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Reactivation of latent HIV by histone deacetylase inhibitors

Kotaro Shirakawa^{1,2}, Leonard Chavez^{1,2}, Shweta Hakre^{1,2}, Vincenzo Calvanese^{1,2}, and Eric Verdin^{1,2}

¹Gladstone Institute of Virology and Immunology, University of California, San Francisco, CA 94158

²Department of Medicine, University of California, San Francisco, CA 94158

Abstract

Latent HIV persists in CD4+ T cells in infected patients under antiretroviral therapy (ART). Latency is associated with transcriptional silencing of the integrated provirus and driven, at least in part, by histone deacetylases (HDACs), a family of chromatin associated proteins that regulate histone acetylation and the accessibility of DNA to transcription factors. Remarkably, inhibition of HDACs is sufficient to reactivate a fraction of latent HIV in a variety of experimental systems. This basic observation led to the shock and kill idea that forcing the transcriptional activation of HIV might lead to virus expression, to virus- or host-induced cell death of the reactivated cells, and to the eradication of the pool of latently infected cells. Such intervention might possibly lead to a cure for HIV infected patients. Here, we review the basic biology of HDACs and their inhibitors, the role of HDACs in HIV latency and recent efforts to use HDAC inhibitors to reactivate latent HIV *in vitro* and *in vivo*.

Keywords

HIV; Latency; HDAC; HDAC inhibitors

Introduction: HIV persistence

With the introduction of combination antiretroviral therapy (ART) in the mid-1990s, HIV-associated mortality and morbidity dramatically decreased, and gave rise to the hope for viral eradication in infected individuals. ART is comprised of several drugs that each target a specific stage of the viral life cycle, and is effective at combating active viral replication of newly infected cells and reducing the viral load down to undetectable levels [1]. However, despite the ability of ART to stop new rounds of infection, HIV persists within the body of infected individuals undergoing therapy and cessation of ART leads to a viral rebound within 3–4 weeks [2]. The observation of viral rebound has given rise to questions surrounding the mechanisms behind this viral persistence, and subsequent studies have led to the discovery of several potential viral reservoirs within the body that may contribute to persistence. These include long-lived cells such as resting CD4+ T lymphocytes, follicular dendritic cells, and hematopoietic stem cells. In addition, the virus may persist in anatomical

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Corresponding author: Verdin, E. (everdin@gladstone.ucsf.edu).

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sanctuaries that are not reached by the ART drugs, such as the male urogenital tract, gut-associated lymphatic tissues and central nervous system (reviewed in [3]).

It is not yet fully clear whether HIV persists in the presence of ART because of ongoing low level replication or because of the episodic reactivation of a stable reservoir. A recent study by Shen and Siliciano used a detailed analysis of residual viremia and a new pharmacodynamic analysis of the effectiveness of ART to show that current therapy is fully capable of suppressing ongoing viral replication [4]. However, the possibility of residual ongoing viral replication cannot be fully excluded [5], and intensification of ART using new agents such as integrase inhibitor, fusion inhibitor, and chemokine antagonists are under clinical trials. If, as we suspect, the majority of residual viremia is the result of viral production from stable reservoirs, novel approaches will be required to target these stable latent reservoir for the eradication of HIV.

Latent infection of CD4+ T cells

Following binding to its main target cell, the CD4+ T lymphocyte, HIV fuses with the cell membrane and releases its contents into the cytoplasm. The virus reverse-transcribes its genomic RNA into double-stranded DNA that then enters into the nucleus using both host and viral factors. At this point, the double-stranded viral DNA, or provirus, integrates into the host genome, where it comes under the control of host transcriptional activation and repression mechanisms. In activated CD4+ T cells, cellular transcription factors such as NF- κ B and Sp1 drive the initial transcription of viral regulatory proteins, including the viral transactivator of transcription, Tat. Once Tat production reaches sufficient levels, viral transcription is increased by greater than 100-fold, which results in virus production and death of the infected cell usually within 24 hours post infection [6].

