Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 678–682, February 1983
Biochemistry

Characterization and partial nucleotide sequence of endogenous type C retrovirus segments in human chromosomal DNA

(retrovirus evolution/human endogenous retrovirus/repetitive human DNA)

ROY REPASKE, RAYMOND R. O'NEILL, PAUL E. STEELE AND MALCOLM A. MARTIN

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

Communicated by Wallace P. Rowe, October 19, 1982

ABSTRACT **Twenty-six different murine leukemia virus (MuLV)-related clones have been isolated from a human DNA library and characterized by restriction enzyme mapping and reciprocal nucleic acid hybridization reactions. The sequence of approximately 2,600 nucleotides, spanning more than 4.0 kilobases, of one of the MuLV-related cloned human DNAs was also determined. The deduced amino acid sequence permitted the alignment of this prototype cloned human DNA segment with the p12 *gag*, p30 *gag*, p10 *gag*, and *pol* regions of Moloney MuLV. A majority of the endogenous type C retrovirus-related segments present in human DNA are approximately 6.0 kilobases in size and appear to contain a deletion of *env* sequences.**

In many vertebrate species, endogenous type C proviruses are vertically transmitted (1–3), have been shown by various nucleic acid hybridization techniques to be integrated into genomic DNA (4–7), have been mapped to certain chromosomal loci (3, 8, 9), and may be expressed as infectious retroviruses (10). Although the spontaneous induction of endogenous proviral DNAs has been associated with disease in some animals, the functional significance of the numerous copies of type C retroviral DNA in most mammalian species remains to be elucidated.

The identification and molecular cloning of murine leukemia virus (MuLV)-like sequences from human DNA was reported recently (11). By using low-stringency hybridization techniques and a radiolabeled DNA probe consisting of a 2.7-kilobase (kb) segment of the polymerase (*pol*) gene of an endogenous African green monkey (AGM) proviral DNA (12), a clone was obtained from a human DNA library that specifically hybridized to internal restriction fragments of AKR ecotropic proviral DNA (11). Because *gag*, *env*, and long terminal repeat (LTR) regions of different type C mammalian proviruses are not as highly conserved as the *pol* gene (11, 12), we were unable to determine precisely the molecular organization of the MuLV-related cloned human DNA by using hybridization techniques.

In this communication we describe the cloning and characterization of 25 additional type C endogenous retroviral DNAs. These cloned segments were screened from a human DNA library under high-stringency hybridization conditions. The sequence of approximately 2,600 nucleotides of one of the MuLV-related clones (51-1) has been determined and the deduced amino acid sequence is compared to that published for Moloney (Mo) MuLV (13). This analysis revealed a colinear conservation of *gag* and *pol* sequences in human DNA and provided definitive data that DNA segments located between 950 and 4,600 base pairs (bp) in the Mo-MuLV genome were present in nearly all of the cloned human DNAs.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Molecular Cloning of Type C Viral DNA from Human DNA. A partial *Alu* I and *Hae* III human DNA library (14) in λ Charon 4A (15) was initially screened for type C retrovirus sequences by using a cloned *pol* segment (pAGM-B6) of an endogenous AGM proviral DNA (λ AGM 1) (11, 12) under nonstringent hybridization conditions. MuLV-related human DNA segments were also isolated from the library under stringent hybridization conditions by using the two subgenomic portions of human clone 51-1 DNA shown in Fig. 1.

Restriction Enzyme Digestions and Nucleic Acid Hybridizations. Cloned DNAs were digested with commercial preparations of restriction enzymes used as recommended by their suppliers. Restricted DNAs were electrophoresed in horizontal agarose slab gels and transferred to nitrocellulose membranes as described (16). "Standard" (17) and nonstringent (11) hybridization conditions have been described.

DNA Sequence Analysis. Subgenomic segments of human clone 51-1 were digested with restriction endonuclease and 5′ end-labeled with [γ-^{32}P]ATP (Amersham; 3,000 Ci/mmol; 1 Ci $= 3.7 \times 10^{10}$ Bq) by using T4 polynucleotide kinase (P-L Biochemicals). The end-labeled fragments were cleaved and separated electrophoretically on 1% agarose gels. Labeled fragments were recovered from gel slices by using the saturated NaI and glass bead procedure of Vogelstein and Gillespie (18). The nucleotide sequence was determined by the partial degradation method (19). The sequence analysis strategy is presented in Fig. 3.

