Bcl11b, a T-cell commitment factor, and its role in human immunodeficiency virus-1 transcription

Woerner, Andrew James

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Thesis

BCL11B, A T-CELL COMMITMENT FACTOR, AND ITS ROLE IN HUMAN IMMUNODEFICIENCY VIRUS-1 TRANSCRIPTION

by

ANDREW J. WOERNER
B.S., San Diego State University, 2011

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First Reader

Andrew J. Henderson, Ph.D.
Associate Professor, Medicine and Microbiology

Second Reader

Theresa A. Davies, Ph.D.
Director, M.S. in Oral Health Sciences Program
Adjunct Professor of Biochemistry
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ADVANCES OF ANTIRETROVIRAL THERAPIES (ART) HAVE MADE SIGNIFICANT STRIDES IN REDUCING HUMAN IMMUNODEFICIENCY VIRUS (HIV) VIRAL LOADS IN PATIENTS TO UNDETECTABLE LEVELS. UPON INTERRUPTION OF ART, VIRAL LOAD REBOUNDS AND AIDS SYMPTOMS RETURN. LATENT RESERVOIRS OF VIRUS ARE RESPONSIBLE FOR THIS PHENOMENON BECAUSE THEY CONTAIN INTEGRATED PROVIRUS, WHICH IS TRANSCRIPTIONALLY SILENT, THUS UNAFFECTED BY ART AND HIDDEN FROM HOST IMMUNE SURVEILLANCE. A COMMONLY PROPOSED MECHANISM FOR HIV LATENCY IS THE PRESENCE OF HOST CELL TRANSCRIPTION FACTORS THAT LEAD TO TRANSCRIPTIONAL SILENCING. CD4+ T CELLS AND OTHER IMMUNE CELLS, WHETHER DUE TO THEIR SUBSET PHENOTYPE, ACTIVATION STATE, OR STAGE IN DEVELOPMENT, WILL VARY IN THEIR BATTERY OF TRANSCRIPTION FACTORS. OF PARTICULAR INTEREST IS BCL11B, A CRITICAL TRANSCRIPTION FACTOR INVOLVED IN THE COMMITMENT TO A T-CELL FATE DURING THYMOCYTE DEVELOPMENT THAT HAS RECENTLY BEEN SHOWN TO PLAY A ROLE IN SILENCING HIV-1 TRANSCRIPTION.

BCL11B IS REQUIRED FOR INHIBITING THE DEVELOPMENT OF NATURAL KILLER CELL-LIKE traits during the early development of T cells. The repressive role of this zinc-finger transcription factor has recently been shown to inhibit HIV-1 transcription in the context of microglial cells via recruitment of chromatin remodeling factors. Also, BCL11B has been shown to interact with other HIV-1 transcriptional silencing
factors such as NuRD and NCoR. Preliminary mass spectrophotometry results have pointed to a physical interaction of Bcl11b with NELF, another proven repressive factor of HIV transcription. We hypothesize that Bcl11b represses HIV transcription and is recruited to the HIV-1 long terminal repeat (LTR) through a paused RNA polymerase II complex, contributing to the establishment and maintenance of latency. Our studies confirm Bcl11b’s repressive role in T cells, and investigate the mechanism with NELF.

Transfection of HEK293T cells with HIV-LUC shows nearly 50% reduction in HIV transcription in the presence of Bcl11b, and analysis of viral protein output by p24 ELISA confirms this result. Furthermore, when co-transfected with NELF-B, the two transcription factors lead to nearly 90% reduction in HIV transcription. Results suggest that these factors cooperate to repress HIV transcriptional elongation. Protein and chromatin immunoprecipitations (ChIP) were also performed to see a direct interaction between the two transcription factors and the HIV LTR. Physical interaction of the two factors was not witnessed, while ChIP analysis shows enrichment of RNA polymerase II at the transcriptional start site suggesting Bcl11b increasing RNA polymerase II pausing. We conclude that Bcl11b plays a repressive role in HIV transcription through promoter-proximal pausing with a synergistic effect with NELF, but a yet to be identified factor is responsible for the coordination of the two factors.

As an important T-cell commitment factor, Bcl11b may play an important role in establishing and maintaining cellular latency through transcriptional
repression via a complex with NELF. Confirming Bcl11b’s role as a repressive transcription factor and providing further support for a synergistic role with NELF, could highlight a new target for therapeutic strategies against the elusive latent reservoir.
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<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
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<td>ART</td>
<td>Antiretroviral therapy</td>
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<tr>
<td>AZT</td>
<td>Azidothymidine</td>
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<tr>
<td>Bcl11b</td>
<td>B-cell lymphoma-leukemia 11b protein</td>
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<tr>
<td>bp</td>
<td>Base pairs</td>
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<tr>
<td>cART</td>
<td>Combined antiretroviral therapy</td>
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<tr>
<td>CCR5</td>
<td>C-C chemokine receptor type 5</td>
<td></td>
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<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
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<tr>
<td>cDMEM</td>
<td>Complete Dulbecco’s Modified Eagle Medium</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
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<tr>
<td>ChIP</td>
<td>Chromatin immunoprecipitation</td>
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<tr>
<td>COUP-TF</td>
<td>Chicken ovalbumin upstream promoter transcription factor</td>
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<td>CXCR4</td>
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<tr>
<td>DN</td>
<td>Double negative</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DP</td>
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<tr>
<td>DRB</td>
<td>DRB sensitivity inducing factor</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>Env</td>
<td>HIV viral envelope protein</td>
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<tr>
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<td>--------------</td>
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</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
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<td>Gp41</td>
<td>Glycoprotein 41</td>
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<tr>
<td>Gp120</td>
<td>Glycoprotein 120</td>
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<td>Gps2</td>
<td>G-protein pathway suppressor 2</td>
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<td>HBS</td>
<td>Hepes-buffered saline</td>
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<td>HDAC</td>
<td>Histone deacetylase</td>
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<tr>
<td>HEK</td>
<td>Human embryonic kidney</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HRP</td>
<td>Horseradish peroxidase</td>
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<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IP</td>
<td>Immunoprecipitation</td>
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<td>ITNK</td>
<td>Induced-T-to-Natural Killer</td>
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<tr>
<td>LSD1</td>
<td>Lysine-specific demethylase 1</td>
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<td>LTR</td>
<td>Long terminal repeat</td>
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<td>LUC</td>
<td>Luciferase</td>
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<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
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<tr>
<td>NCoR</td>
<td>Nuclear receptor co-repressor</td>
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<tr>
<td>Nef</td>
<td>Negative regulatory factor</td>
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<td>NELF-B</td>
<td>Negative elongation factor B</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa-light-chain-enhancer of activated B cells</td>
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<td>NIAID</td>
<td>National Institute of Allergy and Infectious Diseases</td>
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NuRD ................................................................. Nucleosome remodeling deacetylase

