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Chromatin dynamics associated with HIV-1 Tat activated transcription

Rebecca Easley¹, Rachel Van Duyne¹, Will Coley¹, Irene Guendel¹, Sherry Dadgar¹, Kylene Kehn-Hall¹, and Fatah Kashanchi^{1,2,*}

¹ The George Washington University Medical Center, Department of Microbiology, Immunology, and Tropical Medicine, Washington, DC 20037, USA

² The J. Craig Venter Institute, Rockville, Maryland 20850

Summary

Chromatin remodeling is an essential event for HIV-1 transcription. Over the last two decades this field of research has come to the forefront, as silencing of the HIV-1 provirus through chromatin modifications has been linked to latency. Here, we focus on chromatin remodeling, especially in relation to the transactivator Tat, and review the most important and newly emerging studies that investigate remodeling mechanisms. We begin by discussing covalent modifications that can alter chromatin structure including acetylation, deacetylation, and methylation, as well as topics addressing the interplay between chromatin remodeling and splicing. Next, we focus on complexes that use the energy of ATP to remove or secure nucleosomes and can additionally act to control HIV-1 transcription. Finally, we cover recent literature on viral microRNAs which have been shown to alter chromatin structure by inducing methylation or even by remodeling nucleosomes.

Keywords

Tat; HIV-1; chromatin remodeling; RNAi; SWI/SNF; post-translational modifications

1. Introduction

It has been suggested that the role of chromatin regulation upon infection with HIV-1 begins when the proviral DNA integrates into the genome of a host cell. Many studies have shown that the location of the viral integration site on the host genome is directly responsible for either productive HIV-1 infection or a state of post-integration latency. HIV-1 latency describes a cell that contains a fully integrated HIV-1 provirus, however is transcriptionally silent, and therefore not actively producing viral progeny (at least not high titers of virus). In addition, repressive chromatin structure has been proposed as one mechanism to maintain HIV-1 in a latent state. Therefore, the understanding of chromatin remodeling in terms of HIV transcription is critical for understanding latency formation and maintenance. The focus of this review is to discuss the various means that chromatin remodeling complexes are utilized by HIV-1 in order for RNA polymerase II (RNA Pol II) to efficiently produce viral transcripts.

*Corresponding author: Fatah Kashanchi, Ph.D., George Washington University, School of Medicine, 2300 Eye Street, NW, Ross Hall, Rm. 552, Washington, DC 20037, Office: (202) 994-1781, Lab: (202) 994-1782, Fax: (202) 994-1780, bcmfxk@gwumc.edu.

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We will first address the complexes that remodel chromatin via chemical modifications and then look at ATP-dependent chromatin modifying complexes. Next, we will discuss the interplay between splicing and chromatin remodeling, followed by the effect of microRNA (miRNA) on chromatin.

1.1 HIV-1 LTR

The HIV-1 long terminal repeat (LTR) is present on both ends of the viral genome and contains cis-acting regulatory elements necessary for transcriptional initiation on the 5'LTR and those needed for polyadenylation of viral transcripts on the 3'LTR [1]. The LTR functionally contains three regions: a downstream negative regulatory element, an enhancer region, and finally the basal promoter region which holds the transcription start site [2]. The HIV-1 promoter contains numerous transcriptional elements important for regulation including two NF- κ B binding sites, three SP1-binding sites, the TATA box, and ligand-binding protein 1 (LBP-1)/Yin Yang 1 (YY1) site [3,4].

After the virus integrates into the host genome, a high order chromatin structure is formed on the LTR. Five nucleosomes (nuc-0 to nuc-4) are deposited on the 5'LTR, regulating viral gene expression through restricting access to the HIV-1 LTR. When the virus is silent, the HIV-1 promoter is inhibited by nucleosome-1 (nuc-1) which sits at the -2 to +140 position of the viral LTR. Of extensive interest in the HIV-1 transcription field are the location, modification state, and binding partners of nuc-1 as its position regulates the transcriptional start site. The accessibility of the integrated HIV-1 genome due to the effect of chromatin structure plays a direct role in viral transcription. In order for transcription to proceed nuc-1 must be remodeled, which has been experimentally shown when the nucleosome-free region that exists from -255 to -3 was extended with the use of viral activating agents such as TNF- α TPA, or using various HDAC-specific inhibitors [5]. Additionally, chromatin immunoprecipitation assays (ChIPs) showed histone acetylation around nuc-1 following TSA treatment [6], further highlighting the importance of chromatin structure in HIV-1 transcriptional regulation.

1.2 Tat

The HIV-1 transactivator, Tat, plays a major role in assembling a number of required transcription factors, which work together in transcriptional initiation and elongation. Without Tat the HIV-1 provirus does not efficiently undergo activated transcription due to an elongation defect that produces short transcripts that correspond to the first 50 transcribed nucleotides [7,8]. However in the presence of Tat, transcription increases by several hundred-fold, indicating its critical role in activated transcription. Tat interacts with the HIV-1 LTR through the viral RNA element, TAR (transactivation responsive region), which exists on the 5' end of all HIV-1 viral transcripts [9–11]. The presence of Tat allows the recruitment of the transcriptional elongation complex, p-TEFb, which was originally termed Tat-associated kinase (TAK) [12–14]. Further studies on p-TEFb indicated that it can be found in two distinct complexes, a small p-TEFb that contains only cdk9 and cyclin T [15–19], and a large p-TEFb complex that also contains 7SK small nuclear RNA (snRNA) and the Hexamethylene bisacetamide-induced protein 1 (HEXIM1) protein [20–23]. p-TEFb is additionally, responsible for the phosphorylation of the RNA Pol II CTD promoting transcriptional elongation. Once Tat, TAR, and p-TEFb interact, hyperphosphorylation of the C-terminal domain of RNA Pol II occurs and transcription proceeds at a more processive rate.

