Temporal Association of the Herpes Simplex Virus Genome with Histone Proteins during a Lytic Infection

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Previous work has determined that there are nucleosomes on the herpes simplex virus (HSV) genome during a lytic infection but that they are not arranged in an equally spaced array like in cellular DNA. Viral gene expression during lytic infection is controlled by virus-encoded and host proteins. VP16 in the virion tegument activates the immediate-early (IE) genes by association with host cell factor and Oct-1, both of which are cellular proteins. IE viral gene products are detected first during infection and are involved in activating expression of the early (E) genes. E gene-encoded proteins are involved in viral DNA replication and encode mainly structural proteins. This progression of viral gene expression and some of the mechanisms of its control have been extensively reviewed (29, 31). Complete MNase digestion experiments with infected cultured cells have shown a mononucleosome-like band, suggesting that a nucleosomal structure exists within HSV DNA during lytic infection (9). Partial MNase digestion experiments with latently infected tissue culture cells showed a smear band (not a ladder), suggesting that the majority of the HSV DNA was not in an evenly spaced nucleosomal pattern during lytic infection (3, 12, 15). ChIP experiments with mouse trigeminal ganglia showed that viral DNA association with histone H3 keeps increasing with time until establishment of latency (30 days postinfection) (30). ChIP experiments with cultured cells showed that viral DNA is associated with histone H3 or modified H3 during early infection (6, 8, 9). However, it is unclear when this association is formed, because the chromatin structure in virions is not clearly defined.

From the earliest work, encapsidated viral DNA was believed to be without histones (7, 21). However, HSV type 1 (HSV-1) DNA in infected cells is detected in association with histone protein even at 1 h postinfection (hpi) (9). Furthermore, there is little information about the association of chro-

Herpes simplex virus (HSV), a double-stranded DNA virus, infects epithelial cells and eventually establishes a latent infection in neurons. Viral gene expression during lytic infection is controlled by virus-encoded and host proteins. VP16 in the virion tegument activates the immediate-early (IE) genes by association with host cell factor and Oct-1, both of which are cellular proteins. IE viral gene products are detected first during infection and are involved in activating expression of the early (E) genes. E gene-encoded proteins are involved in viral DNA replication and encode mainly structural proteins. This progression of viral gene expression and some of the mechanisms of its control have been extensively reviewed (29, 31).

Finally, newly replicated DNA is packaged in capsids, which are released from the infected cell (4). Cell gene expression regulation is mediated through chromatin, a complex of histone protein and DNA. The exact mechanism of chromatin regulation of gene expression remains to be resolved, although recently the role of chromatin structures on cellular promoter sequences during transcription has received much attention (17). It is clear that chromatin structure controls access to DNA sequences along the genome, during transcription or replication, through posttranslational modifications such as lysine acetylation, lysine methylation, and serine/threonine phosphorylation of histone proteins.

The basic unit of chromatin is the nucleosome, which is a histone octamer composed of an H3-H4 histone protein tetramer associated with two H2A-H2B dimers, which are wrapped around 146 bp of DNA sequence (10). Two mechanisms to alter chromatin structure have been extensively reviewed (29, 31). Finally, newly replicated DNA is packaged in capsids, which are coated with tegument proteins, and a lipid envelope, when exported from the infected cell (4).

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From the earliest work, encapsidated viral DNA was believed to be without histones (7, 21). However, HSV type 1 (HSV-1) DNA in infected cells is detected in association with histone protein even at 1 h postinfection (hpi) (9). Furthermore, there is little information about the association of chro-
mation with newly replicated viral DNA during the course of the infectious cycle. Thus, we have examined the chromatin structure of HSV-1 DNA in capsids and during lytic infection.

