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Chromatin regulation of virus infection

Paul M. Lieberman

The Wistar Institute, Philadelphia, PA 19104, USA

Cellular chromatin forms a dynamic structure that maintains the stability and accessibility of the host DNA genome. Viruses that enter and persist in the nucleus must, therefore, contend with the forces that drive chromatin formation and regulate chromatin structure. In some cases, cellular chromatin inhibits viral gene expression and replication by suppressing DNA accessibility. In other cases, cellular chromatin provides essential structure and organization to the viral genome and is necessary for successful completion of the viral life cycle. Consequently, viruses have acquired numerous mechanisms to manipulate cellular chromatin to ensure viral genome survival and propagation.

A chromatin perspective of virology

Viruses are mobile genetic elements that must navigate through multiple host-cellular compartments to complete their life cycle. The complex and dynamic properties of nuclear chromatin present a formidable challenge to viral gene expression and genome propagation. Recently, a wealth of information has been uncovered regarding the structure, function and regulation of chromatin during complex cellular processes such as differentiation, recombination, ageing and carcinogenesis. Recent studies have also revealed that chromatin has a major role in the life cycle of many viruses, and that viruses have coevolved with numerous strategies for modulating chromatin-related processes. Here, several examples of chromatin regulation of virus infection and viral mechanisms of chromatin modulation are reviewed.

The bulk of eukaryotic nuclear DNA associates with histone-like proteins to form nucleosomal structures, which form the basic unit of cellular chromatin. The nucleosome consists of ~145 bp of DNA wrapped twice around an octamer of the core histones H3, H4, H2A and H2B [1]. The histone tails that project out from the core can be post-translationally modified by lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation and lysine ubiquitylation or sumoylation. Histone tail modifications provide a coding mechanism for protein recognition and signaling at specific locations within chromatin [2,3]. For example, acetylation of histones H3 and H4 promotes transcription activity by providing binding sites for bromo domains that are found in several chromatin-modifying and transcription-coactivator complexes. Similarly, methylation of histone H3 on Lys9 (H3mK9) can recruit the chromo domain of heterochromatin protein 1 (HP1) to induce transcription repression. In addition to histone-tail modifications, the position of the nucleosome on the DNA and its ability to form condensed higher-ordered structures such as heterochromatin can have profound effects on the accessibility and activity of the packaged DNA. The ATP-dependent chromatin-remodeling complexes that contain BRG1, BRM1 or SNF2h can alter the nucleosome positions and alter DNA accessibility to promote or repress transcription and replication [4]. Histone chaperones and histone variants can also regulate chromosome functions. For example, histone variant H2AX is phosphorylated in response to DNA double-strand breaks and is thought to be essential for the recruitment of DNA repair proteins [5]. The signaling mechanism and patterns of recognition, referred to as the histone code hypothesis, can be markers for cellular division cycle and cancer prognosis [2,3,6]. It remains to be determined whether a distinct histone modification code or nucleosome organization pattern marks viral genomes, infected cellular genomes or stages of viral infections.

Chromatin organization of viral genomes

The assembly and organization of nucleosomes on viral genomes is thought to be linked to the replication mechanism and life cycle of the virus. Persistent DNA viruses that replicate using cellular replication enzymes tend to have a chromatin structure that is similar to that of cellular chromosomes. Acute infections that use viral-specific replication machinery tend to have heterogeneous nucleosome composition and modifications associated with the replicating or parental genomes. Viruses that integrate their genomes, such as HIV, can use different strategies of chromatin manipulation during integration and reactivation. RNA viruses, which do not form nucleosome structures, can also associate with chromatin during replication and transcription of their genomes. In this section, some examples of chromatin organization on several viral genomes are reviewed and the means by which chromatin can regulate viral gene expression, DNA replication and integration is considered.

Acute and persistent DNA viruses

The genomes of many DNA viruses persist for considerable lengths of time in the host-cell nucleus. The small DNA tumor viruses such as simian virus 40 (SV40) and polyoma virus are assembled into nucleosomal minichromosomes during the DNA replication process. Because these viruses use cellular replication enzymes, it is thought that these viral minichromosomes are assembled through a mechanism that is indistinguishable from nucleosome assembly on replicating cellular chromosomes [7]. SV40 and polyoma viruses associate with core

Corresponding author: Lieberman, P.M. (lieberman@wistar.org).
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histones during virion encapsidation but lose their association with other chromatin proteins, including linker histones and the chromatin-associated high mobility group proteins [8].

Adenoviruses encode their own DNA polymerase and adenoviral core proteins catalyze the genome condensation that is required for encapsidation so there seems to be no opportunity for cellular nucleosome assembly during adenoviral DNA replication [9]. Nevertheless, adenovirus does not escape chromatin assembly during nuclear entry and the early stages of DNA replication. By six hours post infection, ~50% of the adenovirus DNA is associated with nucleosomes [10,11]. Nucleosome assembly might be required for the proper temporal control of viral gene transcription and for the stability of the parent genome during persistent infections but nucleosomes must be removed before encapsidation of newly replicated genomes.

