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Manipulation of the host protein acetylation network by human immunodeficiency virus type 1

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Abstract

Over the last 15 years, protein acetylation has emerged as a globally important post-translational modification that fine-tunes major cellular processes in many life forms. This dynamic regulatory system is critical both for complex eukaryotic cells and for the viruses that infect them. HIV-1 accesses the host acetylation network by interacting with several key enzymes, thereby promoting infection at multiple steps during the viral life cycle. Inhibitors of host histone deacetylases and bromodomain-containing proteins are now being pursued as therapeutic strategies to enhance current antiretroviral treatment. As more acetylation-targeting compounds are reaching clinical trials, it is timely to review the role of reversible protein acetylation in HIV-infected $CD4^+$ T cells.

Keywords

Post-translational modification; epigenetic regulation; virus–host Interactions; viral infection; therapeutic inhibitors; latency

Introduction

The survival and function of cells are critically dependent on their ability to rapidly integrate multiple, intersecting cell-signaling circuits. A key strategy for effectively regulating complex signals is the reversible post-translational modification (PTM) of proteins. Over 200 PTMs are known, many of which are highly conserved among a wide range of organisms (Jensen, 2006). Despite their ubiquitous presence, only a few PTMs have been comprehensively studied including acetylation of lysines. The global role of protein acetylation was initially underappreciated (Verdin & Ott, 2014). Originally found to reversibly modify lysines in the tails of histones, acetylation was thought to regulate gene expression primarily by altering the structural properties of the chromatin environment (Box 1). However, with the identification of acetylation-modifying enzymes and improvements in high-resolution mass spectrometry, it became clear that the regulation of cellular function by

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Declarations of interest

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protein acetylation extends beyond the nucleus. Over 3600 novel acetyl-lysine sites have been identified in a broad range of human proteins in different subcellular compartments (Choudhary *et al.*, 2009). Acetylation of these proteins has been linked to the regulation of diverse cellular pathways, including cell-cycle control, DNA damage response, cytoskeletal organization, and immune signaling (Spange *et al.*, 2009; Shakespear *et al.*, 2011).

Box 1

Translating electrostatic and physical properties of acetylation into the regulation of cellular function. The reversible addition of an acetyl group to the ε-NH2 group of lysines modifies the electrostatic and physical properties of the target protein. The positive charge of the lysine is neutralized by the acetyl group, thereby altering hydrogen bonding capabilities through the formation of a circumferential hydrophobic milieu. At the level of the chromatin, this means that increased acetylation of histone tails will decrease the affinity of the histone for the negatively charged DNA strand. As a result, the chromatin environment becomes less compact, nucleosomes are remodeled, and gene transcription increases as a result of the greater accessibility of transcription factors. Furthermore, transcription factors themselves are often subject to cycles of acetylation and deacetylation. The acetylation of transcription factors modifies their DNA-binding capacity, protein stability, and nuclear translocation, thereby regulating their ability to activate target genes. Lysine acetylation can also alter cellular signaling pathways by modifying the dynamics of protein–protein interactions. Depending on the context, the addition of the acetyl group may create steric hindrance that prevents key interactions. At the same time, acetylated lysines can serve as docking sites for proteins that contain acetyl-lysine recognition domains (e.g. bromodomains), thereby facilitating transient interactions between proteins. Functionally, acetylation of enzymes can modify their catalytic activity through allosteric regulation, restricting access to substrates, or altering interactions with other critical regulatory proteins. Many metabolic enzymes appear to be governed by reversible acetylation—thereby not only ensuring rapid cellular responses to environmental cues, but also flexibly fine-tuning reaction rates. Another level of complexity involves functional crosstalk between acetylation and other PTMs. Because lysine residues are subject to additional PTMs—such as methylation, ubiquitylation, and sumoylation—acetylation can compete with PTMs at the same site. Moreover, by controlling the recruitment of specific enzymes, acetylation can influence PTMs at nearby sites.

Histone acetyltransferases (HATs) are considered the “writers” of acetylation because they transfer an acetyl group from the cofactor, acetyl-coenzyme A, to the target lysine (Figure 1). At least 26 human HATs are known, nine of which are grouped into three major families based on similarities in their structure and sequence: (1) GNAT (Gcn5-related N-acetyltransferases), including PCAF and GCN5; (2) MYST (MOZ, Ybf2/Sas3, Sas2, TIP60), including HBO1; and (3) p300/CBP proteins (E1A-associated protein of 300kDa/CREB-binding protein) (Berndsen & Denu, 2008; Li *et al.*, 2012).

The activity of HATs is counterbalanced by HDACs, which remove the acetyl groups and are therefore considered “erasers” (Figure 1). Thus far, 18 mammalian HDACs are known,

which are categorized into three classes based on distinct catalytic characteristics. Class I and II HDACs (HDACs 1–11) use a Zn^{2+}-dependent deacetylation mechanism and are inhibited by hydroxamic acids such as trichostatin A, vorinostat (SAHA), givinostat (ITF2357), and panobinostat (LBH589) (Shirakawa *et al.*, 2013). Notably, class II HDACs shuttle between the nucleus and cytoplasm; a subset of these HDACs (class IIa) have little *in vitro* HDAC activity unless associated with the class 1 HDAC3/N-CoR complex (Jones *et al.*, 2008). Class III HDACs are NAD^+-dependent sirtuin deacetylases (SIRTs 1–7), which are found in the nucleus, cytoplasm, and mitochondria, and are not responsive to classical HDAC inhibitors (Houtkooper *et al.*, 2012).

Besides HATs and HDACs, so-called reader proteins have been identified that contain protein domains which bind specifically to acetylated lysines (Figure 1). Best known and characterized are proteins containing bromodomains—conserved ~110–amino acid protein modules that form a deep hydrophobic cavity that specifically accommodates acetyl-lysine residues (Filippakopoulos & Knapp, 2014). The human genome is predicted to encode 46 bromodomain-containing proteins, which are usually epigenetic regulators; some of these proteins contain more than one bromodomain (Filippakopoulos *et al.*, 2012).