In this study we have examined the temporal association of histone with viral DNA. We have looked at DNA in virions and during early stages of infection. The genomic location of histone during the lytic phase of infection has also been studied. Histone proteins appear to associate with viral DNA after infection, because they are not detected inside capsids. Viral DNA promoter regions of all gene classes (IE, E, and L) bind with H3 soon after entry to the nucleus. However, after replication initiation, histones appear not to associate with newly replicated viral DNA.

MATERIALS AND METHODS

Cells and viruses. Sy5y cells (human neuroblastoma cell line) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. Vero cells (African green monkey kidney cell line) were grown in Dulbecco modified Eagle medium supplemented with 5% calf serum and antibiotics. The F and KOS strains of HSV-1 were used to infect cells at a multiplicity of infection (MOI) of 5.

Capsid preparation. Five 175-cm² flasks of Vero cells infected with the KOS strain of HSV-1 at an MOI of 5 PFU per cell were processed using previously described methods (24). At 22 hpi, cells were infected by centrifugation at 1,000 × g for 10 min and washed once in Dulbecco’s phosphate-buffered saline (Invitrogen). The cell pellet was resuspended in 8 ml of lysis buffer containing 1 M NaCl, 20 mM Tris (pH 7.6), 2 mM EDTA, 2% Triton X-100, and a complete protease inhibitor cocktail tablet (Boehringer Mannheim). The cell pellet was lysed by freeze-thawing three times, and the lysate was precentrifuged by centrifugation at 8,000 rpm. Capsids were partially purified by two centrifugation steps. In the first step, capsids were centrifuged through a 35% (wt/vol) sucrose cushion prepared in TNE (500 mM NaCl, 20 mM Tris [pH 7.6], 1 mM EDTA). The pellet was then resuspended in TNE, and if needed, trypsin or Dnase I was added. The trypsin- or Dnase I-treated capsid pellet solution was incubated at 37°C for 45 min or 30 min, respectively. Capsids were then purified in a 12-ml tube of 20 to 50% (wt/vol) sucrose gradients centrifuged at 24,000 rpm in a Beckman SW41 rotor for 60 min. Capsids were visualized as light-scattering bands and collected by gradient tube side puncture and aspiration of the band into a 1-ml insulin syringe with a 28-gauge needle.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western blots. Samples were mixed with 4× NuPAGE sample buffer (Invitrogen), boiled for 5 min, and separated by electrophoresis in 4 to 12% polyacrylamide gradient gels (Invitrogen). Samples were electrophoretically transferred to polyvinylidene difluoride membranes (Amersham) and blocked for 30 min in blocking buffer (0.1% Tween 20–phosphate-buffered saline plus 3% nonfat dry milk). Primary antibodies were diluted in blocking buffer. Antibodies were added to blots overnight at the following dilutions: monoclonal antibody to VP5 (Advanced Biotechnologies Inc.) at 1:3,000, polyclonal antibody to VP16 (Sigma) at 1:1,000, polyclonal antibody to histone H3 (Abcam) at 1:2,000, monoclonal antibody to ICPS (Santa Cruz Biotech) at 1:200, and horseradish peroxidase (HRP)-conjugated sheep polyclonal antibody to bromodeoxyuridine (BrdU) (Abcam) at 1:2,000. HRP-conjugated anti-mouse or donkey anti-rabbit secondary antibodies (Amersham) were added to blots for 1 h at a 1:3,000 or 1:5,000 dilution in blocking buffer. Secondary antibodies were detected using a chemiluminescent detection kit (Amersham), and blots were exposed to a Fuji Imager as directed by the manufacturer. Protein bands were quantitated by using AlphaEaseFc software (Alpha Innotech). ChIP assays. F-strain-infected and mock-infected Sy5y cells were processed for ChIP assay by previously described methods (9). For double ChIPs, BrdU (In-vitrogen) was added at 0 hpi and left for either 3 or 6 h. In brief, one 175-cm² flask of cells was cross-linked with 1% HCHO at 15 min for room temperature. The cross-linking was stopped by the addition of 0.125 M glycine. Cross-linked cells were lysed with 5 ml IP buffer (20 mM Tris [pH 8]). 200 mM NaCl, 0.5% Triton X-100, 0.05% deoxycholic acid, 0.5% NP-40, and 1 mM phenylmethylsulfonyl fluoride) and then sonicated two times for 10 min each with a Bioruptor (Diagonade). Before IP, 1/10 of the extract was saved for use as the input. The antibodies used for IP were polyclonal anti-histone H3 (Abcam) and HRP-conjugated sheep polyclonal antibody to BrdU (Abcam). For double ChIPs, purified immunoprecipitated DNAs from the first ChIP with antibody to H3 were used for the second ChIP with antibody to BrdU. The second ChIP was performed in the same way, with the first ChIP from the preclearing step, with protein A agarose (Upstate).