Similar to adenovirus, herpesvirus genomes are assembled into nucleosomal structures during the early stages of lytic infection but lack nucleosomes when encapsidated in the virion [12]. During the acute phase of herpes simplex virus (HSV) infection, HSV genomes do not form the extended nucleosome arrays typical of bulk cellular chromatin. However, histones do associate with regulatory regions upstream of HSV genes during the early stages of infection. The histones positioned upstream of active genes have post-translational modifications that are characteristic of actively transcribed cellular genes, including enrichment of histone H3 Lys14 acetylation and Lys4 methylation (H3mK4) and the absence of histone H3 Lys9 methylation at promoter start sites [12]. These modification patterns are thought to form a code or signal that is permissive for transcription.

Formation of permissive chromatin structure and histone modifications is an active process induced by the HSV encoded virion protein 16 (VP16). VP16 functions as a potent transcription activator of viral immediate early (IE) genes. A mutant virus lacking the VP16 activation domain failed to stimulate histone H3 acetylation at lytic cycle promoters [13]. This mutant virus also failed to recruit histone acetyltransferases CBP and p300, nucleosome remodeling factors BRG1 and BRM and general transcription factors TFIID and RNA polymerase II to IE promoters. These studies demonstrate that VP16 recruits the cellular apparatus that is required to modify and remodel nucleosomes that otherwise inhibit transcription of the parental genomes during the early stages of lytic infection (Figure 1).

In contrast to acute infection, the latent HSV DNA forms structures that are more typical of cellular chromatin [14]. During latency, transcription is silenced at most viral genes, with the exception of the latency associated transcript (LAT). It is thought that chromatin structure has a substantial role in separating the active and inactive regions of transcription within the latent genome. The histone-tail modification patterns during latent infection are consistent with this hypothesis [15,16]. The regions that surround the LAT promoter are enriched in histone H3 acetyl Lys9 and Lys14 relative to the majority of nontranscribed genes of the HSV genome. The region downstream of the LAT promoter, within the 5′ exon, is also enriched in histone H3 acetylation. The LAT 5′ exon functions as an enhancer of latent transcription and is crucial for induced reactivation. This suggests that the 5′ exon might regulate viral gene expression by maintaining a permissive chromatin environment for latent transcription (Figure 1).

Latent episomal viruses

Hepatitis B virus (HBV), the papillomaviruses and herpesviruses can form long-term latent infections as non-integrated, nucleosome-associated genomes. These viruses are likely to share many interesting chromatin-based functions, including the ability to maintain their genomes during cellular division and nuclear breakdown.

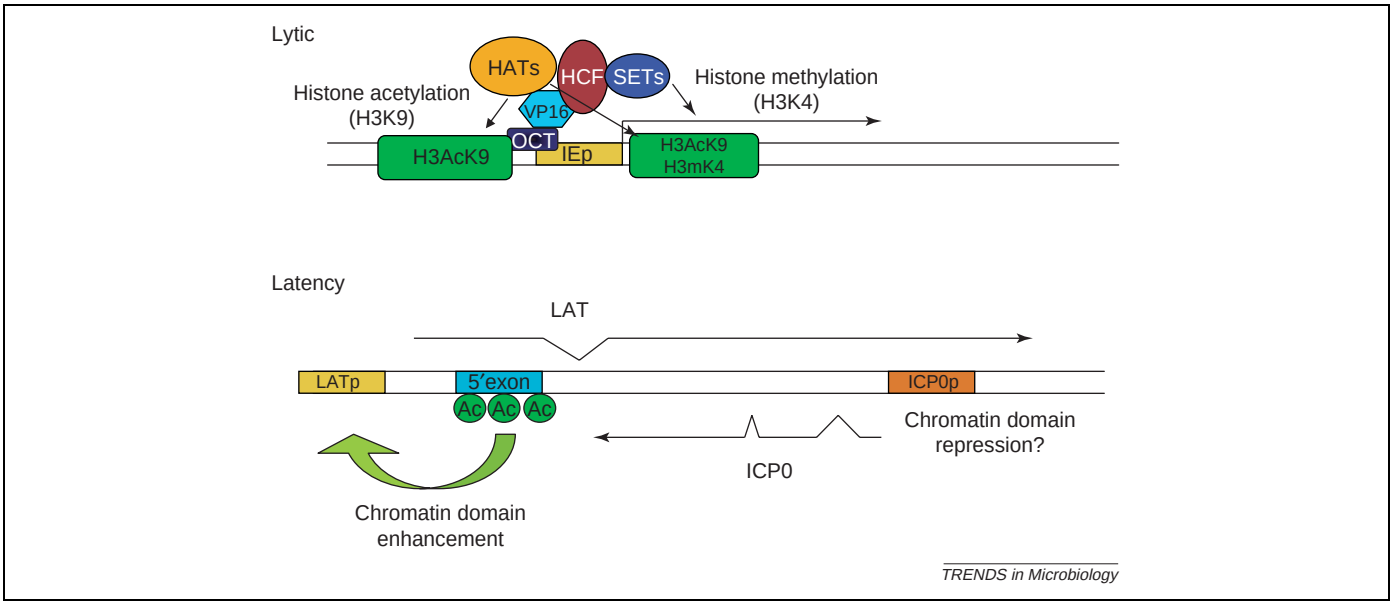

Figure 1. Chromatin regulation of herpes simplex virus. During lytic infection, the virion protein VP16 is required for histone H3 Lys9 acetylation and transcription activation of IE genes. VP16 binds to IE genes through the cellular sequence-specific OCT family of DNA-binding proteins. VP16 also recruits cellular HCF, which, in turn, can associate with SET-family histone H3 Lys4 methyltransferases, which promote transcription initiation. During latency, the primary LAT is activated by the 5′-exon enhancer, which is enriched for acetylated histone H3.

