Recombination of genomic terminus of bovine herpesvirus type 1 with cellular DNA

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Bovine herpesvirus 1 (BHV-1) has a linear DNA genome of about 135 kb which appears as two isomers, resulting from its short unique segment being present in the two possible orientations with respect to the large unique segment. BHV-1 also circularizes its DNA to form replicative molecules. Definition of the target sequences at the genomic termini involved in the recombination events during genomic replication and isomerization, as well as virus maturation, led to the discovery that 10% of the genome molecules have additional DNA sequences attached to the right-hand terminus, as shown by electron microscopy. Three such tails have been cloned molecularly; they differ in length and nucleotide sequence, and hybridization experiments demonstrate the cellular origin of two of the three tails. The evidence presented here is consistent with a proportion of the BHV-1 genomes recombining their DNA with cellular DNA during lytic infection.

Introduction

Infections with bovine herpesvirus type 1 (BHV-1) cause two diseases in cattle: infectious bovine rhinotracheitis, associated with abortion in pregnant cows, and infectious pustular vulvovaginitis, usually transmitted by the venereal route of infection. Although BHV-1 is of economic importance, there is usually only a single episode of disease after primary infection. To our knowledge, natural recurrences have not yet been reported from infected cattle, although the virus does persist and can be reactivated by immunosuppression as well as by cocultivation of explanted tissues of clinically healthy foetuses (Sheffy & Davies, 1982; Snowdown, 1965; Ludwig & Storz, 1973; Davies & Carmichael, 1973).

The physical structure of the BHV-1 genome in latently infected cells is not known. The viral genome could theoretically persist in cells that have yet to be identified in different physical states; (i) in linear form, as virion DNA; (ii) in circular form, as an episome obtained by joining of the genomic ends of the linear virion DNA molecule; (iii) as concatemers built up by the head-to-tail fusions of genome units; (iv) integrated into chromosomes.

Conflicting results have been reported on the physical state of herpesviral DNAs in latently infected cells, but it seems accepted that episomes are the prevalent form of genome maintenance besides genomic integration. The physical state of the latent genome of herpes simplex virus (HSV) seems to be episomal as this DNA, isolated from latently infected mouse brain, was found to band at the density of virion DNA in CsCl buoyant density gradients and was not linked to the cellular chromosome (Mellerick & Fraser, 1987). Thus, the absence of restriction fragments typical for genomic termini of virion DNA in latent HSV-1-infected mouse ganglia and human brain tissue can be interpreted to mean its DNA is circularized (Efstathiou et al., 1986; Fraser et al., 1981; Rock & Fraser, 1983, 1985). In contrast, Puga et al. (1984) detected sequences specific for genomic termini of virion genomes in restriction fragments of non-typical size. Therefore, either a rearrangement of viral sequences must have occurred or the viral genome can become integrated into cellular DNA.

The genome of pseudorabies virus (PRV), which has the same genomic structure as BHV-1, was found to be circular or concatemeric in a small proportion of cases. However, linear and unintegrated PRV DNA has also been observed in different parts of the nervous system (Rziha et al., 1986).

Marek's disease virus (MDV) and Epstein–Barr virus (EBV) genomes have been found in two physical states in latently infected cells. Most EBV-infected human B lymphocytes harbour many episomal copies of the EBV genome, formed by covalent fusion of the terminal direct repeats present at both ends of the virion DNA (Dambaugh et al., 1980), whereas a latently infected
Burkitt's tumour cell line (Namalwa) contains only two copies of the EBV genome, which are integrated into the cellular genome (Lawrence et al., 1988). Viral genome integration and the episomal state of EBV can even coexist in latently infected cell lines IB4 (Henderson et al., 1983) and Raji (Anvret et al., 1984). In the case of transformed T cell lines latently infected with MDV, both integrated and episomal states of genomic DNA have been reported (Hirai et al., 1981; Rziha & Bauer, 1982; Tanaka et al., 1978). Herpesvirus atelae and herpesvirus saimiri genomes are maintained as episomes in latently infected T cell lines (Kaschka-Dierich et al., 1982).

