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Chromatin assembly on herpes simplex virus genomes during lytic infection

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Abstract

The human herpes simplex viruses HSV-1 and HSV-2 infect a significant portion of the human population. Both viruses can undergo lytic infection in epithelial cells and establish lifelong latency in neuronal cells. The large HSV-1 DNA genomes have long been considered to be devoid of histones both inside the virion particle and inside the cell during lytic infection, but to be packaged in repressive chromatin during latency. However, recent reports indicate that many histone and non-histone chromosomal proteins can associate with viral DNA during lytic infection and may influence important events during the HSV-1 lytic cycle. In this article, we summarize recent developments in this field and their implications.

I. Introduction

A. Overview of chromatin structure and its cellular effects

All eukaryotes face the challenge of accommodating their large DNA genomes into the relatively small three-dimensional space of the cellular nuclei. To achieve this goal, a well-conserved mechanism is used to condense DNA into chromatin through a complicated but well-regulated process [1,2]. The basic subunit of chromatin, the nucleosome core particle (NCP), is formed by wrapping approximately 147 base pairs (bp) of DNA around an octameric protein core formed by two copies each of four core histone proteins, H2A, H2B, H3 and H4 [3,4]. Thousands of NCPs along the DNA strand form the nucleosomal array, which is now considered as the primary structure of chromosome organization. These arrays can further condense into secondary and tertiary chromatin structures to eventually form the highly-condensed mitotic chromosomes visible under a light microscope [5]. Although the details are still poorly understood, the entire chromatin condensation process is achieved through the combined action of core histones, linker histones and non-histone chromosomal proteins [1, 2,5].

Chromatin structure, and in particular highly condensed chromatin, is generally inhibitory to transcription, replication and DNA repair. Therefore, all eukaryotes face another challenge, namely, to counteract the inhibitory effects of chromatin in order to accomplish these essential cellular processes [1,2]. An interwoven set of well-conserved mechanisms is employed to

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overcome the inhibitory effects of chromatin compaction, including chromatin remodeling, histone modifications and replacement of canonical histone with histone variants.

Chromatin remodeling is mediated by a family of related protein complexes whose founding member is the yeast SWI/SNF complex [6]. Chromatin remodeling enzymes can disrupt certain DNA and histone interactions within a nucleosome using the energy provided through ATP hydrolysis mediated by their ATPase. *In vitro*, these complexes can alter the position of a nucleosome along the DNA, disrupt a nucleosome, or transfer the histone octamer to a different DNA segment. Conceivably, *in vivo*, these actions could alter the local chromatin structure or even expose a certain portion of DNA for binding of transcription, replication or DNA repair proteins [7,8].

Histones are small, basic proteins, each containing a structural core and a disordered N-terminal domain (NTD). The NTDs and to a lesser extent the structural cores can be post-translationally modified. Examples of such modifications include acetylation, methylation, phosphorylation, ubiquitinylation, and proline isomerization [9]. Modifications can result in direct alternation of chromatin structure [10-12] with consequences for gene expression. For example, extensive acetylation of histone octamers can disrupt formation of highly-condensed chromatin structure and enhance transcription activity *in vitro* [11]. Specific acetylation of H4 K16 was also shown to disrupt formation of condensed chromatin structure and inhibit nucleosome remodeling mediated by ACF [10]. Histone modifications can also provide novel binding surfaces for interacting partners [13-15]. One example is the bromodomain, commonly found in chromatin-associated proteins involved in active transcription, which binds to acetylated lysine [16-21]. Another example is the chromodomain, present in a number of transcriptional repressor proteins, which binds to methylated lysine [13-16,22].

