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Intragenomic Linear Amplification of Human Herpesvirus 6B *ori*Lyt Suggests Acquisition of *ori*Lyt by Transposition

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We identified some passage lineages of human herpesvirus 6 variant B (HHV-6B) strain Z29 that contain as many as 12 tandem copies of a genomic segment that corresponds almost precisely to a previously identified minimal efficient origin of lytic replication (*ori*Lyt). Analysis of nucleotide sequences in the vicinity of the amplified segment suggests that the amplification occurred as a two-step process, with the first step being a rare sequence duplication mediated through directly repeated sequences located near the termini of the amplified segment and the second step occurring via homologous recombination through the duplicated sequence. These results demonstrate that *ori*Lyt has been amplified in some virus stocks and indicate that (i) origin amplification confers a growth advantage on the virus in cell culture and (ii) laboratory-passaged HHV-6B genomes can accommodate additional nucleotide sequences and thus may be useful gene transfer vectors. The structures of the amplified segment and its adjacent sequences together suggest that HHV-6B or a progenitor virus acquired *ori*Lyt by transposition from an unknown source.

Several lines of evidence indicate that an origin of lytic replication (*ori*Lyt) is located approximately 70 kb from the left end of the 162-kb human herpesvirus 6 variant B strain Z29 [HHV-6B(Z29)] genome. First, by nucleotide sequence analysis, a putative origin region was identified that consists of a region of approximately 1,400 nucleotides with no obvious protein-coding capacity located immediately upstream of the HHV-6B(Z29) homolog of the herpesvirus major DNA-binding protein (4). Origins of lytic replication are present in the analogous regions of herpes simplex virus type 1 (HSV-1), human cytomegalovirus, and varicella-zoster virus (1, 16, 18). In HHV-6B this region is A+T rich (over 70%) and contains an imperfect direct repeat (IDR) of approximately 200 nucleotides. Second, DNA fragments containing a 400-bp segment adjacent to the IDR were replicated in HHV-6B-infected cells in a transient transfection assay, indicating that a portion of this region can indeed function as an origin of lytic replication (6). Third, binding sites for the HHV-6B homolog of the alphaherpesvirus origin-binding protein (OBP), exemplified by HSV-1 UL9, have been identified within the minimal origin region. These binding sites have sequences and arrangements similar to those of the OBP-binding sites in alphaherpesvirus *ori*Lyts (9).

Here we describe spontaneous intragenomic linear amplification in some HHV-6B(Z29) passage lineages of a segment of the HHV-6B(Z29) genome that contains the *ori*Lyt region. We mapped the amplified region and identified the boundaries of the amplified region by nucleotide sequencing. The arrangement of directly repeated sequences near the termini of the amplified segment suggested mechanisms for both the amplification and the acquisition of *ori*Lyt by HHV-6B or a primordial herpesvirus via transposition.

The presence of an amplified segment of the HHV-6B(Z29) genome was identified as follows. Wild-type *Hin*dIII fragment L was unexpectedly absent in a digest of nucleocapsid DNA, prepared and analyzed as described previously (11), from a high-passage stock of HHV-6B(Z29) (12) that had been propagated as described previously (2). We identified two additional samples with alterations in *Hin*dIII-L abundance in a set of 36 other HHV-6B(Z29) DNA preparations that had been accumulated over a period of 4 years (data not shown). All of the samples with the *Hin*dIII-L anomalies were from virus that had been independently passaged but were harvested over a 4-month period. Two of these preparations were used in the studies reported here. The anomalous migration of *Hin*dIII-L is shown in Fig. 1A; the fragment is clearly reduced in abundance relative to the wild-type fragment or is nearly absent in the DNA preparations shown in lanes 1 and 2, respectively. A blot of the gel was probed with cloned *Hin*dIII-L (10) by previously described methods (11). Wild-type *Hin*dIII-L was present in the DNA preparations shown in Fig. 1B, lanes 1 and 3, consistent with the results shown in Fig. 1A. The presence of a ladder of fragments that hybridized with *Hin*dIII-L in the DNA preparations with the reduced abundance of wild-type *Hin*dIII L was very striking (Fig. 1B, lanes 1 and 2). Wild-type *Hin*dIII-L is 4.43 kb long, and the fragments in the ladder increased in length from 4.43 kb in increments of approximately 770 bp (Fig. 1B, lane 1) and 860 bp (lane 2). As many as 12 discrete *Hin*dIII-L-hybridizing bands were visible in some autoradiograms. The hybridization pattern, the unit molarity of all other fragments visible in the ethidium bromide-stained gel, and the comigration of all other fragments with their wild-type counterparts suggested the presence of an intragenomically amplified sequence within *Hin*dIII-L in some passage lineages. This possibility is shown schematically in Fig. 1C. The incremental length difference of the third band from the bottom in Fig. 1B, lane 1, is approximately half of that of the other bands in this lane and cannot be easily explained by the model presented below. Sufficient material was not available for further study of this species.

The approximate boundaries of the repeat element were determined by restriction endonuclease mapping. The remain-

