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Are Viral-Encoded MicroRNAs Mediating Latent HIV-1 Infection?

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Abstract

The Human Immunodeficiency Virus type 1 (HIV-1), a member of the lentivirus subfamily, infects both dividing and nondividing cells and, following reverse transcription of the viral RNA genome, integrates into the host chromatin where it enters into a latent state. Many of the factors governing viral latency remain unresolved and current antiviral treatment regimens are largely ineffective at eliminating cellular reservoirs of latent virus. The recent identification of microRNA (miRNA) encoding sequences embedded in the HIV-1 genome, and the discovery of functional virus-derived miRNAs, suggests a role for RNA Interference (RNAi) in the regulation of HIV-1 gene expression. Recently, the mammalian RNAi machinery was shown to regulate gene expression epigenetically by transcriptional modulation, providing a direct link between RNAi and a mechanism for inducing latency. Interestingly, both HIV-1 Tat, and the host TAR RNA-binding protein (TRBP), bind to the transactivating response (TAR) RNA of HIV-1 and affect the function of RNAi in human cells. Specifically, TRBP, a cofactor in Tat-TAR interactions, is a vital component of Dicer-mediated dsRNA processing. These novel observations support a central role for HIV-1 and associated host factors in regulating cellular RNAi and viral gene expression through RNA directed processes. Thus, HIV-1 may have evolved mechanisms to exploit the RNAi pathway at both the transcriptional and posttranscriptional level to affect and/or maintain a latent infection.

INTRODUCTION

Lentiviruses (*lentus* = slow, Latin) such as the Human Immunodeficiency Viruses type 1 (HIV-1) are nononcogenic retroviruses that cause persistent infections, leading ultimately to the demise of immune regulatory cells. In the case of HIV-1, infection is inexorably followed by diseases attributable to acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi *et al.*, 1983; Gallo *et al.*, 1984). Clearance of lentiviral infections by the immune system is inefficient, consequently allowing for integration of proviral DNA into the genome of host cells. This feature represents a necessary prelude for the orchestration of transcriptional shutdown and a latent state in resting CD4+ cells (reviewed in Persaud *et al.*, 2003). Since inactive, intact viral sequences remain hidden within the human genome, conventional therapeutic approaches, which are targeted to actively replicating virus, appear to be severely limited. The underlying cellular mechanisms responsible for establishing and maintaining latency are still unclear. An understanding of these mechanisms remains an important determinant for future prospects of successful long-term treatment. Recently, a large body of evidence has inferred a central role for RNA in controlling viral expression and mediating chromatin remodeling and/or maintenance (Group *et al.*, 2005; Lu *et al.*, 2005). In mammalian

biology, as with the lower eukaryotes, the endogenous RNA interference (RNAi) pathway is likely to play an integral role in regulating viral gene expression (Seitz *et al.*, 2003; Soifer *et al.*, 2005). Conversely, viruses such as HIV-1 have developed appropriate counter measures, which include viral-encoded factors that inhibit the antiviral properties of RNAi (Omoto *et al.*, 2004; Bennasser *et al.*, 2005; Couturier and Root-Bernstein, 2005; Omoto and Fujii, 2005). However, viruses such as HIV-1 may have adopted a more subtle approach, in which the host RNAi machinery is usurped to ensure a survival advantage. In this review we hypothesize that the establishment and maintenance of HIV-1 latency may be mediated by viral expressed microRNAs (miRNAs) that operate at the transcriptional level by remodeling promoter associated chromatin, specifically nucleosomes (0), (+1), and (+2), to inactivate processive transcription from the viral long terminal repeat (LTR) promoter.

HIV-1 LTR promoter

All lentiviruses utilize reverse transcriptase to convert their RNA genome into a DNA provirus (Fig. 1). Following integration and establishment of infection, the provirus exploits the cellular transcription machinery, through interactions with the 5'-LTR, to express viral RNAs. This includes the genomic RNA for incorporation into nascent virions as well as messenger RNAs for production of viral proteins (reviewed in Coffin, 1998).

