JOURNAL OF VIROLOGY, June 1984, p. 864–871
0022-538X/84/060864-08$02.00/0

Vol. 50, No. 3

Nucleotide Sequence Analysis Establishes the Role of Endogenous Murine Leukemia Virus DNA Segments in Formation of Recombinant Mink Cell Focus-Forming Murine Leukemia Viruses

ARIFA S. KHAN

Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

Received 6 December 1983/Accepted 13 March 1984

The sequence of 363 nucleotides near the 3′ end of the *pol* gene and 564 nucleotides from the 5′ terminus of the *env* gene in an endogenous murine leukemia viral (MuLV) DNA segment, cloned from AKR/J mouse DNA and designated as A-12, was obtained. For comparison, the nucleotide sequence in an analogous portion of AKR mink cell focus-forming (MCF) 247 MuLV provirus was also determined. Sequence features unique to MCF247 MuLV DNA in the 3′ *pol* and 5′ *env* regions were identified by comparison with nucleotide sequences in analogous regions of NFS-Th-1 xenotropic and AKR ecotropic MuLV proviruses. These included (i) an insertion of 12 base pairs encoding four amino acids located 60 base pairs from the 3′ terminus of the *pol* gene and immediately preceding the *env* gene, (ii) the deletion of 12 base pairs (encoding four amino acids) and the insertion of 3 base pairs (encoding one amino acid) in the 5′ portion of the *env* gene, and (iii) single base substitutions resulting in 2 MCF247-specific amino acids in the 3′ *pol* and 23 in the 5′ *env* regions. Nucleotide sequence comparison involving the 3′ *pol* and 5′ *env* regions of AKR MCF247, NFS xenotropic, and AKR ecotropic MuLV proviruses with the cloned endogenous MuLV DNA indicated that MCF247 proviral DNA sequences were conserved in the cloned endogenous MuLV proviral segment. In fact, total nucleotide sequence identity existed between the endogenous MuLV DNA and the MCF247 MuLV provirus in the 3′ portion of the *pol* gene. In the 5′ *env* region, only 4 of 564 nucleotides were different, resulting in three amino acid changes between AKR MCF247 MuLV DNA and the endogenous MuLV DNA present in clone A-12. In addition, nucleotide sequence comparison indicated that Moloney- and Friend-MCF MuLVs were also highly related in the 3′ *pol* and 5′ *env* regions to the cloned endogenous MuLV DNA. These results establish the role of endogenous MuLV DNA segments in generation of recombinant MCF viruses.

Mink cell focus-forming (MCF) murine leukemia viruses (MuLVs) have been isolated from preleukemic and leukemic mouse tissues (9, 10). Heteroduplex mapping (5, 7) oligonucleotide fingerprinting (23, 28), as well as restriction enzyme mapping and Southern blot analyses (4, 17) indicate that MCF viruses are recombinants between ecotropic MuLVs and nonecotropic sequences. Since ecotropic viruses lack characteristics of MCF MuLVs such as dualtropism, mink cell focus-forming activity, and ability to accelerate leukemias in newborn mice (6), it is most likely that the nonecotropic sequences acquired during recombination contribute to expression of some of these properties unique to MCF viruses. I have previously reported the characterization by restriction enzyme mapping and Southern blot analyses of several endogenous MuLV proviruses isolated from AKR/J and BALB/c mouse DNAs (17). The cloned endogenous MuLV DNA segments were distinct from known infectious MuLV proviruses due to the insertion of a 190-base-pair (bp) transposon-like segment in the long terminal repeats (LTR) (15) and by the presence of unique restriction sites in the *gag* and *pol* regions (17). However, the *env* segment associated with the endogenous MuLV DNAs was related to the *env* region in MCF MuLV proviruses based on similar-sized restriction fragments and dual-reactive hybridization properties. Furthermore, restriction enzyme and Southern blot analyses suggested that the *env* region in one cloned endogenous MuLV DNA segment, designated as A-12, might represent the nonecotropic progenitor of AKR MCF247 MuLV DNA. To establish this relationship between endogenous MuLV DNAs and MCF proviruses, the nucleotide sequence in the 3′ end of the *pol* gene and in the 5′ portion of the *env* gene in clone A-12 and AKR MCF247 MuLV DNAs was determined. Nucleotide sequence comparison of AKR MCF247, NFS xenotropic, and AKR ecotropic MuLV DNAs permitted the identification of sequences unique to MCF247 in the 3′ *pol* and 5′ *env* regions. The MCF247-specific sequence features were conserved in the endogenous MuLV DNA present in clone A-12, which distinguished the latter DNA from both xenotropic and ecotropic MuLV proviruses. In fact, almost total nucleotide sequence identity was seen in the 3′ *pol* and 5′ *env* regions between MCF247 MuLV provirus and the cloned endogenous MuLV DNA. In addition, the nonecotropic sequences present in recombinant Moloney- (M) (2) and Friend- (F) (A. Adachi, K. Sakai, N. Kitamura, S. Nakanishi, O. Niwa, and A. Ishimoto, submitted for publication) MCF MuLVs were also virtually identical in the 3′ *pol* and 5′ *env* regions to the endogenous MuLV provirus present in clone A-12.