The molecular basis of virus-host interactions that lead to the establishment and maintenance of a virus in infected cells in a latent state is poorly understood. Taking this lack of information into account, we have investigated the processing of genomic termini of BHV-1. Preliminary studies indicated that, during the lytic cycle, a minor proportion of viral genomes of BHV-1, isolated from nucleocapsids of infected cell culture supernatants, displayed unexpected tail-like additional sequences at the right genomic terminus. The objective of this study was to investigate the occurrence of these terminal extensions, which seem to result from recombination of BHV-1 with bovine cellular DNA. This interaction of viral and cellular DNA could be an essential step during an abortive infectious cycle, which could eventually lead to the establishment of integrated herpesvirus DNA.

**Methods**

**Cell and virus strains.** The BHV-1 strain B4 used in this study was a generous gift from O. C. Straub (Federal Research Center for Virus Diseases of Animals, Tübingen, F.R.G.). This strain is a tissue-culture-adapted derivative of an isolate (strain B1, Schönböken) from the vagina of a cow infected with infectious pustular vulvovaginitis. BHV-1 strain B4 was propagated in Georgia bovine kidney (GBK) cells with a low m.o.i. (10⁻³), as described earlier (Hammerschmidt et al., 1986).

**Preparation of viral and cellular DNA.** Nucleocapsids were collected from the supernatants of infected cell cultures and purified by centrifugation through a linear 20% to 70% sucrose gradient. After collection, the nucleocapsids were treated with DNase prior to lysis with SDS and proteinase K (Serva), further purified by phenol-chloroform extraction and collected by ethanol precipitation. The right genomic terminus of BHV-1 was molecularly cloned in pUC8 (Messing, 1983). For this purpose, whole virion DNA was treated with DNA polymerase I large fragment in the presence of the four deoxyribonucleoside triphosphates to generate blunt ends. Decamer HindIII linkers (New England Biolabs) were ligated to virion DNA, the DNAs were cleaved with EcoRI and HindIII in combination and the terminal EcoRI E fragment was isolated from a preparative gel and cloned in an appropriately cleaved pUC8 plasmid as described earlier (Hammerschmidt et al., 1986, 1988).

**DNA sequencing and identification of terminal size heterogeneity of the EcoRI E fragments.** HindIII-cut plasmids carrying the EcoRI fragment E were labelled at their 5' ends with T4 polynucleotide kinase (Stehekin) and [γ-³²P]ATP. After digestion of the end-labelled clones with SstI, the DNA fragments were separated on non-denaturing polyacrylamide slab gels (8% w/v polyacrylamide). Autoradiographs of XAR-5 films (Eastman Kodak) allowed the detection of the labelled heterogeneous terminal HindIII/SstI subfragments. Once identified, these labelled HindIII/SstI fragments were subjected to specific chemical degradation according to the sequencing protocols of Maxam & Gilbert (1977, 1980).

**Subcloning sequences from the right genomic terminus.** In order to make specific hybridization probes, the subcloning of certain unusual fragments derived from the right genomic terminus of BHV-1 was necessary. This was performed using M13 mp18 or mp19 vectors (Messing, 1983). To check whether cloning had been successful, all subclones were sequenced by the chain termination method (Sanger et al., 1980).

**Hybridization experiments.** After electrophoresis on agarose gels, restriction fragments were transferred (Southern, 1975) to GeneScreen-Plus membranes (New England Nuclear). Radioactive probes, with a specific activity of about 5 x 10⁸ to 2 x 10⁸ c.p.m./µg DNA, were obtained by using the appropriate M13 clone in its single-stranded form as a template for in vitro DNA synthesis starting from the M13 hybridization probe primer (New England Biolabs) in the presence of [α-³²P]dCTP (Amersham) (Hu & Messing, 1982).

The membranes were prehybridized and hybridized at 60 °C in solutions containing 1% SDS, 1 M-NaCl, 10% dextran sulphate (Pharmacia) and 150 µg/ml salmon sperm DNA (Boehringer Mannheim) for 24 to 48 h as recommended by the manufacturer of the nylon membranes. The membranes were washed at room temperature (2 × SSC, 2 × 10 min), at 65 °C (2 × SSC, 1% SDS, 2 × 30 min) and again at room temperature (0.2 × SSC, 2 × 30 min). The dried membranes were exposed to XAR-5 films.