However, in most patients, one can detect rare CD4+ T cells (frequency of 1 per 10^6 cells) that contain an integrated and transcriptionally silent HIV provirus [2, 7]. These proviruses can be reactivated and are replication competent. It is not clear how latency is established, particularly whether latency is established by infection of resting T cells or by infection of activated CD4+ T cells that escape the cytotoxic effects of circulating CD8+ T cells and revert back to a quiescent state. Latent HIV is primarily found in long-lived central memory and transitional memory CD4+ T cells [8]. The absence of viral evolution in latently infected cells supports the model that this reservoir is seeded early in infection and likely maintained by T cell survival and low levels of antigen-driven homeostatic proliferation.

Because of the failure of ART to eradicate HIV from infected individuals, new strategies are being developed with the aim of curing HIV. One such strategy is to purge, or reactivate, the pool of latently infected cells in the presence of ART. To reach this goal, a comprehensive understanding of the mechanisms that govern HIV latency is required.

Several mechanisms have been identified that play a role in the establishment and maintenance of HIV latency. These include a variety of cis- and trans-acting mechanisms. Cis-acting mechanisms include the site of integration of the provirus in the genome and the possible role of the local chromatin environment [9, 10], or possible transcriptional interference mediated by an adjacent gene [11]. Trans-acting mechanisms reflect the state of activation of the CD4+ T lymphocyte and include the level of transcriptional activators such as NF- κ B, and Tat cofactors [12] or the presence of transcriptional repressors [13, 14].

Both cis- and trans-acting mechanisms affect the chromatin environment at the HIV promoter and the transcriptionally active and inactive HIV promoters show significant differences in terms of their chromatin organization. For example, the nuc-1 nucleosome is present immediately downstream of the HIV promoter under latency conditions and is

disrupted in the activated provirus [15]. Supporting a role for chromatin in the establishment and maintenance of HIV latency, treatment of HIV latently infected cells with small molecule inhibitors of histone deacetylases (HDACs), a family of histone modifying enzymes, is sufficient to reactivate latent HIV [16]. These early observations led to current studies of the role of HDAC inhibitors in patients with the aim of eradicating latently infected cells.

Here, we review the role of HDACs and their inhibitors in HIV transcription, focusing specifically on transcriptional repression leading to latency.

Histone acetylation and characteristics of HDACs

Histone acetylation consists in the addition of an acetyl group to the ϵ -amino group of lysine residues. Histone lysine acetylation, on lysine 9 or 14 of histone H3 (H3K9, H3K14) or on lysine 16 of histone H4 (H4K16), is generally associated with active gene transcription. The positively charged ϵ -amino group of lysine residues on the histone tails are thought to regulate the degree of compaction of chromatin and thereby its accessibility to transcriptional factors [17]. In addition, a protein domain known as the bromodomain specifically binds to acetylated lysines in proteins and thereby enables the docking of transcriptional co-activators, such as ATP-dependent chromatin-remodeling complexes, to acetylated chromatin [18].

Histones and other proteins are reversibly acetylated by lysine acetyltransferases (KATs) and deacetylated by lysine deacetylases (KDACs) [19]. Because these activities were initially identified and studied in the context of histone proteins in chromatin, these enzymes are commonly referred to as histone acetyltransferases (HATs) and HDACs, a practice that we will follow here. HDACs are grouped into four classes based on their homology to the yeast deacetylases RPD3 (class I, IV), HDA1 (class II) and Sir2 (class III) [20] (Figure 1). Class I includes HDAC1, 2, 3 and 8 and represents a subset of mostly nuclear and ubiquitous enzymes [21]. Class II HDACs are divided in two subclasses: class IIa includes HDAC4, 5, 7, 9, while class IIb contains HDAC6 and 10. Class II HDACs are found both in the nuclei and cytoplasm and are expressed in a more tissue-specific manner and regulate tissue differentiation in a variety of organs [22]. Class IIa HDACs contain a large regulatory N-terminal domain while class IIb HDACs have two tandemly arranged deacetylase domains (Figure 1). Class IV contains only HDAC11 and is related to both RPD3 and HDA1. HDACs from classes I, II, and IV HDACs all share some sequence homology and are Zn^{2+} -dependent enzymes. They harbor a catalytic pocket with a Zn^{2+} ion at its base that can be inhibited by Zn^{2+} -chelating compounds. In contrast, the class III HDACs, which are also called sirtuins, are nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases. The seven mammalian sirtuins (SIRT1-7) are important and widely expressed enzymes that target histone and non-histone proteins for deacetylation [23].

