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Structure of the baboon endogenous virus genome: nucleotide sequences of the long terminal repeat

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ABSTRACT

The entire nucleotide sequence of the long terminal repeat (LTR) of baboon endogenous virus (BaEV) M7 was determined, which consisted of 554 base pairs (bp). At both ends of the LTR, 13 bp sequences, AAATGAAAAGTAA and TGATTCTAACATC, were detected to be inverted repeats. The structure with these inverted repeats resembles those of other retroviruses and transposable elements. A Hogness box, TATAAAA, and a putative poly(A)-addition signal, AGTAAA, were present within the right-hand half of the LTR, where the initiation and termination of the viral RNA synthesis seems to occur in the integrated BaEV genome. The primer-binding site of at least 14 bp long was found just outside of the LTR where the strong stop DNA started, and the primer for reverse transcription in BaEV seemed to be $tRNA^{Pro}$. Several structural features are commonly detected in the LTRs of BaEV and other retroviruses. Our studies suggest that BaEV has evolved from a common ancestor with other mammalian type C viruses. Close relationships between BaEV and a feline endogenous virus, RD114, are demonstrated.

INTRODUCTION

In an early period of retroviral infection, the viral RNA genome is converted to three major forms of double-stranded viral DNAs, prior to integration of the proviral DNA into the host DNA (1). A linear duplex form of the viral DNA is slightly longer than the viral RNA subunit and contains terminal repeated sequences, i. e., long terminal repeats (LTRs) (2), derived from the 3'- and 5'-termini of the RNA genome. The linear molecule of the viral DNA enters into the nucleus, whereupon two closed circular forms of the viral DNAs carrying either one or two copies of the LTR are detected in the nucleus. However, it is not clear which form of the viral DNAs is the immediate intermediate of the integration. Finally, the integrated provirus exists in a form colinear with the unintegrated linear molecule of the viral DNA and serves as the template for the progeny viral RNA. The LTR seems to play important roles in integration and expression of the viral genome.

The M7 strain of baboon endogenous virus (BaEV) (3) is a primate retro-

virus which grows well in human and dog cells. The DNA of these cells lack sequences related to BaEV so that the integration and expression of the BaEV DNA can be studied in the absence of the cellular DNA background. In the previous report (4), we detected the three forms of the unintegrated BaEV DNAs and cloned the two circular forms of the BaEV DNA in bacteriophage λ. We describe here the entire nucleotide sequence of the BaEV LTR which is 554 bp long. The functions of the retrovirus LTR suggested from the nucleotide sequence are discussed.

MATERIALS AND METHODS

Materials. Polynucleotide kinase from T4-infected E. coli was a gift of M. Takanami, Kyoto University. Other enzymes were purchased from New England BioLabs, Bethesda Research Laboratories or Takara Shuzo Co., Kyoto, and used as recommended. ^{32}P-γATP (specific activity: 5,000 Ci/mmole) was purchased from Amersham Corp. Hydrazine and dimethylsulfate were obtained from Eastman Organic Chemicals. All glasswares were treated with SCILICONIZE (Fuji System Corp., Tokyo).

Subcloning of Bam HI Fragments of BaEV DNA. A recombinant phage, λ BEV-11, carrying BaEV DNA of a full size, 8.6 kilobase pairs (kb), with two tandem LTRs (4), was used. The phage DNA (5 μg) cleaved with Bam HI plus Eco RI, and pBR322 DNA (0.5 μg) treated with Bam HI and bacterial alkaline phosphatase, were incubated with T4-ligase (2.5 units) in a 40 μl reaction mixture for 12 hr at 4° C. A thy$^-$ mutant of E. coli KM723 (5) was transformed with the ligated DNA and tetracycline-sensitive and ampicillin-resistant colonies were selected. One recombinant plasmid designated as pBE-B8, which contained the two Bam HI fragments of BaEV DNA as shown in Fig. 1, was isolated.