One common, shared mechanism of these genomes is the ability to tether to metaphase chromosomes. Recent studies have identified the cellular bromodomain-containing factor Brd4 as a chromatin-bound receptor for the human papillomavirus (HPV) E2 protein [17,18]. E2 is responsible for papillomavirus plasmid maintenance and chromosome attachment. Because bromodomains are implicated in binding to acetylated histones and transcription activation, it is not clear how Brd4 mediates metaphase chromosome attachment or whether it functions primarily in E2 transcription regulation.

Similar to the papillomaviruses, the gammaherpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) establish stable latent infections as multicopy episomes that tether to metaphase chromosomes [19,20]. During latency, the episomes are assembled into nucleosomal structures that are similar to bulk cellular chromatin, with some important exceptions. The regions surrounding the EBV origin of plasmid replication (OriP) have a deviant chromatin structure in latently infected cells [21]. The viral protein EBNA1 binds to tandem repeats in OriP that confer plasmid maintenance and metaphase chromosome attachment [19]. EBNA1 can displace nucleosomes from a single EBNA1 binding site *in vitro* and might have a crucial role in chromatin organization of the viral genome during latency [22]. EBNA1 also stimulates DNA replication within OriP by recruiting cellular replication proteins but chromatin organization could also contribute to this activity.

The origins of DNA replication are also subject to cell-cycle changes in chromatin structure during latency [23,24]. For both EBV and KSHV origins, nucleosomes are positioned immediately adjacent to the origin-binding proteins EBNA1 (in EBV) and LANA (in KSHV). The flanking nucleosomes are elevated in histone H3 acetylation and Lys4 methylation. These histone-tail modifications oscillate with the cell cycle in a pattern distinct for each virus. These changes in histone modifications might reflect some unique differences in the local chromatin environment of the EBV and KSHV origins [25]. Despite the differences in particular modification, both viral origins are subject to cell-cycle-associated nucleosome remodeling and histone modifications (Figure 2a).

The EBV latent chromosome is also subject to complex epigenetic regulation of the LMP1 and EBNA2 genes, which have central roles in EBV pathogenesis. OriP is located upstream of the diverging promoters for LMP1 and EBNA2 and might regulate their transcription through a chromatin-based mechanism. Analysis of histone-tail modifications indicates that the regions surrounding OriP are constitutively elevated for histone H3 Lys4 methylation [26]. The spreading of H3 Lys4 methylation from OriP correlates with enhanced expression of the LMP1 and EBNA2 transcripts. In latent infections in which EBNA2 and LMP1 are repressed, the H3 mK4 domain is restricted by DNA cytosine methylation and by the chromatin boundary factor CTCF. The mechanisms for generating high levels of H3mK4 at OriP are not known but could be linked to the inherent disruption of nucleosome structure and stimulation of DNA replication at OriP. It is also unclear whether the restriction of H3mK4 spreading by CTCF and DNA methylation actively regulates or passively correlates with transcription of LMP1 and EBNA2 (Figure 2).

Chromatin structure also contributes to the maintenance of latency by suppression of lytic cycle gene activation. The IE genes of both KSHV and EBV can be stimulated by treatment with histone deacetylase inhibitors. The KSHV IE promoter is repressed, in part, by a nucleosome positioned over a Sp1 binding site