MATERIALS AND METHODS

MuLV DNA clones used for nucleotide sequencing. A recombinant plasmid DNA clone containing a 6.8-kilobase-pair (kb) DNA segment from AKR MCF247 MuLV provirus which extended from an *Eco*RI site in the 5′ LTR to an *Eco*RI site in the *env* region (at 6.9 kb) was used for nucleotide sequencing. The construction and characterization of this DNA clone (previously designated as pMCF-1) has been described previously (16). The nucleotide sequence of an endogenous MuLV DNA segment was determined with a recombinant lambda Charon 4A DNA clone designat-

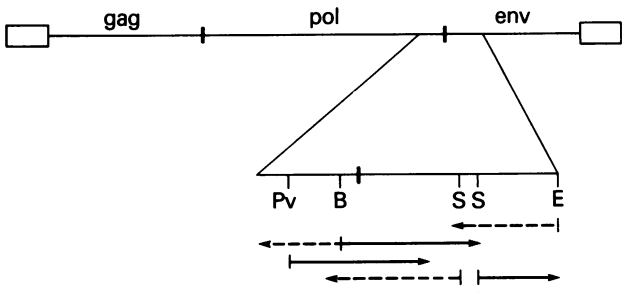

FIG. 1. Strategy used for determining nucleotide sequences in the 3′ *pol* and 5′ *env* regions of AKR MCF247 DNA and endogenous MuLV DNA in clone A-12. Arrows indicate regions sequenced from minus strand (←--) or plus strand (—→) DNA. Restriction enzyme designations are as follows: Pv, *Pvu*II; B, *Bam*HI; S, *Sma*I; E, *Eco*RI.

ed as A-12. The A-12 DNA clone, which was isolated from AKR/J mouse DNA as described previously (17), consisted of a 15-kb DNA segment and contained endogenous MuLV proviral sequences extending from the 5′ LTR to the end of the clone, which was an *Eco*RI site at 6.9 kb in the *env* region.

DNA sequence determination. Due to the presence of identical restriction sites in the 3′ *pol* and 5′ *env* regions of AKR MCF247 provirus and the endogenous MuLV (clone A-12) DNA, a similar nucleotide sequencing strategy could be used for both DNAs (shown in Fig. 1). Recombinant lambda and plasmid DNAs or fragments isolated from preparative agarose gels were restricted with enzymes and labeled at their 5′ termini with [γ-^{32}P]ATP and polynucleotide kinase. Labeled segments were recleaved and isolated on agarose gels and their nucleotides sequenced by the partial degradation method of Maxam and Gilbert (19).

RESULTS

Identification of sequences unique to AKR MCF247 MuLV DNA. The sequence of 363 nucleotides near the 3′ end of the *pol* gene and 564 nucleotides from the 5′ terminus of the *env* gene in a cloned AKR MCF247 MuLV DNA segment designated previously as pMCF-1 (16) was determined by the strategy shown in Fig. 1. A comparison of the nucleotide sequence in MCF247 MuLV DNA with that previously reported for NFS-Th-1 xenotropic (22) and AKR ecotropic (12) MuLV proviruses permitted the identification of MCF247-specific sequences.

***pol* region.** Nucleotide sequence comparison in the 3′ end of the *pol* gene of MCF247, NFS xenotropic, and AKR ecotropic MuLV DNAs is shown in Fig. 2. A splice acceptor site for *env* mRNA (27), located from nucleotides 16 to 24, is conserved in the three MuLV proviruses. The MCF247 MuLV DNA could be distinguished from xenotropic and ecotropic MuLV proviral sequences due to the insertion of 12 bp (designated as X in Fig. 2) extending from nucleotides 292 to 303 and located 60 bp from the 3′ end of the *pol* gene. It is interesting to note that the adenine residue, which is the last nucleotide in the MCF247-specific 12-bp insert, becomes the first nucleotide of an ATG codon (indicated by an asterisk in Fig. 2), which is a potential amino terminus of an *env* gene product. The location of the 12-bp sequence in the *pol* reading frame predicts that the 3′ *pol* gene-coded protein product of MCF247 DNA would be four amino acids larger than the protein encoded from the corresponding regions in xenotropic or ecotropic MuLVs. The nucleotide sequence on either side of this 12-bp insert in MCF247 DNA is more homologous to the NFS xenotropic provirus (96%) than to AKR ecotropic MuLV DNA (85%). In addition, single base substitutions in the 3′ region of *pol* result in only two heterologous amino acids (positions 43 and 78) between xenotropic and MCF247 MuLV DNAs (resulting in 95% homology), whereas 12 amino acids differ between ecotropic and MCF247 MuLV DNAs (resulting in 87% homology). It should be noted that in the 3′ end of the *pol* gene, the region of greatest sequence heterology in MCF247, NFS xenotropic, and AKR ecotropic MuLV DNAs extends from nucleotides 280 to 312 and includes the 12-bp insert.

***env* region.** The nucleotide sequence of the 5′ portion of the *env* genes of AKR MCF247, NFS xenotropic, and AKR ecotropic MuLV proviruses is compared in Fig. 3. MCF247 MuLV DNA could be distinguished from xenotropic and ecotropic MuLV proviruses in the 5′ *env* region based on the following unique features: (i) 12 bp encoding four amino acids were deleted in MCF MuLV DNA (designated as Y in Fig. 3), (ii) 3 bp (from 499 to 501) were inserted which resulted in the insertion of an MCF247-specific alanine at position 167 (indicated as Z in Fig. 3), and (iii) other single base substitutions resulted in 23 MCF247-specific amino acids, 3 in the leader peptide (8) and 20 in the gp70-coding region. Comparison of nucleotide sequences in the 5′ *env* regions of MCF247, NFS xenotropic, and AKR ecotropic MuLV DNAs indicated 89% nucleotide sequence homology between MCF247 and NFS xenotropic MuLV DNAs and only 48% polynucleotide sequence homology between MCF247 and AKR ecotropic MuLV proviruses. The MCF247 MuLV DNA is further related to NFS xenotropic proviral DNA on the basis of conservation of a series of nucleotide deletions (such as the region between nucleotides 315 and 316 in Fig. 3) and insertions (such as the sequence between nucleotides 477 and 523 in Fig. 3) relative to the AKR ecotropic MuLV provirus in the 5′ *env* region (22). A glycosylation site (20, 24) at nucleotide positions 127 to 135 (Fig. 3) is conserved between the three classes of MuLV DNAs; in addition, a second potential glycosylation site located at positions 172 to 180 is shared between MCF247 and NFS xenotropic MuLV DNAs. It is interesting to note that the greatest nucleotide sequence divergence in the 5′ *env* regions of MCF247, NFS xenotropic, and AKR ecotropic MuLV proviruses occurs in the region of the 12-bp deletion (Y in Fig. 3) and the 3-bp insertion (Z in Fig. 3) present in MCF247 DNA.

