viruses revealed that five of the seven isolates from 5- to 6-month-old mice were class II polytropic MuLVs and that the remaining two were class I viruses (Fig. 5). The class II viruses corresponded to those which exhibited in vitro properties similar to those of polytropic isolates from young mice, whereas the class I viruses corresponded to the two CPE-positive MuLVs (M60P-T and M73P-S; Table 1). We have also included a more complete analysis of the previously reported class II polytropic MuLV from the spleen of a 3- to 4-week-old mouse (M81P-S) (11). Four additional polytropic MuLVs from spleens of 3- to 4-week-old mice were analyzed and found to be class II polytropic MuLVs with only minor differences (e.g., different points of recombination) from the class II viruses shown in Fig. 5 (data not shown).

Several additional, more salient features were also suggested by our analyses. It is apparent from the oligonucleotide maps (Fig. 5) that much of the heterogeneity among the isolates was a result of different points of recombination of nonecotropic sequences with the ecotropic MuLV. Recombination at the 3′ end appeared very conserved and occurred very near the 3′ end of the *env* gene in five of six class II isolates. One of the four additional class II isolates from young mice exhibited recombination within the p15E sequence similar to that of M72P-S (Fig. 5). In contrast, the 5′ ecotropic-nonecotropic sequence junctions varied greatly, ranging from within the leader sequences of the *env* gene (M62P-S) to within the 5′ region of the *pol* gene (M72P-S). The oligonucleotides of nearly all the class II polytropic MuLVs were consistent with their derivation from a single endogenous nonecotropic species. For the class II isolate M79P-T, allelic differences in the 3′ region of the *pol* gene suggested that this recombinant originated from a different endogenous sequence. With the exception of M79P-T, all the class II viruses in which the nonecotropic sequence extended into the *pol* gene (M72P-S, M75P-T, and M75P-S and two additional class II isolates from young mice) contained the nonecotropic oligonucleotide 103. Our oligonucleotide mapping data suggested that M79P-T was derived from an endogenous sequence which did not contain oligonucleotide 103, but contained the oligonucleotides 13C and 19B as well as a new oligonucleotide, X74.

In our depiction of the class II viruses we assumed that a single substitution of nonecotropic sequences occurred. In some instances oligonucleotides identified in the ecotropic virus RNA (no. 13C, 19B, 22, 27, and 36) were flanked by nonecotropic oligonucleotides and were thus included in the nonecotropic region. The same pattern of ecotropic and nonecotropic oligonucleotides was repeated in several isolates (e.g., oligonucleotides 22 and 27 were flanked by the same nonecotropic oligonucleotides in four class II isolates). It is unlikely that this pattern of oligonucleotides was generated independently in several viruses by a similar series of smaller recombinations. Furthermore, four of the five ecotropic oligonucleotides (no. 13C, 22, 27, and 36) which we assigned to the nonecotropic regions of some viruses have previously been identified in polytropic viruses derived after inoculation of ecotropic viruses that do not contain these oligonucleotides (12, 13). Those results indicate that these oligonucleotides are sequence elements contained in ecotropic viruses of AKR mice (AKV) and in endogenous nonecotropic sequences.

The nonecotropic oligonucleotides contained in the 5′ gp70 substitution of the class I polytropic MuLV M73P-S were identical to the corresponding oligonucleotides of the class II viruses (Fig. 5). The other class I MuLV (M60P-T) varied in only one oligonucleotide (X85) in this region. The RNase A digestion products of X85 and its electrophoretic migration suggested that it differed from X10 by a single base change (data not shown). Thus, our data suggested a common origin for the nonecotropic gp70 sequences of the class

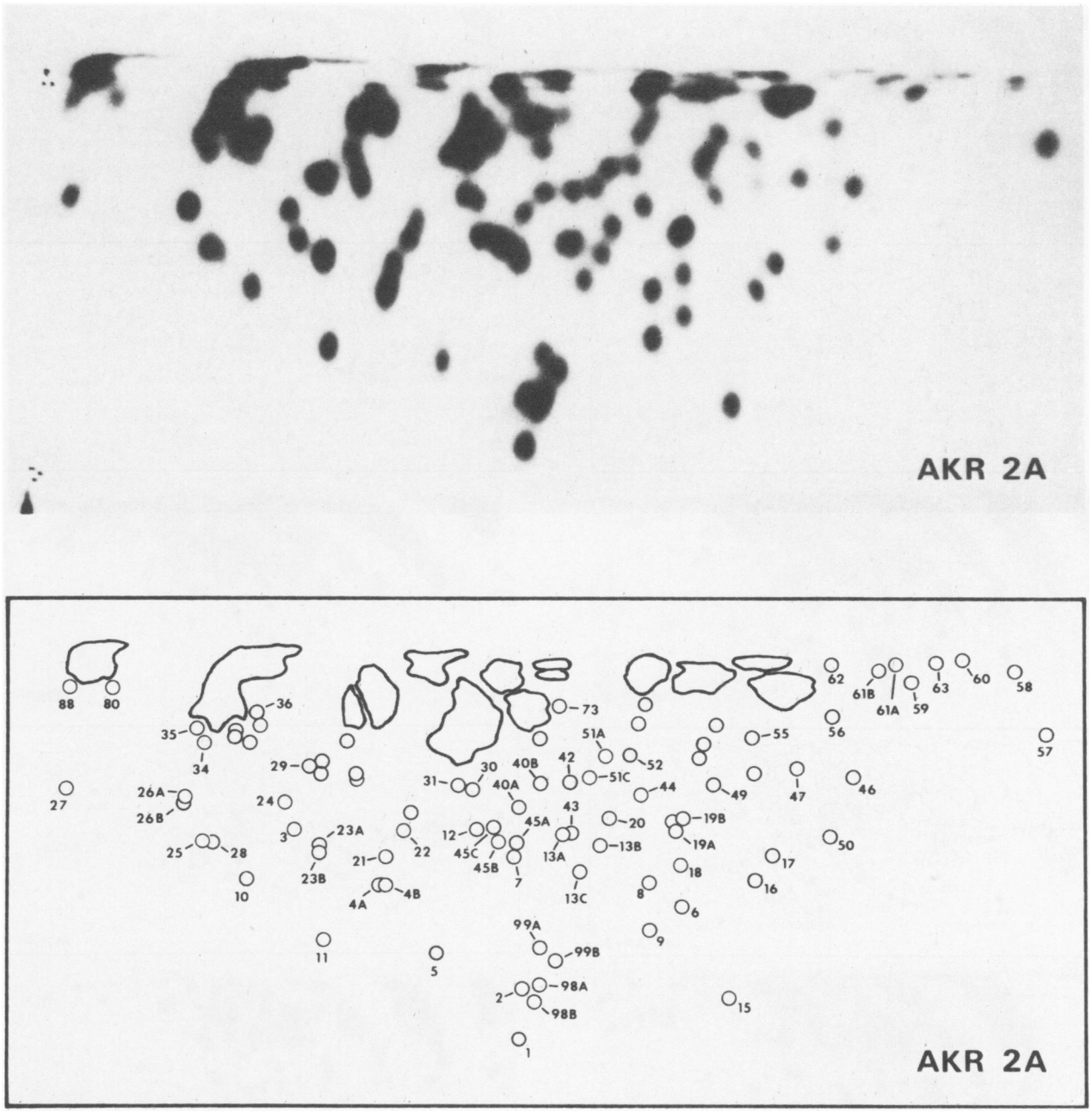
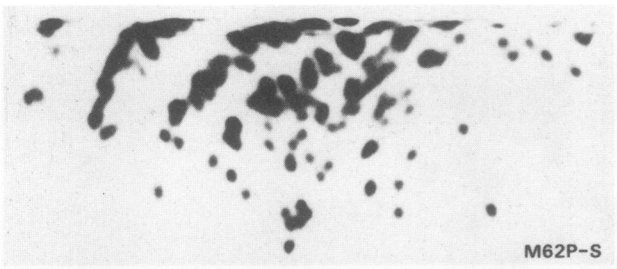