RESULTS

Isolation of MuLV-Related Segments from Human DNA. The previously reported (11) identification and molecular cloning of MuLV-reactive sequences in human DNA was accomplished by hybridizing human DNA with an endogenous retroviral DNA probe derived from AGM liver DNA, under low-stringency nucleic acid hybridization conditions. Of the two clones (51-1 and 71A) of human DNA isolated in this manner (Fig. 1A), the former was extensively characterized and shown to contain a region that hybridized to *gag* and *pol* segments of cloned MuLV DNA (11). An internal 1.8-kb *Hin*dIII fragment of human clone 51-1, which maps in the *pol* region (heavy lines in Fig. 1A), was subcloned in pBR322 and used to screen a partial *Alu* I and *Hae* III human DNA library in λ Charon 4A. For this second cloning of MuLV-related sequences from human DNA, stringent hybridization conditions were used. Thirteen clones were obtained; a representative sample is grouped in Fig. 1*B*. One of these 13 new clones was identical to clone 71A (originally isolated from human DNA by using the cloned en-

Abbreviations: MuLV, murine leukemia virus; kb, kilobase(s); AGM, African green monkey; bp, base pair(s); Mo, Moloney; LTR, long terminal repeat.

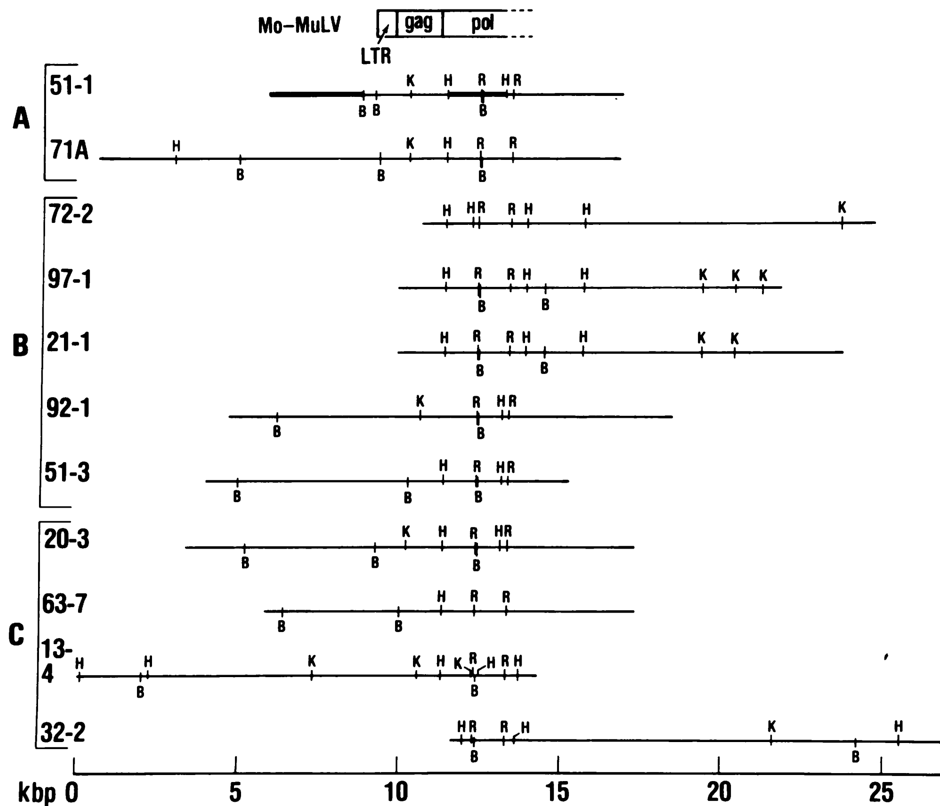

FIG. 1. Representative MuLV-related DNA segments cloned from human DNA. A human DNA library (14) was screened for type C retrovirus-reactive sequences by using a 2.7-kb *Bam*HI internal fragment of an endogenous AGM proviral DNA containing *pol* sequences (12) under low-stringency hybridization conditions (*A*) or with the 1.8-kb *Hin*dIII (*B*) or the 2.7-kb *Eco*RI/*Bam*HI (*C*) fragments of clone 51-1 under high-stringency conditions. Each horizontal line represents the DNA insert present in a Charon 4A recombinant phage isolated from the human