Epigenetic Repression of Herpes Simplex Virus Infection by the Nucleosome Remodeler CHD3

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ABSTRACT **Upon infection, the genome of herpes simplex virus is rapidly incorporated into nucleosomes displaying histone modifications characteristic of heterochromatic structures. The initiation of infection requires complex viral-cellular interactions that ultimately circumvent this repression by utilizing host cell enzymes to remove repressive histone marks and install those that promote viral gene expression. The reversion of repression and activation of viral gene expression is mediated by the cellular coactivator HCF-1 in association with histone demethylases and methyltransferases. However, the mechanisms and the components that are involved in the initial repression remain unclear. In this study, the chromatin remodeler chromodomain helicase DNA binding (CHD3) protein is identified as an important component of the initial repression of the herpesvirus genome. CHD3 localizes to early viral foci and suppresses viral gene expression. Depletion of CHD3 results in enhanced viral immediate early gene expression and an increase in the number of transcriptionally active viral genomes in the cell. Importantly, CHD3 can recognize the repressive histone marks that have been detected in the chromatin associated with the viral genome and this remodeler is important for ultimately reducing the levels of accessible viral genomes. A model is presented in which CHD3 represses viral infection in opposition to the actions of the HCF-1 coactivator complex. This dynamic, at least in part, determines the initiation of viral infection.**

IMPORTANCE **Chromatin modulation of herpesvirus infection is a dynamic process involving regulatory components that mediate suppression and those that promote viral gene expression and the progression of infection. The mechanisms by which the host cell employs the assembly and modulation of chromatin as an antiviral defense strategy against an invading herpesvirus remain unclear. This study defines a critical cellular component that mediates the initial repression of infecting HSV genomes and contributes to understanding the dynamics of this complex interplay between host cell and viral pathogen.**

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Herpes simplex virus (HSV) infection results in disease ranging from recurrent lesions to more significant neurological complications. Additionally, HSV remains the leading cause of virus-mediated blindness (herpetic keratitis) in the developed world. The initial lytic infection leads to the establishment of a lifelong reservoir of virus that is maintained in a quiescent or latent state in neurons of sensory ganglia. Periodically, this latency is interrupted and the virus reenters the lytic replication cycle to produce recurrent disease (1, 2).

The lytic replication cycle is determined by the sequential expression of three major classes of viral genes. The regulated pattern of viral transcription is controlled by specific viral and cellular transcription factors and coactivators (1, 3). However, in addition to these, the viral genome is also subject to the regulatory control that results from the modulation of the viral chromatin structure (4–21). While the genome is nonnucleosomal within the viral capsid (22), it becomes rapidly assembled into chromatin upon infection (6, 15). This initial stage is dynamic and involves a complex interplay of host cell and pathogen regulatory factors that ultimately determine the progression of infection.

Similar to the lytic replication cycle, the pattern of viral latency and recurrent reactivation is also determined by the chromatin state of the virus (12, 23–30). In latency, the genome is quiescent, and nucleosomes associated with lytic genes bear repressive histone marks (12, 24, 25, 28, 30, 31). This state transitions to transcriptionally permissive chromatin upon viral reactivation (12, 23, 26, 27, 29). Thus, the dynamic chromatin modulation of the viral genome is a critical regulatory determinant of both the lytic and latency reactivation cycles of the virus.

Immediately postinfection of a host cell, the nucleosomes that are assembled on the viral genome exhibit histone marks characteristic of repressive heterochromatin (histone H3-lysine 9 and 27 methylation) (17, 18, 21). This initial cell-mediated antiviral repression can be circumvented by viral (VP16) and cellular (e.g., GABP, Sp1, Oct-1) transcription factors that recognize the immediate early (IE) promoter domains. These factors recruit an essential cellular coactivator complex that contains HCF-1 coupled with two histone H3K9 demethylases (LSD1 and JMJD2 proteins) and a histone H3K4 methyltransferase (Setd1A or MLL1) (Fig. 1) (9, 16–18). Thus, the HCF-1 coactivator complex contains the