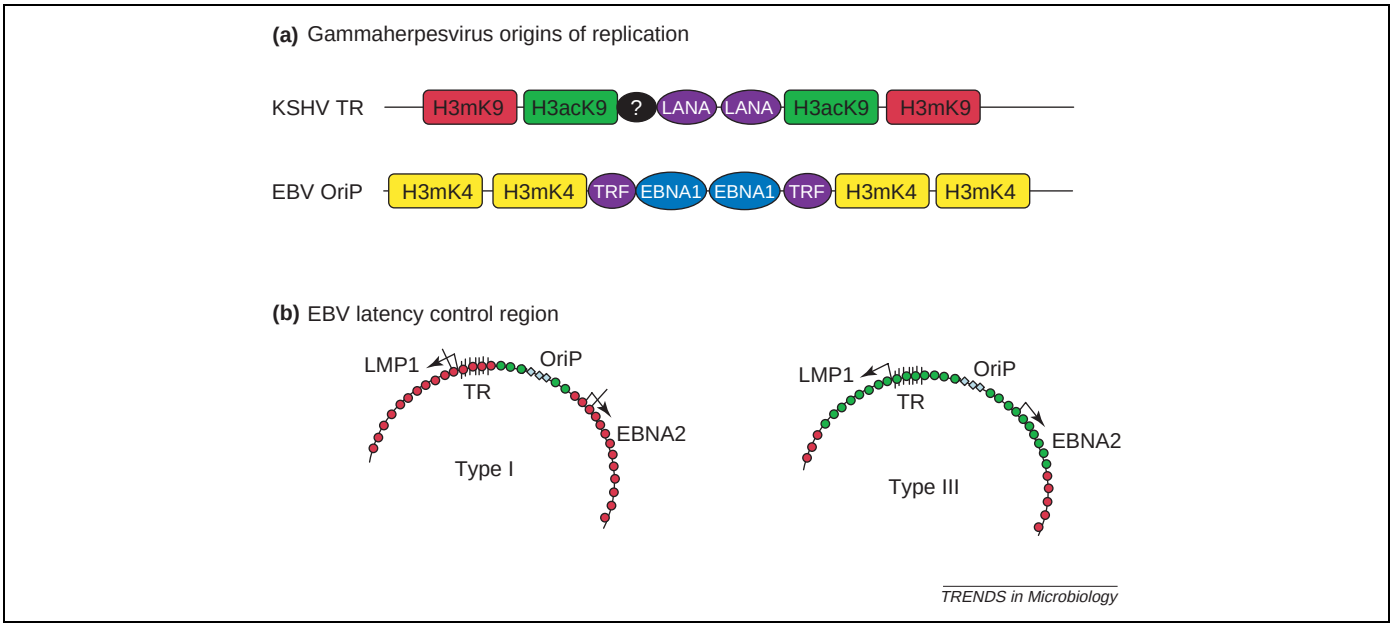

Figure 2. Chromatin regulation of gammaherpesvirus latency. **(a)** The latent cycle origins of DNA replication for KSHV and EBV are flanked by nucleosomes. The KSHV origin is in the terminal repeats (TR), and nucleosomes immediately adjacent to the LANA binding sites are enriched in acetylated histone H3 and H4. Histones more distal to the LANA binding sites are enriched in Lys9-methylated H3 and HP1. The EBV OriP nucleosomes are enriched in Lys4-methylated H3. **(b)** The histone modifications at the EBV latency control region form a domain that correlates with transcription activity (type III latency) or inactivity (type I latency) of the viral-encoded oncogenes LMP1 and EBNA2. Green spheres represent transcriptionally permissive H3mK4 and red spheres represent transcriptionally repressive H3mK9.

and the transcription initiation site [27,28]. In EBV, the IE promoter is repressed partly by the recruitment of histone deacetylases by transcription factor MEF2D [29,30]. Thus, in both EBV and KSHV, the IE genes seem to be inhibited by histone deacetylation and nucleosome occlusion of transcription initiation sites, similar to many cellular promoters with rapid responses to environmental cues.

Integrating viruses

The integrating viruses (e.g. retroviruses, lentiviruses and adeno-associated viruses) must disrupt cellular chromatin during integration and reactivation in unique ways. For lentiviruses and retroviruses, integration is directed primarily through the viral-encoded integrase (INT). Although integration is sensitive to DNA sequence and nucleosome position [31–33], a complete understanding of the epigenetic factors that regulate integration has not yet been reached. HIV integrase binds to the INI1 (also referred to as SNF5) subunit of the BRG1 nucleosome-remodeling complex that alters nucleosome interactions with DNA and stimulates viral integration [34,35]. HIV integrase also binds to the p300 histone acetyltransferase and is a substrate for it, which suggests that both histone and integrase acetylation are required for integration [36]. Based on these and other studies, it is thought that compact chromatin restricts HIV integration. However, compacted chromatin enhances integration of the avian sarcoma virus [37], which indicates that different integrases might prefer different chromatin environments (Figure 3).

Integration also involves the formation of a double-strand break in the cellular chromosome. The chromatin environment that surrounds a double-strand break is typically modified by phosphorylation of histone H2AX (γH2AX) [5]. γH2AX is enriched at sites of retroviral integration, similar to the enrichment that is observed at other double-strand breaks [38]. However, retroviral integration is not dependent on H2AX because integration occurs efficiently in mouse cells that lack H2AX [5]. It is not clear whether additional DNA double-strand break-response proteins assemble and participate during viral integration.

Chromosome condensation and nuclear-envelope breakdown might also inhibit viral integration [39]. At least one cellular factor associated with the nuclear envelope can modulate HIV integration. The barrier-to-autointegration factor (BAF) facilitates HIV integration by preventing suicidal autointegration. BAF is a highly conserved 10 kDa protein that binds to the LEM domains of several nuclear envelope associated proteins, including lamina-associated protein (LAP) 2α, emerin and MAN1. The precise mechanism of BAF inhibition of autointegration is thought to reside in its ability to 'cross-bridge' DNA in the pre-integration complex and facilitate intermolecular integration. Whether BAF has additional functions in limiting HIV integration to certain regions of the chromosome or particular chromatin domains remains to be determined [40,41].