Most nucleosomes are assembled with canonical histone proteins, which are only expressed in the S phase of the cell cycle in concert with DNA replication [1,2]. However, both H3 and H2A (but not H4 or H2B) have variants that are specialized for certain cellular functions. Histone variants are expressed and incorporated into chromatin throughout the entire cell cycle. The most prominent variants are CenH3, H3.3, H2A.Z, H2AX H2A.Bbd and macroH2A [23,24]. CenH3 localizes to centromeres and is essential for both their structure and function [25]. H3.3, which differs from the canonical H3 (H3.1 or H3.2) by only a few amino acid substitutions, is typically associated with transcriptionally active regions of chromatin. Among all histone variants, macroH2A is most distinctive in its size and structure because of its large carboxyl-terminal domain. MacroH2A usually localizes to inactive, highly-condensed heterochromatin, particularly the inactivated X chromosomes [26]. In contrast, H2A Barr-body deficient, or H2A.Bbd, is essentially excluded from inactivated X chromosomes, and localizes to active chromatin [27]. About 10% of nucleosomes are assembled with H2AX. It is unclear whether unmodified H2AX plays any specialized structural and/or functional roles. However, upon DNA damage, H2AX is rapidly phosphorylated at Ser139, which triggers a signaling pathway leading to DNA damage response and DNA repair. By far the most complicated histone variant is H2A.Z, which has been implicated in many different and often contradictory functions [28]. For example, in budding yeast, Tetrahymena, and worm, H2A.Z has been associated with active transcription, whereas in some cases in fly and mouse, H2A.Z has been associated with heterochromatin formation. In human cells, H2A.Z is enriched at promoter regions near transcription start sites [29], and at functional upstream regulatory elements [30].

B. Overview of chromatin structure on DNA virus genomes

To complete their life cycles, all viruses depend to certain extent on the molecular machinery of their host cells. DNA viruses, like their hosts, face the challenges of neutralizing the negative charge on the DNA and condensing their DNA genomes both inside their virion particles and

inside the cells, while also retaining the ability to accommodate the processes of transcription, DNA replication and recombination which are required for viral propagation. Different families of DNA viruses use very different mechanisms to address this challenge, ranging from almost completely mimicking the host DNA condensation pathway to almost completely abandoning it. This topic has been reviewed recently [31]. Here we provide several typical examples. The DNA of the small DNA tumor viruses (papillomaviruses and polyomaviruses) is associated with core histones both inside the virion particles and within infected cells. Two such examples are simian virus 40 (SV40) and polyoma viruses. Organizing their DNA to mimic the structure of host chromatin may be required for their DNA replication, which relies on the host DNA replication machinery. In contrast, adenoviruses compact their DNA in their virion particles using virally-encoded histone-like proteins. At 6 hours post infection (hpi), about 50% of viral DNA was assembled into nucleosome-like structures [32]. At the other end of the spectrum, herpesviruses contain little or no protein associated with DNA inside their capsids. During lytic infection by herpes simplex virus (HSV-1), histones are associated to a limited extent with viral DNA although the presence of intact nucleosomes and the nucleosome density on the viral DNA remain as unresolved issues. During latency, HSV-1 DNA is assembled into classical nucleosomes with nucleosome density similar to that of the host genome. HSV-1 DNA-associated histones and their effects on HSV-1 lytic infection are the focus of this review article.

C. Overview of HSV-1 life cycle

HSV-1 and its closely related virus HSV-2 are important human pathogens. HSV-1 infection typically causes oral cold sores, whereas HSV-2 infection results in genital lesions. Both viruses infect a very significant portion of human population. By age 60, close to 90% of American adult population displays serological evidence of prior HSV-1 infection, whereas 20-30% are HSV-2 positive by age 40 [33]. Despite the difference of their typical infection sites, these two viruses share a similar life cycle [34].

All herpesviruses manifest infections in two distinct ways; a productive lytic infection and a non-productive latency. For HSV-1, lytic infection takes place in epithelial cells, after entry of HSV-1 through mucosal surfaces, and latency is established in the sensory neurons innervating that initial site of lytic infection. Lytic infection involves sequential transcription and translation of the immediate-early (IE), early (E) and late (L) genes. HSV-1 does not encode its own RNA polymerase and thus solely relies on the host RNA polymerase II (pol II) for transcription. The virion protein VP16 is a potent transcription activator that is essential for efficient IE gene expression. The products of IE genes regulate subsequent expression of other viral genes, including the E genes whose products are necessary for viral DNA replication. DNA replication and continued activity of IE proteins are required for efficient expression of L genes, whose products, together with newly-synthesized viral DNA, are eventually packaged into new virion particles. Progeny virions are exported through a poorly understood mechanism and can infect surrounding cells, including the sensory neurons in which latency is established. During latency, only the latency-associated transcript (LAT) is actively expressed. In response to any of a number of stressors, latent virus can be re-activated and establish another round of lytic infection around the primary infection site [34].

