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Snapshots: Chromatin Control of Viral Infection

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Abstract

Like their cellular host counterparts, many invading viral pathogens must contend with, modulate, and utilize the host cell's chromatin machinery to promote efficient lytic infection or control persistent-latent states. While not intended to be comprehensive, this review represents a compilation of conceptual snapshots of the dynamic interplay of viruses with the chromatin environment. Contributions focus on chromatin dynamics during infection, viral circumvention of cellular chromatin repression, chromatin organization of large DNA viruses, tethering and persistence, viral interactions with cellular chromatin modulation machinery, and control of viral latency-reactivation cycles.

Keywords

Chromatin; Virus; Herpesvirus; Adenovirus; Papillomavirus; Human Immunodeficiency Virus; Influenza virus

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Introduction

Epigenetics, the supra-regulatory overlay of nucleosomes and chromatin structure on the genome has emerged as a critically important determinant of cellular transcription, replication, and differentiation state. Advances in the field have revealed families of proteins that modulate the assembly, placement, recognition, modification, and remodeling of nucleosomes; thus regulating and modulating access to the genome.

In recent years, investigations into the role of chromatin in the regulation of viral infection have revealed the significance of dynamic viral-host chromatin interactions in determining viral infection as well as the interplay of viral encoded proteins in modulating chromatin. While advances in chromatin biology have accelerated studies on the role of chromatin in regulation of viral infection, viral model systems have also elucidated novel mechanisms that impact the larger cellular chromatin arena. Significantly, chromatin control of viral infection also represents a new area with potential targets for the development of novel antiviral therapies. This compilation review focuses on concepts in chromatin biology in various viral model systems.

Chromatin regulation of alpha-herpesvirus immediate early gene expression by the HCF-1 coactivator complex

Thomas M. Kristie—Productive alpha-herpesvirus (HSV, herpes simplex virus; VZV, varicella zoster virus) infection requires a tightly controlled program of viral gene expression. Upon infection, transcription of the viral immediate early (IE) genes is initiated based upon a complex combinatorial set of both viral and cellular DNA-binding transcription factors (HSV IE, Figure 1A). High level induced expression is dependent upon the assembly of enhancer core complexes (EC) composed of the cellular POU-homeo domain factor OCT-1, the viral IE activator (VP16 or ORF10) and the cellular coactivator HCF-1 (reviewed in (Kristie et al., 2010)). However, while multiple factors contribute to the level of IE transcription, the transcription potential of these factors is dependent upon the essential coactivator HCF-1 (Narayanan et al., 2005).

While HCF-1 is an essential component of the HSV and VZV IE regulatory paradigm, the mechanisms by which the protein functions have only recently been described. Based upon advances in epigenetic regulation and proteomics, HCF-1 has been found to be a critical modulatory platform in multiple chromatin modulatory complexes (Figure 1B) including histone methyltransferases (Set1 and MLLs (Wysocka et al., 2003; Yokoyama et al., 2004)), demethylases (LSD1, PHF8, JMJD2s (Liang et al., 2009; Liu et al., 2010)), and acetyltransferases (ATAC, MOF (Cai et al., 2010; Guelman et al., 2006; Suganuma et al., 2008)). Additionally, HCF-1 interacts with histone chaperones (Asf1 (Peng et al., 2010)) and other chromatin modulatory components.

Strikingly, many DNA viruses including the herpesviruses are devoid of nucleosomes in the capsid and become rapidly chromatinized upon infection. While not completely understood, it is clear that this process is a dynamic interplay of cell-mediated deposition of repressive chromatin (histone H3K9 and H3K27 methylation) and viral directed modulation of histone modification and nucleosome remodeling. For the alpha-herpesviruses, the recruitment of an HCF-1 coactivator complex containing both histone H3K9 demethylases (LSD1 and JMJD2 family members) and histone H3K4 methyltransferases (Set1 and/or MLL family members) presents a model in which this complex plays an integral role in limiting the accumulation of repressive chromatin (H3K9-me) and promoting the installation of the positive-transcriptional activating marks (H3K4-me) (Liang et al., 2009; Narayanan et al., 2007) (Figure 1C). The model is supported by recent studies in which inhibition of the activity of

the HCF-1 associated H3K9 demethylases (LSD1 and JMJD2s) results in continued accumulation of repressive chromatin on the viral IE gene promoters and silencing of the viral genome. Importantly, given the central role of HCF-1 in chromatin modulation, this regulatory paradigm is likely to be more general and extend to the regulation of infection by other DNA viruses. While many questions remain concerning epigenetic regulation of viral gene expression, it is also clear that this research front has potential to expose novel targets for the development of antivirals directed against stages of infection distinct from the present replication inhibitors.

Chromatin Model for Regulation of Herpes Simplex Virus Lytic versus Latent Infection

David Knipe—During acute infection of mucosal epithelium, herpes simplex virus (HSV) undergoes a productive infection in which the more than 80 viral lytic genes are expressed (Roizman et al., 2013). The virus spreads into sensory neurons where it establishes a latent infection in which the lytic genes are silenced and only the latency-associated transcript (LAT) and miRNAs are expressed. During lytic infection the viral genome shows a limited chromatin association and the histones show eukaryotic modifications (reviewed in (Knipe and Cliffe, 2008); Figure 2) while during latent infection the HSV genome is associated with chromatin bearing heterochromatic modifications. In 2008 we proposed that epigenetic regulation of viral chromatin by viral gene products plays an important role in determining whether the outcome of infection is a lytic or latent infection (Knipe and Cliffe, 2008).

A number of lines of evidence are accumulating to support the regulation of lytic infection by chromatin regulation: (i) The viral genome shows a progressive removal of chromatin and euchromatic modification of the remaining histones during the course of infection (Cliffe and Knipe, 2008; Oh and Fraser, 2008); (ii) Viral mutants defective for VP16 or ICP0 show increased heterochromatin association with viral lytic promoters (Cliffe and Knipe, 2008; Herrera and Triezenberg, 2004), showing that viral proteins in the tegument or expressed as IE proteins promote lytic gene expression by promoting euchromatic modifications on histones during lytic infection (Figure 2); (iii) the VP16-HCF1-Oct1 complex recruits a number of histone modification enzymes to viral IE genes to promote gene expression, as described above, and inhibitors of one of those, LSD1, inhibit viral lytic gene expression and reactivation from latent infection (Liang et al., 2009)(iv) the immediate-early ICP0 protein disrupts HDAC1 complexes (Gu and Roizman, 2007) and disrupts inhibitory ND10 structures (Everett et al., 1998); (v) host functions such as the nuclear lamina define intranuclear targeting of the viral genome and the level of heterochromatin on the viral genome, which correlates with silencing of the viral genome (Silva et al., 2008); (vi) Host chromatin remodeling proteins are required for optimal levels of viral gene expression (Bryant et al., 2011).

A number of lines of evidence are accumulating to support the regulation of latent infection by heterochromatin: (i) The viral genome shows a progressive accumulation of heterochromatin as latent infection is established (Wang et al., 2005); (ii) the viral genome shows low levels of euchromatic marks on viral chromatin on lytic genes (Kubat et al., 2004a; Kubat et al., 2004b) but instead is associated with facultative heterochromatin, from which it can be readily reactivated (Cliffe et al., 2009; Kwiatkowski et al., 2009); (iii) LAT expression leads to silencing of viral lytic genes (Garber et al., 1997) and increased levels of heterochromatin (Cliffe et al., 2009; Wang et al., 2005)In these studies comparison of two LAT deletion mutants and their respective rescued viruses showed statistically significant increases in heterochromatin when LAT was expressed. However, comparison of one mutant with the wild type parent showed decreased levels of heterochromatin with LAT expression (Kwiatkowski et al., 2009). Although the effects of LAT may differ in different reports, there is a clear correlation between heterochromatin and silencing of the viral

genome in all of the LAT mutant studies; (iv) Treatment of latently infected animals with HDAC inhibitors leads to reactivation of latent virus (Neumann et al., 2007).

The potential role of chromatin in the lytic-latent infection decision was first defined with HSV, and similar stories have developed for other herpesviruses. In some cases, it is easier to study latent infections by other viruses where a cell culture system can be used to establish latent infection, such as KSHV (Toth et al., 2010). These systems have been amenable to rapid gains in our knowledge of the mechanisms of epigenetic control of latent viral genomes. A neuronal cell culture system for HSV latency could also enable many important experiments in this area.