**Results**

**Electron microscopy of self-annealed single strands of BHV-1 virion DNA.**

We investigated genomic BHV-1 DNA by electron microscopy under conditions that allowed self-annealing
of DNA single strands after melting of the dsDNA. Considering the genome arrangement of BHV-1, which consists of large unique (UL) and short unique (Us) segments as non-reiterated segments and two large inversely orientated reiterations TR and IR (Fig. 1), one would expect self-annealing of ssDNA molecules to take place between the TR and IR sequences, due to sequence complementarity forming intrastrand base pairing. In contrast, UL and Us are not able to anneal intra-stranded part representing UL, a double-stranded stretch inversely orientated reiterations TR and IR (Fig. 1), one place between the TR and IR sequences, due to sequence contrast, UL and Us are not able to anneal intra-
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**Characterization of recombinant clones**

Fragments containing the right genomic terminus of BHV-1, as represented by the EcoRI fragment E, were cloned several times independently, as outlined in Methods. The fragments were cloned with the aid of an external synthetic HindIII site linked to the genomic terminus and the closest EcoRI site within the viral genome. This enabled us to clone such a fragment with a defined orientation into a plasmid vector at a reasonable efficiency. The identity of cloned EcoRI E fragments was verified by restriction enzyme digestion and agarose gel electrophoresis, combined with Southern blot hybridization of viral DNA and cloned DNA fragments (data not shown). A physical map was established for the 8.2 kb fragment E shown in part in Fig. 1. In order to identify any size heterogeneity in the terminal genomic fragments of BHV-1 which might be due to additional sequences added to the right genomic terminus, we labelled the unique HindIII site of recombinant plasmid clones by

![Map of BHV-1 and cloning strategy for the right-hand genomic terminus.](image)

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*Fig. 1.* Map of BHV-1 and cloning strategy for the right-hand genomic terminus. 

(a) The scale of map units of the genome. (b) The characteristic segments of the genome. (c) An EcoRI restriction map; the fragments A to E are indicated, with their sizes given in kb. (d) A fine map of the EcoRI E fragment, as it is present in plasmid clone E16, bearing a synthetic HindIII linker at the genomic terminus. (e) A further expansion of the standard 160 bp SstI/HindIII fragment covering the genomic terminus; the position of the structurally important c-element is indicated by a boxed arrow.

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**Recombination of BHV-1 with cellular DNA**

To our surprise, a minor proportion of molecules (six of 64 complete molecules belonging to both classes and randomly taken in one series of microphotographs) revealed an additional structure at the right genomic terminus. A single-stranded tail-like elongation occurred at the junction of the single-/double-stranded regions (Fig. 2b and d). As these single-stranded tails were not capable of participating in the formation of the TR/IR DNA duplex, they were not part of the inverted repeats, TR and IR. Thus, they represent short stretches of DNA added to the right genomic terminus. In additional series of micrographs, more tails were detected in self-annealed complete DNA molecules showing single-stranded terminal extensions of variable lengths. The micrographs shown in Fig. 2(e, f) are enlargements taken from complete TR/IR homoduplexes in which the relevant region could be identified unambiguously. The TR/IR homoduplexes were considered to be complete only if the size of the ss Us plus the ds TR/IR segment was of the correct length. The appearance of these single-stranded tails was indicative of elongations at the right genomic terminus that are not part of the inverted repeats TR and IR.

Tails were detected with a frequency of about 10% in the measured molecules. The length of tails differed remarkably (mean 0.23 kb, standard deviation 0.15 kb). However, the limits of resolution of the electron microscopic examination we performed would have failed to detect extensions shorter than 80 base pairs.
Fig. 2. Homoduplex analysis of denatured BHV-1 virion DNA by electron microscopy. BHV-1 virion DNA was heat-denatured and allowed to form double-stranded regions between the homologous sequences of TR and IR. Two representative molecules are shown as electron micrographs and as schematic drawings. The heavy lines in the drawings indicate double-stranded regions formed by the annealing of TR and IR. Thin lines indicate the ss UL and Us segments forming a large loop. The TR/IR duplex region is interspersed with small single-stranded internal loops (and/or hairpin-like structures). These structures stand for heterogeneities at distinct sites of the hybridized TR/IR DNA duplex. They occur at three sites within TR/I R and are located proximal to the right genomic terminus of BHV-1. Sequences are given starting from the ends, not showing the start of the TR/IR DNA duplex. These enlarged micrographs were derived from complete TR/IR homoduplexes in which the relevant portions could be identified unambiguously.