Quantitative real-time PCR. Purified immunoprecipitated DNAs were amplified by previously described methods (9). In brief, standard curves were made for each of the virus-specific and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) primer sets using known dilutions of viral or cellular DNA. Primers were previously described (9). The relative PCR values for each amplified region were normalized to GAPDH. The normalized values for the immunoprecipitated samples were then compared to the normalized input values and presented as a percentage ratio of immunoprecipitated to input.

Sucrose gradient. Five 175-cm² flasks of Sy5y Cells infected with the F strain of HSV-1 at an MOI of 5 were collected at 3 hpi and 6 hpi by centrifugation at 1,000 × g for 10 min and washed once in Dulbecco’s phosphate-buffered saline (Invitrogen). BrdU (Invitrogen) was added at 1 hpi, a time at which cell DNA replication will start to be inhibited by viral infection. The cell pellet was resuspended in 1 ml of radioimmunoprecipitation assay buffer containing 150 mM NaCl, 50 mM Tris (pH 8.5), 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and a complete protease inhibitor cocktail tablet (Boehringer Mannheim). The lysate was precentrifuged by centrifugation at 12,000 rpm for 10 min. The precentrifuged lysate was centrifuged in a 12-ml tube of 5 to 50% (wt/vol) sucrose gradients at 28,000 rpm in a Beckman SW41 rotor for 2 h. Samples were fractionated from the top of the tube using a Labconco Auto Densiflow. One half of each fraction was mixed with SDS-polyacrylamide gel electrophoresis sample buffer for Western blotting, and the other was used to purify viral and cell DNA by phenol-ethanol precipitation.

RESULTS

No histone is detected inside HSV-1 capsids. The association of modified histone protein with viral DNA can be detected at 1 hpi. However, it is not clear whether this association is formed after infection or whether it is maintained from the virion. Historically, polyanines such as spermine were thought to neutralize the charge on HSV virion DNA. Although spermine sufficient to neutralize only 40% of the virion DNA was detected in the nucleocapsid, no histone was reported (5). To determine whether any histone protein is present inside HSV-1 capsids, A, B, and C capsids were purified by sucrose gradient isokinetic centrifugation and used in Western blot experiments.

There are three types of viral capsids in the nuclei of HSV-1-infected cells (22, 25) (Fig. 1A). “A” capsids lack both scaffold proteins and viral DNA. “B” capsids lack viral DNA but contain scaffolding proteins. “C” capsids contain viral DNA and can mature into infectious virions. The B form of capsid is considered a precursor to both A and C capsids (22, 25). The A capsid is considered an abortive attempt at DNA packaging. Genome-size viral DNA is packaged in preformed capsids through proteolytic processing, resulting in the release of scaffold proteins and cleavage of newly replicated concatameric viral DNA.

When capsid fractions were prepared by sucrose density gradient ultracentrifugation, there was a possibility of copurifying contaminating viral DNA and histone proteins sticking to the outside of the capsids. To remove these proteins, capsids were treated with various concentrations of trypsin prior to sedimentation through a 20 to 50% sucrose gradient as described in Materials and Methods (Fig. 1). In this manner, trypsin was used to test whether a protein (histone) is on the outside or inside of the capsid shell (18).