gene library and contains *Eco*RI linkers at both termini. The thick-line portions of clone 51-1 indicate the *Hin*dIII or *Eco*RI/*Bam*HI fragments used to select MuLV-related segments presented in *B* and *C*, respectively. The various retrovirus-reactive human clones have been aligned with the Mo-MuLV genome on the basis of nucleic acid hybridization experiments and the nucleotide sequence analyses presented in Fig. 3. B, *Bam*HI; K, *Kpn* I; H, *Hin*dIII; R, *Eco*RI.

dogenous AGM retroviral DNA segment and low-stringency hybridization conditions). All of the new clones as well as 51-1 and 71A contained an internal 1.0-kb *Eco*RI fragment (mapping between 12.3 and 13.3 kb on the scale used in Fig. 1) as well as a *Bam*HI site at 12.4 kb. Several of the MuLV-reactive cloned human DNAs also contained a *Hin*dIII site at 11.3 kb.

Preliminary characterization of the clones obtained from these first two forays into the human DNA library included electron microscopic analyses of heteroduplex structures formed after the hybridization of different cloned DNAs to one another. When clone 51-1 was hybridized to clone 71A, an 8.5-kb region of homology containing three small substitution loops was observed (C. Garon, personal communication). This homologous segment mapped from 6.0 to 14.5 kb on the scale used in Fig. 1. Because this result suggested that MuLV-related sequences might extend to the left of the *Bam*HI site of clone 51-1 located at 8.7 kb and only a few of the newly obtained human clones shown in Fig. 1*B* contained sequences extending into this region, we decided to obtain additional clones from the human library by using the 5′ 2.8-kb *Eco*RI/*Bam*HI fragment of 51-1 DNA (mapping between 5.9 and 8.7 kb; heavy line in Fig. 1*A*) as a hybridization probe under stringent reaction conditions.

