Core Histones H2B and H4 Are Mobilized during Infection with Herpes Simplex Virus 1[▽]

Kristen L. Conn,¹ Michael J. Hendzel,² and Luis M. Schang^{1,3*}

*Departments of Biochemistry,¹ Experimental Oncology,² and Medical Microbiology and Immunology,³
University of Alberta, Edmonton, Alberta, Canada*

Received 17 August 2011/Accepted 30 September 2011

The infecting genomes of herpes simplex virus 1 (HSV-1) are assembled into unstable nucleosomes soon after nuclear entry. The source of the histones that bind to these genomes has yet to be addressed. However, infection inhibits histone synthesis. The histones that bind to HSV-1 genomes are therefore most likely those previously bound in cellular chromatin. In order for preexisting cellular histones to associate with HSV-1 genomes, however, they must first disassociate from cellular chromatin. Consistently, we have shown that linker histones are mobilized during HSV-1 infection. Chromatinization of HSV-1 genomes would also require the association of core histones. We therefore evaluated the mobility of the core histones H2B and H4 as measures of the mobilization of H2A-H2B dimers and the more stable H3-H4 core tetramer. H2B and H4 were mobilized during infection. Their mobilization increased the levels of H2B and H4 in the free pools and decreased the rate of H2B fast chromatin exchange. The histones in the free pools would then be available to bind to HSV-1 genomes. The mobilization of H2B occurred independently from HSV-1 protein expression or DNA replication although expression of HSV-1 immediate-early (IE) or early (E) proteins enhanced it. The mobilization of core histones H2B and H4 supports a model in which the histones that associate with HSV-1 genomes are those that were previously bound in cellular chromatin. Moreover, this mobilization is consistent with the assembly of H2A-H2B and H3-H4 dimers into unstable nucleosomes with HSV-1 genomes.

Herpes simplex virus 1 (HSV-1) is a nuclear replicating DNA virus that establishes latent and lytic infections. Latent HSV-1 genomes are largely transcriptionally silent, whereas lytic HSV-1 genomes are transcriptionally active. Like cellular DNA, latent and lytic HSV-1 genomes associate with histones to form chromatin. The basic unit of chromatin, the nucleosome, consists of two molecules each of histones H2A, H2B, H3, and H4 wrapped with approximately 146 bp of DNA (28). Histone H1 binds to nucleosomes at the DNA entry and exit points, stabilizing them and helping the formation of higher order chromatin structures (16).

The assembly into chromatin poses a physical barrier to DNA access. More compact heterochromatin is less accessible to proteins, such as transcription complexes, than less compact euchromatin. Genes that are heterochromatinized are consequently typically silenced, whereas those that are euchromatinized are typically expressed. The specific proteins associated with chromatin, the posttranslational modifications (PTMs) to the histones in chromatin, and the histone variants assembled in chromatin, all impact the structure of the chromatin and therefore regulate access to DNA. Consequently, all these factors regulate gene expression. Such epigenetic regulation can promote gene silencing or expression.

As with the expression of cellular genes, the expression of HSV-1 genes is also epigenetically regulated (reviewed in reference 33). Silenced (latent or quiescent) viral genomes are regularly chromatinized, with histones that have PTMs typi-

cally associated with silenced chromatin (3, 5, 8, 51). Moreover, the nonhistone proteins that associate with the mostly transcriptionally silent latent HSV-1 chromatin are those that typically associate with cellular heterochromatin (1). In contrast, transcriptionally active (lytic) HSV-1 genomes are assembled into unstable nucleosomes, with histones that have PTMs typically associated with transcribed cellular chromatin (12, 15, 19, 20, 34, 37). Moreover, the nonhistone proteins that associate with lytic HSV-1 chromatin are those that typically associate with cellular euchromatin (10, 12, 54).

HSV-1 transcription transactivators, such as VP16, ICP0, and ICP4, interact with cellular chromatin modifying proteins (reviewed in reference 33). These interactions are thought to epigenetically favor HSV-1 gene expression such that viral gene expression is preferred over silencing. Consistent with this model, HSV-1 strains with mutations in one or several viral transactivators have delayed replication kinetics or are silenced in certain cell types (31, 38, 40), although small interfering RNA (siRNA) depletion of cellular histone acetyltransferases (HATs) did not impair lytic HSV-1 replication (18).

The infecting parental HSV-1 genomes are not chromatinized within virions (9, 34). Furthermore, late HSV-1 replication compartments occupy nuclear domains depleted of histones, and progeny genomes are packaged into virions as naked DNA (complexed with spermine) (30, 45, 46). The processes whereby histones first interact with HSV-1 genomes and are later displaced away from them are unknown. Moreover, the sources of the histones that bind to HSV-1 genomes have also yet to be addressed. However, it is unlikely that these histones are newly synthesized. Histone synthesis is tightly regulated (32). Most histones are synthesized along with DNA replication during S phase, whereas HSV-1 can infect cells in

* Corresponding author. Mailing address: Department of Biochemistry, University of Alberta, 6-142G KATZ, Edmonton, Alberta T6G 2E1, Canada. Phone: (780) 492-6265. Fax: (780) 492-3383. E-mail: luis.schang@ualberta.ca.

[▽] Published ahead of print on 12 October 2011.

any stage of the cell cycle. Furthermore, HSV-1 infection inhibits histone synthesis (21, 42, 48, 55). Thus, the histones that associate with HSV-1 genomes are most likely those preexisting in the cell prior to infection.

Cellular chromatin is dynamic. Histones normally disassociate from chromatin, diffuse through the nucleus, and reassociate in chromatin at a different site. Linker H1 histones, which bind peripherally to the core nucleosome, have a much higher rate of chromatin exchange than core histones. Whereas H1 chromatin exchange occurs in minutes, core histone chromatin exchange occurs in hours, with H2A or H2B undergoing faster exchange than H3 or H4 (11, 17, 23, 29, 49). Processes that disrupt chromatin (such as transcription, DNA repair, or DNA replication) increase the rates of histone exchange in chromatin.

We propose that the histones that bind to HSV-1 genomes are those that were previously bound in cellular chromatin. This model requires that the histones be mobilized away from cellular chromatin first, to then be available to bind to HSV-1 genomes. Consistent with such a model, we have previously shown that linker histone H1 is mobilized during infection (7). H1 is the most mobile histone, and, accordingly, it is the histone most likely to be mobilized. Unlike core histones, however, H1 has not been shown to bind to HSV-1 genomes.

Chromatinization of HSV-1 genomes also requires the association of core histones. We therefore now tested whether core histones are also mobilized during HSV-1 infection. We first evaluated the mobilization of H2B and H4. These core histones are in different dimers within the nucleosome and have no variants that could be differentially mobilized during infection. Here, we show that core histones H2B and H4 are indeed mobilized during HSV-1 infection. This mobilization increases the pools of free H2B and H4, concomitantly with a decrease to the rate of H2B fast chromatin exchange. The mobilization of H2B is independent of HSV-1 protein expression and DNA replication and requires only nuclear, non-cross-linked, HSV-1 genomes. Nonetheless, expression of HSV-1 immediate-early (IE) or early (E) proteins mobilizes H2B to a greater degree.

MATERIALS AND METHODS

Cells, viruses, and drugs. African green monkey (Vero) cells were maintained at 37°C in 5% CO₂ in Dulbecco's modified minimum Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS). Osteosarcoma (U2OS) cells, a generous gift from J. Smiley (University of Alberta) and HeLa cells stably expressing H2B-green fluorescent protein (GFP) fusion proteins (13), a generous gift from Z. Wang (University of Alberta), were maintained at 37°C in 5% CO₂ in DMEM supplemented with 10% FBS. Wild-type HSV-1, strain KOS (passage 10), and mutant strains n212 (the late P. Schaffer, Harvard Medical School) and KM110 (J. Smiley, University of Alberta) are all in the KOS genetic background and all have been described previously (4, 31, 47). Viral stocks were prepared and titrated by standard plaque assay as described previously (7). Mutant strains n212 and KM110 were titrated on complementary U2OS cells; KM110 was titrated in the presence of 5 mM *N,N'*-hexamethylene bis(acetamide) (HMB; Sigma). Phosphonoacetic acid (PAA; Sigma) was prepared in DMEM as a 100 mg/ml stock at neutral pH and stored in aliquots at -20°C. PAA was used at a concentration of 400 µg/ml. Following adsorption, PAA was maintained in the medium throughout the course of infection.

Plasmids. The DNA sequence encoding H2B was obtained from cDNA clone PX01012E07 from the Riken Mouse cDNA library (14, 35). The H2B-encoding DNA sequence was isolated by digestion with PstI and BglII for directional cloning into pEGFP-C1 (Clontech). A DNA linker (5'-AAGTCCGAGAGC

TCAAAGATCTCAAA-3') was directionally inserted in the BspEI and BglII sites between the enhanced GFP (EGFP) and H2B coding sequences.

The DNA sequence encoding H4 was PCR amplified from cDNA clone ZX00123P01 obtained from the Riken Mouse cDNA library with the following primers: 5'-TAAAGATCTATGTCTGGTCTGGCAAGGG and 3'-TAACTG CAGTTAACCGCCGAATCCGTAGAG (14, 35). The amplified sequence encoding H4 was digested with BglII (5') and PstI (3') for directional in-frame cloning into similarly digested pEGFP-C1.

Transfection. Vero and U2OS cells were transfected with Lipofectamine 2000 (Invitrogen) as described previously (7). Briefly, cells were seeded in six-well plates and incubated overnight. For each well to be transfected, Lipofectamine 2000 was added to one microcentrifuge tube containing 100 µl of DMEM, and plasmid DNA was added to another. The plasmid-DNA mix was added to the Lipofectamine mix after a 10-min incubation at room temperature. Following an additional 30-min (U2OS) or 60-min (Vero) incubation, the volume in each microcentrifuge tube was brought to 1 ml with room temperature DMEM. Medium was removed from the cells, which were then overlaid with the transfection mix. Cells were incubated at 37°C in 5% CO₂ for 4.5 (U2OS) or 6.5 (Vero) h, and then 1 ml of 37°C DMEM supplemented with 10% FBS was added to each well. Cells were further incubated at 37°C for at least 12 (GFP-H2B) or 24 (GFP-H4) h before any other procedure.