Different HDACs have been implicated as relevant drug targets in cancer, inflammation, cardiovascular and neurological conditions [24]. Two HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA)/vorinostat and romidepsin/istodax, have been approved for the treatment of cutaneous T cell lymphoma. Furthermore, a large number of other HDAC inhibitors are currently at different preclinical and clinical stages as novel therapies for various conditions [25].

HDAC inhibitors are classified into four major structural families (Figure 2): short-chain aliphatic acids (including valproic acid and butyric acid), hydroxamic acids (such as trichostatin A and vorinostat), benzamides (including entinostat), and cyclic tetrapeptides and depsipeptides (such as trapoxin B and romidepsin). Different HDAC inhibitors share the same molecular structure consisting of a cap, which targets the surface of the enzyme, a

hydrophobic aliphatic chain called the linker (optimal length equals 5–6 carbons) and a head or functional group, which can be either a hydroxamic acid, a benzamide group, a phenylene diamine, a carboxylic acid group or an epoxide group. Most of these groups chelate the catalytic Zn²⁺ ion in different manners (hydroxamic acid or benzamide) or become covalently linked at the active site (epoxide) [26] (Figure 2).

Importantly, recent large-scale mass spectrometry efforts have shown that 10 to 15% of the mammalian cell proteome undergoes reversible acetylation [27]. In parallel, evidence has accumulated that HDAC proteins target both histone and non-histone proteins for deacetylation [28]. These recent observations suggest that inhibition of HDACs by small molecules is likely to modify not only histone but also non-histone protein acetylation and their biological functions. Understanding the true mechanism of action of HDAC inhibitors will therefore have to take this into account.

HDACs and HIV transcriptional regulation

HDACs regulate HIV latency directly by inducing histone deacetylation at HIV integrated sites (Figure 3A) and indirectly through non-histone protein modification such as NF- κ B (Figure 3B). HDAC inhibitors also induce global histone acetylation resulting gene expression changes, and possibly affect HIV latency (Figure 3C). In this section, we discuss mechanisms how HDACs may contribute to the latent state of HIV and mechanism by which HDAC inhibitor treatment may lead to the reactivation of latent HIV.

Recruitment of HDACs to the HIV promoter via transcription factors

The HIV promoter is packaged in chromatin, and nucleosomes are deposited at defined positions within the HIV long terminal repeat [29]. Importantly, a single nucleosome positioned downstream of the HIV transcription start site, called nuc-1, is present on the latent proviral genome and displaced when it is transcriptionally activated. Nuc-1 remodeling is an early event in the reactivation of latent HIV and modification of histone acetylation alone by treatment with an HDAC inhibitor is sufficient to activate latent HIV, indicating that chromatin plays a dominant role in the control of HIV latency [15, 16]. Several transcription factors can recruit HDACs to the HIV long terminal repeat (LTR) promoter under basal conditions (Figure 3A). These include Ying-Yang 1 (YY1) [30], late SV40 factor (LSF) [31], COUP-TF interacting protein (CTIP2) [32], c-promotor-binding factor (CBF-1) [33], NF- κ B p50 homodimer [34], and c-myc and Sp1 [35]. These transcription factors recruit HDAC1 to the HIV promoter, locally deacetylate histone proteins locally and lead to transcriptional silencing. HDAC2 is also recruited to the HIV promoter via CTIP2 and Sp1 [32]. HDAC3 also associates with the HIV promoter [36] (Figure 3B).

Importantly, the relative contribution of each HDAC to HIV transcriptional silencing during latency has not been fully elucidated, particularly in primary lymphocytes models of latency or in patients' cells. It is also not fully clear which of the transcription factors described above is critical for HDAC recruitment to the HIV promoter. In recent experiments, Margolis and colleagues showed that short hairpin RNA (shRNA)-mediated knockdown of YY1 caused an increase in HIV expression in latent cell lines [37]. However, knockdown of YY1 alone or in combination with c-myc showed no change in HDAC occupancy on the HIV LTR indicating that other factors than HDACs contribute to the suppression of HIV expression mediated by YY1 [37].

