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Latent Herpes Simplex Virus Type 1 DNA Contains Two Copies of the Virion DNA Joint Region

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Southern blot analysis of latent herpes simplex virus DNA detected in mouse brain and digested with a restriction enzyme revealed two copies of the virion DNA joint fragment. Thus, the absence of free ends noted previously in latent herpes simplex virus type 1 DNA is due to joining of the termini.

Herpes simplex virus (HSV) is known for its ability to form latent infections (16, 18). The genome of HSV type 1 (HSV-1) is well characterized as a linear double-stranded DNA molecule of ca. 150 kilobases in size (15). Previously, we showed that the HSV-1 genome residing in the latent state in mouse brain and trigeminal ganglia lacks free ends (14), a characteristic that we proposed might result from (i) deletion of the virion terminal sequences, (ii) integration of the virion DNA via its ends, or (iii) joining of these ends to form long linear concatemers or circles which may or may not then integrate. Because of the structure of the HSV-1 genome, concatemerization or circularization produces two joint fragments—the normal virion joint (IR_L-IR_S) and a second joint from the joining of the termini (TR_L-TR_S) (see Fig. 1). We report here the detection of two joint fragments in latent HSV-1 DNA by Southern blot hybridization and conclude that joining accounts for the lack of free ends in the majority of latent HSV-1 DNA.

Because of the inherent difficulty of studying HSV-1 latency in humans, model systems must be used (16, 18). Mice infected after corneal scarification have been used by us and others to study the latent state and are particularly useful because spontaneous reactivation of virus does not occur at any measurable frequency in these animals. Virus can be reactivated from the ganglia of latently infected animals but not (or with very low efficiency) from the central nervous system by explant cocultivation (3). This difference in reactivation may reflect differences in either latently infected cells or in the latent virus (or both). Because there is a comparatively large amount of brain tissue compared with ganglion tissue and because we have not detected any difference in viral DNA from brain or ganglia during the latent state, we used latently infected brain DNA for this study.

BALB/c mice were infected with ca. 10^6 PFU of HSV-1 strain F per eye after corneal scarification, which induces acute infection resulting in 30 to 50% mortality at 8 to 10 days postinfection. Survivors are considered to be latently infected with HSV-1. During the course of the infection, the virus travels from the eye along the ophthalmic branch of the trigeminal nerve to the trigeminal ganglia and then along the trigeminal root to the brain stem. Viral DNA can be demonstrated in both the ganglia (peripheral nervous system) and brain stem (central nervous system) of these latently infected animals, usually at 2 months postinfection, when no infectious virus can be detected (14).

To determine the number of copies of the junction or joint region of the genome relative to the number of unique regions of the genome, Southern blots of *Bam*HI-digested mouse brain DNA were hybridized with a ^{32}P-labeled probe consisting of *Bam*HI PS and B fragments of the HSV-1 strain F genome (Fig. 1). The PS fragment hybridizes to the joint

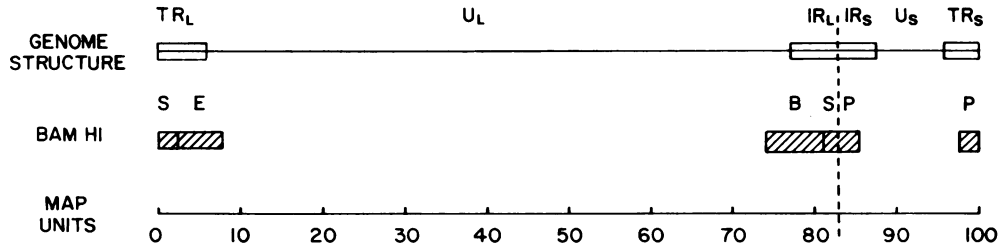

FIG. 1. Map of HSV-1 genome. The genome of HSV-1 is a linear double-stranded DNA molecule consisting of two unique sequence regions bounded by inverted terminal repeat sequences. This structure is shown in the upper line of the figure. The position of the *Bam*HI fragments of HSV-1 strain F used in this study are shown under the genome structure. These clones were obtained from B. Roizman (12). Radioactive probes prepared with the PS fragment hybridize to the joint region and also to the termini. Probes prepared with the B fragment also hybridize to the E fragment because they both contain repeated sequences. In the linear conformation, there is one of each of the fragments. However, on circularization or concatemerization, two copies of PS are produced from the fusion of P and S. Consequently, P and S disappear.