HSV-1 infection. Transfected cells were seeded onto coverslips for fluorescence recovery after photobleaching (FRAP) or immunofluorescence, as described previously (7). Seeded cells were incubated at 37°C in 5% CO₂ for at least 4 h before infection. Inoculum was prepared by diluting purified HSV-1 stocks in 4°C DMEM. For mock infection, 4°C DMEM was used. Cells were overlaid with a minimum volume of inoculum, incubated at 37°C for 1 h, with rocking and rotating every 10 min, and washed twice with 4°C phosphate-buffered saline ([PBS] 150 mM NaCl, 1 mM KH₂PO₄, 3 mM Na₂HPO₄, pH 7.4). Cells were then overlaid with fresh 37°C DMEM supplemented with 5% (Vero) or 10% (U2OS) FBS and incubated in 5% CO₂ at 37°C until they were subjected to FRAP or fixation for immunofluorescence.

Fluorescence recovery after photobleaching. Histone mobilization was evaluated from 4 to 5 or from 7 to 8 h postinfection (hpi) as described previously (7). Briefly, a coverslip was mounted on a slide and put on a heated stage (37°C) on a Zeiss NLO 510 multiphoton microscope. Cells were viewed on a Plan-Neofluar 40× oil immersion objective lens (numerical aperture [NA], 1.3) heated to 37°C. FRAP was performed using a 25-mW Argon laser (488 nm) with a band-pass filter of 505 to 530 nm and the maximum pinhole size. Whole-cell imaging was typically performed at 3 to 5% intensity while photobleaching was typically achieved with 30 iterations at 95 to 100% intensity. A region passing across the cell nucleus was photobleached, and then 60 fluorescent and differential interference contrast (DIC) images were collected for each cell at timed intervals from before bleaching to after bleaching. At each interval, the fluorescence of the photobleached region and of the whole nucleus was measured. The fluorescence of the photobleached region at each time point was then normalized to the total nuclear fluorescence at that time. The normalized fluorescence of the photobleached region at any time is presented as a ratio to the normalized fluorescence of the same region before photobleaching. Fluorescence in the photobleached region is recovered as bleached GFP-histones exchange for non-bleached GFP-histones. The FRAP was measured for only 100 s. The contribution of newly synthesized, and nuclear imported, GFP-core histones to the fluorescence recovery is therefore not a relevant factor.

The normalized fluorescence intensity of the photobleached nuclear region at the first time point after photobleaching was used as a surrogate measure for the levels of histones available in the free pools (i.e., not bound in chromatin) (17). The slope between the normalized fluorescence at the first and second time points after photobleaching, representing the initial rate of fluorescence recovery, was used as a surrogate measure for the rate of fast chromatin exchange.

Immunofluorescence. All washes and incubations were performed at room temperature unless otherwise indicated. Infected cells were washed with 4°C PBS, fixed with 5% formaldehyde for 15 min, and then washed again with 4°C PBS. Cells were permeabilized with -20°C MeOH for 1 min and then incubated in blocking buffer for 1 h with slow rocking. Mouse monoclonal anti-ICP4 (antibody 1101-897; Goodwin Institute for Cancer Research Inc., Plantation, FL) diluted 1:15,000 in blocking buffer was added to the wells, and the cells were incubated for 2 h with slow rocking. Cells were washed three times with PBS, and then Alexa Fluor 594-labeled goat anti-mouse secondary antibody (Molecular probes) diluted 1:1,000 in blocking buffer was added. The cells were incubated with secondary antibody for 1 h with slow rocking. Following three PBS washes, nuclei were counterstained with 1 µg/ml Hoechst 33258 for 5 min. Coverslips were rinsed with double-distilled H₂O (ddH₂O), mounted onto slides with

FIG. 1. GFP-H2B or GFP-H4 is incorporated in mitotic chromosomes and does not adversely affect HSV-1 replication, as evaluated by ICP4 expression and its accumulation into replication compartments. (A) Digital fluorescent (left panels) and DIC (right panels) micrographs showing Vero cells expressing GFP-H2B or GFP-H4. Cells were transfected with plasmids expressing GFP fused to H2B or H4. (B) Dot plots representing the levels of GFP-core histone in the free pools in individual cells against their normalized nuclear fluorescence intensities. Vero cells were transfected with plasmids encoding GFP-H2B (H2B) or GFP-H4 (H4). Transfected cells were mock infected or infected with 30 PFU/cell of HSV-1 strain KOS, and the levels of free GFP-H2B or GFP-H4 were evaluated from 4 to 5 (4 hpi) or 7 to 8 (7 hpi) hpi by FRAP. H2B $r^2 = 0.002, 0.001, \text{ or } 0.012$ for mock, 4 hpi, or 7 hpi, respectively; H4 $r^2 = 0.008, 0.047, \text{ or } 0.023$ for mock, 4 hpi, or 7 hpi, respectively. (C) Bar graphs showing the percentage of HSV-1-infected cells transfected or not with either GFP-H2B or GFP-H4 that express ICP4 as a nuclear diffuse pattern or in replication compartments. Cells were transfected with plasmids expressing GFP fused to H2B or H4, infected with 6 (U2OS) or 30 (Vero) PFU/cell of HSV-1 strain KOS or 30 PFU/cell of HSV-1 strain KM110, fixed at 4.5 (4) or 7.5 (7) hpi, and stained for ICP4. Nuclear expression of ICP4 and its accumulation in replication compartments in cells in which GFP-H2B or GFP-H4 were expressed (+) or not (-) were evaluated by fluorescence microscopy.

Vectashield mounting medium (Vector) and sealed with clear nail enamel, or mounted with Mowiol (G. Barron; Cross Cancer Institute, Edmonton, Alberta, Canada). The cells were viewed on a Leica DM IRB microscope. A minimum of 200 transfected or nontransfected cells from at least two experiments were counted at 4 and 7 hpi, with the following exceptions: for Vero H2B KOS-infected transfected cells counted at 4 hpi and KM110-infected transfected cells counted at 4 hpi and nontransfected cells counted at 7 hpi, a minimum of 100 cells from two experiments were counted; for KM110-infected cells counted at 7 hpi, 58 transfected cells from two experiments were counted.

Image preparation. Fluorescent images (512 by 512 pixels; 12-bit) were analyzed with Zeiss LSM software. Image contrast and brightness were adjusted for figure preparation using Adobe Photoshop.

RESULTS

Core histones H2B and H4 are mobilized during HSV-1 infection. To test whether core histones H2B and H4 were mobilized by HSV-1, we evaluated the kinetics of fluorescence recovery after photobleaching of GFP-H2B and GFP-H4 fu-

sion proteins. However, we first had to test the potential cellular effects of these GFP-histone fusion proteins. Expression of these fusion histones did not appear to affect cells in any obvious manner in that they were incorporated into chromatin, the cells were viable, and no chromosomal aberrations were displayed during mitosis. Moreover, cells expressing GFP-H2B or GFP-H4 displayed no extranuclear fluorescence during interphase or extrachromosomal fluorescence during mitosis (Fig. 1A). The levels of GFP-H2B or GFP-H4 expression could nonetheless affect the percentage of GFP-H2B or GFP-H4 not bound in chromatin. However, the percentage of free GFP-H2B or GFP-H4 in any given cell did not correlate with its expression level, as measured by its relative nuclear fluorescence ($r^2 = 0.002$ or 0.008 for H2B or H4, respectively) (Fig. 1B, mock).

The effects of GFP-H2B or GFP-H4 expression on HSV-1

infection were unknown. We therefore evaluated the expression of ICP4 and its accumulation in replication compartments in cells expressing or not expressing GFP-H2B or GFP-H4. Expression of GFP-H2B or GFP-H4 did not inhibit the progression of HSV-1 infection (Fig. 1C, Vero KOS). Expression levels of ICP4 and its accumulation in replication compartments were similar in cells expressing or not expressing GFP-H2B or GFP-H4 at 4 or 7 hpi (Fig. 1C, Vero KOS). As expected (19, 30), GFP-H2B was observed sometimes associated with, and sometimes depleted from, accumulations of ICP4 (data not shown).

We therefore proceeded to evaluate H2B and H4 mobilization during HSV-1 infection (Fig. 2). As the initial test, we evaluated the FRAP of GFP-H2B and GFP-H4 at early (4 h) and late (7 h) times after infection. The normalized fluorescence after photobleaching was recovered faster in infected than that in mock-infected cells (Fig. 3). At any give time, the fluorescence in the photobleached region of cells infected with 30 PFU/cell of HSV-1 strain KOS for 4 or 7 h normalized to the total nuclear fluorescence at that time was greater than that in the photobleached region of mock-infected cells normalized to their total nuclear fluorescence at that time (Fig. 3A and B). H2B and H4 are therefore mobilized in HSV-1-infected cells.

Most H2B and H4 are stably incorporated in chromatin and undergo slow exchange, reflected in the overall slow fluorescence recovery (Fig. 2 and 3B) (17). Such stably incorporated histones are not likely to be available to bind to HSV-1 genomes in a timescale relevant to HSV-1 infection. We therefore analyzed the mobilization of the population of H2B and H4 that would be available, i.e., those histones in the free pools or weakly bound in chromatin and therefore undergoing fast chromatin exchange (Fig. 2B). The normalized level of fluorescence in the photobleached region at the first time evaluated after photobleaching (approximately 1 s) is a surrogate measure of the level of GFP-histones in the free pool (Fig. 2B). Only freely diffusing (i.e., free) histones move throughout the nucleus at a rate fast enough to move into the previously photobleached region in less than 1 s (17). As a surrogate measure for the percentage of histones participating in fast chromatin exchange, we analyzed the initial rate of normalized fluorescence recovery after photobleaching. The initial rate of fluorescence recovery is calculated as the slope of the normalized fluorescence recovery between the first and second times after photobleaching (Fig. 2B).