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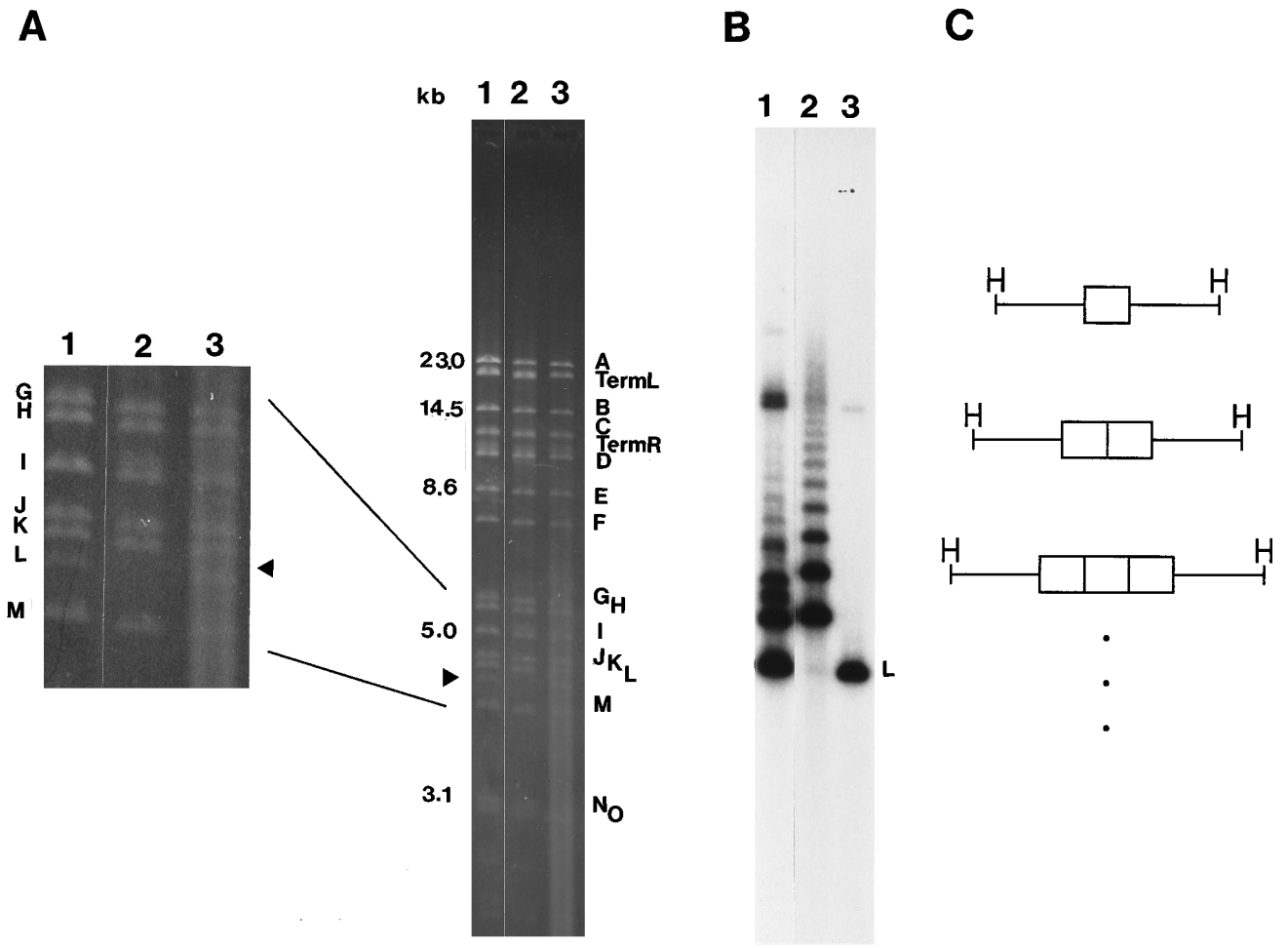
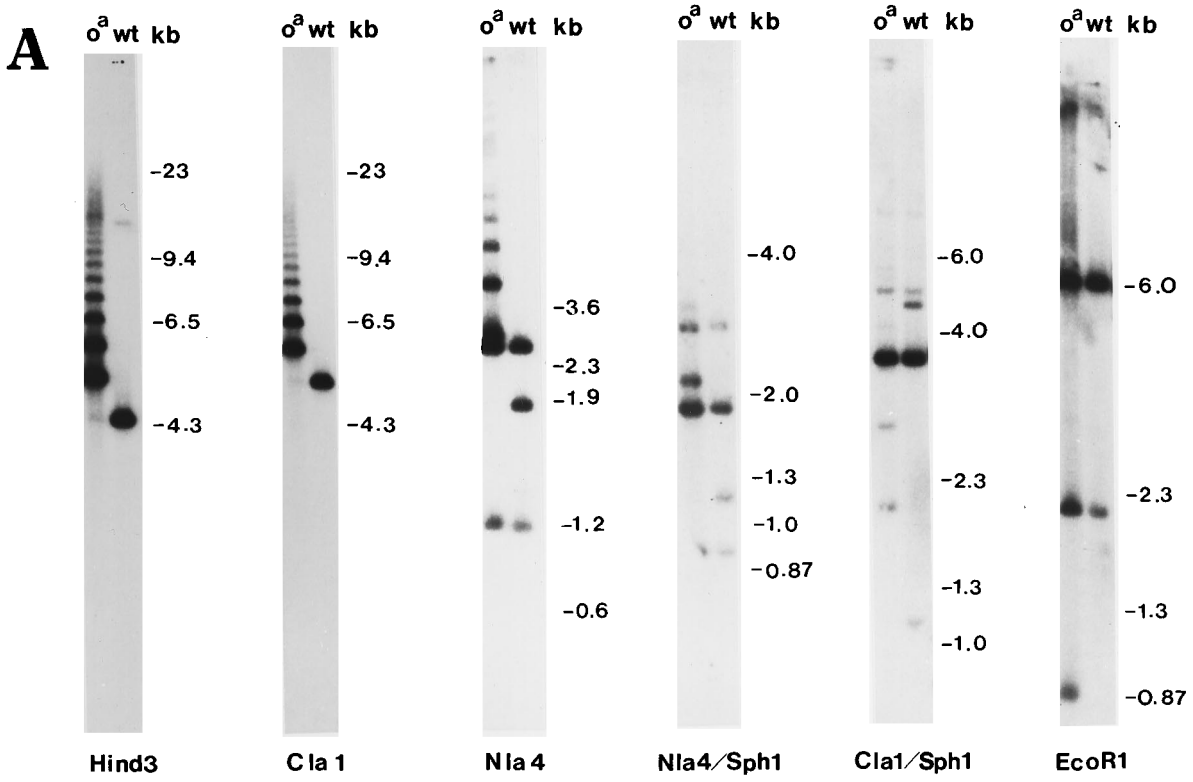