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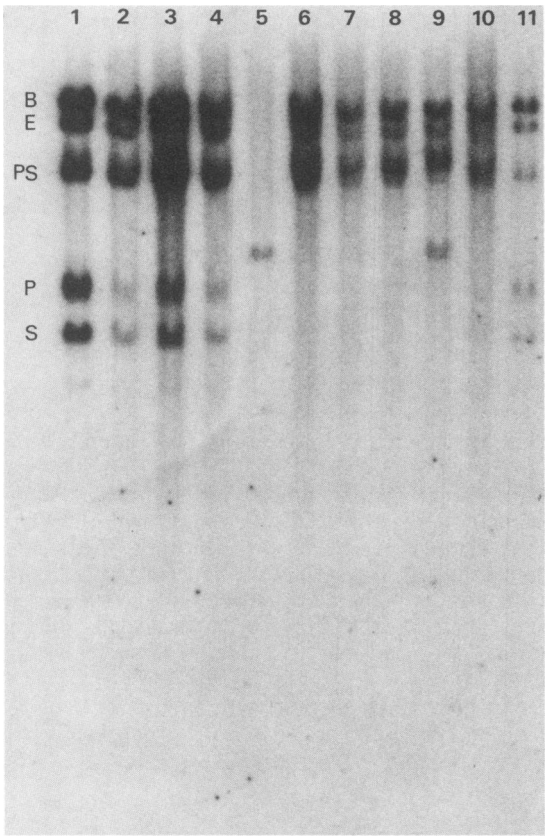

FIG. 2. Blot hybridization of nick-translated ^{32}P-labeled *Bam*HI PS and B fragment DNA to *Bam*HI digests of brain and ganglion DNA taken from acutely and latently infected mice. Mice were killed and tissue was excised within 2 min. Lanes 1 and 11, 10 and 1.0 ng of HSV-1 virion DNA, respectively, plus 10 μg of uninfected mouse brain DNA. Lanes 2 to 4, 20 μg of acutely infected mouse brain DNA. Lane 5, 20 μg of uninfected mouse brain DNA. Lanes 6 to 9, 20 μg of latently infected mouse brain DNA. Lane 10, 20 μg of latently infected mouse trigeminal ganglion DNA.

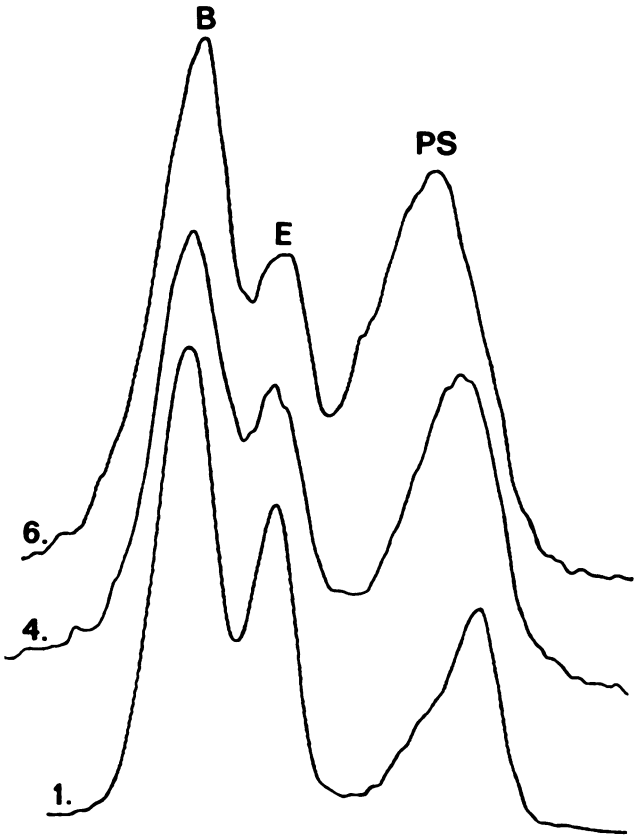

FIG. 3. Densitometer scans of autoradiographs. Autoradiographs such as those seen in Fig. 2 were scanned on a Corning model 750 scanning densitometer. Lanes 1, 4, and 6 from Fig. 2 are shown as examples of virion, acute, and latent DNA, respectively. The peaks from left to right correspond to fragments B, E, and PS (P and S are not shown).