FIG. 2. RNase T_1-resistant oligonucleotide fingerprint of the ecotropic MuLV AKR 2A. 70S ^{32}P-labeled virion RNA was digested with RNase T_1 and fingerprinted. Electrophoresis was from left to right, and homochromatography was ascending. Large oligonucleotides resolved in the fingerprint were analyzed by secondary digestion with RNase A, and the products were compared with the predicted products of large oligonucleotides identified in the sequence of an ecotropic MuLV from AKR mice (AKV) (10, 20). A schematic of the fingerprint is displayed immediately below it. Only those oligonucleotides previously identified in fingerprints of AKV were assigned numbers. The numbering system used was that of Etzerodt et al. (10).

II polytropic viruses and at least one, and perhaps both, of the class I viruses. In contrast, the 3′ nonecotropic sequences of the class I viruses encompassing the 3′ region of the p15E gene were clearly of a different origin from those of the class II polytropic viruses. Since the precise positions of the ecotropic oligonucleotides (and many of the nonecotropic oligonucleotides) are known from nucleotide sequencing data (10, 20, 22, 23, 26), it was clear that the nonecotropic substitution of most of the class II polytropic viruses overlapped with the 3′ p15E/U_3 substitution of the class I polytropic MuLVs. Two oligonucleotides (X62 and 114) of the class II MuLVs were identified in the 3′ p15E sequences of all isolates which contained nonecotropic sequences in this region. The corresponding region of both of the class I polytropic MuLVs contained the oligonucleotides X73 and 119. In this regard, oligonucleotides which very likely correspond to X73 and X62 have been identified as alternative sequences in two molecularly cloned polytropiclike genomic sequences from HRS mice (J. Stoye, personal communication). X73 differs from X62 by a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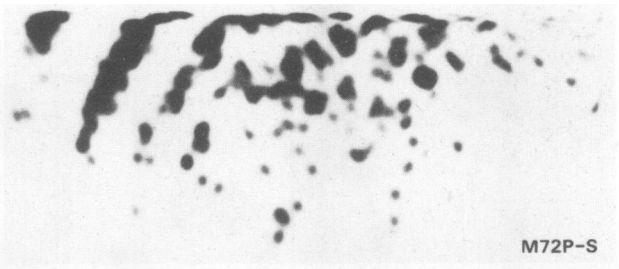