The 5'-LTR contains three structural sequence elements: the U3 (-453 to -1), R (+1 to +98), and U5 (+99 to +180) regions. The basal promoter (-78 to -1) includes the TATAA box and binding sites for the transcription factor Sp1. The enhancer (-105 to -79) and modulatory regions (-454 to -104) contain NF- κ B and NF-AT binding sites, respectively. The latter includes a negative regulatory element (NRE), where cellular factors such as Ap1, USF, IL-2, or c-Myb bind to and affect functions in the viral modulatory region (Fig. 1), where they have been shown to be essential for optimal promoter function (Pereira *et al.*, 2000). Within the R region, a structured RNA element, namely, the TAR RNA (transactivation-response region), present at the 5' end of the pregenomic mRNA sequence (+1 to +59), binds to the viral encoded transactivator protein, Tat, to induce transcriptional activation of the provirus and concomitant modification of chromatin at the integration site (Barboric and Peterlin, 2005).

Regardless of the site of viral integration, the relative regulatory domains and 5' LTR are always packaged by two nucleosomes (Nuc), Nuc-(0) (-413 to -253) and Nuc-(+1) (+1 to +155). Nuc-(+1) is located close to the transcriptional start site, and the region between Nuc-(0) and Nuc-(+1) is bound by many of the cellular factors involved in regulating viral expression (Steger and Workman, 1997) (Fig. 2). The binding of cellular transcription factors upstream of Nuc-(+1) induces remodeling of Nuc-(+1) and subsequent transcription (Sheridan *et al.*, 1995; Pazin *et al.*, 1996; Steger and Workman, 1997). The importance of Nuc-(+1) for viral expression and latency is underscored by a dynamic regulation of chromatin structure surrounding the LTR promoter, which exerts an additional layer of controlled expression (Knezetic and Luse, 1986).

Latency and viral expression

Upon successful infection in CD4+ T-cells, HIV-1 enters into a state of latency, a process which has been argued to be multifactorial, but which may be due to the natural progression of a CD4+ T-cell subset (whether infected or not) to shift to memory T cells (reviewed in Lassen *et al.*, 2004). Of particular interest, is the role of the viral LTRs in establishing, maintaining, and overriding the latent state. In the absence of activation-dependent nuclear transcription factors NF- κ B and NF-AT, RNA polymerase II (RNA Pol-II) associates with the 5'-LTR and transcribes relatively inefficiently. Without Tat, transcription is stalled by the presence of unphosphorylated serines 2 and 5 on the C-terminal domain (CTD) of RNA Pol-II (Zhou *et al.*, 2004) (Fig. 2A). Basal transcription is largely non-processive when Tat is absent,

resulting in very small amounts of full-length transcripts and mostly in the release of short RNAs which include the ~59 nucleotide TAR region (Fig. 2) (Kao *et al.*, 1987).

Following Tat expression and its subsequent acetylation by p300/CBP (cAMP response element [CREB] binding protein), p300/CBP-associating factor (PCAF) and hGCN5 (Kiernan *et al.*, 1999; Ott *et al.*, 1999) the stalled RNA Pol-II can be activated by CTD hyperphosphorylation, which is mediated by a complex containing Tat and the positive transcription elongation factor-b (P-TEFb). P-TEFb is composed of cyclin-dependent kinase 9 (CDK9) and cyclin T1 (Zhou *et al.*, 2004). Once activated, RNA Pol-II-mediated transcriptional elongation can commence, resulting in full-length viral mRNAs (Fig. 2B). Provirus-infected CD4⁺ T cells require activation in order to express suitable quantities of Tat, which is usually mediated by the expression of cellular transcription factors such as NF- κ B and NF-AT (reviewed in Lassen *et al.*, 2004). Cytoplasmic NF- κ B and NF-AT localize to the nucleus following T-cell receptor (TCR) activation of resting CD4⁺ T cells by antigen and/or cytokines (IL-2 or TNF- α ; reviewed in Lassen *et al.*, 2004).

