JOURNAL OF VIROLOGY, Feb. 1984, p. 471–478
0022-538X/84/020471-08$02.00/0

Vol. 49, No. 2

Nucleotide Sequence of AKV Murine Leukemia Virus

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Received 22 July 1983/Accepted 4 October 1983

AKV is an endogenous, ecotropic murine leukemia virus that serves as one of the parents of the recombinant, oncogenic mink cell focus-forming viruses that arise in preleukemic AKR mice. I report the 8,374-nucleotide-long sequence of AKV, as determined from the infectious molecular clone AKR-623. The 5′-leader sequence of AKV extends to nucleotide 639, after which lies a long open reading frame encoding the *gag* and *pol* gene products. The reading frame is interrupted by a single amber codon separating the *gag* and *pol* genes. The *pol* gene overlaps the *env* gene within the 3′ region of the AKV genome. The nucleotide sequence of the 5′ region of AKV reveals the following features. (i) The 5′-leader sequence lacks any AUG codon to initiate translation of $gPr80^{gag}$, suggesting that $gPr80^{gag}$ is not required for the replication of AKV. (ii) A short portion of the leader region diverges in sequence from the closely related Moloney murine leukemia virus and appears to be related to a sequence highly repeated in eucaryotic genomes. (iii) As in Moloney murine leukemia virus, there is a potential RNA secondary structure flanking the amber codon that separates the *gag* and *pol* genes. This structure might function as a regulatory protein binding site that controls the relative levels of synthesis of the *gag* and *pol* precursors. The nucleotide sequence of the 3′ region of AKV is compared with sequences reported previously from both infectious and noninfectious molecular clones of AKV.

The murine leukemia virus (MLV) AKV resides in the germ line of AKR mice and is implicated in the high incidence of thymic leukemias that arise when these mice are 6 to 9 months old. AKV is expressed in AKR mice from birth (41) and is distinguished from the other MLV endogenous to AKR mice because it is ecotropic; i.e., it can infect mouse cells. Low-leukemic strains of mice (e.g., NIH/Swiss) which have acquired a germ line AKV provirus from AKR mice become viremic and subsequently contract leukemia, albeit with a considerably longer latency period (39, 40). Nevertheless, AKV itself does not appear to be directly oncogenic because it is unable to induce leukemia in healthy mice (18, 29).

During the development of AKR mice, AKV recombines with germ line nonecotropic MLV to produce recombinant MLVs (4, 5, 9, 17, 19, 23, 38) not found in the germ line of AKR mice (3, 17, 36). The recombinants, called MCF, appear in the preleukemic thymus (14) and are thought to be the proximal oncogenic agent in AKR leukemogenesis because they can accelerate the onset of leukemia if injected into young AKR mice (6, 31), and they are found integrated in the genomes of leukemic thymocytes (3, 16, 36). Because the nonecotropic parent of MCF viruses has not been identified, the nonecotropic sequences within MCF genomes have been localized by comparison to AKV.

The nucleotide sequence of the 3′ region of the AKV genome has been reported previously. Lenz et al. (21) and Van Beveren et al. (56) determined the nucleotide sequence of the envelope (*env*) gene and the long terminal repeat (LTR), respectively, from an infectious molecular clone of AKV called AKR-623 (22). We previously reported the nucleotide sequence of the majority of the *pol* gene and the entire *env* gene from a noninfectious molecular clone of AKV (15). These reports concentrated on the 3′ region of AKV because the *env* gene and LTR sequences of oncogenic MCF viruses consist of a complicated mosaic of ecotropic and nonecotropic MLV sequences (4, 19, 23, 38). Nevertheless, sequences within the 5′ half of the MCF genome frequently appear to be derived from the nonecotropic parent MLV (4, 23). To define fully the AKV genomic structure, I have determined the nucleotide sequence of the 5′ region of AKV and redetermined the nucleotide sequence of the 3′ region from the infectious AKR-623 clone.