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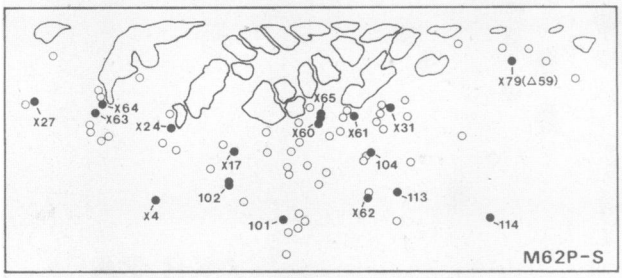
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X63
X24
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X79(Δ59)
X17
104
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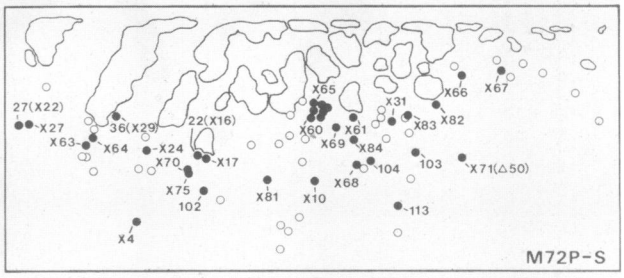
113
X4
101
X62
114
M62P-S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X66
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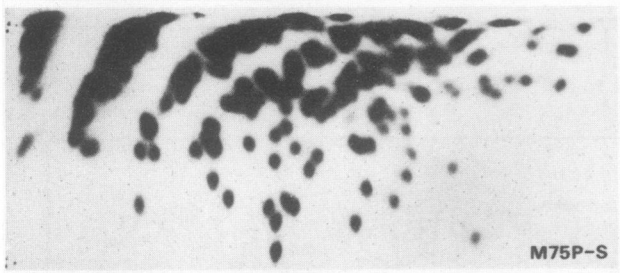
M72P-S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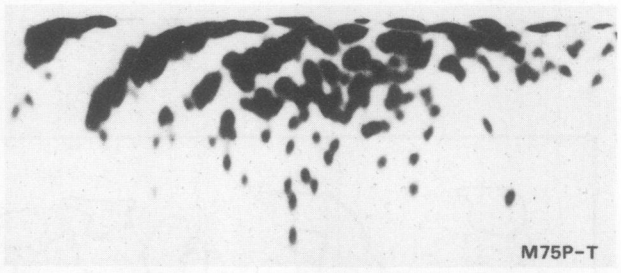
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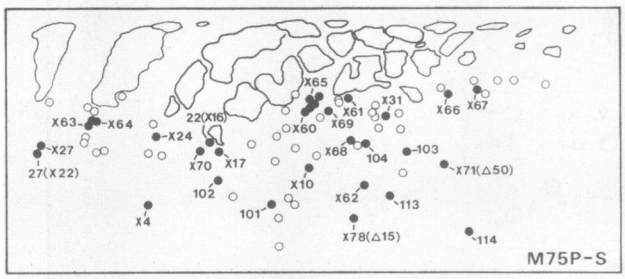
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X62
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X4
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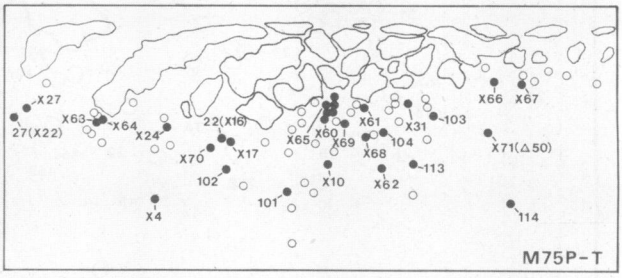
114
M75P-S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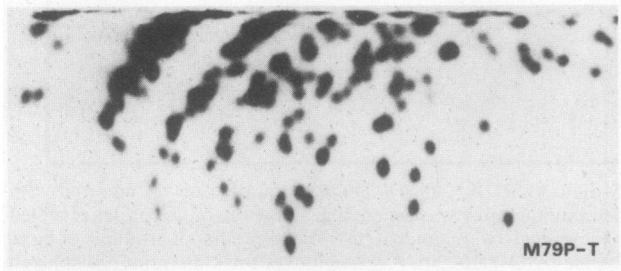
M75P-T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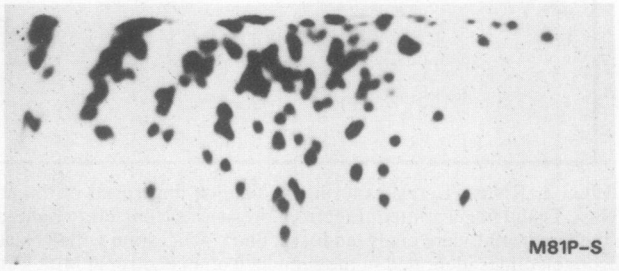
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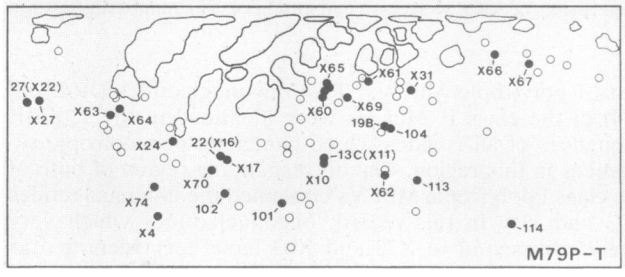
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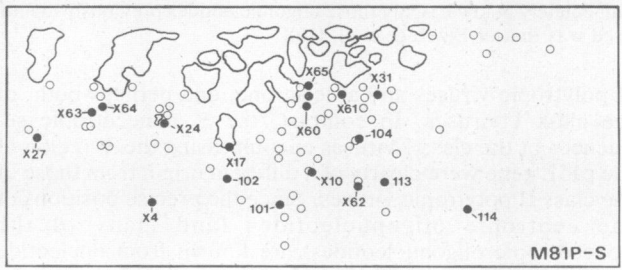
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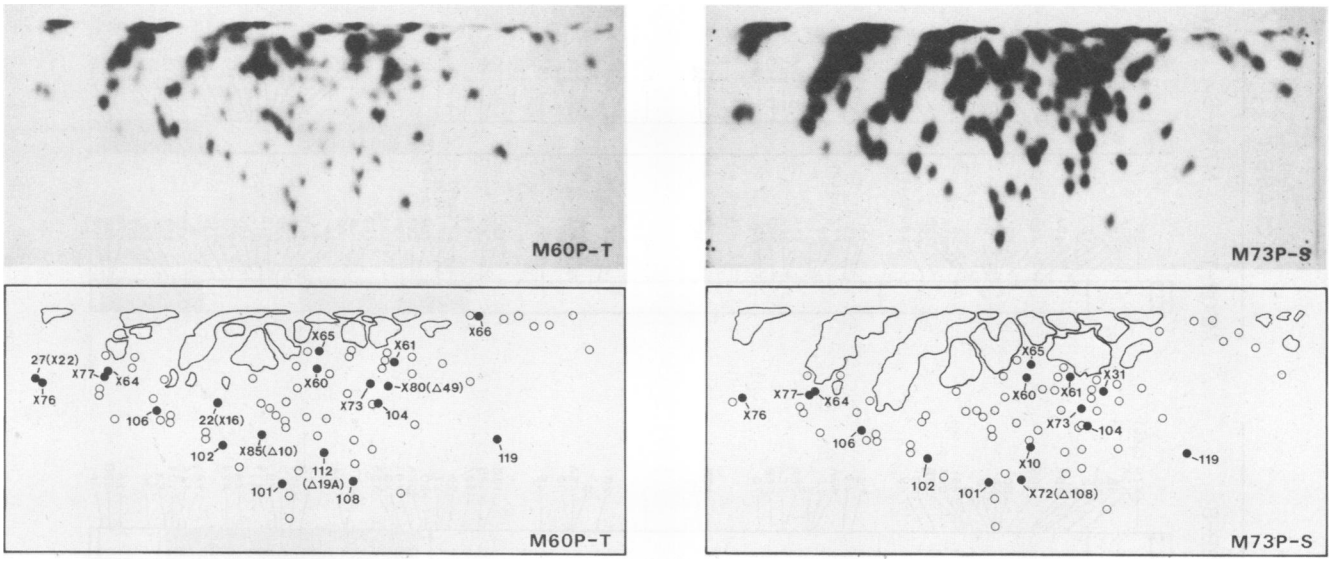

FIG. 4. RNase T_1-resistant oligonucleotide fingerprints of class I polytropic MuLVs from AKR mice. 70S ^{32}P-labeled virion RNA was digested with RNase T_1 and fingerprinted as described in the legend to Fig. 2. The identities of the isolates are indicated in the bottom right-hand corner of the fingerprints and also in the schematics of the fingerprints located directly below each autoradiogram. Numbering of nonecotropic oligonucleotides was as described in the legend to Fig. 3.

single base (G versus A) resulting in a truncated oligonucleotide.