Control of Gammaherpesvirus Latency by Chromosome Conformation

Paul M. Lieberman—The human gammaherpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated virus (KSHV) establish stable latent infections where only a small subset of viral genes are expressed. During latency, the viral genome is maintained as a multicopy minichromosome that adopts chromatin structures similar to that of the host cell chromosome. The mechanisms that restrict expression of some genes and permit expression of others remain an important question in gammaherpesvirus biology. To better understand the transcription factor binding and chromatin organization that controls viral gene expression during latent infection, we have used genome-wide chromatin-immunoprecipitation (ChIP) combined with next generation sequencing (ChIP-Seq) (Arvey et al., 2012; Chen et al., 2012; Kang et al., 2011; Tempera et al., 2011). We have found that the cellular chromatin boundary factor CTCF colocalizes with cohesins at several sites throughout the latent genomes (Figure 3). Both EBV and KSHV share the common feature of having a major CTCF-cohesin binding site located ~15 kb from the terminal repeats and located within an intron of a highly regulated latency transcript. For KSHV, the major CTCF-cohesin peak resides in the first intron of the LANA-vCyclin-vFLIP multicistronic transcript. For EBV, the major CTCF-cohesin peak resides in the overlapping first intron of LMP2 and the 3′ UTR of LMP1. Depletion of CTCF or cohesins, as well as mutations of these CTCF binding sites results in deregulated gene expression. These chromatin-organizing factors play an important role in epigenetic patterning of the viral chromosome, coordinating histone tail modifications and DNA methylation with gene expression programs.

CTCF and cohesin have been implicated in forming higher-order chromatin conformations that control gene expression. To explore the chromatin conformation of the latent viral genomes of EBV and KSHV, we used chromosome conformation capture (3C) assays. We found that CTCF-cohesin binding sites are involved in DNA-loop formation with other regions of the viral genomes. For KSHV, CTCF-cohesin mediates a loop between the latent and lytic control regions, and depletion of cohesin subunits leads to a robust reactivation of lytic cycle gene transcription. For EBV, CTCF-cohesin mediates a loop between the LMP1/LMP2 region and the OriP enhancer controlling EBNA2 transcription. Depletion of cohesin or mutation of the key CTCF-cohesin binding sites results in a deregulation of EBV latency transcripts. Our data suggest that both EBV and KSHV use chromatin organizing factors and chromosome conformation to maintain stable gene programs during latent infection.

Epigenetic regulation of KSHV gene expression

Jae U. Jung—Kaposi's sarcoma-associated herpesvirus [KSHV, human herpesvirus 8 (HHV-8)] establishes persistent infection, which can lead to the development of Kaposi's sarcoma and B cell lymphomas in infected individuals with compromised immune system. Latent KSHV infection is characterized by the repression of lytic genes and the constitutive expression of a few genes from the latency-associated locus. During latency, the viral

genome exists in a chromatinized state in the nucleus, where the KSHV genome is distinctively enriched with the activating (acetylated H3 or acH3 and H3K4me3) and repressive (H3K9me3 and H3K27me3) histone modifications (Figure 4A) (Gunther and Grundhoff, 2010; Toth et al., 2010). Latency-associated genes (La) are associated with activating histone marks, exclusively accompanied by transcriptionally active RNA polymerase II (RNAPII) binding, and thereby constitutively expressed during latency. Immediate early (IE) and early genes (E) mainly carry either bivalent chromatin (acH3, H3K4me3 and H3K27me3) or active chromatin (acH3/H3K4me3-rich). In contrast, the majority of late genes are enriched in H3K9me3- and H3K27me3-marked heterochromatin. In addition, two histone modifying enzymes, EZH2 H3K27me3 histone methyltransferase of the Polycomb Repressive Complex 2 (PRC2) and JMJD2A H3K9me3 histone demethylase, are associated with the latent KSHV genome (Figure 4A) (Chang et al., 2011; Toth et al., 2010). While EZH2 occupies most of the viral genome and is involved in the inhibition of lytic gene expressions during latency, JMJD2A-binding is limited to the acH3/H3K4me3 chromatin domains of KSHV genome where it prevents the methylation of H3K9. As a result, the H3K9 can be swiftly acetylated, which ultimately allows robust expressions of the lytic genes at the early phase of reactivation (Chang et al., 2011; Toth et al., 2010). Collectively, the genome-wide epigenetic analysis indicates that the chromatinization of KSHV genome is involved in the regulation of latent gene expression as well as the temporally ordered progression of lytic gene expression.

Surprisingly, a number of IE and E genes carry a euchromatin structure similar to the latent genes during latency but their gene expressions are tightly repressed, suggesting that mechanisms other than heterochromatin-mediated transcription inhibition must participate in blocking their expression during latency. RNA polymerase II (RNAPII) ChIP-on-chip has revealed that RNAPII binds to the promoters of OriLytL, K5, K6 and K7 lytic genes during latency. However, the productive transcription of these genes is blocked by cellular regulatory factors that specifically repress the transcription elongation step (Figure 4B) (Toth et al., 2012). Thus, the control of transcription elongation is an additional regulatory mechanism of lytic gene expressions during latency. Importantly, the presence of paused RNAPII on the promoters of K5, K6 and K7 genes allows their expression independently of the canonical RTA-dependent lytic reactivation. As these lytic proteins function in immune evasions and cell survivals, the transient expression of K5, K6 and K7 genes, in an RTA-independent manner, may protect latently infected cells from host immune attacks.

The switch from latency to reactivation is principally mediated by an IE protein of KSHV, called RTA (Figure 4C). Although the RTA promoter is associated with bivalent chromatin, it lacks paused RNAPII and is tightly repressed by PRC2 and histone deacetylases during latency (Lu et al., 2003; Toth et al., 2010). Upon reactivation, the JMJD3 and UTX H3K27me3 histone demethylases and H3K4me3 histone methyltransferase are recruited to the RTA promoter via KSHV-encoded PAN RNA (Rossetto and Pari, 2012; Toth et al., 2010). Then, RTA binds to its own promoter through the cellular transcription factor CBF1 and subsequently recruits histone acetyltransferases (CBP/p300) and chromatin remodeling factors (SWI/SNF2), modifying the KSHV chromatin structure to a transcriptionally permissive state and thereby allowing a complete cycle of viral reactivation (Gwack et al., 2003).

Association of papillomavirus genomes with host chromatin

Alison McBride—Papillomaviruses are a ubiquitous and ancient group of DNA viruses and, to date, there are over 240 completely sequenced and characterized papillomavirus genomes (McBride et al., 2012b). Each virus is species specific and tropic for particular anatomical regions of the host mucosal or cutaneous epithelium. Papillomavirus infection is

persistent and can be asymptomatic or result in a wide variety of papillomas, and is associated with several human cancers. Despite the diversity of disease cause by papillomaviruses, each virus has a very similar genomic organization and encodes just a handful of similar gene products. Hence, papillomaviruses must use and exploit cellular mechanisms.

Papillomaviruses initiate infection in the dividing basal cells of a stratified epithelium and within these cells they establish the viral genome as a low copy, extrachromosomal element. When the infected basal cells divide the viral genome is partitioned to the daughter cells, which can either remain in the lower layers of the epithelium or can begin the process of differentiation, moving up through the different layers towards the surface of the epithelium. Papillomaviruses take advantage of this process, and rely on the differentiating cellular environment to switch on vegetative viral DNA replication and late capsid protein synthesis. Viral particles are assembled in the upper layers of the epithelium and are released in viral-laden squames that naturally slough from the surface of the epithelium. Thus, the infected basal cells provide a reservoir of infected cells that can persistently shed viral particles into the environment.

Papillomavirus genomes are packaged in host nucleosomes, both in persistently infected cells and in virion particles and, for the most part, use cellular factors to regulate viral gene expression and replication. But papillomavirus proteins (mainly E6 and E7) also modulate host global gene expression by altering the function and activity of cellular chromatin modifying and regulating factors (McLaughlin-Drubin et al., 2011) and reviewed in (McLaughlin-Drubin and Munger, 2009). These global changes can modulate cell growth and differentiation and help the virus escape detection. Papillomaviruses also exploit host chromatin by associating their genomes with specific regions to facilitate viral transcription, replication and genome partitioning. In this review, we will focus on how this association sustains the papillomavirus life-cycle.

Papillomavirus genomes are associated with active regions of chromatin in the host nucleus and this is probably important for escape from host silencing mechanism and to guarantee that the appropriate transcription factors are easily accessible (reviewed in (McBride et al., 2012a; You, 2010)). The viral E2 proteins (full-length E2 and E8^E2 repressor) modulate viral transcription by recruiting chromatin binding proteins to the viral promoters. For example, E2 recruits the Brd4 chromatin adaptor protein to viral promoters to both repress and activate viral transcription (reviewed in (You, 2010)). The E8^E2 protein enlists recruits chromatin modifying complexes such as NCoR1/HDAC3 to repress viral transcription (Powell et al., 2010).

At least one of the chromatin binding factors used to regulate viral transcription, is also important for viral genome partitioning. The double-bromodomain protein, Brd4, in complex with the viral E2 protein, binds to regions of acetylated cellular chromatin throughout the cell cycle (reviewed in (McBride et al., 2012a; You, 2010)). The E2 protein binds directly to specific consensus motifs in the viral genome and links the viral genomes to the host chromatin. This linkage can serve to associate the viral genomes with specific regions of viral chromatin to ensure that the genome is located in an active domain of the nucleus. Because this association persists during mitosis, it also guarantees that the genomes will be partitioned to daughter cells and will be located in the correct nuclear location when the nucleus reforms after mitosis. Notably, the gamma-herpesviruses also use this mechanism to maintain their genomes in dividing cells and the HHV-8 LANA and EBV EBNA tethering proteins have many structural and functional similarities with the papillomavirus E2 protein (reviewed in (McBride, 2008)). Figure 5 depicts a model of the viral E2 protein tethering the viral genome to mitotic chromosomes.