In summary, much of the current literature suggests that chromatin must be remodeled in order for HIV-1 transcription to succeed. While this review is focused on the role of HIV-1 Tat in chromatin remodeling, the interaction between viral proteins and chromatin remodeling factors is complex and not limited to Tat. For example, Vpr interacts with histone acetyltransferases (HATs) such as p300/CBP, resulting in activated HIV-1 transcription [24,25]. In addition,

HIV-1 integrase interacts with INI1, a component of the SWI/SNF chromatin remodeling complex [26–28]. The following references provide an overview of the role of integrase and Vpr in chromatin remodeling [29–33].

2.0 Epigenetic regulation of HIV-1 transcription

2.1 HATs and HDACs

The N-terminal tails of histones are subject to multiple forms of post translational modifications (PTM) such as phosphorylation, methylation, and acetylation. In particular, HATs function to acetylate core histone tails, whereas histone deacetylases (HDACs) function to remove the acetyl group. The manipulation of PTMs as applied to histones and chromatin assembly is critically linked to the availability of promoters for transcription. In particular, the recruitment of HATs and HDACs by Tat to the HIV-1 LTR facilitates viral transcription.

The interaction of Tat with HATs can be examined by looking at HIV-1 transcriptional activation. Tat has been extensively shown to target at least five different histone acetyltransferases in HIV-1 infected cells: CBP/p300, p/CAF, GCN5, Tip60, and TAF_{II}250 [18,19,34–38]. CBP and p300 are close homologs of each other and act as overall transcriptional coactivators; they function by interacting with promoter binding transcription factors such as CREB, as well as HATs such as p/CAF [39]. CBP/p300 can additionally acetylate all lysine residues, but preferentially acetylates lysines 14 and 18 of histone H3 and lysines 5 and 8 of histone H4. p/CAF is closely related to GCN5 (70–80% sequence homology) and tends to acetylate free histones or nucleosomes on lysine 14 of histone H3 or lysine 8 of histone H4 [39]. Relief of chromatin repression at the HIV-1 LTR by Tat can first occur prior to the onset of transcriptional initiation [36,40]. Tat forms a complex with both CBP/p300 and p/CAF which both stimulates Tat to activate transcription and targets the HATs to the viral promoter and the subsequent nucleosomes [41]. The presence of Tat induces the acetylation of histones H3 and H4 by CBP/p300 and p/CAF to induce the activation of the HIV-1 LTR [17,35,38,41]. The presence of Tat also promotes a conformational change in CBP/p300 and significantly increases the HAT activity of p300 on histone H4 [39,42]. Assisting with transcriptional activation is the interaction of Tat with TAF_{II}250, which interrupts transcription of other cellular genes [34]. The interaction of Tat with Tip60 inhibits its histone-acetyltransferase activity resulting in a completely negating effect on Tip60-dependent genes [43,44]. This interaction also results in the interference of normal cellular genes in order to preferentially transcribe the virus [44]. The recruitment of CBP/p300 and p/CAF to the HIV-1 LTR assists in chromatin remodeling through the formation of a permissive environment that allows the binding of basal transcription machinery such as TFIIB and TBP. In the absence of Tat, the LTR-bound nucleosomes are hypoacetylated (repressed), however, recruitment of Tat-associated HATs resulted in hyperacetylation (activated) of the nucleosomes [40].

Viral transactivation requires Tat to interact with many different cellular proteins and even RNA transiently. Tat accomplishes this through post-translational modifications, such as phosphorylation, methylation, and acetylation. In particular, Tat can be acetylated by HATs at lysines 28, 50, and/or 51, all of which impart different functions. CBP/p300 and GCN5 can acetylate Tat at lysine 50 and 51 respectively, within the TAR binding domain, which promotes the dissociation of Tat from TAR RNA indicating the start of transcriptional elongation [15–19,42]. The acetylation of lysine 50 of Tat also provides a binding site specific for the bromodomain of p/CAF which competes with the Tat/TAR interaction; therefore promoting the dissociation of Tat from TAR RNA [45]. Unacetylated and acetylated Tat have been shown *in vitro* to have the same affinity for TAR RNA, however acetylated Tat has a decreased ability to bind TBP, CBP, Core-Pol II, and cyclin T [42,46]. Tat acetylation is specifically involved in dissociating Cyclin T1 from TAR at the point of transcriptional elongation [46]. Interestingly, Tat is capable of autoacetylation at lysine residues 41 and 71 [39]. Another recent

study showed that GCN5 is also able to acetylate the catalytic core of cdk9, rendering the kinase inactive especially during low levels of viral transcription [47]. Upon activation, cdk9 is unmodified and forms a complex with Cyclin T1 [47].

The post-translational modifications of Tat add complexity to the transcriptional atmosphere at the viral promoter; however, without acetylation, the levels of viral transactivation would be minimal. To account for this, Tat uses its interaction with cellular acetylases to its advantage to enhance viral gene expression and divert cellular resources to viral transcription [18]. Recently, Tat has also been shown to have a direct interaction with a histone chaperone protein hNAP-1 [48]. Though previously uncharacterized, this interaction seems to reinforce the importance of histone manipulation during viral transcriptional activation. hNAP-1 has been shown to cooperate with ATP-dependent chromatin remodeling complexes as well as being involved with CBP/p300.