Nucleotide sequence analysis of the 3′ *pol* and 5′ *env* regions of endogenous MuLV DNA. The *env* region present in a cloned endogenous MuLV DNA, A-12, was previously shown by restriction enzyme mapping and Southern blot analysis to be a potential progenitor of AKR MCF247 MuLV DNA (17). To establish this relationship, the nucleotide sequence in the 3′ *pol* and 5′ *env* regions of the cloned endogenous MuLV DNA was determined by the strategy shown in Fig. 1 and compared with sequences in an analogous region in AKR MCF247 DNA. In the 3′ *pol* region, total nucleotide sequence identity existed between the endogenous MuLV DNA segment in clone A-12 and MCF247 MuLV DNA (Fig. 4). This included conservation of the splice acceptor site located from nucleotides 16 to 24 and the 12-bp MCF247-specific insert designated as X (nucleotides 291 to 303) and located 60 bp from the 3′ end of the *pol* gene. In addition, the two amino acids specific to the MCF247 MuLV provirus (positions 43 and 78) were also conserved in the cloned endogenous MuLV DNA.

Nucleotide sequence comparison of the 5′ *env* regions of the cloned endogenous and AKR MCF247 MuLV DNAs is shown in Fig. 5. All the MCF247-specific sequence hallmarks (Fig. 3) could be identified in the endogenous MuLV DNA. These included the 12-bp deletion with respect to xenotropic and ecotropic proviruses between nucleotides 261 and 262 (indicated as Y in Fig. 5) and the 3-bp insertion (nucleotides 499 to 501) encoding alanine at position 167 (designated as Z in Fig. 5). In fact, nucleotide sequences in the 5′ *env* region of the endogenous MuLV provirus in clone A-12 and AKR MCF247 MuLV DNA were virtually identical; only 4 of 564 bases were different, resulting in three amino substitutions, two of which (positions 34 and 157) were present in the gp70-coding region and one of which (position 22) was present in the leader region. Moreover, 21 of the 23 MCF247-specific amino acids in the 5′ *env* region were also present in the cloned endogenous MuLV DNA.

Nucleotide sequences in the 3′ *pol* and 5′ *env* regions of the endogenous MuLV DNA in clone A-12 were also compared with those present in the analogous regions in M-MCF (2) and F-MCF (Adachi et al., submitted for publication) MuLV proviruses (Fig. 4 and 5). The MCF247-unique sequences found in the cloned endogenous MuLV DNA, which included the 12-bp insertion in the 3′ region of the *pol* gene, the 12-bp deletion, and the 3-bp insertion in the 5′ portion of the *env* gene, could also be identified in the same nucleotide positions in the M-MCF and F-MCF MuLV proviruses. Furthermore, a high degree of polynucleotide sequence homology was observed in the *pol* and *env* regions of M-MCF and F-MCF proviral DNAs and comparable segments of the cloned endogenous A-12 DNA. In the 3′ *pol* region, M-MCF and the endogenous MuLV DNAs were different in only 8 of the 249 bp present in the nonecotropic portion of M-MCF DNA (Fig. 4). These nucleotide changes did not lead to amino acid alterations. Ten base substitutions were noted between F-MCF and the cloned endogenous