In vitro properties of polytropic MuLVs from thymuses of 2- to 3-month-old AKR/J mice. In the analyses presented above, we determined that all our polytropic isolates from the spleens of young mice and many of our polytropic isolates from both the spleens and thymuses of older preleukemic mice were class II polytropic MuLVs. Furthermore, the detection of polytropic MuLVs in the spleen at 3 to 4 weeks of age preceded their detection in the thymus by over 1 month. These results were consistent with the generation of class II polytropic MuLVs in the spleen and the subsequent spread of the virus to the thymus. Thus, it was of interest to determine the predominant type of polytropic virus initially detected in the thymuses of mice 2 to 3 months of age. As described earlier, the class I and class II polytropic MuLVs were easily distinguished by their in vitro host range (Table 1). The class I polytropic MuLVs exhibited approximately equal titers on SC-1 and mink cells and slightly higher titers on *M. dunni* cells. In contrast, the class II polytropic MuLVs exhibited higher titers (ca. 10-fold) on SC-1 cells than on mink cells and 100-fold-higher titers on *M. dunni* cells than on SC-1 cells. Using their in vitro host range as an indication of the polytropic MuLV class, we examined six polytropic isolates obtained from the thymuses of different 2- to 3-month-old mice (Table 2). Five of the six isolates exhibited in vitro tropisms characteristic of class II polytropic MuLVs. The remaining isolate (M110P-T; Table 2) exhibited an in vitro tropism similar to the class I polytropic MuLVs but did not induce obvious CPE on mink cells. These analyses suggested that class II polytropic MuLVs are the predominant polytropic viruses in the thymuses of most mice of this age.

DISCUSSION

In this report we described the appearance of class II polytropic MuLVs in the spleens of young mice and their subsequent expression in the spleens and thymuses of older preleukemic mice. Only class II polytropic viruses were identified among five isolates from the spleens of 3- to 4-week-old mice. Similarly, the in vitro host ranges of polytropic isolates initially detected in the thymuses of 2- to 3-month-old mice were consistent with the predominant expression of class II polytropic MuLVs in the thymuses of most (five of six) mice of that age. In older preleukemic mice (5 to 6 months of age) class I and class II polytropic MuLVs

FIG. 3. RNase T_1-resistant oligonucleotide fingerprints of class II polytropic MuLVs from AKR mice. 70S ^{32}P-labeled virion RNA was digested with RNase T_1 and fingerprinted as described in the legend to Fig. 2. The identities of the isolates are indicated in the bottom right-hand corner of the fingerprints and also in the schematics of the fingerprints located directly below each autoradiogram. Five class II polytropic MuLVs isolated from 5- to 6-month-old mice (M62P-S, M72P-S, M75P-T, M75P-S, and M79P-T) and one isolated from a 26-day-old mouse (M81P-S) are shown. Only the nonecotropic oligonucleotides (black circles) were numbered in the schematic of each fingerprint. In some cases oligonucleotides identified in the ecotropic virus were believed also to be elements of endogenous nonecotropic sequences (oligonucleotides 22 and 27 of M72P-S, M75P-T, M75P-S, and M79P-T; oligonucleotides 13C and 19B of M79P-T; and oligonucleotide 36 of M72P-S). All but one of these oligonucleotides (19B) have been previously identified in other polytropic MuLVs unrelated to AKV. The nonecotropic designations previously assigned to these oligonucleotides are included in parentheses to the right of the ecotropic oligonucleotide designation. Other oligonucleotides not found in the ecotropic virus were believed to be the result of point mutations in ecotropic-derived sequences, based on RNase A digestion products and electrophoretic mobility. In those instances the related ecotropic oligonucleotide is indicated with a preceding delta (Δ) in parentheses to the right of the nonecotropic oligonucleotide designation. Nonecotropic oligonucleotides which had previously been identified in other polytropic isolates were numbered according to their original designations (12, 13). Oligonucleotides not previously identified in other polytropic isolates (X60 to X85) were assigned numbers prefixed with an X in accordance with our previous convention (12, 13).