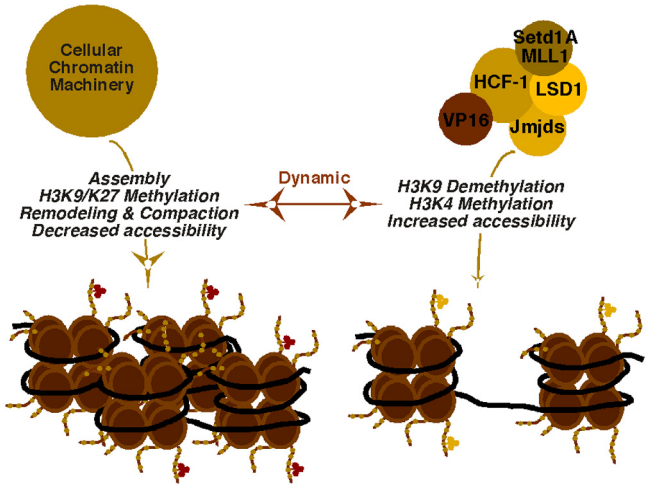

FIG 1 Repression and activation of the HSV genome through opposing chromatin modulation activities. Upon infection, the HSV genome is subject to impacts of cellular chromatin modulation machinery that suppresses the virus by the assembly of the genome into heterochromatic structures. In opposition to this, the HCF-1 coactivator complex contains histone modification activities that circumvent the accumulation of repressive marks and promote the installation of positive marks.

activities required to remove repressive H3K9 methylation and install activating H3K4 methylation marks at IE promoters. Inhibition of the activities of either of the HCF-1-associated demethylases results in enhanced epigenetic suppression of the viral genome and a block to the progression of infection (16, 17, 32).

It is clear that the recruitment of HCF-1 chromatin modulation activities is critical to the initiation of viral IE expression and the progression of infection. However, the components and mechanisms involved in the dynamic chromatin regulation remain unknown, including those that mediate the stages of nucleosome assembly, modification, and remodeling that are the basis for the initial cell-mediated suppression of the viral genome.

Here, members of the chromodomain helicase DNA-binding (CHD) nucleosome remodeler family were assessed for their potential role in mediating HSV early chromatin dynamics. This family is characterized by the presence of two tandem chromodomains (chromatin organizing domains) and an SNF2-related helicase/ATPase domain (33–35). While specific functions or targets of many of the members are unknown, some have been linked to chromatin remodeling, leading to either transcriptional activation or repression.

Of the family members, CHD3 but not the highly related CHD4 nucleosome remodeler was found to be specifically involved in initial suppression of HSV-1. Depletion of CHD3 resulted in enhanced accessibility of the viral genome with a concomitant increase in viral IE gene transcription. Strikingly, CHD3 appears to play a significant role in mediating repression of a large percentage of the infecting viral genomes. The data support the model whereby CHD3 functions in opposition to the activities of the HCF-1 coactivator complex in the chromatin dynamics of the infecting virus.

RESULTS

Chromatin-mediated repression of the HSV genome and reversion by the HCF-1 coactivator complex. Upon infection, the HSV-1 genome is repressed by the assembly of nucleosomes that exhibit heterochromatic histone signatures. Initiation of infection depends upon modulating this repressive chromatin to allow expression of the viral immediate early genes and the progression of infection. This initial interaction suggests a dynamic process between the cellular epigenetic machinery that promotes silencing of the genome and the factors that function to counteract this suppression.

It is clear that an important aspect of the viral regulatory paradigm that results in reversing the accumulation of repressive chromatin on the viral genome is the recruitment of the HCF-1 coactivator complex to the viral IE gene enhancer-promoter domains. In contrast, the components and mechanisms involved in the initial cell-mediated repression of the genome remain unclear. The initial dynamic would require nucleosome remodelers that could function to arrange the genome into ordered and compacted heterochromatin or, conversely, would provide access for the DNA binding factors and coactivators that promote viral gene expression.

Depletion of CHD3 but not the highly related CHD4 nucleosome remodeler enhances HSV IE gene expression. The family of CHD remodelers consists of nine related members that share domains involved in chromatin recognition (histone modifications) and ATP-dependent nucleosome remodeling (Fig. 2A) (33–35). However, little is known of the functions of this family, especially with respect to modulation of chromatin associated with infecting viral genomes. To investigate the potential roles of CHD proteins in the initial dynamics of chromatin associated with the HSV genome, cells were depleted of each member of the family using small interfering RNA (siRNA) pools and subsequently infected with HSV-1 for 2 h. The levels of the viral IE ICP27 and cellular control GAPDH mRNAs were determined by quantitative reverse transcription-PCR (qRT-PCR). Of the CHD family, depletion of CHD3 resulted in the consistent increase in viral ICP27 expression without any significant impact on the cellular control GAPDH (Fig. 2B; see also Fig. S1A in the supplemental material).