Histone H3 was observed in all sucrose gradient fractions of mock- and 22-hpi-infected samples, including C capsid fractions (Fig. 1C). After trypsin treatment, the light-scattering
band intensities of each capsid were not changed (by visual inspection), suggesting that each capsid structure is still intact. Nevertheless, histone H3 was significantly decreased in the C capsid fraction following treatment with 5 µg/ml trypsin and was eliminated after treatment with 10 µg/ml trypsin (Fig. 1C). VP16 (which is in the tegument surrounding the outside of the capsids) was removed from all C capsid fractions after trypsin treatment, confirming that the treatment was effective (Fig. 1C). VP5 (the major capsid protein) was maintained in all fractions following trypsin treatment, confirming the maintenance of the capsid shell (Fig. 1C).

The C capsid fraction had more viral DNA than the A, B, and 0 capsid fractions (Fig. 1B). The 0 capsid fraction was a negative control for encapsidated DNA which was collected above the A capsid band in the sucrose gradient tube (Fig. 1A). However, after subtraction of the 0 capsid fraction DNA, the A and B capsid fractions still had a significant amount of viral DNA. The fact that there should be no viral DNA in A and B capsids led us to think that the viral DNA observed in the A and B capsid fractions was due to nonspecific sticking to the outside of the capsids or was an artifact of the preparation technique. To remove DNA sticking to the outside of capsids, capsids were treated with various concentrations of DNase I before sedimentation through a 20 to 50% sucrose gradient as described in Materials and Methods (Fig. 2).

Viral DNA observed in the A and B capsid fractions was significantly reduced to the level in mock-infected fractions after treatments with both lower and higher concentrations of DNase I (Fig. 2). However, viral DNA observed in the C capsid fraction was maintained at a level higher than that in the mock-infected fraction, confirming that DNase I does not affect the viral DNA within capsids (Fig. 2A). Histone H3 was decreased but did not disappear in the C capsid fraction after DNase I treatment.

In summary, using DNase we could remove contaminating DNA from the outside of capsids and most of the histone (presumably attached to the contaminating DNA); however, some remained (Fig. 2B). However, histone protein could be completely removed by trypsin treatment (Fig. 1C), supporting the lack of histone, and hence nucleosomes, on encapsulated viral DNA.

Viral DNA promoter regions bind histone H3 at the start of the infectious cycle but do not associate during progression of the infectious cycle. Previous studies have indicated that viral DNA is associated with modified nucleosomal structures that correlated with active transcription early during infection (6,

FIG. 1. Viral DNA and histone protein in HSV capsids treated with trypsin. Capsids from KOS strain-infected Vero cells were purified by sucrose gradient centrifugation. Crude capsids were prepared from cell lysates on a 35% sucrose cushion and treated with 5 µg/ml or 10 µg/ml trypsin as described in Materials and Methods. (A) Treated capsids were then subjected to 5 to 50% sucrose gradient centrifugation and capsids visualized as light-scattering bands. (B) DNA purified from capsid bands was used for quantitative PCR, using HSV-1 TK promoter and GAPDH primers. Error bars indicate standard deviations. (C) Purified capsid fractions were used for Western blots with histone H3, late viral protein VP16, and capsid protein VP5 antibody.
In fact, viral DNA can be shown to associate with histone H3 even at 1 hpi (Fig. 3A). Because there is no histone detected inside capsids (see above), this early association of viral DNA from virions with histone must occur after viral infection.

The relative levels of viral genome copy were determined at 1, 2, 3, and 6 hpi for three genes representing the three major kinetic gene classes. HSV viral replication appeared to start between 3 and 6 hpi in our infections, because the amount of viral DNA had doubled after 3 hpi and before 6 hpi (Fig. 3B). After replication initiated, the ratio for association of histones with viral DNA decreased significantly (Fig. 3A). This may suggest that histones do not associate with newly synthesized viral DNA. Previous work by others has indicated that host chromatin is marginalized and excluded from viral replication compartments in late infection, supporting the hypothesis that histones are not available for binding to viral DNA due to exclusion (26).