FIG. 1. Amplification of a sequence within *Hin*dIII-L. (A) Ethidium bromide-stained *Hin*dIII profiles of three preparations of HHV-6B(Z29) nucleocapsid DNA after separation in a 0.8% agarose gel. Lane 3 contains wild-type HHV-6B(Z29) DNA, and lanes 1 and 2 contain DNAs with anomalous *Hin*dIII profiles. The portion of the gel containing *Hin*dIII-L is enlarged to the left of the complete profile. Fragment lengths are as described previously (11). (B) Identification of *Hin*dIII-L hybridizing fragments in the profiles shown in panel A. After being blotted to nitrocellulose, the DNA was hybridized as described in the text with cloned *Hin*dIII-L (pH6Z-225) that had been radiolabeled by nick translation. The prominent upper band in each lane is due to partial *Hin*dIII digestion. (C) Schematic diagram of the linear amplification of a segment of DNA internal to *Hin*dIII-L.

ing experiments described in this paper were done with the DNA preparation shown in lanes 2 of Fig. 1 because it had very little detectable wild-type *Hin*dIII-L. Two predictions based on the model diagramed in Fig. 1C are (i) that a novel ladder will be present in DNA containing the amplified origin region after digestion with restriction endonucleases that cut outside the amplified region and (ii) that smaller supramolar bands will result from restriction endonuclease digestion within the amplified region. On the basis of these considerations, the novel ladders present in the *Hin*dIII, *Cla*I, *Nla*IV, *Nla*IV-*Sph*I, and *Cla*I-*Sph*I digests of amplified origin DNA (Fig. 2) define the minimal external boundaries of the amplified region as the *Nla*IV and *Sph*I sites at positions 4270 and 5455, respectively (nucleotide sequence coordinates throughout this paper are as in reference 4) (GenBank accession number L16947). Similar ladders were also seen in digests with *Bam*HI and *Sal*I (data not shown). The generation of a novel supramolar 860-bp *Eco*RI fragment is a consequence of digestion at a single site within each repeat element and indicates that the right end of the repeat element is to the right of the *Eco*RI site at position 5362. On the basis of mapping the right end of the repeat between the *Eco*RI site at 5362 and the *Sph*I site at 5455, and given the repeat unit length of approximately 860 bp, the repeat element is composed of sequences located between positions 4502 and 5455.

We employed PCR to obtain materials suitable for precisely determining the boundaries of the repeat unit and their juxtaposition with flanking sequences. PCRs used approximately 40 ng of nucleocapsid DNA amplified in 100-μl PCR mixtures with a thermostable DNA polymerase (AmpliTaq; Perkin-Elmer Cetus, Norwalk, Conn.), using the manufacturer's recommended conditions. Thirty-five amplification cycles were performed; each consisted of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min. Aliquots (25 μl) from the amplification reaction mixtures were separated by electrophoresis for 3 h at 3 V/cm in composite agarose gels consisting of 3% agarose (Nu-Sieve; FMC, Rockland, Maine) and 1% agarose (Sea-Kem; FMC). Gels were stained with ethidium bromide, and DNA was visualized by UV fluorescence.

For amplification of the junction between repeat elements, primers were chosen, with orientations suitable for priming