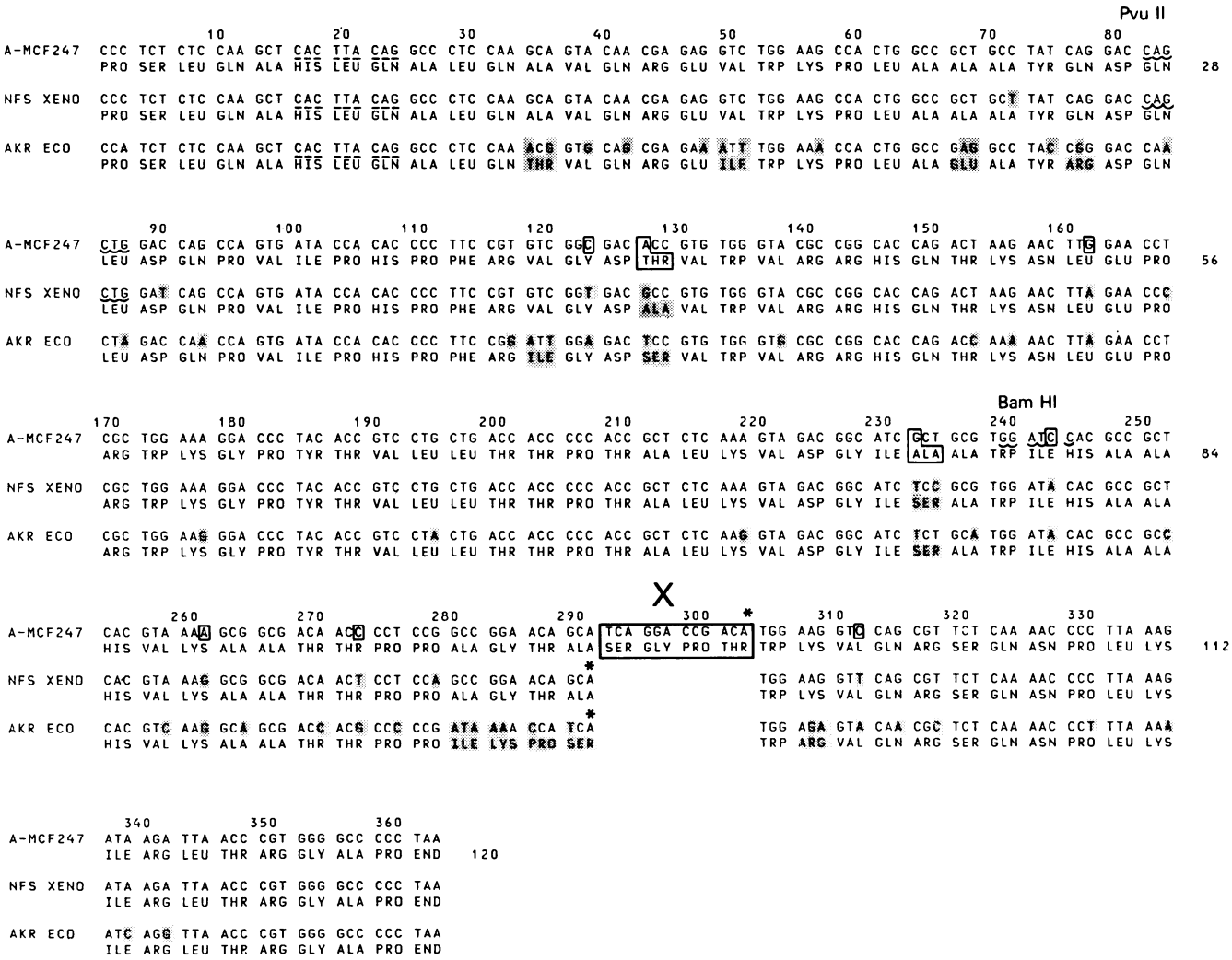

FIG. 2. Identification of MCF247-specific sequences in the 3′ *pol* region. Comparison of the nucleotide and deduced amino acid sequences of AKR MCF247 (A-MCF247) MuLV DNA with those previously published for NFS-Th-1 xenotropic (NFS XENO) (22) and AKR ecotropic (AKR ECO) (12) MuLV proviruses is presented. Sequences unique to MCF247 DNA are enclosed within solid lines; the MCF247-specific 12-bp insert is enclosed within heavy solid lines and indicated as X; differences in nucleotides and deduced amino acids in the xenotropic and ecotropic MuLV DNAs with respect to MCF247 DNA are shaded; the gaps in sequences indicate absent bases; the splice acceptor site is indicated with a broken line; the restriction sites are indicated with wavy lines; the asterisk indicates the position of initiation codon for the translation of the envelope precursor polypeptide in the *env* gene reading frame (see Fig. 3).