P-TEFb .............................................................. Positive transcription elongation factor b

PBS ................................................................. Phosphate-buffered saline

PBST .............................................................. Phosphate-buffered saline with Tween 20

Pcf11 .............................................................. Pre-mRNA-cleavage complex 11 factor

PMA ................................................................. 12-myristate 13-acetate

PVDF .............................................................. Polyvinylidene fluoride

RNA ................................................................. Ribonucleic acid

RPMI .............................................................. Roswell Park Memorial Institute

RT-PCR .......................................................... Reverse-transcription polymerase chain reaction

SDS-PAGE ............... Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

shBcl11b ................................................................. Small hairpin-Bcl11b

TAR ................................................................. Tat activating region

Tat ................................................................. Trans-activator of transcription

TCR ................................................................. T-cell receptor

WHO .............................................................. World Health Organization

X-OMAT ............................................................. Automatic processor for X-ray film
INTRODUCTION

There are over 34 million individuals worldwide currently infected with HIV, the virus that leads to acquired immunodeficiency syndrome (AIDS), and HIV is responsible for over 25 million deaths (“Global Statistics,” n.d.). The number of annual fatalities peaked in 2005 at 2.3 million, and has been in a steady decline ever since. The decline is attributed to a decrease in newly infected individuals, and more importantly, the increased availability of antiretroviral therapy (ART) (“WHO | Number of deaths due to HIV/AIDS,” n.d.). HIV infection is becoming a more manageable chronic condition; however, average life expectancy is still over 15 years less than the general population and 6,300 individuals continue to be infected each day (“amfAR: Statistics: Worldwide: The Foundation for AIDS Research: HIV / AIDS Research,” n.d.; Samji et al., 2013). Given these continued drastic statistics, there is no doubt to the global impact of this disease from a public health and research standpoint.

The global AIDS epidemic came onto the scene in 1981 via the presentation of immunosuppressed individuals with depletion of CD4+ T lymphocytes suffering from a variety of opportunistic infections and diseases, such as Kaposi’s sarcoma and Pneumocystis pneumonia. Two years later, researchers found the cause of AIDS to be HIV, and in 1987 zidovudine (or azidothymidine (AZT)) was introduced as the first ART (Adler, Edwards, & Miller, 2012). HIV belongs to the Retroviridae family of viruses, characterized by the
reverse transcription of a ribonucleic acid (RNA) viral genome into proviral deoxyribonucleic acid (DNA). The process of reverse transcription is dependent on the virally encoded enzyme, reverse transcriptase ("Human Immunodeficiency Virus (HIV)," 2007). The activity of this enzyme serves as the active target of the previously mentioned AZT. Further development of ART, with greater efficacy and a lower pill-burden has significantly reduced AIDS-related morbidities and mortalities; however, silent reservoirs of the virus exist in infected individuals that remain insensitive to ART provide a barrier to complete eradication (Lint, Bouchat, & Marcello, 2013).

**Characteristics and Life Cycle of HIV**

HIV is an 80-120 nm enveloped icosahedral sphere (a solid with 20 plane faces) containing a cone-shaped protein core, the surrounding capsid, and a two-layered envelope ("Human Immunodeficiency Virus (HIV)," 2007) (see Figure 1). The viral genetic material, ribonucleic acid (RNA), necessary viral enzymes (reverse transcriptase, integrase, and protease), and other viral proteins are included within the capsid. The virus’ envelope is a host-derived phospholipid bilayer that is acquired when it egressed from the previously infected cell. Within the envelope are important viral glycoproteins necessary to propagate infection ("HIV/AIDS Fact Sheets," n.d.).

HIV entry is the first phase of the replication cycle, step 1 (see Figure 2). This process starts with the adhesion of the virus to a host cell, and concludes
with fusion of viral and host membranes primed for delivery of the viral capsid. Initial interaction with the cell is mediated through the viral envelope (Env) protein, or host cell proteins that were incorporated into the virion’s membrane from a previous round of replication. Interactions may be nonspecific, such as Env interacting with negatively charged cell-surface heparin sulfate proteoglycans (Saphire, Bobardt, Zhang, David, & Gallay, 2001), or specific through attachment to pattern recognition receptors (Geijtenbeek et al., 2000). Initial interactions of the virus to host cells are neither essential nor necessary for infection, but the weak binding events can facilitate the proximity Env to CD4 receptor and chemokine receptors, the primary receptor complex for HIV entry, increasing the overall efficiency of transmission. Env is a trimer of gp120 and gp41 heterodimers. The gp120 subunit, which contains the CD4 binding site, is composed of five conserved domains and five variable loops; these variable loops assist in immune evasion and coreceptor binding. When Env binds CD4, there is a rearrangement of the variable regions of gp120, facilitating coreceptor binding (Wilen, Tilton, & Doms, 2012). Env interacts with the coreceptors CXCR4 or CCR5, depending on the tropism of the virus. Interaction with the coreceptor induces exposure of the gp41 fusion peptide that then inserts into the host cell membrane and facilitates fusion (step 2) (Wilen et al., 2012). Cell fusion allows for viral capsid entry into the cytoplasm.
Figure 1. HIV Structure: Overview of the virus structure, including: the viral capsid with associated viral RNA, reverse transcriptase, and integrase; and the viral envelope containing gp41 and gp120. Original image from: National Institute of Allergy and Infectious Diseases (NIAID).