Isolation of DNA Fragment. DNA fragments generated by digestion with restriction enzymes were separated by electrophoresis in a cylindical gel of 3-8% acrylamide (acrylamide:bis-acrylamide = 20:1), in 75 mM TBE buffer (75 mM Tris, 75 mM boric acid, 2.5 mM EDTA-2Na, pH 8.3), at 100-200V for a 10 cm gel. To separate complementary DNA strands, alkali-denatured DNA was electrophorezed at 4° C by the method of Maxam and Gilbert (6). DNA fragments were extracted from gels with TE buffer (10 mM Tris-HCl, pH 8.1, 1 mM EDTA-2Na) and purified by passage through Sephadex G-50 column. Samples were finally solubilized with small amounts of Te buffer (10 mM Tris-HCl, pH 8.1, 0.1 mM EDTA-2Na).

Labeling of 5'-Ends of DNA Fragment. Labeling of the 5'-ends of DNA fragments with ^{32}P-γATP by T4 polynucleotide kinase was carried out by the method of

Sugisaki et al. (7). Five to twenty picomoles of DNA fragment as protruding 5'-ends whose terminal phosphates had been removed by bacterial alkaline phosphatase, were labeled with 100 μCi of ^{32}P-γATP by 10 units of T4 polynucleotide kinase for 60 min at 37°C, in presence of 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$ and 10 mM 2-mercaptoethanol. Unincorporated nucleotides were removed by gel filtration.

DNA Sequencing. The two labeled ends of each restriction fragment were separated by secondary cleavage with a different enzyme or by strand separation, and the sequences from the 5'-labeled ends were determined by the chemical methods of Maxam and Gilbert (6). Gels for sequencing studies were 0.3-1.0 mm thick and 30 cm wide by 40 cm long. Concentrations of acrylamide (acrylamide:bis-acrylamide = 20-30:1) in 75 mM TBE were varied from 6 to 20% according to the length of DNA fragments. Electrophoresis was carried out at 750-1,300 V. After electrophoresis, gels were directly overlaid on Sakura X-ray film type A or AO and the films were exposed for 5-25 days at -20°C.

RESULTS

Sequencing Strategy. The structure of a recombinant phage carring the unintegrated BaEV DNA, λBEV-11 (4), is illustrated in Fig. 1. Tandem repeats of the two LTRs are located in the Bam HI fragment of 3.1 kb, which was subcloned in pBR322 for this study. One of the subclones, pBE-B8, shown in Fig. 1, was used as a source of DNA for sequencing the LTR of BaEV, in which the LTR-containing fragment of 3.1 kb and an adjacent Bam HI fragment of 1.0 kb invertedly joined. Each recognition site of various restriction endonucleases was completely repeated at an interval of 0.56 kb in the tandem repeat of the LTRs and Xho I made a single cut in one stretch of the LTR. Therefore, the Xho I fragment of pBE-B8 covers the entire sequence of the LTR. First, we sequenced both strands of the 0.56 kb Xho I fragment. We also sequenced the right-hand end region of the tandemly repeated LTRs, to determine the termini of the LTR. In this report, the "right" and "left" LTRs are tentatively designated as shown in Fig. 2. The detailed strategy used for sequencing is summarized in the figure.

Nucleotide Sequences of the Xho I Fragment of BaEV LTR. The results of the complete base analyses of the Xho I fragment are shown in Fig. 3. The LTR of BaEV is 554 nucleotides long and the sequence contained the restriction sites shown in Fig. 3. The GC contents in the nucleotide sequence were 49%. A lot of AT-rich or GC-rich clusters were found. There were three long GT-rich stretches in the middle of the fragment; gt-1, -2 and -3. The sequence from