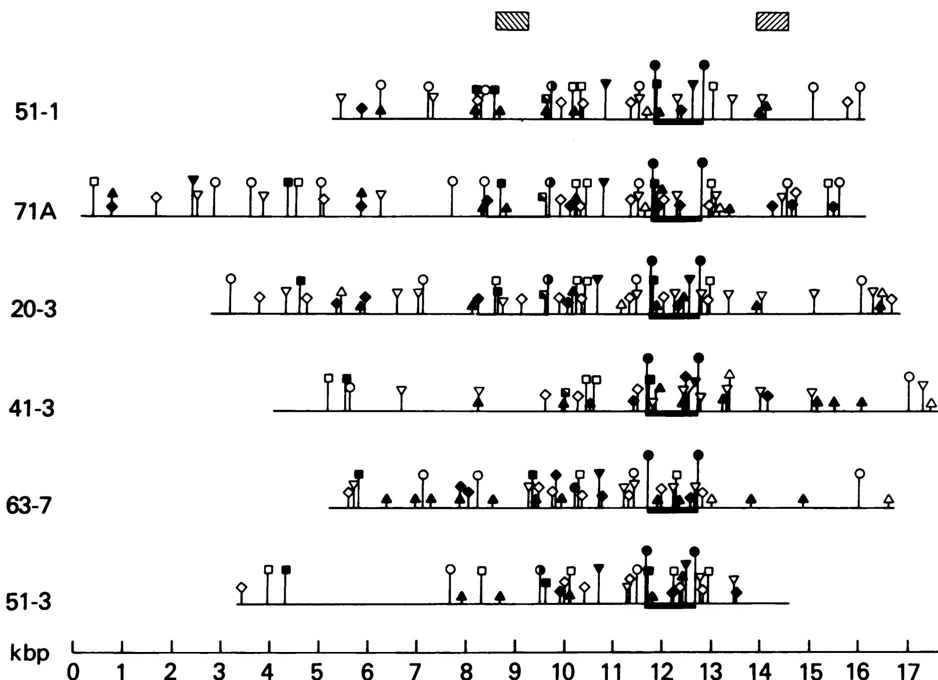

FIG. 2. Restriction maps of six different MuLV-related cloned human DNA segments. Six human DNA clones containing *gag*- and *pol*-reactive regions as well as cross-hybridizing, LTR-like segments (hatched rectangles) were cleaved with various restriction enzymes. The common 1-kb *Eco*RI segment is shown by a heavy line. Clones 51-1 and 71A were acquired in the first foray into the human gene library; 51-3, in the second foray; and 20-3, 41-3, and 63-7, in the third foray. Clone 41-3 is identical to 92-1 (Fig. 1). Additional restriction sites present within some of the clones could not be precisely mapped. Their positions (in kb) were: 51-1 *Acc* I, 6.1–8.0; 71A, *Taq* I, 2.5–3.5 and *Pvu* II, 2.3–4.3; 20-3, *Ava* I, 3.3–6.9 and *Acc* I, 2.7–5.7; 41-3, *Taq* I, 4.0–6.5 and *Acc* I, 4.0–8.2; 63-7, *Taq* I, 12.7–16.7; 51-3, *Taq* I, 13.9–14.4. ▲, *Acc* I; ○, *Ava* I; ■, *Bam*HI; △, *Bgl* II; ●, *Eco*RI; ◆, *Hin*cII; ▼, *Hin*dIII; ◪, *Kpn* I; □, *Pst* I; ◇, *Pvu* II; ◑, *Sal* I; ▽, *Taq* I.