The hyperacetylation of histones results in chromatin accessibility and transcriptional activation and consequently, the removal of acetyl groups via HDACs results in transcriptional repression. Although the mechanism for HDAC regulation of nucleosomal structure is not yet clear, these enzymes interact efficiently with DNA binding proteins that tether the enzymes to DNA resulting in gene silencing by deacetylation of histone tails.

Repression of the viral LTR and viral production is mediated by the recruitment of two transcription factors, YY1 and LSF (late simian virus 40 [SV40] transcription factor) to the LTR. LSF binds specifically to the -10 to +27 region of the HIV-1 LTR and recruits and binds YY1 via a zinc-finger domain. These two transcription factors in complex, recruit HDAC1 to repress transcription of the HIV-1 LTR [4,49]. Tat expression results in nuc-1 histone acetylation and downregulation of HDAC1, whereas the recruitment of HDAC1 to nuc-1 by YY1 and LSF results in the hypoacetylation of nuc-1 and transcriptional repression. This counter-regulation of chromatin remodeling and HAT/HDAC occupancy at the HIV-1 promoter implies a complex mechanism of viral expression and repression in response to various cellular signaling pathways.

The HDAC family of proteins has been attributed in part to the establishment of HIV-1 latency and quiescence. Recently, a connection between HIV-1 latency and the presence of an NF- κ B p50-HDAC1 complex has been established [50]. This complex can bind the HIV-1 LTR and suppress recruitment of RNA Pol II and induce histone deacetylation. This complex requires the presence of HDACs at the viral LTR. However, it is unclear at this point whether both 5' and 3' LTRs contain this complex in latent viruses. Multiple studies have established that HIV-1 transcription can be activated by the addition of HDAC inhibitors (HDACi), such as TSA, trapoxin, valproic acid or sodium butyrate, in latently infected cells [51–55]. This mechanism is important for *in vitro* biochemical studies, however can also play a role as a potential therapeutic. The administration of HDACi to HIV-1 infected patients undergoing HAART treatment could potentially activate the latent reservoirs of virus, allowing the anti-retrovirals to treat the emerging virus production as if an active infection was occurring [56]. Indeed, HDACi induced activation of virus without enhancing the existing infection [57].

2.2 Protein methylation

There is a growing body of literature that characterizes methylation as a regulator of HIV-1 replication and latency. Protein methylation, specifically methylation of nucleosomal histones, works synergistically with other types of PTMs in the regulation of transcription by the induction of chromatin conformational changes and the consequential regulation of proviral gene expression [58–60]. Protein methylation can occur on arginine, lysine, histidine, and proline as well as carboxyl groups [61,62]. This PTM can confer activation or repression of various cellular processes depending on the amino acid modified and the number of these

methyl modifications, especially on lysine and arginine residues [63]. Of particular interest, lysine and arginine methylation have been greatly documented in the last decade, beginning with works that correlated histone H3 lysine and arginine methylation with transcriptional regulation [64,65]. Lysine methylation occurs mainly through the SET domain family of proteins. These enzymes were originally termed histone methyltransferases due to their ability to methylate various histone protein residues [66], but in light of the identification of many non-histone protein substrates they are now referred to as protein lysine methyltransferases (PKMTs). Protein methylation has gained new insight and interest due to the identification and characterization of protein arginine methyltransferases (PRMTs) which catalyze methyl additions to arginine residues [58,62]. PRMT1 is the major arginine methyltransferases in mammalian cells [67]. The histone-modifying enzymes that catalyze reversible lysine acetylation and methylation are central to the epigenetic regulation of chromatin remodeling [68].

Over the last several years, emerging evidence of methylation-regulated HIV-1 replication has been thoroughly illustrated [60,69–76]. HIV-1 replication was inhibited by multiple adenosine analogues providing initial notions that protein methylation may play a paramount role in the HIV-1 life cycle [77]. Later, through the use of a general methyltransferase inhibitor and adenosine analog (adenosine periodate), Willemssen *et al.* demonstrated that HIV-1 infected CEM and HEK293T cells displayed increased levels of virions in the culture supernatant, despite the fact that they were less infective due to a miss-processing of the Gag-Pol gene [76]. More recently, the importance of epigenetic changes in the regulation of HIV-1 has been depicted by the description of Tat methylation [78–80]. PRMT6 methylates Tat on arginines 52 and 53 resulting in decreased Tat-dependent transcription [70,78]. Likewise, knockdown of PRMT6 increases viral production and faster replication [78]. Tat is also methylated on lysines 50 and 51 by SETDB1, a member of the SUV39-family of SET domain containing proteins [80]. Functional analysis of lysines 50 and 51 methylation indicated that methylation of these residues inhibited Tat-dependent transcription.

2.3 DNA and RNA methylation

DNA methylation-mediated transcriptional regulation is indispensable in the process of human development and disease and is known to occur largely at CpG dinucleotides. Five percent of cytosines in the mammalian genome account for methylated cytosines (or 5-methylcytosines) present principally in CpG sites (displaying more than sixty percent methylation) [81]. Works in the early nineties established the effect of DNA methylation on gene silencing by demonstrating the correlation between gene-specific methylation patterns [82,83]. Furthermore, heterochromatin regions of mammalian DNA are associated with high levels of CpG methylation [82,84], depicting chromatin structure and thus, gene activity in the area. This is important because it has been shown that due to chromatin heterogeneity, the site of integration of the HIV-1 genome can affect its transcriptional activity, making local chromatin environment important for proviral gene expression [85]. Preferential selection of integration sites between simple retrovirus (MuLV) and lentivirus (HIV-1) derived vectors has been demonstrated. For example, 17% of MuLV integration sites are found within 1kb of CpG islands in contrast to only 2% of HIV-1 integrations [86,87]. In addition, HIV-1 favors integration near transcriptionally active regions of the host genome [88]. A lentiviral transgenesis study in higher mammals found lentiviral transgenesis of similar lentiviral vectors, including HIV-1 derived, to be affected by varying degrees of epigenetic modifications. Factors that recruit silencing machinery to some but not all lentiviral proviruses could therefore be potential targets for future HIV-1 therapy [87].