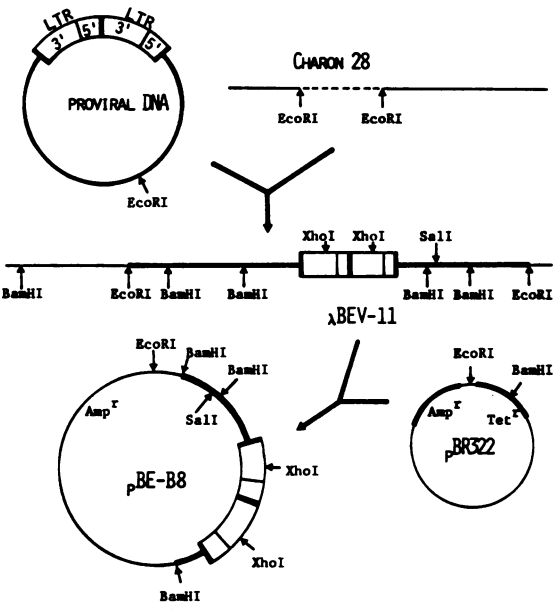


Fig. 1 The construction and the structure of pBE-B8, a plasmid pBR322 carrying the two LTRs of BaEV. The Bam HI-fragment of λBEV-11 (4), a clone of the unintegrated circular DNA of BaEV, was subcloned in pBR322. The arrows show restriction sites and the rectangles indicate the LTRs. The 3'- and 5'-domains are shown in the LTRs of the proviral DNA.

157 to 174, designated as gt-1, contained only G and T. The other two GT-rich regions, gt-2 and gt-3, were located from 255 to 300 and from 328 to 347, respectively, where more than 70% of the nucleotides were G or T. Interestingly, in a region from 87 to 100, seven stretches of CT were located. Long direct repeats of 22 bp, DR-1, were observed from 367 and 531. Several inverted repeats were also found.

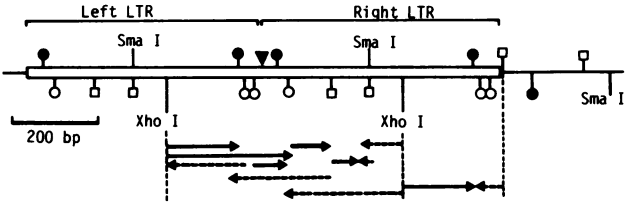
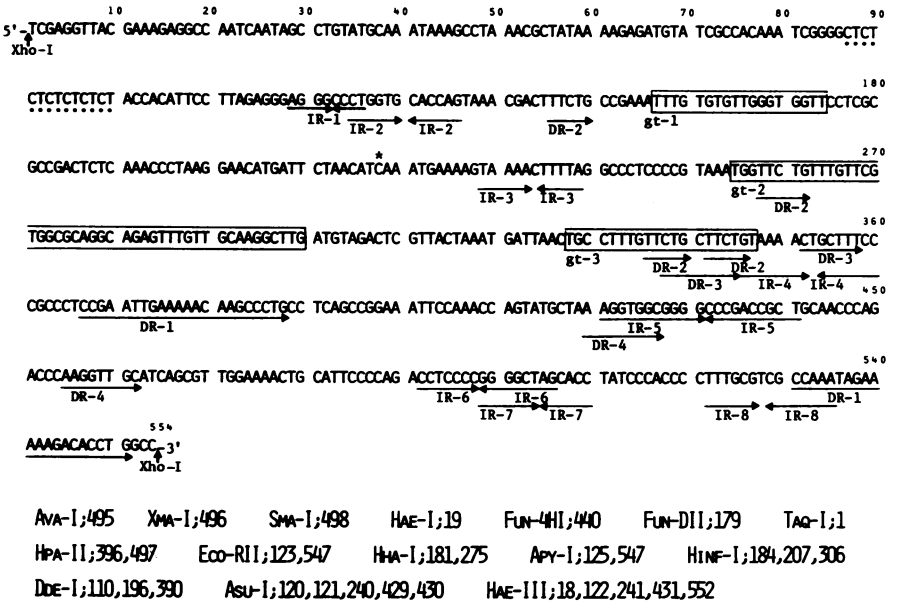


Fig. 2 A fine restriction map of the BaEV LTR and the strategy used for sequencing of the LTR. The rectangle shows the two LTRs of pBE-B8 and the site indicated by ▼ is the junction of the two LTRs, determined in this study. Restriction sites for Hha I (●), Hpa II (□) and Hinf I (○) are shown by vertical lines. The thick lines or broken lines with arrows, shown below the map, indicate the sequences of each strand of the DNA fragments determined in this study, by the Maxam-Gilbert chemical modification procedure (6). The "left" and "right" LTRs are designated as shown in the figure.