The three groups of acetylation-associated proteins engage in regulatory crosstalk. Many HATs, including p300/CBP and GNAT enzymes, contain bromodomains. Since they can both write and recognize acetylation marks, HATs can be recruited to acetylated sites and promote spreading of the mark (Josling *et al.*, 2012). Because class IIa HDACs have negligible intrinsic deacetylase activity, they might function as acetyl-lysine readers rather than erasers and recruit other chromatin-modifying enzymes to sites of transcription (Bradner *et al.*, 2010). Moreover, HAT and HDAC activities are regulated by acetylation of the enzymes themselves. p300/CBP proteins bind and regulate the activity of several HDACs (e.g. HDAC1, HDAC6, and SIRT2) by directly acetylating lysines (Qiu *et al.*, 2006; Han *et al.*, 2008, 2009). Conversely, SIRT2 can regulate the autoacetylation of p300 and thereby modulate its ability to bind to transcription pre-initiation complexes (Black *et al.*, 2006, 2008). HATs and HDACs not only regulate each other, but they are also intimately tied to the metabolism of cells through their cofactors acetyl-coenzyme A (HATs) and NAD^+ (Class III HDACs). This regulatory crosstalk serves to maintain a dynamic equilibrium between the acetylation and deacetylation of specific substrates within cells and to rapidly translate environmental cues into shifts in complex cellular processes.

Viruses have evolved intricate strategies to usurp complex cellular processes in support of their own propagation. HIV-1 is a complex lentivirus that reverse transcribes its RNA genome into cDNA and integrates into the host chromatin of $CD4^+$ T lymphocytes and macrophages. Through recruitment of the host transcriptional machinery, HIV promotes high-level transcription of its viral genome or becomes transcriptionally silenced in a subset of latently infected memory T cells (Ott *et al.*, 2011).

Acetylation of the chromatin environment near the viral integration site can affect HIV transcription (Shirakawa *et al.*, 2013). However, acetylation of non-histone proteins is also important in the viral life cycle. Multiple interactions exist between HIV and HATs, HDACs, and bromodomain-containing proteins encoded by the host. These interactions can alter the

function of these epigenetic regulators, thereby disrupting the host acetylation network. Viral proteins, including the virally encoded integrase enzyme and transactivator of transcription (Tat), serve as substrates for cellular HATs and HDACs and require timely acetylation and deacetylation events for their proper function. In this review, we discuss the known mechanisms by which HIV taps into the host acetylation network as a basis for our understanding of how acetylation-targeting strategies interfere with the HIV life cycle.

HIV and the host acetylation machinery

Recent system-wide mass spectrometry approaches identified a large number of interactions of acetylation-related proteins with HIV (Gautier *et al.*, 2009; Fahey *et al.*, 2011; Jäger *et al.*, 2012), many of which have yet to be experimentally confirmed. Importantly, because these proteomic strategies rely on affinity purifications, it is unclear whether the interactions are direct or indirect via a larger protein complex. The majority of human HATs (19 of 26) interact with at least one HIV protein; some, such as p300 and p160, interact with up to five (Figure 2). As much as one third of human HDACs (6 of 18) have been formally identified as HIV interaction partners. Notable HDACs that can interact with up to three HIV proteins include HDAC1 (Tat, Vpr, and integrase) and HDAC6 (Tat, gp41, and gp120). Similarly, about one third of bromodomain-containing proteins (16 of 46) display HIV-binding potential. Interestingly, the majority of these interactions are made with HIV Tat (Tat binds to 11 HATs, 4 HDACs, and 14 bromodomain-containing proteins), underscoring the importance of reversible acetylation in the function and regulation of this accessory HIV protein. Below, we focus on confirmed interactions and modifications of critical regulators of the HIV life cycle.

Acetylation of host and viral factors during HIV entry and integration

Although studies of the effect of acetylation on HIV replication have focused traditionally on transcriptional regulation, mounting evidence suggests that acetylation is critical in early steps of the viral life cycle. After HIV-1 attaches to the plasma membrane of the host cell, the viral envelope (Env) proteins gp120 and gp41 interact with the host receptor CD4 and one of two coreceptors—CXC chemokine receptor type 4 (CXCR4) and CC chemokine receptor type 5 (CCR5)—to facilitate viral fusion and entry (Figure 3) (Loetscher *et al.*, 2000). Once within the cytoplasm, the viral nucleocapsid uses the host microtubule network to move toward the nuclear pore complex by manipulating cytoplasmic factors such as dynein (McDonald *et al.*, 2002). During this time, the core of the HIV-1 particle progressively disassembles (viral uncoating), and the viral RNA genome is reverse transcribed by the viral reverse transcriptase enzyme to form a pre-integration complex with the proviral double-stranded cDNA at its center. The pre-integration complex, composed of viral and host factors, mediates the transport of double-stranded cDNA into the nucleus, where it integrates into the host chromatin with the assistance of host factors such as the lens epithelium-derived growth factor (LEDGF/p75) and the virally encoded integrase enzyme. During these early steps of HIV infection, several host and viral factors undergo reversible acetylation. Here, we will discuss how these early steps are regulated by the acetylation of α-tubulin, HIV-1 integrase, and cyclophilin A (Figure 3).

Microtubules are composed of α/β tubulin heteropolymers, which form key structures in cell division, vesicular trafficking, and multiple signaling pathways. These dynamic filaments are stabilized by acetylation of the α-tubulin polymer—a highly conserved mechanism that is increasingly recognized as a key factor in human health and disease (Piperno *et al.*, 1987; Perdiz *et al.*, 2011). As shown by Valenzuela-Fernández *et al.*, the interaction between the HIV gp120 protein and the CD4 T-cell surface receptor induces α-tubulin acetylation, leading to microtubule stabilization necessary for fusion of the virus to the host cell (Valenzuela-Fernández *et al.*, 2005). By altering the activity of HDAC6, one of two reported tubulin deacetylases (North *et al.*, 2003)–either through overexpression of the wildtype protein or a dominant-negative mutant—the authors linked α-tubulin acetylation to early viral fusion (Figure 3).

