TRANSCRIPTION AND CHROMATIN ANALYSIS OF HUMAN RETROVIRUSES

by

Yao A. Akpamagbo

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Committee:

_____	Dr. Fatah Kashanchi, Thesis Director
_____	Dr. Yuntao Wu, Committee Member
_____	Dr. Karl Fryxell, Committee Member
_____	Dr. Iosif Vaisman, Acting Director, School of Systems Biology
_____	Dr. Donna M. Fox, Associate Dean, Office of Academic Affairs and Special Programs, College of Science
_____	Dr. Peggy Agouris, Dean, College of Science
Date: _____	Fall Semester 2016 George Mason University Fairfax, VA

Transcription and Chromatin Analysis of Human Retroviruses

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

by

Yao A. Akpamagbo
Bachelor of Science
Hampton University, 2014

Director: Fatah Kashanchi, Professor
Department of Biology

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Fairfax, VA

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DEDICATION

This thesis is dedicated to the best parents ever, Amavi Akpamagbo and Adjovi Akpamagbo, the best support group, uncle Kodjo Apetoh and his family, especially Ophelia Apetoh. To the many friends, relatives, and supporters who have encouraged me and championed me to make this happen, thank you.

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LIST OF ABBREVIATIONS

Human Immunodeficiency Virus ..... HIV
Human T-Lymphotropic Virus ..... HTLV
Ribonucleic Acid ..... RNA
Deoxyribonucleic Acid ..... DNA
long non-coding RNA ..... lncRNA
Chromatin Remodeling Complex ..... CRC
Trans-activation Response ..... TAR
Trans-activator of transcription ..... Tat
group-specific antigen ..... gag
Cyclin-dependant kinase ..... cdk
SWIth/Sucrose NonFermental ..... SWI/SNF
Brg-1 Activated Factor ..... BAF
Histone Deacetylase ..... HDAC
P-element Induced Wimpy Testis ..... PIWI
Histone ..... H
Polymerase ..... Pol
positive-Transcription Elongation Factor ..... p-TEF
Circulating Recombinant Forms ..... CRF
Nuclear Factor of Activated T-cells ..... NFAT

ABSTRACT

TRANSCRIPTION AND CHROMATIN ANALYSIS OF HUMAN RETROVIRUSES

Yao A. Akpamagbo, M.S.

George Mason University, 2016

Thesis Director: Dr. Fatah Kashanchi

During HIV infection, a provirus is integrated into the host genome where it is protected from transcription activators resulting in viral latency. Cellular long non-coding RNAs (lncRNA) and chromatin remodeling complexes (CRC) have recently emerged as key regulators in inhibitory pathways of infected cells. Here, we studied the activity of transcription inhibitors in HIV-infected cells to demonstrate that a novel HIV-1 RNA transcript, TAR-gag, is involved in HIV-1 latency. We also described a PBAF complex found only in HIV cells. Our results suggest that transcription inhibitors, CR8#13 and F07#13, independently regulate transcription machinery in infected cells via the pTEF-b complex. We also observed the presence of a BAF 170 complex only found in HIV-infected cells. The results indicate that a component of the pTEF-b complex, cdk9, phosphorylates both activator and inhibitor forms of BAF, and PBAF in the same cell. We then showed that TAR-gag is bound to the msin3A/HDAC CRC. Additionally, treatment of HIV-infected cells with F07#13 favored an interaction between TAR-gag,

HDAC and PIWI proteins, whereas CR8#13 favored an interaction between TAR-gag, HDAC and mSin3A proteins. Given that TAR-gag is not translated and is increased by HIV transcription inhibitors in T-cells, the data suggest that it is a viral non-coding RNA that contributes to viral latency.

INTRODUCTION

Widely known for their minuscule size of 20- 400 nanometers, viruses are a unique and less understood class of infectious agents (Lycke & Norrby, 1983). Aside from some complex virions, most viruses have a helical or icosahedral capsid that contains either a naked or an enveloped nucleic acid, DNA or RNA, which can be single or double stranded. Some RNA viruses that are enveloped positive-sense viruses are termed retroviruses because they enclose reverse transcriptase, an enzyme that produces complementary DNA (cDNA) from RNA (Becker & Hadar, 1983; Palmenberg & Sgro, 2005). During an infection, they introduce the new cDNA into the genome creating a provirus that is transcribed into an RNA, which subsequently translates into a protein. A virion introduced into one cell will cause functional changes in the cell it infects and invade other cells, causing a spread of the infection (Modrow, et Al, 2013). Preventing these changes require a good understanding of the genetic alterations viral particles may cause in infected cells.

Human Immunodeficiency Virus (HIV) is a retrovirus that affects 1.2 million people in the United States. Patients infected with HIV primarily receive a combination of antiretroviral drugs, c-ART, which to date, is the most efficient treatment for HIV/AIDS. This therapy works by preventing four major viral processes; entry, reverse transcription, integration, and maturation of new viruses. To stop entry of new HIV

virions into host cells, entry inhibitors and fusion inhibitors are used to prevent fusion and docking of the virus. The treatment also prevents the process of reverse transcription, which changes viral RNA into DNA. Inside the nucleus, c-ART inhibits the action of the viral enzyme integrase, blocking integration of the virus into the host genome. Furthermore, the treatment inhibits viral proteases responsible for newly formed viral protein from becoming mature or infectious (Clercq, 2007; Le Douce, Herbein, et Al, 2010). Nevertheless, the current therapies are ineffective at eliminating the virus and are the leading cause of drug-resistant variants. In resting T cells, quiescence of the provirus allows the virus to exist in a latent state as part of the host genome (Archin, et Al, 2014a). HIV-1 will remain a chronic viral infection with a strong potential to cause and spread AIDS if the persistent latent infection is not eradicated, primarily because latently infected cells escape the effect of c-ART and produce viral RNA and infectious virus (De Crignis & Mahmoudi, 2014).

The latency of HIV is maintained by several mechanisms (figure 1). In an infected cell, nuclear transcription factors NF-κB and NFAT have the ability to enter the nucleus, and loosen the chromatin to transcribe the hidden virus. However, these transcription factors are sequestered in the cytoplasm, which leads to transcriptional silencing. Similarly, the positive transcription elongation factor b (p-TEFb) complex is sequestered inside the nucleus by the HEXIM1–7SK small nuclear RNA (snRNA) resulting in an inactive ribonucleoprotein complex. p-TEFb also binds to the Trans-Activator of Transcription (Tat) protein to initiate transcription (Contreras, et Al, 2007). However, p-TEFb is down-regulated when the Bromodomain-containing protein 4 (Brd4) competes

with it by binding-Tat. In HIV latent cells, the HIV-1 long terminal repeat (LTR) serves as a linker DNA to nucleosomes that can recruit epigenetic silencing inducing proteins. In the host gene, in the event where the viral DNA is inserted into an intron upstream, the RNA polymerase II (Pol II) moves transcription factors on the HIV-1 LTR in a process known as promoter occlusion. On the other hand, convergent transcription occurs when

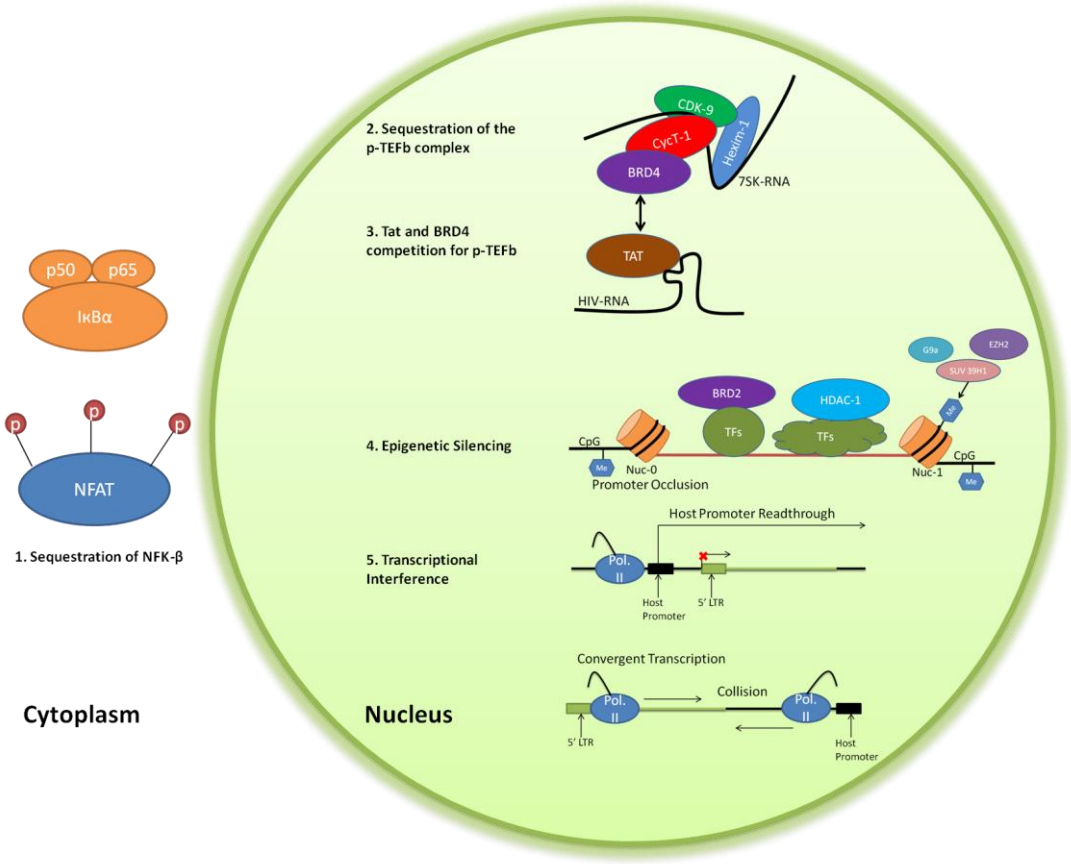

Figure 1. Mechanism of latency in HIV cells.
The five understood mechanisms for HIV latency. 1) Transcription factor NFK-b is sequestered in the cytoplasm preventing its movement to the nucleus. 2) p-TEF-b complex is sequestered by the HEXIM-1 and 7SK-RNA complex. 3) BRD4 competes with pTEF-b to avoid binding of pTEF-b to Tat. 4) HIV-LTR serves as a linker DNA to recruit transcription silencing factors. 5) Transcription interference through promoter occlusion or convergent transcription prevents Pol II to transcribe the virus.

the virus is integrated opposite to the host gene. Under these conditions, the host RNA Pol II can prematurely terminate the HIV-1 transcript promoting latency (Archin, et Al, 2014b).