AVA-I;495 XMA-I;496 SMA-I;498 HAE-I;19 FUN-4HI;440 FUN-DII;179 TAQ-I;1
HPA-II;396,497 ECO-RII;123,547 HHA-I;181,275 APY-I;125,547 HINF-I;184,207,306
DDE-I;110,196,390 ASU-I;120,121,240,429,430 HAE-III;18,122,241,431,552

Fig. 3 Nucleotide sequences of the fragment between the two Xho I sites of the two tandem LTRs of BaEV. The nucleotides are numbered from the 5'-end of the plus strand of the fragment and the polarity is identical to that of the viral genome RNA. Direct repeats, DR-1, -2, -3 and -4, and inverted repeats, IR-1, -2, -3, -4, -5, -6, -7 and -8 are shown by arrows. GT-rich regions, gt-1, -2 and -3 are enclosed by solid lines. Seven CT stretches are underlined by dots. The asterisk indicates the extreme 3'-end base of the left LTR. The restriction sites deduced from the sequences are listed below the nucleotide map.

Sequencing the Region around the Right-Hand End of BaEV LTR. We sequenced the junction of the right LTR and the unique sequences of the viral DNA to determine the end of BaEV LTR. A 232 bp fragment from the right Xho I site to the next Hpa II site was prepared and sequenced (Fig. 2). The sequence of 218 bp from the Xho I site in this fragment (data not shown) was completely identical with those of the first 218 bp in the fragment between the two Xho I sites shown in Fig. 3. However, the sequence of the next 14 bp to the Hpa II site was different from that of the Xho I fragment (Fig. 4). Therefore, we concluded that the C at position 218 in Fig. 3 was the extreme right-hand terminus of the LTR. Interestingly, these 14 bp described above were complementary to those of the OH-terminus of $tRNA^{Pro}$ (Fig. 5).