Recently, the matrix region of HIV Gag was shown to recruit the EB1-binding protein Kif4 to the ends of microtubules, thereby regulating the formation of acetylated α-tubulin necessary for early stages of HIV-1 infection (Sabo *et al.*, 2013). Occurring specifically at the postentry stage of infection, this recruitment affects nuclear import, initiation of reverse transcription, and viral cDNA synthesis. Human herpes virus 8 also induces microtubule acetylation during early stages of viral infection, pointing to a common phenomenon conserved among different viruses (Naranatt *et al.*, 2004). However, the molecular mechanism by which HIV uses the host acetylation machinery to promote α-tubulin acetylation remains unclear. Although tubulin was one of the first non-histone proteins shown to undergo acetylation, the α-tubulin acetyltransferase αTAT-1 (or MEC17 in worms) was not identified until recently (Akella *et al.*, 2010; Shida *et al.*, 2010). Further studies are required to determine whether HIV regulates microtubule acetylation by directly recruiting αTAT-1, by inhibiting the α-tubulin deacetylases HDAC6 and SIRT2, or by alternative mechanisms.

Integration occurs within the large pre-integration nucleoprotein complex, which consists of the viral cDNA, viral proteins (integrase, matrix, Vpr, nucleocapsid, and reverse transcriptase), and several host factors. p300 can directly acetylate the viral integrase at three carboxy-terminal lysines: K264, K266, and K273 (Cereseto *et al.*, 2005). Acetylation increases the affinity of integrase for genomic DNA and enhances strand transfer activity (Figure 3). Conversely, point mutations in acetylation sites or inhibition of p300 inhibited viral integration and replication (Ceresto *et al.*, 2005). An additional report confirmed the acetylation of integrase by p300, but could not find a replication defect of point mutants when an untagged viral construct was used (Topper *et al.*, 2007). A second integrase acetyltransferase, human GCN5, has partially overlapping specificity for integrase lysine residues, suggesting that integrase acetylation is more complex than originally assumed (Terreni *et al.*, 2010).

The acetylated residues in HIV integrase are interaction sites for host bromodomain-containing proteins. Data from a tethered catalysis yeast two-hybrid screen identified host TRIM28 (also known as KAP1 and Tif-1B) as a bromodomain factor that binds preferentially to acetylated integrase (Allouch & Cereseto, 2009). TRIM28 recruits HDAC1, thus triggering deacetylation of HIV integrase and restricting viral integration (Allouch *et al.*, 2011). This highlights the evolution of cellular mechanisms to counter infection by

exploiting viral dependence on protein acetylation. Interestingly, HDAC1 was previously identified as a component of the integrase complex (Sorin *et al.*, 2009). Here, the authors showed, using the yeast two-hybrid system, that integrase interacts with SAP18, a component of the cellular Sin3a/HDAC complex. They further showed that this complex, packaged into HIV-1 virions, is critical for postentry viral infection. In sum, these studies suggest that HDAC1 can regulate HIV infection either positively or negatively, depending on the context of viral interaction.

Cyclophilin A (CypA), a highly conserved host peptidyl-prolyl *cis-trans* isomerase, has complex functions in diverse cellular processes such as protein folding, signal transduction, and cell-cycle regulation. During HIV infection, CypA is recruited by the group-specific antigen (Gag) precursor polyprotein, which consists of important components of the HIV virion such as matrix and capsid proteins, and is packaged into budding virions (Figure 3) (Franke *et al.*, 1994; Thali *et al.*, 1994). After entering target cells, CypA is associated with multiple steps of early infection, including uncoating, reverse transcription, and nuclear trafficking (Fassati, 2012). These functions are regulated by acetylation (Lammers *et al.*, 2010). The authors used a new *in vitro* system to generate large amounts of acetylated CypA protein using synthetically evolved acetyl-lysyl-tRNA synthetase/$tRNA_{CUA}$ pair system in *E. coli.*, which co-translationally directs the incorporation of acetyllysine into a target protein in response to specifically encoded amber codons (Neumann *et al.*, 2008). They showed that acetylation of lysine 125 (K125) inhibits the catalytic activity of the CypA enzyme and disrupts its interactions with the HIV Gag protein. It is possible, although not shown, that the positive function of HDAC1 in early viral infection is associated with deacetylation of K125 in CypA by the co-packaged integrase/Sin3a/HDAC complex, thereby promoting optimal CypA activity (Sorin *et al.*, 2009).

Reversible protein acetylation and HIV transcription

Once integrated into the host chromatin, the HIV genome, like a human protein coding gene, is subject to transcriptional regulation by the host RNA polymerase II (Pol II) enzyme. During the first phase of HIV-1 transcription, short incomplete viral transcripts accumulate that cannot support full viral replication (Kao *et al.*, 1987). These incomplete transcripts results from pausing of the Pol II complex shortly after transcription starts. This elongation block is not unique to HIV and is found in many human genes (Core *et al.*, 2008). To overcome it, HIV encodes the transcriptional transactivator Tat, an RNA-binding protein required for elongation of HIV transcription. Tat recruits a critical multicomponent host factor, the positive transcription elongation factor b (P-TEFb), to the 5′ extremities of elongating HIV transcripts, specifically to a conserved RNA stem-loop structure called TAR (Figure 4). The recruitment of P-TEFb to TAR promotes transcriptional elongation through its intrinsic serine/threonine kinase activity, enhancing the processivity of Pol II and dissociating negative elongation factors that physically obstruct transcription. Subsequent splicing of these elongated HIV RNA transcripts fuel the viral life cycle and give rise to novel Tat molecules which, in an autoregulatory loop, activate HIV transcription (Weinberger *et al.*, 2005).

Throughout this process, viral transcription is regulated at multiple levels by reversible acetylation (Figure 4). The integrated HIV provirus is fully chromatinized, controlling access of host transcription factors to the HIV promoter in the 5′ long-terminal repeat (LTR) (Verdin *et al.,* 1993). This chromatin structure is under the control of HATs and HDACs, as first shown in studies in which the HDAC inhibitor trichostatin A potently remodeled the chromatin structure at the HIV LTR in cells (Van Lint *et al.*, 1996) and in *in vitro* reactions (Sheridan *et al.*, 1997). Since then, interest has grown to identify which HATs and HDACs play distinct roles in regulating HIV transcription. These efforts were recently comprehensively reviewed (Hakre *et al.*, 2011; Shirakawa *et al.*, 2013) Briefly, HDACs 1–3 are known to reside at the HIV LTR in cells with transcriptionally inactive (latent) HIV through interactions with various transcription factors, including YY1, LSF, CTIP2, CBP-1, NF-κB p50, c-myc and Sp1 (Shirakawa *et al.*, 2013). In contrast, cellular HATs such as p300, CBP, PCAF, and GCN5 are recruited to the LTR by Tat and activating transcription factors such as NF-κB p65, AP-1, Myb, GR C/EBP, NFAT, Ets-1, LEF-1 and IRF (Hakre *et al.*, 2011). Interestingly, increased histone acetylation during HIV activation appears to be associated with chromatin modifications during G_2 arrest of the cell cycle—demonstrating the ability of HIV to manipulate critical cellular processes through the host acetylation network (Thierry *et al.*, 2004).

