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During Latency, Herpes Simplex Virus Type 1 DNA Is Associated with Nucleosomes in a Chromatin Structure

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Latent herpes simplex virus type 1 DNA has a nucleosomal structure similar to that of cellular chromatin, as determined by micrococcal nuclease digestion. All of the major regions, including the transcriptionally active region of the genome, were found to be associated with nucleosomes. Such a chromatin structure is likely to be an important element in the control of herpes simplex virus type 1 gene expression during latency.

After primary infection, herpes simplex virus type 1 (HSV-1) undergoes latency in ganglionic neurons, in which it persists throughout life (11, 12, 14, 41). Although most of the HSV-1 virion DNA is in linear form (21, 30, 31), HSV-1 DNA during latency exists either in circular form or as a large, unintegrated concatemer (8, 22, 28, 29) which is not extensively methylated (7). Furthermore, during acute infection most of the genome is actively transcribed (15, 31, 42), whereas during latency transcription of the latent HSV-1 genome is limited to a small region of the genome (4, 6, 27, 38, 39, 40). It is not clear which factors or events are responsible for such stringent transcriptional control of HSV-1 DNA during latency.

Proteins associated with DNA in the chromatin complex are thought to play an important role in regulation of the pattern of gene activity (1, 44, 46). In an effort to understand the limited transcription pattern of latent HSV-1 DNA, we explored the possible association of latent HSV-1 DNA with nucleosomes. One way to demonstrate nucleosomal structure is to digest chromatin with micrococcal nuclease, which attacks the linker region between nucleosomal units (17). The histone core provides a degree of protection against this nuclease, and after partial digestion a series of fragments, each an integral multiple of the nucleosomal repeat unit, is produced. These fragments can be separated by agarose gel electrophoresis. With the results presented here, we show that during latency HSV-1 DNA is associated with nucleosomes in a type of chromatin structure similar to that of cellular chromatin.

Female BALB/c mice (cBYJ; Jackson Laboratory) were infected by corneal scarification as described previously (28). All of the mice tested were latently infected as determined by explant reactivation assay (3). Nuclei from brain stems were prepared as described by Weintraub and Groudine (45). The nuclei were digested with micrococcal nuclease as described earlier (24), and DNA was extracted by a standard protocol (20). A dot hybridization procedure described by Kafatos et al. (16) was used; 5 μg of DNA was spot blotted onto nitrocellulose filters with a manifold apparatus (Schleicher & Schuell, Inc.) as previously described (22). In most of the brain stems, the concentration of latent HSV-1 DNA was ≤1 pg/μg of cellular DNA (data not shown). Only samples containing >3 pg of latent HSV-1 DNA per μg of micrococcal nuclease-digested cellular DNA were further analyzed by gel electrophoresis. It is assumed that samples with lower concentrations of HSV-1 DNA would give similar results if our method had the sensitivity to detect them. Gels consisting of 2% agarose were used. The technique described by Southern (37), modified as described by Wahl et al. (43), was used to transfer DNA onto either nylon-based filters (Nitran; Schleicher & Schuell) (see Fig. 1 and 3) or nitrocellulose (see Fig. 2).

For hybridization probes, total HSV-1 virion genomic DNA and subgenomic cloned fragments were used. Among subgenomic regions, *Xba*I fragments C (33 kilobases [kb]), F (29 kb), E (27 kb), and D (28 kb) of the KOS strain of HSV-1 (as described by Challberg [2]) and *Bam*HI fragment B of the F strain of HSV-1 were used. DNA probes labeled with ^{32}P by the random-priming method as described by Feinberg and Vogelstein (10) were used. Filters were hybridized with 0.5 × 10^8 to 1 × 10^8 cpm of a radioactive probe as described earlier (22).