The role of CHD3 in repression of viral gene expression was confirmed by depletion of CHD3 or the highly related CHD4 using siRNA pools and two independent individual siRNAs (Fig. 2C to F; see also Fig. S1B to D in the supplemental material). Depletion of CHD3 but not CHD4 resulted in enhanced viral IE (ICP0, ICP4, ICP22, ICP27; 1.5- to 2.4-fold) and E (UL29, UL30, UL52; 2.0- to 3.5-fold) expression. The expression of ICP27 in cells transfected with individual CHD3 or CHD4 siRNAs paralleled that in cells transfected with the respective siRNA pools. No impact of either CHD3 or CHD4 depletion was seen on the expression of the cellular control TATA binding protein (TBP). In addition to mRNA analyses, quantitative Western blots confirmed clear increases in levels of viral IE proteins upon transfection of CHD3 siRNAs (2.5- to 5-fold), relative to that of control siRNAs or CHD4 siRNAs (Fig. 2G).

CHD3 mediates repression of a large population of HSV genomes upon infection. Enhanced expression of viral IE genes upon depletion of CHD3 suggests that this CHD family member may function in the initial chromatin-mediated repression of the viral genome. As shown in Fig. 3A, enhanced viral IE gene expression in CHD3-depleted cells can be readily detected at the earliest time postinfection (30 min) compared to control or CHD4- or CHD6-depleted cells. No significant impacts were seen on the cellular control gene (TBP gene; see Fig. S2 in the supplemental

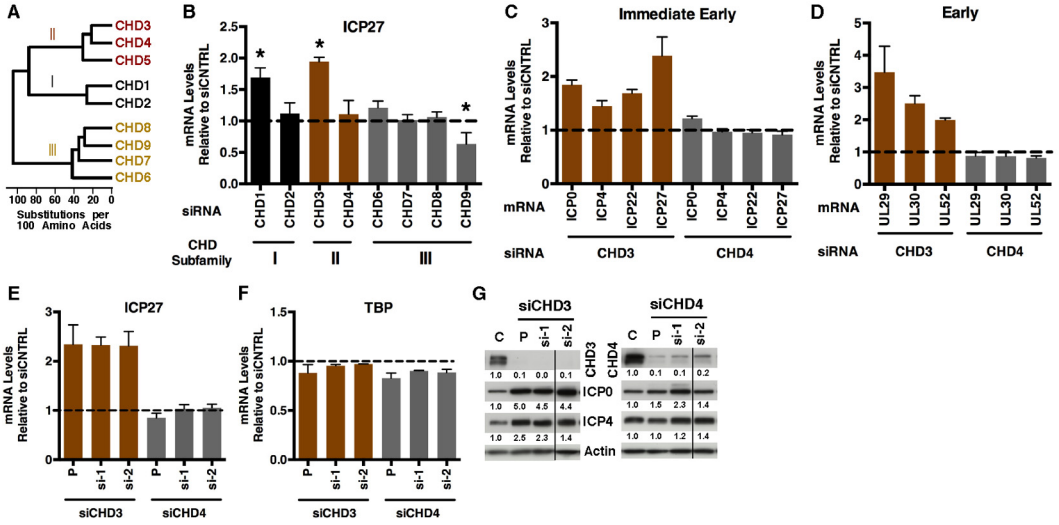

FIG 2 Depletion of chromodomain helicase DNA binding (CHD) proteins identifies CHD3 as a repressor of HSV-1 IE gene expression. (A) Phylogenetic tree of human CHD proteins generated by the Clustal W method. (B) HeLa cells were depleted of CHD proteins using siRNA pools and infected with HSV-1 (1.0 PFU/cell) for 2 h. The levels of viral IE ICP27 mRNA are expressed as ratios to the levels in control siRNA (siCntrl)-treated cells. *, significant difference relative to siRNA control (2-tailed *t* test, $n = 4$). (C and D) HeLa cells were depleted of CHD3 or CHD4, followed by infection with HSV-1 (1.0 PFU/cell) for 1.5 h. The levels of viral IE (ICP0, ICP4, ICP22, ICP27) and E (UL29, UL30, UL52) mRNAs are expressed as ratios to the levels in siCntrl-treated cells. (E to F) HeLa cells were depleted of CHD3 and CHD4 proteins using siRNA pools (p) or individual siRNAs (si-1, si-2) and infected with HSV-1 (1.0 PFU/cell) for 1.5 h. The levels of viral (ICP27) and cellular (TBP) control mRNAs are expressed as ratios to the levels in control siRNA-treated cells. (G) Western blot of IE proteins (ICP0, ICP4) in CHD3- or CHD4-depleted cells. The ratios to levels in control siRNA-treated cells are shown and are normalized to the actin-loading control.