Three of 37 clones showed an unusual larger size of the HindIII/SsrI terminal fragment which varied in length between 200 and 260 bp.
nucleotide sequence of TR. From the structure of the BHV-1 genome, two deduced features are noteworthy with respect to the right genomic terminus. Firstly, it was inferred (Hammerschmidt et al., 1988) that the terminus itself bears a single base extension (a guanosyl residue) at the 3' end of the molecule. Fusion of the right and left ends of the linear genome has to occur during DNA replication and happens by complementary base-pairing of the right 3' guanosine with the single 3' cytosine located at the left terminus, followed by a covalent linkage and resulting in a circular molecule. The single base extensions at the 3' ends were artificially removed by the 3'→5' exonuclease activity of DNA polymerase I large fragment prior to attachment of the linkers, but they were deduced by sequencing the fusion fragment itself (Hammerschmidt et al., 1988). The second feature to note is an 18 bp motif, located 3 bp proximal to the right end, which is called an α-element (over- and underlined in Fig. 3). This α-element occurs four times in the virion DNA, twice within U_L (at the genomic terminus and at the junction with I_R), within T_R at the genomic terminus and, therefore, also within I_R at the junction with U_L. The α-element consists of C and G residues only and displays a nearly palindromic structure with one discontinuity [at position 7 (G) and 12 (G) respectively, of the palindrome]. This discontinuity permits definition of the different orientations of the α-element (Hammerschmidt et al., 1988). The α-element is boxed in Fig. 1 and lined in Fig. 3, and its relative orientation is given.

By comparison of the prototype nucleotide sequence of the right genomic terminus with the terminal sequences as present in the three clones with longer E fragments, it became obvious that these clones had acquired an additional stretch of nucleotides ranging in size from 37 bp (clone E53) to 101 bp (clone E265) (Fig. 3). These tail-like sequences revealed neither sequence identity with one another nor homology to the standard E clone sequence of the extreme right end of the genome. The nucleotide sequence of the synthetic HindIII linker that was attached during the cloning procedure could be confirmed in each case (not shown), which implies that authentic tails have been cloned. However, it should be noted that all three junctions of the right terminus with heterologous sequences lack the unpaired 3' guanosine residue present at the authentic right genomic terminus.

**Hybridization experiments**

For detection of the origin of these additional tail-like sequences at the right genomic end of a minor proportion of the virion molecules, the tails were individually subcloned in M13 mp8 or mp19. The terminal HindIII/MnlI fragment in the case of the clone E53, the HindIII/FnuDII fragment of the clone E65 and the terminal HindIII/HaelIII fragment in the case of the clone E265 (Fig. 3), were subcloned into the HindIII/HindII-cleaved multiple cloning site of the vectors M13 mp8 and M13 mp19. Sequencing of the recombinant single-stranded M13 phage DNA confirmed the presence of the tail sequences. The M13 subclones of E53, E65 and E265 were composed of 30 bp, 45 bp, 101 bp of the original clones (Fig. 3), respectively. The recombinant M13 phage DNAs were used as single-stranded templates to synthesize partially double-stranded 32P-labelled probes that were used in Southern blot hybridizations. EcoRI-cut DNA fragments of BHV-1 and HindIII-cleaved DNA of three uninfected cells (Vero, GBK cells and BFL) were separated on analytical agarose gels, transferred to nylon membranes (Southern, 1975) and hybridized to those probes. By use of this experimental approach, two predictions can be made. Firstly, if the terminal extensions were to originate from the right-hand terminus of the BHV-1 genome, the radioactive probes would give rise to a detectable signal by hybridizing to the right terminal fragment of restricted BHV-1 virion DNA (fragment E in Fig. 1 and 4). Secondly, if the terminal extensions were to originate from cellular DNA, the length of the radioactive probes, ranging from 30 to 101 bp in M13 subclones, would not permit the detection of a single cellular DNA fragment by Southern blot hybridization, because of the limits of detection under these conditions, but, if the terminal extensions were to originate from highly repetitive cellular DNA (> 10^4 copies/genome), the length of the probes would enable them to be detected.