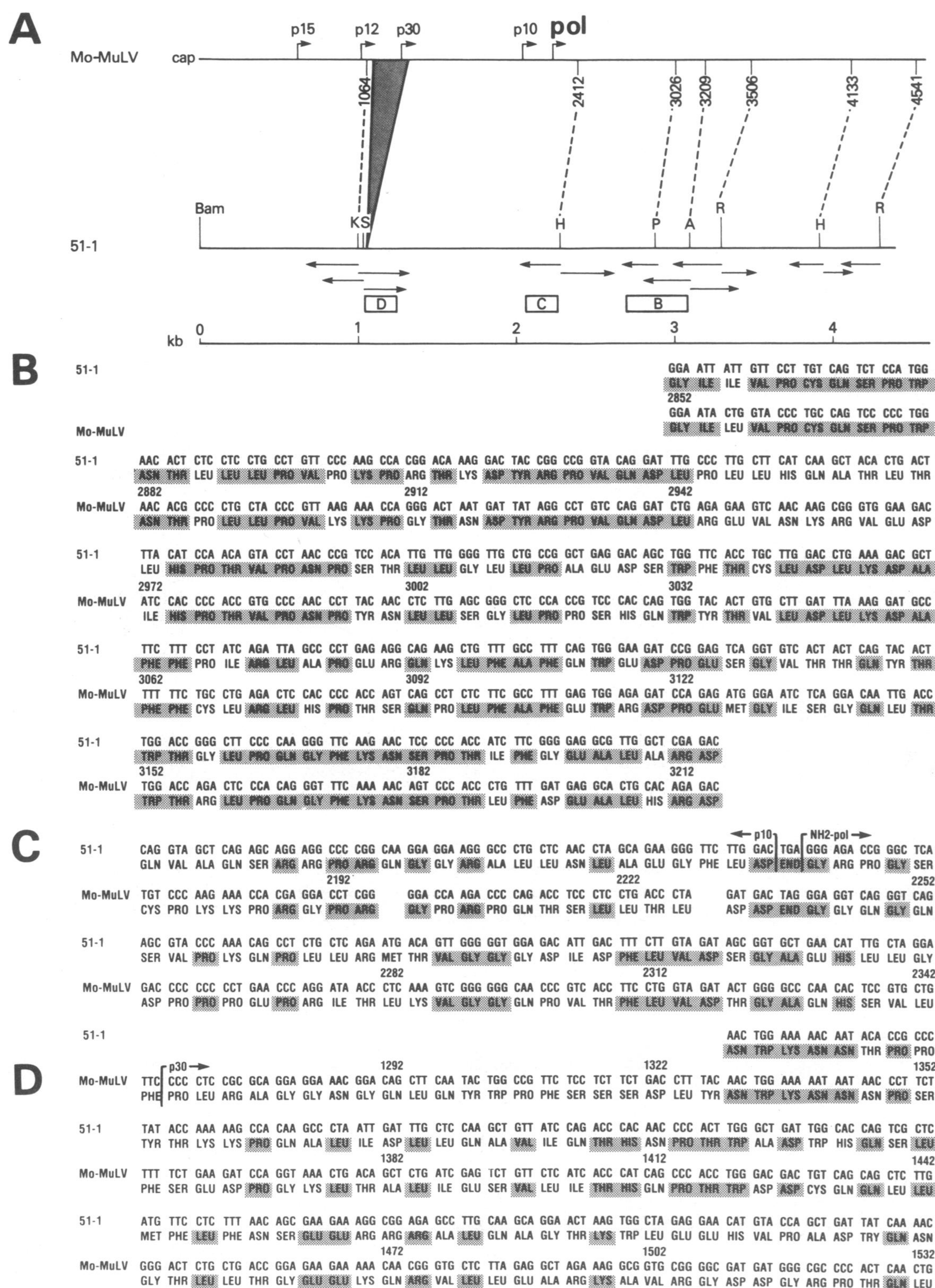

FIG. 3. Sequence analysis of portions of clone 51-1 DNA and its alignment with Mo-MuLV. (*A*) Strategy used for analysis of 51-1 DNA and alignment of 51-1 DNA with the Mo-MuLV genome as deduced from the nucleotide sequence. Sequences at the principal restriction sites of 51-1 are connected by dashed lines to the analogous base number of Mo-MuLV. The numbers refer to the numbering scheme of Shinnick *et al.* (13) for Mo-MuLV. A deletion in 51-1 DNA relative to Mo-MuLV, shown by the stippled wedge, occurs 3′ to the *Sal* I restriction site in the junction region encoding p12 and p30 *gag* proteins. Letters enclosed in the boxes refer to the panels in which particular nucleotide sequences and derived amino acids are presented (in the subsequent panels, homologous amino acids are stippled). Restriction sites: A, *Ava* I; Bam, *Bam*HI; H, *Hin*dIII; K, *Kpn* I; P, *Pvu* II; R, *Eco*RI; S, *Sal* I. (*B*) Nucleotide sequence and deduced amino acids of 51-1 and Mo-MuLV proviral DNAs in a segment of the *pol* region. Mo-MuLV base numbers are shown for reference. (*C*) Nucleotide sequence and deduced amino acids of 51-1 and Mo-MuLV proviral DNAs located around the carboxy terminus of p10 *gag* and the amino terminus of *pol*. Gaps occur in the Mo-MuLV sequence wherever nucleotides are inserted into 51-1 DNA relative to Mo-MuLV. (*D*) Sequence homology near the amino terminus of the p30 *gag* region of 51-1 and Mo-MuLV proviral DNAs.

Fifteen additional clones were subsequently isolated; four of these are shown in Fig. 1*C*. Three of the clones isolated from this third foray into the human library were indistinguishable from clone 51-1 and one was identical to clone 92-1. The 11 new MuLV-related cloned human DNA segments contained the 1.0-kb internal *Eco*RI fragment and the highly conserved *Hin*dIII and *Bam*HI sites at 11.3 and 12.4 kb, respectively, previously noted in most of the other clones.