Papillomaviruses exploit host chromatin-related processes in yet another way. The viral E1 and E2 proteins induce a cellular DNA damage response in specific nuclear foci on host chromatin to replicate viral DNA (Fradet-Turcotte et al., 2011; Sakakibara et al., 2011). This involves activation of the ATM/ATR pathways, phosphorylation of histone H2AX and recruitment of DNA repair machinery.

Papillomaviruses vegetatively amplify viral DNA in differentiated cells that are most likely in the G2 phase and so recruitment of DNA repair machinery will enable the virus to synthesize DNA without S-phase. In addition, late DNA replication has been shown to require the ATM pathway (Moody and Laimins, 2009). Activation of the cellular DNA damage response in nuclear foci is an ingenious strategy as it induces an influx factors required for viral DNA replication with very little effort on the part of the virus. Although there is most evidence at this point that this is important for late viral replication, it is likely that similar mechanisms are involved in the initial establishment of the viral genome upon infection and for long-term genome maintenance and stability.

In summary, papillomaviruses are well versed in the chromatin-related processes of the cell. At each step of the viral life-cycle they use and exploit these processes for transcription and replication, to maintain a long-term infection and to escape detection by the host cell.

Transcriptional activation of HIV-1

Kevin V. Morris—Infection of both dividing and nondividing cells by the Human immunodeficiency virus type 1 (HIV-1) results in integration of the viral genome into host chromatin, followed by a period of latent infection. Latency in HIV-1 infection has immense importance because infectious virus can remain protected from the infected individual's immune system and emerge several years after infection. The underlying cellular mechanism(s) responsible for establishing and/or maintaining latency are still unclear (reviewed in (Lassen et al., 2004)).

What is clear however is that the proviral genome functions in a manner similar to the host DNA and interacts with chromosomal proteins and is thus subject to the host epigenetic regulatory machinery. Two components seem to be operative in affecting viral latency, (1) the location of the integrated provirus and (2) a location independent mechanism of host gene regulatory machinery. Several observations point to the fact that the local state of the chromatin at the integrated provirus plays a significant role in virus expression and latency. These reports have shown a role for chromatin and chromatin remodeling in latency. Histone acetyltransferases (HATs) have been implicated in remodeling the chromatin at the provirus as well as recruiting histone deacetylase complex 1 (HDAC-1) (reviewed in (Mok and Lever, 2007)).

The reactivation of latent virus has been shown to require HATs, which operate in the acetylation of the histone tails specifically at the HIV-1 promoter/LTR. ATP-dependent chromatin remodeling proteins have also been shown to be recruited to the activated viral promoter/LTR (Henderson et al., 2004). Conversely, silent state chromatin marks, such as histone 3 lysine 9 trimethlyation, and HDAC-1 recruitment to the viral promoter/LTR have been observed when the provirus is repressed (Marban et al., 2007). Surprisingly such a molecular cascade of epigenetic based regulatory mechanisms has also been observed in human cells with those loci that are regulated by long non-coding RNAs (lncRNAs) (reviewed in (Malecova and Morris, 2010)), possibly suggesting a similar mechanism is operative in HIV-1 infected human cells.

Emerging evidence suggests that lncRNAs can exhibit regulatory functions ranging from active scaffolding to moieties involved in *trans* targeting of silent state epigenetic complexes

to particular loci (reviewed in (Koziol and Rinn, 2010; Morris, 2011)). In particular are observations whereby long antisense ncRNAs have been found to target the epigenetic regulation of gene expression (Figure 6). In this model antisense lncRNAs containing sequence homology to particular loci function as recruiters of silent state epigenetic complexes to the intended target. The result of this antisense lncRNA targeting is ultimately transcriptional gene silencing (TGS) of the targeted loci due to silent state epigenetically related changes at the targeted loci (Figure 6). Interestingly, similar to endogenous genes in human cells, HIV-1 has also been observed to express long antisense transcripts (Kobayashi-Ishihara et al., 2012; Landry et al., 2007; Ludwig et al., 2006). These transcripts emanate from the 3′ LTR, similar to previous observed regulatory antisense lncRNAs, have been observed to implement silencing of HIV-1 expression (Kobayashi-Ishihara et al., 2012). Collectively, a notion is emerging that suggests the previously observed modes of lncRNA based epigenetic regulation may also be functional for HIV-1 in viral infected cells (Figure 6). Such a mechanism would be expected to be functional during particular stages of the viral life cycle, namely when the 3′ LTR is transcribed in the antisense orientation. The genesis of the 3′ LTR expressed HIV-1 specific antisense lncRNA could then feedback to target epigenetic silencing complexes to the 5′ LTR, ultimately resulting in some level of TGS (Figure 6). While it remains to be determined if HIV-1 expressed antisense lncRNAs are functional as transcriptional modulators of HIV-1 and viral latency, it can be ascertained, based on recent studies that a mechanism is functional in human cells whereby antisense lncRNAs act to direct epigenetic based silencing complexes to target loci (Figure 6). As such the targeted destruction of this HIV-1 expressed antisense transcript in HIV-1 infected cells may prove useful in obstructing the progression to viral latency or inducing the emergence of HIV-1 from latency.

Acetylated Lysine/Bromodomain Interactions Regulate HIV Transcription

Melanie Ott—Transcription of the HIV provirus is regulated by reversible acetylation of histones and nonhistone proteins. Upon integration into the human genome, the HIV proviral cDNA is organized into higher order chromatin, a process that is dictated by the provirus itself and is independent from the integration site. Inhibitors of histone deacetylases (HDACs) were shown early on to re-activate HIV from transcriptional latency in cell culture cells, and the concept of a potential “flush-out” approach to latency in patients was developed (Van Lint et al., 1996). These findings led to clinical approaches to reduce the latent reservoir in HIV-infected patients under highly active antiretroviral therapy with different HDAC inhibitor cocktails (Archin et al., 2012).

In addition to histones, several nonhistone factors central to HIV transcription are subject of reversible acetylation, including the viral transactivator Tat and its essential cellular cofactor, the positive transcription elongation factor b (P-TEFb). Tat is acetylated at lysines 28 and 50 by histone acetylases PCAF/KAT2B and p300/KAT3B, respectively (Ott et al., 2011). Both acetylation events regulate the interaction of Tat with TAR RNA and P-TEFb, in the case of K28 acetylation, in a positive way, while acetylation of K50 terminates this interaction. In addition to Tat, both components of P-TEFb, cyclin T1 and CDK9, are subject to reversible acetylation (Cho et al., 2010).

Acetylated lysines serve as specific interaction platforms for bromodomain-containing proteins. As such, acetylated K50 in Tat specifically interacts with the bromodomain of PCAF/KAT2B, a proposed mechanism to recruit the acetyltransferase activity of PCAF to elongating HIV transcripts (Dorr et al., 2002; Mujtaba et al., 2002) (Figure 7). Structural analysis of the acetylated K50/PCAF bromodomain interaction identified several additional residues in Tat and PCAF that interacted in a K50-acetylation-dependent manner and strengthened binding between the two factors. Mutations in the PCAF bromodomain that

suppressed interactions with acetylated Tat or treatment with small molecules that bind the PCAF bromodomain effectively suppressed Tat transactivation, supporting the concept that this interaction could serve as a specific drug target in HIV transcription.

A second bromodomain-dependent interaction exists between acetylated cyclin T1 and the second bromodomain in the double-bromodomain-containing protein Brd4 (Schröder et al., 2012; Vollmuth et al., 2009). Cyclin T1 is acetylated at four defined residues within a predicted coiled-coil region of the protein (Cho et al., 2010). Three of the four residues (K380, K386, K390) bind the second bromodomain of Brd4, defining a new modification-specific P-TEFb interaction domain in Brd4 besides a previously identified C-terminal P-TEFb-interacting domain (PID) (Bisgrove et al., 2007). Interestingly, while this acetylation-dependent interaction is required for basal HIV LTR activity and cellular gene expression, it is not necessary for Tat-mediated transactivation of HIV transcription, supporting the model that Tat- and Brd4-mediated activities at the HIV promoter are mutually exclusive (Bisgrove et al., 2007; Yang et al., 2005) (Figure 7).

In summary, acetylated lysine/bromodomain interactions of nonhistone proteins play versatile roles in the regulation of HIV transcription and latency, and the prospect of targeting these interactions with specific bromodomain inhibitors in the future is compelling.

Translating epigenetics into therapeutics for latent HIV infection

David Margolis—Current antiretroviral therapy suppresses human immunodeficiency virus type 1 (HIV-1) replication, however, proviral infection still persists in a small pool of latently infected cells, primarily resting $CD4^+$ T cells. Although advances in therapy have led to marked improvements in patient longevity and quality of life, chronic lifelong antiretroviral therapy is complex and costly. Therefore clearance of HIV infection has become an important, albeit difficult therapeutic goal, and targeting this reservoir of latently infected cells is a key approach to eradication strategies.