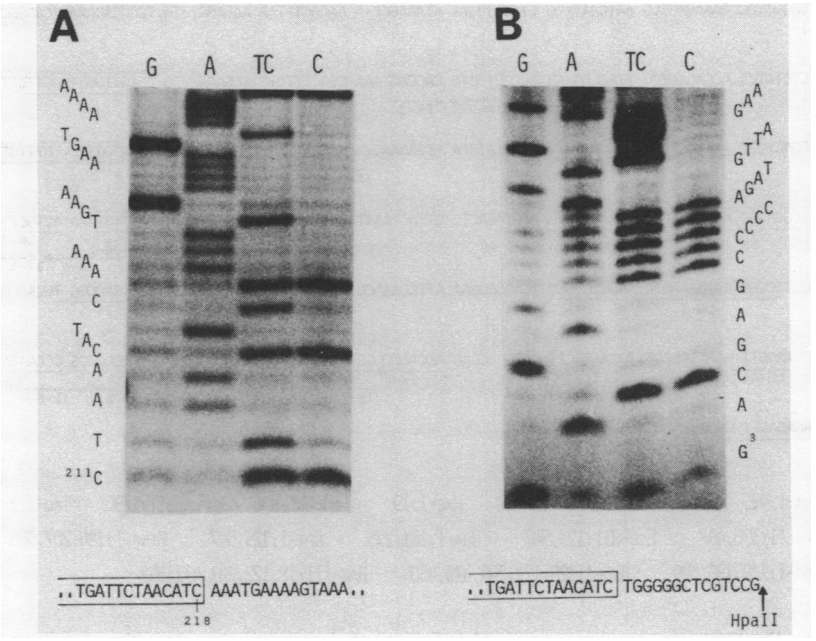


Fig. 4 Autoradiograms of the gels sequencing the termini of BaEV LTR and the nucleotide sequences deduced from the autoradiograms.
A. Those around the juction of the two LTRs at position 218. The sequence is also shown at the site with the asterisk in Fig. 3. The nucleotide numbers are those used in Fig. 3. ^{32}P-labeled Hinf I subfragment (99 bp) of the Xho I -fragment (see Fig. 2) was cleaved with Hha I and sequenced.
B. Those around the right-hand end of the two LTRs. A 232 bp fragment cleaved from the insert of pBE-B8 by Xho I and Hpa II was end-labeled and digested with Hha I. The shorter fragment (51 bp) of the Hha I digest was sequenced from the Hpa II site. The nucleotide sequence shown below the autoradiogram is presented as that of the complementary strand, to compare with the results shown in A.
The sequences commonly found in A and B are enclosed by solid lines.

DISCUSSION

The Nucleotide Sequences and the Structure of BaEV LTR. The entire nucleotide sequence of BaEV LTR was determined. This is the first report on sequencing of a retroviral LTR other than those of murine- or avian-origins. The nucleotide numbers of the BaEV LTR, 554 bp, are close to those of the LTRs of murine type C retroviruses which contain 550 ± 40 nucleotides (8, 9, 10). The Xho I fragment, whose sequences shown in Fig. 3, covers the entire sequence of the LTR and the junction of the two LTRs is clearly defined to be located between 218 and 219 from the results shown in Fig. 4. From these

findings, the schematic presentation of the structure of BaEV LTR is illustrated in Fig. 5. The unique sequence of BaEV DNA seems to be about 7,500 bp long.

Two Domains of BaEV LTR Derived from 3'- and 5'-Ends of the Viral Genome. Retroviral LTR consists of two parts, 5'- and 3'-domains, derived from the 5'- and the 3'-ends of the viral genome (2). The complementary strand of the 5'-domain is so-called "strong stop DNA" which is predominantly synthesized by viral reverse transcriptase (1). From our results, the exact position of the boundary of the two domains is not defined. However, the G at position 86 is assumed to be the first nucleotide of the 5'-domain by the following reasons: First, G is the first base of the 5'-domain of LTR in various retroviruses as far as reported so far (8, 10, 11, 12, 13). Second, Lovinger and Schochetman (14) reported that the strong stop DNA of BaEV was 133 nucleotides long and the 3'-terminus ended with C. The nucleotide sequence of the strong stop DNA of BaEV (14) is well matched with those reported here, even though differences in several bases are found. Haseltine and Kleid (15) reported that the strong stop sequences of BaEV M7 showed minor heterogeneity in each virus stock. Ju and Skalka (13) also reported the heterogeneity of the nucleotide