Many transcription factors involved in recruiting HATs or HDACs to the HIV promoter are themselves targets of acetylation. For example, the p65 subunit of NF-κB is acetylated at multiple sites. One of which is K310, a target site of SIRT1 and SIRT2; this acetylation event is critical for full transcriptional activity of p65 (Yeung *et al.*, 2004; Rothgiesser *et al.*, 2010). The p50 subunit of NF-κB is also acetylated at multiple sites, including K431, K440, and K441; these acetylation events enhance the DNA-binding activity and transcriptional activity of the heterodimeric NF-κB complex (Furia *et al.*, 2002; Deng *et al.*, 2003). Similarly, acetylation of Sp1 at K703 increases affinity of the transcription factor for DNA (Ryu *et al.*, 2003). Interestingly, both components of the Tat-associated P-TEFb complex, cyclin T1 and CDK9, also undergo reversible acetylation—a modification that alters their association with inhibitory ribonucleoprotein complexes and the kinase activity of CDK9 directly (Fu *et al.*, 2007; Sabò *et al.*, 2008; Cho *et al.*, 2009, 2010). Three acetylation sites in the cyclin T1 subunit also serve to bind the second bromodomain of the double bromodomain and extraterminal domain (BET) protein BRD4, a process associated with activation of the P-TEFb complex (Schröder *et al.*, 2012). Interestingly, eight lysines in the C-terminal domain of Pol II undergo reversible acetylation by p300 (Schröder *et al.*, 2013). Acetylation of the Pol II C-terminal domain is specifically enriched downstream of polymerase-occupied gene promoters and is required for optimal activation of genes carrying paused Pol II. However, a direct connection with HIV has not yet been established (Figure 4).

Tat itself interacts with HAT enzymes including TIP60, PCAF, CBP, p300, $TAF_{II}250$, and human GCN5 (Kamine *et al.*, 1996; Yamamoto & Horikoshi, 1997; Benkirane *et al.*, 1998; Hottiger & Nabel, 1998; Marzio *et al.*, 1998; Weissman *et al.*, 1998; Col *et al.*, 2001). These interactions can target the individual enzymes to specific transcription factor complexes (e.g. TBP, TF_{II}, NF-κB), recruit them to the HIV LTR, or modulate their catalytic activities (Caron *et al.*, 2002). In certain cases, interactions between Tat and HATs promote extra-

transcriptional effects, such as neuronal cell death (by disrupting neurotrophin signaling) or increased neoplasia (by impairing p53 tumor suppressor function) (Harrod *et al.*, 2003; Wong *et al.*, 2005). However, understanding the relationship between these individual interactions (i.e. temporal and spatial kinetics) within the context of the viral life cycle still remains a major challenge in the field.

Tat has at least two acetylation sites. Acetylation of lysine 28 (K28) by PCAF supports the cooperative interaction of Tat with its target RNA structure TAR and the P-TEFb cofactor, thereby promoting Pol II phosphorylation and efficient transcript elongation (Kiernan *et al.*, 1999; D'Orso & Frankel, 2009). Acetylation of K50/51 by p300/CBP and human GCN5 terminates the P-TEFb-dependent step in Tat transactivation, mediates dissociation of Tat from TAR/P-TEFb, and recruits instead the PCAF HAT via the PCAF bromodomain (Kiernan *et al.*, 1999; Ott *et al.*, 1999; Deng *et al.*, 2000; Dorr *et al.*, 2002; Mujtaba *et al.*, 2002; Kaehlcke *et al.*, 2003). Other interactions of acetylated Tat with host bromodomain-containing proteins include its recruitment of Brg-1—a component of the SWI/SNF nucleosome remodeling complex—and the HAT and transcription initiation factor $TAF_{II}250$ (Weissman *et al.*, 1998; Mahmoudi *et al.*, 2006). Bromodomain-containing protein BRD2 has also recently emerged as a potential regulator of the HIV LTR; this mechanism, however, appears to occur independently of Tat involvement (Boehm *et al.*, 2013).

Tat also interacts with host HDACs, such as SIRT1 and HDAC6, to undergo lysine deacetylation (Figure 4) (Pagans *et al.*, 2005; Huo *et al.*, 2011). Deacetylation of Tat by SIRT1 is necessary for optimal transactivator function—supporting a model in which timely and balanced acetylation/deacetylation events are important to fully support Tat function during HIV transcription. Tat serves as a super-substrate for SIRT1, associating avidly with the SIRT1 HDAC domain and thereby preventing other substrates (e.g. p65 K310) from accessing the enzyme (Kwon *et al.*, 2008). By effectively inhibiting SIRT1 activity on other substrates, Tat induces hyperacetylation of p65, rendering it more active and activating infected $CD4^+$ T lymphocytes. Thus, Tat is not only a *bona fide* substrate and recruitment module for HATs, HDACs, and bromodomain-containing proteins, it also directly manipulates the activity of HATs and HDACs, resulting in reprogramming of infected T cells and manipulation of the infection rates of neighboring lymphocytes. Besides Tat, the accessory HIV protein Vpr also binds to p300/CBP HAT proteins and supports HIV transcription (Kino *et al.*, 2002).

Acetylation during late stages of HIV infection

It remains to be determined whether acetylation also regulates the late stages of viral replication. However, it is clear that the changes in HIV entry, integration, and transcription described above will also indirectly alter the rates of virion assembly and budding. Furthermore, because stable microtubules are important for virion assembly and budding, it is likely that altering the acetylation of α-tubulin will also directly affect these later stages of the viral lifecycle (Jolly *et al.*, 2007). Similarly, the co-packaging of integrase with the Sin3/HDAC complex into virions is likely associated with more widespread acetylation/deacetylation processes during assembly, budding, and maturation of HIV virions (Sorin *et*

al., 2009). However, the exact nature of the host or viral factors critical for these late steps of the viral life cycle remains unclear at this stage.