Activated and nuclear localized NF-AT and NF- κ B interact with sites in the proviral LTR promoter to induce transcription (Fig. 1). Of significance is the observation that NF- κ B can also directly interact with and activate brahma-related gene (BRG)-1 (Holloway *et al.*, 2003). In mammals the Swi/SNF complex (Fig. 2A versus 2B) functions to remodel chromatin by utilizing the hydrolysis of ATP to recruit transcription factors to target promoter regulatory sequences (reviewed in Muchardt and Yaniv 1999). The human Swi/SNF is a large (~2 MDa) multisubunit complex that is directly involved in binding to acetylated nucleosomes *in vitro* and in the recruitment of gene specific activators (Henderson *et al.*, 2004). All Swi/SNF complexes have a subunit with homology to known DNA helicases (Eisen and Lucchesi 1998), one of which has been identified in mammalian cells as BRG-1 (Filetici *et al.*, 2001). The DNA-dependent ATPase activity associated with BRG-1 is most likely the main mechanism involved in nucleosomal perturbation leading to gene expression (Tsukiyama and Wu, 1995; Varga-Weisz *et al.*, 1995; Zhang *et al.*, 1998) (Fig. 2). In addition, Tat has also been shown to function in the derepression of chromatin via interactions with p300/CBP, PCAF, and hGCN5, followed by subsequent enhanced histone acetyl-transferase (HAT) activity (Benkirane *et al.*, 1998; Hottiger and Nabel, 1998; Marzio *et al.*, 1998). Activation of HATs result in modulating chromatin to an “active” state, which correlates with enhanced transcriptional activity and methylation of lysine positions 4 and 36 on histone 3 (H3K4^{ME+} and H3K36^{ME+}, respectively) (Kawasaki *et al.*, 2005).

It is clear that the integrated provirus controls viral transcription by repressing or activating the 5'-LTR, specifically at Nuc-(+1), via the expression and access of soluble Tat to the LTR (Verdin *et al.*, 1993). Nuc-(+1) is consistently positioned downstream of Nuc-(0) and Nuc-(-1) at a preset equilibrated distance, regardless of where the virus integrates (Verdin *et al.*, 1993; He *et al.*, 2002). This positional effect, along with data on HIV-1 transcriptional activation (Verdin *et al.*, 1993; He *et al.* 2002), suggests that Nuc-(+1) is critical in viral replication and control. The activation of Nuc-(+1) depends not only on viral factors such as Tat but also on the acetylation of Nuc-(+1)-specific histones and other cellular transcription factors (He and Margolis, 2002). This has been recently expounded in a pilot clinical study where Margolis and colleagues used valproic acid (VPA), a histone deacetylase (HDAC) -1 inhibitor, to activate HIV-1 transcription in resting CD4⁺ T cells, allowing the HIV-1 fusion inhibitor enfuvirtide to inhibit the spread of activated HIV-1 from latent pools (Lehrman *et al.*, 2005). However, there remains considerable skepticism regarding the potential of VPA/antiretroviral treatment for the readication of viral reservoirs, since a relatively small sample size was used in this study (Smith, 2005). The order of events in the transactivation of HIV-1 from the latent/integrated isoform is depicted in Figure 2A and B. However, a mechanistic explanation for the establishment and maintenance of latency remains enigmatic. The

possibility of a role for the endogenous RNAi machinery in affecting latent viral infection in CD4⁺ T cells is explored below.

RNA interference pathway

The recent discovery and characterization of a vast array of small (21–26 nt) noncoding RNAs is dramatically changing the classical understanding of gene regulation (Zamore and Haley, 2005). In particular, short interfering RNAs (siRNAs) and microRNAs (miRNAs), which are effector sequences of the RNAi pathway, are estimated to regulate up to a third of all human genes, many of which are implicated in higher order regulatory activities. These include (but are not limited to): cellular differentiation, apoptosis, developmental timing, and regulation of transposable elements (reviewed by Kim, 2005). MicroRNAs derived from viruses have been recently identified for SV40 (Sullivan *et al.*, 2005), Herpesvirus family members (Pfeffer *et al.*, 2004; Cai *et al.*, 2005; Samols *et al.*, 2005) and HIV-1 (Omoto *et al.*, 2004; Bennasser *et al.*, 2005). Host-encoded miRNAs are also known to affect retrovirus primate foamy virus type 1 (PFV-1) (Lecellier *et al.*, 2005) and Hepatitis C Virus (Jopling *et al.*, 2005). The ever-increasing number of viral-associated miRNAs being characterized indicates an important function for small noncoding RNAs in viral regulation, adding to the complexity of host–viruses interactions.