Restriction Mapping of Selected Clones. The restriction maps of six Mo-MuLV-related cloned human DNAs were determined in greater detail (Fig. 2). These six were selected because each contained segments (indicated by the hatched rectangles at the top of Fig. 2) which cross-hybridized in a manner typical of LTRs (unpublished data). The putative 5' LTR-like segment was located at an appropriate position relative to a p30 *gag*-related region (determined from nucleotide sequence analysis shown in Fig. 3*D*) and, with the cross-hybridizing 3' segment, defined a proviral DNA about 6 kb in size. The results presented in Fig. 2 indicate that many restriction sites are conserved among the six clones although microheterogeneity with respect to their existence and alignment was observed. For example, the 1.0-kb *Eco*RI segment (heavy line in Fig. 2) was present in all six clones. Five of the six contained the *Hin*dIII and *Bam*HI sites at, respectively, 10.7 and 11.8 kb (Fig. 2 scale). Two closely spaced *Pst* I sites in the 10.0- to 10.6-kb region were present in four clones whereas only a single *Pst* I site was detected in this region of the other two. Furthermore, the exact alignment of *Pst* I sites varied between different clones. Four clones contained a *Sal* I site at 9.6 kb; an adjacent *Kpn* I site was present in three of the four. This heterogeneity apparently reflected the presence of microdeletions in each clone relative to the others, as judged by the pattern of hybridization between correspondingly positioned clone segments (data not shown). The overall pattern is consistent with a multimembered family of related sequences characteristic of endogenous retroviruses (20).

Fine mapping of the human endogenous retroviral DNAs permitted localization of the *Alu* family of reiterated sequences within some of the clones presented in Fig. 2 (data not shown). In all cases, an *Alu* family probe (BLUR8) (21) hybridized to regions of different clones outside of the 6-kb segment encompassed by the putative LTRs.

Nucleotide Sequence of Clone 51-1 DNA. To assess more fully its molecular organization and to align the type C retroviral sequences present in 51-1 DNA precisely with the Mo-MuLV provirus, portions of the cloned human DNA were subjected to sequence analysis. The sequence of more than 2,600 nucleotides of clone 51-1 DNA was determined (Fig. 3*A*). Although conservation of nucleotide sequences was readily demonstrable in different regions of the two DNAs, a more striking alignment could be made when the deduced amino acid sequences were compared (Fig. 3 *B–D*). On this basis, clone 51-1 DNA and the Mo-MuLV genome were colinear over a region of at least 3.5 kb. Thus, nucleotides and predicted amino acids located immediately 5' to the *Kpn* I (1.0 kb), *Hin*dIII (2.3 kb), and two highly conserved *Eco*RI (3.3 and 4.3 kb) sites of 51-1 DNA could be aligned with homologous regions of Mo-MuLV mapping at 1,064, 2,412, 3,506, and 4,541 bp, respectively (Fig. 3*A*).

The results of previously published hybridization experiments assessing polynucleotide sequence homology among different type C mammalian retroviral DNAs indicated that the sequences encoding reverse transcriptase were the most highly conserved (12). In the case of Mo-MuLV and clone 51-1 proviral DNAs, this was confirmed by direct nucleotide sequence analysis. The region of greatest homology consisted of a 366-bp segment of 51-1 DNA that was strikingly similar to a portion of the *pol* region of Mo-MuLV mapping between 2,850 and 3,215 bp. Of the first 31 amino acids encoded in this segment, 26 were identical (Fig. 3*B*). Similarly, 20 of the 25 amino acids encoded by the 3' end of the *pol* segment presented in Fig. 3*B* including a run of 10 consecutive amino acids are also identical. In the 5' direction, the sequence of clone 51-1 DNA revealed conservation of the carboxy-terminal amino acid of the p10 *gag* protein (aspartic acid), the translation terminator, and the amino-terminal amino acid of *pol* (glycine) (Fig. 3*C*). These sequences were positioned at the appropriate distance [510 bp (Mo-MuLV) vs. approximately 500 bp (51-1 DNA)] to the 5' side of the region shown in Fig. 3*B*. Even further in the 5' direction of clone 51-1 DNA, the sequence of a 204-bp segment revealed conservation of nucleotides encoding the p30 *gag* protein (Fig. 3*D*) at the appropriate location in the human DNA segment.

Knowledge of the sequences of different portions of clone 51-1 DNA allows definitive alignment with the Mo-MuLV genome. Fine mapping of different MuLV-related human DNA clones (Fig. 2) suggested the existence of small deletions when they were compared to one another in reciprocal hybridization experiments. In this regard, sequence analysis of 51-1 DNA in the region of the *Kpn* I site at 1.0 kb (Fig. 3*A*) revealed a deletion of 234 nucleotides present in Mo-MuLV and mapping between 1,095 and 1,329 bp. If this segment of the cloned human DNA is expressed, the deletion would result in a fused p12–p30 *gag* protein.