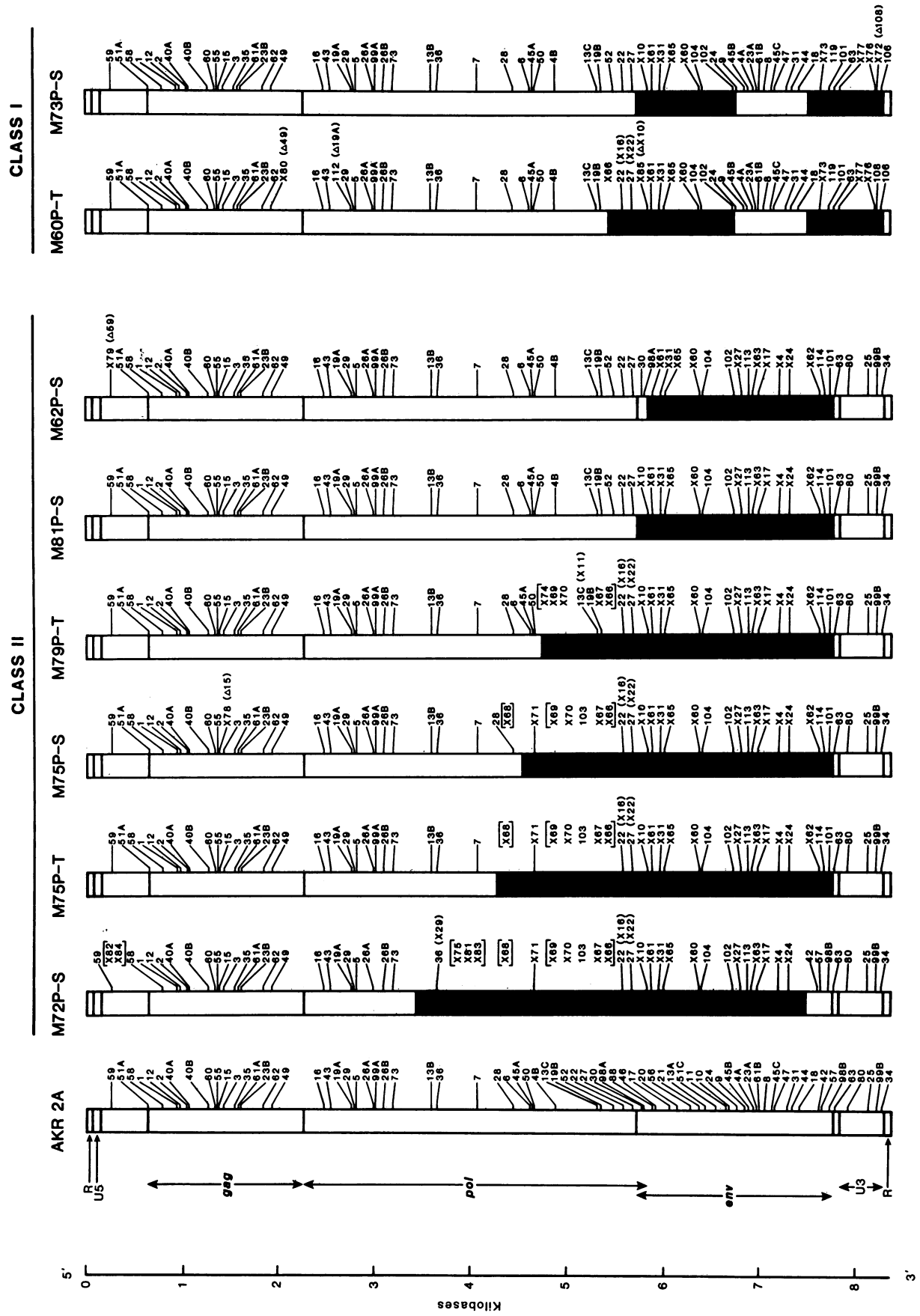
CLASS I
CLASS II
M60P-T
M73P-S
M62P-S
M81P-S
M79P-T
M75P-S
M75P-T
M72P-S
AKR 2A
R
U5
gag
pol
env
U3
R
5'
3'
0
1
2
3
4
5
6
7
8
kilobases

FIG. 5. T_1 oligonucleotide maps and bar diagrams representing 35S genomic RNAs of ecotropic AKR 2A, class II polytropic MuLVs, and class I polytropic MuLVs from AKR/J mice. The T_1 oligonucleotides of AKR 2A which were identified in the published nucleotide sequence (10) were ordered according to their locations in the genome. The precise location of each oligonucleotide is indicated by a line drawn from the oligonucleotide designation to a point on the bar diagram. The precise locations of oligonucleotides of the polytropic isolates identified in AKV or polytropic MuLV nucleotide sequences (10, 22, 23, 26; J. Stoye, personal communication) are indicated in an identical fashion. In some cases oligonucleotides identified in the ecotropic virus are believed also to be elements of endogenous nonecotropic sequences (oligonucleotides 22 and 27 of M72P-S, M75P-T, M75P-S, and M79P-T; oligonucleotides 13C and 19B of M79P-T; and oligonucleotide 36 of M72P-S). The nonecotropic designations previously assigned to these oligonucleotides are included in parentheses to the right of the ecotropic oligonucleotide designation. Some other oligonucleotides identified only in polytropic MuLVs are believed to be the result of point mutations in ecotropic-derived sequences. In those instances the related ecotropic oligonucleotide is prefixed by a delta (Δ) and enclosed in parentheses to the right of the nonecotropic oligonucleotide designation. The locations of nonecotropic oligonucleotides which were not identified in nucleotide sequences were deduced from oligonucleotide-mapping experiments and are enclosed in brackets to indicate that their assigned locations are approximate. White areas on the bar diagrams correspond to regions of the genome derived from sequences of the ecotropic parental virus. Black areas correspond to regions derived from endogenous nonecotropic sequences. Retroviral gene positions are indicated by arrows and also by horizontal lines on the bar diagrams. In the interest of clarity, the genomes of all polytropic viruses are depicted as colinear with AKR 2A, even though moderate deviations from colinearity of polytropic MuLV genomes with the ecotropic MuLV genome have been described (22, 26).

were identified in both the spleens and thymuses. These results are consistent with the expression of class II polytropic MuLVs in the spleen and their subsequent spread to the thymus, before the expression of class I polytropic MuLVs. Cloyd and Chattopadhyay (7) have also described the isolation from young AKR/J mice of MuLVs which have a class II genotype; however, their isolates were reported to exhibit an ecotropic host range. We have not identified recombinant viruses with an ecotropic host range, and the relationship of their viruses to those described here is currently unclear.

In previous studies we have successfully differentiated polytropic isolates derived from different endogenous sequences on the basis of oligonucleotide differences in the 5′ gp70 region (12, 13). However, in this study the 5′ gp70 oligonucleotides of most polytropic MuLVs examined (class I and class II) were identical. Only one oligonucleotide difference was identified in this region and it was found in a class I isolate (X85 in M60P-T). This oligonucleotide was likely the result of a single base change in oligonucleotide X10, which resides in the leader sequence of the *env* gene (Fig. 4), and may have occurred subsequent to the generation of this recombinant virus. The other class I isolate (M73P-S) contained oligonucleotides in the 5′ gp70 sequences which were identical to those of the class II polytropic MuLVs. In contrast to the close correspondence of oligonucleotides in the 5′ gp70 sequences of the polytropic viruses, the 3′ p15E sequences of the class I and class II viruses were obviously derived from different endogenous sequences.