material). Thus, CHD3-mediated suppression of viral gene expression must occur rapidly, prior to any significant expression of IE genes. Additionally, the data suggest that CHD3 would be found proximal to early viral foci. This was confirmed by confocal imaging of cells infected with HSV-1 for 1.5 h and stained for CHD3 and the IE protein ICP4, a marker for early viral foci. As shown in Fig. 3B, CHD3 localizes adjacent/juxtaposed to early punctate (Fig. 3B, top) and more developed ICP4 foci (Fig. 3B, bottom). This localization pattern is more clearly evident in the three-dimensional (3-D) volume reconstruction of an infected cell nucleus (Fig. 3C).

As an additional approach to visualize the association of CHD3 with early foci, cells were infected with HSV-1 at a low PFU (0.001) for 24 h. This allows for the completion of one round of the viral lytic cycle and detection of nascent viral foci at the nuclear periphery of the adjacent cell (21). As shown in Fig. 4A, CHD3 was highly localized proximal to the early viral foci.

The impact of CHD3 on viral IE gene expression and the association with early viral foci suggest that CHD3 is an important component of the initial repression of the viral genomes. Thus, CHD3 could be responsible for suppression of a population of the viral genomes that enter a cell. To investigate this, cells were transfected with CHD3, CHD4, or control siRNAs and infected with HSV-1 for 2 h. The cells were stained for ICP4 viral foci, and the numbers of small, medium, and large foci were counted ($n > 270$ cells). Strikingly, depletion of CHD3 but not CHD4 resulted in an increase primarily in the number of small and medium-sized viral foci per cell (Fig. 4B). The substantial increase in the number of small viral foci in CHD3-depleted cells further indicates that this protein is a key component involved in repression of a large percentage (52%) of the infecting viral genomes.

CHD3 depletion compensates for inhibition of the HCF-1-associated histone demethylase LSD1. Reversal of the initial

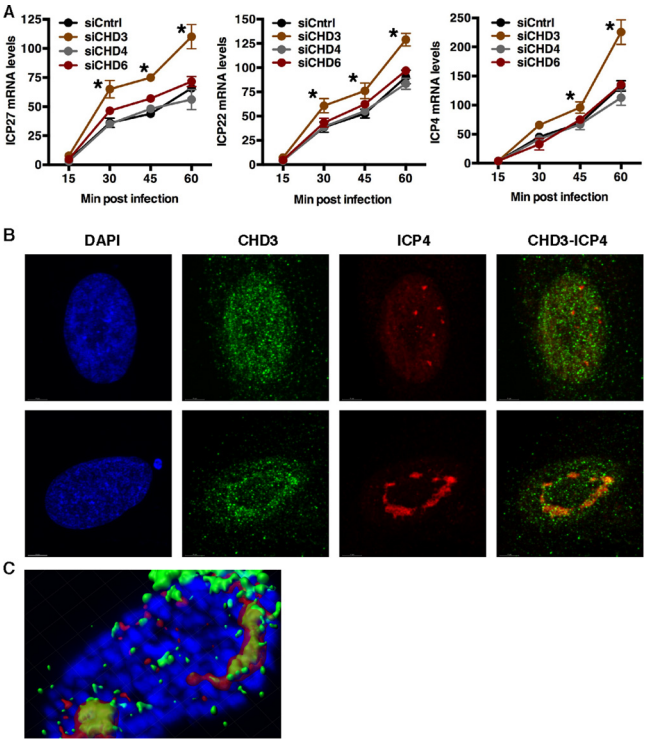

FIG 3 CHD3 localizes to early viral foci during initiation of infection. (A) MRC-5 cells were depleted of CHD3, CHD4, or CHD6 and infected with HSV-1 (1.0 PFU/cell) for the indicated times. The levels of viral IE (ICP27, ICP22, ICP4) mRNAs are shown relative to siRNA control-treated cells. *, significant difference relative to siRNA control (multiple *t* test, $n = 3$). (B and C) Confocal images of MRC-5 cells infected with HSV-1 (10.0 PFU/cell) for 1.5 h. Cells were stained for DAPI, CHD3, and ICP4. The IE protein ICP4 was used as a marker for early viral foci. (C) 3-D volume of an infected cell illustrating the colocalization of CHD3 (green) with ICP4 (red). DAPI, blue.