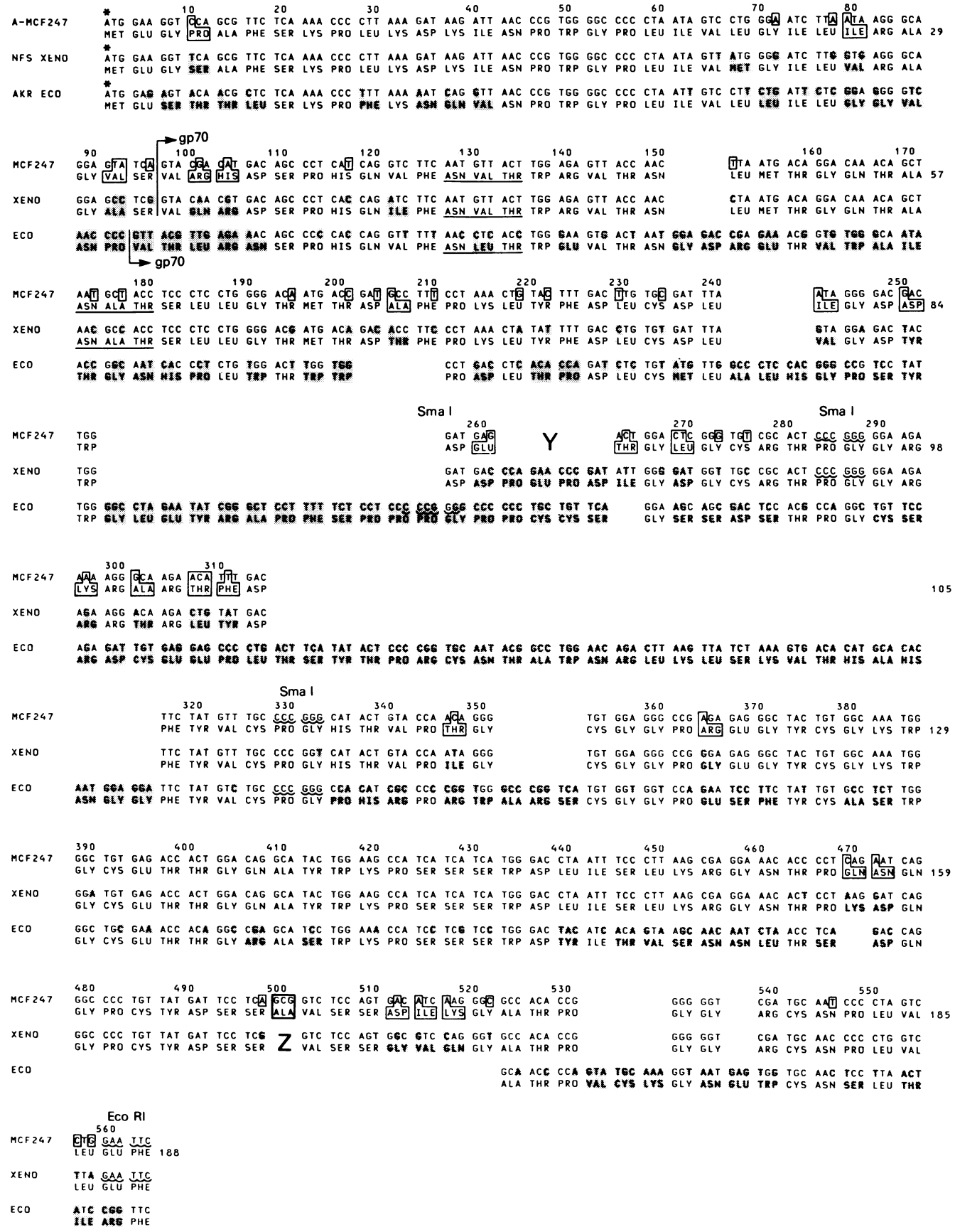

FIG. 3. Comparison of the nucleotide and deduced amino acid sequences in the 5′ *env* regions of AKR MCF247 (A-MCF247 and MCF247), NFS-Th-1 xenotropic (NFS XENO and XENO) and AKR ecotropic (AKR ECO and ECO) MuLV proviruses. Nucleotide and predicted amino acid sequences which are unique to AKR MCF247 DNA are enclosed within solid lines; the MCF247-specific 12-bp deletion and 3-bp insertion (enclosed within heavy solid lines) are indicated as Y and Z, respectively; the nucleotide and deduced amino acid sequences in the shaded regions in NFS-Th-1 xenotropic and AKR ecotropic proviruses represent substitutions with respect to the sequence of AKR MCF247 DNA; the gaps represent absent nucleotides; the asterisk indicates the initiation codon for translation of the *env* gene-coded precursor polypeptide; the amino terminus of gp70 is indicated in AKR MCF247 DNA based on amino acid sequence homology to Rauscher MCF MuLV DNA (26); the potential glycosylation sites are underlined; and the restriction sites are indicated with wavy lines.

MuLV DNAs in the 3′ *pol* region which resulted in only a single substituted amino acid at position 29. In the 5′ portion of the *env* gene, M-MCF and the endogenous MuLV DNAs were different in three nucleotides which resulted in two altered amino acids: one (position 59) was located in the second potential glycosylation site and the second was located at position 171. In the same region, F-MCF DNA contained 18 different nucleotides with respect to the cloned

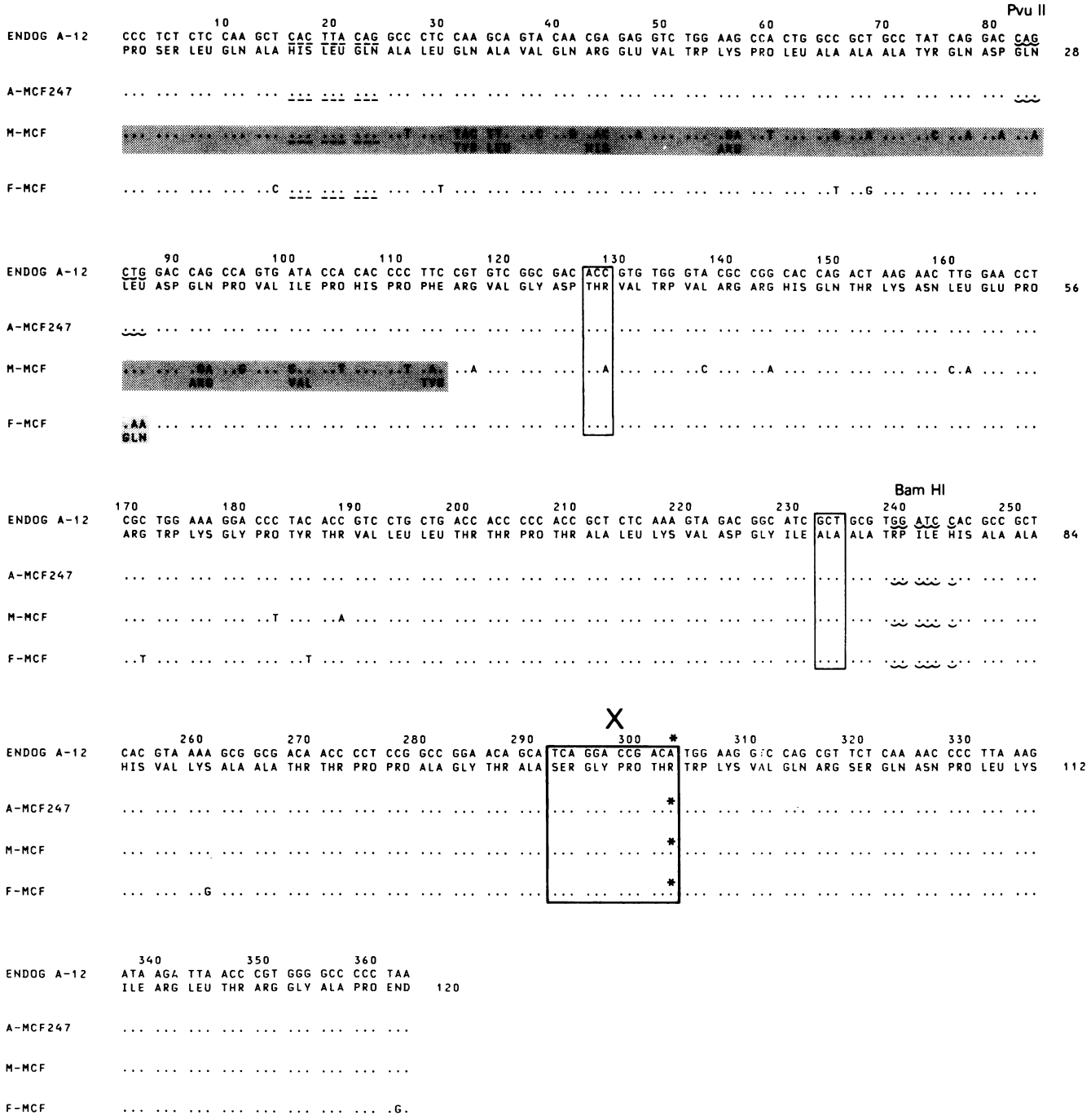

FIG. 4. Comparison of sequences in the 3′ *pol* regions of cloned endogenous and MCF MuLV DNAs. Nucleotide and deduced amino acid sequences of an endogenous MuLV DNA segment in clone A-12 (17) (ENDOG A-12) are compared with sequences in an analogous region in AKR MCF247 (A-MCF247), M-MCF (2), and F-MCF (Adachi et al., submitted for publication) MuLV DNAs. Nucleotides which are identical in the endogenous A-12 and the MCF DNAs are represented by dots; the nucleotide differences are indicated; the 12-bp MCF247-specific insert is enclosed within heavy solid lines and designated as X; the sequences conserved in the cloned endogenous and MCF MuLV DNAs which are different from those present in ecotropic or xenotropic proviruses are enclosed within solid lines; the light-shaded region represents the nucleotides and amino acid unique to F-MCF; the dark-shaded region indicates M-MuLV ecotropic sequences (29), which are present in the M-MCF recombinant MuLV DNA; the splice acceptor site is indicated with a broken line; restriction sites are indicated with a wavy line; the asterisk marks the position of the initiation codon for translation of *env* gene precursor polypeptide.