DISCUSSION

Determination of the nucleotide sequences of different MuLV-related human DNA segments extends the previous report (11) which described the identification and molecular cloning of such sequences from human chromosomal DNA. Comparison of endogenous human retroviral sequences with those of Mo-MuLV has facilitated the alignment of more than 3.5 kb of cloned human DNA with the proviral DNA of a known murine leukemia virus. Previously reported nucleic acid hybridization experiments indicated that the *pol* region was highly conserved when the proviral DNAs of murine and simian type C retroviruses were compared (11, 12). The nucleotide sequence analysis described in this report indicates extensive polynucleotide and deduced amino acid sequence homology involving the *pol* segments of Mo-MuLV and 51-1 DNAs. Within a block of 366 nucleotides located in the *pol* region (Fig. 3*B*), which maps 612 bp downstream from the amino terminus of *pol*, 66% of the amino acid sequence (80 of 122 amino acids) is conserved. This segment is located well within the portion of the *pol* gene encoding reverse transcriptase (T. M. Shinnick and J. G. Sutcliffe, personal communication). Within the *pol* region of clone 51-1 DNA analyzed (2,000 nucleotides), 34% of the deduced amino acids are identical. As indicated in Fig. 3, regions of the Mo-MuLV genome other than *pol* gene are also conserved in cloned human DNA. An interesting feature of the homologous p30 *gag* segment shown in Fig. 3*D* is the presence of Asn-Trp-Lys as the first three amino acids in the human DNA. This tripeptide is located 28–30 amino acids from the amino terminus of all known mammalian type C virus p30 *gag* proteins and is a component of a highly conserved heptapeptide mapping between positions 24 and 30 of p30 (22).

The results of nucleotide sequence analyses have provided definitive data indicating that DNA segments located between 950 and 4,600 bp in the Mo-MuLV genomes were represented in the cloned human DNAs. However, many clones, including 51-1 and the others shown in Fig. 2, are truncated relative to Mo-MuLV because the position of LTR-like elements within them defines an overall length no greater than 6.2 kb. Recent

nucleotide sequence analysis indicates that most of the shortening of the human endogenous type C retroviral DNAs relative to Mo-MuLV results from a deletion of *env* and some *pol* sequences from these particular human clones (unpublished data). Clone 51-1 may be like several MuLV proviral DNAs that have been characterized and shown to contain *env* deletions of various sizes (23, 24). In this regard, we have recently ascertained that some of the clones isolated during the second foray into the human library [such as 72-2, 97-1, and 21-1 (Fig. 1*B*)] contain type C retroviral DNA sequences that are absent from clone 51-1. The 51-1 family of clones hybridizes weakly, if at all, to subgenomic segments positioned in the 3′ terminal 3.5-kb of baboon or AGM endogenous proviral DNAs (unpublished data).

On the other hand, the second family of Mo-MuLV-related human DNA segments (5 of the 26 different type C retroviral clones obtained to date) reacts strongly with portions of type C simian proviruses mapping between 5.5 and 6.2 kb and has a nucleotide sequence closely related to that of Mo-MuLV proviral DNA between nucleotides 5,400 and 6,000 (unpublished data). The second class of human endogenous retroviral DNAs thus contains the 3′ portion of the *pol* gene and possibly *env* gene sequences that are absent from the 51-1 family of clones. Preliminary restriction mapping of one member of this second category of cloned human DNAs has revealed the presence of closely associated *Acc* I and *Sac* I sites that are located 8.4 kb apart and map at positions appropriate for LTRs (at 8.6 and 17.0 kb on the scale used in Fig. 1) (A. Rabson, personal communication).

An unexpected finding which became apparent once the LTR-like regions in clone 51-1 were localized was that the 5′ 2.7-kb *Eco*RI/*Bam*HI segment of that clone, which was used to select several additional MuLV-related clones in the third foray into the human library (Fig. 1*C*), lies entirely 5′ to type C retroviral sequences. Twelve new clones containing retroviral sequences were obtained when the human DNA library was screened with this "flanking" cellular DNA probe. Thirteen of the 26 MuLV-related human DNA clones that have been characterized contain this conserved flanking sequence; in contrast, several of the clones isolated by using the 1.8-kb internal *Hin*dIII *pol* DNA probe (Fig. 1*B*) had different flanking cellular sequences.