More recent studies have suggested that cellular long non-coding RNAs (lncRNA) can be a source of latency. lncRNAs are known to be involved in immune regulation and pathogenesis of certain infections. In fact, they can bind genetic materials and proteins to influence epigenetic modifications, transcription, signal transduction and much more (Geisler & Coller, 2013). For example, NRON, a lncRNA in T-cells, contributes to latency by inducing the degradation of the Tat protein. Currently, there is no known lncRNA latency mechanism. Our lab recently discovered the presence of a novel viral transcript that might play a critical role in HIV-1 latency (Li et al., 2016). Presently, lncRNAs have been shown to interact with CRCs like the SWI/SNF to promote latency, but little is known about how they prevent transcription (Geisler & Coller, 2013; Imam, et Al, 2015).

CRCs are rapidly emerging as therapeutic candidates to shut down the virus completely. In infected cells, the viral gene expression is based on the state of the chromatin structure within the nucleus. Structurally, chromatin is folded into compact fibers (30 to 400 nm thick), which physically prevents the transcriptional machinery from accessing the cellular promoters (Coffin, et Al, 1997). When chromatin is in its more condensed, heterochromatin form, the level of transcription is reduced since transcription cannot proceed efficiently. Therefore, a variety of enzymes and protein complexes are

present in the nucleus to intercede the opening and closing of the chromatin structure. Upon infection and integration of the provirus into the host genome, an obstruction in transcription occurs when the nucleosomes are deposited along the viral chromatin (Easley et al., 2010). Overcoming this barrier requires a remodeling of the chromatin structure. Consequently, the right CRC has to be recruited to the viral LTR to either promote or repress transcription of the virus. BRG-1 is the core catalytic subunit of most SWI/SNF chromatin remodeling complexes. To date, the well-known CRCs include the activator PBAF, and the transcription inhibitors BAF. Other BAF complexes include WINAC, NCoR, NUMAC, and mSin3A/HDAC (Fan, Narlikar, & Kingston, 2004; Liu, Balliano, & Hayes, 2011; Narlikar, Fan, & Kingston, 2002; Van Duyne et al., 2011a) (Figure 2).

In this paper, we studied the activity of transcription inhibition drugs in HIV-infected cells to investigate CRC proteins interaction with lncRNA. We show that the novel HIV-1 RNA transcript, TAR-gag, could be involved in HIV-1 latency. CR8#13 and F07#13, which are third generation drugs of roscovitine, individually control the transcription machinery in infected cells through the p-TEFb complex. In fact, we observed that both drugs move the small complex of p-TEFb differently to promote transcription inhibition. We also noted that TAR-gag is bound to the msin3A/HDAC type CRC. Additionally, treatment of infected cells with CR8#13 favors TAR-gag, HDAC, mSni3A interaction, when F07#13 favors a TAR-gag, PIWI, HDAC interaction for transcription inhibition. We later showed that TAR is mostly expressed in the brain, and the lung after treatment with CR8#13 and that TAR-gag is not translated in the cell. Our

data suggests that HIV transcription inhibiting drugs increase the viral non-coding RNA TAR-gag, which binds to CRCs to promote latency

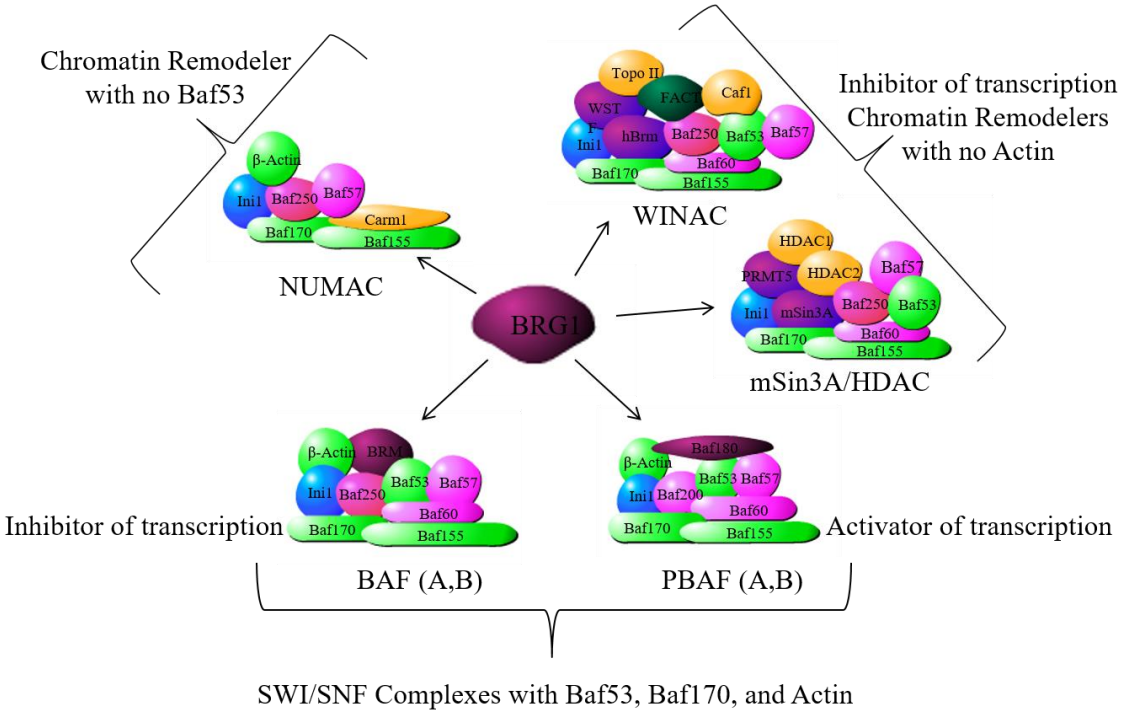

Figure 2. Organization of BAF associated chromatin remodeling complexes.
CRCs share subunits that have a variety of functions. All complexes shown contain Ini1, Baf170, and Baf155. The two BAF and PBAF complexes differ only by the incorporation of Brm and Baf180, respectively; all four complexes contain Baf53, Baf170, and actin. The WINAC and mSIin3A/HDAC complexes do not contain actin, and the NUMAC complex does not contain Baf53. Subunits are organized as follows: green, core proteins; pink, nuclear receptor-associated; gold, DNA replication associated; and light green, Arp's. As chromatin remodelers, these complexes can act as either activators or repressors depending on the association of subunits(Van Duyne et al., 2011b).

MATERIALS AND METHODS

Cell Culture and Antibodies

Jurkat and CEM cell line are uninfected T-cell line. ACH2 and Jurkat-E4 cell lines are latently HIV-1 infected T-cells, J1.1 is an HIV-1 infected T-cell line, U1 is a latently HIV-1 infected monocytic cell line, and OM10.1 is a latently infected promyelocytic cell line. Cells are grown in RPMI-1640 media containing 10% FBS, 1% L-glutamine, and 1% streptomycin/penicillin (Quality Biological). For cell lines used in Flow Cytometry and RT assays, cells were grown in RPM1-1640 medium containing 5% FBS, 1% L-glutamine, and 1% streptomycin/penicillin (Quality Biological). All cells were incubated at 37°C and 5% CO_2. cyclinT1 (H245), p300 (C-20), Pol II (N-20), α-Cdk9 (phosphor T186) ab79178, α-Baf200 (H-300), α-Baf170 (H-116) and α-Actin (C-11) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Preparation of whole cell extract and size exclusion chromatography

The pellets of uninfected Jurkat and HIV-1 infected J1.1 cell were washed with PBS without Ca and Mg, resuspended in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 120 mM NaCl, 5 mM EDTA, 0.5% NP-40, 50 mM NaF, 0.2 mM Na3VO4, 1 mM DTT, and one complete protease inhibitors cocktail tablet/50 ml (Roche, Mannheim, Germany), and incubated on ice for 20 min with gentle vortexing. Lysates were centrifuged at 4°C at 10,000 rpm for 10 min, and protein concentrations in the supernatants were determined using the Bradford protein assay (BioRad, Hercules, CA).

For each cell line, 2.5 mg protein was equilibrated in chromatography running buffer [(0.2 M Tris–HCl (pH 7.5), 0.5 M NaCl, and 5% glycerol)].