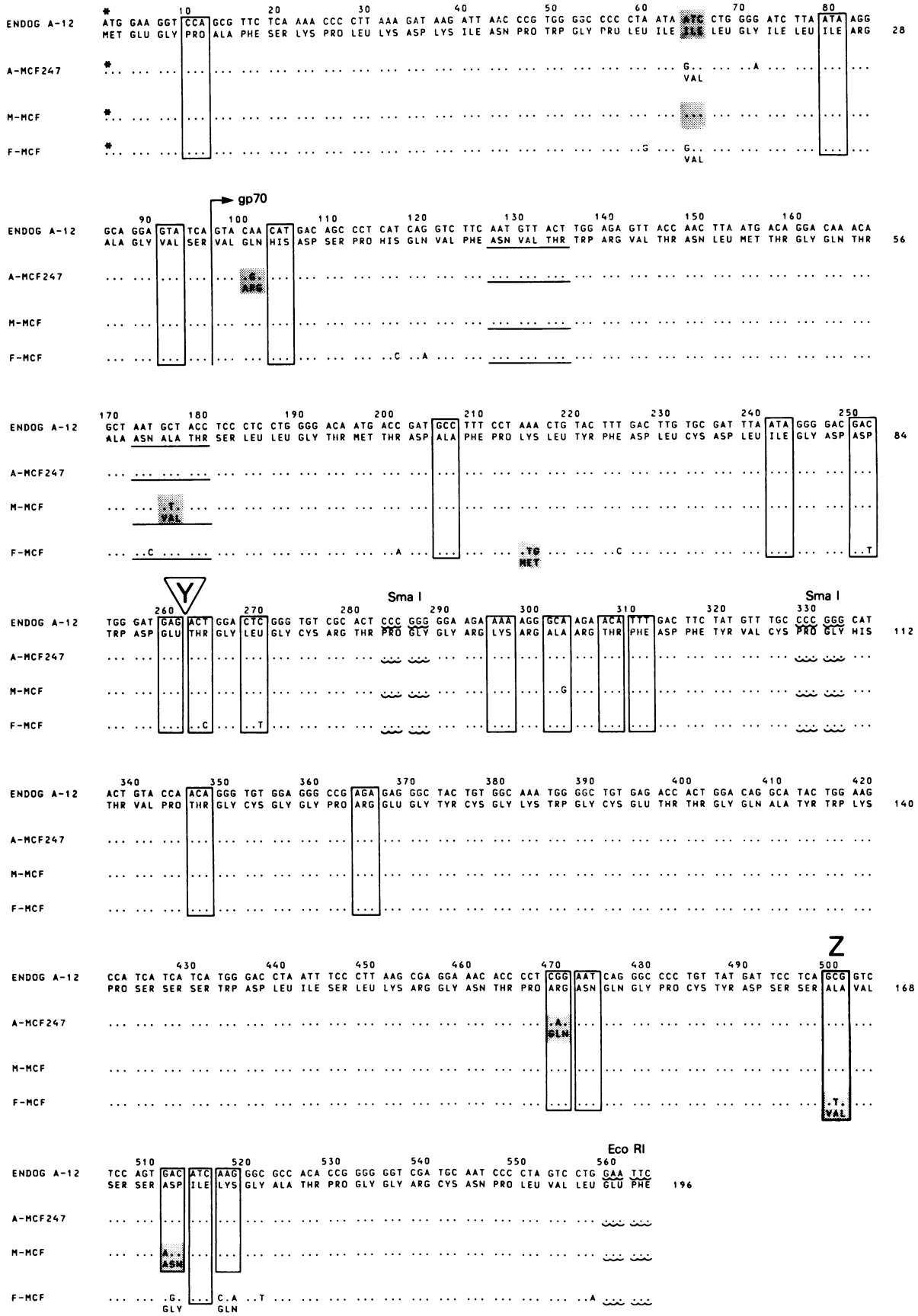

FIG. 5. Comparison of sequences in the 5′ *env* regions of cloned endogenous and MCF MuLV DNAs. Nucleotide and deduced amino acid sequences of AKR MCF247 (A-MCF247), M-MCF, and F-MCF MuLVs are compared with the sequences present in the cloned endogenous A-12 DNA (ENDOG A-12). The designations used in this figure are the same as described in the legend to Fig. 4. The MCF247-specific 12-bp deletion (▽) and 3-bp insertion (enclosed within heavy solid lines) are designated as Y and Z, respectively. The shaded areas indicate nucleotides and amino acids which are unique to each MCF isolate and different from sequences in corresponding positions in ecotropic and xenotropic proviruses; the amino terminus of the gp70 in the cloned endogenous and MCF DNAs is indicated based on amino acid homology to Rauscher MCF gp70 (26); potential glycosylation sites are underlined.

endogenous MuLV DNA sequences, which resulted in five substituted amino acids in the *env* region: one in the leader segment (position 22) and four in the gp70-coding region (positions 72, 167, 171, and 173). The substitution of alanine by a valine at position 167 in F-MCF DNA resulted from a single base change at nucleotide 500 in the 3-bp MCF247-specific insertion (designated as Z in Fig. 5).