DNA methylation and histone methylation

DNA methylation of two CpG islands in the HIV promoter has been observed in latently infected cell lines and in patients samples and can mediate the recruitment of the NuRD

complex via the methyl binding protein MBD2 [38, 39] (Figure 3A). Importantly, the NuRD complex exists in vertebrates in different compositions in a cell-type-dependent manner and contains HDAC1 and HDAC2 [40]. Histone methylation is linked to DNA methylation. In the HIV latent cell line, 2D12, which has a hypermethylated HIV promoter, histone H3 lysines 9 (H3K9) and 27 (H3K27) are methylated. H3K9 methylation is mediated by Suv39H1 (di- and tri-methylation) and G9a (mono- and di-methylation) while H3K27 methylation is partly mediated by EZH2 (di- and tri-methylation) [41]. These histone methyltransferases are recruited to latent HIV and have been both implicated in HIV latency [42–44]. Recent reports have questioned whether latent HIV is methylated in patients [45]. However, since defective proviruses outnumber latent proviruses by a factor of 100:1 [7], and since latent cells can only be identified *a posteriori*, methylation analysis of total patient DNA is likely to reflect the defective HIV pool rather than the latent pool. The exact role of DNA methylation in patients therefore remains undefined and a challenge for future investigations.

Remodeling of nuc-1 in the HIV promoter by the BAF/PBAF complexes and protein acetylation

Remodeling of nuc-1 occurs in response to a variety of agents that activate latent HIV, including tumor necrosis factor- α (TNF- α), phorbol esters and the HIV transactivator Tat [15, 46]. The chromatin region encompassing the nuc-1 region becomes acetylated in a transcription factor and HAT-dependent manner [47, 48]. Remarkably, treatment of latently infected cells with HDAC inhibitors is sufficient to induce the chromatin remodeling of nuc-1 [16]. A SWI/SNF chromatin remodeling complex called BAF is associated with the latent HIV promoter and may help to stabilize nuc-1 downstream of the transcriptional start site, on a region of DNA that is thermodynamically unfavored for nucleosome incorporation (Figure 3A) [14]. Interestingly, the BAF complex and HDAC1 and 2 interact [49].

Regulation of trans-acting factors by acetylation: NF- κ B, Sp1 and HIV Tat

Transcription factors are also regulated by reversible lysine acetylation. Lysine acetyltransferase p300/CBP acetylates the RelA subunit of NF- κ B and enhances its transcriptional activity [50]. Acetylation of lysine residues 210 and 218 of RelA controls its interaction with its endogenous inhibitor I κ B [51], and acetylated RelA persists longer in the nucleus. Acetylation of lysine 310 is required for full activation of its transcriptional activity [52]. HDAC3 deacetylates RelA and negatively regulates its activity (Figure 3B) [51]. Sp1 is also acetylated at lysine 703 in its DNA binding domain leading to increase DNA affinity (Figure 3B) [53]. Inhibition of HDACs causes hyperacetylation of these factors, and likely enhances their ability to activate transcription and cause reversal of repression on the HIV LTR.

HIV-1 Tat is also regulated by posttranslational modifications including reversible acetylation (Figure 3B) [54]. Lysine 28 in the cysteine-rich, transactivation domain of Tat is acetylated by the acetyltransferase PCAF, and this modification is critical for high affinity binding to TAR and P-TEFb. HDAC6 deacetylates lysine 28 of Tat in the nucleus and inhibits Tat transcriptional activity by triggering its export to the cytoplasm [55]. Lysines 50 and 51 in the arginine-rich RNA binding domain, which is required for TAR binding, are acetylated by acetyltransferases p300/CBP and GCN5/PCAF. Acetylation of lysines 50 and 51 is recognized by the bromodomain of the PCAF or SWI/SNF chromatin-remodeling complex, and further facilitate HIV transcription [56]. The class III HDAC, SIRT1 deacetylates Tat [57] and inhibits Tat transactivation in the latently infected cell model [58].