Western blots

Chromatography fractions and input lysates were resolved by SDS-PAGE on a 4–20% Tris-Glycine gel (Invitrogen, Carlsbad, CA, USA). Proteins were transferred to PVDF membranes overnight. Western blot visualization and analysis was performed with the Odyssey IR imaging system (LI-COR Biosciences) following the manufacturer's instructions. Briefly, membranes were blocked with 5 mL of LI-COR blocking buffer (LI-COR Biosciences, Cambridge, UK) for 1 hour at room temperature and incubated overnight at 4°C with primary antibodies in LI-COR buffer containing 0.1% Tween-20. Membranes were washed three times with PBS+0.1% Tween-20 and incubated with donkey α-goat IRDye 800CW (LI-COR) or goat α-rabbit IRDye 680 (LI-COR) for 30 min. Membranes were washed four times with PBS+0.1% Tween-20 and once with PBS prior to scanning. Membranes were stripped and re-processed with appropriate antibodies when needed.

Immunoprecipitation

Cells were harvested at 4°C, and cell pellets were washed with PBS. Cell lysates were prepared as previously described (Zhang, Scadden, & Crumpacker, 2007). Control antibodies (5 μg) (anti-IgG) were incubated with whole-cell lysates overnight at 4°C with rotation. The overnight-incubated mixture was then cleared by centrifugation, and protein A/G beads (30% slurry) were added for 2 h at 4°C. The immunoprecipitated complex was washed with buffer K (150 mM KCl, 20 mM HEPES [pH 7.4], 5 mM $MgCl_2$) and was

then resuspended in SDS-PAGE loading Laemmli buffer. Samples were separated on a 4 to 20% SDS-PAGE gel and were subjected to Western blotting.

Kinase assay

Immunoprecipitation (IP) and in vitro kinase assays were carried out using either whole cell extract or column fraction from infected or transfected cells. Briefly, for immunoprecipitation, low and molecular weight complex fractions were immunoprecipitated at 4 °C overnight with IKK- β antibody. The next day, complexes were precipitated with A/G beads (Calbiochem) for 4 h at 4 °C. Immunoprecipitated samples were washed 3X with appropriate TNE buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA) and kinase buffer. The reaction mixtures (30 μl) contained the following final concentrations: 40 mM β-glycerophosphate (pH 7.4), 7.5 mM MgCl2, 7.5 mM EGTA, 5% glycerol, [γ- 32P] ATP (0.4 mM, 1 μCi), 50 mM NaF, 1 mM orthovanadate, and 0.1% (v/v) β- mercaptoethanol. Phosphorylation reactions were performed with immunoprecipitated material and [γ- 32P] -labeled GST-IκBα (0.5 μg) or histone H1 (0.5 μg) as a substrate in TTK kinase buffer containing 50 mM HEPES (pH 7.9), 10 mM MgCl2, 6 mM EGTA, and 2.5 mM dithiothreitol. Reactions were incubated at 30°C for 1 hr, stopped by the addition of 1 volume of Laemmli sample buffer containing 5% β-mercaptoethanol, and run on a 4–20% SDSPAGE. Gels were subjected to autoradiography and quantification using Amersham Biosciences PhosphorImager software (Amersham Biosciences) (Narayanan et al., 2012).

c-ART treatment

ACH2, U1, and OM10.1 cells were pre-treated with cART for three days before exosome addition. Indinavir (protease inhibitor), lamivudine, tenofovir, and emtricitabine (all NRTI) were added together in an equal ratio to form a cocktail. The cocktail was then added to the cells in a way that allowed for each drug to be present at 10 μM within the cells.

Nanoparticle capture of exosomes

1 mL of cell supernatant was taken and centrifuged at 25,000 X *g* for 5 minutes to remove cells, and subsequently filtered through a 0.22 μm filter. Thereafter, 30ul of a 30% slurry of NT80/82 (Ceres Nanoscience) was added to the filtrate and rotated at 4° C overnight. After this, the filtrate was centrifuged at 25,000 X *g* for 10 minutes, and the supernatant was removed. The NT pellet was washed and resuspended in 50 μL of PBS.

RT-PCR

Total RNA was extracted from different samples including nuclear and whole cell lysates via trizol-chloroform method. Total RNA was used to generate cDNA with the GoScript Reverse Transcription System (Promega, Madison, WI) using specific reverse primers, Gag Reverse: (5′-GCT GGT AGG GCT ATA CAT TCT TAC- 3′; *T*m = 54°C), and TAR- Reverse: (5’- CAA CAG ACG GGC ACA CAC TAC -3’, *T*m = 58°C). Quantitative real-time PCR analysis was performed with 2μl of undiluted aliquots of cDNA using iQ Supermix (Bio-Rad) with the following pair of primers specific for target TAR sequences, primers were as follows: TAR- Reverse: (5’- CAA CAG ACG GGC ACA CAC TAC -3’, *T*m = 58°C) and TAR-Forward (5'- GGT CTC TCT GGT TAG ACC AGA TCT G -3', Tm= 58°C). Serial dilutions of DNA from 8E5 cells (a CEM T

cell line containing a single copy of HIV-1 LAV provirus per cell) were used as the quantitative standards. The PCR conditions were: one cycle at 95°C for 2 min, 41 cycles at 95°C for 15 s and 58°C or 54°C (depending on primer set) for 40 s. The absolute quantification of the samples was determined based on the cycle threshold (Ct) value relative to the standard curve. Real-time PCR reactions were carried out in triplicate using the PTC-200 Peltier Thermal Cycler with Chromo4 Continuous Fluorescence Detector (both from MJ Research) and Opticon Monitor 2.03 software.

RESULTS

Effect of CR8#13 and F07#13 on HIV-1 variants

HIV is a virus prone to mutations each time it replicates. Consequently, different forms of the virus have been isolated over the years. Through surveys from various geographical areas, researchers have classified HIV-1 gene sequences in four major groups M, N, O, and P with M being most prevalent. Within the group M, exist 9 distinctive subtypes A, B, C, D, F, G H, J, and K. These subtypes can further exchange genetic material to form circulating recombinant forms (CRFs), which are essentially hybrids (Koulinska et al., 2001; Peeters, et al., 2003). Most of the subtypes were shown to contain nucleotide variations in their promoter region or Tat open reading frame (Jeeninga et al., 2000; Roof et al., 2002). We previously observed that CR8#13 is an effective inhibitor of Tat-activated transcription. We are now asking whether the transcription inhibition drug CR8#13 will have the same effect on various subtypes of HIV-1 clades. In **figure 3**, we infected PBMC cells with subtypes A, B, C and CRF. Two hours post-adsorption/ infection, the samples were treated with CR8#13 and collected after 7 days for RT assay. The clade C subtype of the virus replicates better than any other clade, especially the CRFs. These results indicate that the clades have different kinetics of replication. More interestingly, the titration of CR8#13 showed inhibition in clades A, B, and CRF (AB:KAL153) as compared to clade C (lane 10-12). This may indicate that the transcription inhibition effect of CR8#13 is clade-specific. Collectively,

our data suggest that the transcriptional machinery in clades A, B, and CRF might be conserved, but different in clade C.

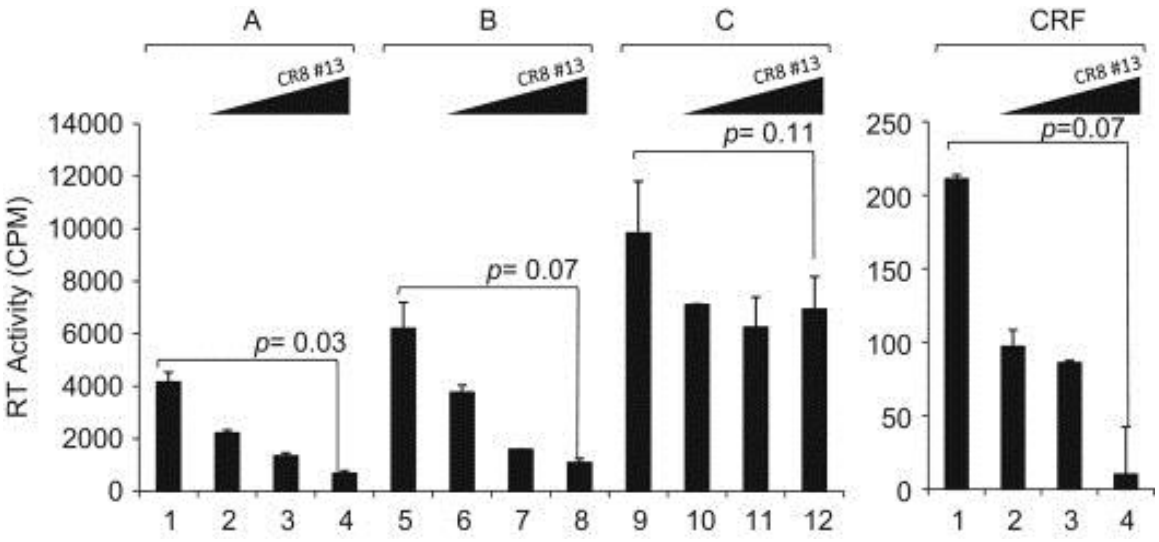

Figure 3. The varying response of viral clades to CR8#13 treatment.
Approximately 5×10^6 PBMCs were infected with Subtype A: 92UG037.1; Subtype B: HxB2; Subtype C: 96BW05.02; and CRF AB: KAL153 (5 ng of p24 gag antigen/virus). After 2 h of infection, cells were washed, and fresh medium was added. Drug treatment (CR8#13; 0.1, 1, and 10 nM) was performed once immediately after the addition of fresh medium. Samples were collected at day 7 for reverse transcription assay.