Chromatin silencing of the integrated genomes could be essential for the establishment of a latent reservoir of infected cells [42] and might be linked to early integration events. The avian sarcoma virus integrase protein associates with cellular Daxx, a protein that is typically associated with transcription repression and histone deacetylation [43]. Latency could also be established by cellular mechanisms of chromatin silencing. Completion of the viral life cycle requires reactivation from latency and disruption of chromatin silencing. The human T-cell leukemia virus I (HTLV-I) transcriptional activator protein TAX and the HIV transcriptional activator TAT are known to interact with numerous cellular chromatin remodeling and histone-modifying activities. This observation reflects the importance of nucleosome manipulation during reactivation [44]. HIV TAT also recruits the pTEFb kinase, which is required for phosphorylation of RNA polymerase and transcription elongation [45,46]. In addition, pTEFb seems to stimulate methylation of histone H3 Lys4 and Lys36 during reactivation of latent HIV genomes [46] (Figure 3). These findings suggest that histone H3 Lys4 methylation is linked to RNA polymerase II phosphorylation and transcription elongation through chromatin-repressed domains that limit HIV reactivation.

RNA viruses

Although RNA viruses are not packaged into nucleosomes, there is some evidence that RNA viruses that replicate in the nucleus do interact with the nuclear matrix and must,

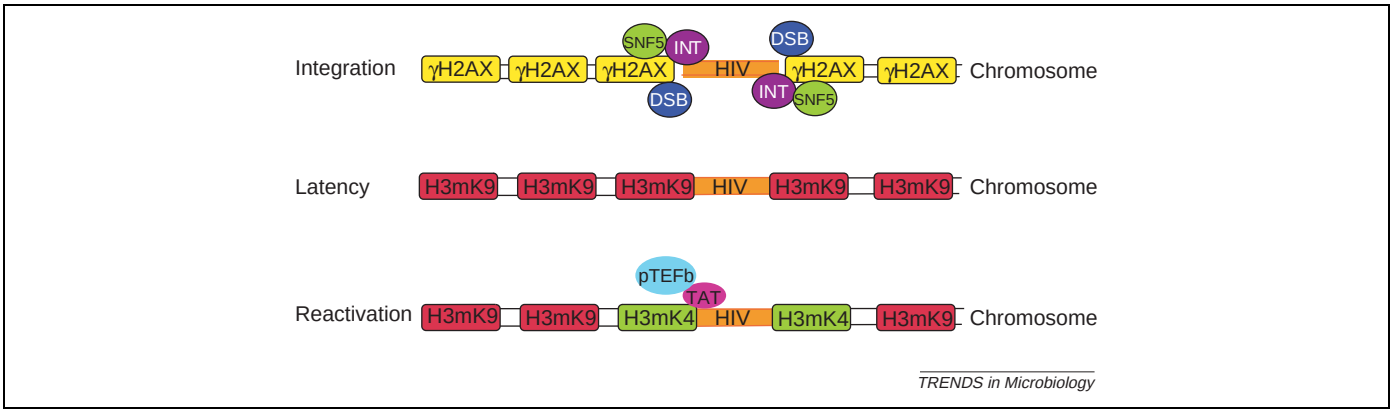

Figure 3. HIV chromatin dynamics. The integration of the HIV genome involves the generation of a double-strand break on the cellular chromosome. Phosphorylation of H2AX occurs at integration sites and chromatin-remodeling factor SNF5 interacts with viral integrase. During latency, the HIV genome is thought to be repressed at heterochromatin sites. The role of H3mK9-modified heterochromatin in HIV repression has not been experimentally established and is, therefore, speculative. Reactivation correlates with expression of TAT, recruitment of pTEFb and methylation of histone H3 Lys4 at the HIV long terminal repeat and genome sequence.

therefore, contend with the cellular chromatin environment. One recent example is provided from studies of the influenza virus ribonucleoproteins (vRNPs) and matrix protein (M1), both of which can interact with histones [47]. Influenza virus enters the cytoplasm as a segmented negative strand and vRNP and M1 are actively transported to the nucleus where viral transcription and replication occurs. Influenza vRNPs are tightly associated with the nuclear matrix and associate with chromatin 1 h after infection. After newly synthesized vRNPs have been generated, M1 binds to the vRNPs and facilitates their transport back to the cytoplasm. M1 has been reported to bind to nucleosomal histones [48], which suggests that M1 could be involved in the release of vRNPs from chromatin. In these studies, vRNP bound to histone tails whereas M1 bound to the globular domain of the histone octamer. It is speculated that interaction with histone tails could influence cellular gene transcription and modulate the release of vRNP from chromatin. These studies suggest that both RNA viruses and DNA viruses must negotiate with the chromatin environment to complete their life cycles.

Viral targeting of cellular chromatin-modifying proteins

Viral regulatory proteins have profound effects on chromatin structure and formation. The variety of viral interactions with chromatin reveals a range of different strategies for maneuvering through the nuclear environment during viral infection. In this section, some representative examples of virus-host interactions that modulate chromatin structure of the host and viral genomes are reviewed.

Histone acetyltransferases (HATs)

HATs have a central role in the activation of transcription and DNA replication. It has been well established that viruses encode proteins that recruit cellular HATs to stimulate cellular and viral gene expression [49]. Numerous viral proteins have been found to associate with cellular HATs. In the majority of interactions, viral-encoded proteins direct HAT activity towards the viral or cellular gene promoters to stimulate transcription. In other cases, viral factors can stimulate acetylation of non-nucleosomal proteins [50] or can be substrates for protein acetylation themselves [51]. Viral proteins can also inhibit HAT activity. For example, in some circumstances, adenovirus E1A protein can inhibit the p300 and CBP-associated factor (P/CAF) and p300 histone acetylation [52], and HPV E6 can bind to and inhibit p300 acetylation of tumor suppressor p53, which prevents p53 stabilization [53].