To find out whether nucleosomes form on the newly synthesized viral genome, two consecutive ChIP assays were performed with anti-H3 and anti-BrdU antibodies (Fig. 3C). The first ChIP with anti-H3 antibody was followed by the second ChIP with anti-BrdU antibody. The first ChIP was performed in the same way as described for Fig. 3A, and the second ChIP was done with the product of the first ChIP. The second ChIP, with anti-BrdU antibody, showed the association of histones with newly replicated viral DNA and was compared to total viral DNA. The association of histones with newly replicated promoter regions of the ICP0, TK, and VP16 genes was not detected at 6 hpi, while a low level was detected at 3 hpi (Fig. 3C). However, the association of histones with the GAPDH cellular gene was detected and did not change between 3 and 6 hpi (Fig. 3C). To use GAPDH as a positive control for the second ChIP assay, the data for the second ChIP in the double ChIP assay were not normalized against GAPDH (in contrast to the GAPDH normalization for the single ChIP data in Fig. 3A). The association of histones with newly replicated viral DNA at 3 hpi could be explained by the slight increase in the level of viral DNA at 3 hpi compared to 1 hpi (Fig. 3B), which may be due to very early DNA replication or possibly to a DNA repair mechanism labeling prereplication DNA. The association was very low (about 0.05%).

Interestingly, although the H3 histone level is not higher on the IE (ICP0) gene promoter than that on the E (TK) or L (VP16) gene promoter region at 1 hpi, the H3 level on the E (TK) gene promoter is significantly higher than that on the IE or L promoter at 2 hpi, and the L gene H3 level is higher than the IE gene level at 3 hpi (Fig. 3A and C). The different sensitivities of each gene’s primer sets were normalized as described in Materials and Methods. F strain-infected HeLa cells showed a result similar to that for Sy5y cells (data not shown).

The decrease of histone association with viral DNA at 6 hpi is due to the lack of association of histone proteins on newly synthesized viral DNA and not to a shortage of histone proteins in the replication compartment. In Fig. 3, the association...
of histones with viral DNA is seen to decrease at 6 hpi. There are several possibilities to explain this. First, the level of histone protein could be decreased. However, we know that the level of histone H3 is constant up to 10 hpi (1). Second, histone may be excluded from the site of viral DNA replication, based on immunofluorescence data which showed that histone H1 did not colocalize with ICP8 at 8 hpi (5). Last, even though they are available, the mechanism to place histones on newly replicated viral DNA may not be active during viral DNA replication. To investigate these possibilities, we isolate replication compartments using sucrose gradient sedimentation.

F strain-infected Sy5y cell lysates were fractionated on 5% to 50% (wt/vol) sucrose gradients (as described in Materials and Methods). Purified DNA and histone proteins were measured by real-time PCR and Western blotting (Fig. 4).

Viral DNA was detected at two positions on the gradient profiles. In the 3-hpi and 6-hpi samples, fractions 2 to 5 (lighter viral DNA complex) had 20% and 24% of the total viral DNA, respectively, while fractions 14 and 15 (heavier viral DNA complex) had 63% and 65%, respectively (Fig. 4A). Cellular DNA was also detected in the heavier viral DNA complex (Fig. 4B). Because the amount of GAPDH DNA at 3 hpi is similar to that at 6 hpi (Fig. 3C), we do not think that the difference in histone protein levels on the Western blot is due to differences in cellular chromatin levels.