Once inside the host cell, the viral RNA genome must be reverse transcribed into DNA (step 3). HIV reverse transcriptase carries out the process of reverse transcribing a single stranded RNA into a double stranded DNA, which will be translocated into the host cell nucleus. For step 4, the viral DNA is inserted into the host genome via another viral enzyme of the viral capsid, integrase. Once integrated into the host cell DNA, HIV can be replicated as part of the host genome during cell division or transcribed to generate viral RNA (step
5). In step 6 an HIV protease is responsible for post-translationally modifying the translated viral Gag polypeptides; this is a critical step in maturation of the budding virion, enhancing its infectivity (Kohl et al., 1988). The viral particle now buds from the cell, capturing fragments of the host cell membrane as it egresses from the cell (step 7).

Most of the aforementioned steps have been potential therapeutic targets. For example, the first approved HIV therapy, AZT, is a nucleoside base analog of thymine that, when incorporated during reverse transcription, will cause premature chain termination (Bozzi, D’Andrea, & Brisdelli, 2008). Other drugs include, non-nucleoside reverse transcriptase inhibitors, entry inhibitors, fusion inhibitors, integrase inhibitors, and protease inhibitors. Having multiple targets in the HIV life cycle has opened the door to combined antiretroviral therapy (cART). cART is the use of two or more antiretroviral therapies from more than one class of target sites. This strategy provides a more efficient method of decreasing patient viral load and preserving immune function (Wood, 1996). The use of more than one approved drug also increases the difficulty for the virus to develop drug resistance (“What Is Antiretroviral Therapy (ART)? | aidsinfonet.org | The AIDS InfoNet,” n.d.)
Figure 2: HIV Life Cycle. Depicted above is the life cycle of the HIV through a host cell. The progression of the life cycle is as follows: attachment to host cell, fusion and entry of virus particles, reverse transcription, provirus integration, viral transcription/translation, post-translational modification, and budding of new virion particle. Original image from: NIAID.
**Viral Latency**

A significant hurdle facing a cure to HIV infection is the latent reservoirs of virus that are not targeted by ART. Viral latency is defined by the establishment of a state of reversible nonproductive infection of cells (Siliciano & Greene, 2011). Several weeks following the onset of ART, patients enter an asymptomatic period (depicted as the 3rd phase in Figure 3), and clinicians originally termed this phase as a period of latency. However, the advent of sensitive reverse transcription-polymerase chain reaction (RT-PCR) viremia assays allowed for detection of HIV replication even during the asymptomatic period (Piatak et al., 1993). Also confirming the status of a stable reservoir of latent virus is the depiction of the viral rebound of patients (Figure 3) after the cessation of ART.

Activated CD4+ T-cells are the main target of HIV infection and the cytopathic effects of the virus and host immune responses leads to significant death of these cells (1st phase of Figure 3). An interesting phenomenon to the establishment of latent reservoirs is the finding that HIV does not effectively infect resting CD4+ T-cells (Zack et al., 1990; Zhou, Zhang, Siliciano, & Siliciano, 2005). Instead, the virus exploits the foundational mechanism to the establishment of immunological memory. When activated CD4+ T cells are infected, a small number of these cells may survive long enough to revert back to a resting state, which will then provide a reservoir for stably integrated and transcriptionally silent virus.
There are two types of cellular viral latency: pre-integration and post-integration latency. Pre-integrated latency resulting from unintegrated reverse transcripts does not persist more than one day in CD4+ T cells, so it does not account for the long-term latent reservoir and is less clinically relevant (Durand, Blankson, & Siliciano, 2012). Post-integration latency displays a multifactorial basis and is the foundation of our current research. In most latently infected cells, HIV replication is blocked at the transcriptional level, and mechanisms include: the chromatin structure of the HIV promoter; a lack of nuclear transcription factors; the presence of transcriptional repressors, such as Bcl11b and NELF-B (Lint et al., 2013; Schiralli Lester & Henderson, 2012). Elucidating these mechanisms of transcriptional repression will bring insight into possible methods of activating and targeting these reservoirs by subsequent ART in a “binge and purge” manner.
Figure 3: Plasma virus levels in cART treated HIV+ individuals. At the onset of cART, individuals see a decline in plasma viral load in an initial rapid phase and a second slower phase. During the third phase, the plasma viral load is too low for clinical detection. Cessation of cART results in a viral rebound to the original plasma virus levels. Original image from: Lint et al., 2013. Retrovirology.
HIV Transcription in the Context of Latency

Latent proviruses from patients undergoing ART tend to be found within host genes that are actively transcribed (Han et al., 2004). Actively transcribed genes are found in “relaxed” chromosomal regions, termed euchromatin, and the non-transcribed genes found in a much more compact chromosomal structure referred to as heterochromatin. This fact supports that latent virus is integrated into heterochromatic regions; however, in patients undergoing ART HIV preferentially integrates into the actively transcribed genes of resting CD4+ T cells (Han et al., 2004). This leads to the idea that maintenance of latent reservoirs is dependent on a multifactorial mechanism, including: transcription initiation, RNA polymerase II recruitment, transcriptional elongation, and chromatin structural organization.