II. Association of histones with HSV-1 DNA during lytic infection

A. HSV-1 DNA inside the virion particle

The HSV-1 virion particle contains three major sections. The outermost section is the envelope, a lipid bilayer membrane embedded with about a dozen glycoproteins that are important for viral binding and entry. Underlying the envelope is the tegument, a structurally amorphous region comprising approximately 14 proteins, some of which, e.g., VP16 and VP22, are

important or even essential for HSV-1 infection. Inside the tegument is the icosahedral capsid, composed of 10 proteins, in which is packaged the linear double-stranded DNA viral genome of 152 kilobasepairs [35].

The HSV-1 DNA inside the capsid is not associated with histones [36-38] nor, apparently, with substantial amounts of any other DNA-binding proteins. Instead, the positive charge of the DNA phosphates is balanced by the presence of the polyamine spermine. Interestingly, spermine was the only polyanion found inside the capsid, whereas spermidine was restricted to the tegument [39]. This DNA compaction strategy might provide a selective advantage for HSV. Probably due to its higher positive charge density for the same mass, spermine has better DNA compaction ability than histones [40], and thus provides an advantage over histones for compacting the large DNA genome of HSV-1 into the small volume of the capsid. Interestingly, a similar compaction pathway is utilized in sperm cells of many animal species, in which chromatin is highly condensed by the unusually high concentration of spermine inside the nuclei, in conjunction with specific histone and non-histone chromosomal proteins [41].

B. Association of canonical histones and nucleosomes with viral DNA during HSV-1 lytic infection

Micrococcal nuclease (MNase) digestion of nucleosomal DNA typically yields a series of DNA fragments with lengths representing multiples of approximately 160 bp. This nuclease digestion approach has been used to study the nature of HSV-1 DNA during lytic infection [38,42-44]. A ladder of DNA bands originating from the host genome was typically observed upon agarose gel electrophoresis, indicating that much of the host genome was densely packed into nucleosomes. However, the digestion products of viral DNA do not resolve into discrete bands or ladders in the agarose gel. Upon extensive digestion, a DNA species of ~ 160 bp was produced with the host DNA, but viral DNA probes hybridize to smeared fragments of smaller sizes. These results indicate that in lytic infection, the viral and host DNA have much different structures, and that little if any viral DNA is assembled into nucleosomes.

With the development of the chromatin immunoprecipitation (ChIP) technique, the association of histones (most often represented by H3) was explored on HSV-1 DNA. Several studies showed that H3 was associated with viral DNA, albeit at a much lower density than that on host DNA [45,46], although one study gave a higher estimate [47]. The other core histones, H4, H2A and H2B have also been found to be associated with viral DNA [48], raising the possibility that intact nucleosomes may indeed assemble on HSV-1 DNA. However, alternative interpretations of the data should be considered. For example, alternative forms of nucleosomes, particularly tetrasomes and/or hexasomes, have been inferred to be present in cellular chromatin (reviewed in [49]). A tetrasome usually refers to a DNA and histone complex that only contains an H3/H4 tetramer, whereas a hexasome contains a complete H3/H4 tetrasome and one H2A/H2B dimer. Both structures are associated with regions of DNA engaged in active transcription [49]. Therefore, the presence of such structures on the HSV-1 genome during lytic infection might be quite likely since HSV-1 genomes are engaged in very active transcriptional and replication activities in lytic infection. However, the demonstration of the presence of hexamers or tetramers on viral DNA will be technically challenging. One potential approach is to use cross-linking and gel electrophoresis, which has been applied to identify the existence of a special histone tetramer, composed by a copy each of CenH3, H4, H2A and H2B in centromeres [50]. In this study, the authors cross-linked MNase-digested chromatin and then immuno-purified those nucleosomes containing CenH3. Purified products were subjected to SDS-PAGE and their molecular weights were found to be consistent with the presence of the aforementioned tetrasomes.

In host cells, the canonical histones are synthesized only in S phase, but HSV-1 lytic infection induces cell cycle arrest at the G1/S or G2/M boundaries [51,52]. This leads to the question of

the source of core histones for assembly on viral DNA. One possibility is that the histones that associate with viral DNA come from a residual pool of canonical core histones in the cells. Given that the amount of viral DNA is far less than the host genome and that the nucleosome density on viral DNA is much lower, a very small histone pool might be sufficient. Moreover, core histones are actively displaced from host chromatin during transcription and DNA repair, a process that likely continues during HSV-1 lytic infection. Therefore, the HSV-1 DNA-associated histones could come from the host chromosomes. However, it will be rather difficult to test this hypothesis.