Initial studies by Verdin demonstrated that a strictly positioned nucleosome was found at the viral RNA start site, and increased accessibility of chromatin near this nucleosome was associated with transcriptional activation (Van Lint et al., 1996). Further, histone deacetylase inhibitors were shown to up-regulate LTR expression, and recruitment and occupancy of histone deacetylase 1 (HDAC1) at the HIV LTR was shown to directly mediate transcriptional silencing (Margolis, 2011). Further study of the role of HDACs in LTR regulation revealed multiple cellular DNA-binding protein complexes that could recruit HDACs to the integrated provirus (Margolis, 2010).

Recently it has been found that the class I HDACs 1, 2, and 3 predominate at the HIV LTR in primary CD4+ T cells, and that viral outgrowth could be induced from the resting CD4+ T cells of HIV-infected, aviremic, ART-treated patients by selective HDAC inhibitors (HDACis) targeting these same class I HDACs (Archin et al., 2009; Keedy et al., 2009; Margolis, 2011). Other epigenetic modifications, such as methylation of histones or of DNA itself contribute to the regulation of proviral latency, and may be targets for therapy. CpG methylation of HIV promoter DNA has been shown to contribute to establish a durable, “locked” state that is difficult to reactivate. Histone methyltransferases such as EZH2 and SUV39H1 can regulate HIV-1 transcription by inducing histone H3 at lysine 9 (H3K9) methylation, and other repressive proteins can accumulate on transcriptionally inactive proviruses (Blazkova et al., 2009; Kauder et al., 2009). Pearson and colleagues further corroborated those findings and showed that progressive iterative histone modifications drive a proviral promoter into latency in primary CD4+ T cells (Pearson et al., 2008).

We directly tested the hypothesis that a clinically tolerable dose of the class I-selective HDAC inhibitor suberoylanilide hydroxamic acid (SAHA or vorinostat (VOR), could induce expression of HIV within resting CD4+ T cells of HIV-infected patients *in* vivo (Archin et al., 2012). Eight participants (CD4 count 432-1147/μl, mean 713/μl) in whom an induction of HIV RNA expression following *in vitro* exposure to VOR was measured continued their successful, suppressive, long-term antiretroviral therapy (tenofovir, emtricitabine, and efavirenz in all). Global cellular histone acetylation in PBMCs was unchanged up to 24 hours after a single 200 mg oral dose, and histone acetylation at the human p21 gene promoter was modestly increased in only two participants. However, a single 400 mg dose achieved peak plasma concentrations of 244 (median, range 153–301) ng/mL a median of two hours (range 0.5–4) after dosing, with rapid clearance as reported in other populations. This exposure was invariably correlated with median 1.6-fold (range 1.2–2.0) increase in acetylation of total cellular histone H3 ($p < 0.01$), an increase in acetylation of the human p21 gene as measured by chromatin immunoprecipitation, and of most interest a universal induction of 1.5 to 10.0-fold (mean 4.8) in expression of unspliced HIV-1 *gag* RNA within circulating resting CD4+ T cells. Throughout this limited exposure, VOR was well tolerated with no adverse events greater than Grade I.

Therefore a single, clinically tolerable dose of VOR induces an increased level of HIV RNA expression detected within resting CD4+ T cells, demonstrating that, at least for a period of time in some infected cells, all of the restrictions that limit the expression of latent proviral genomes have been overcome. These cells may therefore be cleared due to cytopathic effects of viral expression, or may become targets for immune-mediated clearance.

Efforts are now focused on determining whether combination therapy targeting multiple epigenetic restrictions to the expression of HIV may more rapidly or universally ablate the latent state of HIV infection, and allow fuller clearance of HIV genomes that persist for years in patients despite suppressive antiretroviral therapy. Although evidence in favor of this approach has been reported in cell line experiments, and in assays of bulk cell populations from patients (Bouchat et al., 2012), the pharmacodynamic conditions used cannot be replicated in the clinic with currently available drugs (Figure 8). Challenges to be overcome include the possible side effects of inhibition of multiple post-translational modifications of cellular proteins, and the possible negative effects of such inhibition on the full expression of viral antigens or virions, a process that is likely to be required to render latently infected cells targets for clearance.

CHD6 chromatin remodeler, a cellular repressor of influenza virus replication that relocates to inactive chromatin upon infection

Amelia Nieto—The influenza virus genome consists of eight segments of single-stranded and negative sense RNAs (vRNAs. For genome expression, vRNAs form ribonucleoprotein complexes (vRNPs) together with four essential viral proteins: the nucleoprotein (NP) and the three subunits of the polymerase (PB1, PB2 and PA) (Elton et al., 2006). While the influenza virus does not integrate into the host genome, vRNPs are actively transported into the nucleus where transcription and replication occur early in infection, and they are found to be tightly associated with chromatin (Lopez-Turiso et al., 1990).

Influenza virus transcription requires a functional coupling with the cellular transcription machinery since it involves the synthesis of capped and polyadenylated viral mRNAs, primed by short-capped oligonucleotides that are scavenged from newly synthesized RNA polymerase II transcripts (Figure. 9B). Indeed, the viral polymerase interacts with the C-terminal domain of the largest subunit of the RNAP II complex (Engelhardt et al., 2005) as well as with a large number of cellular proteins related to transcription (Fig. 9B). Despite this coupling between viral and cellular transcription, influenza virus infection causes the

degradation of RNAP II, with a concomitant inhibition of cellular mRNA synthesis, once viral transcription is finished and therefore cellular transcription in not longer required (Rodriguez et al., 2007) (Figure 9C).

Viral replication has been proposed to take place in DNAse insensitive nuclear fractions that include chromatin and/or cellular matrix (Takizawa et al., 2006). Accordingly, it has been described that viral ribonucleoproteins are closely bound to the nuclear matrix or to chromatin components. This binding may be mediated at least in part through interaction of the NP with nucleosomes, since NP interacts with histone tails *in vitro* (Garcia-Robles et al., 2005). Similarly, *in vitro* binding experiments suggest that viral matrix protein interacts non-specifically with core histones (Zhirnov and Klenk, 1997).

CHD6 protein belongs to the relatively unknown third subfamily of the ATP-dependent chromatin remodeler CHD proteins. The relation of CHD6 with the mRNA transcription machinery is known since it colocalizes with the RNAP II and is present at sites of mRNA synthesis (Lutz et al., 2006) (Figure 9A). Accordingly, *in vivo* CHD6 mainly associates with markers of active (H3K4me3) chromatin, but also with repressed (H3K9me3 and H3K27me3) chromatin, suggesting that CHD6 may act as both activator and repressor (Alfonso et al., 2011).

Influenza virus RNPs colocalize with repressed chromatin (H3K9me3 and H3K27me) at late time post-infection and CHD6 is relocated from active to inactive chromatin together with the viral RNPs (Alfonso et al., 2011) (Fig. 9C). The association of CHD6 with the PA viral polymerase subunit and with the entire viral polymerase complex might mediate the change of CHD6-chromatin association that takes place during infection. Silencing experiments indicate that CHD6 is a negative modulator of influenza virus replication (Alfonso et al., 2011).

The recruitment of CHD6 to inactive chromatin (Figure 9C) could represent a viral mechanism to avoid its negative modulation on viral replication. CHD6 can function as a positive transcriptional activator, therefore, in addition to the RNAP II degradation, the recruitment of CHD6 to inactive chromatin at late time post-infection could function as a cooperative and additional mechanism to decrease the host-cell gene expression, highjack its metabolism and favor viral RNA replication. Hence, these viral-induced activities could help to generate an efficient host cell shut-off by suppressing the establishment of the host antiviral defense against viral pathogens.

Nucleosome dynamics in human cytomegalovirus chromatin

Michael Nevels—The large double-stranded DNA genomes of herpesviruses, including human cytomegalovirus (CMV), do not carry histones when encapsidated. However, upon release from virus capsids into cell nuclei, CMV genomes rapidly associate with host-derived histones to form nucleosome-occupied episomes (Figure 10A–C) reviewed in (Nevels et al., 2011; Paulus et al., 2010).

It has been reported that the temporal sequence of CMV transcription concurs with changes in histone modifications associated with the viral chromatin (Cuevas-Bennett and Shenk, 2008; Groves et al., 2009; Murphy et al., 2002; Nevels et al., 2004; Nitzsche et al., 2012). In fact, the histone marks in CMV chromatin initially resemble a heterochromatin signature but gradually switch to a euchromatin-like pattern over the course of a productive (lytic) infection (Figure 10C). This switch is characterized by a general decrease in H3K9 methylation and a concomitant increase in histone tail acetylation (affecting all four classes of core histones) and H3K4 methylation (reviewed in (Nevels et al., 2011; Reeves, 2011)).

At least one histone mark (H3K4me2) preferentially associates with post-replicative CMV chromatin (Figure 10B) (Nitzsche et al., 2012).