Indirect effects of HDAC inhibitors on latent HIV

HDAC inhibitors induce histone acetylation and affect gene expression in a small proportion (2%–20%) of genes [59, 60]. Genes induced by HDAC inhibitors may regulate the state of HIV latency (Figure 3C).

HDAC inhibitors in basic and clinical research

Various HDAC inhibitors have been shown to reactivate latent HIV in a variety of experimental systems and in cells from HIV-infected individuals. These studies are described next. Current ongoing and completed clinical trials of HDAC inhibitors in HIV infected patients are summarized in Table 1.

Valproic acid

Valproic acid is an FDA-approved anti-epileptic agent that inhibits class I and II HDACs at millimolar concentrations. In a pilot clinical study, valproic acid was administered to four patients on ART [61]. These patients were also given the fusion inhibitor drug enfuvirtide to intensify ART. The frequency of latent infection was measured and significantly declined in three of four patients [61]. However, several follow up studies of valproic acid have failed to detect reduction in latent reservoir size (Table 1) [62–65].

SAHA/vorinostat

The most studied HDAC inhibitor in the context of HIV latency is SAHA, an FDA-approved HDAC inhibitor named vorinostat currently approved for the treatment of cutaneous T cell lymphoma [66]. It reactivates latent HIV in a variety of *in vitro* experimental models including J89, ACH-2, U1 and J-LAT [67]. Vorinostat also reactivates latent HIV in primary CD4+ T cell models of HIV latency and in CD4 T+ cells isolated from patients on ART [68, 69]. Vorinostat activates the HIV promoter, induces hyperacetylation of the HIV promoter nucleosomes and suppresses HDAC binding to the HIV promoter [70]. In an important pilot experiment (Table 1), a group of 11 ART-treated HIV infected patients were selected based on the absence of measurable viral plasma RNA and increased HIV RNA production *ex vivo* in response to vorinostat. Treatment of this select group of patients with a single dose of vorinostat triggered a median 4.8-fold increase of HIV mRNA expression in resting CD4+ T cells (range: 1.5 to 10.0) [68, 69]. Among these patients, vorinostat was well tolerated with no significant adverse event. However, it should be noted that the patients were selected based on responsiveness to vorinostat, that we do not know whether this treatment resulted in a decrease in reservoir size, and that we do not know what fraction of latent HIV responds to HDAC inhibitor.

Other hydroxamic acid HDAC inhibitors: givinostat (ITF2357) and panobinostat (LBH-589)

Two other promising HDAC inhibitors are givinostat and panobinostat. Givinostat is currently in phase II clinical trials for relapsed hematological malignancies [71]. Givinostat also induces HIV transcription in latently infected T cells and monocytic cells [72]. Although givinostat needs to be further evaluated for efficacy in aviremic patients on ART, experiments in ACH2 cells and U1 latent cells showed that this inhibitor effectively induces HIV transcription at therapeutically relevant concentrations. Importantly, givinostat decreases the expression of the HIV co-receptors CXCR4 and CCR5 on the cell surface of primary CD4+ T cells [72]. Another hydroxamic acid HDAC inhibitor, panobinostat is a pan-HDAC inhibitor in phase I/II clinical trials for relapsed hematological malignancies and solid tumors. Panobinostat targets class I, II and IV HDACs at low nanomolar range except

for HDAC4, 7 and 8 [73]. Panobinostat is at least 10 times more potent than vorinostat and is a good candidate to test in reactivation of latent HIV.

Benzamide HDAC inhibitors: entinostat (MS275) and mocetinostat (MGCD0103)

Entinostat induces HIV expression in latently infected cell lines and in primary cell models of latency [67]. Entinostat preferentially targets class I HDACs (HDAC1, 2 and 3) [69], and shows little toxicity in the models tested [67]. Since entinostat is selective for only HDAC1, 2 and 3, which are most relevant to HIV regulation [74], entinostat might be more efficacious and less toxic. Of note, mocetinostat is another benzamide HDAC inhibitor that also targets class I HDACs and is undergoing clinical trials on Hodgkin lymphoma and other cancers [75].