CpG methylation of other retroviruses including the HTLV-1 LTR has been found to be linked to functional proviral latency [89]. This property may be characteristic of viral latency

establishment since evidence exists that DNA methylation influences HIV-1 replication, in particular focusing on CpG hypermethylation of the provirus 5' LTR [71,73,90–92]. This mechanism of latency establishment is a plausible effect of LTR-CpG methylation explained by the masking effects of methylation on LTR sites that bind essential transcription factors such as Sp1 and NF- κ B [71,91–94]. Methylated DNA is known to recruit important factors that aid in chromatin remodeling, such as the repressor protein HP1 [95,96], linking DNA methylation to silencing complexes and highlighting the importance of DNA methylation as an epigenetic mechanism in the maintenance of repressive chromatin structure. Viruses are capable of altering the expression of human DNMTs as has been reported for HIV-1 induced expression of DNMT1 [97,98]. Overexpression of DNMT1 correlated with increased genomic methylation as well as specific methylation of the p16/INK4A promoter [97]. Current efforts in the understanding and application of de novo methylation effects highlight promoter-targeted siRNA transcriptional silencing of HIV-1, suggesting a novel system that brings about viral silencing through the induction of epigenetic changes as well as targeting of conserved promoter elements to evolve less pathogenic variants of HIV-1 [99,100].

Although it is accepted that DNA methylation inhibits or decreases levels of transcription, its role in the control of retroviral gene expression is controversial. First, DNA methylation has been detected in silenced, transfected HIV-1 constructs [92,96]. However, other studies have noted that cells with transiently transfected plasmids display different repression responses due to DNA methylation than cells harboring integrated vectors [96,101]. Finally, partial relief of methylated HIV-1 5' LTR after induction of proviral activity with cytokine treatment has been observed [71,96]. These inconsistencies could be explained by DNA methylation and viral gene silencing having a dynamic relationship that depends on the degree of methylation [96, 102]. DNA methyltransferase 3A (DNMT3A) has been shown to interact with SETDB1 [103]. Therefore, it is possible that SETDB1 methylation of Tat results in the recruitment of members of the transcriptional silencing machinery to the HIV-1 genome, hence decreasing viral transcription [80].

The mid-nineties saw methylated DNA linked to chromatin remodeling by the observation that methyl-binding proteins interact with HDACs [87,104,105]. In addition, heterochromatin formation by methylation of histone 3 lysine 9 (H3K9) was accomplished through the direct endogenous association of SETDB1 with DNMT3A and DNMT3B at promoter methylated CpG regions of cancer cell lines [103]. Similarly, the formation of a transcriptional repression complex on methylated DNA occurs through the ability of methyl-DNA binding domain (MBD) proteins to interact with both methylated DNA as well as HDAC silencing complexes [61,62,106]. In addition, the MDB protein MDB2 is methylated on arginine residues by PRMT1 and PRMT5, decreasing the ability of MDB2 to recognize and bind methylated DNA, stalling the formation of the repression complex [106].

While it is universally accepted that HIV-1 transcription relies on host cellular factors, the complex interactions between viral and host proteins through pleiotropic effects needs to be established [107–109]. Studies investigating the relationship between viral proteins and host transcription and signaling machinery, DNMTs and protein methyltransferases in particular, have suggested an ever increasing role of epigenetic modulation in transcriptional silencing of HIV-1 [99,100,109]. In 2007, two independent studies demonstrated inhibition of HIV-1 gene expression in microglial cells by the recruitment of SUV39H1, HDACs, HP1, as well as the tri-methylation of H3K9 (histone 3 at lysine 9) at the HIV-1 LTR. Moreover, it was also shown that siRNA knockdown of the SUV39H1 and HP1 resulted in an incremental increase of viral gene expression [96,110,111].

In addition to DNA and protein methylation, it is interesting to consider the less explored RNA methyl modifications which are known to be involved in functional maturation of many species

of cellular RNAs including mRNA, rRNA, tRNA, snRNA and snoRNA. Yedavalli *et al.* reported their discovery of a novel cellular RNA methyltransferase whose activity appears to upregulate HIV-1 gene expression [60]. In addition, the RNA component of the large inactive p-TEFB complex, 7SK, is mono-methylated, which aids in the stabilization of 7SK [112]. To date, the true significance of RNA methylation in relation to HIV-1 transcription remains unknown.

3.0. ATP-dependent chromatin remodelers

3.1 SWI/SNF and HIV-1 Tat

In 2006 a number of groups published data discussing the necessity of the SWI/SNF chromatin-remodeling complex in Tat transactivation of HIV-1 promoter [109,113,114]. Our group specifically showed that Tat-activated transcription not only takes place at the G₁/S border of the cell cycle, but also that the SWI/SNF complex facilitates transcriptional elongation. Other colleagues, including the Verdin group, showed that knocking down two key components of the SWI/SNF complex, BRG1 and INI-1 (integrase interactor-1), significantly represses Tat-mediated transactivation. BRG1 has also been shown to co-localize with nuclear factor 1 (NF1) family of site-specific DNA-binding proteins (CTF) as well as RNA Pol II [115]. The interaction of Tat with CTF/NF1 downstream of the promoter can act to mediate viral transcription [116].