H2B and H4 mobilization resulted in increases to their average levels in the free pools at 4 hpi to $133\% \pm 5\%$ and $140\% \pm 7\%$, respectively ($P < 0.01$, one-tailed Student's *t* test) (Fig. 3C). These pools remained increased to an average of $169\% \pm 7\%$ or $157\% \pm 9\%$ for H2B or H4, respectively, at 7 h after infection ($P < 0.01$, one-tailed Student's *t* test) (Fig. 3C). Increases in the average levels of free H2B or H4 may reflect a general increase in the levels of free histones in all cells in the population or an extreme increase in the levels of free histones in one subpopulation of cells without affecting the levels of free histones in the remaining population. To differentiate between these two possibilities, we replotted the data to analyze the percentage of free H2B or H4 in individual cells. The increases to the free pools of H2B or H4 occurred throughout the cell population, as shown by a general rightward shift of the uni-

FIG. 2. FRAP of GFP-core histone fusion proteins. Cells were transfected with plasmids expressing GFP-H2B. A region passing across the long axis of the nucleus was photobleached, and the recovery of fluorescence in the photobleached region was evaluated over time. Fluorescence in the photobleached region recovers as the photobleached GFP-H2B molecules in this region exchange with the non-photobleached molecules from outside the photobleached region. (A) Digital fluorescent micrographs showing a Vero cell expressing GFP-H2B before and after photobleaching. The enlargements at the bottom highlight the photobleached region. (B) Line graph presenting representative FRAP of GFP-H2B. The fluorescence intensity of the photobleached region and that of the entire nucleus were measured prior to and at selected intervals after photobleaching. The fluorescence of the photobleached region was then normalized relative to the total nuclear fluorescence at that time. The normalized fluorescence intensity of the photobleached region is then expressed as a fraction of the normalized fluorescence of the same region before photobleaching and plotted against time after bleaching. The normalized fluorescence at the first data point after photobleaching is a surrogate measure for the level of GFP-core histone in the free pool. The subsequent fluorescence recovery is biphasic. The initial faster phase represents those histones that are undergoing faster chromatin exchange at any given time (i.e., they are weakly bound in chromatin). As a surrogate measure for this population, we calculated the initial rate of normalized fluorescence recovery, i.e., the slope of the graph between the normalized fluorescence at the first and second data points after photobleaching. The second slower phase of fluorescence recovery represents those histones that are undergoing slower chromatin exchange at any given time (i.e., they are tightly bound in chromatin). This population is likely not available to undergo chromatin exchange with HSV-1 chromatin in a timescale that is relevant to infection.

modal frequency distributions of infected cells (Fig. 3D). At 7 h after infection, 88% or 70% of cells had increased their individual pools of free H2B or H4, respectively, to a large degree (defined as greater than 1 standard deviation [SD])

above the average level in mock-infected cells and indicated by the dotted line in Fig. 3D). Only 16% of cells in a normal population would be expected to have such high levels of free histones.

The average increase to the pools of free H2B or H4 could also have resulted from different levels of GFP-H2B or GFP-H4 expression. However, the level of free GFP-H2B in a given infected cell did not correlate with its level of GFP-H2B expression either ($r^2 = 0.001$ or 0.012 at 4 or 7 hpi, respectively) (Fig. 1B, H2B), and neither did the levels of free GFP-H4 ($r^2 = 0.047$ or 0.023 at 4 or 7 hpi, respectively) (Fig. 1B, H4). The increases to the free pools of H2B or H4 were therefore not a consequence of increases in their expression levels. Also consistent with this conclusion, HSV-1-infected cells tended to have more uniform levels of GFP-H2B or GFP-H4 expression than mock-infected cells (Fig. 1B, compare the x axis distributions of mock, 4 hpi, and 7 hpi) but a broader range in the percentage of free H2B or H4 (Fig. 1B, compare the y axis distributions). Taken together, these data show that HSV-1 infection alters the relative rates of H2B and H4 association with, and disassociation from, chromatin such that their levels in the free pool are increased at any given time.

The increased levels of H2B or H4 in the free pools indicate changes to the relative rates of their association with, and disassociation from, chromatin. We therefore next evaluated whether HSV-1 infection altered the rates of H2B or H4 fast chromatin exchange. The average rate of fast H2B chromatin exchange tended to be decreased (to $90\% \pm 12\%$; $P > 0.1$, one-tailed Student's t test) relative to that in mock-infected cells at 4 h after infection, and it was decreased to $69\% \pm 6\%$ of that in mock-infected cells at 7 h ($P < 0.01$, one-tailed Student's t test) (Fig. 3E). The tendency toward a decreased rate of H2B fast chromatin exchange occurred throughout the cell population, shown by the leftward shifts in the unimodal frequency distribution plots of the same primary data (Fig. 3F). At 7 h after infection, 34% of cells had large decreases in their individual rates of H2B fast chromatin exchange (defined as more than 1 SD lower than the average rate of H2B fast chromatin exchange in mock-infected cells and indicated by the dotted lines in Fig. 3F), approximately twice the 16% expected in a normal population.

In contrast to H2B, the average rate of H4 fast chromatin exchange in HSV-1-infected cells was not significantly altered relative to that in mock-infected cells at either time evaluated

(Fig. 3E). However, reevaluation of the rate of H4 fast chromatin exchange per individual cell in frequency distribution plots revealed a bimodal distribution in mock-infected cells (Fig. 3F). The subpopulation of cells with the highest rate of H4 fast chromatin exchange was notably absent in HSV-1-infected cells, and, consequently, the rate of fast H4 chromatin exchange displayed a unimodal frequency distribution (Fig. 3F).

Taken together, these data demonstrate that H2B and H4 are mobilized at early and late times during HSV-1 infection. This mobilization decreases the average rate of H2B fast chromatin exchange and increases the average levels of H2B and H4 in the free pools.

Stably expressed GFP-H2B is mobilized during HSV-1 infection. The histone mobilization could have been an artifact of transient GFP-histone expression. We therefore evaluated the mobility of H2B-GFP stably expressed from a construct integrated into the genome of HeLa cells (13). As for the transiently transfected cells, H2B-GFP levels in stably expressing cells did not correlate with free H2B-GFP levels ($r^2 = 0.040$) (Fig. 4A, mock). As expected, the stably transfected cells had a more homogenous distribution of H2B-GFP levels than the transiently transfected cells (compare x axis distributions of Fig. 4A, mock, and 1B, H2B mock). However, the average absolute level of free H2B-GFP was higher than that in transiently transfected Vero cells ($P < 0.01$, one-tailed Student's t test).

As for transiently expressed GFP-H2B, stably expressed H2B-GFP was mobilized at 4 and 7 h in HSV-1-infected HeLa cells (Fig. 4B). The average level of free H2B-GFP increased to $115\% \pm 4\%$ or $143\% \pm 5\%$ of the average level in mock-infected cells at 4 or 7 h, respectively, after infection with 30 PFU/cell of HSV-1 strain KOS ($P < 0.01$, one-tailed Student's t test) (Fig. 4C). At either time, greater than 50% of cells had large increases in their individual pools of free H2B (Fig. 4D). Moreover, the levels of free H2B in any given cell did not correlate with its level of H2B-GFP expression, as evaluated by nuclear fluorescence intensity ($r^2 = 0.006$ or 0.002 at 4 or 7 h, respectively) (Fig. 4A, KOS 30). As in mock-infected cells (and as expected), H2B-GFP levels were also more homogeneous in stably transfected than in transiently transfected cells infected with HSV-1 (compare the x axis distribution of Fig. 4A, KOS 30, and 1B, H2B 4 and 7 hpi).

We next evaluated whether HSV-1 infection altered the fast chromatin exchange rate of H2B. The average rate of H2B fast

FIG. 3. Core histones H2B and H4 are mobilized in HSV-1-infected cells. Vero cells were transfected with plasmids expressing GFP fused to H2B or H4. Transfected cells were mock infected or infected with 30 PFU/cell of HSV-1 strain KOS. (A) Digital fluorescent micrographs showing Vero cells expressing GFP-H2B or GFP-H4 at 4 h after mock or HSV-1 infection, taken before or at 1 or 100 s after photobleaching. The insets (below the HSV-infected cells or above the mock-infected ones) are enlargements of the photobleached regions. (B) The mobilities of GFP-H2B and GFP-H4 were examined by FRAP from 4 to 5 (4) or 7 to 8 (7) hpi. Line graphs show the normalized fluorescence intensity of the photobleached nuclear region expressed as a ratio to the normalized fluorescence intensity of the same region before photobleaching and plotted against time after photobleaching. Error bars, standard errors of the means. (C) Bar graphs presenting the average normalized levels of GFP-H2B or GFP-H4 in the free pools at 4 or 7 hpi, expressed as a ratio to the average normalized level in mock-infected cells at 4 or 7 hpi, respectively (dashed line). Error bars, standard errors of the means. (D) Frequency distribution plots showing the percentage of free GFP-H2B or GFP-H4 in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized levels of free GFP-H2B or GFP-H4 in mock-infected cells. (E) Bar graphs presenting the average initial rate of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars indicate standard errors of the means. (F) Frequency distribution plots showing the initial rate of normalized fluorescence recovery per individual cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. **, $P < 0.01$; *, $P < 0.05$; n.s., not significant.

FIG. 4. H2B-GFP stably expressed in HeLa cells is also mobilized during HSV-1 infections. (A) Dot plots representing the level of H2B-GFP in the free pools in individual cells against their normalized nuclear fluorescence intensities. HeLa cells were mock infected or infected with 30 PFU/cell of HSV-1 strain KOS ($r^2 = 0.040$, 0.006, or 0.002 for mock, 4 hpi, or 7 hpi, respectively). (B) Nuclear mobility of H2B-GFP was examined from 4 to 5 (4) or 7 to 8 (7) hpi by FRAP. Line graphs show the normalized fluorescence intensity of the photobleached nuclear region expressed as a ratio to the normalized fluorescence intensity of the same region before photobleaching and plotted against time after photobleaching. Error bars, standard errors of the means. (C) Bar graphs presenting the average normalized levels of H2B-GFP in the free pools at 4 or 7 hpi expressed as a ratio to the normalized average level in mock-infected cells at 4 or 7 hpi, respectively (dashed line). Error bars, standard errors of the means. (D) Frequency distribution plots showing the percentage of free H2B-GFP in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized level of free GFP-H2B in mock-infected cells. (E) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars, standard errors of the means. (F) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. **, $P < 0.01$; *, $P < 0.05$.

chromatin exchange was decreased to $83\% \pm 3\%$ ($P < 0.05$, one-tailed Student's *t* test) at 4 h after infection with 30 PFU/cell of HSV-1 strain KOS and to $65\% \pm 4\%$ ($P < 0.01$, one-tailed Student's *t* test) at 7 h (Fig. 4E). As evidenced by replotting the data as a frequency distribution, the decrease in the rate of H2B fast chromatin exchange occurred throughout the cells in the population (Fig. 4F). Greater than 80% of cells had large decreases in their individual rates of fast H2B chromatin exchange at 7 h after infection. The decrease in the rate of fast H2B chromatin exchange in HeLa cells was inversely proportional to the increase in free H2B, suggesting that the increase in the pool of free H2B is largely a result of the decrease in the rate of fast chromatin exchange.