Romidepsin (FK228)

Romidepsin was originally discovered as an antibiotic produced by *Chromobacterium violaceum* and is another FDA-approved HDAC inhibitor for the treatment of recurrent cutaneous T cell lymphoma. Romidepsin targets class I HDACs more potently than vorinostat with IC₅₀ of 1 nM in *in vitro* assays [76]. Unlike vorinostat, romidepsin is Ames test negative. Both basic and clinical studies are required to test this HDAC inhibitor in the context of HIV reactivation.

Novel class I selective HDAC inhibitors

Recent efforts in HDAC inhibitor development have focused increased specificity against unique HDACs. Several of these compounds that selectively target class I HDACs induce HIV transcription in resting T cells from patients receiving ART [77]. This observation suggests that class I HDACs represent promising targets for anti-HIV-latency therapies.

Other compounds to reactivate HIV latency

Chemical compounds that target unique HIV transcription factors and co-factors have been identified for their ability to reactivate latent HIV. Prostratin is a PKC activator from mamala tree bark that induces nuclear accumulation of the NF- κ B p65-p50 heterodimer [78]. Synergy is observed when it is used with an HDAC inhibitor [69]. Recently, the acetaldehyde dehydrogenase inhibitor disulfiram was identified in a screen for small molecules that reactivate latent HIV [79]. Disulfiram activates the Akt signaling pathway through downregulation of a phosphatase called PTEN, resulting in latent HIV reactivation [80]. Interestingly, it has been proposed that vorinostat may also activate latent HIV via phosphatidylinositol 3-kinase/Akt pathway activation [81]. Furthermore, other epigenetic modifiers such as histone methyltransferases and DNA methyltransferases are also involved in HIV transcriptional repression. The DNA cytosine methylation inhibitor 5-aza-2'-deoxycytidine synergizes with either prostratin or TNF- α to reactivate latent HIV in J-LAT cell lines [38, 39]. Histone methyltransferase inhibitors such as the Suv39H1 inhibitor chaetocin [82], G9a inhibitor BIX01294 [43], and EZH2 inhibitor DZNep [44] are also reported to reactivate latent HIV in a latent cell line model or primary CD4⁺ T cell model. Combining these small molecules with HDAC inhibitors may prove to be an even more effective inducer of latent HIV.

Concluding remarks: shock and kill approach and future directions

The shock and kill approach combines the induction of latent HIV, by HDAC inhibitors and other drugs, in combination with ART therapy. It is hoped that reactivation of latent HIV

will lead to the killing of the productively infected cells by the virus itself, or by the host immune system, and that the ART will block novel infection from the released virus. If effective, this approach might lead to the elimination of the latent viral reservoir and represent a first step in our search for an HIV cure. The recently reported vorinostat study is the first promising example of this shock and kill approach [68], but it raises many new questions. First, as a proof of concept, further clinical studies are required to test whether the addition of vorinostat increased virus production, whether it induced cell death in the latent reservoir and whether it is sufficient to reduce the size of the latent reservoir. Second, additional clinical studies are required to test other drugs and other combinations of drugs and measure their effect on the latent reservoir. Since vorinostat is a toxic anticancer drug and is mutagenic (Ames test-positive), safety issues must also be clarified. In particular, there is concern that HDAC inhibitors might lead to the reactivation of endogenous viruses in the host genome that are under the control of HDACs as well [83]. Finally, a recent report from the Siliciano lab, using an *in vitro* model of HIV latency in primary lymphocyte, showed that reactivated latently infected resting T cells survive viral cytopathic effects [84]. They further showed that antigen-specific stimulation of cytotoxic T cells from patients on ART was required to efficiently kill latently infected cells that contained reactivated provirus. A combination of virus inductive therapy, such as HDAC inhibitors or prostratin, along with immune stimulators might therefore be necessary for this type of therapeutic approach to be successful.