CR8#13 inhibits kinase activity and phosphorylation of H1 and Pol II from clades A and B (PTEF-b) but not clade C

We have previously observed that HIV can be inhibited by CR8#13 in activated PBMC cells infected with HIV-1 subtypes clades A, B, and CRF but not clade C. These results hinted that the transcriptional units used in all clades might be similar (Narayanan, Sampey et al., 2012) except in clade C. We have also shown that a small PTEF-b complex present exclusively in infected cells is possibly targeted by CR8#13 or other

cdk9 inhibitors (Van Duyne et al., 2011). Therefore, we followed the activity of this small PTEF-b from PBMC infected cells with clades A, B, and C. In figure 4-A, we treated different clades with increasing concentrations of CR8#13 (lane 3-8). An untreated sample of Clade A was used as a positive control. We immunoprecipitated these infected PBMC samples with Cyclin T1 antibody followed by an in-vitro kinase assay with two substrates including histone 1 (H1) and carboxyl terminal domaine (Gst-CTD). The results show that CR8#13 effectively inhibited the kinase activity and phosphorylation of H1 and Pol II from both clade A and B but not clade C. This result possibly explains why CR8#13 is not very efficient against clade C, although similar PTEF-b complex may be used for transcription. In clades A and B, CR8#13 prevents elongation from proceeding all the way to completion. However, this function is not present in clade C where H1 phosphorylation is not inhibited. The fact that CR8#13 does not have an effect on histone H1 in our clade C kinases means that the pTEF-b complex or more precisely the cdk9 might be different in clade C. In Figure 4-B we followed the same protocol with the addition of an immunoprecipitation with IgG to assess for nonspecific background (lane 1). We noticed a background issue with clade C H1 kinase. After measuring pixel intensity of clade C's bands, we noticed that phosphorylation decreases, but in a small amount. As a result, we believe that the effect of the drug on clade C might take longer to occur or may require higher concentrations of the drug. In all, we confirmed that CR8#13 has no effect on H1 in clade C. As a follow-up, it will be necessary to treat the PBMC cells infected with different clades with F07#13. It will be important also to try higher concentrations of the drugs on clade C.

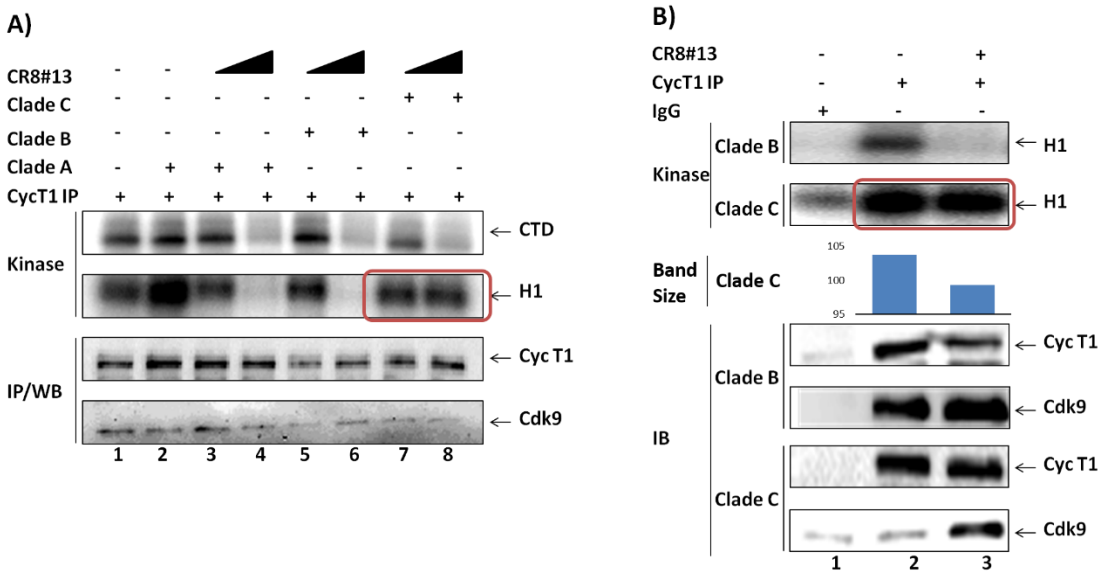

Figure 4. Kinase activity of nuclear PTEF-b from clades A, B, and C, infected cells.
A) PBMC cells treated with PHA+IL-2 were infected with MOI: 3 of 3 viral clades for 24 hrs, followed by incubation for 10 days. Nuclear extracts were isolated and immunoprecipitated with CyclinT1 antibody. The IP complexes were divided in half and used for WB for the presence of cdk9 and kinase reaction using purified histone H1 and Gst-CTD as substrates. B) The previous experiment was repeated with IgG to detect background signals in our kinase and immunoblots.

Transcription machinery in HIV-infected cells is unstable in the presence of CR8#13 and F07#13

On the basis of the previous observation, we next sought to understand how the pTEF-b complex gets phosphorylated. We used transcription inhibitors with chromatin to achieve transcription in vitro. We performed a streptavidin DNA pulldown. We used a biotin pull-down assay where the HIV-1 LTR (U3/R/U5) was chromatinized with all four histones and subsequently used for in vitro transcription (IVT) using active extracts. The extracts were from cells treated with increasing concentrations of Flavo, F07#13, CR8#13, and DMSO. Cells treated with Tat (lane 2) were used as a positive control and the HIV-1 DNA in lane 1 was used as negative control. We observed that the acetylation

of histone increased with Tat. This is due to p300 joining the promoter complex. Then we noticed that increased concentration of Flavo removed the acetylation, showing that the drug is inhibiting cdk9, which phosphorylates p300 causing a better acetylation. Amounts of bound Baf170 increases in the presence of Tat, but all our drugs were able to inhibit the recruitment of Baf170 except the DMSO control. Ini-1, cdk9, and RNA-Pol2 are serving as a control.

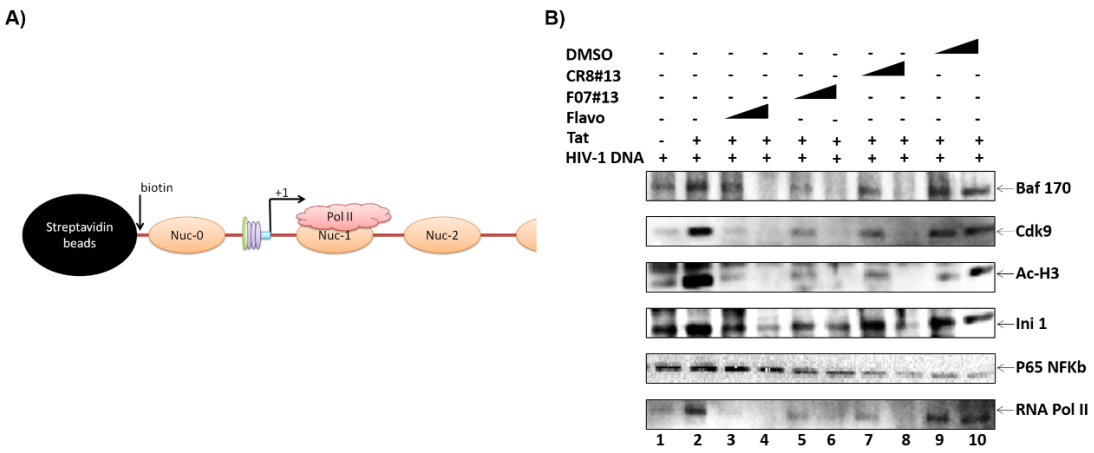

Figure 5: In vitro transcription using immobilized HIV chromatin LTR.
A) In vitro transcription was performed with CEM whole-cell G1/S extracts (50 µg total) on immobilized HIV-1 LTR chromatin templates. The DNA fragments were biotinylated, gel purified, and reconstituted with core histones by step dilution. In vitro transcription reactions in the presence or absence of Tat (500 ng) were incubated for 1 hr at 30°C and contained the nucleoside triphosphates cold ATP (0.05 µM), GTP, UTP, and CTP at a final concentration of 50 µM and [32P] γ-ATP (20 µCi; 400 Ci/mmol; Amersham, Piscataway, NJ) in buffer D. B) Western blot of transcription factors upstream (NFK-b), downstream (Pol II, cdk9), chromatin factors (Ini 1, Baf 170) and modified histone (H3). flavopiridol was used as positive control (10, 100nm), F07#13 (10, 100nM) and CR8#13 (10, 100nM) was used to inhibit transcription.

We see that Baf170 is initially present. The fact that it is not present when cdk9 is inhibited indicates that cdk9 regulates it. In short, cdk9/Cylcin-T phosphorylates Baf170 or one of the components in its complex. Consequently, the phosphorylated Baf170 complex is the form bound to the DNA. This is unique in the sense that no one has shown that cdk9 is capable of phosphorylating components of SWI/SNF complex.