region, and the B fragment hybridizes to two of the unimolar fragments of the genome. The *Bam*HI-B probe hybridizes to the *Bam*HI E fragment in addition to the B fragment, because it contains part of the repeat region of the genome. Figure 2 shows an autoradiogram of a blot hybridized in this way. The intensity of the bands corresponding to the P- and S-terminal fragments from acutely infected mice (lanes 2 to 4) is decreased relative to the other fragments when compared with the virion samples (lanes 1 and 11). No P or S fragment can be detected in latently infected mouse brain DNA (lanes 6 to 9). No difference between the latently infected ganglia and brains can be detected (cf. lane 10 with lanes 6 to 9). The band of cross-hybridization in uninfected (lane 5) and latently infected (lane 9) mouse brain DNA might represent a bacterial DNA contaminant in some of the mouse samples which hybridizes with residual pBR plasmid or *Escherichia coli* sequences in the probe.

Densitometer scanning was done to verify the change in the molar ratio of the joint (PS) fragment relative to the unimolar (B or E) fragments suggested by visual inspection of Fig. 2. Figure 3 shows the traces of lanes 1, 4, and 6, representing virion, acute, and latent DNA, respectively. Peak areas were then cut out and weighed to quantitate the fragments (Table 1). The B and E fragments should be present at 1 M concentrations, regardless of the conformation (linear, circular, or integrated) of the genome. Furthermore, the physical form of the virion DNA is known to be linear and unit length; thus, B, E, and PS in virion DNA are each present at 1 M. By measuring the amount of the PS fragment relative to the B and E fragments for the virion DNA and knowing that one copy of each exists, one can

TABLE 1. Quantitation of DNA fragments

Lane no.	Viral state	Relative amt of:		Ratio (PS/BE)	Molar ratio (PS/B or E)[b]
		PS	BE[a]		
1	Virion	0.084	0.213	0.394	1.07
2	Acute	0.096	0.190	0.505	1.36
3	Acute	0.130	0.187	0.695	1.88
4	Acute	0.117	0.198	0.590	1.59
6	Latent	0.055	0.067	0.821	2.23
7	Latent	0.121	0.201	0.602	1.63
8	Latent	0.153	0.207	0.739	2.00
9	Latent	0.162	0.194	0.835	2.26
10	Latent	0.194	0.254	0.764	2.07
11	Virion	0.041	0.119	0.344	0.93

[a] Fragments B and E were cut from the densitometer trace and weighed as one peak.

[b] The molar ratios are normalized to a value of 1 for the average of the two virion state DNAs (lanes 1 and 11).

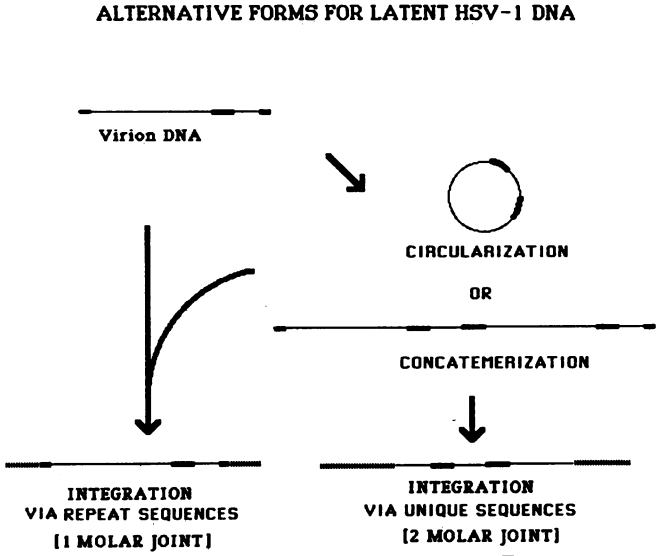

FIG. 4. Alternate forms for latent HSV-1 DNA. Integration via the repeat regions of the genome from linear, circular, or concatemeric DNA generates 1 copy of the joint region per genome. Integration via the unique regions of the genome of circular or concatemeric DNA gives rise to 2 copies of the joint region, as does the presence of nonintegrated circular or concatemeric HSV-1 DNA.