It is interesting to note that no single alteration was shared between the three MCF DNAs with respect to the endogenous MuLV DNA in clone A-12. The high degree of nucleotide sequence conservation between the retroviral DNA in clone A-12 and the three MCF proviruses establishes the role of endogenous MuLV DNA segments in formation of recombinant MuLVs.

DISCUSSION

Oligonucleotide fingerprinting and restriction enzyme mapping analyses of MCF MuLV isolates indicate that they differ from one another in the amount of nonecotropic sequences incorporated into their *pol* and *env* regions (4, 23). These nucleotide substitutions invariably involve the 5′ portion of the *env* gene. It has previously been demonstrated that the novel sequences which are ultimately incorporated into MCF viruses preexist in mouse chromosomal DNA (3, 17). Some of these segments, which contain MCF-related *env* regions on the basis of restriction mapping and Southern blot analyses, have recently been cloned from AKR/J and BALB/c mouse DNAs (17). One of the cloned endogenous MuLV DNA segments, which was isolated from AKR/J mouse DNA and designated A-12, was identified as a potential *env* gene progenitor of AKR MCF247 MuLV. To confirm this relationship between the endogenous and infectious MuLV DNAs, the nucleotide sequence of 363 bases near the 3′ end of the *pol* gene and 564 bases in the 5′ region of the *env* gene in the cloned endogenous and AKR MCF247 MuLV DNA was determined.

Recently, Holland et al. have reported the nucleotide sequence of the *env* gene in cloned AKR MCF247 MuLV DNA (14); that sequence differs by one nucleotide from the one reported here (position 249; Fig. 3). This single base change does not result in an amino acid alteration. The nucleotide sequence comparison of the cloned endogenous MuLV DNA segment and MCF247 MuLV DNA reported here indicates that all of the hallmarks of MCF proviruses, identified after the sequencing of MCF247, xenotropic, and ecotropic MuLV proviral DNAs (Fig. 2 and 3), were present in the cloned AKR mouse cellular DNA segment. These unique structural features, which were lacking in both xenotropic and AKR ecotropic MuLV proviruses, included (i) a 12-bp insertion (designated as X in Fig. 2) located 60 bp from the 3′ end of the *pol* gene (and immediately preceding the 5′ terminus of the *env* gene) and (ii) a 12-bp deletion and a 3-bp insertion in the 5′ *env* region (indicated with a Y and Z, respectively, in Fig. 3). The comparison of the cloned endogenous and MCF247 MuLV DNAs revealed total nucleotide sequence identity in the 3′ *pol* region (Fig. 4) and only 4 of 564 mismatched bases in the 5′ *env* region, resulting in the substitution of three amino acids (Fig. 5). In addition, the unique structural features present in the cloned endogenous and MCF247 MuLV DNAs could also be identified in analogous regions of M- and F-MCF MuLV proviruses. The high degree of nucleotide sequence conservation between the cloned endogenous MuLV DNA and MCF proviruses corroborates the role of mouse cellular DNA sequences in the generation of *env* gene recombinant MuLVs.

Southern blot analyses of restricted mouse DNAs have indicated the presence of 18 to 28 xenotropic MuLV-related DNA segments (13). Of the six *env*-containing cloned endogenous MuLV proviral segments isolated from BALB/c and AKR/J mouse DNAs, only one contained purely xenotropic *env* specificity (17). The remaining five cloned endogenous MuLV DNAs had *env* regions possessing restriction maps and hybridization properties typical of MCF proviruses. Such results suggest that the majority of MuLV-related DNAs in the mouse genome contain an MCF-type *env* region. It is interesting to note that each of the MCF-related *env* segments associated with cloned endogenous MuLV DNAs could be distinguished from one another after *Alu*I or *Hpa*II digestion (17). It is unclear at this time whether these endogenous MCF-type *env* DNA segments constitute a family of highly related MuLV DNAs or whether each isolate represents a different class of endogenous MuLV DNA. The relationship of the endogenous MCF-type *env* DNA segments to each other and to known MCF MuLV proviral *env* regions remains to be elucidated by nucleotide sequence analysis. In view of the existence of a large number of endogenous MuLV DNAs containing distinctive *env* regions, it was quite unexpected to learn that the *env* regions of three different MCF MuLV (AKR MCF247, M-MCF, and F-MCF) were nearly identical to the *env* region present in a single endogenous MuLV DNA (namely, in clone A-12). Several possibilities can be entertained to explain the incorporation of *pol* and *env* sequences similar to those present in clone A-12 into three different MCF proviruses. (i) The proviral DNA in clone A-12 is located in a region of the chromosome in which a high frequency of recombination occurs; such a "hot spot" for recombination could even be located within the retroviral sequences in clone A-12. (ii) The endogenous MuLV provirus associated with clone A-12 may represent a member of a highly related multimembered MuLV family which contains *env* genes capable of donating a functional gp70 to an ecotropic MuLV to generate MCF viruses.

It has been demonstrated in viral interference studies that the host range of MuLVs is defined by the recognition of distinct cell surface receptors by the viral *env* gene-coded protein products, such as gp70, of the three classes of MuLVs, namely, ecotropic, xenotropic, and dualtropic MCFs (1, 21). The results presented here indicate that the amino acid sequence of the 5′ portion of the *env* gene in MCF247 MuLV is significantly different from that in the analogous region of AKR ecotropic MuLV. Furthermore, MCF247 MuLV can also be distinguished from xenotropic MuLV in its 5′ *env* region based on 23 amino acid substitutions, deletion of 4 amino acids, and insertion of 1 amino acid (Fig. 3). Thus, the amino-terminal portions of the gp70s associated with the three classes of MuLVs may very well be involved in determining host range by recognizing different receptors on the cell surface. Recombinant MCF MuLVs have been shown to infect mouse cells via receptors distinct from those used by ecotropic MuLVs (21). The MCF-encoded gp70 might, thereby, allow the entry of MCF viruses into tissues that are inaccessible to prototype ecotropic MuLVs. After entry into such cells, other regions of the viral genome may then function to initiate the leukemogenic process. For example, in the case of MuLV-induced thymic lymphomas (25), the LTR associated with MCF proviruses may facilitate enhanced expression of viral genes in thymus cells. The resulting increase in the number of progeny virions could then lead to multiple integration events, one of which might activate a "*leuk*" gene in a manner analogous to the promoter-insertion model (11). Alternatively, enhanced

expression of a viral gene product (e.g., gp70) mediated by an LTR now associated with a recombinant MuLV *env* region and located in a cell type previously inaccessible to it could be pathogenic.