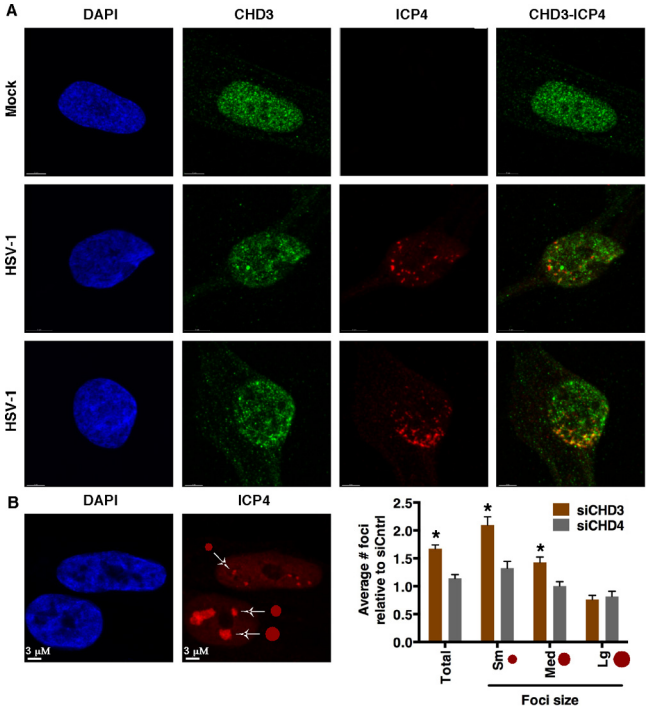

FIG 4 A large percentage of infecting HSV genomes are repressed by CHD3. (A) HFF cells were infected with HSV-1 (0.001 PFU/cell) for 24 h followed by CSK treatment as described in Materials and Methods. Cells were stained for DAPI, CHD3, and ICP4. (B) MRC-5 cells were depleted of CHD3 or CHD4 and infected with HSV-1 (10.0 PFU/cell) for 2 h. The numbers and sizes (small, 0.4 to 1.0 μM; medium, 1.0 to 2.5 μM; large, >2.5 μM) of ICP4 foci are expressed as ratios to those in siRNA control-treated cells (siCntrl) ($n > 270$ cells). *, significant relative to siRNA control (ANOVA with Dunnett's *post hoc* multiple comparisons test, $n = 270$).

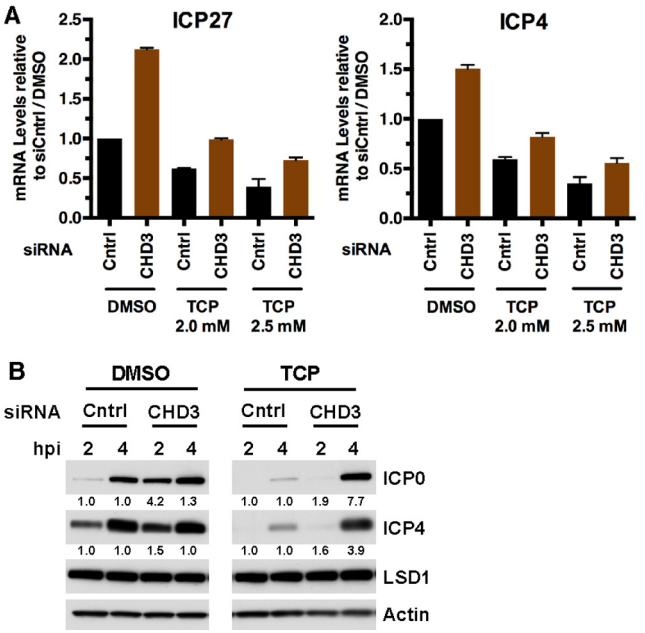

FIG 5 Depletion of CHD3 compensates for TCP-mediated repression of IE gene expression. Control and CHD3-depleted HeLa cells were treated with DMSO or TCP and subsequently infected with HSV-1 (1.0 PFU/cell) for 1.5 h (A) or 2 and 4 h (B). The levels of viral (ICP27, ICP4, ICP0) and cellular control (TBP, LSD1, actin) mRNAs (A) and proteins (B) are expressed as ratios to the levels in siRNA control-treated cells.