When nuclei were digested for 2 and 5 min with micrococcal nuclease, a ladder of DNA fragments (multiples of 164 base pairs [bp]) typical of nucleosome-protected cellular DNA was produced (Fig. 1A, lanes 1, 5, and 8 and 2, 6, and 9, respectively). However, after 30 min of digestion, most of the cellular chromatin was completely digested to a mononucleosomal form (Fig. 1A, lanes 3, 7, and 10). Hybridization of Southern blots of gels containing latently infected tissue samples with radiolabeled HSV-1 DNA resulted in detection of a ladder of latent HSV-1 DNA bands (Fig. 1B, lane 6). The average repeat size of these bands was 164 bp, similar to that of the cellular nucleosome-associated DNA seen in ethidium bromide-stained gels (Fig. 1A, lanes 2, 6, and 9). Although the smallest DNA fragment corresponding to the mononucleosomal size was not retained efficiently on the blot, there was no difficulty in detecting larger DNA fragments.

Although most of the cellular chromatin was digested to monomeric form within 30 min of digestion, as seen in ethidium bromide-stained gels (Fig. 1A, lanes 3, 7, and 10), latent HSV-1 DNA still produced an oligomeric ladder with an average repeat unit of about 150 bp (Fig. 1B, lane 7). As a result of excessive digestion of linker DNA between the nucleosomes (17, 26), the size of the monomeric nucleosome produced under these conditions was smaller (150 bp) than that calculated from the ladder produced under conditions of partial digestion (164 bp). This was also evident from the pattern of DNA in ethidium bromide-stained gels (see Fig. 2 and 3), in which Southern blots revealed more bands in the ladder than could be seen in the stained gels. The delay in digestion of latent HSV-1 DNA in comparison with cellular chromatin may imply a different conformational structure for

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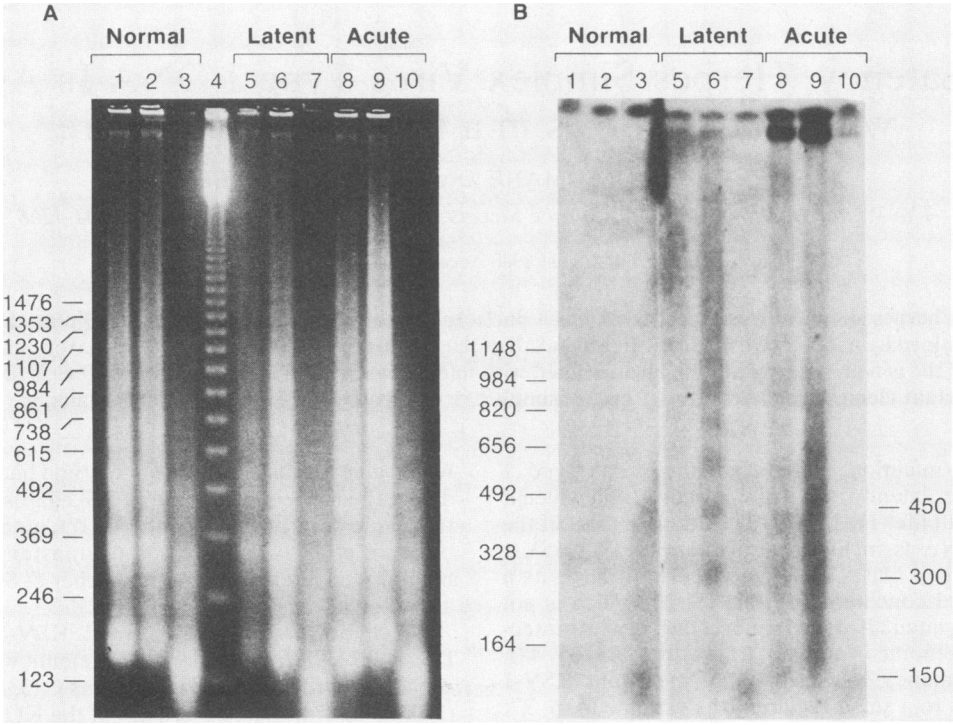

FIG. 1. Time course analysis of micrococcal nuclease digestion. Nuclei were digested for 2, 5, and 30 min. DNA samples (about 40 μg) containing about 150 to 200 pg of latent HSV-1 DNA were analyzed. The samples digested for 2 min were in lanes 1, 5, and 8, those digested for 5 min were in lanes 2, 6, and 9, and those digested for 30 min were in lanes 3, 7, and 10. (A) Ethidium bromide-stained gel; (B) Southern blot hybridized with a total HSV virion probe. A 123-bp ladder used for molecular weight markers (lane 4) was removed before autoradiography. The numbers to the sides indicate molecular sizes in base pairs.