MATERIALS AND METHODS

Sequence determination. The nucleotide sequence was derived from the infectious molecular clone of AKV, AKR-623 (22), generously provided by D. Lowy and S. Chattopadhyay. The nucleotide sequence was determined by the chain terminator method of Sanger et al. (43), after cloning random fragments into the single-stranded bacteriophage M13 (1, 42). The sequence of the 5′ region of AKV was derived from the large 4.5-kilobase *Xma*I (*Sma*I) fragment spanning this region. This fragment was purified by agarose gel electrophoresis, concatenated and circularized with T4 ligase, and sheared randomly by sonication (7). The sonicated ends were repaired with T4 polymerase and nucleotide triphosphates. Fragments 400 to 1,000 nucleotides long were selected by agarose gel electrophoresis and cloned into the *Sma*I site of the bacteriophage M13 vector mp8 (24). A total of 182 M13 clones were randomly chosen for sequencing. The inserts were sequenced by the chain terminator method, and the samples were analyzed by electrophoresis through buffer gradient gels as described by Biggin et al. (2). The sequences were compiled and ordered by computer, using the automatic DB system (51). Each nucleotide was sequenced an average of six times, and the entire region was sequenced at least once on both DNA strands.

The nucleotide sequence of the 3′ region of AKV was redetermined by using the same strategy as above except that the AKR-623 *Xho*I/*Eco*RI fragment stretching from the middle of the AKV genome into the 3′ cellular DNA sequences was used to generate the random M13 clones; in general, only one of the DNA strands was sequenced to

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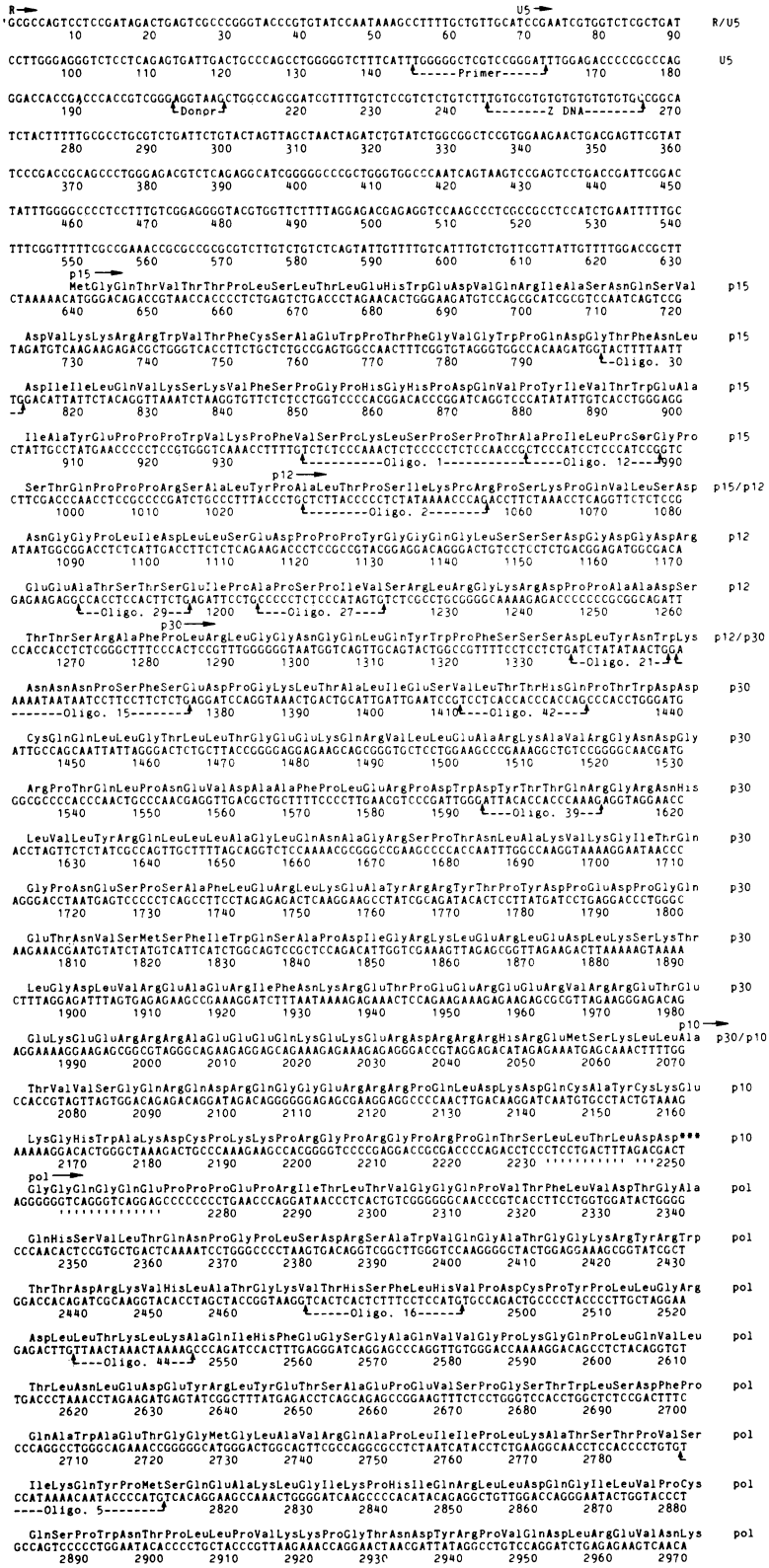