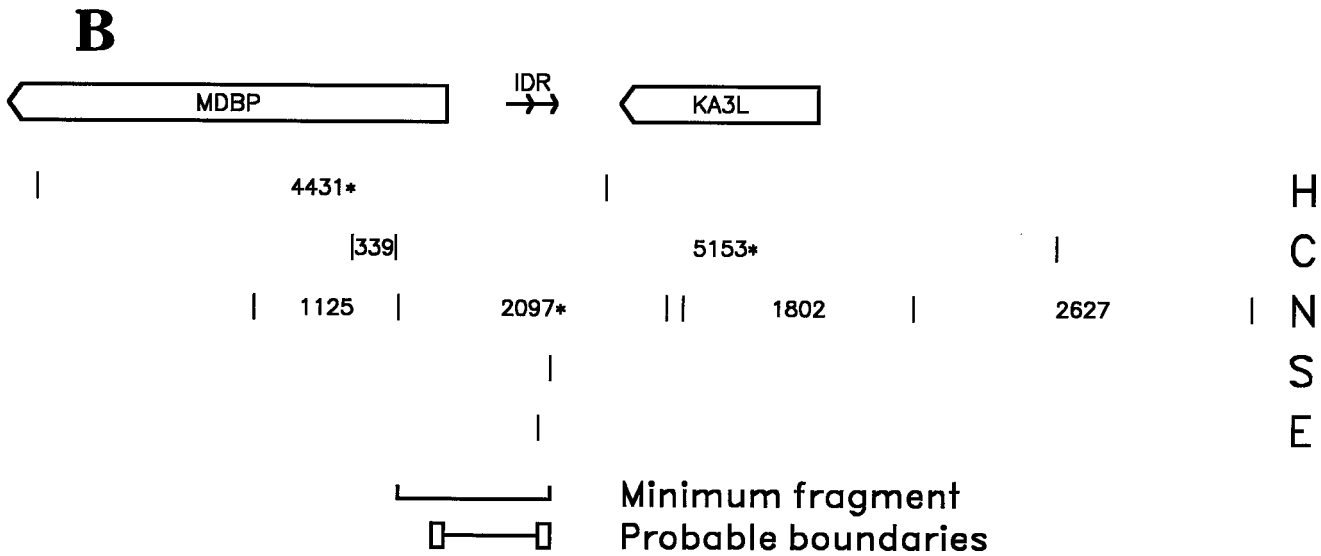
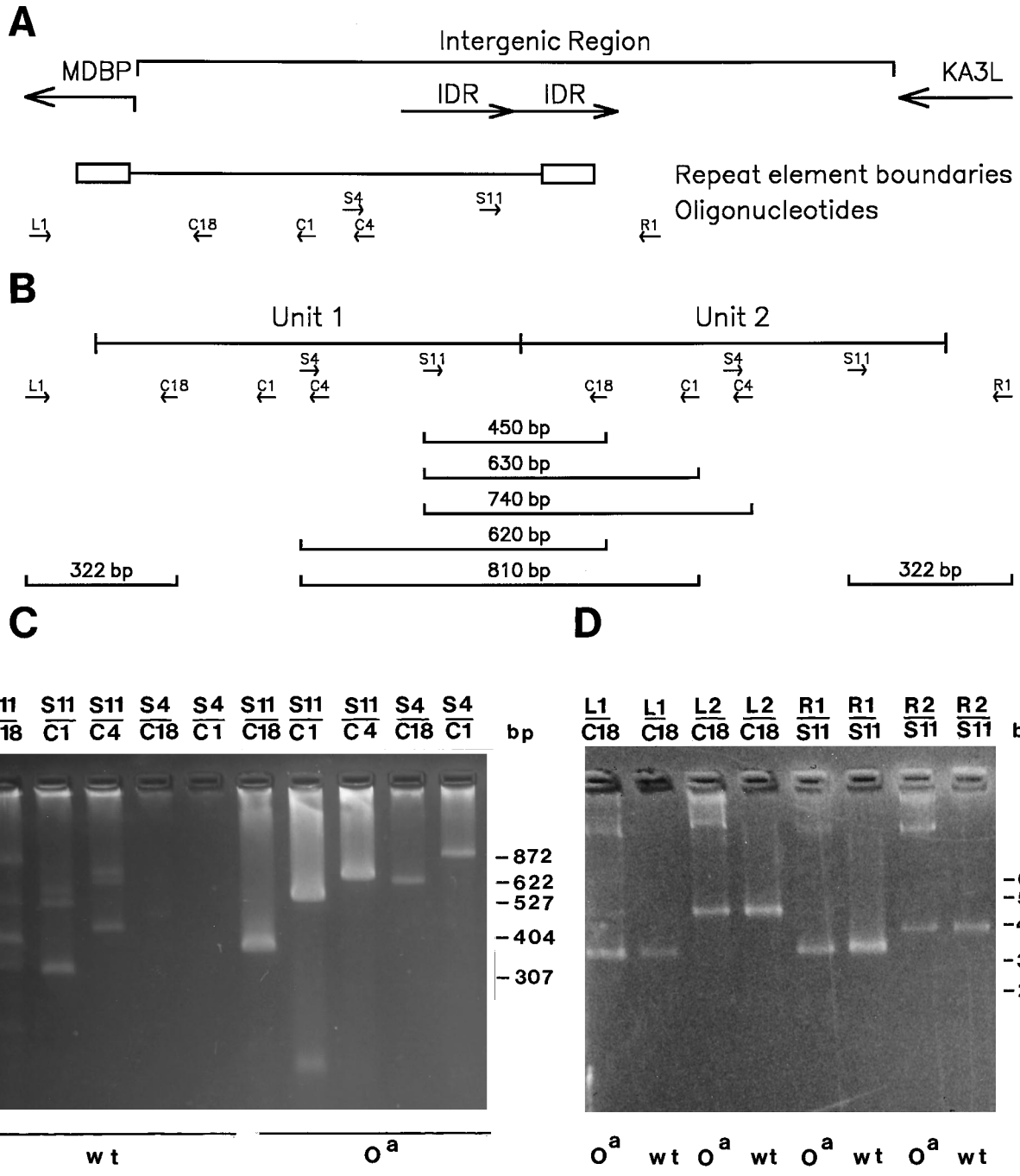


FIG. 2. Restriction endonuclease mapping of the amplified region. (A) Digestion of HHV-6B(Z29) nucleocapsid DNA preparations with restriction enzymes that cut within *Hin*dIII-L. Wild-type (wt) and amplified origin (o^a) DNA preparations are indicated. Hybridizations were with cloned *Hin*dIII-L (*Hin*dIII, *Cla*I, and *Nla*IV digests) or with cloned *Cla*I-J (pH6Z-603). Positions of size standards (lambda DNA-*Hin*dIII or ϕX174 DNA-*Hae*III) are shown. (B) Diagram showing the mapping of the amplified region relative to *Hin*dIII (H), *Cla*I (C), *Nla*IV (N), *Sph*I (S), and *Eco*RI (E) maps and genetic features in the vicinity of *Hin*dIII-L. The locations of the open reading frame encoding the HHV-6B major DNA-binding protein homolog (MDBP), the IDR structure, and the HHV-6B(Z29) KA3L open reading frame (4) are shown. Boundaries of the amplified segment as determined by restriction endonuclease mapping are shown. Asterisks indicate wild-type fragments that are replaced by higher-molecular-weight ladder rungs in origin-amplified DNA and thus contain the amplified region. Numbers indicate restriction endonuclease fragment lengths (in base pairs).



E

5442 4587

5403 TCACGGTATTTTTGAAATTAATAAATTTTAAATCGGGTAA|CAGCAGTAGAAACAGGAGCAGAAACCACCGTTTCGTTTTC 4626

4547 tccaaaagttctttctcttttggaaaaacatagatccaag|CAGCAGTAGAAACAGGAGCAGAAACCACCGTTTCGTTTTC 4626
5403 TCACGGTATTTTTGAAATTAATAAATTTTAAATCGGGTAA|atggtaaagcatgctgttaaattcacgttaacagagcaag 5482

DNA synthesis in opposing directions from within the predicted repeat unit, e.g., oligonucleotides C18 and S11 (Fig. 3A). Thus, from a wild-type template, which contains a single copy of the repeat unit, no chain reaction would occur, just a linear amplification of the nonoverlapping portions of opposite strands. A chain reaction would occur only in the presence of two or more tandemly duplicated copies of the repeat unit and would result in the predicted fragments shown in Fig. 3B. As shown in Fig. 3C, no specific double-stranded amplification of wild-type DNA was observed, and amplification of DNA containing the duplicated region resulted in fragments of approximately the predicted sizes.