Our results demonstrating the early appearance of class II polytropic MuLVs in spleens, the subsequent appearance of class II and class I polytropic MuLVs in thymuses, and the structural relationships between the class I and class II polytropic MuLVs of AKR/J mice suggest that the class II polytropic MuLVs are intermediates in the generation of oncogenic class I polytropic MuLVs in a stepwise mechanism of recombination (Fig. 6). In this model, endogenous ecotropic MuLVs in the spleen would undergo recombination with a nonecotropic endogenous species giving rise to the class II polytropic viruses. For reasons discussed below, we suggest that the nonecotropic endogenous species in the spleen is an endogenous polytropiclike provirus which contains functional gene sequences encoding retroviral proteins, but is defective in its LTR with respect to its ability to infect and replicate in in vitro cell lines. In the thymus the endogenous ecotropic MuLV would undergo recombination with a xenotropic MuLV containing the p15E/U_3 nonecotropic sequences found in class I polytropic MuLVs to generate the ecotropic recombinant intermediate, as proposed by Thomas and Coffin (42). Infection of thymocytes harboring the ecotropic recombinant virus with the class II polytropic viruses would result in secondary recombination between the two intermediates to generate the class I polytropic viruses.

There are a number of alternative possibilities, in addition to the model presented in Fig. 6, for the generation of class I polytropic MuLVs. It is possible that the spread of class II polytropic MuLVs from the spleen to the thymus is not necessary but that class II viruses are generated independently in the spleen and, somewhat later, in the thymus. Furthermore, although our data suggest that class II polytropic MuLVs precede the appearance of class I polytropic MuLVs in both the spleen and thymus, it is difficult to exclude the possibility that some or all class I polytropic MuLVs arise independently by recombination

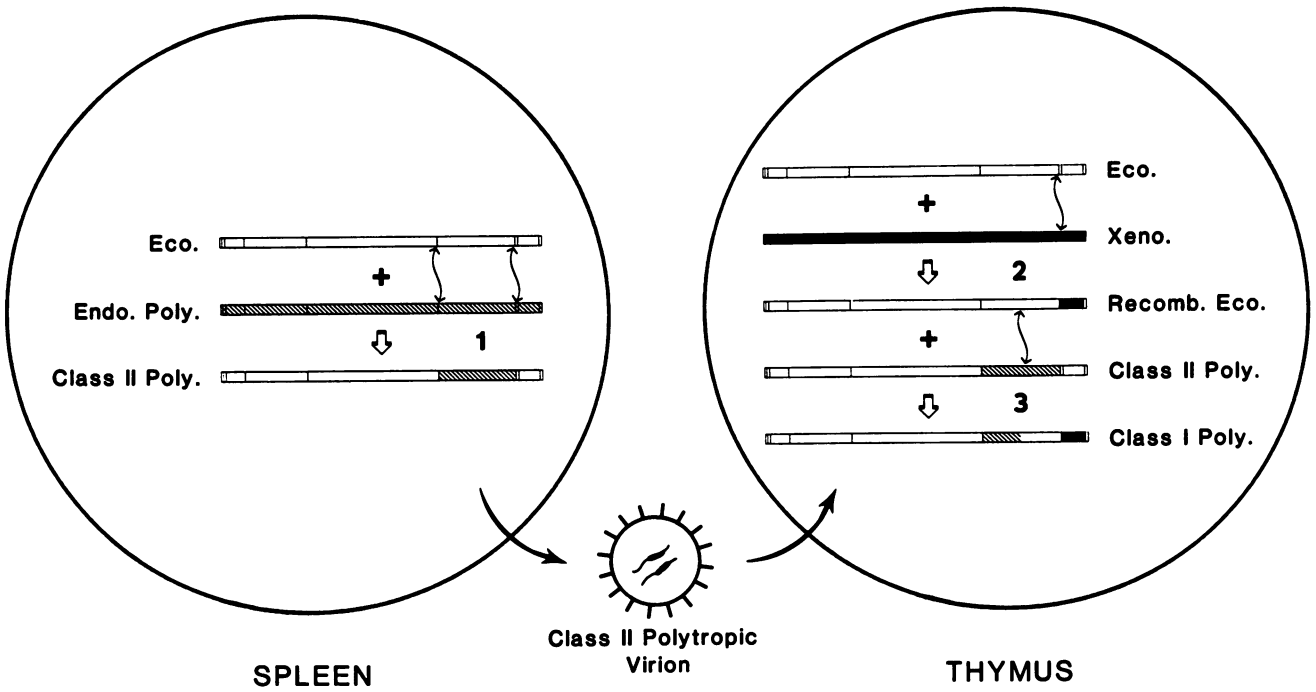

FIG. 6. Proposed model for the generation of class I polytropic MuLVs in AKR/J mice. The proposed model includes three recombination steps (1, 2, and 3) involving three endogenous proviruses and two intermediate recombinant MuLVs. The mouse ages at which the recombination steps and infection of the thymus occur are indicated below and refer to the earliest detection of the resulting recombinant MuLVs (Fig. 1) (40). Although the recombination may proceed via the formation of virion RNA heterodimers and secondary infection, in the interest of clarity those steps have not been included. In the spleen the endogenous ecotropic MuLV (open bar) and an endogenous polytropiclike MuLV (slashed bar) are expressed soon after birth. The polytropiclike MuLV is presumed to contain an LTR sequence which precludes the productive infection of in vitro cell lines (see Discussion). Recombination between the ecotropic MuLV and the polytropiclike MuLV proceeds in the spleen (step 1, 3 weeks of age) to yield an infectious recombinant class II polytropic MuLV. In the thymus the ecotropic MuLV and a xenotropic MuLV (black bar) containing the 3′ P15E/U_3 sequence of class I polytropic MuLVs are expressed at an early age. Recombination between the ecotropic and xenotropic viruses proceeds in the thymus (step 2, 6 weeks of age) to yield recombinant ecotropic MuLVs containing the xenotropic p15E/U_3 sequence. Superinfection of thymocytes with a spleen-derived class II polytropic MuLV (2 months of age) ultimately results in recombination of the recombinant ecotropic MuLV with the class II polytropic MuLV (step 3, 3 months of age) to yield a class I polytropic MuLV.

with the same, or very similar, endogenous polytropiclike retroviral species which give rise to the class II polytropic MuLVs. In this regard, our model suggests that the latency of the onset of leukemia in AKR/J mice (approximately 6 months) would be due not only to the multiple recombinational events required, but also due to compartmentalization of the recombinational events giving rise to the two penultimate intermediates in the generation of the class I polytropic viruses. Since we observed a substantial lag in the detection of class II MuLVs in the thymus compared with the spleen (Fig. 1), some degree of disease acceleration might be expected upon intrathymic inoculation of class II polytropic viruses in neonatal mice. We are currently investigating this possibility.