As shown in Fig. 4, Southern blot hybridization with the subcloned part of E65 used as a radioactive probe, demonstrated that this tail hybridized to repetitive sequences found in the bovine, but not the monkey, genome. No signal was detected from the right-hand terminal EcoRI fragment of BHV-1 DNA (Fig. 4, E fragment). The probe did not hybridize to other BHV-1 EcoRI fragments, nor did it hybridize to the φX174 and λ Mλ standards. The failure to detect hybridization between E65 and the right terminal fragment of BHV-1 DNA, and the strong hybridization with bovine cell DNA only, is consistent with the tails being derived randomly from repetitive elements in bovine cell DNA. Similar hybridization signals were obtained when the tail in E265 was used as a radioactive probe for parallel Southern blots. In the case of clone E53, no signal could be detected with immobilized viral or cellular DNA except for hybridization to its positive control (E53 replicative form DNA; data not shown).

The hybridization results strongly suggest that the tails at the right genomic end were not part of the BHV-1 prototype genome, that two tails had their origin in...
Recombination of BHV-1 with cellular DNA

Fig. 4. Detection of the origin of tail-like sequences. (a) BHV-1 DNA digested with EcoRI and total cellular DNA of bovine cell lines (GBK, BFL) and monkey cell line (Vero) were digested with HindIII and the fragments separated on an agarose gel stained with ethidium bromide. E indicates the right-hand terminal fragment of the BHV-1 virion DNA. Since the purpose of running this gel did not require a highly purified BHV-1 virion DNA preparation, crude BHV-1 DNA was used and shows some background fragmented DNA. (b) Autoradiography, after transfer of the DNA fragments to a membrane and hybridization to 32P-labelled DNA, of the tail sequence found in plasmid clone E65 and subcloned in an M13 vector. Cellular DNA of bovine origin (BFL, GBK) is heavily marked, whereas DNAs from Vero cells, BHV-1 and the size markers (2, ~bX174) do not give signals. Asterisks and arrows point to some bands originating from a family of related HindIII repeats in BFL and GBK cell DNA which appear to be detected by the E65 probe. Lanes 1, 2, ~bX174; lanes 2, BHV-1; lanes 3, BFL; lanes 4, GBK; lanes 5, Vero.

highly repetitive bovine genomic sequences that did not share homology with monkey (Vero) cell DNA and that bovine cellular DNA contained repetitive DNA elements. Failure to detect a signal with subcloned tail sequences of the clone E53 indicated that this tail is neither part of these bovine repetitive elements nor part of the BHV-1 genome itself. This tail is most likely to be derived from a single-copy nucleotide sequence, not detectable in 5 μg of cleaved bovine DNA (representing approximately 10^6 genome copies) by a 30 bp long target region.

Discussion

The investigation by electron microscopy and cloning of the genomic terminal restriction fragments of BHV-1 showed that about 10% of BHV-1 genomes isolated from virions carried additional nucleotide sequences at the right genomic terminus when compared with the standard molecule. Hybridization experiments indicate that these tail sequences are not part of the prototype BHV-1 genome but originate from the host cell genome. These experiments provide evidence that the viral genome recombines with cellular DNA during the lytic cycle. However, it should be emphasized that this recombination event is not necessarily an essential step in latent or lytic infection.

There are several possibilities for the means by which BHV-1 DNA could have acquired additional cellular DNA sequences. One explanation could simply be a cloning artefact since the ligation of contaminating unrelated cellular DNA fragments onto virion DNA could have happened during the attachment of the synthetic HindIII linker molecules. As this is a potential artefact, we have tried to circumvent this problem by eliminating cellular DNA from the virion DNA preparations by preparing virion capsids over a sucrose gradient and treating with DNase prior to their lysis. Since there is no measure for absolute purity of virion DNA, we cannot entirely exclude a cloning artefact. However, since the electron microscopy of homoduplex virion DNA which has not been otherwise modified clearly revealed additional sequences at the right genomic terminus, we think that we have cloned three of these tails molecularly.

There are two mechanisms by which cellular DNA could become attached to the genomic ends of BHV-1 virion DNA. Although it is speculative, one explanation could be that the cellular sequences are gained by the integration of viral genomes into the host chromosome, followed by an imprecise excision. As about 10% of virion molecules have an extension of cellular DNA, if our explanation is correct, recombination of BHV-1 DNA with cellular DNA during lytic infection must be frequent. Considering that the virion DNA analysed was amplified by two rounds after plaque purification, the observed heterogeneity of the tails supports this idea. On
the other hand, an effective mechanism to eliminate those tail-like sequences, or the whole viral genome carrying such a tail, is required to limit the tails to only 10% of the packaged DNA molecules. One possible mechanism would be that packaged virion DNA molecules with tails cannot be circularized after a cell is infected because the typical structure at the right genomic terminus (Hammerschmidt et al., 1988), necessary for fusion of both termini, is missing. In this case, genomes with tail sequences would not be able to enter a replicative cycle since circularization is a prerequisite for herpesviral DNA replication via the rolling circle model. Therefore, it is not clear whether a productive infection can result from the expression and replication of excised genomes that might have been formerly integrated.