To precisely determine the repeat unit boundaries, the repeat unit junction-spanning amplimers generated by primer pairs S11 and C18, S11 and C1, S11 and C4, and S4 and C18 were used as templates in nucleotide sequencing reactions that were primed by either oligonucleotide S11 or C18, thus allowing multiple determinations of the nucleotide sequence across the repeat unit junction on both strands. Briefly, PCR products were purified (Centricon 100; Perkin-Elmer) and sequenced by cycle sequencing with a dye-labeled chain terminator sequencing kit (Applied Biosystems, Foster City, Calif.). Twenty-five sequencing cycles were performed, consisting of a rapid ramp to 96°C for 30 s, a hold at 96°C for 15 s, a rapid ramp to 50°C for 1 s, and a rapid ramp to 60°C for 4 min. Product separation in a 6% polyacrylamide gel and sequence reading were done in an Applied Biosystems model 373A DNA sequencer. The nucleotide sequence in the vicinity of the repeat unit junction is shown in Fig. 3E and indicates that nucleotide positions 5442 and 4587 are juxtaposed at the junction of the repeat units, giving a repeat length of 856 bp. This result both is consistent with the approximate size estimates obtained by restriction endonuclease mapping and leads to greater agreement with the apparent lengths of the PCR amplimers (not shown).

The following two experiments were designed to address the question of whether the amplified segment is present in a genomic context similar to that of the nonamplified segment. First, wild-type and origin-amplified DNAs were both amplified by using primer pairs consisting of one primer located in the flanking region and one within the repeat element, oriented to prime toward each other, e.g., L1 and C18 (Fig. 3A). In all cases, the amplified fragments were of the predicted sizes (Fig. 3D). To eliminate the possibility that the amplification seen in the amplified-origin template was due to the presence of a small amount of wild-type DNA, similar PCRs were done with serial dilutions of wild-type and amplified-origin DNAs (data not shown). Equivalent dilution end points were found with both templates, indicating that even if a small amount of wild-type template was present in the amplified-origin DNA preparation, the bulk of the amplified product came from amplified-origin templates.

Second, to obtain the nucleotide sequence across the junction of the flanking region and the amplified segment, the flanking region amplimers generated by primer pairs L1-C18 and R1-S11 were cloned into the pGEM-T vector system (Promega, Madison, Wis.). The resulting plasmids were purified with a QIAwell-8 plasmid kit (Qiagen, Chatsworth, Calif.) and used as templates in nucleotide sequencing reactions as described above with M13/pUC forward (−20) and reverse (−24) sequencing primers (New England Biolabs, Beverly, Mass.). Both strands were sequenced from at least three clones of wild-type and amplified-origin templates from each end of the repeat element. There was no evidence for rearrangement in any of the templates, and no base changes were noted. These results, along with the absence of differences between wild-type and amplified-origin DNAs in the migration of fragments other than *Hin*dIII-L (Fig. 1) and comigration of the 2- and 6-kb *Eco*RI flanking fragments in both wild-type and amplified-origin DNAs (Fig. 2), provide strong evidence that the amplification occurs within the normal genomic location of these sequences.

One plausible mechanism for generation of the arrays of tandemly duplicated nucleotide sequences observed here can be envisioned to consist of two steps. In the first step, two separate identical molecules containing a segment of unique sequence bounded by directly repeated sequence elements would become aligned through the repeats in a staggered manner (Fig. 4B, Duplication). Intermolecular recombination through the repeat elements would result in a sequence duplication in one progeny molecule and a deletion in the other. In the second step, molecules with the duplicated element would recombine via intermolecular homologous recombination with other such molecules to result in species with three tandem copies (Fig. 4B, Amplification). Further rounds of homologous recombination between such molecules would result in a mixed population of molecules containing variable numbers of the repeated element. In the case of a herpesvirus genome, the length limits on packaging DNA into capsids would impose a constraint on the number of reiterations.

A set of directly repeated sequences compatible with this mechanism is present in the wild-type HHV-6B(Z29) genome in the vicinity of the boundaries of the amplified region (Fig. 4A). The A/a pair has 13 of 16 identical bases, and the B/b, C/c, and D/d pairs are each identical in 9 of 11 bases. The A and B elements are part of one arm of an 88-bp palindrome in which 54 (61%) of the residues can base pair. This could be important in generating single strands that would facilitate initiation of the recombination event. Other, less significant palindromic

FIG. 3. Identification of repeat unit junctions and flanking region boundaries. (A) Diagram showing the orientations and locations of five oligonucleotides within the predicted repeat element boundaries and two oligonucleotides that flank the repeat element boundaries that were used for PCR. The locations of the HHV-6B major DNA-binding protein homolog (MDBP) and HHV-6B(Z29) KA3L open reading frame, the intergenic region, and the IDR are shown. The five oligonucleotides within the repeat element boundary were originally prepared for use in determining the binding site of the HHV-6B OBP (9). (B) Diagram showing PCR amplimers that could be generated between various oligonucleotide pairs in the event of a sequence duplication, as predicted on the basis of restriction endonuclease mapping of the repeat element boundaries. (C) PCR amplification products from wild-type (wt) and amplified-origin (o^a) templates generated from primers within the repeat unit boundaries. Primer combinations were as shown (see below for primer sequences), and size standards are indicated. (D) PCR amplification products from wild-type and amplified-origin templates generated from primers that flank and are within the repeat unit boundaries. Primer combinations were as shown. The locations of primers L2 and R2 are not shown in panel A or B but were predicted to produce amplified fragments of 423 and 385 bp, respectively, in the combinations used. PCR amplification and detection are described in the text. Primer sequences are as follows (5′ to 3′): S11, AGTAATAAAGCATACTATTAGATTCTTCACGTTAA (positions 5251 to 5285); C1, GACGTAAACGCCACGCCTTCGTTCTCCCGTCCTCA (4921 to 4955); C4, ATCATTTTCAGTCAAACGTCTCAAGTCGTAC CCCA (5026 to 5060); S4, CCGGCTGTTCAGAGGGACGCTGGGGTACGACTTGA (5006 to 5040); C18, TATAGAAAACGGTATATTTCAACTTGTGGT (4741 to 4770); L1, ACCGTGGTCGAGAAATCGTTTTCTACAGTCAAATTC (4447 to 4483); L2, TCGAATAAGATCACCATGATCTTCGGCCATACCAA (4318 to 4352); R1, TTTTACACGGACTTATATCTTTACCGAACCATGATC (5538 to 5573); and R2, GAGCTAAACCATCGACTTCAAACACCTCACAGTCTA (5600 to 5635). (E) Nucleotide sequence across the repeat unit boundary. The nucleotide sequence across the junction between amplified segment boundaries is shown above segments of wild-type sequences that span the ends of the repeat unit. Wild-type sequences that flank the amplified segment are shown in lowercase letters.