TABLE 2. Relative infectivity of in vitro cell lines by polytropic MuLVs from thymuses of 2 to 3-month-old AKR/J mice

Virus designation[a]	Relative infectivity[b]			Induction of CPE on mink cells
	M. dunni	SC-1	Mink	
M31P-T	1,510	10	1	–
M35P-T	1,870	13	1	–
M103P-T	650	14	1	–
M108P-T	1,300	12	1	–
M109P-T	1,050	21	1	–
M110P-T	9	3	1	–

[a] Designation of the viruses is as follows. The first three or four characters identify the mouse from which the isolate was obtained. The letter before the hyphen (P) indicates the virus class (polytropic), and the last character (T) identifies the organ (thymus).

[b] Titers on *M. dunni* and SC-1 cells were normalized to the titers on mink cells.

Several studies have addressed the genomic origin of the 5′ gp70 sequences found in the class I polytropic MuLVs (2, 3, 16, 23, 25, 33, 42). Numerous endogenous sequences homologous to polytropic MuLVs exist in a variety of inbred mouse strains which could give rise to the 5′ sequences of the polytropic *env* genes (1, 2, 23, 25, 33). Most or all of the endogenous polytropic proviruslike sequences examined to date are thought to be defective, owing to deletions or terminations within the protein-coding regions (25) or to a 190-base insertion identified in their LTRs (24, 34). In this regard we have previously reported the isolation of several polytropic viruses from NFS mice after inoculation with the Friend erythroleukemia virus (F-MuLV) (12). All these isolates were competent for replication even though some retained very few, if any, protein-coding sequences derived from the ecotropic parent. Of 36 oligonucleotides identified in the coding sequences of one of the isolates, 30 were of endogenous origin, and 5 of the remaining 6 oligonucleotides were identified in a polytropic virus unrelated to F-MuLV

(12). Our present analyses of the class II polytropic MuLVs of AKR/J mice defined functional endogenous nonecotropic sequences extending well into the 5′ region of the *pol* gene, and it is likely that analyses of additional recombinants would define even more extensive nonecotropic substitutions. Moreover, with the exception of the class I viruses described in this study, we have never encountered, among the many polytropic viruses we have isolated and characterized, an isolate with nonecotropic LTR oligonucleotides (12, 13). In consideration of these findings we have postulated that the parental source(s) of the gp70 sequences of polytropic MuLVs may be an endogenous provirus(es) with competent structural genes, but containing an LTR which does not favor replication in currently employed in vitro detection systems. Alternatively, the absence of nonecotropic LTRs in the class II isolates may reflect the mechanism of recombination giving rise to those viruses, irrespective of the LTR function in in vitro cell lines.