Figure 4C shows a Western blot probed with antibody to ICP8, a single-stranded viral DNA binding protein which is indicative of viral replication compartments (26). In gradients loaded with 3-hpi and 6-hpi samples, both lighter and heavier viral DNA complexes were positive for ICP8 proteins. However, the intensity of ICP8 bands in the 6-hpi sample was much higher at fraction 14 than that in the 3-hpi sample, while the intensity at fractions 2 to 5 remained relatively similar. This suggests that the heavier viral DNA complex contains newly replicated DNA indicative of the viral replication compartment (Fig. 4C).

To determine which fractions contain the newly replicated DNA, BrdU (a proliferation marker) was added to the infected cells and thus incorporated into newly replicating DNA. The newly replicated DNA was measured by Western blotting with anti-BrdU antibody. In gradients loaded with 3-hpi and 6-hpi samples, only the heavier viral DNA complex at 6 hpi was positive for BrdU, confirming that fraction 14 contains the viral replication compartment (Fig. 4C).

The level of histone H3 proteins in the Western blots was measured on a Fuji Imager and calculated as a percentage of the total H3 protein. As shown in Fig. 4D, the percentage of histone was not decreased in the heavier viral DNA complex, suggesting that histones are available for deposit on viral DNA during replication.

**DISCUSSION**

The HSV-1 virion is considered to be composed of four different parts: the envelope (important in entry and exit from the infected cell), the tegument (important in the earliest stages of gene expression), an icosahedral capsid (important for protection of the viral genome), and the DNA genome (7).

Seven viral genes (UL6, UL15, UL17, UL25, UL28, UL32, and UL33) are known to participate in the processing of viral DNA (18). The gene products of UL6, UL17, and UL25 are thought to be minor capsid proteins (7), and that of UL6 is believed to be the subunit of the portal through which DNA enters the capsid (19). The gene products of UL15 and UL28 are components of a terminase enzyme complex, which cleave the viral DNA concatamer into a genome length (18).

Simian virus 40 (SV40) is a member of the papovavirus family. It has a DNA genome in a icosahedrally symmetrical capsid, is nonenveloped, and contains histones (28). The SV40
Virion is reported to enter the nucleus through the nuclear pore complex (32). Histones, which have a nucleus-targeting signal, enter the nucleus through the nuclear pore complex, suggesting that histones could contribute to the nuclear entry of SV40 viral DNA (16, 23).

Previous evidence that packaged HSV DNA is free of bound histones included the measurement of the distance between condensed viral duplexes, which was shown to be similar to that for a double-stranded DNA bacteriophage which does not have bound histones (2), and the determination of spermidine and spermine in the capsids (presumably to substitute for histones in neutralizing the charge on the encapsidated DNA) (5). MNase digestion of HSV-1 DNA in [3H]thymidine-labeled nucleocapsids has shown a smear of DNA fragment (12). Also, no histone was detected in nucleoprotein complexes in autoradiographic electrophoresis (21). The nucleoprotein complex was considered to be a natural intermediate in virion assembly and finally assembled into nucleocapsids.

To directly test whether packaged viral DNA includes nucleosomes, the presence of histone proteins on encapsidated viral DNA was determined (Fig. 1, 2). In our experiments, no histone protein was detected on viral DNA in capsids, though histone proteins could be detected sticking to the outside surface of the capsid. These histone proteins could be removed by mild digestion with trypsin or DNase. The DNase did not have access to viral DNA, which was protected within the icosahedral capsids. Because histone H3 was completely removed under trypsin digestion conditions in which VP5 (the major capsid protein) was not removed (Fig. 1C), our data support the hypothesis that histone proteins (and thus nucleosomes) are not present inside HSV viral capsids.

![FIG. 4. Free and DNA-bound H3 histone protein profiles from sucrose gradient centrifugation. At 3 or 6 hpi Sy5y cells were harvested and cell lysates fractionated on 5 to 50% (wt/vol) sucrose gradients. Viral DNA, cell (GAPDH) DNA, and H3 histone protein levels were measured by real-time PCR (A and B) and Western blotting (C), respectively. The percentage of total gradient histone protein in Western blot fractions was determined by densitometry (D).](http://jvi.asm.org/)
viral capsids, we looked at the association of histones with viral DNA after entry into the nucleus of an infected cell. The data from our ChIP experiments (Fig. 3A), showed that the promoter regions of every kinetic class of viral gene associate with histone H3 as early as 1 hpi. Acetylated H3 was shown to associate with every kinetic class of viral gene at 1 hpi (9).