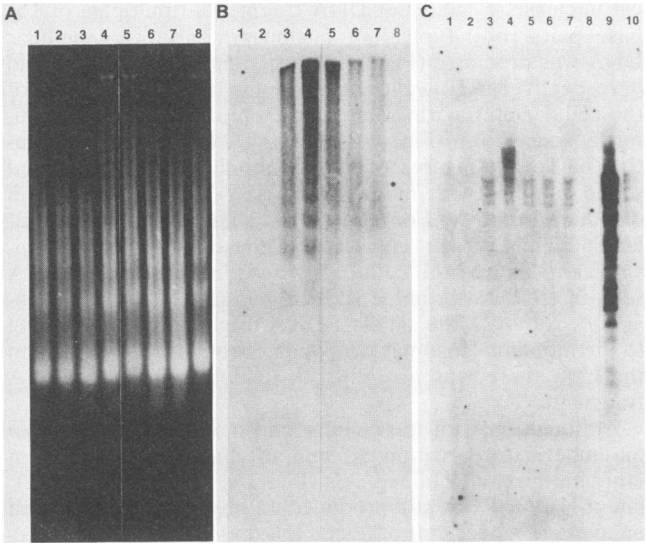

FIG. 2. Nuclei isolated from brain stems of individual uninfected and latently infected mice were divided into two portions, one of which was digested with micrococcal nuclease. DNA was extracted from both portions. The micrococcal nuclease-digested samples were electrophoresed on a 2% agarose gel. (A) Ethidium bromide-stained gel; (B) Southern blot hybridized with an HSV-1 virion DNA probe. Lanes: 1 and 2, uninfected mouse DNAs; 3 to 8, latently infected mouse DNAs. DNA was extracted from the portions of the undigested set of samples, digested with restriction enzyme *Bam*HI, and analyzed by agarose gel electrophoresis (10 μg per sample). (C) Southern blot of *Bam*HI digests hybridized with an HSV-1 total virion DNA probe. Lanes: 1 and 2, uninfected mouse DNAs; 3 to 8, latently infected mouse DNAs; 9 and 10, 10 μg of uninfected mouse brain DNA mixed with 1 and 0.1 ng, respectively, of purified HSV-1 virion DNA.

latent HSV-1 DNA or may be related to the high G+C content of HSV-1 DNA.

In contrast to latently infected brain stems, analysis of acutely infected brain stems revealed that some of the HSV-1 DNA was present in an encapsidated, nuclease-resistant form near the sample well at the top of the gel, and the remaining part appeared as a smear, indicating that it was randomly digested by nuclease and therefore nonnucleosomal (Fig. 1B, lanes 8, 9, and 10). It is possible that a small fraction of the DNA from acutely infected tissue was present in nucleosomal form. These results confirm our earlier findings (24).

Normal cellular chromatin from uninfected brain stems did not hybridize significantly with the HSV-1 DNA probe (Fig. 1B, lanes 1, 2, and 3). However, a variable level of cross-hybridization was seen with different control animals. The high level shown in Fig. 1B, lane 3, was not seen in duplicate experiments (data not shown).