In humans, the SWI/SNF complex exists in two forms: BAF (BRG1-associated factor) and PBAF (polybromo-associated BAF). The only difference between these two complexes is BAF's subunit Baf250 and PBAF's subunits Baf180 (polybromo), Baf200, and Brd7 [117–121]. The two complexes both contain the core subunits BRG1 (sometimes BRM), Baf170, Baf155, Baf47/INI1 as well as Baf60, Baf57, and Baf53. BRG1 is not only the essential ATPase subunit for the BAF/PBAF complexes, but also for a number of other chromatin-remodeling complexes including WINAC (WSTF including nucleosome assembly complex), NCoR (nuclear receptor corepressor), NUMAC (nucleosomeal methylation activation complex), and mSin3A/HDAC (histone deacetyltransferase) (Figure 1) [122]. It is generally assumed that the BAF/PBAF (SWI/SNF) complex is the essential complex for HIV-1 Tat transactivation; however, papers published to date have only studied the subunits BRG1, INI1, Baf170, and Baf155 none of which are BAF or PBAF specific.

SWI/SNF complexes have a diverse impact on chromatin and while studies in HIV-1 are not yet as extensive as those in various cancer and embryonic studies, much can be learned from these studies. For instance, SWI/SNF can act to regulate alternative splicing by creating barriers to transcriptional elongation, which affects RNA Pol II phosphorylation [123]. SWI/SNF's BRM subunit has been found to regulate alternative splicing [124]. Because many of the subunits that belong to the BAF/PBAF complexes are similar, it is important to look at the subunits that differ between the two complexes as well as those of other BRG1 complexes.

3.2 SWI/SNF Subunits

The core subunits of the SWI/SNF complex include the ATPase subunits BRM or BRG1 as well as the subunits, Ini1, Baf170, and Baf155. Nucleosome remodeling cannot occur efficiently without all four of these subunits [125,126]. The ATPase subunit for the SWI/SNF complexes can be either BRM or BRG1; these two ATPase subunits are 70% identical and differ mainly at their amino termini allowing for the binding of different transcription factors [127]. Interestingly, BRG1 is required in the development of early embryos and T-cells in mice, while BRM is not. BRG1 is also believed to play a role in V(D)J rearrangement because BRG1 stimulates recombination of chromatin templates *in vitro* and binds selectively to regions in the T-cell receptor [128–131].

The other core subunits also have their own, unique role in chromatin remodeling. While BRM or BRG1 can remodel chromatin independent of other SWI/SNF subunits, the addition of subunits Baf155, Baf170, and INI1 dramatically increases chromatin remodeling activity [126]. Baf155 and Baf170 are not only core subunits of SWI/SNF, but are also part of many other BRG1 driven chromatin-remodeling complexes. These two subunits are believed to form heterodimers through leucine-zipper domain interactions [127]. They also have SANT domains which are histone-DNA binding motifs. INI1 (also known as hSNF5, Baf47, SMARCB1), was originally isolated as an HIV-1 integrase interacting protein and is now known to be a tumor suppressor biallelically deleted/mutated in most rhabdoid tumors [132]. INI1 additionally interacts with c-Myc, and this relationship is required for c-Myc mediated transactivation [133]. c-Myc regulates HIV-1 DNA nuclear import via cell cycle interactions and is essential for infection in activated T cells [133,134].

The non-essential subunits of the SWI/SNF complex include actin, Baf53, Baf57, and Baf60 [127]. Baf53 plays a role in neuron-specific chromatin remodeling [135]. Baf53 also acts as an actin-related protein; both actin and Baf53 are believed to assist in regulation of transcription and act to disrupt chromatin structure [136]. Baf57 is essential for CD4 repression and CD8 expression [127,137]. Baf60 exists in a number of forms including Baf60a, Baf60b, and Baf60c [138]. Baf60s act differently between cell types; for example, Baf60c interacts directly with nuclear hormone PPAR in adipocytes to initiate SWI/SNF remodeling activity.

3.3 BAF and PBAF specific subunits

As discussed earlier, the human SWI/SNF complex can be one of two complexes: BAF or PBAF which only differ by a few subunits [117]. It has been shown in embryonic stem cells (ESC) that the BAF and PBAF complexes differentially act on genes in either a repressive or active way. It is generally believed that, and has also been shown in ESC, that the two complexes are antagonists in cells. The helicase/SANT-associated (HSA) domain of BRG1 has been shown to interact with Baf250 on both mouse mammary tumor virus as well as endogenous promoters *in vivo* [139]. This interaction further shows that Baf250 induces transcriptional activation in the two systems, indicating that, in some cases, the BAF complex is responsible for transcriptional activation. If the BAF and PBAF complexes are indeed antagonists, this can mean that PBAF acts to repress transcription. In a 2001 study, however, one group found that PBAF – not BAF – assisted in ligand-dependent transcriptional activation via nuclear receptor *in vivo* [118].