RNAi functions largely through posttranscriptional gene silencing (PTGS) which is defined by the homology-dependent targeted degradation and/or translational suppression of mRNA (Montgomery *et al.*, 1998; Nishikura, 2001; Sharp, 2001). siRNAs and miRNAs are short ~22-nt duplexes with 2-nt 3'-OH overhangs that are processed from precursor, longer, dsRNA triggers by the endonuclease activity of Dicer (Zamore *et al.*, 2000; Bernstein *et al.*, 2001; Elbashir *et al.*, 2001). Although the pathways for generation of mature siRNAs and miRNAs are largely interchangeable, in the case of miRNAs, longer hairpin precursors of ~70 nt, or pre-miRNAs, are derived by RNA Pol II primary (pri-) miRNA transcripts that are processed by the endonuclease Drosha/DGCR8 (Lee *et al.*, 2003) and subsequently exported from the nucleus to the cytoplasm by Exportin 5 (Lund *et al.*, 2004) (Fig. 3). In mammalian cells, functional Dicer activity *in vivo* has recently been shown to require the TAR RNA binding protein (TRBP) (Chendrimada *et al.*, 2005; Haase *et al.*, 2005). TRBP is a host cell protein with three dsRNA binding domains, and was originally shown to bind specifically to the HIV-1 TAR stem-loop and complement Tat-TAR binding interactions (Gatignol *et al.*, 1991; Dorin *et al.*, 2003). TRBP may stabilize Dicer, and appears to provide a functional link between Dicer and the endonuclease Argonut 2 (Ago2), an important component of the RNA-induced silencing complex (RISC) (Chendrimada *et al.*, 2005; Gregory *et al.*, 2005). Additionally, TRBP directly inhibits protein kinase R (PKR) and the dsRNA-mediated interferon (IFN) pathway (Daher *et al.*, 2001), further strengthening the delicate interplay between IFN and RNAi-regulated interactions.

Within RISC, the siRNA guide strand targets mRNA for degradation mediated by Ago2 cleavage (Tuschl *et al.*, 1999; Hammond *et al.*, 2000, 2001; Zeng and Cullen 2002; Song *et al.*, 2004, Liu *et al.*, 2004) (Fig. 3). Similarly, the Dicer/TRBP complex may shuttle miRNA into the RISC-like ribonucleoprotein complex (miRNP), which includes eIF2C2 and fragile X mental retardation protein (FMRP) (Mourelatos *et al.*, 2002; Jin *et al.*, 2004). Since miRNAs possess only partially complementary sequences with the target mRNA, silencing is generally at the level of translation and usually by targeting multiple sites located in the 3'(untranslated region (UTR) (Ambros, 2004; Bartel, 2004; Kawasaki *et al.*, 2004). MicroRNAs are found to be conserved across the eukaryotes; however, in plants miRNAs function mainly as siRNAs, guiding the cleavage of targeted mRNAs (Llave *et al.*, 2002; Tang *et al.*, 2003; Mansfield *et al.*, 2004; Yekta *et al.*, 2004).

Effector sequences of the RNAi pathway may additionally induce transcriptional gene silencing (TGS), which is characterized by the targeting of RNAi effector sequences to direct epigenetic modifications of DNA, resulting in transcriptional inhibition (Sijen *et al.*, 2001; Pal-Bhadra *et al.*, 2002). Small interfering RNA-mediated transcriptional suppression was first observed in transformed plants (Matzke *et al.*, 1989), where careful analysis indicated that methylation of the targeted gene was involved in silencing (reviewed in Sijen *et al.*, 2001; Matzke *et al.*, 2004). RNA-dependent methylation of DNA (RdDM) within promoter regions was shown to require short RNAs which are processed by the RNAi machinery from longer dsRNA precursors in viroid-infected plants (Wassenegger *et al.*, 1994; Mette *et al.*, 2000).

In *S. pombe* RNAi-mediated TGS has been implicated in regulating heterochromatic silencing through actions of the RNA induced initiation of transcriptional silencing (RITS) complex, which ultimately results in histone 3 lysine 9 methylation (H3K9^{ME+}; Volpe *et al.*, 2002; Noma *et al.*, 2004). TGS has only recently been reported in human cells (Morris *et al.*, 2004; Buhler *et al.*, 2005; Castanotto *et al.*, 2005; Janowski *et al.*, 2005; Ting *et al.*, 2005; Zhang *et al.*, 2005) and reviewed in Morris (2005). However, the underlying mechanism of TGS in mammalian cells is not yet completely clear but appears to involve DNA methylation and histone deacetylation/methylation (Morris *et al.*, 2004; Buhler *et al.*, 2005; Castanotto *et al.*, 2005; Janowski *et al.*, 2005; Morris, 2005; Suzuki *et al.*, 2005; Ting *et al.*, 2005). In human cells, TGS may also involve RITS associated factors (Noma *et al.*, 2004); silencing has been associated with the presence of a H3K9 methylation mark at the targeted site (Buhler *et al.*, 2005; Ting *et al.*, 2005; Weinberg *et al.*, 2005).

