Latent Herpes Simplex Virus Type 1 DNA Is Not Extensively Methylated in vivo

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SUMMARY

The methylation pattern of herpes simplex virus type 1 (HSV-1) DNA, present in the central nervous system of latently infected mice, was examined by digestion of the DNA with methylation-sensitive restriction endonucleases and Southern blot hybridization. Using the enzymes $\text{SmaI}$, $\text{XmaI}$, $\text{SalI}$ and $\text{SacII}$, the data indicate no extensive methylation of latent HSV-1 DNA in vivo. Thus, extensive methylation of the viral genome is not a necessary condition for, or a consequence of maintaining, the latent state in vivo.

Herpes simplex virus type 1 (HSV-1) can establish latent infections in peripheral nerve ganglia and the central nervous system (CNS) of man and experimental animals (Stevens & Cook, 1971; Baringer & Swoveland, 1973; Cabrera et al., 1980; Fraser et al., 1981; Rock & Fraser, 1983). Studies on latently infected ganglia and CNS tissue indicate that: (i) the HSV genome is primarily reactivated from neurons, although it is not known whether other cell types also harbour the viral DNA (McLennan & Darby, 1980); (ii) the physical structure of latent HSV-1 DNA is in a form other than linear unit length molecules (Rock & Fraser, 1983, 1985); and (iii) limited viral transcription occurs during latency, with some evidence of protein synthesis (Green et al., 1981; Tenser et al., 1981; Galloway et al., 1982; Stroop et al., 1984). Little is known about the virus–host interactions during the establishment and maintenance of latency, although they undoubtedly involve mechanisms regulating viral gene expression.

DNA methylation at the dinucleotide 5'-CG-3' has been correlated with transcriptional inactivity and appears to be a factor regulating eukaryotic gene expression (for review, see Ehrlich & Yang, 1981; Yisraeli & Szyf, 1984). In general, hypomethylation is a necessary, but not sufficient, precondition for gene transcription. In several in vitro viral systems, including adenoviruses, herpesviruses and retroviruses, transcriptionally active or replicating viral genomes are consistently hypomethylated, whereas inactive/latent viral genomes, or specific inactive regions of viral genomes are relatively heavily methylated (Doerfler, 1981; Youssoufian et al., 1982; Szyf et al., 1985).

In order to determine whether extensive DNA methylation is correlated with the maintenance and selective repression of the latent HSV-1 genome in vivo, we examined HSV-1 DNA from the CNS of latently infected mice for the presence of methylated sequences.

Six- to ten-week-old BALB/c mice were infected with $10^6$ p.f.u. of HSV-1 strain F following corneal scarification. The resulting infection was as described previously (Rock & Fraser, 1983); infectious virus was present in all regions of the CNS (pons–medulla, cerebrum and cerebellum) at 6 days post-infection (p.i.), but could not be detected in cell-free homogenates from the CNS tissue of latently infected animals at 2 months p.i. DNA was extracted from the brains of acutely infected animals at 6 days p.i., sonicated and digested with methylation-sensitive restriction endonucleases. The digested DNA was fractionated on an agarose gel and transferred to nitrocellulose, where it was hybridized with a probe coding for the early or late HSV-1 gene. The data demonstrated no extensive methylation of latent HSV-1 DNA in vivo. Thus, extensive methylation of the viral genome is not a necessary condition for, or a consequence of maintaining, the latent state in vivo.
Fig. 1. Blot hybridization of $^{32}$P-labelled HSV-1 virion DNA to SmaI (S) or XmaI (X) digests of brain DNA taken from acutely and latently infected mice (6 and 60 days p.i., respectively). Lane 1, 100 pg HSV-1 DNA in 20 μg BHK cell DNA; lane 2, 20 μg DNA from the brains of mice acutely infected with HSV-1; lanes 3 to 6, 20 μg DNA from the brains of latently infected mice; lanes 7 and 8, 20 μg DNA from the brains of uninfected mice.

and latently infected mice, digested with restriction endonucleases, electrophoresed on 0.6% or 1.2% agarose gels, transferred to nitrocellulose and hybridized with $^{32}$P-labelled nick-translated HSV-1 virion DNA as described previously (Fraser et al., 1981; Rock & Fraser, 1983).

To assess the degree of methylation of the latent HSV-1 genome, we initially used the isoschizomer pair of enzymes, SmaI/XmaI. The restriction endonuclease SmaI will not cleave the recognition sequence 5'−CCCGGG−3' if the internal cytosine is methylated, whereas XmaI will cleave both methylated and unmethylated forms of this sequence (McClelland, 1981). Fig. 1 shows results obtained following XmaI and SmaI digestion of virion DNA and DNA taken from the brains of mice that were acutely or latently infected with HSV-1 or were uninfected. Within the limits of detection, there are no apparent differences in HSV-1-specific restriction enzyme patterns between SmaI- and XmaI-digested DNA from the brains of latently infected mice (lanes 3 to 6). Furthermore, similar HSV-1 restriction patterns for both SmaI and XmaI are seen with DNA from the brains of acutely infected mice (lane 2) and purified virion DNA (lane 1). Similar results were obtained when SmaI/XmaI-digested virion DNA, DNA from the brains of acutely infected mice and DNA from the brains of latently infected mice were hybridized with cloned DNA probes representing BamHI restriction fragments Q, PS and Y of HSV-1 strain F.
Fig. 2. Blot hybridization of $^{32}$P-labelled HSV-1 virion DNA to SalI (a) and SacII (b) digests of brain and ganglion DNA taken from acutely and latently infected mice (6 and 60 days p.i., respectively). (a) Lane 1, 100 pg HSV-1 DNA in 20 µg BHK cell DNA; lanes 2 and 3, 20 µg DNA from the brains of acutely infected mice; lanes 4 to 9, 20 µg DNA from the brains of latently infected mice; lane 10, 20 µg pooled DNA from latently infected trigeminal ganglia. (b) Lane 1, 100 pg HSV-1 DNA in 20 µg BHK cell DNA; lane 2 and 3, 20 µg DNA from the brains of acutely infected mice; lanes 4 to 8, 20 µg DNA from the brains of latently infected mice.