Thus, either modified histones are added to the DNA or unmodified histones in newly formed virion nucleosomes are quickly modified. Our data do not distinguish between these two possibilities, but nucleosomes added to cellular DNA are thought to undergo modification after assembly on the DNA.

The virion protein VP16 is reported to recruit chromatin-modifying coactivators such as HATs and SWI/SNF complexes to IE promoters at 2 hpi (6). These complexes may then modify nucleosomes present on the viral DNA. Taken together, our data suggest that virion DNA enters the nucleus of a cell in a naked state and then associates with histone proteins (modified or unmodified) prior to viral gene expression. The assembly of transcriptional complexes may further modify the viral nucleosomes.

In our ChIP experiments, the increase of viral DNA associated with histone H3 peaked at 3 hpi, a time before viral replication starts. This suggests that histones and hence nucleosomes increase in density on the viral DNA until the initial stages of viral DNA replication. However, this association dramatically decreases at 6 hpi, a time when viral replication is in high gear, because the newly synthesized viral DNA was not associated with histones at 6 hpi (Fig. 3C). In our sucrose gradient experiment, histones were found present with replicating DNA even at 6 hpi (Fig. 4C, H3 6 hpi, fraction 14). This supports a model in which histones do not associate with newly replicated viral DNAs even though they are available.

This model is supported by the results of two MNase digestion studies, both of which suggested that newly replicated viral DNA is not organized in nucleosomes. An MNase digestion study of progeny HSV-1 DNA ([3H]thymidine pulse-labeled for 1 h at 6 hpi) found random DNA cleavage (14). Another MNase digestion study of progeny HSV-1 DNA ([3H]thymidine pulse-labeled for 1 h at 12 hpi) found major heterogenous fragments and a minor nucleosomal one, which may have originate from cellular DNA (11).

Taken together, the data suggest that histones are deposited only on input viral DNA and are absent from newly replicated DNA. Since we have shown that nucleosomes are not packaged in virions, this would simplify the problem of stripping nucleosomes from the newly replicated DNA prior to packaging. Thus, only newly replicated (nucleosome-less) DNA is packaged.

Fluorescence microscopy studies have shown that host chromatin starts to be marginalized and dispersed out of replication compartments at 8 hpi (26). Furthermore, in those studies histone protein is not detected in the viral replicating compartments. Thus, the microscopy data support the lack of histone on newly replicated viral DNA and suggest a mechanism: that it is due to exclusion of histone protein from the newly replicated DNA inside the viral replication compartment after 8 h. However, our data suggest that another mechanism may be operating, as histones are excluded from newly replicated viral DNA as early as 6 h.

It is well known that HSV infection down regulates host gene expression. This is called the early shut off or early destabilization response by the virion host shutoff protein (13). This effect on histone synthesis could also be a factor in the lack of availability of histone to form nucleosomes on newly replicated HSV DNA. However, the in vitro half-life of histone mRNA is not changed during HSV infection (27). Also, histone protein levels have been shown to be constant up to 10 hpi, compared with mock infection (9).

In conclusion, our studies suggest that following infection of a cell, input (nucleosome-less) HSV DNA, from virions docked at the nuclear pore, is complexed with histones to form nucleosomes within 1 h. These histones may be modified, as is found on active cellular chromatin, at this time. Following the onset of viral DNA replication after 3 h, the newly replicated viral DNA does not appear to have any nucleosomes covering it, and none are found on packaged DNA (Fig. 5). This is in contrast to the case for another DNA virus, SV40, which has been shown to contain nucleosomes inside its capsids.

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