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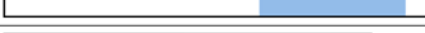



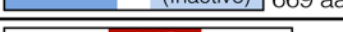








CLASS	ISOFORM	YEAST COUNTERPART	SIZE	CO-FACTOR	LOCATION	EXPRESSION	CATALYTIC DOMAIN
I	HDAC1	RPD3	58	Zn ⁺⁺	N	Ubiquitous	 482 aa
	HDAC2		59		N		 488 aa
	HDAC3		50		N, C		 428 aa
	HDAC8		44		N		 377 aa
IIa	HDAC4	HDA1	120	Zn ⁺⁺	N, C	Specific	 1084 aa
	HDAC5		130		N, C		 1122 aa
	HDAC7		110		N, C		 952 aa
	HDAC9		160		N, C		 1011 aa
IIb	HDAC6	HDA2	160	Zn ⁺⁺	N, C	Specific	 1215 aa
	HDAC10		70		N, C		 (Inactive) 669 aa
III	SIRT1	Sir2	120	NAD ⁺	N	Variable	 747 aa
	SIRT2		45		C		 389 aa
	SIRT3		28		M		 399 aa
	SIRT4		35		M		 314 aa
	SIRT5		36		M		 310 aa
	SIRT6		39		N		 355 aa
	SIRT7		48		N		 400 aa
IV	HDAC11	RPD3/HDA1	39	Zn ⁺⁺	N	Ubiquitous	 347 aa

Figure 1. Classification of HDAC isotypes

18 HDACs have been identified in mammalian cells, and classified into four groups based on sequence similarity of the catalytic domain to yeast prototypes. Class I HDACs include the yeast RPD3 homologues, HDAC1, 2, 3, 8. Class II HDACs include the yeast HDA1/2 homologues, HDAC4, 5, 6, 7, 9, 10, and are further subclassified as IIa (HDAC4, 5, 7, 9) and IIb (HDAC6, 10). Class III HDACs (also known as sirtuins) include the yeast Sir2 orthologues, SIRT1, 2, 3, 4, 5, 6, 7. Class IV HDACs include HDAC11, which has sequence similarity to both RPD3 and HDA1. N, M and C in localization indicate nuclear, mitochondrial and cytoplasmic, respectively. Sizes are in kDa.