Histone deacetylases (HDACs)

HDACs are also targets of viral regulatory proteins. The GAM1 protein of avian adenovirus binds to the N terminus of class I HDACs (HDAC1, 2 and 3) and inhibits histone deacetylation activity [54]. GAM1 is essential for viral replication and transcription activation and deletions can be suppressed by addition of HDAC inhibitor TSA. Viral-encoded promiscuous transcriptional activators can also bind to and inactivate HDACs. Bovine herpesvirus ICP0 binds to and inactivates class I HDACs [55], whereas herpes simplex virus ICP0 can bind to class II HDACs 4, 5 and 7 [56]. HSV1 ICP0 can also disrupt HDAC interactions with the co-repressor of neuronal-specific genes, CoREST [57]. A domain in HSV ICP0 mimics the surface of the CoREST transcription repressor that interacts with HDACs 1 and 2 [57]. HSV ICP0 causes the dissociation of HDAC1 from CoREST and other viral factors are involved in the phosphorylation and nuclear export of the CoREST–HDAC1 complex during productive infection. Disruption of the CoREST–HDAC nuclear complex correlates with enhanced viral replication at low multiplicities of infection [57,58].

Human cytomegalovirus (CMV) IE1 (72 kDa, UL123) and IE2 (86 kDa, UL122) also function as promiscuous transcriptional activators. IE1 was found to interact with HDAC3, which correlates with increased histone H4 acetylation at viral major IE promoters [59]. Murine CMV IE1 binds to and inactivates HDAC1, and IE1 mutants have replication delays that can be rescued by HDAC inhibitors [60]. CMV is known to associate with histones during the early stages of replication [61–63] and it is likely that deacetylation of these histones blocks viral gene expression and DNA replication, perhaps promoting viral latency. Thus, modulation of chromatin-modifying enzyme activity might initiate changes in viral gene expression and replication programs.

Linker histones

Histone H1 has a crucial role in chromosome condensation during metaphase and the formation of higher-order structures during anaphase [64]. The adenovirus 2 (Ad2) capsid-hexon protein was found to bind to mono-acetylated histone H1.2 [65]. Histone H1 helps in the processes of nuclear-pore docking and hexon disassembly. Ad2 hexon might have selected H1 for several advantageous reasons, including elimination of the linker histone from interaction with viral DNA and avoidance of viral genome compaction, and transcriptional repression. H1 nuclear import is thought to contribute to the mechanism of capsid disassembly at the nuclear pore and release of adenovirus DNA into the nucleus. H1 might also provide import signaling for the viral capsid after disassembling the hexons [65].

Poly-ADP ribose polymerase (PARP) is the founding member of an expanding family of protein-modifying enzymes that have profound effects on chromatin structure and gene expression [66–68]. PARP can condense chromatin in a similar manner to linker histones and has a poly-ADP-ribosylation activity that reverses chromatin condensation [69]. PARP1 binds to several regulatory regions on viral genomes including the HIV transactivation response region RNA [70] and the replication origins of both EBV [71] and KSHV [72]. PARP is also a target of several viral proteins. KSHV IE transcriptional activator protein RTA has been isolated in a complex with PARP1, and inhibitors of poly-ADP ribosylation can stimulate viral reactivation [73]. The SV40 capsid protein VP3 binds to PARP1 to enhance viral survival through a mechanism that limits PARP-dependent cellular necrosis [74]. In some of these cases, PARP1 can function to inhibit viral gene activity by initiating

chromosome condensation. Stimulation of PARP enzymatic activity might relieve PARP repression through its automodification but PARP modification of viral proteins could also inhibit essential viral processes.

Other chromatin targets

Chromatin-remodeling proteins such as SWI/SNF alter nucleosome position and histone interactions with DNA [4]. INI1/SNF5 is a component of the SWI/SNF ATP-dependent chromatin-remodeling complex, which is the yeast ortholog of the human BRG1 and BRM complexes. INI1/SNF5 was found to be a target of HIV integrase and facilitated integration into chromatin. INI1/SNF5 is also a target of the EBV EBNA2 protein, which is a potent transcriptional activator of repressed viral and cellular genes [75]. Other chromatin-remodeling factors have also been found as targets of viral regulatory proteins. HPV E7 binds to the Mi2B ATPase–histone deacetylase complex to inhibit IRF1-dependent transcription [76]. Remarkably, unlike the HATs and HDACs, substantially fewer interactions have been reported for viral proteins with nucleosome-remodeling complexes.

HSV VP16 can be isolated from infected cells in a complex with cellular proteins that include host cell factor (HCF), a protein involved in chromatin modification and cell-cycle progression [77]. HCF has been isolated as a component of a multiprotein complex that contains the Set1 and Ash1 histone H3 Lys4 methyltransferases and mSin3 histone deacetylase [78]. Loss of HCF abrogates histone H4 K20 methylation, which results in blocking of mitotic chromosome alignment and segregation [79]. Whether interactions with VP16 alter the normal function of HCF or whether HCF is required to modulate virus chromatin structure is not completely understood.