C. Histone variants associated with HSV-1 DNA

Histone variants play important roles in a broad range of cellular pathways including transcriptional activation and repression, chromosome segregation and DNA repair (see above, reviewed in [23,24]). Therefore, it is intuitive to hypothesize that histone variants are associated with HSV-1 DNA and might play important roles in HSV-1 lytic infection. ChIP is a technique well-suited to answer such questions, but this method is often hindered by the lack of antibodies that are effective in ChIP experiments.

The only histone variant that has been reported to associate with HSV-1 DNA is H3.3 [53]. Such an association was not surprising given that H3.3 is typically associated with active transcription, a feature of HSV-1 lytic infection. Interestingly, in this study, probably because no H3.3-specific antibody was available, the authors established cell lines stably expressing either Flag-tagged H3.3 or Flag-H3.1 (the canonical H3), and utilized antibody against the Flag tag in ChIP experiments. In this study, the authors found that the levels of Flag-H3.3 associated with HSV-1 viral DNA remained fairly constant during the course of lytic infection, while the levels of Flag-H3.1 increased significantly as the infection progressed. The authors conclude that shortly after entry, HSV-1 DNA was incorporated into nucleosomes mostly containing H3.3, while at least some of the newly synthesized DNA was associated with H3.1. Moreover, the association of Flag-H3.3 with HSV-1 DNA depended on HIRA, a H3.3-specific histone chaperone. Intriguingly, disruption of HIRA expression by RNA interference decreased the amount of Flag-H3.3 that was associated with HSV-1 DNA and decreased the levels of viral mRNAs, suggesting that H3.3 and thus its chaperone HIRA was required for viral transcription. Although the epitope-tagging strategy can circumvent the problem of lack of suitable antibodies in ChIP experiments, the results from such experiments should be interpreted carefully. One concern is that overexpressing an otherwise endogenous protein may alter its cellular functions. Another concern is that the expression of the Flag-tagged histones in this case was accomplished using the constitutive CMV-IE gene promoter, which does not recapitulate the normal cell cycle-regulated expression of core histones; this unnatural pattern of expression may also affect the results.

Given their known functions (see above) [23,24], other histone variants, including H2A.Z, H2A.X, or H2A.Bbd, might also associate with HSV-1 DNA. Unlike the canonical histones, the expression of these variants is not controlled by the cell cycle, and so the variants might be preferentially incorporated into HSV-associated nucleosomes during infection. However, because macroH2A is typically associated with inactive heterochromatin, and CenH3 is centromere-specific, the presence of these variants on HSV-1 DNA during lytic infection seems unlikely. Nevertheless, such hypotheses should be tested and ChIP experiments remain the technique of choice. It is important to note that even if one or more of these variants are confirmed to associate with HSV-1 DNA, whether that association has important functional consequences for viral infection will need to be experimentally determined. Mutant cell lines with defects in the protein of interest or disruption by RNA interference might be approaches suitable for such studies.

D. Linker histones on HSV-1 DNA

Members of the linker histone family (H1, for instance) are small basic proteins of about 200 amino acid residues in length. They can bind to nucleosomes and facilitate chromatin condensation, and thus are typically associated with regions of transcriptional repression. The expression patterns of the eight known mammalian linker histone variants are tightly regulated both temporally and spatially, which suggests that specific variants might have distinct functions [1,5]. However, recent *in vivo* and *in vitro* studies indicated that for several functions, including chromatin condensation, linker histone variants functioned quite similarly [55-57].

The mobility of linker histones is enhanced upon HSV-1 lytic infection [53]. This mobilization was dependent on expression of viral IE proteins but not on viral DNA replication, indicating a potential interaction between linker histones and HSV-1 DNA and/or HSV-1 gene expression. However, this study did not define whether linker histones mobilized to (or from) the intranuclear HSV-1 transcription and/or replication compartments, and so the significance of this mobilization remains to be determined.

One relatively simple experiment (if appropriate ChIP-grade antibodies are available) is to test whether linker histones are associated with HSV-1 DNA using ChIP experiments. However, if such association is confirmed, testing the functional significance of this association may be much more difficult, because linker histone variants appear to have redundant functions that would complicate gene knockout or siRNA studies.