Therapeutic manipulation of the acetylation network

These intricate interactions between HIV and host acetylation-associated processes make acetylation-targeting drugs ideal candidates to support current antiretroviral therapy (ART). ART potently inhibits actively replicating HIV, but cannot eradicate the virus from patients (Chun *et al.*, 1997; Wong *et al.*, 1997; Siliciano *et al.*, 2003). The major barrier to curing HIV-1 remains the persistence of long-lived, resting $CD4^+$ memory T cells harboring replication-competent but transcriptionally silenced proviruses (Chomont *et al.*, 2009). These latent reservoirs are established early after infection, are resistant to ART, and trigger viral rebound after ART is stopped (Zhang *et al.*, 1999; Fischer *et al.*, 2004; Kaufmann *et al.*, 2004; Lewin *et al.*, 2008). One current approach is to “shock and kill” latently infected T cells with latency-reversing agents, forcing latent proviruses into active transcription under the protection of ART to eliminate them through the immune system or additional intervention.

HDAC inhibitors and reversal of HIV latency

Since early studies demonstrated that HDAC inhibitors modify the chromatin environment of the integrated provirus and potently activate HIV, considerable effort has focused on identifying HDACs that are important for maintaining the latent state (Hakre *et al.*, 2011; Shirakawa *et al.*, 2013). A growing library of small molecules that inhibit class I and II HDACs reactivate HIV within *in vitro* models of latent HIV infection; some of these compounds, previously approved for the treatment of cancer, have advanced into clinical trials (Sgarbanti & Battistini, 2013; Cillo *et al.*, 2014; Falkenberg & Johnstone, 2014; Campbell *et al.*, 2015). Compounds such as valporic acid, vorinostat, and givinostat showed success in increasing viral RNA levels in latently infected resting T cells from treated patients; however, the results were either not reproducible (Archin *et al.*, 2009; Blazkova *et al.*, 2012; Routy *et al.*, 2012; Wei *et al.*, 2014) or pointed to the finding that repeated intake of HDAC inhibitors desensitizes cells to their latency-reversing activities (Archin *et al.*, 2010).

The findings from studies of select latency-reactivating agents are summarized in Table 1, which also gives the working concentrations for each of the compounds used *in vitro* or *ex vivo* and outlines their mechanism of action. A more extensive list of HDAC inhibitors used *in vitro* can be found in a recent review (Wightman *et al.*, 2012). Various comparative studies indicate that panobinostat and romidepsin are most efficient at targeting class I HDACs (Rasmussen *et al.*, 2013; Wei *et al.*, 2014). Notably, panobinostat, decreased the size of the latent pool in patients in a phase I clinical trial (Rasmussen *et al.*, 2014). Furthermore, prolonged treatment with romidepsin had robust latency-reversing activity in patient-derived cells and induced virion release in a clinical study in patients (Wei *et al.*, 2014). It remains to be shown whether panobinostat or romidepsin treatment will effectively delay viral rebound in patients. A single-drug will likely prove insufficient to overcome latency in all cells, and thus a combination treatment targeting multiple stages of the life cycle may be required (Bullen *et al.*, 2014).

In addition, “shock” therapies like HDAC inhibitors may exert unwanted effects on the “kill” arm of the approach. Notably, HDAC inhibitor treatment caused defects in T-cell development and distorted $CD8^+$ T cell activity, potentially diminishing the potential of these cells to effectively eliminate reactivated cells in patients (Shan *et al.*, 2012; Tschismarov *et al.*, 2014). Furthermore, treatment with vorinostat and panabinostat decreased interferon-γ production in primary activated $CD8^+$ T cells, resulting in impaired elimination of HIV-Gag-positive $CD4^+$ T cells in an *in vitro* model of HIV latency (Jones *et al.*, 2014). Studies outside HIV also point to important roles of HDACs in the effector function of T cells and macrophages (Halili *et al.*, 2010; Bagley *et al.*, 2014; Cheng *et al.*, 2014; Yan *et al.*, 2014). For example, in conditional HDAC1 knockout mice, cytokine production in $CD8^+$ T cells was enhanced, but the ability to fend off a viral challenge was decreased (Tschismarov *et al.*, 2014). It remains to be tested whether the targeted inhibition of individual HDACs is effective in reactivating latent HIV while reducing unwanted effects on T-cell function (Archin *et al.*, 2009; Barton *et al.*, 2014; Klase *et al.*, 2014).

Bromodomain inhibitors and HIV transcription

Recently, novel inhibitors of bromodomain-containing proteins, especially those targeting so-called BET (bromodomain and ET domain) proteins BRD2–4 and BRDT, have shown impressive effects in cancer, immunity, and contraception (French, 2012; Matzuk *et al.*, 2012; Filippakopoulos & Knapp, 2014). These drugs occupy the binding pockets for acetyl-peptides in bromodomains and thereby displace BET proteins from chromatin or other binding partners (Shi & Vakoc, 2014). Much of the work characterizing these compounds, particularly the freely available compound JQ1, has focused on inhibiting BRD4, owing to its relevance in some malignant midline carcinomas (Filippakopoulos *et al.*, 2010; French, 2012). Because BRD4 is a cofactor of the P-TEFb complex and competes with the HIV Tat protein for P-TEFb binding, BET inhibitors were also tested for the ability to reactivate latent HIV. Several BET bromodomain inhibitors, including JQ1, iBET151 and MS417, activate HIV transcription in cell culture models of latency (Table 1) (Banerjee *et al.*, 2012; Li *et al.*, 2012; Boehm, Conrad, *et al.*, 2013), but their effect in patient-derived cells varies (Zhu *et al.*, 2012; Shi & Vakoc, 2014). BET inhibitors do not synergize with HDAC inhibitors to activate HIV transcription, supporting the notion that both drugs target similar molecular pathways (Bartholomeeusen *et al.*, 2012; Boehm *et al.*, 2013; Loosveld *et al.*, 2014). However, strong synergies exists with activators of the cellular protein kinase C pathway in cell culture (Li *et al.*, 2012; Wang *et al.*, 2012; Boehm *et al.*, 2013; Jiang *et al.*, 2014) and with monoclonal antibodies against HIV in humanized mouse models of HIV (Halper-Stromberg *et al.*, 2014). Further studies are still required to better understand the molecular mechanisms of how BET inhibitors activate HIV transcription, which BET protein is targeted, and how best to combine these inhibitors in clinical trials.