(data not shown). The high mol. wt. DNA species seen in lanes 2 to 8 are most likely due to cross-hybridization of HSV-1 to homologous sequences present in mouse DNA, which were evident in uninfected mouse DNA (lanes 7 and 8) and reported previously (Puga et al., 1982). Thus, the data indicate no extensive methylation of HSV-1 DNA in the recognition sequence 5'-CCCGGG-3' during latency.

In order to examine CG dinucleotides in other sequence contexts and to improve the resolution of the viral restriction patterns, two additional methylation-sensitive enzymes were used. The enzyme SacII will not cleave the recognition sequence 5'-CCGCGG-3' if either of the CG dinucleotides are methylated. Similarly, SalI is sensitive to methylation in the recognition sequence 5'-GTCGAC-3' (McClelland et al., 1981). Fig. 2(a and b) show HSV-1-specific hybridization to DNAs digested with SalI and SacII respectively. The restriction patterns obtained with SalI and SacII are indistinguishable between virion DNA (lanes 1), DNA from the brains of acutely infected mice (lanes 2 and 3), DNA from the brains of latently infected mice (Fig. 2a, lanes 4 to 9 and Fig. 2b, lanes 4 to 8) and pooled DNA from latently infected trigeminal ganglia (Fig. 2a, lane 10). Minor differences in the molarity of restriction fragments are observed for some of the samples from latently infected mice. In Fig. 2(a) lanes 7 and 8, a band of approximately 8 kb is over-represented. Similarly, the same DNA samples digested with
SacII (Fig. 2b, lanes 6, 7 and 8) show a band of approximately 500 bp over-represented. No new HSV-1-specific bands unique to DNA from the brains of latently infected mice are seen upon comparison with virion DNA and DNA from the brains of acutely infected mice. The observed over-representation of a single band in some DNAs from the brains of latently infected mice could possibly represent a site-specific methylation event; however, if this were the case, it is unlikely to be of significance in viral latency since it is only observed in a fraction of the DNA samples. Again, there is no evidence of extensive methylation of HSV-1 DNA during latency in the recognition sequences for SalI and SacII.

With the use of methylation-sensitive restriction endonucleases, we have demonstrated that latent HSV-1 DNA is not extensively methylated in the CNS. The enzymes used here, SmaI, XmaI and SacII, are likely to cleave the 67% GC-containing HSV-1 genome (Roizman, 1979) more than 200 times each, generating a molecule of average size 700 bp, and so allowing approximately 3% of all potential CG dinucleotides to be examined. That these restriction enzymes sites are potentially favourable methylation sites is suggested by the following: (i) the enzymes used are known to cut in the 5’ regulatory regions of some HSV genes (for review, see Wagner, 1985), sites that by analogy with other viral systems would be likely to be preferentially methylated as a result of, or a controlling mechanism for, transcriptional inactivity (Doerfler et al., 1984); (ii) the SmaI/XmaI recognition sequence 5’-CCCGGG-Y has been shown to be a favoured methylation site for HSV-1 DNA in a tissue culture system for latency in vitro (Youssoufian et al., 1982) and (iii) methylation in vitro with HeLa cell DNA methylase has shown that CG dinucleotides are preferentially methylated in the context of other CG dinucleotides and that AT-rich flanking sequences inhibit methylation (Bolden et al., 1985).

Although there are no apparent differences in methylation patterns of HSV-1 DNA in digested mouse brain DNAs, we can not totally exclude the possibility that some viral sequences are methylated. Specifically, our method of analysis, which examined only a subset of all viral CG dinucleotides, together with the limited resolution of viral restriction patterns obtained [due to the low copy number of HSV-1 DNA in latently infected tissue (Rock & Fraser, 1983)] would allow subtle specific changes in DNA methylation, low levels of random methylation or methylation at unexamined sites to go undetected.

It is interesting to note that our HSV-1 methylation results in vivo are in contrast to those obtained by Youssoufian et al. (1982) using an HSV-1 latency model system in vitro. Their results, using persistently infected lymphoblastoid T cell lines, indicated near 100% viral DNA methylation in non-producing cell lines and little, if any, methylation in cell lines reactivated to produce virus.

In conclusion, these data demonstrate that extensive methylation of the latent HSV-1 genome is not a necessary condition for, or a consequence of maintaining, the latent state in vivo.

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REFERENCES


Short communication


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