The Baf200 (ARID2) subunit of the PBAF complexes is known to regulate expression of interferon-responsive genes independent of Baf180 expression, making Baf200 a major PBAF targeting subunit [120]. In breast cancer, however, the Baf180 subunit is a major regulator of p21/waf1 induction and it additionally plays a role as a tumor suppressor in breast cancer [140]. p21/waf1 levels are low in HIV-1 infected cells due to the inactivation of p53 by Tat, resulting in the loss of the G1/S checkpoint [141]. The induction of p21/waf1 through p53 activating compounds results in decreased HIV-1 replication [142]. It would therefore be interesting to explore the role of Baf180 in p21/waf1 promoter regulation considering its role in HIV-1 replication.

4.0 Interplay between Splicing and Chromatin Remodeling

The host splicing machinery is essential for HIV-1 replication in infected cells. Alternative splicing of HIV-1 generates more than 40 different mRNAs required for viral replication. Three classes of RNA can be generated through alternative splicing: unspliced (US), singly spliced and doubly spliced [143]. Spliceosome assembly mainly controls this procedure in which spliceosomes are assembled on pre-mRNA in association with the small nuclear ribonucleoprotein particles. Serine/arginine-rich (SR) proteins are essential for spliceosome

assembly, and they play a role in early recognition of splice sites, recruitment of basic splicing factors to the pre-mRNA, and formation of bridging contacts with other RS domain-containing splicing factors [144].

There is evidence that splicing can be influenced by chromatin remodeling complexes. The SWI/SNF subunit BRM regulates promoter activity by facilitating recruitment of transcriptional regulators and affects the quality of the transcripts by favoring inclusion of alternative exons in the mRNA of several genes [124]. BRM may function as a “hub” connecting several required factors of efficient splicing through its interaction with the U1, U5 snRNPs and RNA Pol II. BRM associates with components of the spliceosomes as well as proteins involved in splicing assembly such as Sam68 or PRP6. Since knockdown of other SWI/SNF subunits, such as INI1, affects the differential inclusion of CD44 exons, it can be concluded that, the SWI/SNF subunit BRM affects alternative splicing in the context of a complex rather than as an isolated protein. Mutating the ATPase does not have an impact on splicing activity, demonstrating that chromatin remodeling does not play a role in BRM’s splicing activity [124]. Sam68 interacts with BRM and has been shown to be involved in signal transduction, transcription, RNA metabolism, cell cycle regulation and apoptosis [145]. BRM and Sam68 specifically cooperate to slow RNAPol II transcription and in turn, acts to recruit necessary splicing machinery [124]. Of significant relevance, is Sam68’s role in HIV-1 Rev responsive element gene expression as well as viral production [146]. Inhibition of Sam68 in HIV-1 infected cells leads to inhibition of viral production.

Another line of evidence that links splicing to chromatin remodeling is that siRNAs derived from centromeric repeats have chromatin modeling effects through the RNA-induced transcriptional silencing (RITS) complex. Several specific splicing mutants affect processing of centromeric transcripts into siRNA and impair centromere silencing hence, it has been demonstrated that there might be some links between splicing and chromatin modifications [147].

Cdks influence many cellular processes, including cell cycle, transcription, chromatin remodeling, and neuronal differentiation. Cdk13 promotes viral splicing and increases the production of the doubly spliced mRNA-encoded protein Nef. Absence of cdk13 results in the shift to unspliced HIV-1 transcripts whereas, overexpression of cdk13 leads to the accumulation of spliced viral mRNA [148]. Cdk13 phosphorylates ASF/SF-2, which contributes to the observed increase of viral splicing. Interestingly, HIV-1 Tat interacts with p32, an inhibitor of the splicing factor ASF/SF-2, affecting splicing of HIV-1. Phosphorylation of ASF is inhibited when cdk13 acts in complex with Tat and p32, resulting in decreased viral splicing [149]. Clearly splicing, transcription, and chromatin remodeling are intimately linked and there is much to be learned about how the interaction between chromatin remodeling components and protein modifying enzymes influence these processes.

5.0 Micro-RNA and Gene Silencing via Chromatin Remodeling

Research into miRNAs and development over the past decade has revealed that endogenous miRNAs are capable of genetic regulation in developing and cancerous cells [150]. It was once assumed that miRNAs exerted their influence only at the translational level, but recent publications show that miRNAs can also regulate gene expression at the epigenetic level [151,152]. In particular, new evidence indicates that miRNAs can initiate gene silencing either by specifically inducing methylation along promoter sequences or by directly remodeling the surrounding chromatin by modifying the promoter-associated histones [153]. This emerging mechanism of epigenetic control is sometimes referred to as RNAi-Mediated Transcriptional Gene Silencing (RNAi-TGS).

Since the discovery of RNAi-TGS in plants, *C. elegans*, yeast, and *Drosophila*, there have been efforts to find homologous components of the RITS complex in mammals [154]. Presently, several miRNA-related enzymes such as Drosha, Dicer, and Ago2 are accepted counterparts of the yeast siRNA pathway. Unfortunately, the mechanisms behind RNAi-TGS (as it occurs in yeast or fruit flies) are not completely conserved in mammals. Some of the seemingly essential proteins that are present in yeast, such as Tas3 and RdRP, lack clear homologues in humans. The absence of RdRP (an endogenous RNA-dependent RNA polymerase) is concerning because some groups have suggested that it is needed to amplify miRNAs prior to RNAi-TGS [151]. Nevertheless, the lack of conservation is not proof that this mechanism is lacking in mammals, especially considering that mammals possess several homologues of the yeast effector enzyme Argonaute. Indeed, available evidence has focused on one of the Ago homologues for its apparent role in chromatin remodeling [153]. Furthermore, it has been demonstrated that abortive transcription from the HIV-1 LTR produce TAR molecules at high levels during all stages of the viral life cycle, including during latent infections and in the absence of Tat [155]. Since Dicer can apparently act directly on these abundant TAR RNA molecules, HIV-1 infections may produce enough miRNA to initiate RNAi-TGS with or without any RdRP activity. In essence, the TAR short transcripts that resulted from abortive and non-progressive transcription may resemble the functional analog of RdRP activity in HIV infected cells.