HAT inhibitors and the permanent silencing of HIV transcription

Alternatives to the “shock and kill” approach, which include durable transcriptional silencing of latently infected cells, are so far less developed. Since HIV–HAT interactions have a central role in the activation of viral transcription, a structure-guided approach could be used to develop specific inhibitors against Tat–HAT interactions (Vendel & Lumb, 2004; Zeng *et al.*, 2005; Pan *et al.*, 2007). While compounds such as curcumin and isogarcinol can

inhibit p300/PCAF HAT activity, they also demonstrate low specificity or high levels of toxicities in cell lines (Balasubramanyam *et al.*, 2004a,b). Mantelingu and colleagues demonstrated that chemical manipulation of these naturally derived compounds could increase the specificity of binding to p300, and in the case of one of the derived compounds, LTK14, suppress viral reactivation *in vitro* (Mantelingu *et al.*, 2007). Another, more recent study, has shown that coumarin derivitaves, specifically, BPRHIV001, also inhibits p300 activity and is able to suppress Tat mediated transcription *in vitro* (Lin *et al.*, 2011). While these studies collectively highlight the importance of p300/CBP as a potential target in HIV treatment, it is unclear whether HAT inhibitors can effectively inhibit viral rebound from latency *in vivo*. Further investigation of the molecular role of HATs, HDACs, and bromodomain-containing proteins in the establishment and maintenance of latency are required to develop better and more targeted therapeutic interventions.

Conclusion and outlook

Protein acetylation, a highly conserved regulatory system in a broad range of organisms, can rapidly translate environment signals into critical cellular functions. Viruses such as HIV have evolved intricate strategies to manipulate this system to facilitate viral propagation at multiple steps of the viral life cycle. As a result, there is growing interest in using inhibitors of acetylation-associated proteins to disrupt these interactions. Despite an impressive body of work, much remains to be learned about the complex role of reversible acetylation during the HIV life cycle and within the immune system. The ability to continuously monitor acetyl-stoichiometric changes (i.e. frequency of each acetylated site within a cell)—on both a local and systemic scale—will be critical in assessing the biological significance of the effects of these modifications on different cellular processes. Two studies have begun to address this topic in *Saccharomyces cerevisiae* and *Escherichia coli* (Baeza *et al.*, 2014; Weinert *et al.*, 2014). These studies revealed that significant acetylation alterations occur in distinct subcellular compartments during specific cell-cycle phases or upon deletion of a particular HDAC. Since viruses operate in distinct host compartments at different time points, it will be interesting to use this technology to map acetylation dynamics in an infected cell during different phases of the viral life cycle. This knowledge will promote a more comprehensive understanding of the dynamics of host–virus interactions and highlight critical areas of interest for therapeutic intervention.

In addition, as new players are still continually being added to the acetylation network, novel hypotheses and opportunities for treating HIV will arise. Besides bromodomains, some tandem plant homeodomain zinc-finger proteins may also bind histones in an acetylation-specific manner (Zeng *et al.*, 2010; Ali *et al.*, 2012; Qiu *et al.*, 2012). Furthermore, the tandem pleckstrin-homology domain of Rtt106, a yeast chaperone protein, binds acetylated histone H3 at lysine 56 (Su *et al.*, 2012). Most relevant to HIV, the highly conserved YEATS domain, named for its five founding proteins (Yaf9, ENL, AF9, Taf14, and Sas5), binds acetyl-lysine residues, with a preference for acetylated histone H3 lysine 9 (Li *et al.*, 2014). ENL and AF9 are both members of the so-called super elongation complex (SEC), which is associated with HIV Tat and P-TEFb and critically involved in their function during HIV transcription elongation (He *et al.*, 2010; Sobhian *et al.*, 2010). It remains to be determined

whether these interactions are dependent on the acetylation status of these factors and can be affected by acetylation-targeting drugs.

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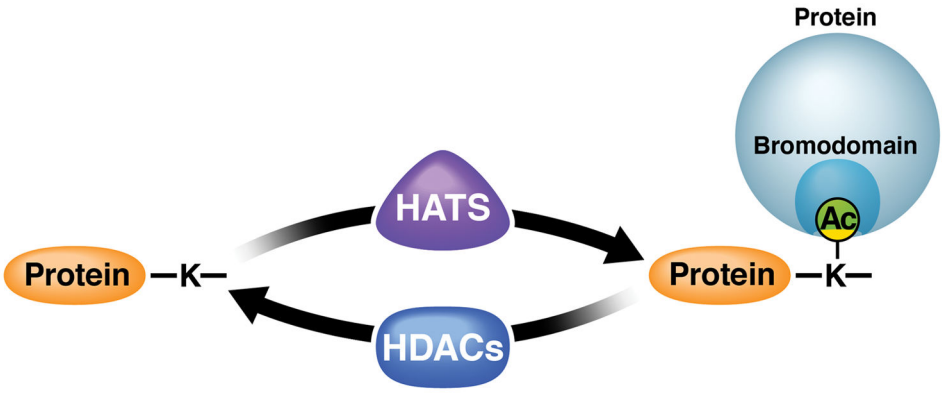

Figure 1. The different players in the host acetylation network

Histone acetyltransferases (HATs) transfer acetyl groups to target lysines in proteins while histone deacetylases (HDACs) remove them. Proteins containing bromodomains bind acetyl-lysines via a distinct structural binding pocket and recruit complexes relevant for the function of the acetylated protein.