If the tail sequences at the termini are reminiscent of an integration and excision event, then integration must occur at the genomic termini, since they are present in mature virions. It should be noted that when EBV has been found to be integrated into the cellular genome, it is integrated at its genomic ends (Matsuo et al., 1984). The sequences at the termini of herpesviruses are known to be involved in circularization and linearization of viral genomes. Integration and excision from the cellular genome are related recombination events with the same viral target sites and probably similar or identical activities for a different pathway. Inversion of segment $U_5$ proves that the large inverted repeats, $T_R$ and $I_R$, are target sites for recombination (Weber et al., 1988). In the case of a viral genome integrated into the host chromosome, the inverted viral segments $I_R$ and $T_R$ could serve as recognition sequences directing the excision of the left terminus. Since this excision does not simultaneously produce the right genomic terminus, as it does when replicative form genomes are the substrate, the inversion activity might be responsible for occasional and imprecise cleavage downstream in the host genome, yielding tailed right termini.

Interestingly enough, in the case of PRV, a similar finding has been reported by Ben-Porat et al. (1979). They showed with self-annealing experiments of single-stranded molecules that, in electron micrographs of PRV genomes, a tail-like structure is present at the right end of the genome. Terminal heterogeneity was also reported at the right end of varicella-zoster virus (Ecker et al., 1984). Both herpesviruses show the same genome arrangement as BHV-1.

The origin of the tail sequences has been identified in two of the three cases tested. Hybridization to uninfected bovine cellular DNA implied that two tails are part of a $HindIII$ repeat family, whereas one tail was not detectable in whole DNA preparations. This result suggests that recombination with cellular DNA might occur at random rather than specific sites.

The left terminus of BHV-1 virion DNA carries a repeat array that shows sequence homology to the IR3 region of EBV (Hammerschmidt et al., 1986). In the latter, cross-hybridizing tandem arrays of repeats in mouse and human cellular DNA have been documented at the nucleotide sequence level (Heller et al., 1982, 1985). Related repeats were detected in a variety of other organisms, including bovine DNA. Therefore, it is tempting to speculate that the 14 bp repeats at the left end of $U_5$ in the genome of BHV-1 could be similar to repeats of bovine cellular DNA. The homology of repetitive cellular DNA with BHV-1 sequences situated closest to the viral genome ends could influence the recombination of BHV-1 with cellular DNA. The finding that at least two of the three clones have picked up these tail-like sequences from repetitive DNA in the host cell is in agreement with this notion, although the small number of clones examined cannot demonstrate it. Sequence comparison of the tails with a published bovine repeat family (Richardson et al., 1986) did not reveal homology and indicates that a bovine DNA different repeat set was detected in this study.

There is an apparent contradiction between the proposed model of herpesvirus DNA maturation (rolling circle DNA replication of a circularized virion DNA molecule, followed by cleavage of a concatemeric molecule into virion DNA units) and our findings. Data presented here may indicate that single virion DNA molecules recombine with cell DNA during a lytic cycle of BHV-1 infection. If this model can be confirmed, it will be of particular interest to know whether BHV-1 recombination or integration takes place shortly after cell infection, as an initial event, or after the onset of virus DNA synthesis. Both aspects are reminiscent of the DNA replication of the Escherichia coli phage Mu, where the Mu genome integrates with the $E. coli$ chromosome for both its lytic cycle and lysogenic state. It is apparent that Mu virion DNA replicates and is unidirectionally packaged from Mu copies randomly integrated in the host chromosome, terminating when the phage head is full and thus encompassing $E. coli$ DNA of variable length (Toussaint & Resibois, 1983). In contrast to Mu, our observations might indicate that only a certain proportion (approximately 10%) of BHV-1 DNA is associated with cell DNA during the lytic cycle, whereas the majority of BHV-1 DNA replicates freely. It remains to be determined whether recombination reflects integration of BHV-1 into the infected cell's DNA and whether this recombination event is crucial for our understanding of BHV-1 latency.

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