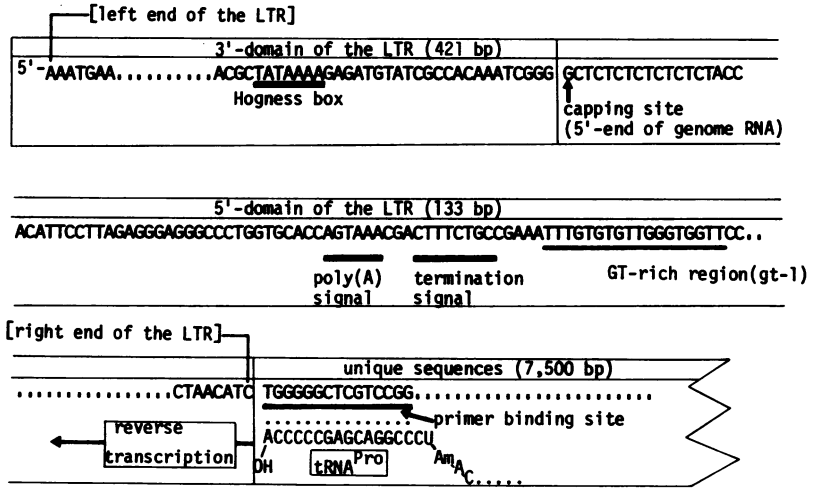


Fig. 5 Schematically presented structure of the BaEV LTR. The results shown in Figs. 3 and 4 are summarized and schematically presented as one unit of the LTR. The 15 bp sequence of the primer-binding site succeeding to the right-hand end of the LTR, which is complementary to the OH-terminal sequence of $tRNA^{Pro}$, is also shown. The sequences suggesting important functions are underlined by thick bars.

sequences in the LTR of cloned avian retroviruses. The strain of BaEV we used in this study may be different from that Lovinger and Schochetman used. Thus, the 3'- and 5'-domains of BaEV LTR are concluded to be 421 and 133 bp long, respectively.

tRNA Primer for Reverse Transcription of the BaEV Genome. In an early step of retrovirus infection, the viral RNA is transcribed into DNA by virion-associated RNA-dependent DNA polymerase (reverse transcriptase) (16). The primer of the reverse transcription is a tRNA of the host cell, which binds to the viral genome at a site near the 5'-end (17). In case of BaEV, the primer tRNA has not yet been identified. In the sequence shown in Fig. 4, the 14 base stretch from the right-hand end of the right LTR to the next Hpa II site, is complementary to the OH-terminal sequences of $tRNA^{Pro}$. At this site, the $tRNA^{Pro}$ primer is suggested to bind to the genome of BaEV.

Signals for Transcriptional Regulation in BaEV LTR. An AT-rich sequence, TATAAAA, is located at position 56 in Fig. 3, i. e., 30 bp upstream from the capping site at position 86. This sequence is known as an eukaryotic promoter signal, Hogness box (18), which is generally found about 30 bp upstream from a capping site. Therefore, the transcription seems to start at the capping site of the LTR in the integrated genome of BaEV. In case of Rous sarcoma virus, this sequence was suggested to act as a promoter by in vitro experiments (19). Other AT-rich regions are also detected in the 3'-domain of LTR, e. g., at positions 533, 7, 21 and 39. These AT-rich sequences might act as promoters, or might cooperate with the Hogness box at position 56, resulting in a strong promoter activity to the BaEV LTR.

The sequences of the regions from the promoter to the capping sites are conserved in various retroviruses (8, 10, 11, 12, 13). A candidate for the promoter, TATAAAG, is commonly detected 28 to 30 bp upstream from the capping sites. In addition, about 15 bp stretches preceding the capping sites are also conserved in these viruses. This region seems to be essential for moving of RNA polymerase from the promoter to the initiation sites of transcription.

Retroviruses have a GT-rich region in the middle of the 5'-domain of the LTRs (8, 10, 12). In case of BaEV, such a GT-rich stretch is located at position 157 to 174 (gt-1 in Fig. 3). This region seems to be important for binding of the viral RNA to ribosomes, in which a trinucleotide, GTG, is the consensus sequence as a ribosome-binding site (20). The initiation sites of the viral protein synthesis have been suggested to be located at a site succeeding to the right terminus of the upstream LTR on the integrated viral

genome (21).

As for the termination control of transcription, three kinds of signals have been proposed (22, 23, 24): poly(A)-addition, termination and polyadenylation site signals. The consensus sequences of these signals are, AATAAA for poly(A)-addition signal; TGCT, TTGC or T cluster for termination signal; and CA for polyadenylation site signal. In the 5'-domain of BaEV LTR, no AATAAA sequences are detected but AGTAAA appears at position 135. However, any typical consensus sequences for termination or polyadenylation sites are not detected. On the other hand, the termination points of transcription are always found 11 to 19 bp downstream from the poly(A)-addition signals in cases of the late messenger RNA synthesis of SV40 (25). If this is the case in BaEV, TGC at position 149, i. e., 14 bp downstream from the AGTAAA might be a termination signal and the C at position 151 might be a polyadenylation site. Alternatively, the C at position 148 cannot be ruled out as a poly(A) site.