Despite the fact that the putative TGS machinery is not completely conserved, there is already persuasive evidence that RNAi-TGS exists in mammals. Effective TGS in humans has been observed for several genes as well as the HIV-1 promoter. Current work on the topic indicates that miRNAs best induce chromatin formation on the promoter region of Pol II transcripts [156,157]. One group was able to demonstrate that an endogenous miRNA recruited Ago1, PcG-member EZH2, and H3K27me3 to a promoter region and suppressed expression of the target gene [158]. This work builds upon a previous observation that DNA-methyltransferase complexes have RNA binding activity [159]. In one notable example of RNAi-TGS, miRNAs that match segments of the HIV-1 LTR promoter induced chromatin remodeling that shifted the nuc-1 nucleosome into a position that occluded the HIV-1 TAR element [160]. In a follow-up to this study, [Yamagishi et al.](#) reported HIV-1 transcriptional silencing for up to one year through retrovirally delivered shRNA against the NF- κ B region of the LTR [161]. This transcriptional silencing could be reversed by the histone deacetylase inhibitor TSA, but not 5-AzaC, a DNA methyltransferase inhibitor. In addition, ChIP assays showed the development of heterochromatin through H3K27 trimethylation and H3K9 methylation, correlating with the ability of TSA to reverse the transcriptional silencing.

Interestingly, RNAi-TGS has also been implicated in maintaining viral latency and host cell proliferation of HIV-infected cells. This implies that viral latency may be mediated by viral miRNAs. Two independent groups have demonstrated that miRNA molecules derived from the stem of the TAR element could be found in latently infected cell lines [162,163]. *Klase et al.* showed that when wild-type and various mutants of TAR were transfected into reporter cells lines, wild-type TAR showed substantially reduced reporter expression compared to TAR-A and TAR-D, mutants with a scrambled stem sequence and shortened stem, respectively. The 5' and 3' arms of the TAR miRNA have been cloned and sequenced from HIV-1 infected cells [155]. Expression of TAR miRNA downregulates multiple cellular genes involved in apoptosis, specifically ERCC1 and IER3, therefore inhibiting apoptosis. The ability of TAR to protect the cell from apoptosis may provide a mechanism for allowing viral latency and increased viral replication. Interestingly, ChIP assays from cells transfected with wild-type TAR showed that HDAC1 was recruited to the HIV-1 LTR. These combined results indicate that miRNAs derived from the TAR element initiated transcriptional silencing response in a sequence specific manner [162]. Interestingly, we have observed that in the absence of Dicer when no TAR miRNA can be produced, there is an increase in HIV-1 transcription

(unpublished data). In contrast, in the presence of Dicer, TAR is processed into a miRNA, which is capable of inhibiting both viral and cellular transcription (Figure 2). Viral transcription inhibition may be mediated by RNAi-TGS via recruitment of RITS to the viral LTR.

At present, there is a rudimentary model of RNAi-TGS in mammals with a short list of essential proteins. First, available evidence lends weight to the idea that mammalian RNAi-TGS begins with a RNA-miRNA hybrid, where an Argonaute protein (either Ago1 or Ago2) loads a miRNA onto a growing mRNA transcript [164]. Hybridizing a near-exact matching miRNA appears to stimulate the recruitment of HDAC1 and methyltransferases (DNMT1, DNMT3A/B, SUV39H1). These enzymes are then believed to deacytlate and methylate histones (at H3K9 and H3K27) as well as CpGs in the associated DNA [158]. These modifying enzymes are known to associate with the chromatin remodelers Swi6 and HP1, which assist in heterochromatin formation [153]. Other potential items required for RNAi-TGS include TRBP1, the PcG-member EZH2, and Pol II transcription across the promoter region [158, 165, 166].

This model forms a rough outline of the proteins and processes that have been observed with RNAi-TGS in humans. Clearly, several mechanistic questions still remain. There is a question as to how or even if miRNAs are processed in the cytoplasm and then imported into the nucleus for use in RNAi-TGS. It is not known if DNA methylation precedes histone modification, if the reverse is true, or if both processes occur simultaneously. Additionally, the respective mechanistic contributions of Ago1 and Ago2 need to be defined. In general, there is small gap between the complexes that associate with miRNAs and the complexes that carry out the chemical modifications of histones and DNA. Nevertheless, there is compelling evidence to suggest that there are as yet unidentified mediators of RNAi-TGS in humans.

6.0 Conclusions and Future Studies

Chromatin remodeling is a rapidly emerging field with critical implications for the control of viral gene expression, especially for viruses with integrated genomes, such as HIV-1. Recent observations indicating that there are as many as eight different BRG1 containing chromatin remodeling complexes highlight the advancement in the field, but also the necessity for future study. As BRG1 is necessary for HIV-1 transcription, future studies are needed to understand which BRG1 chromatin complex(es) are critical or if different complexes are important for certain stages of the viral life cycle (active vs. latent replication). Future studies could also focus on determining the contribution of the less well defined BAF subunits to viral replication as well as the possibility of viral infection creating various stoichiometrically novel complexes of SWI/SNF that benefits the virus over host gene expression.