More recently, we have demonstrated that CMV chromatin is also subject to regulation by global changes in nucleosome occupancy. In latently infected cells, herpesvirus episomes form mostly regular arrays of nucleosomes resembling bulk cellular chromatin, although this has not been experimentally verified for CMV. During lytic infection, however, CMV chromatin is generally characterized by a low content of irregularly spaced nucleosomes, at least in the pre-replicative phase (Figure 10C) (Nitzsche et al., 2008). This unique chromatin state may facilitate accessibility to transcription factors (and other regulatory proteins) and preclude epigenetic silencing, while still allowing for some nucleosome-based regulation. The mechanisms accounting for the conditionally low levels of nucleosomes on CMV genomes have not been elucidated. Conceivably, certain CMV proteins expressed or active only during lytic (but not latent) infection may interact with nucleosomes and/or the cellular histone deposition machinery to limit chromatin formation on the viral DNA (reviewed in (Nevels et al., 2011; Paulus et al., 2010)).

Following initial (replication-independent) nucleosome assembly, herpesvirus chromatin undergoes profound temporal changes over the course of the viral replication cycle. Our work has demonstrated a substantial (up to 10-fold) increase in global histone H3 and nucleosome occupancy on the viral genomes between early and late stages of lytic CMV infection (Nitzsche et al., 2008). This increase proved to largely depend on viral DNA synthesis, implying replication-dependent nucleosome assembly mechanisms (Nitzsche et al., 2008). Thus, we propose that the CMV genome undergoes consecutive replication-independent and replication-coupled stages of chromatinization during productive infection (Figure 10C) (reviewed in (Nevels et al., 2011; Paulus et al., 2010).

Given that herpesvirus genomes exist in a nucleosomal state inside their host cells, how can all viral DNA packaged with virions be entirely devoid of histones? There is evidence from herpes simplex virus type 1 that both naked and chromatinized viral genomes may co-exist during the late stages of viral replication (Cliffe and Knipe, 2008). However, an observed decrease in average histone occupancy on CMV genomes at the end of infection (Nitzsche et al., 2008) points at an unanticipated nucleosome eviction process required for packaging of histone-free viral DNA and production of infectious progeny virions (Figure 10C) (reviewed in (Nevels et al., 2011; Paulus et al., 2010)).

Chromatin structure of adenovirus DNA throughout infection

Robin J. Parks—Human adenovirus (Ad) is a common pathogen with a remarkable capacity to spread. To establish infection in the host cell, the virus must rapidly express its early genes which are responsible for rewriting or disarming numerous cellular pathways and checkpoints, thus reconfiguring the environment for optimal virus replication. Ad has also been developed as a very efficient gene delivery platform, and is the most commonly used delivery system in gene therapy applications. Indeed, the newest “generation” of Ad vector, helper-dependent Ads (hdAd), which are devoid of all viral protein-coding sequences, can mediate life long expression of a transgene in mice and several years of expression in non-human primates after only a single injection. For both wildtype Ad (AdWT) and the replication-deficient vectors, we are only beginning to elucidate the nucleoprotein structure of the viral DNA within the infected cell, and how this contributes to efficient expression of virus-enoded genes (reviewed in (Giberson et al., 2012)).

Within the virion, the ~36 kb dsDNA AdWT genome associates with three highly basic proteins, VII, V and mu. Protein VII is a protamine-like protein, and is the major protein responsible for wrapping and condensing the viral DNA. Protein V is believed to form a

shell around the protein VII-DNA complex, and tethers the protein VII-wrapped DNA to the inner capsid. Mu is speculated to aid in tightly condensing the viral DNA within the capsid, and its cleavage may serve to partially relax this structure prior to its entry into the nucleus.

Cell-free systems developed to study Ad DNA replication have shown that the compacted nature of the VII-wrapped viral DNA allows for only limited transcription and DNA replication, suggesting that this structure must be remodeled to allow these processes to proceed with greater efficiency. Three cellular proteins have been identified that can remodel the Ad core in cell-free systems: template activating factor Iβ (TAF-Iβ) [also known as SET], TAF-II [NAP-1] and TAF-III [B23/nucleophosmin] (Kawase et al., 1996; Matsumoto et al., 1993; Okuwaki et al., 2001). However, the relative contribution of these factors to Ad genome remodeling in infected cells has not been determined conclusively.

To gain insight into the biochemical mechanism of Ad DNA remodeling, we used hdAd (which cannot replicate) as a simplified system to model the early phase of Ad infection (i.e. before the onset of DNA replication) (Ross et al., 2011). For hdAd, protein VII is removed from the vector DNA within a few hours of infection, and is then replaced by cellular histones (Figure 11). Indeed, loss of VII, deposition of histones and onset of gene expression appear coincident, although transcription is not absolutely required to strip VII from the viral DNA. Using chromatin immunoprecipitation assays and cell lines expressing epitope tagged histone H3 variants, we showed that H3.3 is preferentially deposited on the incoming viral DNA, and forms physiologically-spaced nucleosomes within about 6 hr of infection. Knockdown of the H3.3 chaperone HIRA reduced hdAd association with H3, and also reduced expression of an encoded transgene. Taken together, these studies indicate that hdAd DNA is wrapped in nucleosomes through a replication-independent mechanism, and that this event is crucial for optimal expression of vector-encoded genes. As with cellular chromatin, changes in the epigenetic status of the hdAd-associated histones impacts upon regulation and expression of encoded transgenes (Ross et al., 2009).

Using a similar approach as described for hdAd, we and others have begun to characterize chromatinization of AdWT during the various stages of a productive infection (Giberson et al., 2012; Komatsu et al., 2011; Komatsu and Nagata, 2012; Ross et al., 2011). As with hdAd, the majority of protein VII is removed from the viral DNA within the first few hours of infection, and it is then wrapped into physiologically-spaced nucleosomes containing the histone variant H3.3. H3.1 is not found stably associated with the viral DNA to any appreciable level at any point during infection. Since deposition of nucleosomes occurs prior to DNA replication, and involves H3.3, it suggests that AdWT also uses a replication-independent mechanism for chromatin assembly. As the virus transitions to the late stage of replication (i.e. active DNA replication and expression of late genes), association with H3 is dramatically reduced and the repeating nucleosome pattern is no longer evident.

During the final stage of virus replication, the viral DNA must be condensed once again into the compact structure required for packaging within the viral capsid. The histones must therefore be displaced from the Ad DNA and replaced with pre-protein VII, the precursor of the mature protein VII. Reduced association of histones with the Ad DNA is thought to occur either passively, as the cellular stores of histones decline during the course of infection, or actively through exclusion of histones from the viral replication centers by the Ad-encoded DNA binding protein (DBP) (Komatsu and Nagata, 2012). Based on several lines of evidence, it has been suggested that TAF-III/nucleophosmin may be involved in placing pre-protein VII on the viral DNA (Samad et al., 2012). Packaging of the protein VII-condensed Ad DNA into preformed capsids completes the viral lifecycle, forming the progeny Ad virions that can spread to adjacent cells or hosts.

Concluding Remarks

The examples presented in this compilation clearly indicate the significance of chromatin and viral directed modulation of chromatin as a critical component of the viral-host interaction that ultimately dictates the outcome of infection. Directed interactions with cellular chromatin modulation machinery, organization of genomes into microdomains, hitching rides on cellular chromosomes via linkage to chromatin binding proteins, expression of non-coding RNAs that modulate latency and persistence, and subversion of cellular modulation machinery to favor viral replication – all examples of the interactions of diverse viruses with host chromatin and chromatin machinery. The future of this field will continue to define novel paradigms. Additionally, the interactions of virus with cellular chromatin holds promise for identification of targets for the development of novel anti-viral therapeutics.

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Research Highlights

- Chromatin control of viral infection, latency, and reactivation
- Chromatin organization of DNA viruses
- Chromatin modulation complexes regulating viral gene expression
- Epigenetic targets for antiviral therapeutics
- Viral directed modulation of chromatin

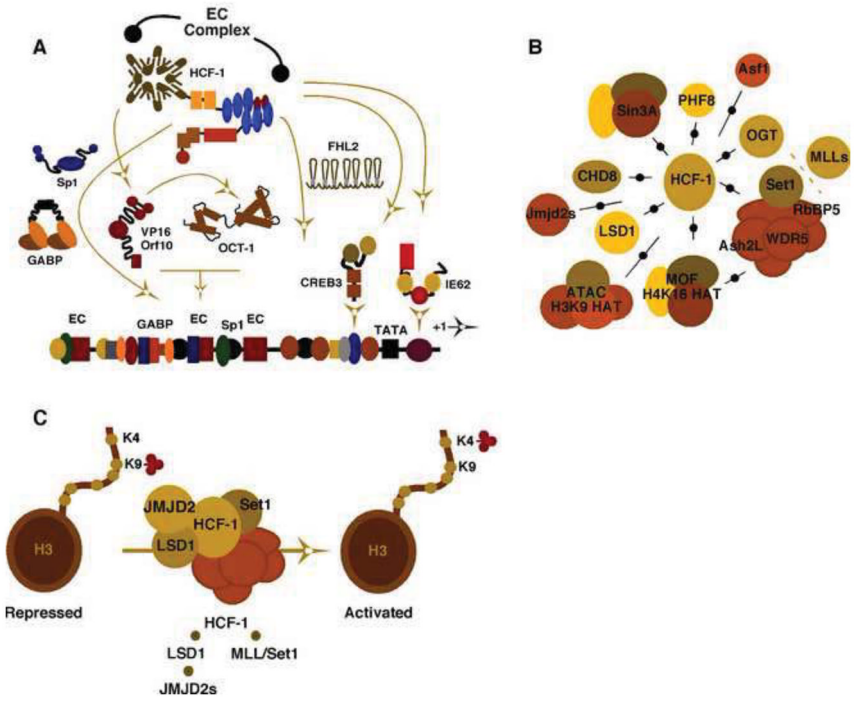

Figure 1. Chromatin regulation by the HCF-1 coactivator complex

(A). Schematic representation of the complex-combinatorial regulation of the alpha-herpesvirus Immediate Early (IE) genes enhancer-promoters. OCT-1, the viral activator VP16 (HSV) or Orf10 (VZV) form an enhancer core complex (EC complex) with the coactivator HCF-1. Additional cellular and viral activators contribute to the high level transcriptional activation of IE genes and are similarly dependent on HCF-1.