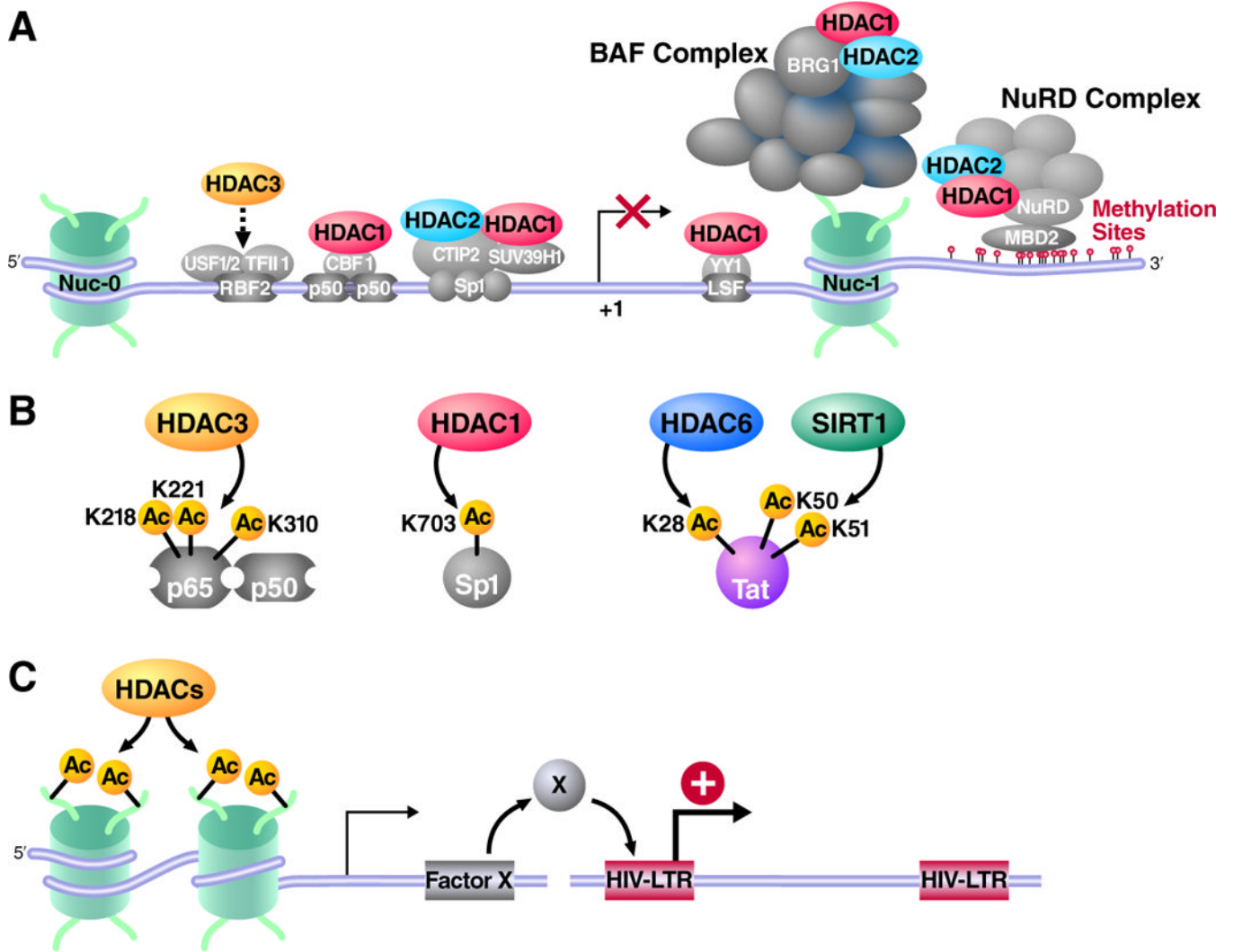


Figure 3. Mechanisms for reactivation of latent HIV by HDAC inhibitors
 (A) Transcription factors such as RBF2, NF- κ B p50, CBF1, Sp1 and YY1 recruit HDACs to the HIV LTR. The BAF complex, which is necessary for the position of the repressor nucleosome nuc-1 at the HIV transcriptional start site, also contains HDAC1 and 2. MBD2 recognizes methylated DNA downstream of nuc-1 and recruits the NuRD complex, which also contains HDAC1 and HDAC2. HDAC inhibitors block these HDACs and directly induce histone acetylation, which leads to the reactivation of the HIV LTR promoter. (B) Full activation of NF- κ B (p65–p50) is negatively regulated by HDAC3 through deacetylation. HDAC3 inhibition induces the hyperactivation of the NF- κ B factor and to latent HIV reactivation. Sp1 is negatively regulated by HDAC1. HIV Tat is also regulated by acetylation. (C) HDAC inhibitors may also activate the expression of a gene whose product positively regulates HIV transcription.

Table 1
Completed and ongoing clinical trials of HDAC inhibitors in HIV-infected patients^a

Agent	Regimen	Intensification	Phase	No.	Results	Refs
Valproic acid	500–750 mg bid 4–6 weeks	Entufviride (90 µg)	I/II	4	Mean 75% reduction in IUPB	[61]
	1000 mg od 16 weeks	None	II	12	More than 50% reduction in IUPB of 4 patients out of 12	[62]
Valproic acid, mean 58 mg/L	None	None	II	11	No significant reduction in HIV DNA copy per million cells	[63]
				(13) ^b		
Vorinostat	500–750 mg bid 96 weeks	Raltegravir (400 mg bid)	II	18	No significant reduction in IUPB	[64]
	500 mg bid 16 or 32 weeks	None	II	56	No significant reduction in IUPB	[65]
Vorinostat	400 mg given 3 times a week 8 weeks	None	I/II	8	Mean 4.8 fold increase in HIV RNA in resting CD4 T cells	[68]

^aAbbreviations: bid, twice a day; od, once daily; IUPB, infectious unit per billion cells.

^bControl.