chromatin-mediated repression of the viral genome is dependent upon the recruitment of the transcriptional coactivator HCF-1 to the IE promoter domains. This coactivator couples two required histone demethylases that function cooperatively to remove the repressive-heterochromatic H3K9-trimethylation mark. Inhibition of the activity of LSD1 results in enhanced epigenetic repression of the viral genome with increased levels of nucleosomes and repressive histone marks. However, it remains unclear how these marks are recognized and translated into chromatin-based suppression of the viral genome.

Strikingly, the CHD3 chromodomains bind both H3K27-trimethyl and H3K9-trimethyl repressive histone marks (36–38). Therefore, the protein is a candidate for an effector that recognizes repressive histone marks associated with the viral genome and promotes the formation of heterochromatin. With respect to the chromatin dynamics during the initiation of HSV infection, CHD3 would represent an opposing effector to the HCF-1--LSD1 coactivator complex.

To test this hypothesis, control and CHD3-depleted cells were treated with the LSD1 inhibitor tranylcypromine (TCP) or dimethyl sulfoxide (DMSO). As previously demonstrated (17), TCP treatment reduced viral IE gene expression without impact on the cellular control (Fig. 5A; see also Fig. S3 in the supplemental material). However, TCP had a significantly reduced impact on viral IE expression in cells depleted for CHD3 relative to that of control cells. These mRNA results were supported by quantitative Western blots of viral IE and cellular control proteins at 2 and 4 h postinfection (Fig. 5B). The data suggest that depletion of CHD3 can partially compensate for the inhibition of the demethylase LSD1. Thus, CHD3 contributes to the initial repression of the viral genome that is circumvented by the HCF-1--LSD1 coactivator complex.

Depletion of CHD3 increases the levels of accessible HSV genomes. Repressive histone marks (H3K9 and H3K27 methylation) have been clearly detected in analyses of chromatin associated with the HSV genome at early times postinfection. The remodeler CHD3 can recognize these marks and can promote the formation of heterochromatic structures by association with corepressors (HDAC1/2, SETDB1) (39–45). To investigate the impact of CHD3 on the "chromatin structure" of the HSV genome during early infection, the accessibility of the viral genome was measured in formaldehyde-assisted isolation of regulatory element (FAIRE) assays (46–48) (Fig. 6; see also Fig. S4 in the supplemental material). As anticipated, inhibition of LSD1 by TCP treatment significantly reduced the level of soluble viral genomes with a parallel increase in the level found in the FAIRE-insoluble fraction (Fig. 6A; see also Fig. S4B to D). In contrast, depletion of CHD3 resulted in an increase in the level of soluble viral genomes with a parallel decrease in the level in the insoluble fraction (Fig. 6B; see also Fig. S4E to G). Furthermore, and complementary to the data described above, depletion of CHD3 partially compensated for the TCP-mediated reduction in the levels of soluble viral genomes (Fig. 6C). It should be noted that alterations in FAIRE solubility do not necessarily translate directly to alterations in transcriptional levels, which depends upon multiple parameters. This is illustrated by the increased FAIRE solubility of the actively transcribed cellular GAPDH loci in the absence of CHD3 without a