FIG. 1

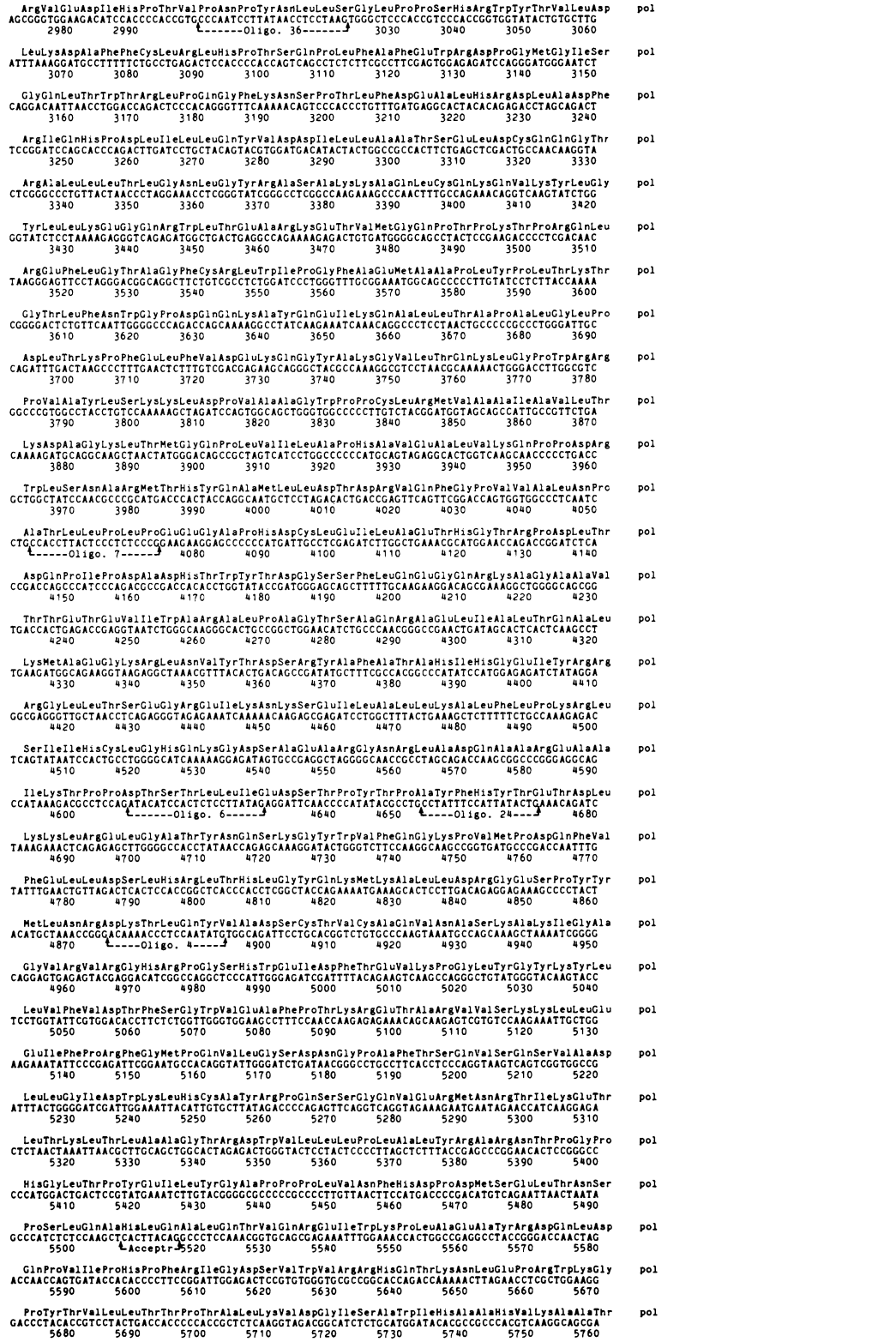

FIG. 1—*Continued*

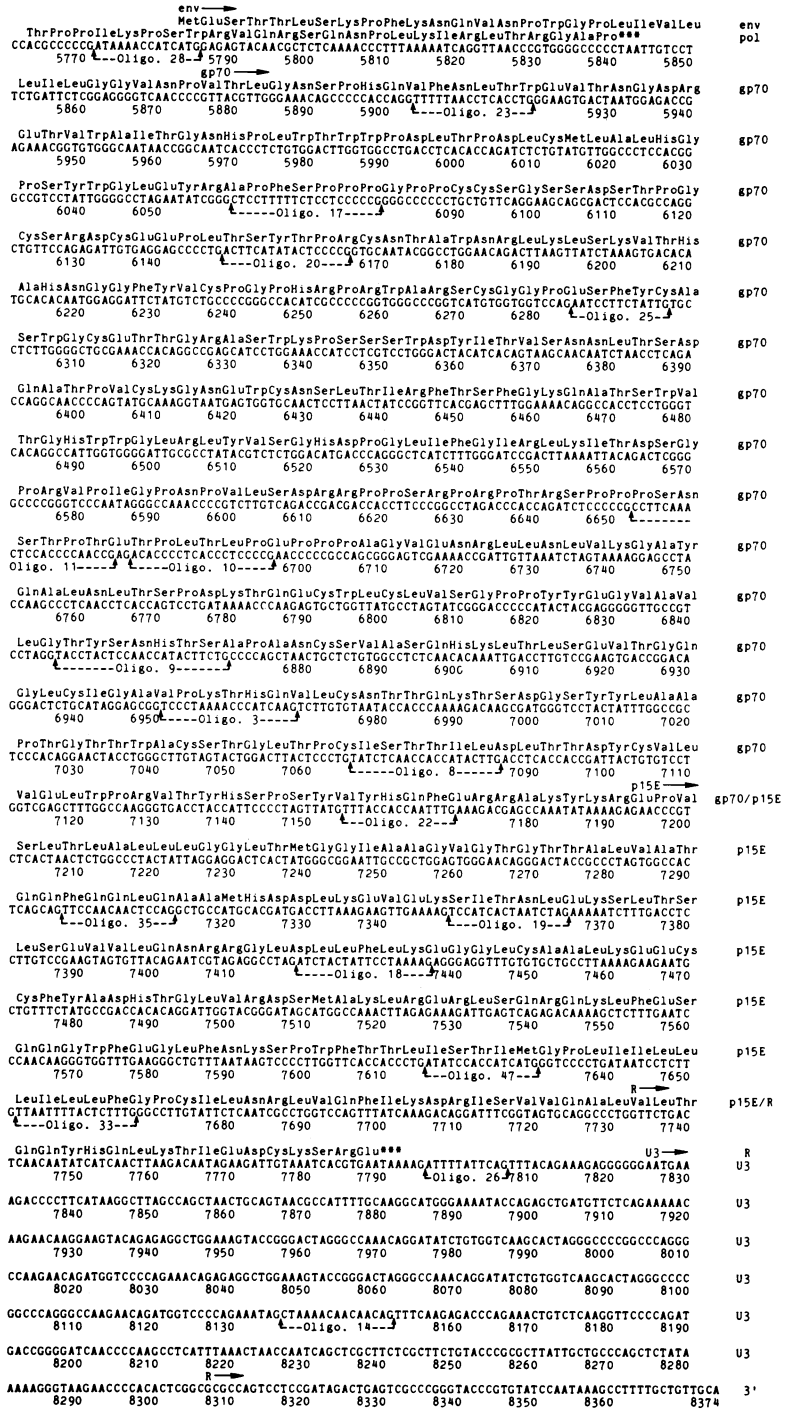

FIG. 1. Nucleotide sequence of AKV MLV. The DNA strand corresponding to the viral plus strand is shown. The amino acid sequences of the *gag*, *pol*, and *env* genes, as well as the beginning of the R, U5, and U3 sequences, are noted above the sequence. RNase T_1 oligonucleotides (23) and features discussed in the text are indicated below the sequence.

identify any differences from the sequences of this region previously reported (15, 21, 56).