Taken together, these data demonstrate that H2B is similarly mobilized during HSV-1 infection in two distinct cell types, Vero and HeLa, regardless of whether GFP-H2B is stably or transiently expressed.

HSV-1 DNA replication is not required to mobilize H2B.

The majority of cellular chromatin assembly, which affects histone mobility, occurs during DNA replication. We therefore next evaluated whether HSV-1 DNA replication affected core histone mobilization. To this end, we evaluated H2B mobility in cells treated with phosphonoacetic acid (PAA), which inhibits the HSV-1 DNA polymerase (41). The mobility of H2B was similar at 7 h after infection with 30 PFU/cell of HSV-1 KOS in untreated cells and in cells treated with 400 $\mu\text{g/ml}$ of PAA ($P > 0.05$, Tukey's honestly significant differences [HSD]) (Fig. 5A). The average level of free H2B was increased to $158\% \pm 7\%$ in cells treated with PAA or to $169\% \pm 7\%$ in untreated cells in comparison to untreated and noninfected cells ($P < 0.01$, one-tailed Student's *t* test) (Fig. 5B). Replotting the data to analyze the frequency distribution indicated that greater than 93% of infected cells treated with PAA had large increases in their individual pools of free H2B (defined as greater than 1 SD above the average level of free H2B in mock-infected cells) (Fig. 5C). Surprisingly, HSV-1 DNA replication is therefore not required to increase the level of H2B in the free pool, and neither are L proteins.

Although the average level of free H2B was also increased in PAA-treated mock-infected cells, it was increased to only $119\% \pm 6\%$ of that in untreated and noninfected cells after 7 h of treatment ($P < 0.05$, one-tailed Student's *t* test) (Fig. 5B), and only 52% of PAA-treated mock-infected cells had large increases to their individual pools of free H2B at this time (Fig. 5C). Moreover, the increase in the average level of free H2B in PAA-treated mock-infected cells was to a lesser degree than that in PAA-treated HSV-1-infected cells ($119\% \pm 6\%$ versus $158\% \pm 7\%$, respectively; $P < 0.01$, Tukey's HSD). PAA treatment itself is therefore sufficient to increase the pool of free H2B to some extent in mock-infected cells; however, HSV-1 infection increases the free pool to a far larger degree (KOS-infected and PAA-treated or nontreated, $P > 0.05$; mock-infected and PAA-treated or nontreated, $P < 0.05$).

The average rate of H2B fast chromatin exchange still tended to be decreased in HSV-1-infected cells treated with PAA (to $88\% \pm 7\%$) although statistical significance was not achieved ($P > 0.1$, one-tailed Student's *t* test) (Fig. 5D). This apparent decrease to the average rate of H2B fast chromatin exchange was nonetheless similar to the decrease in the absence of PAA ($69\% \pm 6\%$) (Fig. 5D), whereas the

average rate was not decreased in PAA-treated mock-infected cells (Fig. 5D).

These data demonstrate that HSV-1 DNA replication or L proteins are surprisingly not required to mobilize H2B. HSV-1 transcription, specific IE or E proteins, or cellular responses to them therefore induce H2B mobilization.

H2B basal mobilization is independent of specific IE or E proteins or HSV-1 transcription. We next evaluated whether other HSV-1 replicative functions affected H2B mobilization. To this end, we tested H2B mobility in Vero cells infected with HSV-1 strain KM110, which has mutations in VP16 and ICP0 (31). IE transcription is not initiated efficiently in Vero cells infected with KM110, and therefore few HSV-1 proteins are expressed. ICP4 was undetectable in more than 80% of Vero cells infected for 4 h with 30 PFU/cell of HSV-1 strain KM110 (Fig. 1C, H2B KM110 Vero). ICP4 was, however, detected in 75% of cells at 7 h although it was mainly dispersed in the nucleus (Fig. 1C, H2B KM110 Vero). Only 1% or 20% of cells showed ICP4 accumulated in replication compartments at 4 or 7 hpi, respectively (Fig. 1C, H2B KM110 Vero). The expression of ICP4 and its accumulation in replication compartments were similar, regardless of whether the cells expressed GFP-H2B or not (Fig. 1C, H2B KM110 Vero).

H2B was still mobilized under such conditions of impaired (though not completely blocked) HSV-1 protein expression (Fig. 6) although the late mobilization occurred to a lesser degree than in cells infected with wild-type HSV-1 (compare Fig. 6 and 3, H2B). The average level of free H2B was increased to $120\% \pm 4\%$ at 4 h ($P < 0.01$, one-tailed Student's *t* test) (Fig. 6A). The increase in free H2B occurred throughout the cell population, with 50% of cells having large increases in their individual pools of free H2B (Fig. 6B). Along with the average increase in free H2B, its average rate of fast chromatin exchange was decreased to $76\% \pm 6\%$ at 4 h ($P < 0.05$, one-tailed Student's *t* test) (Fig. 6C). The magnitude of the average decrease in the rate of H2B fast chromatin exchange was inversely proportional to the average increase in the pool of free H2B. The increase in free H2B that occurs under conditions of impaired (though not completely blocked) HSV-1 protein expression therefore likely results directly from a decreased rate of chromatin exchange of weakly bound H2B.

The average level of free H2B was increased to a similar degree at 4 h after infection with either 30 PFU/cell of KOS or KM110 ($P > 0.05$, Tukey's HSD) (compare Fig. 6 and 3, H2B). The mobilization of H2B at 4 h is therefore independent of the levels of HSV-1 IE protein expression, transcription, VP16, or ICP0 and therefore most likely reflects a cellular response to infection.

The average level of free H2B remained increased at 7 h, to $125\% \pm 5\%$ ($P < 0.01$, one-tailed Student's *t* test) (Fig. 6A). Likewise, the average rate of H2B fast chromatin exchange remained decreased, to $77\% \pm 3\%$, although statistical significance was not achieved at this time ($P > 0.1$) (Fig. 6C). Unlike at 4 h, however, the average increase in free H2B at 7 h was less than that in cells infected with wild-type HSV-1 ($P < 0.01$, Tukey's HSD), whereas the decreases in the average rates of H2B fast chromatin exchange were similar ($P > 0.05$, Tukey's HSD). HSV-1 gene transcription, specific IE or E proteins, or VP16 is therefore required to further increase the level of free

FIG. 5. HSV-1 DNA replication and L proteins are not required to mobilize H2B. (A) Line graphs showing the normalized fluorescence intensity of the photobleached nuclear region expressed as a ratio to the normalized fluorescence intensity of the same region before photobleaching and plotted against time after photobleaching. Vero cells were transfected with plasmids expressing GFP-H2B. Transfected cells were mock infected or infected with 30 PFU/cell of HSV-1 strain KOS in the presence of 400 μ g/ml of PAA or no drug. Nuclear mobility of GFP-H2B was examined from 7 to 8 (7) hpi by FRAP. Error bars, standard errors of the means. (B) Bar graphs presenting the average normalized levels of GFP-H2B in the free pools expressed as a ratio to the average normalized levels in untreated and mock-infected cells at 4 or 7 hpi, respectively (dashed lines). Error bars, standard errors of the means. (C) Frequency distribution plots showing the percentage of free GFP-H2B in each cell

FIG. 6. HSV-1 IE or E proteins, gene transcription, or VP16 is not required for basal mobilization of H2B. (A) Bar graphs presenting the average normalized levels of GFP-H2B in the free pools at 4 or 7 hpi expressed as a ratio to the average normalized levels in mock-infected cells at 4 or 7 hpi, respectively (dashed line). Vero cells were transfected with plasmids expressing GFP-H2B. Transfected cells were mock infected or infected with 30 PFU/cell of HSV-1 strain KM110. Nuclear mobility of GFP-H2B was examined from 4 to 5 (4) or 7 to 8 (7) hpi by FRAP. Error bars, standard errors of the means. (B) Frequency distribution plots showing the percentage of free GFP-H2B in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized level in the free pool of mock-infected cells. (C) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars, standard errors of the means. (D) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. **, $P < 0.01$; *, $P < 0.05$; n.s., not significant.

H2B after 4 h but not to decrease the rate of H2B fast chromatin exchange.

The differences in the average levels of free H2B in KOS- or KM110-infected cells may well reflect the different sizes of the cell populations with large increases in their individual levels of free H2B. Whereas 88% of KOS-infected cells had large increases in their individual pools of free H2B, only 48% of KM110-infected cells had similar increases (Fig. 3D and 6B, compare the area to the right of the dotted line for the frequency distribution curves of HSV-1 infected cells at 7 hpi). However, the population of cells with large increases in their individual levels of free H2B did not correlate with the population of cells with ICP4 expression. ICP4 was detected in 75% of cells infected with KM110 and in approximately 99% of KOS-infected cells. Thus, although HSV-1 transcription, IE or E proteins, or VP16 is required to further increase the level of free H2B, this further increase does not correlate with the level

of ICP4 protein expression in a given cell (or the formation of replication compartments).

Infection of Vero cells with KM110 demonstrated that there are at least two events that mobilize H2B during HSV-1 infection. One event mobilizes H2B to a basal degree, increasing the pool of free H2B and decreasing its rate of fast chromatin exchange independently of specific IE or E proteins, HSV-1 transcription, or VP16. The other event further increases the free pool of H2B without further affecting its rate of fast chromatin exchange and requires specific IE or E proteins, HSV-1 transcription, or VP16.