correct for differences in the specific activity of the probe. Fragment size corrections are not required, as only ratios of molarities are being measured. Thus, assuming a molar ratio of 1:1 for joint to unique fragments (PS to B or E) in virion DNA, average ratios (PS to B or E) for DNA from acute and latently infected brain are 1.6 ± 0.26:1 and 2.0 ± 0.32:1 (mean ± standard deviation), respectively.

Both retrovirus proviral integration and bacteriophage lysogeny share some features with HSV latency (for a review, see references 6 and 7), although neither mimics it exactly. The fact that some phages and proviruses can also exist in a nonintegrated form, however, makes it unreasonable to assume a strict requirement for integration in viral latency. If HSV DNA does in fact integrate, then it seems likely that a specific site on the HSV genome is involved, based on the site specificity of bacteriophage and proviral integration, and that this site is the HSV terminal repeat, the counterpart of the retrovirus proviral long terminal repeat sequences.

If HSV integration occurs at the terminal repeats, then there should be a reduction in the terminal fragments of restriction enzyme digests of DNA from latent HSV-1 genomes. Previously, we showed that there are no virion terminal fragments as such in latent HSV-1 DNA and suggested that they may be linked together to form a second copy of the joint fragment (14). Like λ phage, HSV-1 exists in a linear form when packaged but circularizes within the cell to undergo DNA replication by a rolling circle mechanism (8, 9, 15). Thus, because of the structure of the HSV-1 genome, two copies of the *Bam*HI joint fragment (PS) are generated from each viral genome during replication. Assuming that a copy of the viral genome circularizes within the cell during the establishment of latency and then integrates with the joint regions to form junctions with the cell DNA, the number of copies of the joint would be expected to decrease from 2 to 1, relative to any unique fragment of the viral genome (Fig. 4). The data presented in Table 1 argue against this simple model, as the ratio of joint to unimolar fragments is close to 2. However, this does not rule out the possibility that many HSV-1 genomes linked in tandem are integrated at a single site or random sites within the infected cell genome. In this case, the ratio of cell-virus (integrated) joints to virus-virus (nonintegrated) joints is sufficient to imply a ratio of nonintegrated to integrated joints that approaches 2 (Fig. 4). Although less than 0.1 copy of HSV-1 DNA exists, on average, within latently infected ganglia or brain stem cells (3, 14), the question of how many copies of latent DNA reside within a single cell remains unanswered. Furthermore, it is possible that (as in the case of Epstein-Barr virus) only a small number of viral genomes are integrated and a large number of copies reside in an episomal state (2).

The Epstein-Barr virus genome has been shown by Southern blotting of restriction endonuclease-digested DNA to lack terminal fragments in nonproducer cell lines (5). It is known that most of the Epstein-Barr virus DNA resides intracellularly in an episomal form (1, 2, 4, 5, 10, 11). In the case of HSV-1, we have previously noted the reduction of terminal fragments in acutely infected mice and the lack of terminal fragments in latently infected mice (14). On the other hand, Puga et al. (13), by RPC5 chromatography before analysis of HSV DNA in latently infected mice, demonstrated heterogeneity in the joint fragment, and Wigdahl et al. (17), using an in vitro system, observed normal virion molarities of end fragments in their latency systems. It is possible that the in vitro latency system which utilizes drugs, interferon, and elevated temperatures does not mimic the in vivo state. The data presented in this report indicate that latent HSV-1 DNA exists in infected cells in a form different from that in virions (linear form) and show that the lack of ends in latent viral DNA is due to the joining of ends to form a fragment equivalent to the virion DNA joint fragment. Thus, the loss of the end fragments is not due to their deletion or to integration via these fragments into the host cell genome. Although integration via the joint region occurring at any measurable level is ruled out, the possibility remains of integration at a position in the unique sequences of the circularized genome, or alternatively, integration of a large concatemerized form of the viral genome.