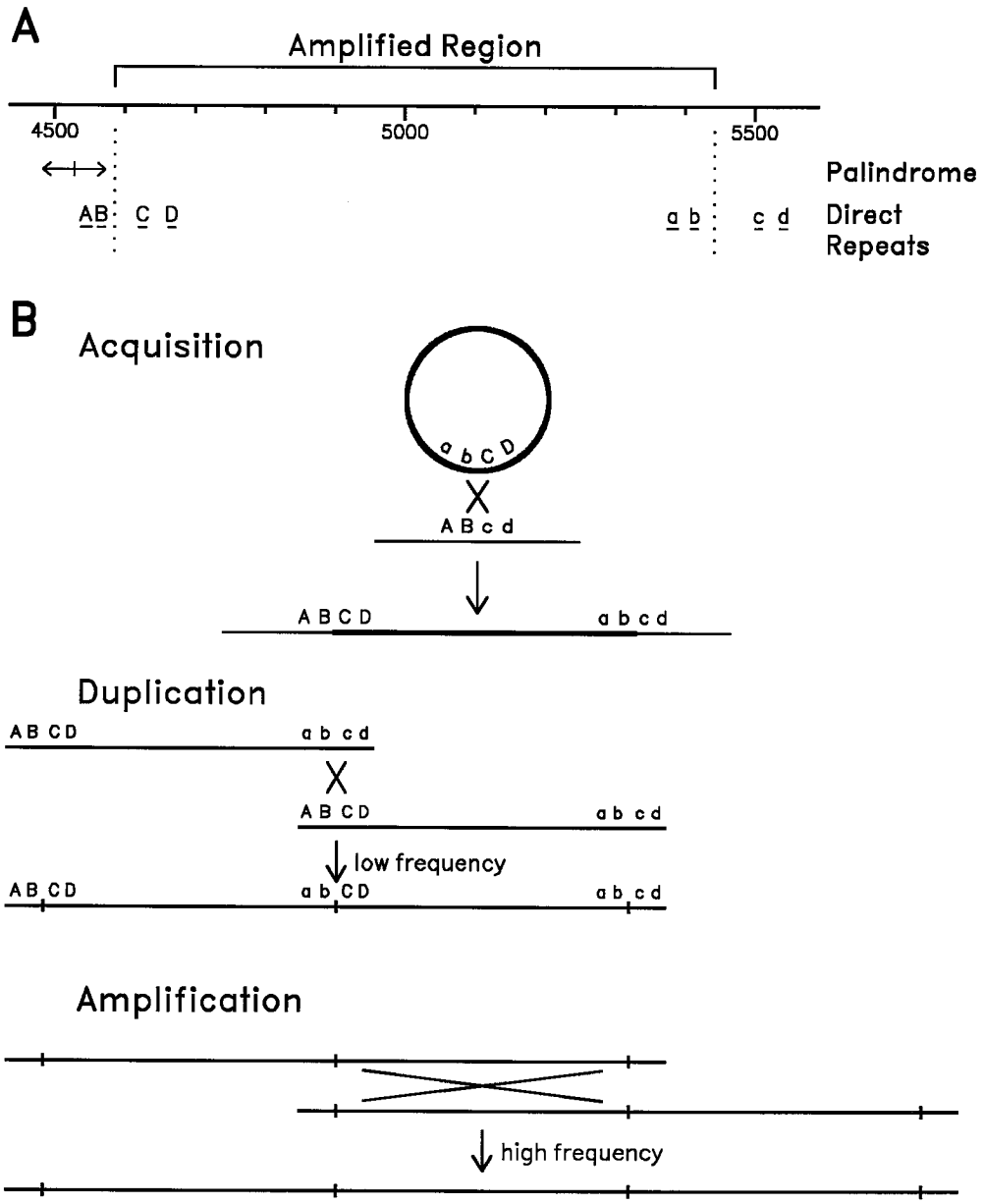


FIG. 4. Possible amplification mechanism. (A) Sequence features near amplification boundaries. Directly repeated sequences (A and a, B and b, C and c, and D and d) that flank the boundaries of the amplified region and an 88-bp palindrome are indicated to scale. Sequence coordinates within Genbank accession number L16947 (4) are as follows: palindrome, 4485 to 4572; A, 4537 to 4553; B, 4562 to 4572; C, 4621 to 4631; D, 4662 to 4672; a, 5374 to 5390; b, 5409 to 5419; c, 5501 to 5511; and d, 5537 to 5547. (B) Model for sequence acquisition, duplication, and amplification (not to scale). Acquisition of the *ori*Lyt element by HHV-6 or a progenitor herpesvirus would be via recombination of a genetic element (circle) with sequence similarity to the direct repeats A, B, c, and d. The duplication event would involve a low-frequency recombination event between two HHV-6 genomes aligned via the direct repeat elements A/a, B/b, C/c, and D/d, with the recombination occurring in between the B/b and C/c elements. The amplification would occur via subsequent homologous recombinations that would occur at higher frequency, and it would result in a mixed population of molecules containing various numbers of sequence reiterations.

sequences are present in the vicinity of the a and b elements (not shown). Figure 4B depicts recombination events employing these repeated sequences that would lead to the duplication and subsequent amplification, resulting in the observed structures. It is important to note that the sequence data indicate that the crossover occurred in the nonhomologous region located between the B/b and C/c repeats. Others have identified similar illegitimate recombination events adjacent to homologous sequences and have termed this "homology-driven nonhomologous recombination" (14), an apt designation in this instance.