CR8#13 and F07#13 independently control the low MW pTEF-b complex

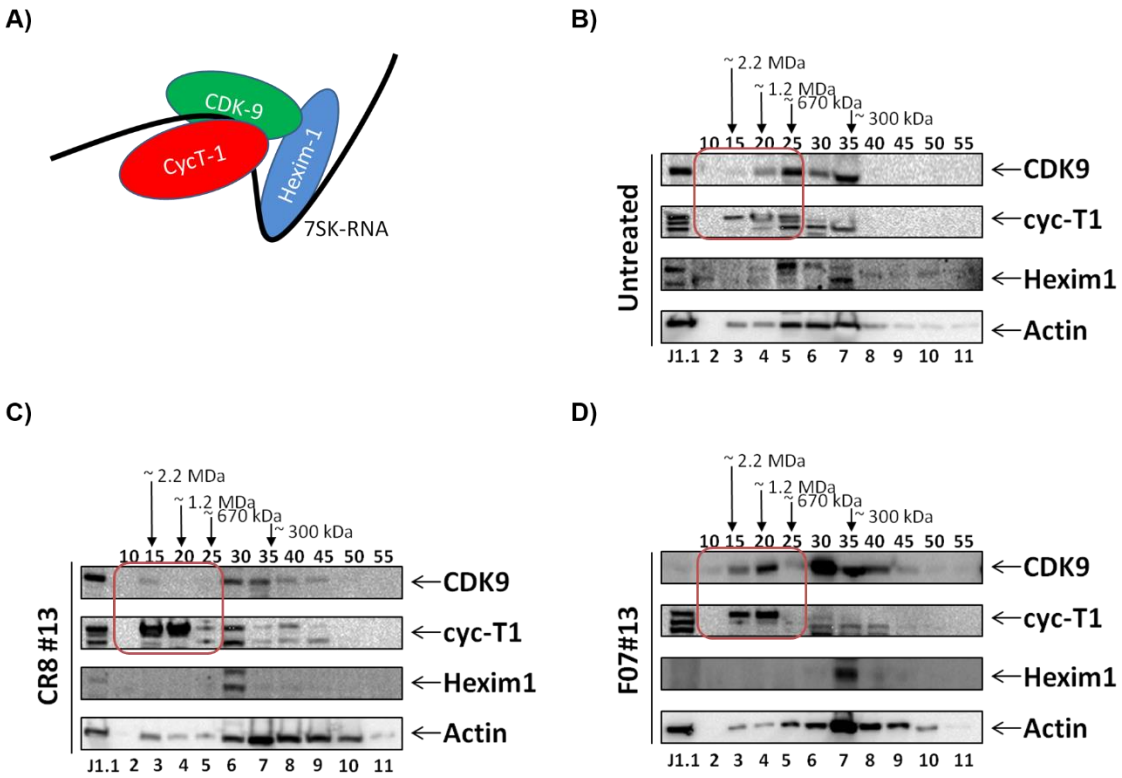

Figure 6: Control of cdk9/cyclin T1 complexes in HIV-1 infected replicating cells.
Total cell extracts from J1-1 untreated, J1-1 F07#13 treated, and J1-1 CR8#13 treated were processed over a sizing column in the presence of 500 mM salt. Every fifth fraction was used to precipitate proteins and western blot for the presence of cyclin T1, cdk9, Hexim1, and β-actin. Molecular weight markers of known positive controls were used to determine the range and size of the complexes.

We next wanted to understand the impact of the drugs on the small pTEF-b complex. In previous studies, pTEF-b is associated with different large molecular weight complexes. In infected cells, we observed the displacement of a small pTEF-b complex when the cells are treated with flavopiridol. Here we used LAI infected Jurkat (J1-1) and J1.1 cells treated with either CR8#13 or F07#13 cells for separation of various cdk9/cyclin T1 complexes, and Hexim1 (inhibitor of P-TEFb) (He et Al. 2010). We used cells at the early mid-log phase of growth and prepared total protein using Lysate buffer. The samples were then loaded onto a sizing column in the presence of high salt to minimize non-specific binding (500 mM NaCl). FPLC fractions were then precipitated and used for western-blot analysis and kinase assays. Results of such an experiment are shown in figure 6, where Jurkat infected fractions showed cdk9 complexes in fractions 15–35. A similar profile was observed for cdk9; however, Hexim1 eluted mostly at fractions 25–35. Upon treatment with CR8#13, cdk9 eluted in fractions 15 and 30-45, while F07#13 treated cells eluted at mostly from 10-50. Cyclin T1 in both cases eluted primarily in fractions 15 and 20, but cyclin T1 also eluted in fractions 25-45 in CR8#13 treated cells. More noticeable, Hexim1 eluted in one fraction when the cells were treated; fraction 30 for CR8#13 treated cells and fraction 35 for F07#13 treated cells. It is also to note that after treatment, the dynamics of the cells change as more actin is expressed in lower molecular weight fractions. The results point to the fact that the pTEF-b complex is sequestered in infected cells by the Hexim-1 containing 7 SK-RNA complex. When treated with transcription inhibitors, the pTEF-b complex is freed causing it to move mostly to the large complex. Since these J1.1 cells continuously produce virus, it is likely

that in some of the cells, the pTEF-b complex is sequestered to promote latency. This might be the reason for the distribution of Hexim1 in various fractions. When the cells are treated, we notice that Hexim1 is pushed to the middle thus freeing the complex to promote transcription. The fact that cdk9 and Cyclin-T1 are pushed into different complexes after treatment means that the drugs are inducing distinct conformational changes to the p-TEFb complex. cdk9 in some instances goes on to phosphorylate other proteins in other complexes, and the p-TEFb is pushed to the large complexes were we found most chromatin remodeling complexes.

The existence of a Novel HIV RNA transcript.

Recent discoveries have shown that cells ~~do~~ have the ability to control gene expression and transcription through short and long RNA molecules that are not transcribed. In HIV-infected cells, many transcription inhibition pathways exist to promote latency of the virus. Recently, a cellular lncRNA, NRON, was described as a key latency promoter (Imam et al., 2015; Li et al., 2016). Here, we try to find possible viral RNA transcripts that could promote HIV latency due to their non-coding activity. Therefore, it is important that the new transcript does not translate in uninfected cells. We have previously shown that HIV-infected cells exosomes contain viral RNA particles (Ahsan et al., 2016). We then grew and isolated exosomes from J1.1 HIV-infected cells to detect the various viral RNA transcripts they contain. We utilized nanoparticles NT080/082 to trap and concentrate exosomes followed by RNA isolation and analysis. Total exosomal RNA was subjected to RT with primers specific for the 3' end, *env*, *pol*, *gag*, U5, and the R region (TAR) (Table-1). The results in figure 7-A show the existence

of a novel RNA transcript, TAR-gag (p17). It represents 13.4% of the RNA transcript found in exosomes and contains 30% of the TAR available in the cell. We investigated further to determine whether the novel transcript is a non-coding RNA. We then grew infected (ACH2) and uninfected (Jurkat, and U937) cells in exosome free media. We treated Jurkat and U937 cells with either ACH2/U1 supernatant alone or ACH2/U1 exosomes concentrated by NT080/082 beads. The cells were treated for 3 days at 37ºC and were lysed and processed for western blot analysis using p17 antibody. It is to note that the ACH2 and U1 cells were under c-ART treatment. As controls, we used uninfected and infected cell lysates (Lanes 1 and 2). Additional controls included the use of infected supernatants either alone or concentrated with nanoparticles for the presence of p17 (Lanes 3 and 4). Results in figure7-B indicate a presence of p17 in ACH2 cells (lanes 2). Supernatants from do not contain much of p17 (lanes 3), but nanoparticle concentration of exosomes from these supernatants reveal the presence of p17 in ACH2 cells. It is to note that our nanoparticles do not concentrate exosomes only, but can also carry some virus. Hence, the levels of p17, p24 in exosomes concentrated are considered as background levels. After treatment of uninfected U937 and Jurkat cells with staright supernatants, we see that p17 and p24 levels were lower than our background. Similarly, the uninfected cells did not increase levels of p17 or p24 (lane 6) when treated with 1ml of nanoparticle concentrated supernatants. We deduce that p17 and p24 are not translated in the uninfected cells when they are treated with exosomes from infected cells. It is clearer in U937 cells where the levels of p17 and p24 actually drop. In all, our results suggest that TAR-*gag* is not translated and is a non-coding RNA.

Table 1. List of primer and Probe Sequence.