(B). Interactions of HCF with multiple chromatin modulation components are illustrated including histone chaperones (Asf1), H3K9 demethylases (PHF8, LSD1, JMJD2s), H3K4 methyltransferases (Set1/MLL complexes), histone acetyltransferases (MOF, ATAC complexes), deacetylases (Sin3A), remodelers (CHD8), and OGT (O-linked N-acetylglucosamine transferase).

(C). An HCF-1 complex consisting of histone H3K9 demethylases and H3K4 methyltransferases limits the accumulation of repressive chromatin marks and promotes the installation of activating marks (H3K4-me) for initiation of alpha-herpesvirus IE genes.

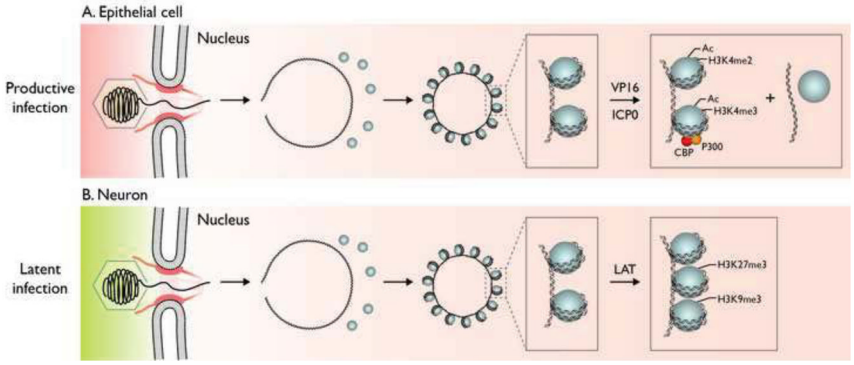

Figure 2. Model for epigenetic regulation of the lytic versus latent infection decision by HSV

(A). Following infection of epithelial cells, the capsid is transported to the nuclear pore where the viral DNA is released into the nucleus where it rapidly circularizes and becomes associated with histones. VP16 from the virion tegument forms a complex with HCF-1 and Oct1 that binds to viral IE promoters and HCF-1 recruits histone modification enzymes and chromatin remodeling complexes that decrease histone association with viral IE genes and increase euchromatin marks on the remaining associated histones. ICP0 is expressed as an IE protein and it promotes the same processes on the rest of the genome.

(B). Following infection of neuronal cells, the capsid is also transported to the nuclear pore where the viral DNA is released into the nucleus where it rapidly circularizes and becomes associated with histones. VP16 cannot be transported into the neuronal nucleus and/or HCF-1 is not localized in the nucleus so viral IE genes are not transcribed efficiently. Instead the latency-associated transcript is expressed and it promotes the association of facultative heterochromatin marks on the viral chromatin (Copyright, Lynne Chang and David Knipe).

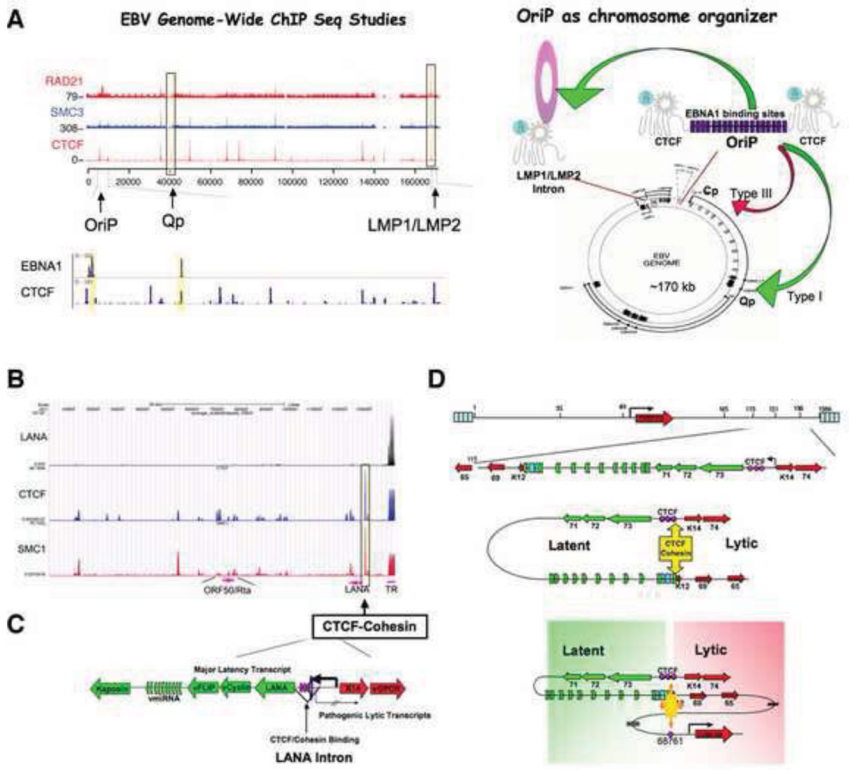

Figure 3. Epstein-Barr Virus and Kaposi's Sarcoma-Associated Herpesvirus (KSHV) chromatin organization and chromosome conformation during latency

(A). EBV genome-wide ChIP-Seq studies revealed colocalization of CTCF with cohesin subunits Rad21 and SMC3 at several key regulatory sites, including the region at OriP and the LMP1/LMP2 control regions. Viral encoded EBNA1 binds to neighboring sites at the OriP and Qp transcription control regions. OriP functions as a transcriptional enhancer that can physically interact by DNA-looping with the proximal promoters of latency transcripts.

(B–C). KSHV genome-wide ChIP-Seq assays reveal CTCF and cohesin co-occupancy at the first intron of the latency transcript encoding LANA-vCyclin-vFLIP multicistronic transcript.

(D). Chromatin conformation capture (3C) revealed that this CTCF-cohesin peak mediates DNA-looping with the 3′ end of the latency transcript at K12, as well as a larger loop structure with the lytic cycle immediate early gene promoter control region.

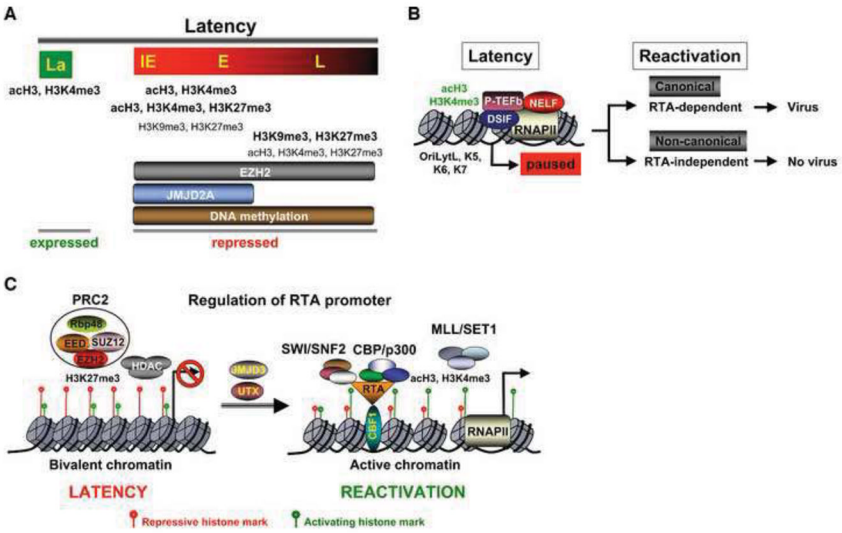

Figure 4. Epigenetic landscape of KSHV during latency

(A) Latent genes (La) are associated with activating histone modifications for their expression. In contrast, immediate early (IE), early (E) and late (L) genes are repressed during latency. IE and E genes possess mainly either acH3/H3K4me3-rich euchromatin or bivalent chromatin (acH3/H3K4me3 and H3K27me3). Heterochromatin characterized by the presence of H3K9me3 and H3K27me3 can also be found on some E genes. Although the majority of late genes are embedded in heterochromatin, some of the late genes have bivalent chromatin. Genome-wide localization of EZH2 H3K27me3 histone methyltransferase of the Polycomb Repressive Complex 2 (PRC2), JMJD2A H3K9me3 histone demethylase and DNA methylation were also mapped onto the KSHV genome during latency.