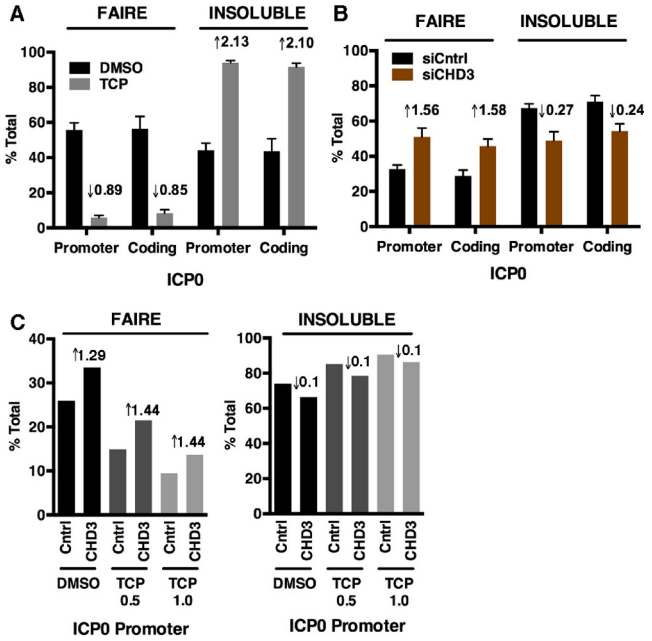

FIG 6 CHD3 promotes FAIRE insolubility of the HSV-1 genome. FAIRE insolubility of the HSV genome is enhanced by TCP treatment and reduced by depletion of CHD3. HeLa cells were infected with HSV-1 (1.0 PFU/cell) for 4 h. The levels of HSV-1 genomes (ICP0) were determined in FAIRE (soluble) and insoluble chromatin (compacted) fractions. (A) Cells were treated with TCP (2.0 mM) for 3 h; (B) cells were transfected with control or CHD3 siRNAs; (C) control or CHD3 siRNA transfected cells were treated with DMSO or TCP. The results are shown relative to the total extracted DNAs.

corresponding increase in transcription (see Fig. S1A and S4F). Thus, in contrast to the viral genome, CHD3 is not a critical component of the regulatory paradigm of this locus. Taken together, the results clearly suggest that the chromatin remodeler CHD3 is a key component of the initial chromatin-mediated repression of the infecting HSV genome.

DISCUSSION

DNA viruses that replicate in the nucleus face the challenge of host cell chromatin modulation machinery that controls access to the viral genome for transcription and DNA replication. In contrast to the cellular genome and the genomes of other small DNA viruses, herpesvirus genomes are packaged in capsids in the absence of nucleosomes. The infecting virus becomes a target of cellular epigenetic machinery that initially suppresses infection through the assembly of a heterochromatic-type chromatin state that limits access to the genome. This process appears to be dynamic, and opposing elements (e.g., viral or cellular transcription factors) can recruit or modulate the epigenetic machinery to promote viral gene expression.

For herpes simplex virus, this dynamic is evident at early times postinfection. Initially, nucleosomes that bear heterochromatic histone marks are readily detected on the viral genome. This repression is countered by the recruitment of a coactivator (HCF-1) complex to the viral IE gene promoters that contains the required enzymes to remove the repressive histone H3K9 methylation along with those that install activating H3K4 methylation. Additionally, the SNF2H remodeler and the CLOCK acetyltransferase also contribute to promoting viral IE gene expression, presumably via early chromatin modulation (4, 10). As viral proteins are expressed, the IE protein ICP0 plays multiple roles that further drive the epigenetic state to the advantage of the virus (5, 7, 8, 20).

Initial suppression of the viral genome appears to be a cellular defense to infection. The process must be multistep, involving initial nucleosome deposition, modification, and the required remodeling to configure the repressive chromatin state of the genome. To more fully understand this dynamic, the members of the CHD family of chromatin remodelers were investigated as potential contributors to viral epigenetic suppression. Of these, CHD3 but not the related CHD4 was found to be important for the initial repression of a large percentage of the infecting viral genomes, as evident by the increase in the number of transcriptionally active viral foci in CHD3-depleted cells. This increase in the number of accessible genomes per cell may account for the enhanced viral IE gene expression in the absence of the remodeler.

Most significantly, CHD3 is responsible, at least in part, for a reduction in the levels of soluble or accessible viral genomes, suggesting its role in the formation of the observed heterochromatic structures. In this respect, it is opposed to the activities of the HCF-1 coactivator complex. It is important to note that the association of CHD3 with developing viral foci might represent vestiges of the initial repression at genomes where activator complexes have successfully shifted the balance to promote viral transcription (Fig. 1). The model suggests a more dynamic picture of the interactions between host and virus-directed epigenetic regulation.

In addition to CHD3, depletion of CHD1 also had some impact on repression of the viral genome, while depletion of CHD9 resulted in modest decreases of IE expression. The data suggest that multiple CHD family members are involved in both repression and activation of initial viral gene expression. Whether CHD1 and CHD3 function cooperatively, function in synergy at distinct stages, or are partially redundant remains to be determined. CHD1 has been linked to complexes involved in nucleosome deposition and remodeling that lead to the canonical spacing required for efficient heterochromatin formation (49–52). CHD3 also plays a role in this process as it recognizes repressive histone marks and recruits additional corepressors. Irrespective, the identification of CHD3 as an important epigenetic repressor of initial infection increases our understanding of the complex dynamics of HSV chromatin. While there is no data to support the supposition, it is tempting to speculate that the CHD family of remodelers may also play important roles in the establishment and maintenance of HSV latency in sensory neurons.