Primer/Probe name	Primer/Probe Sequence	Location in HIV-1 genome or human gene
TAR-Ang-R/TAR+59R*	5'- CAA CAG ACG GGC ACA CAC TAC -3'	99-119
Oligo-dT*	5'-TTT TTT TTT TTT TTT TTT TTT TTT T-3'	5' end
TARfII-F	5'- GGT CTC TCT GGT TAG ACC AGA TCT G -3'	1-25
TAR-Prb	5'- /56-FAM/AGC CTC AAT AAA GCT TGC CTT GAG TGC TTC/36-TAMSp/ -3'	9152-9181
Gag 1483F	5'- AGG GGG AAG TGA CAT AGC AG-3'	1028-1047
Gag 1625R	5'- GCT GGT AGG GCT ATA CAT TCT TAC -3'	1155-1178
GAG1-F	5'- TCA GCC CAG AAG TAA TAC CCA TGT -3'	835-849
GAG2-R	5'- CAC TGT GTT TAG CAT GGT GTT T -3'	886-909
GAG3-Prb	5'- FAM-ATT ATC AGA AGG AGC CAC CCC ACA AGA-TAMRA -3'	857-882
Mf83	5'- GGA TCT GTC TCT GTC TCT CTC TCC ACC -3'	7978-8005
Mf84	5'- ACA GTC AGA CTC ATC AAG TTT CTC TAT CAA AGC A -3'	5558-5590
Ks2-tq	5'- FAM-TTC CTT CGG GCC TGT CGG GTC CC-TAMRA -3'	7945-7969
5T25*	5'- TTT TTT TTT TTT TTT TTT TTT TTT TTG AAG -3'	9178+
LTR+341-R*	5'- ACC CAT CTC TCT CCT TCT AGC C -3'	320-341
Vpr-R208	5'- TAA ACG GCA GTT GTT GCA GA -3'	5293-5312
Env-2187R	5'-TGG GAT AAG GGT CTG AAA CG-3'	7917-7936

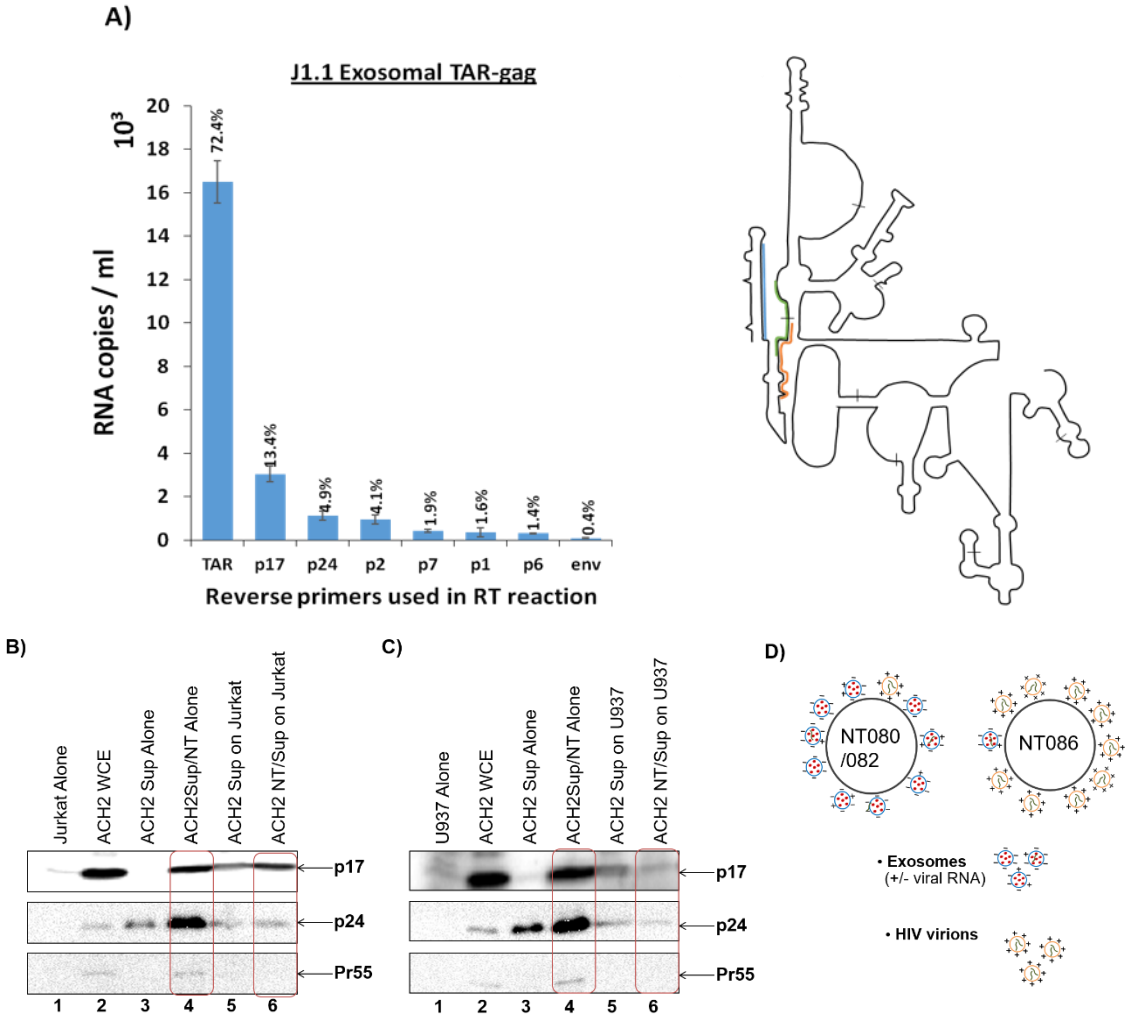

Figure 7. TAR-*gag* is a possible long non-coding RNA.
A) U1, OM10, J1.1, and ACH2 cells were grown for 4 days. The cell supernatant was collected, filtered through 0.22 µm filter, and incubated with NT080/082 overnight. Total RNA was isolated from the nanoparticles, subjected to RT with primers specific for TAR, LTR, *gag*, *pol*, *env*, and the 3' end of the HIV-1 genome. RT-qPCR was performed with primers specific for TAR (with the 3' RT, *gag* primers were used for qPCR). The structure of TAR-gag is represented based on HIV-1 gene structure. B) ACH2 cells and U1 cells were pre-treated for five days with a cART cocktail consisting of equal parts of indinavir, LAM, TDF, and FTC at 10 µM. 1 mL of cell supernatant was collected and treated with a 30% NT080/082 slurry before rotating 16 hours at 4° C to isolate exosomes. 0.750 mL of fresh Jurkat cells and 1 mL of fresh U937 cells were plated and treated with either 1 mL of cell supernatant from ACH2 cell culture or concentrated ACH2 exosomes collected from the NT080/082 particles. The cells were then incubated for 72 hours before being harvested and lysed. The resulting Jurkat and U937 cell lysates were run on a Western blot for p17, p24, Pr55.

TAR-gag is associated with Chromatin remodeling complexes.

From the above experiment, we noticed that TAR-gag is a non-coding RNA. Previous researchers have shown that SWI/SNF CRCs are implicated in the formation of lncRNA-dependant nuclear bodies in mammals (Kawaguchi et al., 2015). Here we wanted to study its interaction of TAR-gag with transcription inhibition proteins of the SWI/SNF CRCs. In figure 8, we immunoprecipitated different chromatin remodeling complex proteins from HIV J1.1 whole cell extracts. We washed the samples twice with TNE50+01%NP40; then, performed RT-PCR for TAR and TAR-gag RNA copies to determine if the RNA transcripts are associated with CRCs and to investigate which of the SWI/SNF BAFs are associated with TAR. Interestingly, we found high levels of TAR and TAR gag in msin3A, HDAC1, PIWI, PRMT-5, Baf200, and BRG-1 samples. The HA-probe was used as negative control for background. Based on background levels, Baf170 and Baf 250 do not directly interact with TAR. We concluded that TAR and TAR-gag are associated with CRCs, but more precisely with the SWI/SNF transcription inhibitor complex msin3A/HDAC. This hints to a possible mechanism involving TAR-gag and chromatin remodeling complexes for controlling gene transcription in HIV-infected cells.

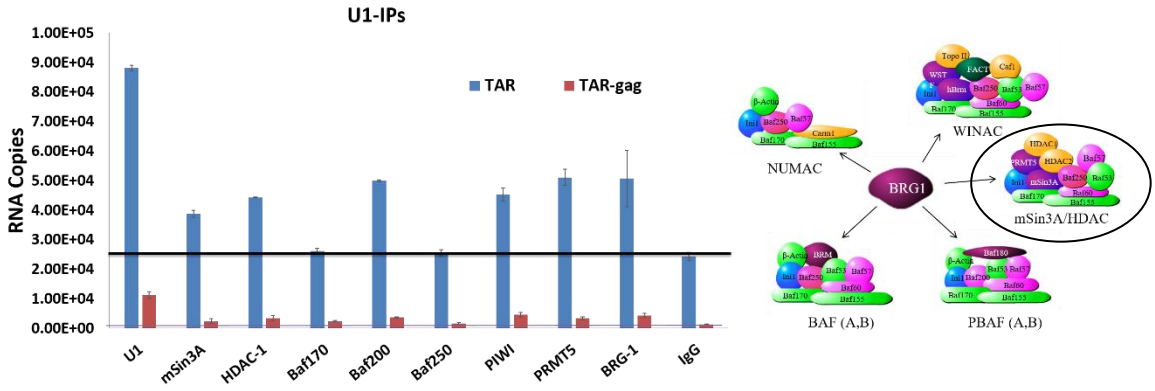

Figure 8. TAR and TAR-gag interaction with CRCs.
Total cell extracts from U1 (500µg) were immunoprecipitated with 25µg of antibodies mSin3A, HDAC-1, Baf170, Baf200, Baf250, PIWI, PRMT5, BRG-1, and IgG using 25 µg of protein A+G. The samples were washed twice with TNE50+0.1%NP40 and processed for total RNA isolation. TAR and TAR-gag reverse primers were used to PCR and find RNA copy numbers. U1 WCE was used as a positive control.

TAR-gag interaction with CRCs after treatment with transcription inhibition drugs.