(B). RTA-independent expression of lytic genes. RNAPII binds to the promoter regions of OriLytL, K5, K6 and K7 genes during latency but its activity is blocked by negative transcription elongation factors (DSIF and NELF), which prevents these lytic gene expressions during latency. Positive transcription elongation factor P-TEFb is also recruited to these genes during latency probably in an inactive state. The paused transcription of these lytic genes can be induced either in an RTA-dependent manner as part of the canonical reactivation pathway or in an RTA-independent manner by dissociation of the negative elongation factors.

(C). Chromatin control of RTA gene expression. The RTA promoter is controlled by several chromatin modifying enzyme complexes during KSHV infection. This promoter is associated with bivalent chromatin (acH3, H3K4me3 and H3K27me3) during latency that is regulated by PRC2 and different histone deacetylases (HDAC). Chromatin-modifying cellular factors for the induction of the RTA promoter are H3K27me3 histone demethylases (JMJD3 and UTX), H3K4me3 histone methyltransferases (MLL/SET1), histone acetyltransferases (CBP/p300), and chromatin remodeling complex, SWI/SNF2.

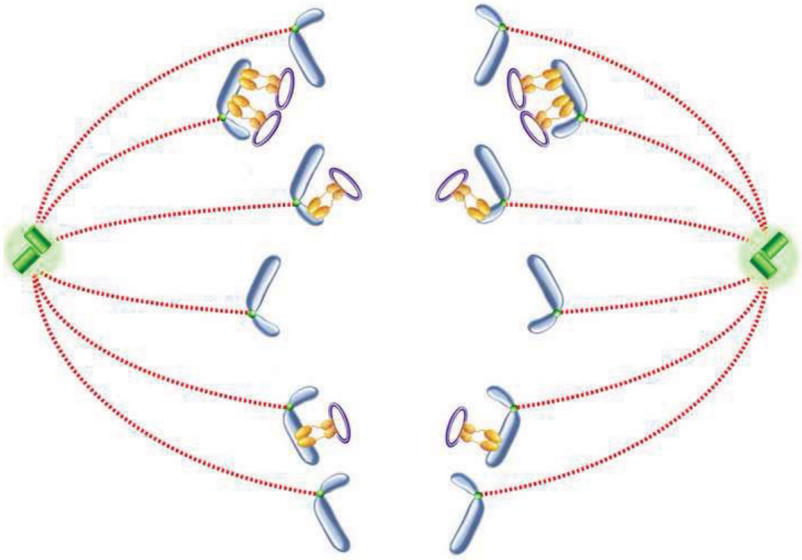

Figure 5. The papillomavirus E2 proteins tethers the viral genomes to host chromatin

The DNA binding domain of E2 binds to specific motifs in the viral genome while other regions interact with host chromatin proteins. This association partitions the viral genome in dividing cells.

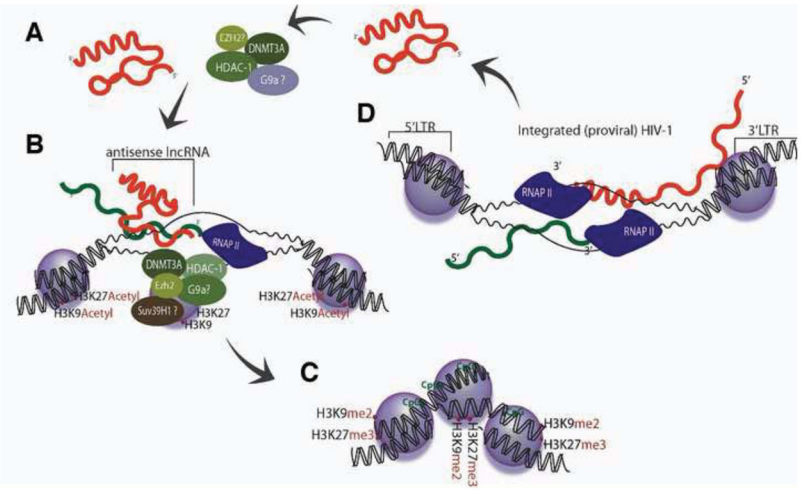

Figure 6. Long non-coding RNA epigenetic regulation of gene transcription

(A) lncRNAs, expressed from pseudogenes or bidirectionally transcribed loci, interact with and recruit various epigenetic remodeling proteins DNA methyltransferase 3A (DNMT3a), Enhancer of Zeste (Ezh2), Histone Deacetylase 1 (HDAC-1) and possibly also G9a. (B) The lncRNA then localizes the epigenetic silencing complex to the homology containing target loci where (C) epigenetic based silencing ensues. (D) HIV-1 expressed antisense lncRNAs, from the 3′ LTR, would also be expected to load into this pathway and guide the epigenetic silencing of integrated forms of HIV-1.

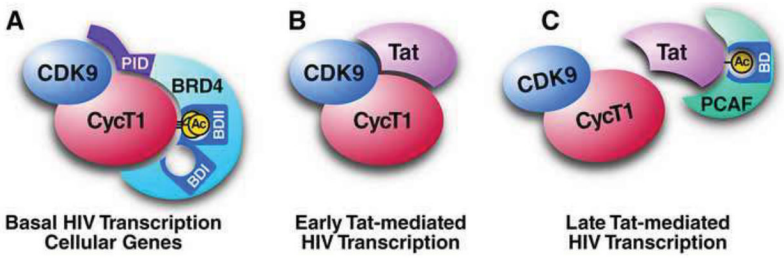

Figure 7. Model of the interactions between acetylated lysines in nonhistone proteins and bromodomain-containing proteins at the HIV promoter

(A) Triacetylated cyclin T1 in P-TEFb interacts with the second bromodomain in Brd4 and regulates basal HIV LTR activity.

(B) The Tat protein competes with Brd4 for P-TEFb binding and interacts with nonacetylated cyclin T1 to mediate early steps in Tat-dependent transactivation of the LTR.

(C) Tat is acetylated at K50 by p300/KAT3B, a step that dissociates P-TEFb and enables interaction with the bromodomain of PCAF/KAT2B during late steps of Tat-mediated HIV transcription. See text for details.

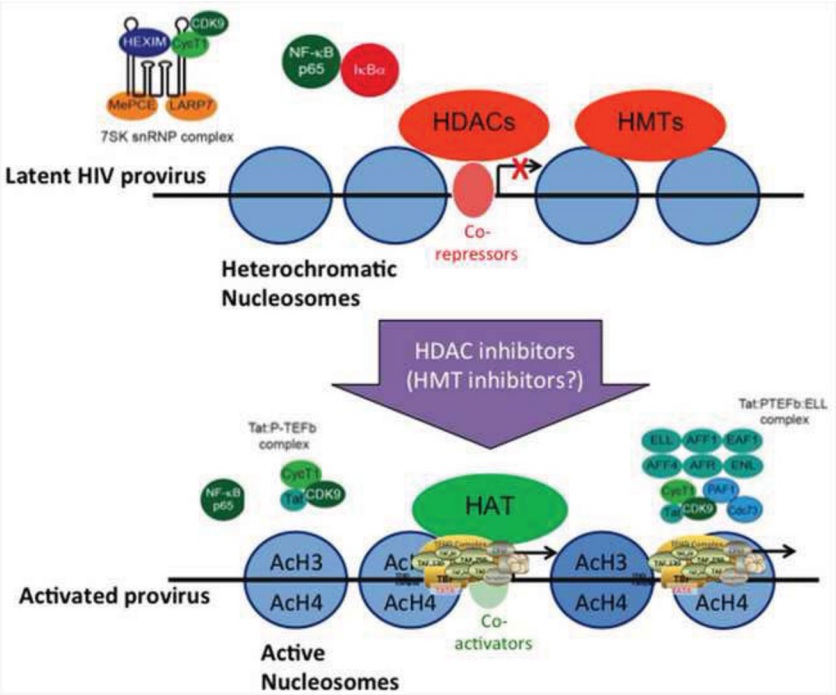

Figure 8. Disrupting HIV latency with epigenetic drugs

Vorinostat, a Class I-selective HDAC inhibitor, can disrupt HIV latency, in association with mobilization of P-TEFb from the HEXIM complex, and acetylation of proviral nucleosomes, resulting processive transcription mediated by the HIV Tat-P-TEFb-elongation complex. Which of the many effects of HDAC inhibitors is necessary and sufficient for viral induction in vivo has yet to be determined. Methyltransferase inhibitors can synergize with HDAC inhibitors in model systems, but this observation may be difficult to test in vivo given the effects on cellular function that might be induced.