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LITERATURE CITED

1. **Adams, A., and T. Lindahl.** 1975. Epstein-Barr virus genomes with properties of circular DNA molecules in carrier cells. Proc. Natl. Acad. Sci. U.S.A. **72**:1477–1481.
2. **Anvret, M., A. Karlsson, and G. Bjursell.** 1984. Evidence for integrated EBV genomes in Raji cellular DNA. Nucleic Acids Res. **12**:1149–1161.
3. **Cabrera, C. V., C. Wohlenberg, H. Openshaw, M. Rey-Mendez, A. Puga, and A. L. Notkins.** 1980. Herpes simplex virus DNA sequences in the CNS of latently infected mice. Nature (London) **288**:288–290.
4. **Dambaugh, T., F. Nkrumah, R. J. Bigger, and E. Kieff.** 1979. Epstein-Barr virus DNA in Burkitt tumor tissue. Cell **16**: 313–322.

5. **Heller, M., T. Dambaugh, and E. Kieff.** 1981. Epstein-Barr virus DNA. IX. Variation among viral DNAs from producer and nonproducer infected cells. J. Virol. **38**:632–648.
6. **Herskowitz, I., and D. Hagen.** 1980. The lysis-lysogeny decision of phage λ: explicit programming and responsiveness. Annu. Rev. Genet. **14**:399–445.
7. **Hughs, S. H.** 1983. Synthesis, integration and transcription of the retroviral provirus. Curr. Top. Microbiol. Immunol. **103**: 23–51.
8. **Jacob, R. J., L. S. Morse, and B. Roizman.** 1979. Anatomy of herpes simplex virus DNA. XII. Accumulation of head to tail concatemers in nuclei of infected cells and their role in the generation of the four isomeric arrangements of viral DNA. Virology **29**:448–459.
9. **Jongeneel, C. V., and S. L. Bachenheimer.** 1981. Structure of replicating herpes simplex virus DNA. J. Virol. **39**:656–660.
10. **Kief, E., D. Given, A. L. T. Powell, W. King, T. Dambaugh, and N. Raab-Traub.** 1979. Epstein-Barr virus: structure of the viral DNA and analysis of viral RNA in infected cells. Biochim. Biophys. Acta **560**:355–373.
11. **Lindahl, T., A. Adams, G. Bjursell, G. W. Bornkamm, C. Kaschka-Dierich, and U. Jehn.** 1976. Covalently closed circular duplex DNA of Epstein-Barr virus in a human lymphoid cell line. J. Mol. Biol. **102**:511–530.
12. **Post, L. E., A. J. Conley, E. S. Mokarski, and B. Roizman.** 1980. Cloning of reiterated and non-reiterated herpes simplex virus 1 sequences as Bam H1 fragments. Proc. Natl. Acad. Sci. U.S.A. **77**:4201–4205.
13. **Puga, A., E. M. Cantin, C. Wohlenberg, H. Openshaw, and A. L. Notkins.** 1984. Different sizes of restriction endonuclease fragments from the terminal repetitions of the herpes simplex virus type 1 genome latent in trigeminal ganglia of mice. J. Gen. Virol. **65**:436–444.
14. **Rock, D. L., and N. W. Fraser.** 1983. Detection of HSV-1 genome in central nervous system of latently infected mice. Nature (London) **302**:523–525.
15. **Roizman, B.** 1979. The structure and isomerization of herpes simplex virus genomes. Cell **16**:481–494.
16. **Stevens, J. G.** 1975. Latent herpes simplex virus and the nervous system. Curr. Top. Microbiol. Immunol. **70**:31–50.
17. **Wigdahl, B. L., A. C. Scheck, E. de Clercy, and F. Rapp.** 1982. High efficiency latency and reactivation of HSV in human cells. Science **214**:1145–1146.
18. **Wildy, P., H. J. Field, and A. A. Nash.** 1982. Classical herpes latency revisited, p. 133–167. *In* B. W. J. Mahy, A. C. Minson, and G. K. Darby (ed.), Virus Persistence. Thirty-third Symposium of the Society of General Microbiology. Cambridge University Press, New York.