The AKV and Moloney MLV (Mo-MLV) (48) nucleotide sequences were compared by using the DIAGON computer program (52).

RESULTS AND DISCUSSION

Figure 1 shows the 8,374-nucleotide sequence corresponding to the plus (sense) strand of the AKV RNA genome, as determined from the infectious molecular clone of AKV, AKR-623 (22). Because the AKV terminal repeat (R) is nearly identical to that of Mo-MLV (56), the 5′ and 3′ termini of AKV have been aligned to match the Mo-MLV genome (48). Figure 1 also shows the deduced amino acid sequence of the AKV *gag*, *pol*, and *env* gene products, the limits of the R sequence, and the locations of the unique 5′ (U5) and 3′ (U3) sequences that are duplicated in the provirus LTR. These structural features were identified by comparing the AKV sequence with the nucleotide sequence of Mo-MLV (48) and the amino acid sequences of Rauscher MLV proteins determined by Orozlan and his colleagues (see reference 58).

Comparison with previously reported AKV sequences. The nucleotide sequence shown in Fig. 1 of the AKR-623 LTR (nucleotides 1 to 144 and 7,825 to 8,374) is identical to that reported by Van Beveren et al. (56). Lenz et al. (21) have reported the nucleotide sequence of the AKR-623 *env* gene (nucleotides 5,674 to 7,865). The sequence shown in Fig. 1 differs at two positions: an additional T residue at nucleotide 5,676 and a C rather than G residue at nucleotide 5,740.

We previously reported the sequence of nucleotides 3,309 through 7,865 from a noninfectious molecular clone of AKV (15). There are six differences within this region between the infectious and noninfectious clones: the noninfectious clone contains an extra G residue within the polypurine tract near the origin of plus-strand strong stop synthesis (nucleotides 7,819 to 7,824) and contains an A residue in place of a G residue at five positions (nucleotides 4,685, 5,240, 5,885, 7,169, and 7,556). Curiously, all five of the base substitutions are identical. Furthermore, the affected guanine residues are surrounded by the consensus sequence TTNGAAA (the consensus nucleotides are each present in at least four of the five sites), suggesting that this sequence is prone to mutation during viral replication. Consistent with this, two differences between the LTRs of AKV and Gross MLV (57) and a change between the 5′-leader sequences of two different molecular clones of Mo-MLV (47) are also G-to-A transitions which share this consensus sequence. The G-to-A transition at nucleotide 5,240 of AKV has mutated a tryptophan codon (UGG) into a UGA termination codon within the *pol* gene of the noninfectious clone, perhaps explaining the lack of infectivity.

Because the 3′ half of AKV has already been described extensively (15, 21, 56), these sequences will not be discussed further here.

AKV 5′-leader sequence. The 5′-leader region of AKV stretches from the 5′ cap site to the first initiation codon (AUG) in the AKV viral genome, at nucleotides 639 to 641, where translation of the *gag* and *pol* precursors is initiated. Within the 5′ leader of AKV, the 5′-terminal 220 nucleotides are 90% homologous to the analogous sequences in Mo-MLV (48). This stretch of nucleotides contains the R (nucleotides 1 to 68) and U5 (nucleotides 69 to 144) sequences and the $tRNA^{pro}$ binding site (nucleotides 145 to 162) where negative-strand synthesis initiates, as well as a putative donor splice site sequence (nucleotides 203 to 209) for generation of the *env* mRNA. A possible *env* mRNA acceptor splice site sequence, identical to Mo-MLV, lies at nucleotides 5,508 to 5,516.