A nucleosome is composed of two tetramers of histone proteins with a 147 base pair (bp) region wrapped around it. They are subject to an array of modifications that alter the accessibility of the super-helically wrapped DNA. Of particular importance is post-translational modifications to histone tails, including acetylation, methylation, ubiquitylation, phosphorylation, sumoylation, and poly ADP-ribosylation (Goldberg, Allis, & Bernstein, 2007). The most significant regulatory element of HIV is the 5’ long terminal repeat (LTR), which is bound to two positioned nucleosomes referred to as nuc-0 and nuc-1 (Verdin, Paras, & Van Lint, 1993). These nucleosomes overlap binding sites of transcription factors that promote gene expression. The status of histone acetylation of nuc-1
seems to play a particularly important role in regulating HIV transcription, and the recruitment of histone deacetylase 1 (HDAC-1) maintains a repressed transcriptional state. HDAC inhibitors are currently being tested in clinical trials for their ability to reactivate the latent reservoirs (Archin et al., 2012)

Transcriptional interference is another possible explanation to the latent reservoirs. Interference is based on the polarity and location of the provirus promoter relative to host genes. For instance, if the HIV promoter with the same polarity is downstream from a host gene, the RNA polymerase II (Pol II) can “readthrough” the HIV promoter causing displacement of key transcription factors located at the promoter (Lenasi, Contreras, & Peterlin, 2008). On the other hand, when the provirus and host gene are of opposite polarity, RNA Pol II collisions can occur that lead to premature terminations (Han et al., 2008). However, this method of interference can be readily overcome by activation of the HIV LTR, by converting it into a strong transcriptional unit (De Marco et al., 2008).

The 5’-LTR is further divided into four functional regions or motif’s: the Tat (trans-activator of transcription) activating region (TAR), the promoter, the enhancer, and the negative/modulatory regulatory element (Schiralli Lester & Henderson, 2012). The latter three elements are responsible for recruiting a wide range of host cell transcription factors with multiple functions that activate and repress HIV transcription. A small group of these transcription factors is highlighted in Figure 3. Host factors, such as Sp1, AP-1, and NF-κB are necessary for HIV transcription, and their availability regulates transcription. For
instance, NF-κB is sequestered in the cytoplasm of resting cells, where it exists as an inactive p50/p65 heterodimer bound to IkBa. Cell activation leads to the heterodimer release from IkBa, and NF-κB translocation to the nucleus and activation of transcription (Nabel & Baltimore, 1987). When NF-κB is unavailable in the nucleus, p50-p50 homodimers are bound to NF-κB’s binding site on the 5’-LTR, which then recruit HDAC complexes to repress transcription (Williams et al., 2006).

Chromatin reorganization and recruitment of factors involved in initiation of transcription are not solely responsible for efficient transcription. Transcriptional elongation plays a significant role in limiting the transcription of the provirus. After association of Tat with TAR, positive transcription elongation factor b (P-TEFb) is recruited to the RNA Pol II elongation complex. P-TEFb, made up of Cdk9 and CycT1, hyperphosphorylates RNA Pol II’s carboxy-terminal domain allowing efficient elongation. Several host-cell factors repress transcription of the provirus by causing promoter-proximal RNA Pol II pausing or premature termination of the transcript. Negative elongation factors, such as 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) and negative elongation factor (NELF) are responsible for promoter-proximal pausing. Pre-mRNA-cleavage complex 11 factor (Pcf11) is responsible for premature termination (Natarajan et al., 2013). These factors are highlighted in Figure 4.

Previous studies performed by our lab, published in Natarajan et al., 2013, showed that NELF has a physical and functional interaction with the nuclear
corepressor (NCoR1)-G protein pathway suppressor 2 (Gps2)-HDAC3 repressor complex. These findings suggest NELF represses HIV transcription by coordinating promoter-proximal pausing, premature termination, and chromatin remodeling. Furthermore, Dr. Chanhyo Lee, in collaboration with Dr. David Gilmour, using mass spectrometry found a physical interaction of several factors with NELF complexes, which included Bcl11b. This leads to the current study which aims to define the interaction of Bcl11b with HIV transcription repressor complexes.
**Figure 4: NF-κB binding site and the promoter-proximal pausing complex.**
Upstream of the transcription initiation site is the NF-κB heterodimer (p50/p65) binding site. When bound, the complex induces transcription initiation. When NF-κB is unavailable, the site is occupied by p50/p50. At the initiation site and associated with RNA Pol II, the TAR stem-loop structure is depicted with its association with the viral Tat and the host-cell’s P-TEFb. Also associated are the negative elongation factors: NELF, DSIF, and Pcf11. Modified image from: Andrew J. Henderson (unpublished)

**Bcl11b and its possible connection to HIV latency**

Bcl11b is part of the Bcl11 family of Kruppel-like C2H2 type zinc finger transcription factors, and has been shown to play a significant role in the maturation of T lymphocytes (Satterwhite et al., 2001). During T cell development, hematopoietic progenitor cells in the thymus (thymocytes) differentiate from a double-negative (DN) phenotype (CD4<sup>+</sup>CD8<sup>+</sup>) to a double-positive (DP) phenotype (CD4<sup>+</sup>CD8<sup>-</sup>), and, finally, to a single-positive (SP) phenotype (CD4<sup>+</sup>CD8<sup>-</sup> or CD4<sup>+</sup>CD8<sup>+</sup>) (Liu, Li, & Burke, 2010). The DN stage is
further subdivided into four stages (DN1-DN4). In the transition from DN1 to DN2, Bcl11b is the most upregulated transcription factor during T lymphopoiesis, and is an important transcription factor in terminating non-T-lineage potential in these progenitor cells (David-Fung et al., 2009).

In studies performed by Wakabavashi et al. (2003), Bcl11b knockout mice were shown to have profound defects in T-cell receptor (TCR) expression. The homozygous Bcl11b knockouts in mice are lethal with increase thymocytes apoptosis, diminished pre-TCR complex expression, and unsuccessful V(D)J rearrangements which are necessary to generate and express functional T-cell receptor genes. The lack of a pre-TCR complex leads to significant apoptosis of thymocytes. Heterozygous Bcl11b +/- mice are at an increased risk for lymphomagenesis, most likely due to blocks in T-cell differentiation and accumulation and outgrowth of T-cell progenitors. Taken together, these findings indicate that Bcl11b is an important T-cell specific factor that is necessary for commitment to the T-cell lineages.