Nevertheless, linker histones are unlikely to perform any active roles on viral DNA, based on existing knowledge regarding linker histone functions and HSV-1 lytic infection. First, linker histones are associated with more condensed chromatin, which is inconsistent with events during HSV-1 lytic infection. Second, linker histones bind to the nucleosome surface; however, as discussed above, few nucleosomes if any associate with HSV-1 viral DNA during lytic infection. Therefore, the linker histones are unlikely to affect HSV-1 gene expression.

E. Histone chaperones and HSV-1 lytic infection

Histone chaperones are required for assembly of nucleosomes with canonical histones, and more so for assembly of nucleosomes with histone variants. Different histone chaperones play different roles in nucleosome assembly, and very often in different stages of the cell cycle. For example, CAF1 supports the assembly of nucleosomes with canonical H3 histones, during S phase, in conjunction with cellular DNA replication. However, since HSV-1 lytic infection causes cell cycle arrest, CAF1 and other chaperones that are only active in S phase are unlikely to deposit nucleosomes on viral DNA during infection [59,60]. A different chaperone, HIRA, assists assembly of nucleosomes with histone H3.3, independent of the cycle control. Another example of a histone variant-specific chaperone is SWR1, which assists in the assembly of nucleosomes containing H2A.Z [61].

As described above, HIRA was found to assemble H3.3 into HSV-1 nucleosomes and to play important roles in HSV-1 gene expression [53]. In contrast, some evidence suggests that other histone chaperones might inhibit HSV-1 lytic infection. Overexpression of TAF-1, a homolog of histone chaperone NAP1, slowed HSV-1 lytic infection. Moreover, the ability of TAF-1 to mediate assembly of nucleosomes *in vitro* was blocked by the viral tegument protein VP22. This study argues that blocking nucleosome deposition on the HSV-1 genome (perhaps through the activity of VP22) is beneficial to HSV-1 lytic infection [62].

III. Chromatin and HSV-1 gene transcription

A. Histone modifications associated with HSV-1 DNA

Covalent post-translational modifications of histones play essential roles in transcription and DNA repair. Some modifications are typically associated with active transcription, and others are most often correlated with transcriptional repression [9]. In recent years, the effects of histone modifications on HSV-1 lytic infection have also been explored. In several studies, histone modifications typically associated with active transcription, such as H3K9/K14Ac [45] and H3K4me3 [46], have been found to associate with HSV-1 DNA. Furthermore, elevated amounts of phosphorylated H2AX (γ H2AX) have been observed upon HSV-1 lytic infection [63]. However, it is unclear whether γ H2AX is associated with host or HSV-1 DNA, and no mechanistic role for γ H2AX in HSV-1 gene transcription or replication has been advanced. The association of many other specific histone modifications with viral DNA and the potential impact of these modifications on viral gene expression or replication have not yet been examined. The increasing availability of ChIP-grade antibodies that recognize histones modifications and of siRNAs that target the modification enzymes makes such studies more feasible.

B. Presence of histone modification and remodeling complexes on HSV-1 chromatin

HSV-1 relies solely on the host RNA polymerase II (RNA pol II) for transcription. The association of general transcriptional factors (GTFs), represented by TBP and RNA pol II, with viral genes is dependent on the binding of VP16 to DNA and on the activity of the VP16 activation domain (AD) [45]. Interestingly, in this process, histone acetyltransferases (HATs) CBP and p300 were recruited to IE gene promoters, with different preferences in that CBP was associated with ICP0, ICP4 and ICP27 promoters whereas p300 was mostly associated with ICP27. ATP-dependent remodeling complexes, represented by their catalytic subunits, BRM and Brg-1 were also recruited to IE gene promoters. However, their dependence on VP16 AD was somewhat different. Specifically, the association of BRM and Brg-1 with the ICP0 promoter entirely depended on the VP16 AD; however, the association of BRM (but not Brg-1) with the ICP4 promoter depended on the VP16 AD, whereas the association of Brg-1 (but not BRM) with the ICP27 promoter depended on the VP16 AD. This VP16 AD-dependent recruitment of coactivators to IE gene promoters suggested that these coactivators are important for efficient IE gene transcription [45]. However, recent developments indicate that this hypothesis may be too simplistic.