Blot-hybridization experiments involving restricted human genomic DNAs with defined *gag* and *pol* segments of cloned human retroviral DNAs produced a complex banding pattern with little variation among the five human DNAs examined (unpublished data). In contrast to the generally heterogeneous MuLV-reactive band pattern seen among different inbred or feral mice (25), remarkably little polymorphism exists among different humans despite the presence of numerous (>25) reactive bands. Insertions or deletions of these type C retroviral sequences are apparently very rare in humans.

We thank Dr. A. Rabson for subcloning segments in pBR322, Mr. Theodore Bryan and Mr. J. Harry Belcher for excellent technical assistance, and Drs. A. Rabson and A. Khan for critically reading this manuscript.

1. Cottral, G. E., Burmester, B. R. & Waters, N. F. (1954) *Poult. Sci.* **33**, 1174–1184.
2. Crittenden, L. B., Smith, E. J., Weiss, R. A. & Sarma, P. S. (1974) *Virology* **57**, 128–138.
3. Rowe, W. P. (1972) *J. Exp. Med.* **136**, 1272–1285.
4. Chattopadhyay, S. K., Lowy, D. R., Teich, N. M., Levine, A. S. & Rowe, W. P. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 1085–1101.
5. Steffen, D. & Weinberg, R. A. (1978) *Cell* **15**, 1003–1010.
6. Chan, H. W., Bryan, T., Moore, J. L., Staal, S. P., Rowe, W. P. & Martin, M. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5779–5783.
7. Buckler, C. E., Staal, S. P., Rowe, W. P. & Martin, M. A. (1982) *J. Virol.* **43**, 629–640.
8. Kozak, C. A. & Rowe, W. P. (1980) *ICN-UCLA Symp. Mol. Cell. Biol.* **18**, 171–175.
9. Kozak, C. A. & Rowe, W. P. (1980) *J. Exp. Med.* **152**, 219–228.
10. Rowe, W. P. (1978) *Harvey Lect.* **7**, 173–192.
11. Martin, M. A., Bryan, T., Rasheed, S. & Khan, A. S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4892–4896.
12. Martin, M. A., Bryan, T., McCutchan, T. F. & Chan, H. W. (1981) *J. Virol.* **39**, 835–844.
13. Shinnick, T. M., Lerner, R. A. & Sutcliffe, J. G. (1981) *Nature (London)* **293**, 543–548.
14. Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. (1978) *Cell* **15**, 1157–1174.
15. Williams, B. G. & Blattner, F. R. (1979) *J. Virol.* **29**, 555–575.
16. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
17. Israel, M. A., Vanderryn, D. F., Meltzer, M. L. & Martin, M. A. (1980) *J. Biol. Chem.* **255**, 3798–3805.
18. Vogelstein, B. & Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615–619.
19. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
20. Hoggan, M. D., Buckler, C. E., Sears, J. F., Chan, H. W., Rowe, W. P. & Martin, M. A. (1982) *J. Virol.* **43**, 8–17.
21. Jelinek, W. R., Toomey, T. P., Leinwand, L., Duncan, C. H., Biro, P. A., Choudary, P. V., Weissman, S. M., Rubin, C. M., Houck, C. M., Deininger, P. L. & Schmid, C. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1398–1402.
22. Oroszlan, S., Copeland, T. D., Gilden, R. V. & Todaro, G. J. (1981) *Virology* **115**, 262–271.
23. Khan, A. S., Repaske, R., Garon, C. G., Chan, H. W., Rowe, W. P. & Martin, M. A. (1982) *J. Virol.* **41**, 435–448.
24. Roblin, R., Young, J. M., Mural, R. J., Bell, T. E. & Ihle, J. N. (1982) *J. Virol.* **43**, 113–126.
25. Hoggan, M. D., Buckler, C. E., Sears, J. F., Rowe, W. P. & Martin, M. A., *J. Virol.* **45**, 473–477.