The suppressive activity of Bcl11b remains prevalent into mature T cells. Expressed in both CD4+ and CD8+ T-cells, although at lower levels in CD8+, Bcl11b expression is lowest in activated T-cells suggesting a role in inhibiting T-cell activation through suppressing activation-associated genes (Li et al., 2010). Further suppressive activity of Bcl11b appears to play a role in maintaining a T-cell identity, as well. When Bcl11b is conditionally deleted using tamoxifen from mature CD4+ T cells, there is reprogramming of the cells to a natural killer-like
(NK-like) cell called induced-T-to-NK-cells (ITNKs). In development, via stimulation by Notch signaling, Bcl11b is responsible for inhibiting the activity of GATA3, therefore limiting the onset of natural killer cell-like properties (Braunstein & Anderson, 2012). Important to this discussion is that the ITNKs from mature T cells demonstrate compromised downstream TCR signaling (Li et al., 2010). Chromatin-immunoprecipitation (ChIP) assays show Bcl11b promotes IL-2 gene expression following TCR activation, as well as increasing NF-κB activity (Cismasiu et al., 2006, 2009). This research contradicts an earlier view of Bcl11b as a repressive transcription factor, and highlight a more complex mechanism behinds its activity. Overall, there is strong support for Bcl11b's critical role in transcriptional regulation of T cells, and this illustrates a potential in HIV transcriptional control.

Bcl11b is also referred to as CTIP2 (COUP-TF-interacting protein-2), because it was independently discovered to be associated with members of the chicken ovalbumin upstream promoter transcription factor (COUP-TF) (Avram et al., 2000). COUP-TF family members typically mediate transcriptional repression through NCoR (Shibata, Nawaz, Tsai, O'Malley, & Tsai, 1997). Bcl11b has also been shown to functionally associate with the nucleosome remodeling deacetylase (NuRD) complex with recruitment of HDAC1 and HDAC2 to the HIV promoter (Marban et al., 2007). In the context of HIV latency within microglial cells, Bcl11b associates with lysine-specific demethylase 1 (LSD1) to promote heterochromatin formation and inhibit transcription (Le Douce et al., 2012).
These relevant associations highlight the significance of the current study in the context of HIV latency. Bcl11b has a critical role in regulating transcription in mature T cells, and specifically HIV transcription, making it a potential target for future therapies against HIV infection.

As discussed, there is a clear need for further elaboration on the pathways for recruitment of host-cell factors to the HIV LTR, and specifically, to identify T-cell specific programs such as those mediated by Bcl11b that negatively regulate HIV transcription. The current study aims to identify a defined role for Bcl11b in the context of HIV latency.
THE ESTABLISHMENT AND MAINTENANCE OF HIV-1 LATENCY PROVIDES A CRITICAL BARRIER TO COMPLETE ERADICATION OF THE VIRUS FROM AN INFECTED INDIVIDUAL. A MAJOR CONTRIBUTION TO HIV-1 LATENCY IS THE CESSION OF VIRAL TRANSCRIPTION. PREVIOUS RESEARCH FROM OUR LAB HAS SHOWN THAT BCL11B INTERACTS WITH NELF-B AS PART OF A PAUSED RNA POLYMERASE II COMPLEX. OUR PRESENT STUDY AIDS TO TEST THE HYPOTHESIS THAT BCL11B RE Presses HIV TRANSCRIPTION AND IS RECRUITED TO THE HIV-1 LTR THROUGH A PAUSED RNA POLYMERASE II COMPLEX, CONTRIBUTING TO THE ESTABLISHMENT AND MAINTENANCE OF LATENCY. TO TEST THE HYPOTHESIS, WE WILL:

a) Determine whether BCL11B inhibits HIV-1 transcription.

b) Examine if there is a synergistic interaction between BCL11B and NELF-B in repressing HIV-1 transcription.

c) Explore the direct interaction between BCL11B and NELF-B.

These studies will define a role, if any, of BCL11B in the maintenance of HIV latency. We hope that this research will broaden the understanding of the transcription factors involved in RNA polymerase II pausing and limiting HIV-1 transcription. Furthermore, these studies are expected to identify potential T-cell specific targets that control HIV-1 transcription and latency.
METHODS

Cell Culture:

HEK 293T cells (ATCC) are a stable line of human embryonic kidney cells. The HEK 293T cells (ATCC) were cultured in complete Dulbecco’s Modified Eagle Serum (cDMEM), supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.2 M L-glutamine. BD Falcon 100mm x 20mm culture dishes were used for cell culture. The Jurkat E6.1 cell line (ATCC) is a stable T-lymphocyte developed by Schneider et al., 1977. Jurkat E6.1 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 0.2 M L-glutamine. All cells were incubated at 37°C and 5% CO₂.

Cell Transfection:

Initial transfection experiments were conducted in HEK 293T cells. First, cells were plated in 6-well plates at a density of 5.0x10⁵ cells in cDMEM and incubated overnight at 37°C and 5% CO₂. Cells were then transfected using calcium phosphate with 2 µg of pNL4-3-Luc(+) Env(-) Nef(-) (HIV-LUC) and 4 µg of the targeted DNA per condition. The four conditions for these transfections were as follows: 4 µg pcDNA3-Vec; 2 µg pcDNA3-Vec and 2 µg Bcl11b-HA; 2 µg pcDNA3-Vec and 2 µg pcDNA3-FLAG-NELF-B; and 2 µg Bcl11b-HA and 2 µg
pcDNA3-FLAG-NELF-B (refer to Table 1). Cells were transfected for 2 hours, at which time the transfection media was changed for fresh cDMEM and incubated for 48 hours at 37° and 5% CO₂. HIV-LUC transcription was measured by luciferase activity (luciferase kit, Promega, Madison, WI), and p24 enzyme-linked immunosorbent assay (ELISA) measured by PerkinElmer Life Sciences ELISA kit.