Since TAR-gag may regulate transcription through interaction with CRCs, we decided to study the impact of transcription inhibition drug on TAR-gag interaction with proteins of the msin3A/HDAC complex. We used LAI infected Jurkat (J1-1) cells at early mid-log phase of growth and prepared total protein using Lysate buffer after the cells were treated with either CR8#13 or F07#13 for 48 hours. The cell extracts were then immunoprecipitated for Dnmt3A, PIWI, HDAC, and mSin3A. They were washed twice with TNE 50+0.1 %NP-40 before RNA isolation. We used RT-PCR to quantify the number of TAR and TAR-gag RNA copies. In figure 9, Dnmt3A, which is not part of any

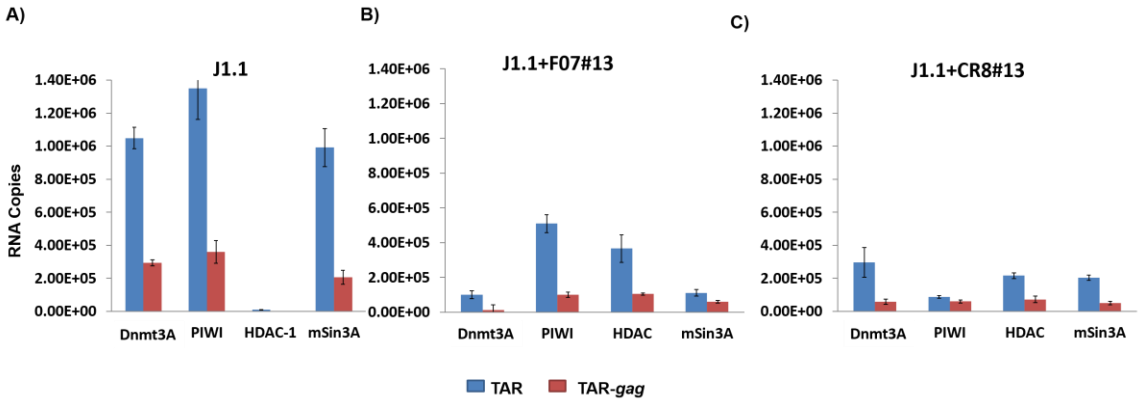

Figure 9. TAR and TAR-gag RNA copies after treatment.
Total cell extracts from J1-1 treated with F07#13, and J1-1 treated with CR8#13 (500µg) were immunoprecipitated with 25µg of antibodies mSin3A, HDAC-1, PIWI, and Dnmt3A using 25 µg of protein A+G. The samples were washed twice with TNE50+0.1%NP40 and processed for total RNA isolation. TAR and TAR-gag reverse primers were used to PCR and find RNA copy numbers.

chromatin remodeling complex recruits different numbers of TAR and TAR-gag RNA copies after treatment. We saw high RNA copies in PIWI and HDAC IPs when the cells are treated with F07#13, but high RNA copy numbers in HDAC and mSin3A IPs. The data indicate that treatment of HIV-infected cells with F07#13 favors an interaction between TAR-gag, HDAC, and PIWI. Alternatively, CR8#13 favors a TAR-gag, HDAC, and mSin3A interaction.

TAR-gag is associated with the large complex of treated HIV cells.

We have previously reported that transcription inhibition drugs CR8#13 and F07#13 independently regulate pTEF-b. We have observed that the complex is pushed toward the left side of the column in treated cells. We then wondered whether TAR and TAR-gag are also present on the left end of the column associated with the large complexes when infected cells are treated. We took J1-1 HIV-infected cells at early mid-log phase of growth, treated them with F07#13 for 48 hours, and prepared total protein

using Lysate buffer. The samples were then loaded onto a sizing column in the presence of high salt to minimize non-specific binding (500 mM NaCl). Total RNA from the FPLC fractions was isolated followed by an RT-PCR for TAR or TAR-gag. Since F07#13 is a transcription inhibitor, we expect lower copies of RNAs in treated cells. We observed a 25% drop in total RNA isolated from all the fractions combined and a 15 % drop in total TAR RNA isolated (data not shown). Results in figure 10 show the presence of TAR in all the fractions. However, they are present in high copies in fractions 15-30. Our data show that TAR is low in fractions 10, 35-55. After treatment with F07#13 the percent of TAR is consistent on the left side of the column, and part of the large complex. We have previously shown that the cdk9 of pTEF-b phosphorylates SWI/SNF BAF complexes. Since the inhibition of pTEF-b by the drug does not disturb TAR distributions, we believe that F07#13 will not prevent recruitment of pTEF-b by TAR, and will not phosphorylate the mSin3A/HDAC SWI/SNF complex that TAR-gag binds.

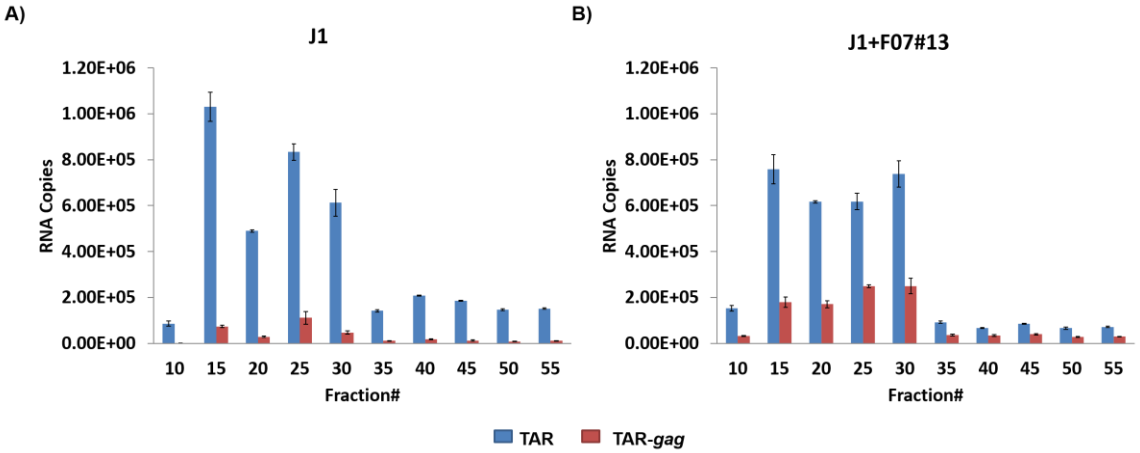

Figure 10. TAR and TAR-*gag* RNA distribution in various fractions of J1-1 untreated and F07#13 treated cells. Total cell extracts from J1-1 and F07#13 treated J1-1 cells were processed over a sizing column in the presence of 500 mM salt. Every fifth fraction was processed for total RNA isolation. TAR reverse primers were used to PCR and find RNA copy numbers. The percent of each copy number in each fraction based on total TAR RNA isolated is graphed.

DISCUSSION

Over the past two decades, remarkable progress has been made in the study of HIV-1 virology and pathogenesis. The understanding of how HIV establishes infection and causes AIDS is crucial to defining strategies for the diagnosis of HIV infection. A better understanding of the virus's structure and mechanisms of infection facilitates the identification and development of effective drugs and vaccines. New concerns have risen due to the appearance of viral subtypes and recombinant forms since some of them have shown differences in the infection cycle. These differences will be a key factor to consider when designing new preventive and therapeutic approaches aimed at the HIV life cycle.

Here, we show that in cell culture systems, the 3rd generation derivatives of R-roscovitine, CR8#13 and F07#13 effectively inhibit HIV-1 replication. We have subjected three different clades and one recombinant form of the virus to a treatment with CR8#13. We noticed that CR8#13 inhibits in most clades, but is more effective in some clades compared others (Figure 3). It is currently known that the LTR and Tat from these clades have slight nucleotide changes and increased lysine residues (except in clade C). Consequently, complexes that are not always dependent on cdk9/T1 activity may be required for regulating the LTR unless the transcriptional machinery is different in some clades like C. This might explain the difference in the rate transcription, splicing events

and varying sensitivity to ATP analogs. We then try to understand the difference in the transcriptional machinery among these clades. Through CR8#13 interaction, elongation does not proceed to completion in clades A and B, contrary to clade C where H1 is not inhibited. Based on Figure 4-B, CR8#13 not having an effect on histone H1 means that the pTEF-b complex or more precisely the cdk9 might be different in clade C.

In most HIV-infected cells, CRCs are also participants in transcription regulation. We then show in figure 5 that on the DNA, Baf170 is initially present. After treatment with p-TEF-b inhibitors, which should inhibit ckd9, Baf170 is not present. This hints that cdk9/Cylcin-T might phosphorylate Baf170 or one of the components in its complex. This is unique because it has not been previously shown that cdk9 is capable of phosphorylating components of SWI/SNF complex. From there, we wanted to find how CR8#13 and F07#13 affect the p-TEF-b complex throughout the cell. To do so, we used FPLC to break up complexes based on their sizes and track the presence of p-TEF-b via western blot. We noticed that the pTEF-b appears in the low molecular weight complexes. On the other hand, little change was observed in the distribution of the TAR-RNA hinting that pTEF-b might no phosphorylate the HDAC/mSin3A complex, but some other complex. Further work will focus on TAR-gag specifically to understand how TAR-gag reacts to F07#13. In fact, when we isolated exosomes from infected cells, concentrated them, and subjected them to RT-PCR, we discovered the existence of a novel viral non-coding RNA named TAR-gag. When uninfected cells were subjected to exosomes containing TAR-gag, they did not translate TAR-gag into a protein.