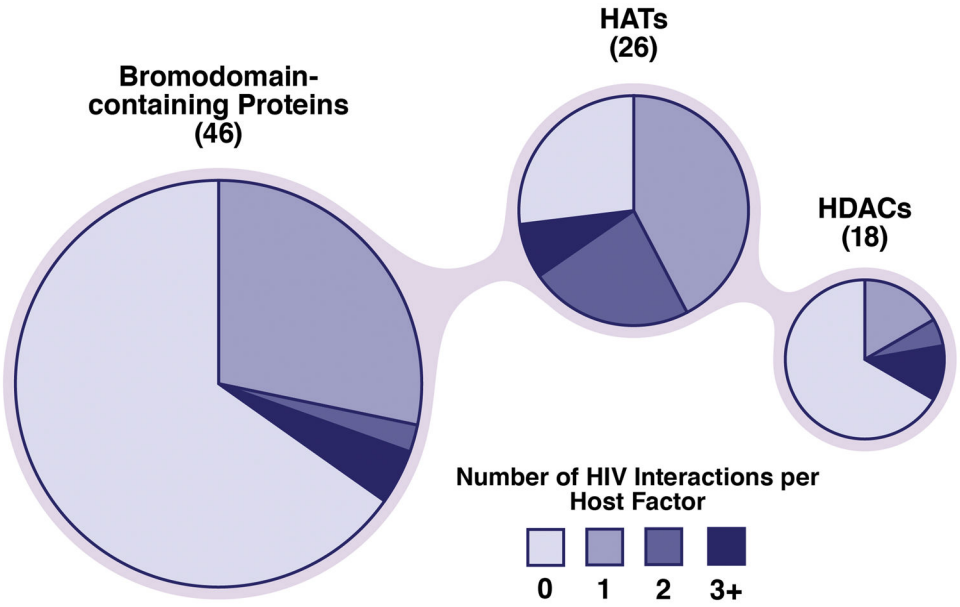

Figure 2. Global interactions of HIV with the host acetylation network

Recent unbiased interaction studies between HIV and host proteins have identified a high degree of interplay between HIV proteins and host acetylation factors: 19 of 26 HATs, 6 of 18 HDACs, and 16 of 46 bromodomain-containing proteins bind diverse HIV proteins. These data were determined with GPS-Prot (http://www.gpsprot.org), a web-based software tool that integrates HIV–host interaction datasets.

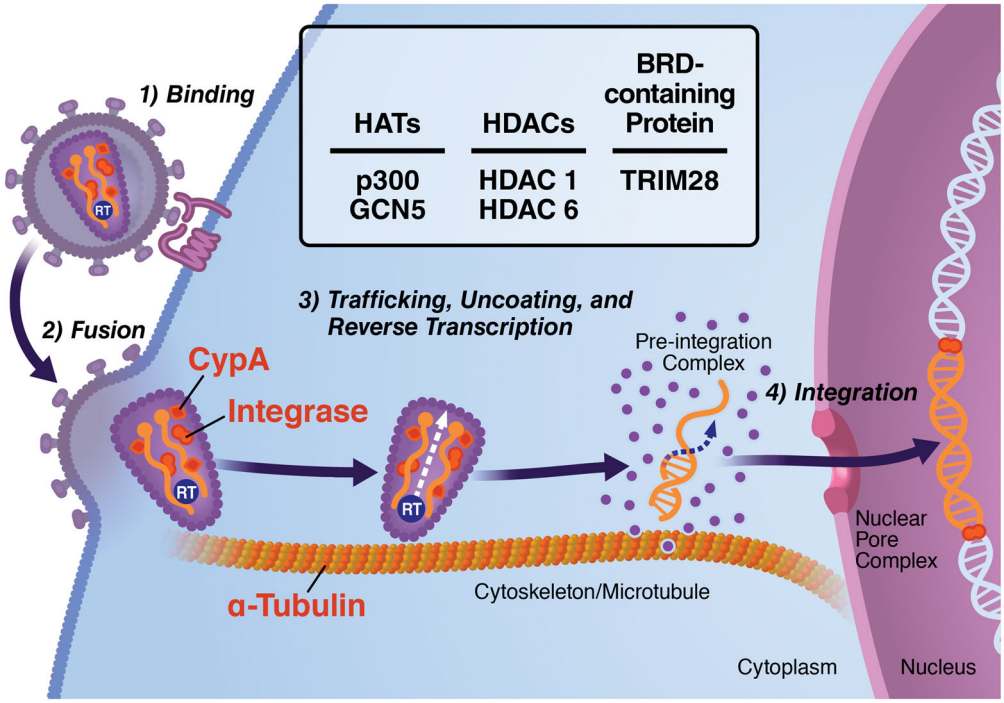

Figure 3. The role of reversible protein acetylation in early HIV infection

The different steps of the early stages of the HIV life cycle are depicted with viral (integrase) and host (cyclophilin A (CypA) and α-tubulin) factors regulated by acetylation depicted in red. Engagement of HIV envelope protein gp120 with its host receptor CD4 induces α-tubulin acetylation and microtubule stabilization, a process required for successful fusion of the virus to the cell. Overexpression of HDAC6, which decreases α-tubulin acetylation, impairs virus–cell fusion and subsequent infection. Cellular CypA, a peptidylprolyl isomerase, is packaged into budding virions and regulates early steps of infection. Acetylation of CypA impairs its catalytic activity and disrupts its interaction with the HIV gag protein. The viral integrase enzyme is critical for the integration of proviral double-stranded cDNA into the host chromatin. Acetylation of the integrase enzyme by p300 enhances its affinity to genomic DNA and its strand transfer activity. It also recruits bromodomain-containing protein TRIM28 and its associated HDAC1 activity.