The link between RNAi and the regulation of HIV-1 latency

Recent experimental evidence has indicated that HIV-1 has evolved mechanism(s) to both suppress and/or usurp the RNAi pathway to maintain a latent state in infected host CD4⁺ cells. TRBP has the dual role of interacting with HIV-1 TAR RNA and in facilitating dsRNA processing. These processes represent an important and, perhaps, profound link between the multifactorial functions of the endogenous RNAi pathway and HIV-1 transcriptional activation. It has been previously noted that the TAR loop resembles a miRNA precursor, implying a functional role for a putative TAR-derived miRNA (Bennasser *et al.*, 2004). However, it remains to be determined experimentally whether the processing of a putative TAR-derived pre-miRNA would be augmented by the recruitment of Dicer to the TAR loop by the Dicer/TRBP complex. Although one cannot rule out the possibility of a host gene target (see Couturier and Root-Bernstein, 2005), it is possible that a TAR-derived miRNA would functionally target the complementary sequence of the TAR region itself, resulting in posttranscriptional or possibly even transcriptional inhibition of HIV-1. MicroRNAs derived from HIV-1 that correspond to sequences in Env and Nef have been detected (Omoto *et al.*, 2004; Bennasser *et al.*, 2005; Omoto and Fujii, 2005). Interestingly, the 3' (end of Nef, which corresponds to the putative HIV encoded Nef microRNA, miR-N367, also overlaps the U3 region of the HIV-1 LTR (Figs. 1 and 4). It is possible that during basal transcription, viral-encoded microRNAs may feedback to regulate the integrated LTR promoter by TGS (histone deacetylation/methylation and/or possibly DNA methylation) (Morris *et al.*, 2004; Castanotto *et al.*, 2005; Morris, 2005; Ting *et al.*, 2005) (Fig. 4). Bennasser *et al.* (2005) have recently added a twist to the story by showing that high intracellular concentrations of HIV-1 Tat inhibits the activity or processivity of Dicer (Bennasser *et al.*, 2005). Although the transactivation function (through the dsRNA binding motif) of Tat does not appear to be responsible for anti-Dicer properties, Tat may function via competitive interactions with TRBP for binding to Dicer (Fig. 4D) when present in high enough concentrations (e.g., following T-cell activation). Moreover, PKR has been shown to phosphorylate Tat (Endo-Munoz *et al.*, 2005), which in turn, is known to inhibit PKR (Cai *et al.*, 2000). Thus, excess Tat may supplement and/or replace the inhibitory effects on PKR by TRBP during viral replication, since any free TRBP

may be utilized for viral replication. Competitive interactions between Dicer and TRBP could also be a mechanism utilized by the virus to relieve the antiviral pressures mediated by RNAi (possibly by viral-derived microRNAs/siRNAs such as TAR) when in a latent state. Interestingly, TAR may function as (1) a decoy to sequester TRBP away from Dicer and as such relieve the RNAi pressures placed on the provirus, and (2) as a microRNA to direct TGS of the provirus and function to assist in the maintenance of viral latency. Thus, following TCR activation, NF- κ B, NF-AT along with other transcriptional activators and chromatin remodeling factors such as BRG-1, are translocated to the nucleus to induce transcription of the previously inactive viral LTR promoter (Fig. 4D). The resulting increase in viral transcription (Nabel and Baltimore, 1987; Tong-Starksen, *et al.*, 1987; Duh *et al.*, 1989) would theoretically provide for enough Tat to drive transcriptional elongation (discussed above) and induce a ~100-fold increase in full-length viral transcripts (Adams *et al.*, 1994) (Figs. 2B and 4E). This would be followed by the production of infectious particles from what was previously a latent virus.