To rule out the unlikely possibility that our data were due to variable levels of cross-hybridization, we compared *Bam*HI restriction enzyme digests of latent DNA samples. Latent HSV-1 DNAs were found to be associated with nucleosomes, and the restriction enzyme digestion patterns of these DNAs were comparable with that of HSV-1 DNA (Fig. 2). Moreover, the relative intensity of the hybridization

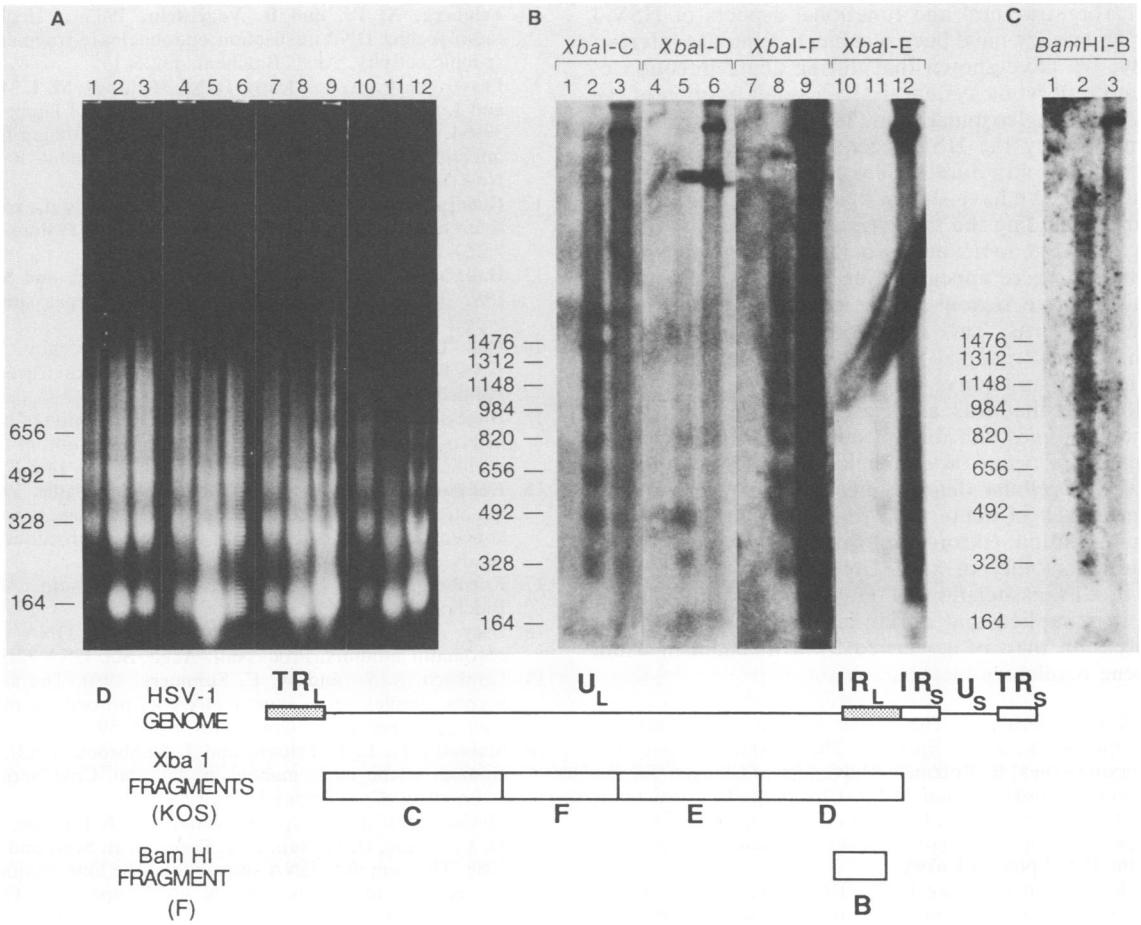

FIG. 3. Hybridization with subgenomic cloned fragments of HSV-1. Lanes: 1, 4, 7, and 10, uninfected mouse DNAs; 2, 5, 8, and 11, latently infected mouse DNAs; 3, 6, 9, and 12, acutely infected mouse DNAs. (A) Ethidium bromide-stained gel. (The striations in lane 6 are from a ruler often used for calibration.) (B) Southern blot hybridized with radiolabeled *Xba*I fragments C, D, E, and F of HSV-1. (C) Southern blot hybridized with radiolabeled *Bam*HI fragment B. The numbers to the sides indicate molecular sizes in base pairs. (D) Locations of *Xba*I and *Bam*HI fragments used as probes on the HSV-1 genomic map. The HSV-1 genome has a molecular weight of about 10^8 (size, about 150 kb). The genome is divided into long unique (U_L) and short unique (U_S) regions, both bracketed by inverted and terminal repeats (IR_L and IR_S and TR_L and TR_S). *Xba*I fragments C (33 kb), F (23 kb), E (27 kb), and D (28 kb) and *Bam*HI fragment B (10.1 kb) were used as radiolabeled probes.