C. Roles of histone modification and remodeling complexes on HSV-1 transcription

H3K9/K14Ac and H3K4me3 have been shown to be associated with HSV-1 DNA during lytic infection, despite the relatively low histone occupancy on viral DNA. Interestingly, their functional subsequences on HSV-1 lytic infection were more complicated than originally thought. A protein methylation inhibitor, 5'-deoxy-5'-methylthioadenosine (MTA), decreased the amount of H3K4me3 and H3K9me2 in both HSV-1 infected and non-infected cells. Moreover, the association of H3K4me3 (but not H3K4me2) to HSV-1 DNA was partially inhibited upon MTA treatment. HSV-1 gene expression and DNA replication were also moderately inhibited in the presence of MTA. These results, as well as the observation that knockdown of H3K4me3 specific methyltransferase Set1 moderately inhibited expression of certain HSV-1 genes and DNA replication, suggested that H3K4me3 might play an active role in HSV-1 infection [46].

A component of the curry spice turmeric, curcumin, was reported to be an inhibitor of specific HAT enzymes (p300 and CBP) [64,65]. Curcumin was found to inhibit HSV-2 [66] and more recently HSV-1 infection [67]. Specifically, curcumin inhibited HSV-1 IE gene transcription by decreasing the association of RNA pol II (but not VP16) with IE genes. However, the

curcumin concentration used in this study was much lower than that required to significantly inhibit HAT activity. Moreover, the amount of acetylated H3 associated with IE genes was not affected by curcumin, suggesting that HAT inhibition might not be the mechanism underlying the antiviral effect [67].

More convincingly, a subsequent study showed that efficient knockdown of HATs, including p300, CBP, PCAF or GCN5, had no detrimental effect on HSV-1 IE gene expression. Similarly, disrupted expression of the chromatin remodeling enzymes Brg-1 or BRM had no substantial effect. These observations were further reinforced by the discovery that HSV-1 IE gene expression was not impaired in cell lines lacking functional p300, or BRM and Brg-1 [68]. Thus, these chromatin-modifying coactivators, although they may be physically proximal to viral DNA, are not required for efficient viral gene expression.

The studies discussed in this section argue that association of a certain coactivator or histone modification with HSV-1 DNA does not always indicate its importance. This observation represents a significant exception to the general rule for cellular genes. For example, in a high throughput study using ChIP followed by massively parallel sequencing (ChIP-seq), p300 was found to be highly enriched at transcriptional enhancers in a tissue-specific manner, suggesting that putative p300 binding sites and transcriptional activity are well correlated [69]. However, this study does not definitively establish whether p300 or its paralog CBP are important for transcription.

V. Concluding remarks

Although recent discoveries indicate many intriguing connections between chromatin structure and HSV-1 lytic infection, most of these studies raised more questions than answers. In particular, although it is now clear that histones, histone modifications, and transcriptional coactivators are associated with HSV-1 DNA, it remains uncertain exactly what roles they play and through which mechanisms. With the development of ChIP techniques, the increasing number of ChIP-quality antibodies, and quantitative PCR assays, now we can ask many questions and test many hypotheses that were unapproachable ten years ago.

It is now well established that histones are associated with viral DNA during lytic infection. However, the implications of these associations remain rather controversial. Most studies indicate that the nucleosome density on viral DNA is much lower than that on host DNA. However, it is not clear how the nucleosomes are distributed on the multiple copies of HSV-1 genome in a typical lytic infection. For instance, we do not yet know whether all viral DNA genomes associated with a low density of histones, or whether some genomes remain entirely nucleosome-free while a small fraction are heavily packaged. It is also unclear whether such chromatin structure and the enzymes that might modulate that structure have any impact on HSV-1 lytic infection. As discussed above, data that are supportive and contradictory to both possibilities have been described. One formal possibility is that HSV-1 gene expression may depend on only a small set of coactivators, whereas other coactivators may be associated with but may not be important for lytic infection.

Finally, the mixed messages that arise regarding the importance of histones and chromatin-modifying enzymes on the progression of HSV-1 lytic infection may reflect vestiges of a more important role of chromatin during establishment, maintenance and reactivation of viral latency. For further insight into recent developments on that topic, readers are referred to other review articles in this same special issue of BBA: Gene Regulatory Mechanisms.

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