In the 5'-domain of mammalian retroviral LTRs, putative poly(A)-addition signals, AATAAA or AGTAAA, are located about 50 bp downstream from the capping sites (8, 10, 12, 14). In cases of avian retroviruses, however, AATAAA is located at sites preceded by 7 bp from the capping sites (12, 13). Moreover, relatively common sequences are conserved around the poly(A)-addition signals in mammalian retroviruses. C-containing T-rich sequences of about 6 to 8 bp long are detected 8 to 9 bp downstream from the poly(A)-addition signals, which can form a hairpin structure with the AATAAA or the AGTAAA.

Thus, the LTR of BaEV seems to carry potent promoters and rather atypical termination signals. In the integrated viral genome, the transcription of the viral genes seems to initiate at the promoter and capping sites of the upstream LTR, and to terminate at the termination signals of the downstream LTR. As both of the LTRs have the identical nucleotide sequences, the discussion described above does not answer a question why the signals for termination in the upstream LTR do not act in the viral RNA synthesis.

Open Reading Frames within BaEV LTR. In a mouse mammary tumor virus, nonstructural proteins have been suggested to be coded by the 3'-terminus of the RNA genome, i. e., by the LTR region of the viral RNA (26, 27). On the other hand, prokaryotic transposons are known to code for a certain enzyme "transposase", by which important steps of the transpositional recombination are catalyzed (28). From similarities of the structure and the basic function, we assumed and surveyed possible open reading frames in the BaEV LTR. Two large ones are detected within the tandem array of the two LTRs: one from

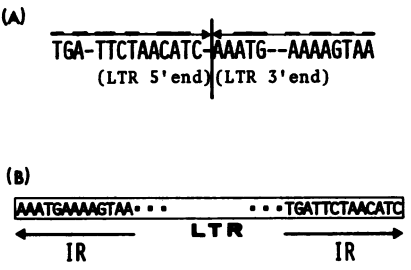


Fig. 6 Terminal inverted repeats in the sequence of BaEV LTR. (A) Thirteen bp sequences are invertedly repeated at the junction of the two LTRs, which appear around position 218 as indicated by asterisk in Fig. 3. (B) The inverted repeats are schematically presented in one unit of the BaEV LTR.

65 to 223 and another from 217 to 25. These frames can code for polypeptides as large as 5,700 and 7,000 daltons, respectively, which could be translated only from the circular form of the unintegrated viral DNA because these frames override the two LTRs. However, neither typical signals of promoters nor those of ribosome-binding sites are found around these frames. Further investigation is needed to provide evidence in support of these possibilities.

Inverted Repeats at the Termini of BaEV LTR. As shown in the results of Fig. 3 and 6, AAATGAAAAGTAA and TGATTCTAACATC are detected at the junction of the two LTRs. The same sequence is also located at the extreme right-hand terminus of the right LTR (Fig. 4 and 5). The matching of these 13 bp is 64%.

Inverted repeats of nucleotide sequences have been reported to be located at the LTR termini of several retroviruses (8, 11, 13, 29) and in other movable genetic elements (30, 31, 32, 33) including prokaryotic transposons. Common features of the repeated sequences in various elements are 5'-..TG... and ...CA..-3' which might play important roles in recombination events denoted by the elements. The terminal sequences of BaEV LTR also carry these features (Fig. 6), although the homology of the sequences at both termini is rather low.

In addition, these sequences of the inverted repeats of the BaEV LTR are coincident with those of an endogenous feline virus, RD114, (14), but not with those of retroviruses in other species. Lovinger and Schochetman (14) have reported that the nucleotide sequences of the strong stop DNA have 71% correspondence between BaEV and RD114. These striking similarities suggest close relationships of the origins of the two viruses. From these findings, retroviruses seem to have evolved from a common ancestor. On the way of evolution, BaEV and RD114 may have diverged from other mammalian retroviruses.

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