Table 1. Conditions for 293T Co-transfection:

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Control (µg)</th>
<th>Bcl11b (µg)</th>
<th>NELF-B (µg)</th>
<th>Bcl11b + NELF-B (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-LUC</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>pcDNA3-Vec</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Bcl11b-HA</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>FLAG-NELF-B</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

A subsequent transfection of HEK 293T cells was conducted using shBcl11b plasmids. The protocol remained the same as previous transfections. The experimental plasmids used were 2 µg of an shBcl11b plasmid (control, or A-D), and 2 µg of Bcl11b-HA. Whole cell lysates from the transfection were analyzed by Western blot analysis.
Establishment of Stable Cell Lines:

Jurkat E6.1 cells were used for a Bcl11b knockdown line. HEK 293T cells were transfected with VSV-G and a puromycin-resistant shBcl11b (Origene) vector (control, or A-D) for 2 hours. After 48 hours incubation at 37\(^\circ\) and 5% CO\(_2\), media supernatants were collected, filtered with a 0.2 mm filter, and used to spinoculate Jurkat E6.1 cells with polybreen (10 µg/ml). Cells were suspended in cRPMI and allowed to recover overnight. Over 4 weeks stable lines were established using cRPMI with 1.0 µM puromycin. Whole cell lysates were collected, and analyzed by western blot analysis and immunoprecipitation.

To establish a stable cell line with overexpression of Bcl11b, Jurkat cells were electroporated with the desired plasmids (pcDNA3-Vec or HA-Bcl11b). Electroporation was done with a BTX electroporator with a cuvet size of 4 mm at 240V, and a low voltage 65 msec gap. Cells were recovered overnight in cRPMI. Positive selection carried out using G418 (Geneticin) over 4-week period to generate a stable population. Whole cell lysates were collected, and analyzed by western blot analysis and immunoprecipitation.

Antibodies:

For Western blot analysis, immunoprecipitation, and chromatin immunoprecipitation, the following antibodies were used: anti-FLAG and anti-β-actin from Sigma-Aldrich, anti-NELF-B (COBRA) (PhosphoSolutions), anti-HA
(Cell Signaling Technologies), anti-Bcl11b (Bethyl Laboratories), anti-Mouse-HRP and anti-Rabbit-HRP (Upstate Biotechnology), anti-Mouse-TrueBlot-HRP and anti-Rabbit-TrueBlot (Rockland Immunochemicals), anti-Rabbit-Ig (Millipore), anti-NCoR1 (Thermo Scientific), anti-Pol II, and anti-p50. The dilutions used during western blot analyses and immunoprecipitation analyses are listed below in Table 2.

Table 2: Antibodies and the associated dilutions used for Western blot or immunoprecipitation detection. All antibodies were dissolved in 5% non-fat milk.

<table>
<thead>
<tr>
<th>Primary Antibody</th>
<th>Dilution</th>
<th>Secondary Antibody</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-FLAG</td>
<td>1:15,000</td>
<td>Anti-Mouse-HRP or Anti-Mouse IgG-TrueBlot</td>
<td>1:30,000</td>
</tr>
<tr>
<td>Anti-NELF-B</td>
<td>1:5,000</td>
<td>Anti-Rabbit IgG-HRP or Anti-Rabbit IgG-TrueBlot</td>
<td>1:15,000</td>
</tr>
<tr>
<td>Anti-HA</td>
<td>1:20,000</td>
<td>Anti-Mouse-HRP or Anti-Mouse IgG-TrueBlot</td>
<td>1:40,000</td>
</tr>
<tr>
<td>Anti-Bcl11b</td>
<td>1:1,000</td>
<td>Anti-Rabbit IgG-HRP or Anti-Rabbit IgG-TrueBlot</td>
<td>1:15,000</td>
</tr>
<tr>
<td>Anti-β-actin</td>
<td>1:5,000</td>
<td>Anti-Mouse-HRP or Anti-Mouse IgG-TrueBlot</td>
<td>1:20,000</td>
</tr>
<tr>
<td>Anti-NCoR1</td>
<td>1:5,000</td>
<td>Anti-Rabbit-HRP</td>
<td>1:20,000</td>
</tr>
</tbody>
</table>
Western Blot Analysis:

Whole cell extracts from transfections were collected by resuspending cells in lysis buffer (10 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1.0 mM EDTA (pH 8.0), 2.0 mM sodium vanadate, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 1% Triton X 100, 1.0 mM phenylmethylsulfonyl fluoride, and protease inhibitor mixture III (Calbiochem). The suspensions were spun down for 5 minutes at 4°C at 13,000 rpm, and the supernatants collected. Extracts were suspended in SDS-PAGE loading buffer and resolved on 8% SDS-PAGE. The proteins were transferred to a PVDF blotting membrane (Millipore) via electroblotting. Membranes were then blocked with a blocking buffer of 5% non-fat milk in PBST for 1 hour. The membrane was incubated in the desired primary antibody with the dilution mentioned in Table 2 in blocking buffer for 1 hour, followed by a final incubation in the necessary secondary antibody. The membrane is then incubated in GE Healthcare’s ECL Prime Western Blotting Detection System for 5 minutes. Film was exposed on membrane and developed with a Kodak X-OMAT.

Immunoprecipitation and Immunoblots:

For immunoprecipitation, whole cell extracts were prepared by the same method for Western blot analysis. After collecting the supernatant from the initial lysis, the samples were pre-cleared with protein A/G beads (Santa Cruz Biotechnology, catalog no. sc-2003) for 1 hour at 4°C. Supernatants were
incubated with anti-NELF-B, ant-Bcl11b, or anti-Rabbit IgG for 1 hour at 4°C. Protein A/G beads were added to each condition and incubated for 1 hour at 4°C. Beads were collected, washed three times with fresh lysis buffer, and suspended in 2x SDS-PAGE loading buffer. Samples were heated at 100°C for 5 mins and then resolved on 8% SDS-PAGE. The proteins were transferred to a PVDF blotting membrane (Millipore) via electroblotting. Membranes were then blocked with 5% non-fat milk and incubated in the appropriate antibodies for protein detection.