MATERIALS AND METHODS

Cells and viral infection. MRC-5 and HeLa cell lines were maintained according to standard procedures. TERT-immortalized human foreskin fibroblast (HFF) cells were a gift from T. Shenk (Princeton University). HSV-1 strain 17 was a gift from N. Fraser (University of Pennsylvania). HSV infections were done in HEPES-buffered Dulbecco's modified Eagle's medium (DMEM) containing 1% fetal bovine serum (FBS) for 1 h at 4°C. Following adsorption, infected cells were washed with phosphate-buffered saline (PBS) and incubated in DMEM containing 10% FBS.

Antibodies, primers, and siRNAs. Antibodies, primer sequences, and siRNAs utilized in these studies are listed in Table S1 in the supplemental material.

LSD1 inhibition. Cells were pretreated with tranylcypromine (TCP; Sigma P8511) or DMSO control for 3 to 4 h prior to infection and maintained throughout the infection as specified in the figure legends.

qRT-PCR and qPCR. cDNA was synthesized from 800 ng of total RNA (NucleoSpin RNAII; Macherey-Nagel) using a Maxima first-strand cDNA synthesis kit (Thermo Scientific) according to the manufacturer's recommendations. cDNA and DNA were quantified by qPCR using SYBR green master mix (Roche) and a Mastercycler ep realplex4 (Eppendorf; realplex 2.2 software).

Western blots. Western blots utilized antibodies listed in Table S1 and were quantitated using a Kodak 4000MM image station.

Immunofluorescence microscopy. Immunofluorescent staining was done according to standard protocols. Where indicated, cells were treated with CSK extraction buffer (0.5% Triton X-100, 10 mM PIPES [pH 6.8], 300 mM sucrose, 100 mM NaCl, 3 mM MgCl2, 2.0 mM NaF, 2.0 mM Na3V04, 10 mM β-glycerophosphate, Complete protease inhibitor) prior to fixation. Cells were visualized using a Leica SP5 confocal microscope with LASAF software (version 2.6.0). Images were assembled from sequential Z-sections using Imaris software (version 7.1.1; Bitplane AG).

FAIRE assays. FAIRE assays were done as described with minor modifications (46, 48). Cells were treated with 4% paraformaldehyde, snap-frozen, and subsequently thawed in LB1 buffer (50 mM HEPES [pH 7.5], 150 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100). Cells were pelleted at 1,500 $\times$ *g* at 4°C, resuspended in LB2 buffer (10 mM Tris-HCl [pH 7.5], 200 mM NaCl, 1 mM EDTA), and incubated for 10 min. Nuclei were pelleted at 1,500 $\times$ *g* at 4°C, resuspended in LB3 (10 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 0.25% Sarkosyl, 0.1% sodium deoxycholate, Complete protease inhibitor) and sonicated (Branson Sonifier 450 D, 19% amplitude, 7 cycles of 40 s) or until chromatin fragments were in the 200- to 800-bp size range. Insoluble chromatin was pelleted by centrifugation at 13,000 rpm for 10 min at 4°C and resuspended in LB3 containing RNase A and proteinase K. The insoluble fraction DNA and the nucleosome-depleted DNA (FAIRE) were purified by sequential phenol-chloroform extractions and precipitated with EtOH. Cross-linking was reversed by incubation in 250 mM NaCl for 12 h at 65°C and purified using a ChIP DNA Clean & Concentrator kit (Zymo Research).

Statistical analyses. Statistical analyses were done using Prism 6.0 (GraphPad Software, Inc.) and included 2-tailed *t* tests ($P < 0.05$) (siRNA analyses) and analysis of variance (ANOVA) with Dunnett's *post hoc* multiple comparisons test (viral foci).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01027-13/-/DCSupplemental.

Figure S1, PDF file, 0.3 MB.
Figure S2, PDF file, 0.1 MB.
Figure S3, PDF file, 0.1 MB.
Figure S4, PDF file, 0.7 MB.
Table S1, PDF file, 0.1 MB.

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