It is worth noting that the rate of viral integration can be affected by mutations in the 3′ region of the *pol* gene (S. Goff, personal communication). Thus, the insertion of 12 bp in the 3′ end of the *pol* gene in MCF MuLV DNAs might affect its integration into cellular DNA and activation of a potential *leuk* gene. A role for this 12-bp *pol* insert in leukemogenesis is supported by the fact that both M-MuLV (29) and F-MuLV (18), which are leukemogenic ecotropic viruses, also have sequences (9 bp) inserted into this portion of their *pol* genes, whereas the nonleukemogenic ecotropic MuLVs, such as AKv, lack sequences in this region.

ACKNOWLEDGMENTS

I am grateful to Paul E. Steele for introducing me to the Maxam and Gilbert sequencing procedure. I also thank Willard W. Davis for excellent technical assistance, Malcolm A. Martin and Paul E. Steele for critical review of the paper, and Laura A. Wilders for typing the manuscript.

LITERATURE CITED

1. **Besmer, P., and D. Baltimore.** 1977. Mechanism of restriction of ecotropic and xenotropic murine leukemia viruses and formation of pseudotypes between the two viruses. J. Virol. **21**:965–973.
2. **Bosselman, R. A., F. van Straaten, C. Van Beveren, I. M. Verma, and M. Vogt.** 1982. Analysis of the *env* gene of a molecularly cloned and biologically active Moloney mink cell focus-forming proviral DNA. J. Virol. **44**:19–31.
3. **Chattopadhyay, S. K., M. W. Cloyd, D. L. Linemeyer, M. R. Lander, E. Rands, and D. R. Lowy.** 1982. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. Nature (London) **295**:25–31.
4. **Chattopadhyay, S. K., M. R. Lander, S. Gupta, E. Rands, and D. R. Lowy.** 1981. Origin of mink cytopathic focus-forming (MCF) viruses; comparison with ecotropic and xenotropic murine leukemia virus genomes. Virology **113**:465–483.
5. **Chien, Y.-H., I. M. Verma, T. Y. Shih, E. M. 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.
6. **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.
7. **Donoghue, D. J., E. Rothenberg, N. Hopkins, D. Baltimore, and P. A. Sharp.** 1978. Heteroduplex analysis of the nonhomology region between Moloney MuLV and the dual host range derivative HIX virus. Cell **14**:959–970.
8. **Engelman, D. M., and T. A. Steitz.** 1981. The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. Cell **23**:411–422.
9. **Fischinger, P. J., S. Normura, 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.
10. **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.
11. **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.
12. **Herr, W., V. Corbin, and W. Gilbert.** 1982. The nucleotide sequence of the 3′ half of Akv. Nucleic Acids Res. **10**:6931–6944.
13. **Hoggan, M. D., C. E. Buckler, J. F. Sears, W. P. Rowe, and M. A. Martin.** 1983. Organization and stability of endogenous xenotropic murine leukemia virus proviral DNA in mouse genomes. J. Virol. **45**:473–477.
14. **Holland, C. A., J. Wozney, and N. Hopkins.** 1983. Nucleotide sequence of the gp70 gene of murine retrovirus MCF 247. J. Virol. **47**:413–420.
15. **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. U.S.A. **80**:2699–2703.
16. **Khan, A. S., R. Repaske, C. F. Garon, H. W. Chan, W. P. Rowe, and M. A. Martin.** 1982. Characterization of proviruses cloned from mink cell focus-forming virus-infected cellular DNA. J. Virol. **41**:435–448.
17. **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.
18. **Koch, W., G. Hunsmann, and R. Friedrich.** 1983. Nucleotide sequence of the envelope gene of Friend murine leukemia virus. J. Virol. **45**:1–9.
19. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:560–564.
20. **Montreuil, J.** 1980. Primary structure of glycoprotein glycans. Basis for the molecular biology of glycoproteins. Adv. Carbohydr. Chem. Biochem. **37**:157–223.
21. **Rein, A.** 1982. Interference grouping of murine leukemia viruses: a distinct receptor for the MCF-recombinant viruses on mouse cells. Virology **120**:251–257.
22. **Repaske, R., R. R. O'Neill, A. S. Khan, and M. A. Martin.** 1983. Nucleotide sequence of the *env*-specific segment of NFS-Th-1 xenotropic murine leukemia virus. J. Virol. **46**:204–211.
23. **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.
24. **Rosner, M. R., L. S. Grinna, and P. W. Robbins.** 1980. Differences in glycosylation patterns of closely related murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **77**:67–71.
25. **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.
26. **Schultz, A., A. Rein, L. Henderson, and S. Oroszlan.** 1983. Biological, chemical, and immunological studies of Rauscher ecotropic and mink cell focus-forming viruses from JLS-V9 cells. J. Virol. **45**:995–1003.
27. **Sharp, P.** 1981. Speculations on RNA splicing. Cell **23**:643–646.
28. **Shih, T. Y., M. O. Weeks, D. H. Troxler, J. M. Coffin, and E. M. Scolnick.** 1978. Mapping host range-specific oligonucleotides within genomes of the ecotropic and mink cell focus-inducing strains of Moloney murine leukemia virus. J. Virol. **26**:71–83.
29. **Shinnick, T. M., R. A. Lerner, and J. G. Sutcliffe.** 1981. Nucleotide sequence of Moloney murine leukemia virus. Nature (London) **293**:543–548.