**Quantitative Real-Time PCR:**

RNA was collected from cell extracts by suspending cells in TRIzol, and cDNA was formed using reverse transcriptase and random primer sets (Invitrogen). 1 ng of cDNA was used for quantitative real-time PCR reactions with SYBR Green reagent (Qiagen). Elongated HIV transcripts (+5396 to +5531 base pairs (bp)) were amplified using 5′-GACTAGAGCCCTGGAAGCA-3′ and 5′-GCTTCTTCCTGCACTAGGAG-3′. NELF-B mRNA was amplified using 5′-GCGAGGTGGGGCTGTACTACG-3′ and 5′-GGCGGTGAGGAAGACCATCG-3′. Bcl11b mRNA was amplified using 5′-GGGCGATGCCAGAATAGAT-3′ and 5′-GGTAGCCTCCACATGGTCAG-3′. For endogenous controls, β-actin mRNA was amplified using Quantitect primer assay (Qiagen). PCR was carried out for 40 cycles, and relative expression was calculated using the ΔΔCt method (Livak & Schmittgen, 2001). Targeted amplification of transcripts was normalized to the
endogenous β-actin amplification of each condition. Amplified products were compared to amplification in pcDNA3-Vec control condition, or shBcl11b-Control, and calculated as fold changes.

**Chromatin Immunoprecipitation:**

Chromatin immunoprecipitation (ChIP) assay was carried out using the protocol described in Zhang et al., 2007. For this study, 4.0x10^7 HEK 293T cells expressing pcDNA3-Vec, Bcl11b-HA, or FLAG-NELF-B were used. The cells were cross-linked by using an 11% formaldehyde solution (prepared from 37% formaldehyde, 10% methanol stock solution (Merck)) in 0.1 M NaCl, 1 mM EDTA, 0.5 mM EGTA, 50 mM Tris-HCl (pH 8), diluting to the final concentration of 1%, and incubated at room temperature for 10 min. Cells were quenched by adding 2 M glycine to a concentration of 240 mM, and then collected by centrifugation at 700 x g for 10 min. Cells were washed with phosphate-buffered saline (PBS) and resuspended in sonication buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5 mM EGTA, 0.5 mM PMSF, 1%SDS) to be sonicated on ice for 30 cycles of 10 s on and 30 s off. Samples were then suspended in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5 mM EGTA, 0.5 mM PMSF, 0.1% SDS, and 1.1% Triton X-100, and incubated with 1 µg of targeted antibodies overnight at 4°C. Protein A/G beads (Santa Cruz Biotechnology, catalog no. sc-2003) were added, incubated for an additional 2 hours, and collected by centrifugation at 3000 rpm for 5 min. Beads were washed three times with a low-salt buffer (0.1% SDS, 1% Triton X-
100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.0), and 150 mM NaCl), two times with a high-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 0.1), and 500 mM NaCl), and two times with a LiCl buffer (0.25 M LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, and 10 mM Tris-HCl, 1 mM EDTA). Samples were eluted with an elution buffer (1% SDS and 0.1 M NaHCO₃), reverse-cross-linked at 65°C for 15 h, and treated with proteinase K at 45°C for 1 h. DNA extracted by two phenol/chloroform extractions and precipitated in ethanol at -80°C for 1 h. Analysis performed by real-time-PCR using SYBR green reagents, and the primers 5’-GAGCCCTCAGATCCTGCATA-3’ and 5’-AGGCTTAAGCAGTGTTGC-3’ to amplify -45 to +72 bp of the HIV LTR, the primers 5’-CTGGGAGCTCTCTGGCTAACTA-3’ and 5’- TTACCAGAGTCACACAACAGACG-3’ to amplify +30 to +134 bp of the HIV LTR, and the primers 5’-ACAGTACTGGATGTGGTGATG-3’ and 5’- AATCCCTGGTGTCTCATTGGTT-3’ to amplify +2415 to +2522 bp of the HIV LTR.
RESULTS

The repressive function of Bcl11b was investigated in HEK 293T cells using an overexpression strategy. Cells were co-transfected with the HIV-LUC provirus, and Bcl11b-HA or FLAG-NELF-B, or both. Protein expression was confirmed by Western blot analysis, and HIV-LUC transcription was measured with a luciferase assay. When co-transfected with Bcl11b or NELF-B, HIV transcription was reduced by approximately 43% and 53%, respectively, compared to the pcDNA3-Vec control (see Figure 5.A). When both factors were transfected together, HIV transcription was reduced by the greatest amount, approximately 76% (P-value = 0.0280). Co-transfection of both factors had a significant decrease in HIV-LUC transcription when compared to NELF-B alone (P-value = 0.0347).

Analysis by p24 ELISA (Figure 5.B) depicts a similar trend as the luciferase activity. The p24 protein is a viral capsid protein targeted for detection of HIV release from cells. In all three of the experimental conditions, p24 expression was significantly reduced. Co-transfection with Bcl11b showed over 50% reduction in p24 production, compared to pcDNA3-Vec control (P-value = 0.0468). NELF-B expression reduced p24 expression by nearly 80% (P-value = 0.00770). Co-transfection of both experimental plasmids showed the greatest reduction in p24 expression at nearly 85% (P-value = 0.0170). Statistical comparison between the experimental conditions (Bcl11b or NELF-B vs. Bcl11b
+ NELF-B) revealed a significant difference between Bcl11b and the co-transfection of both factors (P-value = 0.0187).

Figure 5: 293T HIV-LUC Transfection with Overexpression of Bcl11b and/or NELF-B. (A) HEK293T cells were transfected with HIV-LUC, and either Bcl11b-HA or FLAG-NELF-B, or both. Relative light units were measured as an indicator of HIV-LUC transcription. (B) p24 ELISA measurements of cells transfected with HIV-LUC and indicated transcription factors.
Figure 5: 293T HIV-LUC Transfection with Overexpression of Bcl11b and/or NELF-B. continued (C) Western blot analysis from the transfected cells. Conditions labeled with titles above the lanes, and probed with antibodies shown for the different rows.