To test if the further mobilization of H2B was a direct effect of ICP0 or VP16, we next evaluated its mobilization during KM110 infections of U2OS cells. U2OS cells compensate the phenotype resulting from the VP16 or ICP0 mutations, through yet unknown mechanisms, without functionally replacing either protein (31). As a result, KM110 genes are ex-

at 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized level of free GFP-H2B in mock-infected nontreated cells. (D) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in untreated and mock-infected cells (dashed lines). Error bars, standard errors of the means. (E) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected nontreated cells. KOS data were replotted from Fig. 3 for comparison. **, $P < 0.01$; *, $P < 0.05$; n.s., not significant.

FIG. 7. HSV-1 replication does not decrease the rate of H2B fast chromatin exchange in U2OS cells in the absence of VP16 or ICP0. (A) Bar graphs presenting the average normalized levels of GFP-H2B in the free pools at 4 or 7 hpi expressed as a ratio to the average normalized levels in mock-infected cells at 4 or 7 hpi, respectively (dashed line). U2OS cells were transfected with plasmids expressing GFP-H2B. Transfected cells were mock infected or infected with 30 PFU/cell of HSV-1 strain KM110. Nuclear mobility of GFP-H2B was examined from 4 to 5 (4) or 7 to 8 (7) hpi by FRAP. Error bars, standard errors of the means. (B) Frequency distribution plots showing the percentage of free GFP-H2B in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized level in the free pool of mock-infected cells. (C) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars, standard errors of the means. (D) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. **, $P < 0.01$; *, $P < 0.05$; n.s., not significant.

pressed, and DNA is replicated in U2OS cells, albeit with delayed kinetics. Consistently, $60\% \pm 4\%$ or $76\% \pm 2\%$ of U2OS cells infected with 30 PFU/cell of HSV-1 strain KM110 had ICP4 in replication compartments at 4 or 7 hpi, respectively, in comparison to $80\% \pm 6\%$ or $89\% \pm 4\%$ of cells infected with 6 PFU/cell of HSV-1 strain KOS (Fig. 1C, U2OS). ICP4 expression levels and its accumulation in replication compartments during KOS or KM110 infections were similar regardless of whether the cells expressed GFP-H2B (Fig. 1C, U2OS).

H2B was also mobilized during KM110 infection of U2OS cells (Fig. 7). The mobilization of H2B altered its relative rates of chromatin exchange to increase the average level of H2B available in the free pool to $111\% \pm 3\%$ or $124\% \pm 5\%$ at 4 or 7 h, respectively ($P < 0.05$ or 0.01 at 4 or 7 h, respectively; one-tailed Student's t test) (Fig. 7A). As in Vero cells, the pool of free H2B was increased throughout the cell population, with 64% of infected cells having large increases in their individual levels of free H2B at 7 h after infection (Fig. 7B). Free H2B was increased to a similar degree in U2OS cells infected with KM110 or KOS ($P > 0.05$, Tukey's HSD) although the cells had to be infected with a lower multiplicity of KOS due to the obvious distortion of nuclear morphology at higher multiplicities.

The average levels of free H2B were increased to $114\% \pm 4\%$ or $124\% \pm 5\%$ at 4 or 7 hpi, respectively, in U2OS cells

infected with KOS ($P < 0.01$, one-tailed Student's t test) (Fig. 8A, 6 PFU). The increase in free H2B occurred throughout the cell population (66% of cells had large increases their individual pools of free H2B at 7 h) (Fig. 8B, 6 PFU). HSV-1 transcription or specific IE or E proteins (other than ICP0) are therefore sufficient for this increase, whereas VP16 or ICP0 is not essential. However, free H2B was increased to a lower extent in U2OS cells infected with 6 PFU/cell than in Vero cells infected with 30 PFU/cell of KOS ($124\% \pm 5\%$ compared to $169\% \pm 7\%$). Additionally, the absolute average levels of free H2B were lower in U2OS than in Vero cells (0.23 ± 0.01 compared to 0.27 ± 0.01 , respectively, at 7 hpi). The degree of HSV-1 protein expression may therefore influence the magnitude of increase in free H2B. Alternatively, H2B may just be less mobile in U2OS than in Vero cells. We therefore next analyzed the mobility of H2B in cells infected at a higher multiplicity of KOS. The average level of free H2B was increased to $123\% \pm 5\%$ at 4 h after infection with 15 PFU/cell of KOS, similar to the free H2B level in cells infected with 6 PFU/cell of KOS or 30 PFU/cell of KM110 ($114\% \pm 4\%$ or $111\% \pm 3\%$, respectively; $P > 0.05$, Tukey's HSD) (Fig. 8A, 15 PFU). At early times after infection, therefore, free H2B is increased in the absence of functional VP16 or ICP0 and regardless of the degree of HSV-1 IE or E protein expression.

Unfortunately, the nuclear morphology of cells infected with 15 PFU/cell of KOS was highly distorted at later times after

FIG. 8. The degree of mobilization of H2B in U2OS cells increases with multiplicity of infection. (A) Bar graphs presenting the average normalized levels of GFP-H2B in the free pool at 4 or 7 hpi expressed as a ratio to the average normalized levels in mock-infected cells at 4 or 7 hpi, respectively (dashed lines). U2OS cells were transfected with plasmids expressing GFP-H2B. Transfected cells were mock infected or infected with 6 or 15 PFU/cell of HSV-1 strain KOS. Nuclear mobility of GFP-H2B was examined from 4 to 5 (4) or 7 to 8 (7) hpi by FRAP. Error bars, standard errors of the means. (B) Frequency distribution plots showing the percentage of free GFP-H2B in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized levels in the free pool of mock-infected cells. (C) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars indicate standard errors of the means. (D) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. **, $P < 0.01$; *, $P < 0.05$; n.s., not significant.

infection. Therefore, the analyses of the increases in free H2B at higher multiplicities of infection (MOIs) are constrained by the intrinsic limitations of the system. Nonetheless, the average level of free H2B was increased to $157\% \pm 5\%$ ($P < 0.01$, one-tailed Student's t test) (Fig. 8A, 15 PFU) at 7 hpi. This average increase was larger than that during infection with 6 PFU/cell of KOS or 30 PFU/cell of KM110 ($P < 0.01$, Tukey's HSD). These data suggest that the degree of HSV-1 protein expression influences the magnitude of increase in free H2B after 4 hpi. The difference in the average level of free H2B in

cells infected with 6 or 15 PFU/cell of KOS may also reflect differences in the sizes of the population that had large individual increases in free H2B (66% versus 96%, respectively).

In contrast to the rate of H2B fast chromatin exchange during KM110 infection of Vero cells, the average rate was not altered during KM110 infection of U2OS cells. It remained at $94\% \pm 5\%$ or $91\% \pm 8\%$ of that in mock-infected cells at 4 or 7 hpi, respectively ($P > 0.1$, one-tailed Student's t test) (Fig. 7C). Therefore, the average increase in free H2B in KM110-infected U2OS cells is not largely the result of a decreased

FIG. 9. ICP0 is not required for the enhanced mobilization of H2B. (A) Bar graphs presenting the average normalized levels of GFP-H2B in the free pools at 4 or 7 hpi expressed as a ratio to the average normalized levels in mock-infected cells at 4 or 7 hpi, respectively (dashed line). Vero cells were transfected with plasmids expressing GFP-H2B. Transfected cells were mock infected or infected with 30 PFU/cell of HSV-1 strain n212. Nuclear mobility of GFP-H2B was examined from 4 to 5 (4) or 7 to 8 (7) hpi by FRAP. Error bars, standard errors of the means. (B) Frequency distribution plots showing the percentage of free GFP-H2B in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized levels in the free pool of mock-infected cells. (C) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars, standard errors of the means. (D) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. **, $P < 0.01$; n.s., not significant.

average rate of chromatin exchange of weakly bound H2B. VP16 or ICP0 may therefore be required to alter the fast chromatin exchange rate of H2B in U2OS cells. Alternatively, the fast chromatin exchange rate of H2B may not be variable in U2OS cells or may not be decreased when HSV-1 genes are expressed. To discriminate between these possibilities, we evaluated the fast chromatin exchange rate of H2B in U2OS cells infected with HSV-1 strain KOS. Infection with either 6 or 15 PFU/cell of KOS decreased the average rate of H2B fast chromatin exchange (Fig. 8C) to $85\% \pm 5\%$ or $79\% \pm 6\%$ at 4 h ($P =$ not significant [NS] or < 0.01 for 6 or 15 PFU/cell, respectively; one-tailed Student's t test) or $64\% \pm 3\%$ or $72\% \pm 7\%$ at 7 h, respectively ($P < 0.01$, one-tailed Student's t test) (Fig. 8C). The average rate of H2B fast chromatin exchange in KOS-infected cells was significantly different than that in KM110-infected cells at 7 h ($P < 0.05$ for 6 PFU/cell KOS). Taken together, these data show that the pool of free H2B in U2OS cells increases in the absence of functional VP16 and ICP0 without changes to the fast chromatin exchange rate of the weakly bound H2B population. The free pool of H2B may therefore be increased as a result of changes in the rate of slow H2B chromatin exchange. Alternatively, unbound H2B may only weakly rebind chromatin, may rebind in very unstable nucleosomes, or may be prevented from rebinding chromatin altogether. Additionally, these results indicate that VP16 or ICP0 can reduce the rate of fast H2B chromatin exchange.

These data demonstrate that the basal mobilization of H2B

occurs independently of VP16, specific IE or E proteins, or HSV-1 gene transcription, whereas HSV-1 IE or E proteins are required to enhance H2B mobilization. Additionally, these data show that the rate of H2B fast chromatin exchange is not decreased at early times if HSV-1 proteins are expressed.