It was previously noted that cdk9 interacts with SWI/SNF chromatin remodelers. Similarly, non-coding RNAs were observed to interact with SWI/SNF chromatin remodelers. We then followed up to determine which of the BAF complexes is associated with TAR-gag. We concluded that TAR and TAR-gag are associated with CRCs, but more precisely with the SWI/SNF transcription inhibitor complex msin3A/HDAC. This hinted to the involvement of chromatin remodeling complexes in the gene transcription controlling function of TAR-gag in HIV-infected cells. After treatment of infected cells with F07#13 and CR8#13, we noted that both drugs differentially control TAR-gag's interaction with CRCs. We saw that treatment of HIV-infected cells with F07#13 favors an interaction between TAR-gag, HDAC, and PIWI, while CR8#13 favors a TAR-gag, HDAC, and mSin3A interaction. Given that TAR-gag is not translated and is increased by HIV transcription inhibiting drugs, the data suggest that it is a viral non-coding RNA that contributes to viral latency.

REFERENCES

Ahsan, N. A., Sampey, G. C., Lepene, B., Akpamagbo, Y., Barclay, R. A., Iordanskiy, S., … Kashanchi, F. (2016). Presence of Viral RNA and Proteins in Exosomes from Cellular Clones Resistant to Rift Valley Fever Virus Infection. *Frontiers in Microbiology*, *7*. http://doi.org/10.3389/fmicb.2016.00139

Archin, N. M., Sung, J. M., Garrido, C., Soriano-Sarabia, N., & Margolis, D. M. (2014a). Eradicating HIV-1 infection: seeking to clear a persistent pathogen. *Nature Reviews. Microbiology*, *12*(11), 750–764. http://doi.org/10.1038/nrmicro3352

Archin, N. M., Sung, J. M., Garrido, C., Soriano-Sarabia, N., & Margolis, D. M. (2014b). Eradicating HIV-1 infection: seeking to clear a persistent pathogen. *Nature Reviews Microbiology*, *12*(11), 750–764. http://doi.org/10.1038/nrmicro3352

Becker, Y., & Hadar, J. (1983). Double-Stranded RNA Viruses Made from Single-Stranded RNA: Reoviruses. In J. Hadar (Ed.), *Molecular Virology* (pp. 139–143). Springer US. Retrieved from http://link.springer.com/chapter/10.1007/978-1-4613-3906-9_12

Clercq, E. D. (2007). The design of drugs for HIV and HCV. *Nature Reviews Drug Discovery*, *6*(12), 1001–1018. http://doi.org/10.1038/nrd2424

Coffin, J. M., Hughes, S. H., & Varmus, H. E. (Eds.). (1997). *Retroviruses*. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK19376/

Contreras, X., Barboric, M., Lenasi, T., & Peterlin, B. M. (2007). HMBA Releases P-TEFb from HEXIM1 and 7SK snRNA via PI3K/Akt and Activates HIV Transcription. *PLoS Pathogens*, *3*(10). http://doi.org/10.1371/journal.ppat.0030146

De Crignis, E., & Mahmoudi, T. (2014). HIV eradication: combinatorial approaches to activate latent viruses. *Viruses*, *6*(11), 4581–4608. http://doi.org/10.3390/v6114581

Easley, R., Carpio, L., Guendel, I., Klase, Z., Choi, S., Kehn-Hall, K., … Kashanchi, F. (2010). Human T-Lymphotropic Virus Type 1 Transcription and Chromatin-Remodeling Complexes. *Journal of Virology*, *84*(9), 4755–4768. http://doi.org/10.1128/JVI.00851-09

Fan, H.-Y., Narlikar, G. J., & Kingston, R. E. (2004). Noncovalent modification of chromatin: different remodeled products with different ATPase domains. *Cold Spring Harbor Symposia on Quantitative Biology*, *69*, 183–192. http://doi.org/10.1101/sqb.2004.69.183

Geisler, S., & Coller, J. (2013). RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. *Nature Reviews Molecular Cell Biology*, *14*(11), 699–712. http://doi.org/10.1038/nrm3679

Imam, H., Shahr Bano, A., Patel, P., Holla, P., & Jameel, S. (2015). The lncRNA NRON modulates HIV-1 replication in a NFAT-dependent manner and is differentially regulated by early and late viral proteins. *Scientific Reports, 5*. http://doi.org/10.1038/srep08639

Jeeninga, R. E., Hoogenkamp, M., Armand-Ugon, M., de Baar, M., Verhoef, K., & Berkhout, B. (2000). Functional differences between the long terminal repeat transcriptional promoters of human immunodeficiency virus type 1 subtypes A through G. *Journal of Virology, 74*(8), 3740–3751.

Kawaguchi, T., Tanigawa, A., Naganuma, T., Ohkawa, Y., Souquere, S., Pierron, G., & Hirose, T. (2015). SWI/SNF chromatin-remodeling complexes function in noncoding RNA-dependent assembly of nuclear bodies. *Proceedings of the National Academy of Sciences, 112*(14), 4304–4309. http://doi.org/10.1073/pnas.1423819112

Koulinska, I. N., Ndung'u, T., Mwakagile, D., Msamanga, G., Kagoma, C., Fawzi, W., … Renjifo, B. (2001). A New Human Immunodeficiency Virus Type 1 Circulating Recombinant Form from Tanzania. *AIDS Research and Human Retroviruses, 17*(5), 423–431. http://doi.org/10.1089/088922201750102508

Le Douce, V., Herbein, G., Rohr, O., & Schwartz, C. (2010). Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. *Retrovirology, 7*, 32. http://doi.org/10.1186/1742-4690-7-32

Li, J., Chen, C., Ma, X., Geng, G., Liu, B., Zhang, Y., … Zhang, H. (2016). Long noncoding RNA NRON contributes to HIV-1 latency by specifically inducing tat

protein degradation. *Nature Communications*, *7*, 11730. http://doi.org/10.1038/ncomms11730

Liu, N., Balliano, A., & Hayes, J. J. (2011). Mechanism(s) of SWI/SNF-induced nucleosome mobilization. *Chembiochem: A European Journal of Chemical Biology*, *12*(2), 196–204. http://doi.org/10.1002/cbic.201000455

Lycke, E., & Norrby, E. (1983). 1 - Viruses – a unique kind of infectious agent. In *Textbook of Medical Virology* (pp. 1–3). Butterworth-Heinemann. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780407002531500062

Modrow, S., Falke, D., Truyen, U., & Schätzl, H. (2013). Viruses with Single-Stranded RNA Genomes and Double-Stranded DNA as an Intermediate Product. In *Molecular Virology* (pp. 555–624). Springer Berlin Heidelberg. Retrieved from http://link.springer.com/referenceworkentry/10.1007/978-3-642-20718-1_18

Narayanan, A., Kehn-Hall, K., Senina, S., Lundberg, L., Duyne, R. V., Guendel, I., … Kashanchi, F. (2012). Curcumin Inhibits Rift Valley Fever Virus Replication in Human Cells. *Journal of Biological Chemistry*, *287*(40), 33198–33214. http://doi.org/10.1074/jbc.M112.356535

Narayanan, A., Sampey, G., Van Duyne, R., Guendel, I., Kehn-Hall, K., Roman, J., … Kashanchi, F. (2012). Use of ATP analogs to inhibit HIV-1 transcription. *Virology*, *432*(1), 219–231. http://doi.org/10.1016/j.virol.2012.06.007

Narlikar, G. J., Fan, H.-Y., & Kingston, R. E. (2002). Cooperation between complexes that regulate chromatin structure and transcription. *Cell*, *108*(4), 475–487.

Palmenberg, A. C., & Sgro, J.-Y. (2005). Virus Particle Structures. In *Virus Taxonomy* (pp. 19–22). San Diego: Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780122499517500080

Peeters, M., Toure-Kane, C., & Nkengasong, J. N. (2003). Genetic diversity of HIV in Africa: impact on diagnosis, treatment, vaccine development, and trials. *AIDS (London, England)*, *17*(18), 2547–2560. http://doi.org/10.1097/01.aids.0000096895.73209.89

Roof, P., Ricci, M., Genin, P., Montano, M. A., Essex, M., Wainberg, M. A., … Hiscott, J. (2002). Differential Regulation of HIV-1 Clade-Specific B, C, and E Long Terminal Repeats by NF-κB and the Tat Transactivator. *Virology*, *296*(1), 77–83. http://doi.org/10.1006/viro.2001.1397

Van Duyne, R., Guendel, I., Narayanan, A., Gregg, E., Shafagati, N., Tyagi, M., … Kashanchi, F. (2011). Varying modulation of HIV-1 LTR activity by BAF complexes. *Journal of Molecular Biology*, *411*(3), 581–596. http://doi.org/10.1016/j.jmb.2011.06.001

Van Duyne, R., Guendel, I., Narayanan, A., Gregg, E., Shafagati, N., Tyagi, M., … Kashanchi, F. (2011). Varying modulation of HIV-1 LTR activity by Baf complexes. *Journal of Molecular Biology*, *411*(3), 581–596. http://doi.org/10.1016/j.jmb.2011.06.001

Van Duyne, R., Guendel, I., Narayanan, A., Gregg, E., Shafagati, N., Tyagi, M., … Kashanchi, F. (2011). Varying modulation of HIV-1 LTR activity by BAF

complexes. *Journal of Molecular Biology*, *411*(3), 581–596. http://doi.org/10.1016/j.jmb.2011.06.001

Zhang, J., Scadden, D. T., & Crumpacker, C. S. (2007). Primitive hematopoietic cells resist HIV-1 infection via p21. *The Journal of Clinical Investigation*, *117*(2), 473–481. http://doi.org/10.1172/JCI28971

BIOGRAPHY

Yao A. Akpamagbo graduated from Saint Joseph High School, Lome, Togo. He received his Associates of Science from Tacoma Community College and his Bachelor of Science from Hampton University in 2014.