Although speculative, this model is consistent with our observations. The imperfect homology within the direct repeat pairs and the interspersing of sequences without homologous counterparts suggest that the initial recombination event would be rare (Fig. 4B). This would explain why the duplication was observed only after extensive passaging of the virus and only in some passage lineages. Subsequent homologous recombination events would have generated the mixed population of molecules that we observed. A selective advantage for the reiterated origin would result in the loss of wild-type molecules; few wild-type molecules were present in the mate-

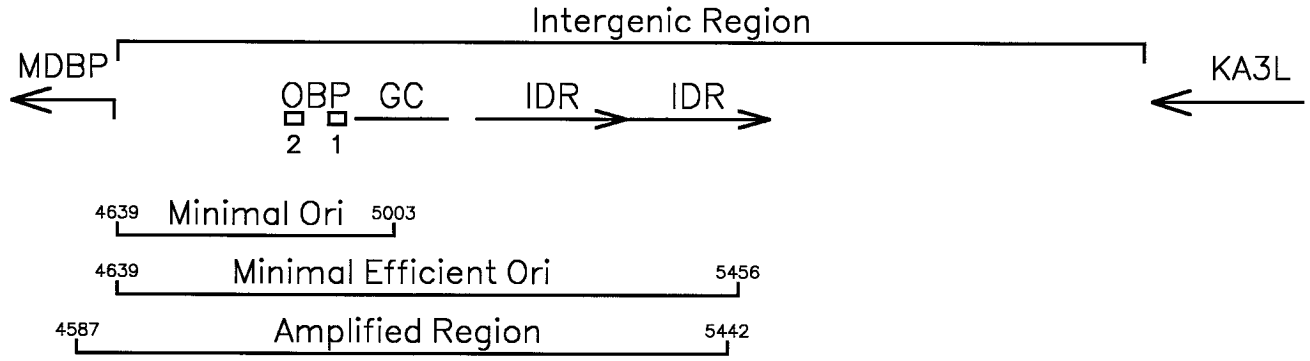


FIG. 5. Summary of genomic features in the vicinity of the amplified region. The amplified region contains binding sites for the HHV-6B OBP homolog (9), a G+C-rich region, and one complete copy of the IDR and corresponds nearly precisely with the region identified by Dewhurst et al. (6) as the minimal efficient origin (Ori) region in transient plasmid replication assays. MDBP, HHV-6B major DNA-binding protein homolog.

rial used in the mapping and sequencing studies (Fig. 1A and B, lanes 2). The use of an alternative crossover point during the initial recombination would result in a different repeat unit length that would then be propagated during the homologous recombination stage. This would account for the difference between the lengths of the repeat shown in Fig. 1 (lanes 1 and 2). Genomes from which *ori*Lyt was deleted during the initial recombination would be nonviable and would not be detected with the primer pairs used in these experiments.

Thus far we have focused on amplification of the genomic segment. However, the presence and location of the duplicated sequences are consistent with acquisition of the origin-containing segment by HHV-6B or a progenitor virus by transposition from an exogenous source (Fig. 4B), with the duplicated flanking sequences possibly diverging in the interim. It is intriguing that the boundaries of this hypothetical mobile element nearly coincide with the boundaries of the minimal efficient HHV-6B *ori*Lyt identified in a transient replication assay (6). The source of this genetic element is unknown, but there are numerous examples of acquisition by herpesviruses of genes from cells and other viruses, including cellular G-coupled proteins, a class I major histocompatibility antigen, and the T-cell receptor gamma chain by human cytomegalovirus (3) and thymidylate synthetase by varicella-zoster virus (5), as well as a parvovirus replication protein gene by HHV-6 (17). Origins of latent viral replication of necessity make use of cellular replication machinery (13) and thus are good candidates for having been acquired from cellular genomes, although there is no evidence for this. The source(s) of lytic origins of viral DNA replication is more speculative.

To summarize our results, in some passage lineages we observed the amplification of an 856-bp DNA segment internal to the wild-type *Hin*dIII-L fragment. The amplified unit is present in as many as 12 tandem arrays in some genomes. As summarized in Fig. 5, the amplified segment corresponds nearly precisely to the minimal origin of lytic replication (*ori*Lyt) identified by Dewhurst et al. (6), a region that also contains binding sites for the HHV-6B OBP (9). We identified a plausible mechanism for the initial recombination event that suggests a mechanism for acquisition of *ori*Lyt by this or a progenitor virus via transposition.

To our knowledge, an origin amplification similar to this has not been described for any other herpesvirus. Amplified origins of replication are present in defective interfering viruses (DIs); an example is the sequence amplification present in HSV-1 DI genomes. HSV-1 DI genomes consist of multimers of units that consist of an origin of replication linked to a cleavage/packaging signal (reviewed in reference 8). Additional sequences can be present within a monomeric unit, and such a system has been used for high-level expression of genes via the HSV-1 amplicon (15). A critical distinction between sequence amplification in standard DIs and the *ori*Lyt amplification observed here is that the HSV-1 DI-based amplicons are dependent on the presence of a helper virus to provide in *trans* the machinery necessary to replicate their DNA. The HHV-6B *ori*Lyt amplification occurred within an otherwise unaltered genome, and while we cannot exclude dependence on wild-type virus, the example that we characterized most completely contained only a trace of wild-type virus.