The region of the AKV genome spanning nucleotides 220 to 270 is characterized by a stretch of 21 alternating purine/pyrimidine residues (nucleotides 245 to 265). This sequence, which is almost entirely composed of repeating TG dinucleotides, is remarkable for three reasons: (i) repeating tracts of TG dinucleotides can exist as a left-handed Z helix under physiological conditions (13, 30); (ii) this sequence is found highly repeated in eucaryotic genomes (11, 25); and (iii) the sequence has been implicated as a hot spot for recombination (35, 49, 54). The analogous region of the Mo-MLV genome bears little resemblance to AKV and lacks the stretch of TG residues, indicating that the latter is not essential for replication. Nevertheless, Mo-MLV and AKV do share within this region two copies of the pentanucleotide TGTCT (nucleotides 227 to 231 and 239 to 243 in AKV), which is also frequently present neighboring other TG tracts identified in mammalian genomes (12, 25, 27, 28). These results suggest that this region of both AKV and Mo-MLV may be related to the TG tract sequences found in eucaryotic genomes.

The remainder of the AKV leader sequence is characterized by an open reading frame beginning at nucleotide 318 in the same frame as the *gag* coding sequences. An analogous open reading frame begins at nucleotide 310 of the Mo-MLV genome. (The two open reading frames are out of frame with respect to one another until nucleotide 363 in the AKV genome.) Cells infected by MLV express on their surface a glycosylated *gag* gene product, referred to as $gPr80^{gag}$ (20, 50, 55). Peptide mapping of $gPr80^{gag}$ (8, 45) indicated that the amino-terminal region of $gPr80^{gag}$ probably derives from part of the open reading frame found in the 5′-leader region. Schwartzberg et al. (47) have shown that Mo-MLV deletion mutants which lack parts of this open reading frame do not produce $gPr80^{gag}$ but are still replication competent. It is perhaps not surprising, therefore, that the AKR-623 AKV clone is infectious even though it lacks an AUG initiation codon to express $gPr80^{gag}$.

AKV *gag* and *pol* genes. The *gag* and *pol* gene products are encoded by a long open reading frame (nucleotides 639 to 5,843) that is interrupted by a single amber (UAG) codon separating the two genes. The MLV *gag* gene is translated as a single polypeptide, $Pr65^{gag}$, which is subsequently cleaved to produce the internal viral proteins p15 (encoded by nucleotides 642 to 1,025), p12 (1,026 to 1,280), p30 (1,281 to 2,069), and p10 (2,070 to 2,237). Comparison of the AKV sequence in this region with the Mo-MLV and Rauscher MLV *gag* sequences (48, 58) shows that the amino acid residues adjacent to the proteolytic cleavage sites are identical in all three MLVs. The AKV p30 and p10 peptides are very highly conserved with respect to Mo-MLV and Rauscher MLV (95% homologous at the amino acid level), whereas the p12 and p15 peptides are not.

The initial translation product of the *pol* gene is the $Pr180^{gag\text{-}pol}$ fusion protein that contains both *gag* and *pol* antigenic determinants. To synthesize such a molecule, the amber codon separating *gag* and *pol* in the viral genome must be either eliminated or suppressed. This could be accomplished by either splicing the genomic RNA to remove the UAG codon from the *pol* mRNA, as appears to be the case with Rous sarcoma virus (46), or translation of the termination codon by a suppressor tRNA (26, 34).

Shinnick et al. (48) proposed a RNA secondary structure

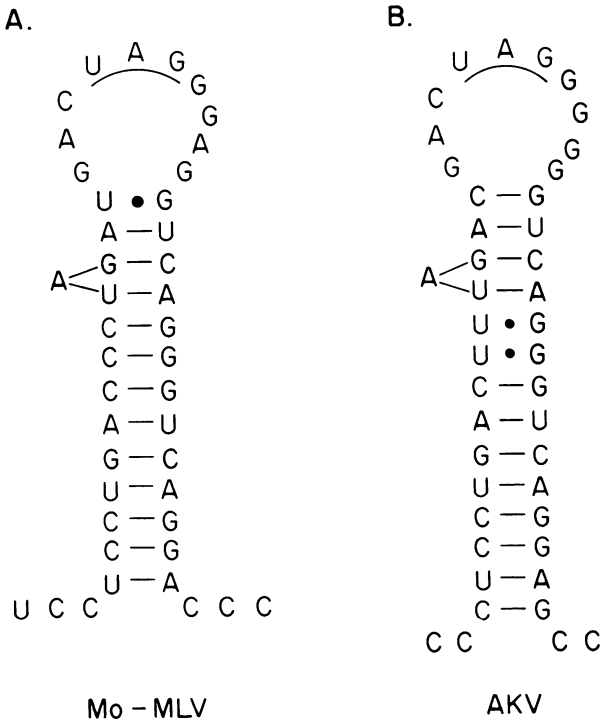

FIG. 2. Comparison of potential RNA secondary structures flanking the amber codon (underlined) which separates the *gag* and *pol* genes of Mo-MLV (A) and AKV (B).