signal between the restriction enzyme-digested samples corresponded to that seen between the micrococcal nuclease-digested samples. These results strongly suggest that the nucleosomal pattern obtained with latently infected brain stems is due to latent HSV-1 DNA.

To determine whether all or only a specific region of the latent viral genome was associated with nucleosomes, various *Xba*I cloned fragments of HSV-1, such as C, D, E, and F covering about 80% of the HSV-1 genome (Fig. 3D), were used as probes. All of these regions were found to be associated with nucleosomes (Fig. 3B, lanes 2, 5, 8, and 11). With all of the probes, the HSV-1 DNA from acutely infected tissue was predominantly nonnucleosomal, present as both a nuclease-resistant, encapsidated form and a nuclease-sensitive, naked form; the latter appeared as a smear along the length of the lane (Fig. 3B, lanes 3, 6, 9, and 12). Shorter exposures of lanes 9 and 12 still appeared as a smear (data not shown). To determine whether the transcriptionally active region of latent HSV-1 DNA is associated with nucleosomes, blots were hybridized with *Bam*HI fragment B of HSV-1 strain F. Most of this fragment has previously been shown to be transcriptionally active during latency by in situ hybridization (6). This region was also found to be associated with nucleosomes (Fig. 3C, lane 2). It should be noted that the technique used can yield limited information about the overall nucleosomal status of a particular gene. Small stretches of DNA not associated with nucleosomes, if present, might not have been detected by the technique used. Such stretches of DNA, having increased sensitivity to DNase 1, are known to be present in the promoter regulatory regions of cellular genes (9). Analysis of DNase-hypersensitive sites within HSV-1 latent DNA is currently under way.

During lytic infection of cultured cells (19, 23, 25, 32, 35) and during acute infection of mice (24), a very small fraction (10 to 15%) of viral DNA is associated with nucleosomes (19, 32), whereas 37% of replicating HSV-2 DNA has been estimated as being associated with nucleosomes in productively infected cells (13). Furthermore, the structure of Epstein-Barr virus DNA in a producer cell line, P3 HR-1, is predominantly nonnucleosomal (34). This percentage dramatically changes during latent infection. With Epstein-Barr virus, 80% of the DNA has been shown to become nucleosome associated (5, 33). The data presented here show that most latent HSV-1 DNA is associated with a nucleosomelike

structure. The structural and functional aspects of HSV-1 DNA during latency have been a subject of intense interest. Previously, we have shown that during acute infection of mouse central nervous system tissue a small fraction of the viral DNA is in nucleosomal form (24). We have now shown that during latency the HSV-1 genome is associated with nucleosomes in a structure similar to that of chromatin in eucaryotic cells. We have shown that most of the regions of the genome, including the transcriptionally active region of the latent HSV-1 genome, are associated with nucleosomes. During latency, there appears to be no difference between active and inactive regions of the genome with regard to nucleosome patterns. This is in good agreement with the finding that a nucleosomal structure does not distinguish between inactive and active genes (18, 36, 44).

It is possible that this association of viral DNA with cellular nucleosomes can disrupt acute viral transcription, which appears to occur on nonnucleosomal DNA, and can be an effective cellular defense mechanism. Alternatively, disruption or lack of acute viral transcription may lead to chromatin formation. Chromatin formation may be essential for long-term stability of viral DNA in the cell nucleus. Because of this association of latent DNA with cellular histones, it is implied that during latency, control of viral gene expression may be governed by a mechanism using cellular gene regulatory factors.

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