In addition to VP16, HSV encodes an abundant virion protein, VP22, which also modulates chromatin structure [80]. VP22 binds to the cellular template activating factor (TAF)-Iα and TAF-Iβ. TAF-Iα and -Iβ were identified originally as host factors required for adenovirus replication [81]. TAF-Iα is also referred to as SETa and is identical to the INHAT activity that can bind to histones and inhibit histone-tail acetylation [82]. VP22 inhibits the ability of TAF-Iα to function as a histone chaperone and, thus, prevents nucleosome assembly *in vitro* [80]. It has been speculated that VP22 prevents ordered nucleosome deposition on incoming viral DNA.

One other inhibitor of viral replication that seems to be a common target of several viruses is the cellular protein Daxx. Daxx is associated with chromatin, transcriptional repression and the nuclear domain 10 (or PML bodies) that are dissociated during productive viral replication [83]. Daxx is the target of several viral regulatory proteins including CMV IE1 [60], HSV ICP0 [84], HPV L2 [85] and avian sarcoma virus integrase [43]. It has been speculated that Daxx and PML-associated proteins provide an antiviral response by assembling foreign DNA into repressed heterochromatin and that productive infection requires the inactivation of Daxx [86].

Concluding remarks

From this review, it is apparent that viruses have co-evolved multiple mechanisms and strategies to interface with host chromatin (Figure 4; Table 1). Some of these mechanisms are unique to the virus family and the particular life cycle of the virus but in other cases the virus can use strategies that are nearly identical to cellular processes. Replication origins of latent episomal viruses might have chromatin structures that are similar to some cellular replication origins but it is too early to know if these structures are unique to viruses because only a small handful of cellular origins have been characterized at a detailed molecular level. Similarly, chromatin regulation of viral integration might resemble cellular recombination and transposition mechanisms. Transcription regulation also shares many features with cellular chromosomal gene activation. However, other processes such as virion encapsidation and lytic cycle replication could require a complete suspension of cellular chromatin formation or an active disassembly of chromatin that is not found in normal cellular processes. What is clear, however, is that a diverse and complex array of chromatin-based regulatory mechanisms controls DNA accessibility and stability for both cellular and viral genomes.

Although viruses recruit many cellular chromatin-modulating activities, it is notable that viral proteins rarely contain chromatin-associated activities or binding motifs such as HAT, bromo, plant homology domains (PHD), chromo or Su(var)3-9, enhancer-of-zeste, trithorax (SET) domains. One exception is the identification of a viral SET-domain protein in the large *Paramecium bursaria* chlorella virus (PBCV-1), which encodes an active histone methylase specific for Lys27 [87]. It is possible that additional viral proteins encode cryptic chromatin-modifying activities similar to the recently discovered de-ubiquitinating enzymes in the herpesvirus family that might also function in modifying chromatin [88]. However, the limited coding capacity of most viruses could restrict the incorporation of these highly specialized enzymatic functions dedicated exclusively to chromatin functions.

A better understanding of the molecular details of chromatin regulation could provide new opportunities for controlling viral infections and viral-associated pathogenesis. The identification of unique histone modification patterns associated with viral genomes or stages of virus infection might provide insight into virus–host interactions. For instance, the histone modifications that regulate EBV latent gene expression affect host-immune recognition and cellular proliferation associated with malignant growth. Similar chromatin regulation enables the persistence of a reservoir of HIV-infected cells that remain intractable to anti-viral treatment. Therapeutic strategies to stimulate the reactivation of latent viruses (referred to as 'lytic therapies') have been explored for treating latent infections with antiviral drugs such as gancyclovir for EBV [89]. Alternatively, stabilization of chromatin structure could suppress pathogenic reactivation or leaky latent gene expression. Clearly, a more complete understanding of the epigenetic control