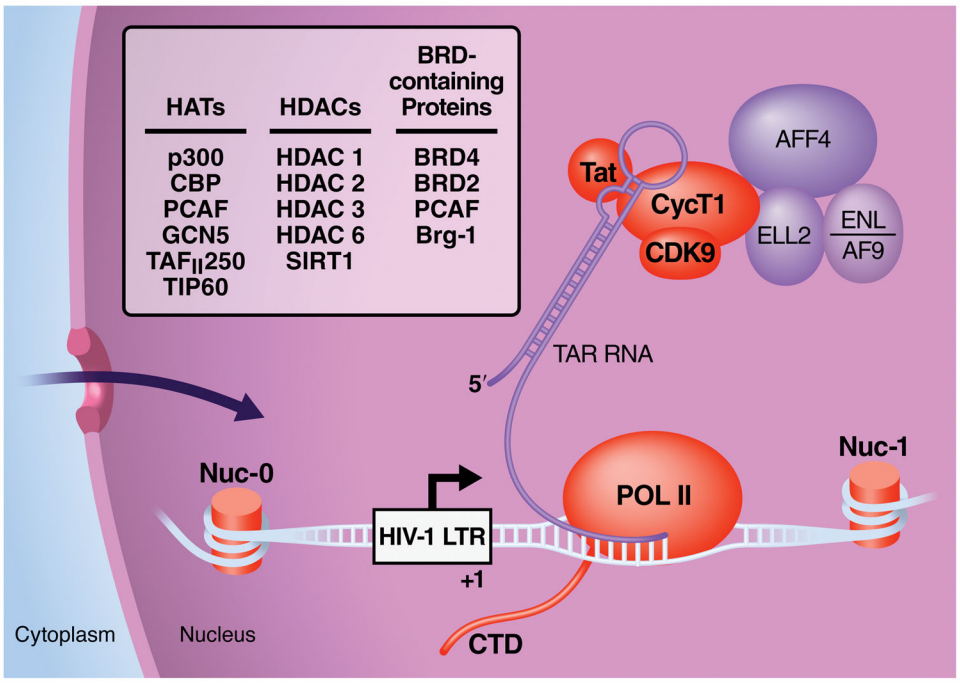

Figure 4. Regulation of HIV transcription by protein acetylation

HIV transcription is closely associated with the host acetylation machinery and is currently a target for acetyllysine-targeting drug regimens. Viral (Tat) and host factors (histones in nucleosomes (nuc), the Tat cofactor P-TEFb, RNA polymerase II (POL II)) that are targets of acetylation are depicted in orange. HATs, HDACs and bromodomain (BRD)-containing factors associated with HIV transcription are listed in the box. Proteins in purple depict factors of the super elongation complex (SEC) recently identified as interacting with P-TEFb and Tat. See text for details.

Table 1

Inhibitors of HDACs, bromodomain-constaining proteins and HATs used as regulators of HIV transcription

Summary of emerging or clinically relevant acetylation-based therapeutic strategies to combat HIV-1 latency by reactivation or sustained suppression of the HIV-1 LTR. Clinical trial information was obtained through https://clinicaltrials.gov/.

Compound Concentration	Target	Effects on HIV-1$^+$ Latent Cells	Status in Trial Trial ID	References
Romidepsin 0.1 nM-45nM	**Class I HDAC Inhibition**	• Increase in intracellular and extracellular HIV-1 RNA in addition to increase in viral outgrowth using *ex vivo* assays • Transient increase in plasma viremia levels in patients	**On-going Phase I Trial** NCT01933594	Bullen *et al*, 2014; Campbell *et al*, 2014; Wei *et al*, 2014; Bertino and Otterson, 2011
Panobinostat 5nM–30nM	**Pan-specific HDAC Inhibition**	• Increase in intracellular HIV-1 RNA of HIV-1 *ex vivo* • Decrease in size of detectable latent reservoir	**Completed Phase I Trial** NCT01680094	Bullen *et al*, 2014; Rasmussen *et al*, 2013; Rasmussen *et al*, 2014; Wei *et al*, 2014
Vorinostat 100nM–5μM	**Pan-specific HDAC Inhibition**	• Increase in HIV-1 RNA observed in patients • No detectable reduction of viral reservoir • Reactivation of HIV-1 transcription using *in vitro* and some *ex vivo* models	**Completed Phase I Trial** NCT1365065 **On-going Phase II Trials** NCT01933594 NCT01319383	Archin *et al*, 2014; Archin *et al*, 2012; Bullen *et al*, 2014; Cillo *et al*, 2014; Del Prete *et al*, 2014; Lucera *et al*, 2014; Elliott J, 2013
Valproic Acid 100μM–5mM	**Pan-specific HDAC Inhibition**	• Induced transient increase in viral production in patients • High levels of toxicity in patients • Weak reactivation of latent HIV-1 transcription using *in vitro* J-Lat models	**Terminated Phase I Trial** NCT00289952	Archin *et al*, 2010; Bullen *et al*, 2014; Routy *et al*, 2012
Givinostat 30nM–250nM	**Class I and Class II HDAC Inhibition**	• Increase in extracellular HIV-1 RNA with corresponding increase in	**Completed Phase I Non-HIV Trials** NCT00792467	Rasmussen *et al*, 2013; Furlan *et al*, 2011; Matalon *et al*, 2010

Compound Concentration	Target	Effects on HIV-1$^+$ Latent Cells	Status in Trial Trial ID	References
		supernatant p24 *in vitro*	NCT00570661	
JQ-1 100nM–10μM	**BET Protein Inhibition**	• Reactivates HIV-1 transcription using *in vitro* J-Lat models, but not primary cells using *in vitro* or *ex vivo* models	**Tested *in vitro* and *ex vivo***	Bullen *et al,* 2014; Boehm *et al,* 2013; Bisgrove *et al,* 2007; Li *et al,* 2012; Filippakopoulos *et al,* 2010
I-BET, I-BET151 100nM–10uM	**BET Protein Inhibition**	• Reactivates HIV-1 transcription using *in vitro* J-Lat models, but not primary cells	**Tested *in vitro***	Nicodeme *et al,* 2010; Boehm *et al,* 2013; Seal *et al,* 2012
MS417 50nM-5μM	**BET Protein Inhibition**	• Reactivates HIV-1 transcription using *in vitro* J-Lat models, but not primary cells	**Tested *in vitro***	Boehm *et al,* 2013; Zhang *et al,* 2012
Compound 8 1 μM–140μM	**Distruption of PCAF/Tat Interaction**	• Inhibits transcription from LTR-luciferase reporter, but not tested with models that express full-length virus	**Tested *in vitro***	Pan *et al,* 2007
LTK14 10μM–50μM	**p300 Inhibition**	• Suppresses viral transcription *in vitro* with decreased cytotoxicity compared to parent compound isogarcinol	**Tested *in vitro***	Balasubramanyam *et al,* 2004a; Mantelingu *et al,* 2007
BPRHIV001 1 μM–140μM	**p300 Down-Regulation**	• Inhibits transcription from LTR-luciferase reporter, but not tested with models that express full-length virus	**Tested *in vitro***	Lin *et al,* 2011