flanking the amber codon separating the *gag* and *pol* genes in Mo-MLV. This and an analogous secondary structure from the AKV sequence are shown in Fig. 2. The nucleotides in AKV that are base paired in the secondary structure are marked by apostrophes below the sequence shown in Fig. 1 (between nucleotides 2,232 and 2,270). Although there are several base differences between the Mo-MLV and AKV structures (one of which extends the AKV stem by 1 base pair), none of these destroy any base pairs, providing further evidence that such a structure exists in vivo. The stem of each structure contains a single, unpaired, "bulged" adenine residue. Double-stranded RNA helices containing a bulged nucleotide have been implicated as RNA-protein contact sites (32); the stem-and-loop structure shown in Fig. 2 might thus serve as a binding site for a regulatory protein that controls the relative amounts of $Pr65^{gag}$ and $Pr180^{gag\text{-}pol}$, whether this occurs by splicing of the genomic RNA or tRNA suppression.

The *pol* gene in AKV extends from nucleotide 2,253 to 5,843 and can encode 1,196 amino acids. As noted previously in Mo-MLV (48), this reading frame is much larger than required to encode the 80,000-dalton reverse transcriptase polypeptide. Because the *pol* gene of avian retroviruses encodes a double-stranded DNA endonuclease (10, 44), Shinnick et al. (48) suggested tha the extra coding potential of the MLV *pol* gene might encode an analogous endonuclease; recent evidence (T. Shinnick, personal communication) indicates that an endonuclease activity is in fact encoded by the 3′ region of the *pol* gene in Mo-MLV. Comparison of the AKV and Mo-MLV *pol* amino acid sequences shows that the amino-terminal two-thirds (encoded by nucleotides 2,253 to 4,500) of the *pol* gene product is 96% homologous, whereas the carboxy-terminal third is only 77% homologous. The conserved region is the expected size to encode reverse transcriptase, suggesting that it is the endonuclease activity which is not highly conserved and might, therefore, be virus specific.

Structure of oncogenic MCF MLV. The structure of recombinant MCF viruses has been extensively studied by both restriction site mapping of proviral DNA (4) and RNase T_1 oligonucleotide mapping (23, 38). Using the RNase T_1 oligonucleotide sequences determined by Pedersen and Haseltine