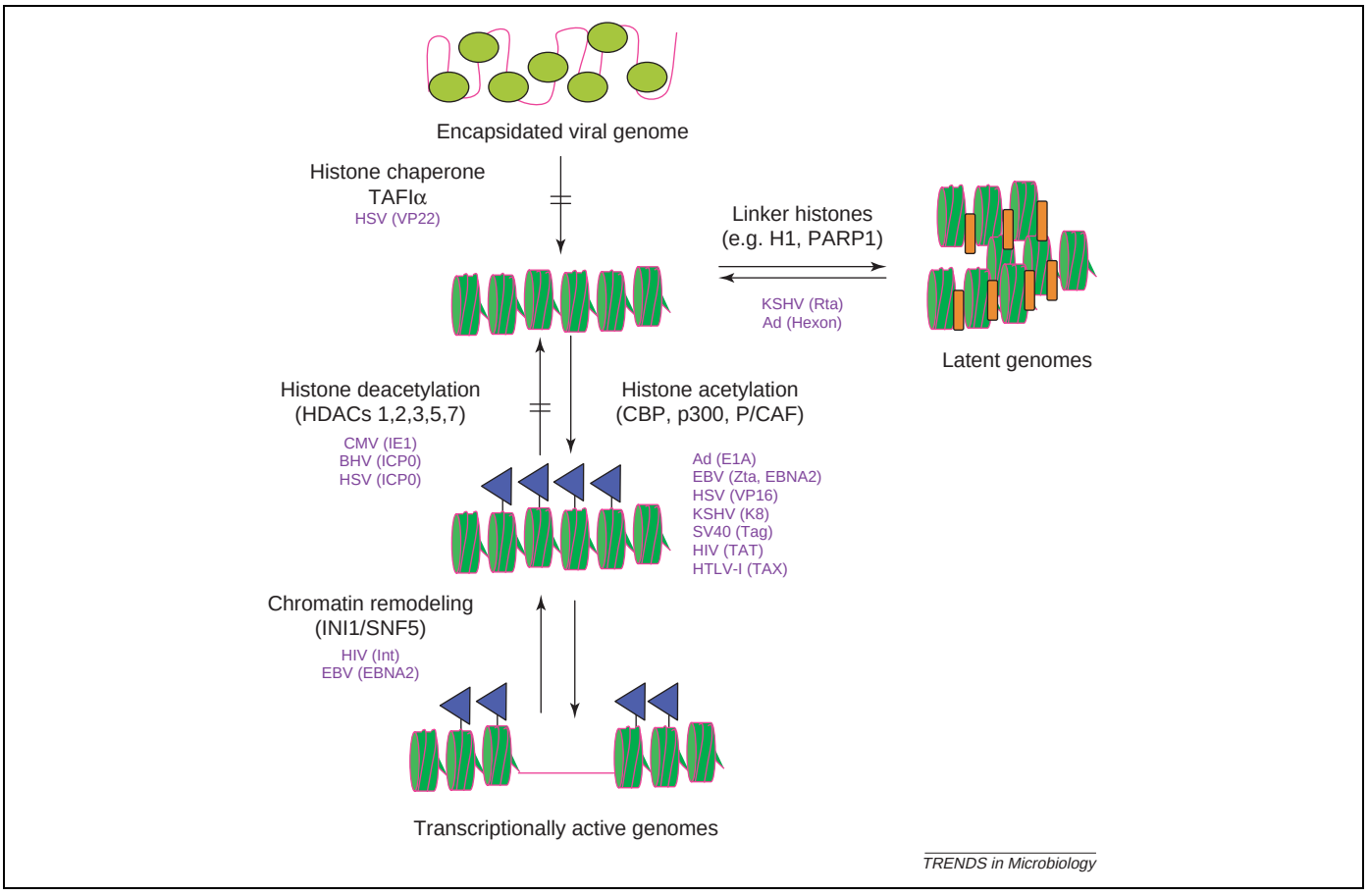

Figure 4. Modulation of chromatin dynamics by viral-encoded proteins. Viral genomes in capsids typically lack nucleosome structure (except for SV40) and acquire cellular chromatin organization during nuclear entry. Histone modifications (primarily acetylation) and ATP-dependent nucleosome remodeling are required to activate viral transcription. Linker histones can induce higher-ordered chromatin compaction and histone chaperones might regulate the assembly of viral genomes into nucleosomes. Many steps of chromatin assembly and disassembly and histone modification can be modulated by viral proteins. Abbreviations: Ad, adenovirus; BHV, bovine herpesvirus; Tag, T antigen of SV40; Zta, EBV IE gene.

Table 1. Examples of viral proteins and their chromatin-associated targets[a]

Virus	Gene	Cellular targets	Function or structure	Refs
SV40	T antigen	p300, CBP	HAT, bromo	[49]
Adenovirus	E1A	p300, CBP	HAT, bromo	[49]
	E1A	P/CAF	HAT	[49]
	GAM1	HDACs 1, 2 and 3	Inhibition of HDACs	[54]
HPV	E7	Mi2 (NURD)	Chromatin remodeling	[76]
	E7	p300, CBP	HAT	[49]
	E2	BRD4	Bromo	[17,18]
HSV	VP16	HCF	H3 Lys4 methylation	[77–79]
	VP22	SETa, TAF-Iα	Histone chaperone	[80–81]
	ICP0	Daxx	Heterochromatin formation?	[84]
EBV	Zta (ZEBRA/EB1)	p300, CBP	HAT	[49]
	EBNA2	INI1/SNF5	Chromatin remodeling	[75]
KSHV	Rta	PARP1	Linker histone, PARP	[72]
	LANA	Histone H1	Linker histone	[90]
	LANA	BRD2	Bromo	[91]
CMV	IE1	HDACs	Inhibition of HDACs	[49]
	IE1	Daxx	Heterochromatin	[83]
HIV	INT	INI1/SNF5	Chromatin remodeling	[34,35]
	INT	p300	HAT	[36]
	TAT	pTEFb	Histone methylation	[45,46]
	TAT	p300, CBP, GCN5, P/CAF, TIP60	HAT	[49]
HTLV-I	TAX	p300, CBP	HAT	[49]
Influenza	vRNPs	Histone tails	Nuclear matrix	[47,48]
	M1	Histones	Interactions?	[47,48]

[a]Abbreviations: GCN5, GCN5 histone acetyltransferase protein; Mi2, Mi2 autoantigen; NURD, nucleosome remodeling and histone deacetylase complex; TIP60, TAT-interacting protein 60.

of virus infections will lead to new opportunities for pharmacological control and intervention in viral-associated disease.

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