HSV-1 transcription or specific IE or E proteins enhance H2B mobilization. To further test the requirement for VP16 or ICP0, we next evaluated H2B mobility during infections with n212. Strain n212 has the same ICP0 mutation as strain KM110 but carries wild-type VP16 (4). Therefore, IE protein expression initiates, and n212 replicates in Vero cells, although with delayed kinetics. H2B was mobilized at early and, even more so, at late times in Vero cells infected with 30 PFU/cell of n212 (Fig. 9). The average level of free H2B was increased at 4 h after infection to $120\% \pm 6\%$ ($P < 0.01$, one-tailed Student's t test) (Fig. 9A), the same extent as during infections with KOS or KM110 ($P > 0.05$, Tukey's HSD), as expected. The average level of free H2B was then further increased to $167\% \pm 7\%$ at 7 h after infection with n212 ($P < 0.01$, one-tailed Student's t test) (Fig. 9A), similar to the level with wild-type KOS infections ($169\% \pm 7\%$; $P > 0.05$, Tukey's HSD) and greater than that during KM110 infections ($125\% \pm 5\%$; $P < 0.01$, Tukey's HSD). ICP0 itself is therefore not required for the enhanced mobilization of H2B, whereas HSV-1 transcription or expression of other IE or E proteins is.

The mobilization of H2B during n212 infection tended to alter the average rate of H2B fast chromatin exchange, but the

FIG. 10. VP16 and HSV-1 gene transcription decrease the rate of H2B fast chromatin exchange in U2OS cells. (A) Bar graphs presenting the average normalized levels of GFP-H2B in the free pools at 4 or 7 hpi expressed as a ratio to the average normalized levels in mock-infected cells at 4 or 7 hpi, respectively (dashed line). U2OS cells were transfected with plasmids expressing GFP-H2B. Transfected cells were mock infected or infected with 30 PFU/cell of HSV-1 strain n212. Nuclear mobility of GFP-H2B was examined from 4 to 5 (4) or 7 to 8 (7) hpi by FRAP. Error bars, standard errors of the means. (B) Frequency distribution plots showing the percentage of free GFP-H2B in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized level of free GFP-H2B in mock-infected cells. (C) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars, standard errors of the means. (D) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. **, $P < 0.01$; *, $P < 0.05$.

changes were not significant. The average rate of H2B fast chromatin exchange was only marginally lower than that in mock-infected cells at 4 or 7 hpi ($96\% \pm 6\%$ or $90\% \pm 8\%$, respectively; $P > 0.1$, one-tailed Student's *t* test) (Fig. 9C). Thus, the increase in free H2B during n212 infections is not largely due to altered rates of fast chromatin exchange of the weakly bound H2B population.

U2OS cells also complement the replication defects of n212. Accordingly, H2B was also mobilized in U2OS cells infected with 30 PFU/cell of HSV-1 strain n212 (Fig. 10). The average level of free H2B was increased to $141\% \pm 6\%$ or $162\% \pm 7\%$ at 4 or 7 h, respectively, after infection with n212 ($P < 0.01$, one-tailed Student's *t* test) (Fig. 10A). Surprisingly, the average increase in free H2B in U2OS cells was greater during n212 infection than during KOS infections (at 4 hpi, $P < 0.05$ or 0.01 for 15 or 6 PFU/cell KOS, respectively; at 7 hpi, $P < 0.01$ for 6 PFU/cell KOS; Tukey's HSD) although the cells were infected with lower multiplicities of KOS due to the nuclear distortion at later times after infection with higher multiplicities. The progression of infection may therefore contribute to the degree of increase in free H2B. Alternatively, ICP0 may partially counteract the relative increase to the pool of free H2B.

The average rate of H2B fast chromatin exchange was decreased to $66\% \pm 5\%$ at 4 or to $69\% \pm 7\%$ at 7 h after infection of U2OS cells with HSV-1 strain n212 ($P < 0.05$,

one-tailed Student's *t* test) (Fig. 10C). ICP0 is therefore not required to decrease the rate of fast H2B chromatin exchange in U2OS cells. This suggests that VP16, in conjunction with HSV-1 transcription or IE or E proteins, decreases H2B fast chromatin exchange.

Taken together, these data are most consistent with the enhanced late mobilization of H2B requiring HSV-1 transcription or IE (other than ICP0) or E proteins. Additionally, ICP0 diminishes the relative increases to the pools of free H2B. However, ICP0 does not do so by directly altering the fast chromatin exchange rate of the weakly bound H2B population.

H2B basal mobilization requires nuclear native HSV-1 genomes. H2B was still mobilized to a basal degree under conditions of little HSV-1 protein expression (KM110 infection of Vero cells). Virion fusion with the host cell, capsid and tegument proteins (other than VP16 or ICP0), or nuclear delivery of HSV-1 genomes is therefore sufficient to induce this basal mobilization. We next evaluated whether infection with UV-inactivated virions was sufficient to mobilize H2B. H2B was not mobilized at 4 h and was only slightly mobilized at 7 h in Vero or U2OS cells infected with the equivalent of 30 PFU/cell of UV-inactivated HSV-1 strain KOS (Fig. 11). The average level of free H2B was marginally increased to $106\% \pm 4\%$ or $115\% \pm 4\%$ at 7 h in U2OS or Vero cells, respectively ($P < 0.05$, one-tailed Student's *t* test) (Fig. 11A). In Vero cells, the later increase in the average level of free H2B is less than that

FIG. 11. Transcription-competent HSV-1 genomes are required to mobilize H2B. (A) Bar graphs presenting the average normalized levels of GFP-H2B in the free pools at 4 or 7 hpi expressed as a ratio to the average normalized levels in mock-infected cells at 4 or 7 hpi, respectively (dashed lines). Vero or U2OS cells were transfected with plasmids expressing GFP-H2B. Transfected cells were mock infected or infected with 30 PFU/cell of UV-inactivated HSV-1 strain KOS. Nuclear mobility of GFP-H2B was examined from 4 to 5 (4) or 7 to 8 (7) hpi by FRAP. Error bars, standard errors of the means. (B) Frequency distribution plots showing the percentage of free GFP-H2B in each cell at 4 or 7 hpi. The vertical dotted lines indicate 1 SD above the average normalized level of free GFP-H2B in mock-infected cells. (C) Bar graphs presenting the average initial rates of normalized fluorescence recovery expressed as a ratio to the average initial rate of normalized fluorescence recovery in mock-infected cells at 4 hpi. Error bars, standard errors of the means. (D) Frequency distribution plots showing the initial rate of normalized fluorescence recovery in each cell. The vertical dotted lines indicate 1 SD below the average initial rate of normalized fluorescence recovery in mock-infected cells. *, $P < 0.05$; n.s., not significant.

which occurs under conditions of little HSV-1 protein expression (KM110 infections; $P < 0.01$, Tukey's HSD) and was achieved without significant alteration in the average rate of H2B fast chromatin exchange (Fig. 11A and C, Vero). Nonetheless, the population of cells with a large increase in their individual pools of free H2B was still greater than expected for a normal distribution, i.e., 40% or 32% in Vero or U2OS cells, respectively. Virion fusion, capsid and tegument proteins, or cross-linked HSV-1 genomes therefore marginally mobilize H2B, a mobilization that becomes evident only at late times.

However, infection with UV-inactivated HSV-1 strain KOS is not sufficient to induce even the degree of H2B basal mobilization that occurs during infections with HSV-1 mutants impaired (though not completely defective) in expression of all genes (KM110).

These data demonstrate that virion fusion, capsid and tegument proteins, or cross-linked HSV-1 genomes alter H2B mobility, without significantly altering the rate of H2B fast chromatin exchange, such that the pool of free H2B is marginally increased and only at late times. However, they are not

sufficient to induce the full early basal mobilization of H2B. Non-cross-linked (transcription competent) nuclear HSV-1 genomes are therefore required to induce the minimal basal mobilization of H2B during HSV-1 infection.

DISCUSSION

In summary, here we show that core histones H2B and H4 are mobilized during HSV-1 infection. This mobilization increases the free pools of H2B and H4 and decreases the rate of H2B fast chromatin exchange. Basal mobilization of H2B requires only nuclear non-cross-linked HSV-1 genomes and occurs independently of HSV-1 transcription, specific HSV-1 proteins, or HSV-1 DNA replication. Specific IE or E proteins, however, enhance H2B mobility. This mobilization of the core histones is most consistent with the previously observed mobilization of linker histone H1 (7) and occurs under the same conditions. The mobilization of histones during HSV-1 infection is also consistent with a model in which the histones that first associate with HSV-1 genomes are those that were previously bound in cellular chromatin. The observed mobilization of core histones H2B and H4 is also most consistent with the assembly of histones and HSV-1 genomes into unstable nucleosomes (20). Additionally, unstable histone binding provides a possible mechanism for the disassociation of histones from progeny HSV-1 genomes before encapsidation.

The FRAP kinetics of the GFP-H2B and GFP-H4 fusion proteins used in this study were similar to those previously reported for core histones (11, 17). Vero cells transiently expressing GFP-H4 had a level of free H4 (approximately 16%) similar to that previously reported for HeLa cells stably expressing it (just less than 16%) (17). Vero cells transiently expressing GFP-H2B also had similar levels of free H2B (17%). This level was slightly less than that observed for U2OS cells transiently expressing GFP-H2B (21%) or HeLa cells stably expressing H2B-GFP (24%). These levels of free H2B that we observed were all similar to those reported for other core histones, which typically range between 10 and 20% (11, 50), but greater than the level reported for another HeLa cell line stably expressing H2B-GFP (17). However, the level of free H2B-GFP (4%) in this particular cell line is the lowest ever reported for any core histone. The nuclear expression and chromatin incorporation of the GFP-H2B and GFP-H4 fusion proteins used in this study (Fig. 1A), in combination with their consistent FRAP kinetics, support the suitability of our approach to study histone mobility during HSV-1 infection. Although it would be interesting to evaluate histone mobility by other techniques, FRAP is currently the only method that permits the evaluation of the highly mobile or most unstably bound histone populations.

Although linker H1 (7) and core H2B are most similarly mobilized, it is not feasible to distinguish whether mobilization of one results in mobilization of the other or whether they are mobilized independently. Mobilization of H2B and destabilization of its chromatin binding would also destabilize H1 chromatin binding, thus mobilizing it. Likewise, mobilization of H1 and destabilization of its chromatin binding result in a more flexible nucleosome, which in turn promotes H2B mobilization.