To further understand the suppression of activated gene transcription and the establishment of latency, Han *et al.* investigated the integration sites of HIV-1 in resting CD-4 T-cells from HAART patients [167]. Surprisingly, 93% of the integrated virus was found in transcription units, usually within introns. This study surprised the scientific community and led to studies investigating how latency could be maintained within actively transcribed genes. Beginning with the Siliciano group, a number of investigators have data indicating that transcriptional interference is responsible for the establishment of latency within actively transcribed genes [168–170]. Transcriptional interference refers to the process where transcription is initiated at the cellular promoter upstream of the integrated viral promoter; therefore the elongating RNA Pol II transcribes through the downstream viral promoter. This process results in the physical exclusion of preinitiation complex formation on the 5'-LTR and thus no viral transcription is allowed to occur. It is thought that the integrated virus would be transcribed along with the other intronic regions of the cellular genes, but would merely be spliced out and the viral RNA never translated. Recently Duverger *et al.* demonstrated that silent integration (i.e. when the

virus never actively expresses its genes) is required for the establishment of latency, while transcriptional interference is then required to maintain latency [169]. Clearly, transcriptional interference is an alternative mechanism to explain latency in the absence of heterochromatin formation on the viral LTR.

Since the seminal paper on the “histone code” [171], it is now clear that many other proteins, including transcriptional factors and cofactors, are post-translationally modified in a similar fashion. In fact, a protein code hypothesis has recently been suggested where PTMs serve to induce the formation of various protein interaction surfaces that would therefore allow different protein complex formations and downstream effects [172]. This regulatory mechanism would help explain the numerous protein-protein interactions observed with Tat, as well as the ability of Tat to be involved in various viral and cellular events (i.e. transcription, splicing, chromatin remodeling) in a regulated manner. Finally, the interplay between PTMs (such as methylation and acetylation) highlights the intricacy of transcriptional regulation and hence the ability of a cell to respond to various stimuli as needed. Future studies should focus on the description of how multiple tandem PTMs influence protein function, rather than looking at PTMs in isolation. We also suggest a coordinated effort to clearly define what modifications (i.e. acetylation or methylation) or acquisition of new machineries (i.e. RNAi-TGS) on the HIV-1 promoter or the open reading frames that can create a “memory” for transcription or DNA replication machineries. This newly acquired “memory” would have to be defined in terms of its persistence through various stage of the cell cycle, differing cell types involved in latency, and whether it is a long-term memory as the infected cell divide *in vitro* or *in vivo*.

Based on the current literature we propose a model where the interaction of Tat with various chromatin factors dictates the flow of HIV-1 Tat dependent transcription (Figure 3). Transcription initiation occurs in the presence of unmodified Tat binding to BAF and p-TEFB, which allows access to nuc-1. However, initiation only allows for basal transcription. For elongation and rapid transcription to transpire, Tat must become acetylated and recruit p/CAF and PBAF complexes. This formation induces chromatin remodeling allowing rapid transcription and decreased splicing to occur. Decreased splicing is likely due to acetylated Tat binding to p32, an inhibitor of splicing factor ASF/SF-2 [149]. Finally, to induce silencing, Tat becomes methylated on both lysine and arginine residues, binding to the PKMT SETDB1, which allows recruitment of DNMT3A and HDAC1. At this stage it is also possible that the RNAi machinery is recruited to aid in transcriptional silencing. This model is simplified due to the large number of known proteins implicated in this process. However, we believe that it represents the major players that allow for the intricate regulation of HIV-1 transcription.

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Figure 1. BRG1-associated chromatin-remodeling complexes

The Brahma-related gene-1 (BRG1) is a catalytic subunit found in a variety of chromatin remodeling complexes including the SWI/SNF complexes BAF and PBAF as well as WINAC, NCoR, mSin3A/HDAC, and NUMAC. The multiple complexes share other subunits that have a number of activities. Green: core components; Pink: nuclear receptor associated; Gold: DNA replication; Light Green: actin-related complexes; Blue: Ini1/Baf47/SNF5; Purple: other functions. As chromatin remodellers, these complexes can either act as transcriptional activators, repressors, or both. For example, NUMAC acts as a transcriptional activator while the mSin3A/HDAC complex acts as a transcriptional repressor. SWI/SNF complexes can act as either activators or repressors.

Figure 2. HIV TAR miRNA influences viral transcription and latency

In the absence of Dicer, no TAR miRNA will be produced resulting in increase HIV-1 transcription. In the presence of Dicer, TAR is cleaved into a miRNA, which is capable of inhibiting both viral and cellular transcription. Viral transcription can be inhibited through RNAi-TGS via recruitment of RITS (the RNA-induced transcriptional silencing) to the viral LTR.

Initiation Complex (Unmodified Tat) **Elongation Complex (Acetylated Tat)** **Inhibitory Complex (Methylated Tat)**

Figure 3. Interaction of Tat with chromatin factors regulates HIV-1 Tat dependent transcription

Initiation complex: Unmodified Tat binds to the chromatin-remodeling BAF complex and p-TEFB making nuc-1 accessible and allowing for basal transcription as well as increased splicing. *Elongation complex:* Acetylated Tat recruits p/CAF and PBAF complexes consequentially inducing chromatin remodeling and allowing rapid transcription and decreased splicing to occur. *Inhibitory complex:* Methylated Tat binds to PKMT SETDB1 allowing recruitment of DNMT3A and HDAC1, leading to the possible methylation of histone H3 lysine 9 (H3K9) and silencing. (??) indicates unpublished results for the presence of the HDAC complex at the 3' end of the genome and the methylation of H3K9 leading to silencing of the genome.