The amplified-origin genomes are relevant to several lines of research. First, binding sites for the HHV-6B homolog of the alphaherpesvirus OBP are present in the amplified segment. The amplified-origin viruses will be useful for studying the interaction of the OBP with viral DNA. Second, during tissue culture passage, HHV-6B(Z29) genomes have lost approximately 6 kb from the direct repeat elements (DR) that are present at both genomic termini (3 kb from each copy) (11). The DR length reductions may have provided the capsid space necessary to accommodate the as much as 10 kb of additional DNA found in some amplified-origin genomes. The ability of HHV-6B genomes to tolerate the presence of additional nucleotide sequences, regardless of their source, is relevant to the possibility of genetic manipulation of HHV-6B genomes for the expression of heterologous genes, either for genetic studies or as a gene delivery vector. Third, we will conduct studies to assess the effect of the amplified origin region on viral replication during growth in tissue culture. The predominance of the amplified-origin genomes in the preparations that contain them suggests that the amplification confers a growth advantage in the cell culture growth system. In recent related experiments, Dewhurst and colleagues (7) found that constructs containing six tandemly repeated copies of a sequence corresponding to the amplified region described here were much more efficiently replicated in a transient replication assay in HHV-6-infected cells than were constructs containing a single unit. Finally, we will attempt to identify an exogenous source for this herpesvirus *ori*Lyt.

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REFERENCES

1. **Anders, D. G., M. A. Kacica, G. Pari, and S. M. Punturieri.** 1992. Boundaries and structure of human cytomegalovirus *ori*Lyt, a complex origin for lytic-phase DNA replication. J. Virol. **66:**3373–3384.
2. **Black, J. B., K. C. Sanderlin, C. S. Goldsmith, H. E. Gary, C. Lopez, and P. E. Pellett.** 1989. Growth properties of human herpesvirus-6 strain Z29. J. Virol. Methods **26:**133–145.
3. **Chee, M. S., A. T. Bankier, S. Beck, R. Bohni, C. M. Brown, R. Cerny, T. Horsnell, C. A. Hutchison, T. Kouzarides, J. A. Martignetti, E. Preddie, S. C. Satchwell, P. Tomlinson, K. M. Weston, and B. G. Barrell.** 1990. Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. Curr. Top. Microbiol. Immunol. **154:**125–169.
4. **Dambaugh, T. R., J. J. O'Brian, E. D. Anton, C. A. Greenamoyer, G. J. Lindquester, and P. E. Pellett.** A 20.9 kb domain of human herpesvirus 6B (Z29) DNA colinear with human cytomegalovirus encodes homologs of genes from discontinuous loci in alpha- and gammaherpesviruses and contains a putative origin of HHV-6B DNA replication. Submitted for publication.
5. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67:**1759–1816.
6. **Dewhurst, S., S. C. Dollard, P. E. Pellett, and T. R. Dambaugh.** 1993. Identification of a lytic-phase origin of DNA replication in human herpesvirus 6B strain Z29. J. Virol. **67:**7680–7683.
7. **Dewhurst, S., D. M. Krenitsky, and C. Dykes.** 1994. Human herpesvirus 6B origin: sequence diversity, requirement for two binding sites for origin-binding protein, and enhanced replication from origin multimers. J. Virol. **68:**6799–6803.
8. **Frenkel, N.** 1981. Defective interfering herpesviruses, p. 91–120. *In* A. J. Nahmias, W. R. Dowdle, and R. S. Schinazi (ed.), The human herpesviruses—an interdisciplinary perspective. Elsevier Science Publishing, New York.
9. **Inoue, N., T. R. Dambaugh, J. C. Rapp, and P. E. Pellett.** 1994. Alphaherpesvirus origin-binding protein homolog encoded by human herpesvirus 6B, a betaherpesvirus, binds to nucleotide sequences that are similar to ori regions of alphaherpesviruses. J. Virol. **68:**4126–4136.
10. **Lindquester, G. J., N. Inoue, R. D. Allen, J. W. Castelli, F. R. Stamey, T. R. Dambaugh, J. J. O'Brian, R. M. Danovich, N. Frenkel, and P. E. Pellett.** Human herpesvirus 6B genome: restriction endonuclease mapping, cloning, and identification of colinearity with human cytomegalovirus and human herpesvirus 6A genomes. Submitted for publication.
11. **Lindquester, G. J., and P. E. Pellett.** 1991. Properties of the human herpesvirus 6 strain Z29 genome: G + C content, length, and presence of variable-length directly repeated terminal sequence elements. Virology **182:**102–110.
12. **Lopez, C., P. Pellett, J. Stewart, C. Goldsmith, K. Sanderlin, J. Black, D. Warfield, and P. Feorino.** 1988. Characteristics of human herpesvirus-6. J. Infect. Dis. **157:**1271–1273.
13. **Roizman, B., and A. E. Sears.** 1987. An inquiry into the mechanisms of herpes simplex virus latency. Annu. Rev. Microbiol. **41:**543–571.
14. **Sakagami, K., Y. Tukinaga, H. Yoshikura, and I. Kobayashi.** 1994. Homology-associated nonhomologous recombination in mammalian gene targeting. Proc. Natl. Acad. Sci. USA **91:**8527–8531.
15. **Spaete, R. R., and N. Frenkel.** 1986. The herpes simplex virus amplicon: a new eucaryotic defective-virus cloning-amplifying vector. Cell **30:**295–304.
16. **Stow, N. D., and A. J. Davison.** 1986. Identification of a varicella-zoster virus origin of DNA replication and its activation by herpes simplex type 1 gene products. J. Gen. Virol. **67:**1613–1623.
17. **Thomson, B. J., S. Efstathiou, and R. W. Honess.** 1991. Acquisition of the human adeno-associated virus type-2 rep gene by human herpesvirus type-6. Nature (London) **351:**78–80.
18. **Weller, S. K., A. Spadaro, J. E. Schaffer, A. W. Murray, A. M. Maxam, and P. A. Schaffer.** 1985. Cloning, sequencing, and functional analysis of oriL, a herpes simplex virus type 1 origin of DNA synthesis. Mol. Cell. Biol. **5:**930–942.