The observed increases in the pools of free H2B and H4

were somewhat surprising. The number of potential H2B or H4 chromatin binding sites is actually greater in HSV-1-infected cells than in noninfected ones. At an MOI of 30, the total DNA in HSV-1 virions adds up to almost 20% of the cellular genome (assuming a particle-to-PFU ratio of 100, cells infected at an MOI of 30 are presented with approximately 4.5×10^8 base pairs of HSV-1 DNA). The increases to the pools of free H2B and H4 during HSV-1 infection indicate that there is, however, a decrease in the number of effective binding sites for H2B and H4. Once unbound, histones in infected cells are therefore more likely to remain unbound than they are to stably rebind chromatin. These results are consistent with histones in infected cells being assembled into most unstable nucleosomes (20) or even being prevented from rebinding viral or cellular chromatin altogether.

Given the strong association between DNA replication and core histone chromatin exchange (reviewed in reference 39), it was surprising that HSV-1 DNA replication is not required to mobilize H2B. The pool of free H2B was increased, and the rate of H2B fast chromatin exchange was decreased, by a similar degree at late times regardless of whether HSV-1 DNA replication was inhibited or not (Fig. 5). H2B was also mobilized by PAA in mock-infected cells (free H2B increased to $119\% \pm 6\%$) (Fig. 5B). However, it is unlikely that the mobilization in HSV-1-infected cells was exclusively the result of this direct effect of PAA on the cell. Free H2B increased far less in mock-infected than in HSV-1-infected cells treated with PAA (119% or 158%, respectively; $P < 0.01$, Tukey's HSD) (Fig. 5B). Moreover, H2B was mobilized to basically the same extent in HSV-1-infected untreated cells or cells treated with PAA. Consistently, the mobilization of H1 and the decrease in histone (H3) occupancy on HSV-1 genomes at late times are also independent of HSV-1 DNA replication (6, 7).

HSV-1 infection induces DNA damage responses, which promote H2B chromatin exchange and could therefore mobilize H2B. During HSV-1 infection, certain proteins in the homologous recombination (HR) and nonhomologous end-joining (NHEJ) DNA repair pathways are activated and even recruited to HSV-1 replication compartments (2, 24, 43, 52, 53), whereas others are sequestered away from replication compartments or even degraded (22, 24–26, 36, 53). As a result, conventional signaling through both the HR and NHEJ DNA repair pathways is disrupted. Whether signaling through disjointed DNA repair pathways can mobilize histones is yet unknown.

H2B is largely not mobilized by HSV-1 transcription-dependent mechanisms. Free H2B was increased to a similar degree at 4 h after KOS or KM110 infections of Vero cells despite much higher levels of HSV-1 transcription (as indirectly evaluated by ICP4 expression) in KOS-infected cells. ICP4 was detected in only 13% of KM110-infected cells, whereas it was detected in 75% of KOS-infected ones. Furthermore, the individual pools of free H2B were increased by a large degree in 50% of KM110-infected cells, whereas only 13% of cells had detectable ICP4 expression. Therefore, HSV-1 transcription (as indirectly evaluated by ICP4 expression) does not correlate with the degree of increase in free H2B, nor is it required to increase free H2B. The mobilization of H2B independently of viral gene transcription is also consistent with previous results. Histones are associated with HSV-1 genes of all kinetic classes

FIG. 12. A model for the mobilization of core histones during HSV-1 infection. (A) In noninfected cells, core histones normally undergo chromatin exchange, some of which is associated with transcription. (B) Following entry of HSV-1 genomes, the pools of free core histones increase. These free core histones are available for assembly into unstable nucleosomes with parental HSV-1 genomes and promote HSV-1 gene silencing. (C) HSV-1 IE proteins counteract the cellular responses, perhaps to promote HSV-1 gene expression. ICP0 likely degrades free core histones, which decreases the population available to bind to HSV-1 genomes. Other IE or E proteins increase the rates of core histone chromatin exchange, perhaps only from HSV-1 genomes, to promote HSV-1 gene expression. (D) Core histones are removed from HSV-1 genomes as they are encapsidated, further increasing their free pools. The chromatin exchange of core histones also decreases, possibly due to an inability to undergo exchange with condensed cellular chromatin or to rebind to the encapsidated HSV-1 genomes.

as early as 1 h after infection (10, 15, 19, 37). The assembly of nucleosomes on E and L genes at these early times still requires histones to be available even though these genes are not yet transcribed to any significant extent. H2B is not mobilized as a result of indirect effects mediated by cellular transcription either. Most cellular transcription is inhibited during HSV-1 infection, and core histones are typically mobilized by ongoing transcription. H2B is therefore mobilized independently of HSV-1 DNA replication, conventional DNA repair pathways, HSV-1 protein expression (as indirectly evaluated by ICP4 expression), or active cellular transcription.

The average pool of free H2B was increased to a greater degree during infection of U2OS cells with an ICP0 mutant strain than with wild-type HSV-1 (141% versus 114% or 162% versus 124% at 4 or 7 h, respectively) (Fig. 10A and

8A). Even during n212 infection of Vero cells, in which ICP0 mutants replicate with delayed kinetics, the average pool of free H2B was increased to a similar degree as during wild-type infections (120% versus 133% or 167% versus 169% at 4 or 7 h, respectively) (Fig. 3B, H2B, and 9A). These results are consistent with ICP0 decreasing the levels of H2B in the free pools. ICP0 is an E3 ubiquitin ligase that promotes proteasome-mediated degradation of, among others, a variant of histone H3, centromeric protein A (27). It is not unreasonable to envision that ICP0 may also promote the degradation of other histones. If degradation preferentially involved free histones, then it would result in lower levels of H2B in the free pools. Normally, however, ubiquitination of H2B regulates gene expression rather than targeting it for proteasomal degradation (reviewed in refer-

ence 44). Moreover, the degradation of ubiquitinated H2B during infection occurs independently of ICP0 (26).

Under conditions of robust HSV-1 replication, the pools of free H2B are increased, whereas the rate of H2B chromatin exchange is decreased. Such effects support a model in which a population of free histones is unable to stably rebind chromatin. HSV-1 genomes are also somehow stripped of their associated histones prior to, or during, encapsidation. Such removal of histones from encapsidating HSV-1 DNA would increase the level of histones in the free pool. Encapsidated HSV-1 DNA is no longer available, resulting in fewer potential binding sites for the removed histones. Furthermore, cellular chromatin is marginalized at these late times (30), which is expected to decrease the accessibility of cellular chromatin, further decreasing the binding sites available to free histones. The probability that histones undergo further chromatin exchange after becoming part of the free pool is, consequently, likely negligible at these late times.

Based on the results presented here and those previously published by other investigators, we propose the following model (Fig. 12). Upon nuclear entry of the infecting HSV-1 genomes, cellular responses increase the level of free histones, making them available for binding to HSV-1 genomes. Such cellular responses decrease the rate of core histone chromatin exchange, perhaps in an attempt to minimize the disassociation of histones with HSV-1 genomes and thus promoting HSV-1 silencing (Fig. 12B). HSV-1 IE proteins counteract these cellular silencing responses. ICP0 likely promotes the degradation of free histones (H2B), decreasing the population available to bind to, and silence, HSV-1 genomes. Other IE or E proteins increase the rates of core histone chromatin exchange, probably from the viral genomes, promoting HSV-1 gene expression (Fig. 12C). This promotion of histone chromatin exchange is consistent with the observed instability of the HSV-1 chromatin at 5 hpi (20). Late during HSV-1 infection, the pools of free histones further increase as the rates of histone chromatin exchange decrease (Fig. 12D). This later increase would correspond with the removal of core histones from HSV-1 genomes for their encapsidation and the consequent decrease in the number of effective binding sites. The free histones at these late times are prevented from reassociating with naked HSV-1 genomes, potentially by VP16, and are also likely unable to undergo exchange with condensed cellular chromatin. Overall, these effects result in reduced core histone chromatin exchange and an increase in free core histones at such late times.

ACKNOWLEDGMENTS

This work was supported by the Canadian Institute for Health Research and the Burroughs Wellcome Fund (BWF). L.M.S. is a BWF Investigator in the Pathogenesis of Infectious Disease.