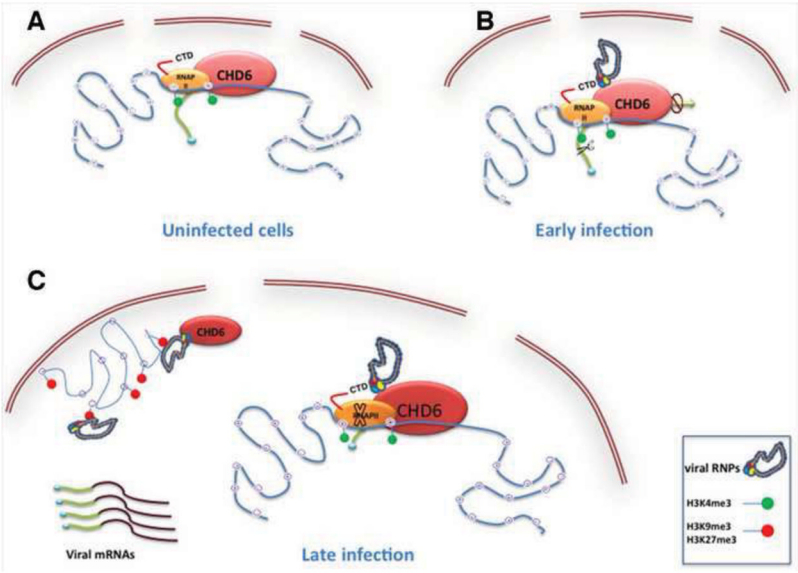

Figure 9. Representative model for influenza virus-host cell transcriptional coupling and the CHD6 chromatin remodeler

(A). In uninfected cells there is active transcription and CHD6 is together with the RNAP II bound to active chromatin marks (H3K4me3).

(B). At early time post-infection, the influenza virus polymerase through its interaction with the RNAP II would couple viral and cellular transcription since viral transcription requires short-capped oligonucleotides from newly synthesized RNAP II transcripts as primers. Once viral transcription is finished, degradation of the RNAP II and inhibition of cellular transcription take place. CHD6 also interacts with the viral polymerase and negatively modulates viral replication.

(C). At late time post-infection, viral RNPs are bound to inactive chromatin together with CHD6. The interaction of vRNPs with H3K9me3 and H3K27me3 could cooperate to reorganize cellular chromatin in order to promote the efficient release of newly synthesized vRNPs. At the same time the relocalization of CHD6 to inactive chromatin would inactivate the function of CHD6 since it negatively modulates viral replication.

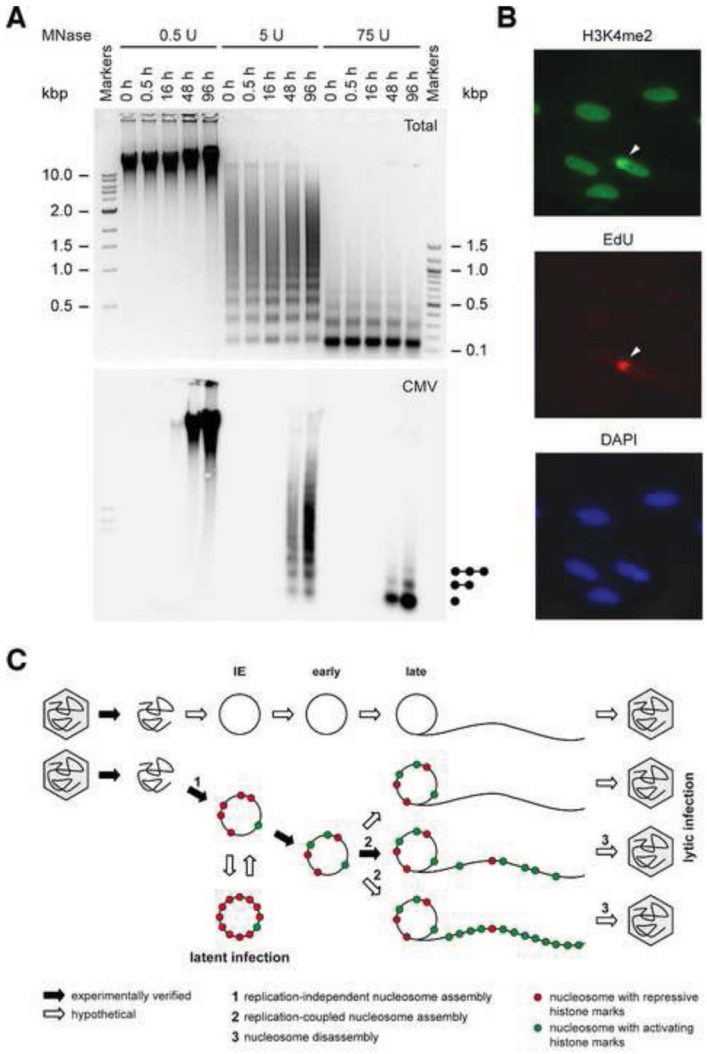

Figure 10. Nucleosome occupancy and histone modifications in CMV chromatin

(A) Presence of nuclear CMV DNA in nucleosomes during productive infection. MRC-5 cells were infected with CMV Towne at a high multiplicity for 0–96 h. Nuclei were reacted with 0.5–75 U micrococcal nuclease (MNase), and purified total DNA was separated in a 1.2% agarose gel stained with ethidium bromide (top). The same DNA samples were subjected to Southern blotting using a ^{32}P-labeled whole genome probe derived from a Towne bacterial artificial chromosome (bottom). Figure provided by Christina Paulus (University of Regensburg).

(B) Preferential association of H3K4me2 with replicated CMV chromatin. MRC-5 cells were infected with CMV Towne at a low multiplicity. H3K4me2, nascent DNA, and total DNA were co-stained using appropriate antibodies (rabbit polyclonal antibodies for H3K4me2 and Alexa Fluor 488-coupled goat antibodies for rabbit immunoglobulins), 5-ethynyl-2′-deoxyuridine (EdU)-based “click chemistry”, and 4′,6-diamidino-2-phenylindole (DAPI), respectively. Arrowheads point at a nucleus showing accumulation of H3K4me2 at the site of viral DNA synthesis. Figure provided by Alexandra Asbach-Nitzsche (University of Regensburg).

(C) Nucleosome and histone modification dynamics in CMV chromatin. Following release from CMV capsids into the cell nucleus, all or a subset of initially “naked” parental viral genomes undergo rapid *de novo* nucleosome formation by DNA replication-independent

mechanisms resulting in viral chromatin with low nucleosome content. The viral genome-associated nucleosomes are targets of repressive histone modifications that, together with additional nucleosome deposition, may contribute to establishment of viral latent infection. As lytic infection progresses, repressive histone marks are gradually replaced by activating marks including H3K4me2. Newly synthesized viral DNA is subject to replication-coupled nucleosome deposition. Unless a subset of replicated viral genomes stays histone-free, a nucleosome disassembly process must exist to enable DNA packaging and infectious progeny production.

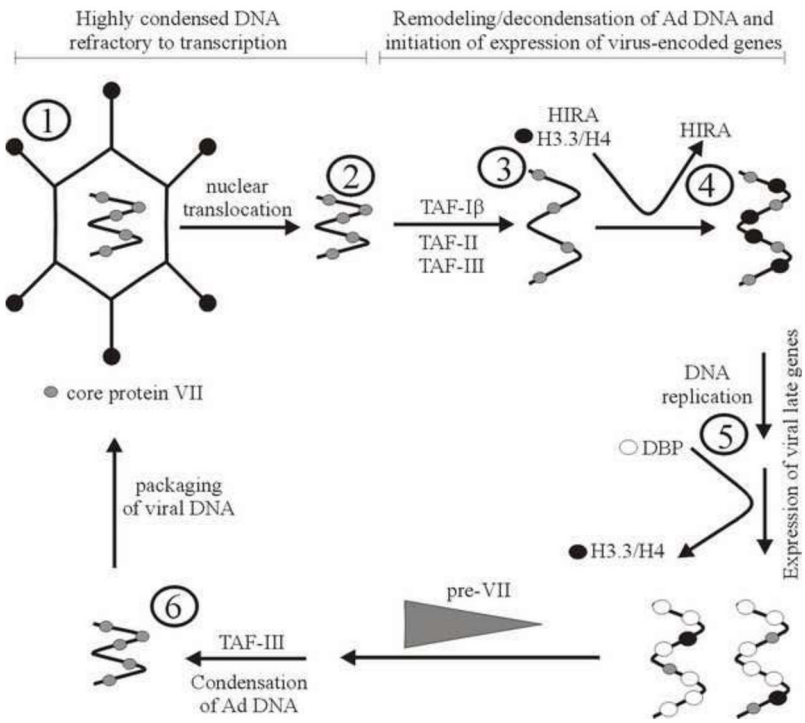

Figure 11. Working model of adenovirus DNA chromatin structure throughout infection

DNA in the Ad capsid is highly condensed with core protein VII (1), along with protein V and mu (not shown). The protein VII-DNA complex transits to the nucleus (2), and undergoes remodeling to decondense the core before transcription of early genes can begin (3). Remodeling may involve loss of at least some VII. At the time when viral gene expression is first detected, histones can be found bound to the viral DNA along with VII. Histone variant H3.3 is preferentially deposited on the viral DNA, through the action of HIRA (4). Onset of viral DNA replication is accompanied by reduced association of the viral DNA with histones, either passively through a reduction in cellular stores of these proteins or actively through exclusion of histones from the viral replication centres by the Ad-encoded DNA binding protein (DBP). Late in infection, the viral DNA must once again associate with pre-protein VII, possibly mediated by TAF-III (6). The viral DNA condensed with pre-protein VII is packaged into the Ad capsid (1). (Adapted from Giberson et al. 2012).