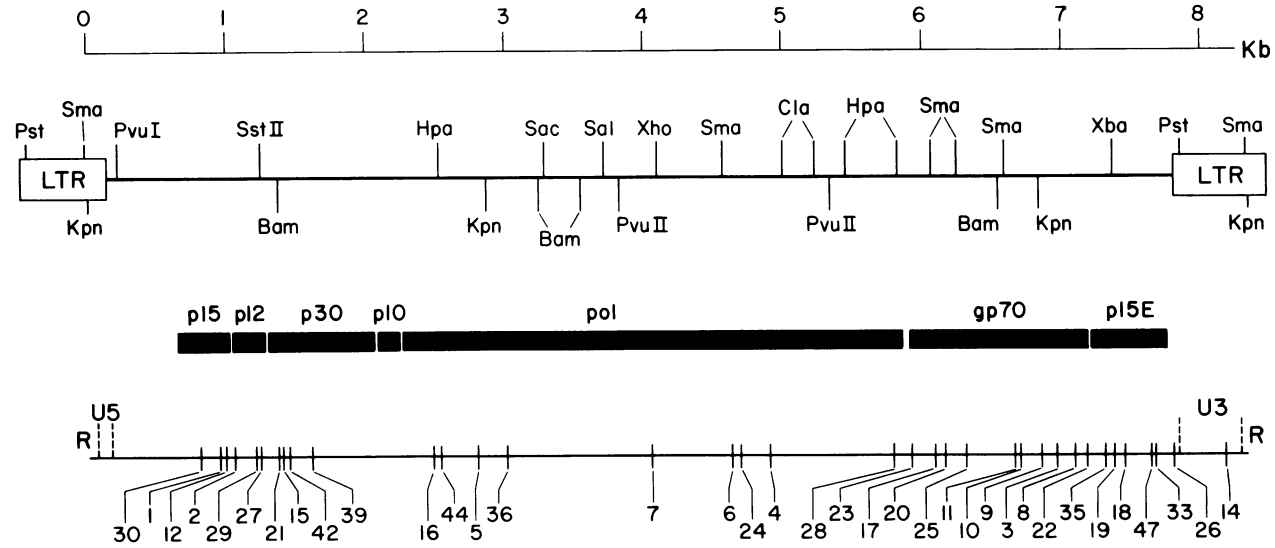

FIG. 3. Correlation of AKV restriction sites and RNase T_1 oligonucleotides with the AKV gene map. Upper portion shows the location of the provirus restriction enzyme sites mapped by Chattopadhyay et al. (4) and confirmed by the sequence. Unless otherwise specified all enzymes are I. Wild-type AKV contains a *Hin*dIII site approximately 2.5 kilobases from the 5′ cap site (53) that is missing from the AKR-623 clone. AKV does not contain the recognition sites for the following enzymes: *Ava*III, *Bcl*I, *Eco*RI, *Mlu*I, *Mst*I, *Nde*I, *Nru*I, *Sph*I, and *Xma*III. Middle portion shows the AKV gene map. At the bottom is shown the location of the RNase T_1 oligonucleotides used by Lung et al. (23) to study the structure of MCF genomes. Oligonucleotide 39 is the N-tropic specific oligonucleotide identified by Rommelaere et al. (37).

(33) and the pancreatic RNase digestion products identified by Rommelaere et al. (38), we have located within the AKV sequence the RNase T_1 oligonucleotides used by Lung et al. (23) to study the recombination patterns of MCF viruses. These RNase T_1 oligonucleotides are identified under the sequence in Fig. 1. Figure 3 correlates the location of the RNase T_1 oligonucleotides (bottom) with both the AKV provirus restriction sites (top) and the AKV gene map (middle).

The sequence from the 5′ half of AKV shows that all of the RNase T_1 oligonucleotides analyzed by Lung et al. (23) from this region derive from either the p15, p12, and amino-terminal half of the p30 coding sequences or a short region in *pol*. The exact nature of large regions of the 5′ half of MCF genomes were thus ignored in the oligonucleotide studies. Furthermore, because the restriction site map of the 5′ half of AKV is more similar to that of nonecotropic proviruses than is the AKV 3′ half (4), the restriction site analyses of Chattopadhyay et al. (4) were likewise not able to detect recombination as readily within the 5′ half of the MCF genomes as within the 3′ half.

Nevertheless, Lung et al. (23) showed that many of the oncogenic MCF viruses (e.g., MCF 13) lack specific RNase T_1 oligonucleotides found in AKV, for example, the *pol* oligonucleotides 44 and 5 (see Fig. 3). Consistent with this result Chattopadhyay et al. (4) showed that some of the MCF proviruses (such as MCF 13) lack AKV restriction sites (e.g., *Sac*I and *Hpa*I) which lie within the *pol* gene. Taken together, these results suggest that sequences within the 5′ half of MCF viruses are frequently derived from the non-AKV parent virus. The new 5′ AKV sequences presented here should aid in identifying the exact locations of the nonecotropic sequences within the 5′ region of MCF genomes.

ACKNOWLEDGMENTS

This work was done in the laboratory of F. Sanger, whom I thank for support and encouragement. I thank D. Lowy and S. Chattopadhyay for the AKR-623 clone of AKV; A. Bankier, B. Barrell, M. Biggin, and T. Gibson for advice on dideoxy sequencing; C. Keller, R. Roberts, and R. Staden for help with the computer analyses; J. Abraham, S. Hughes, and J. Sambrook for critical readings of the manuscript; and P. Barkley for typing the manuscript.

I was supported by a fellowship from the Helen Hay Whitney Foundation.

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