REFERENCES

- Amelio, A. L., P. K. McAnany, and D. C. Bloom. 2006. A chromatin insulator-like element in the herpes simplex virus type 1 latency-associated transcript region binds CCCTC-binding factor and displays enhancer-blocking and silencing activities. *J. Virol.* **80**:2358–2368.
- Balasubramanian, N., P. Bai, G. Buchek, G. Korza, and S. K. Weller. 2010. Physical interaction between the herpes simplex virus type 1 exonuclease, UL12, and the DNA double-strand break-sensing MRN complex. *J. Virol.* **84**:12504–12514.
- Bloom, D. C., N. V. Giordani, and D. L. Kwiatkowski. 2010. Epigenetic regulation of latent HSV-1 gene expression. *Biochim. Biophys. Acta* **1799**: 246–256.
- Cai, W. Z., and P. A. Schaffer. 1989. Herpes simplex virus type 1 ICP0 plays a critical role in the de novo synthesis of infectious virus following transfection of viral DNA. *J. Virol.* **63**:4579–4589.
- Cliffe, A. R., D. A. Garber, and D. M. Knipe. 2009. Transcription of the herpes simplex virus latency-associated transcript promotes the formation of facultative heterochromatin on lytic promoters. *J. Virol.* **83**:8182–8190.
- Cliffe, A. R., and D. M. Knipe. 2008. Herpes simplex virus ICP0 promotes both histone removal and acetylation on viral DNA during lytic infection. *J. Virol.* **82**:12030–12038.
- Conn, K. L., M. J. Hendzel, and L. M. Schang. 2008. Linker histones are mobilized during infection with herpes simplex virus type 1. *J. Virol.* **82**: 8629–8646.
- Deshmane, S. L., and N. W. Fraser. 1989. During latency, herpes simplex virus type 1 DNA is associated with nucleosomes in a chromatin structure. *J. Virol.* **63**:943–947.
- Gibson, W., and B. Roizman. 1971. Compartmentalization of spermine and spermidine in the herpes simplex virion. *Proc. Natl. Acad. Sci. U. S. A.* **68**:2818–2821.
- Herrera, F. J., and S. J. Triezenberg. 2004. VP16-dependent association of chromatin-modifying coactivators and underrepresentation of histones at immediate-early gene promoters during herpes simplex virus infection. *J. Virol.* **78**:9689–9696.
- Higashi, T., et al. 2007. Histone H2A mobility is regulated by its tails and acetylation of core histone tails. *Biochem. Biophys. Res. Commun.* **357**:627–632.
- Huang, J., et al. 2006. Trimethylation of histone H3 lysine 4 by Set1 in the lytic infection of human herpes simplex virus 1. *J. Virol.* **80**:5740–5746.
- Kanda, T., K. F. Sullivan, and G. M. Wahl. 1998. Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. *Curr. Biol.* **8**:377–385.
- Kawai, J., and Y. Hayashizaki. 2003. DNA book. *Genome Res.* **13**:1488–1495.
- Kent, J. R., et al. 2004. During lytic infection herpes simplex virus type 1 is associated with histones bearing modifications that correlate with active transcription. *J. Virol.* **78**:10178–10186.
- Kepert, J. F., J. Mazurkiewicz, G. L. Heuvelman, K. F. Toth, and K. Rippe. 2005. NAP1 modulates binding of linker histone H1 to chromatin and induces an extended chromatin fiber conformation. *J. Biol. Chem.* **280**:34063–34072.
- Kimura, H., and P. R. Cook. 2001. Kinetics of core histones in living human cells: little exchange of H3 and H4 and some rapid exchange of H2B. *J. Cell Biol.* **153**:1341–1353.
- Kutluay, S. B., S. L. DeVos, J. E. Klomp, and S. J. Triezenberg. 2009. Transcriptional coactivators are not required for herpes simplex virus type 1 immediate-early gene expression in vitro. *J. Virol.* **83**:3436–3449.
- Kutluay, S. B., and S. J. Triezenberg. 2009. Regulation of histone deposition on the herpes simplex virus type 1 genome during lytic infection. *J. Virol.* **83**:5835–5845.
- Lacasse, J. J., and L. M. Schang. 2010. During lytic infections, herpes simplex virus type 1 DNA is in complexes with the properties of unstable nucleosomes. *J. Virol.* **84**:1920–1933.
- Latchman, D. S., J. F. Partidge, J. K. Estridge, and L. M. Kemp. 1989. The different competitive abilities of viral TAATGARAT elements and cellular octamer motifs, mediate the induction of viral immediate-early genes and the repression of the histone H2B gene in herpes simplex virus infected cells. *Nucleic Acids Res.* **17**:8533–8542.
- Lees-Miller, S. P., et al. 1996. Attenuation of DNA-dependent protein kinase activity and its catalytic subunit by the herpes simplex virus type 1 transactivator ICP0. *J. Virol.* **70**:7471–7477.
- Lever, M. A., J. P. Th'ng, X. Sun, and M. J. Hendzel. 2000. Rapid exchange of histone H1.1 on chromatin in living human cells. *Nature* **408**:873–876.
- Lilley, C. E., C. T. Carson, A. R. Muotri, F. H. Gage, and M. D. Weitzman. 2005. DNA repair proteins affect the lifecycle of herpes simplex virus 1. *Proc. Natl. Acad. Sci. U. S. A.* **102**:5844–5849.
- Lilley, C. E., M. S. Chaurushiya, C. Boutell, R. D. Everett, and M. D. Weitzman. 2011. The intrinsic antiviral defense to incoming HSV-1 genomes includes specific DNA repair proteins and is counteracted by the viral protein ICP0. *PLoS Pathog.* **7**:e1002084.
- Lilley, C. E., et al. 2010. A viral E3 ligase targets RNF8 and RNF168 to control histone ubiquitination and DNA damage responses. *EMBO J.* **29**: 943–955.
- Lomonte, P., K. F. Sullivan, and R. D. Everett. 2001. Degradation of nucleosome-associated centromeric histone H3-like protein CENP-A induced by herpes simplex virus type 1 protein ICP0. *J. Biol. Chem.* **276**:5829–5835.
- Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent, and T. J. Richmond. 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**:251–260.
- Misteli, T., A. Gunjan, R. Hock, M. Bustin, and D. T. Brown. 2000. Dynamic binding of histone H1 to chromatin in living cells. *Nature* **408**:877–881.
- Monier, K., J. C. Armas, S. Etteldorf, P. Ghazal, and K. F. Sullivan. 2000. Annexation of the interchromosomal space during viral infection. *Nat. Cell Biol.* **2**:661–665.

31. Mossman, K. L., and J. R. Smiley. 1999. Truncation of the C-terminal acidic transcriptional activation domain of herpes simplex virus VP16 renders expression of the immediate-early genes almost entirely dependent on ICP0. *J. Virol.* **73**:9726–9733.
32. Nelson, D. M., et al. 2002. Coupling of DNA synthesis and histone synthesis in S phase independent of cyclin/cdk2 activity. *Mol. Cell. Biol.* **22**:7459–7472.
33. Nevels, M., A. Nitzsche, and C. Paulus. 2011. How to control an infectious bead string: nucleosome-based regulation and targeting of herpesvirus chromatin. *Rev. Med. Virol.* **21**:154–180.
34. Oh, J., and N. W. Fraser. 2008. Temporal association of the herpes simplex virus genome with histone proteins during a lytic infection. *J. Virol.* **82**:3530–3537.
35. Okazaki, Y., et al. 2002. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature* **420**:563–573.
36. Parkinson, J., S. P. Lees-Miller, and R. D. Everett. 1999. Herpes simplex virus type 1 immediate-early protein Vmw110 induces the proteasome-dependent degradation of the catalytic subunit of DNA-dependent protein kinase. *J. Virol.* **73**:650–657.
37. Placek, B. J., et al. 2009. The histone variant H3.3 regulates gene expression during lytic infection with herpes simplex virus type 1. *J. Virol.* **83**:1416–1421.
38. Preston, C. M., and M. J. Nicholl. 1997. Repression of gene expression upon infection of cells with herpes simplex virus type 1 mutants impaired for immediate-early protein synthesis. *J. Virol.* **71**:7807–7813.
39. Ransom, M., B. K. Dennehey, and J. K. Tyler. 2010. Chaperoning histones during DNA replication and repair. *Cell* **140**:183–195.
40. Samanigo, L. A., L. Neiderhiser, and N. A. DeLuca. 1998. Persistence and expression of the herpes simplex virus genome in the absence of immediate-early proteins. *J. Virol.* **72**:3307–3320.
41. Schang, L. M., A. Rosenberg, and P. A. Schaffer. 2000. Roscovitine, a specific inhibitor of cellular cyclin-dependent kinases, inhibits herpes simplex virus DNA synthesis in the presence of viral early proteins. *J. Virol.* **74**:2107–2120.
42. Schek, N., and S. L. Bachenheimer. 1985. Degradation of cellular mRNAs induced by a virion-associated factor during herpes simplex virus infection of Vero cells. *J. Virol.* **55**:601–610.
43. Shirata, N., et al. 2005. Activation of ataxia telangiectasia-mutated DNA damage checkpoint signal transduction elicited by herpes simplex virus infection. *J. Biol. Chem.* **280**:30336–30341.
44. Shukla, A., P. Chaurasia, and S. R. Bhaumik. 2009. Histone methylation and ubiquitination with their cross-talk and roles in gene expression and stability. *Cell. Mol. Life Sci.* **66**:1419–1433.
45. Simpson-Holley, M., J. Baines, R. Roller, and D. M. Knipe. 2004. Herpes simplex virus 1 *U_L31* and *U_L34* gene products promote the late maturation of viral replication compartments to the nuclear periphery. *J. Virol.* **78**:5591–5600.
46. Simpson-Holley, M., R. C. Colgrove, G. Nalepa, J. W. Harper, and D. M. Knipe. 2005. Identification and functional evaluation of cellular and viral factors involved in the alteration of nuclear architecture during herpes simplex virus 1 infection. *J. Virol.* **79**:12840–12851.
47. Smith, K. O. 1964. Relationship between the envelope and the infectivity of herpes simplex virus. *Proc. Soc. Exp. Biol. Med.* **115**:814–816.
48. Sorenson, C. M., P. A. Hart, and J. Ross. 1991. Analysis of herpes simplex virus-induced mRNA destabilizing activity using an in vitro mRNA decay system. *Nucleic Acids Res.* **19**:4459–4465.
49. Th'ng, J. P., R. Sung, M. Ye, and M. J. Hendzel. 2005. H1 family histones in the nucleus. Control of binding and localization by the C-terminal domain. *J. Biol. Chem.* **280**:27809–27814.
50. Vogler, C., et al. 2010. Histone H2A C terminus regulates chromatin dynamics, remodeling, and histone H1 binding. *PLoS Genet.* **6**:e1001234.
51. Wang, Q. Y., et al. 2005. Herpesviral latency-associated transcript gene promotes assembly of heterochromatin on viral lytic-gene promoters in latent infection. *Proc. Natl. Acad. Sci. U. S. A.* **102**:16055–16059.
52. Wilkinson, D. E., and S. K. Weller. 2004. Recruitment of cellular recombination and repair proteins to sites of herpes simplex virus type 1 DNA replication is dependent on the composition of viral proteins within prereplicative sites and correlates with the induction of the DNA damage response. *J. Virol.* **78**:4783–4796.
53. Wilkinson, D. E., and S. K. Weller. 2006. Herpes simplex virus type I disrupts the ATR-dependent DNA-damage response during lytic infection. *J. Cell Sci.* **119**:2695–2703.
54. Wysocka, J., M. P. Myers, C. D. Laherty, R. N. Eisenman, and W. Herr. 2003. Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3-K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1. *Genes Dev.* **17**:896–911.
55. Yager, D. R., and S. L. Bachenheimer. 1988. Synthesis and metabolism of cellular transcripts in HSV-1 infected cells. *Virus Genes* **1**:135–148.