Undoubtedly, understanding the mechanism involved in establishing and maintaining viral latency remains an important objective for the successful eradication of HIV-1 infection. A number of recent findings have been juxtaposed in this review to reveal a possible role for viral encoded microRNAs in affecting latency of HIV-1. Hopefully, future experiments delving into the parameters of dsRNA-induced transcriptional gene silencing will provide further clues to the mechanism of RNA-mediated regulation of transcription, and provide new insight for future therapeutic interventions aimed at targeting latent HIV-1 infection.

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FIG. 1. The LTR of HIV-1. Genomic organization of HIV-1 is shown with the dashed lines delineating the 5'-LTR promoter. The transcription factor binding sites, TATA box, and transactivation-response region (TAR) in the LTR are depicted.

FIG. 2.

Swi/SNF interactions with Nuc(+1) of integrated provirus. **(A)** The integrated provirus can remain silent, most likely due to histone deacetylation and/or inaccessibility of host factors to the LTR NF- κ B and/or SP1 sites. SP1 can also function in a negative fashion to suppress promoter expression. **(B)** LTR-mediated transcription of the integrated virus is a direct result of T cell receptor (TCR) activation, which causes coactivator expression of SP1 and NF- κ B (He, *et al.*, 2002). The increase in transcription from the LTR results in a buildup of Tat, which when acetylated by p300/CBP, PCAF, and hGCN5, can functionally interact with CDK9 and CycT1 to phosphorylate serines 2 and 5 of RNA Pol-II. Enhanced NF- κ B expression can also recruit chromatin modifying factors such as BRG-1 resulting in Swi/SNF, SNF5 complex formation. This is followed by the recruitment of host cell factors (and RNA Pol II) that are involved specifically in chromatin remodeling of Nuc(+1) (Henderson *et al.*, 2004). Nuc(+1) remodeling results in the recruitment of histone acetyltransferases (HAT complex) by interactions between Tat and p300/CBP, PCAF, and hGCN5. This leads to chromatin hyperacetylation, H3K4 and H3K46 methylation, and transcriptional activation.

FIG. 3.

MicroRNA processing pathway in mammalian cells. The RNA Pol-II expressed miRNAs are bound by Drosha/DGCR8 (Lee *et al.*, 2003; Lund *et al.*, 2004) (1), shuttled out of the nucleus by Exportin 5 (2), where Dicer/TRBP (3) processes the pre-miRNA into an siRNA/miRNA-like precursor that can then be loaded into RNA induced silencing complex (RISC) or RISC-like miRNA processing complex (miRNP) ultimately leading to slicing of the target mRNA (4), or translational inhibition, which likely takes place within P bodies (5) (Liu *et al.*, 2005). Alternatively, siRNAs/miRNAs can be shuttled back into the nucleus where targeting of homologous genomic sequences can occur by transcriptional gene silencing (TGS) (6).

FIG. 4.

A putative pathway for the establishment and reactivation of HIV latency. (A) the integrated provirus could produce microRNAs either within the viral genome (miR-N367) or from the unelongated TAR element (Omoto *et al.*, 2004; Bennasser *et al.*, 2005; Omoto and Fujii, 2005). Next, (B) the microRNAs could be processed by Dicer and TRBP and thus capable of targeting the LTR in a transcriptional fashion. (C) The targeting of the LTR by siRNAs can lead to histone 3 lysine 9 dimethylation (H3K9^{ME2+}) (Morris *et al.*, 2004; Kawasaki *et al.*, 2005; Ting *et al.*, 2005; Weinberg *et al.*, 2005) and subsequent gene silencing, that is, the induction of a latent state. (D) When the TCR is stimulated by IL-2 or TNF-α, NF-AT and NF-κB can become activated. NF-κB can activate BRG-1, which can function with other factors to remodel the chromatin at the viral LTR and produce a favorable environment for transcription. Moreover, NF-AT and NF-κB can activate the LTR and induce Tat expression, which, when present in high concentrations, results in feedback to enhance the elongation of viral transcription from the TAR element, as well as inhibit Dicer by competitive interactions with TRBP. The end result (E) would be a reversal of the silent state H3K9 methylation (due to the inhibition of Dicer/TRBP by Tat), an induction of viral expression (due to NF-AT, NF-

κ B, and Tat), which leads to H3K4 and H3K36 methylation (Zhou *et al.*, 2004) and a transcriptional active chromatin state. This ultimately results in the production of full-length viral products and the emergence of HIV-1 from latency.