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LITERATURE CITED

1. **Blatt, C., K. Mileham, M. Haas, M. N. Nesbitt, M. E. Harper, and M. I. Simon.** 1983. Chromosomal mapping of the mink cell focus-inducing and xenotropic *env* gene family in the mouse. Proc. Natl. Acad. Sci. USA **80**:6298–6302.
2. **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.
3. **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.
4. **Chesebro, B., W. Britt, L. Evans, K. Wehrly, J. Nishio, and M. Cloyd.** 1983. Characterization of monoclonal antibodies reactive with murine leukemia viruses: use in analysis of strains of Friend MCF and Friend ecotropic murine leukemia virus. Virology **127**:134–148.
5. **Chesebro, B., K. Wehrly, M. Cloyd, W. Britt, J. Portis, J. Collins, and J. Nishio.** 1981. Characterization of mouse monoclonal antibodies specific for Friend murine leukemia virus-induced erythroleukemia cells: Friend-specific and FMR-specific antigens. Virology **112**:131–144.
6. **Chesebro, B., K. Wehrly, J. Nishio, and L. Evans.** 1984. Luekemia induction by a new strain of Friend mink cell focus-inducing virus: synergistic effect of Friend ecotropic murine leukemia virus. J. Virol. **51**:63–70.
7. **Cloyd, M. W., and S. K. Chattopadhyay.** 1986. A new class of retroviruses present in many murine leukemia systems. Virology **151**:31–40.
8. **Cloyd, M. W., B. Chesebro, J. L. Portis, and M. Weir.** 1982. MCF-specific murine monoclonal antibodies made against AKR-247 MCF virus recognize a unique determinant associated with the gp70-p15(E) complex. J. Virol. **41**:1112–1117.
9. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1980. Lymphomagenicity of recombinant mink cell focus-inducing virus. J. Exp. Med. **151**:542–552.
10. **Etzerodt, M., T. Mikkilsen, F. S. Pedersen, N. O. Kjeldgaard, and P. Jorgensen.** 1984. The nucleotide sequence of the Akv murine leukemia virus genome. Virology **134**:196–207.
11. **Evans, L. H.** 1986. Characterization of polytropic MuLVs from three-week-old AKR/J mice. Virology **153**:122–136.
12. **Evans, L. H., and M. W. Cloyd.** 1984. Generation of mink cell focus-forming viruses by Friend murine leukemia virus: recombination with specific endogenous proviral sequences. J. Virol. **49**:772–781.
13. **Evans, L. H., and M. W. Cloyd.** 1985. Friend and Moloney murine leukemia viruses specifically recombine with different endogenous retroviral sequences to generate mink cell focus-forming viruses. Proc. Natl. Acad. Sci. USA **82**:459–463.
14. **Fischinger, P. J., J. M. Ihle, S. deNoronha, and D. P. Bolognesi.** 1977. Oncogenic and immunogenic potential of cloned HIX virus in mice and cats. Med. Microbiol. Immunol. **164**:119–129.
15. **Fischinger, P. J., S. Nomura, and D. P. Bolognesi.** 1975. A novel murine oncornavirus with dual eco- and xenotropic properties. Proc. Natl. Acad. Sci. USA **72**:5150–5155.
16. **Green, N., H. Hiai, J. H. Elder, R. A. Schwartz, R. 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.
17. **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.
18. **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. USA **74**:789–792.
19. **Henderson, I. C., M. M. Lieber, and G. J. Todaro.** 1974. Mink cell line MvlLu (CCL-64). Focus formation and the generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. Virology **60**:282–287.
20. **Herr, W.** 1984. Nucleotide sequence of AKV murine leukemia virus. J. Virol. **49**:471–478.
21. **Hoggan, M. D., R. R. O'Neill, and C. A. Kozak.** 1986. Nonecotropic murine leukemia viruses in BALB/c and NFS/N mice: characterization of the BALB/c Bxv-1 provirus and the single NFS endogenous xenotrope. J. Virol. **60**:980–986.
22. **Holland, C. A., J. Wozney, and N. Hopkins.** 1983. Nucleotide sequence of the gp70 gene of murine retrovirus MCF 247. J. Virol. **45**:413–420.
23. **Khan, A. S.** 1984. Nucleotide sequence analysis establishes the role of endogenous murine leukemia virus DNA segments in formation of recombinant mink cell focus-forming murine leukemia viruses. J. Virol. **50**:864–871.
24. **Khan, A. S., and M. A. Martin.** 1983. Endogenous murine leukemia proviral long terminal repeats contain a unique 190-base-pair insert. Proc. Natl. Acad. Sci. USA **80**:2699–2703.
25. **Khan, A. S., W. P. Rowe, and M. A. Martin.** 1982. Cloning of endogenous murine leukemia virus-related sequences from chromosomal DNA of BALB/c and AKR/J mice: identification of an *env* progenitor of AKR-247 mink cell focus-forming proviral DNA. J. Virol. **44**:625–636.
26. **Kelly, M., C. A. Holland, M. L. Lung, S. K. Chattopadhyay, E. R. Lowy, and N. H. Hopkins.** 1983. Nucleotide sequence of the 3′ end of MCF 247 murine leukemia virus. J. Virol. **45**:291–298.
27. **Lander, M. R., and S. K. Chattopadhyay.** 1984. A *Mus dunni* cell line that lacks sequences closely related to endogenous murine leukemia viruses and can be infected by ecotropic, amphotropic, xenotropic, and mink cell focus-forming viruses. J. Virol. **52**:695–698.
28. **Li, Y., C. A. Holland, J. W. Hartley, and N. Hopkins.** 1984. Viral integration near *c-myc* in 10–20% of MCF 247-induced AKR lymphomas. Proc. Natl. Acad. Sci. USA **81**:6808–6811.
29. **Lung, M. L., J. W. Hartley, W. P. Rowe, and N. H. 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.** 1980. 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. **O'Donnell, P. V., E. Fleissner, H. Lonial, C. F. Koehne, and A. Reicin.** 1985. Early clonality and high-frequency proviral integration into the *c-myc* locus in AKR leukemias. J. Virol. **55**:500–503.
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. **O'Neill, R. R., A. S. Khan, M. D. Hoggan, J. W. Hartley, M. A. Martin, and R. Repaske.** 1986. Specific hybridization probes demonstrate fewer xenotropic than mink cell focus-forming murine leukemia virus *env*-related sequences in DNAs from inbred laboratory mice. J. Virol. **58**:359–366.
34. **Ou, C.-Y., L. R. Boone, and W. K. Yang.** 1983. A novel sequence segment and other nucleotide structural features in the long terminal repeat of a BALB/c mouse genomic leukemia virus-related DNA clone. Nucleic Acids Res. **11**:5603–5620.
35. **Quint, W., W. Boelens, P. van Wezenbeek, T. Cuypers, E. R. Maandag, G. Selten, and A. Berns.** 1984. Generation of AKR mink cell focus-forming viruses: a conserved single-copy xenotrope-like provirus provides recombinant long terminal repeat sequences. J. Virol. **50**:432–438.
36. **Rommelaere, J., D. V. Faller, and N. Hopkins.** 1978. Characterization and mapping of RNase T1-resistant oligonucleotides derived from the genomes of Akv and MCF murine leukemia viruses. Proc. Natl. Acad. Sci. USA **75**:495–499.
37. **Rowe, W. P., M. W. Cloyd, and J. W. Hartley.** 1980. The status of the association of MCF viruses with leukemogenesis. Cold Spring Harbor Symp. Quant. Biol. **44**:1265–1268.
38. **Ruscetti, S., L. Davis, J. Feild, and A. Oliff.** 1981. Friend murine leukemia virus-induced leukemia is associated with the formation of mink cell focus-inducing viruses and is blocked in mice expressing endogenous mink cell focus-inducing xenotropic viral envelope genes. J. Exp. Med. **154**:907–920.
39. **Sitbon, M., J. Nishio, K. Wehrly, and B. Chesebro.** 1985. Pseudotyping of dual-tropic recombinant viruses generated by infection of mice with different ecotropic murine leukemia viruses. Virology **140**:144–151.
40. **Sitbon, M., J. Nishio, K. Wehrly, D. Lodmell, and B. Chesebro.** 1985. Use of a focal immunofluorescence assay on live cells for quantitation of retroviruses: distinction of host range classes in virus mixtures and biological cloning of dual-tropic murine leukemia viruses. Virology **141**:110–118.
41. **Thomas, C. Y., B. J. Boyken, N. G. Famulari, and M. A. Coopola.** 1986. Association of recombinant murine leukemia viruses in the class II genotype with spontaneous lymphomas in CWD mice. J. Virol. **58**:314–323.
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.
43. **Troxler, D. H., and E. M. Scolnick.** 1978. Rapid leukemia induced by a cloned strain of replicating murine type C virus: association with induction of xenotropic-related RNA sequences contained in spleen focus-forming virus. Virology **85**:17–27.
44. **van der Putten, H., W. Quint, J. van Raaij, E. R. Maandag, I. M. Verma, and A. Berns.** 1981. M-MuLV-induced leukemogenesis: integration and structure of recombinant proviruses in tumors. Cell **24**:729–739.
45. **Van Griensven, L. J. L. D., and M. Vogt.** 1980. Rauscher "mink cell focus-forming" (MCF) virus causes erythroleukemia in mice: its isolation and properties. Virology **101**:376–388.
46. **Vogt, M.** 1979. Properties of "mink cell focus-inducing" (MCF) virus isolated from spontaneous lymphoma lines of BALB/c mice carrying Moloney leukemia virus as an endogenous virus. Virology **93**:226–236.
47. **Yanagihara, K., D. Hamada, T. Seyama, N. Imamura, and K. Yokoro.** 1982. In vitro studies of the mechanism of leukemogenesis. II. Characterization of endogenous murine leukemia viruses isolated from AKR thymic epithelial reticulum cell lines. J. Virol. **41**:360–366.