Based on the Western blot analysis (Figure 5.C), the expression of Bcl11b when co-transfected with NELF-B appeared markedly decreased. qPCR was performed in order to compare transcription of the experimental plasmids to the protein expression seen from the western blots. As seen in Figure 6, the qPCR was clearly flawed, most likely in the collection of the RNA from the transfection samples, or the preparation of the cDNA. Measured transcription levels of HIV elongated transcripts and Bcl11b do not follow the trends shown in the luciferase assay and p24 ELISA. However, transcription levels of NELF-B do show a significant increase in the two conditions transfected with FLAG-NELF-B.
Figure 6: qPCR Analysis of 293T Overexpression. Depicted is the relative increase/decrease in transcription of the targeted transcript. (A) HIV elongated transcripts shown for cells transfected with HIV-LUC and the indicated transcription factors. (B) Measurement of Bcl11b transcription between transfection conditions. (C) Measurement of NELF-B transcription between transfection conditions.
In order to investigate an interaction between Bcl11b and NELF, immunoprecipitations (IP) were performed. Using whole cell lysates co-expressing Bcl11b-HA and FLAG-NELF-B, the IP was executed using an anti-Bcl11b antibody followed by Western blot analysis. This strategy successfully displayed enrichment of the targeted protein (Bcl11b); however, NELF-B was not found to be associated with Bcl11b in this experiment. Figure 7.A shows this IP probed with anti-Bcl11b antibody, confirming successful pull-down of Bcl11b in the 293T sample. Figure 7.B shows the same IP, now probed with anti-NELF-B antibody. NELF-B does not appear in the anti-Bcl11b IP lane, but is shown in the 10% input and flow through lanes.

**Figure 7: 293T anti-Bcl11b Immunoprecipitation.** (A) Bcl11b was pulled down using an anti-Bcl11b antibody; detection with anti-Bcl11b (B) Blot is probed with anti-NELF-B detection antibody.
To explore the potential physical interaction of Bcl11b and NELF, we expanded our search into the T lymphocyte derived cell line: Jurkat E6.1 cells. As opposed to HEK293T cells, Jurkat E6.1 cells endogenously express Bcl11b, so no overexpression strategy was used prior to collecting whole cell lysates. The same IP strategy was performed as in HEK293T cells for the Jurkat E6.1 whole cell lysates (Figure 8). In addition to the anti-Bcl11b IP, an anti-NELF-B IP was also performed. Consistent with the previous IP in HEK293T cells, the anti-Bcl11b IP did not show an endogenous complex with NELF-B. For the anti-Bcl11b IP, the result was the same, with no NELF-B and Bcl11b complex detected. Each protein appeared in a 10% input sample, and showed successful enrichment in their experimental IP lanes.

**Figure 8: Immunoprecipitations in Jurkat E6.1 Cells.** Immunoprecipitations in Jurkat E6.1 cells performed using anti-Bcl11b or anti-NELF-B in order to pull down possible protein complexes. Western blot probed with anti-Bcl11b or anti-NELF-B detection antibody (labeled on the left). Conditions are labeled above the respective lanes, and protein expression labeled on the right of the blot.
After investigating the impact of overexpressing Bcl11b overexpression, we focused on the effect of knocking down Bcl11b expression. As a transcription factor potentially linked to the maintenance of HIV latency, but also critical in the maintenance of T-cell activation; researching the effects of diminishing Bcl11b are important for understanding its role as a potential therapeutic target. To determine which shRNAs could reduce Bcl11b most efficiently, knockdown studies were initially performed in HEK293T cells followed by Western blot analysis. The study was accomplished by transfecting an shBcl11b plasmid (control, or A-D) with a Bcl11b-HA plasmid. Western blot analysis was performed on the whole cell lysates to view the varying expression of Bcl11b between the conditions (Figure 9). As shown, the plasmids shBcl11b-A, shBcl11b-B, and shBcl11-D appeared to limit the expression of Bcl11b compared to the shBcl11b-Control. Using this knowledge, these three plasmids were chosen to be packaged with a VSV-G pseudotyped virus and spinoculated into Jurkat E6.1 cells.
Figure 9: Co-transfection of Bcl11b and shBcl11b plasmids in HEK293T cells. HEK293T cells were transfected with Bcl11b-HA and varying shBcl11b plasmids. Whole cell lysates collected after 48 hours, and Western blot analysis is shown. Condition of the transfection is labeled above the respective lane, with the detection antibodies marked by the rows.

We then used shRNA to limit Bcl11b expression in T cells, and for these experiments we used Jurkat E6.1 cells. In Figure 10, Western blot analysis, Bcl11b was successfully reduced in the Jurkat E6.1 cells. Similar to the HEK293T co-transfection, the shBcl11b-D plasmid was able to limit Bcl11b expression to the greatest extent. Knowledge of this knockdown efficiency will allow us to establish a stable Bcl11b knockdown line for future HIV infection studies in Jurkat E6.1 cells and other T-cell lines.
Figure 10: Bcl11b knockdown in Jurkat E6.1 cells. Using a VSV-G pseudotyped virus containing three different shBcl11b plasmids (A, B, or D), Bcl11b expression was inhibited in Jurkat E6.1 cells. Western blot analysis of whole cell lysates is shown with the indicated conditions above the lanes, and probed with antibodies indicated in the rows.

To investigate the interaction of our experimental factors with particular regions of the HIV provirus, chromatin immunoprecipitation (ChIP) was performed on HEK293T cells. An HIV LTR -45 to +72 primer set was used in order to see association of the transcription factors with the transcriptional start site. Figure 11 below depicts the results of the ChIP. The most striking finding of the ChIP is the relative increase in RNA Pol II found at the HIV transcriptional start site, measured by anti-Pol II antibody pull down in the Bcl11b-HA condition. This increase over the other conditions could suggest a promoter-proximal paused RNA Pol II complex associated with overexpression of Bcl11b. Seeing
no significant difference in measured transcripts pulled down by anti-Bcl11b when overexpressing Bcl11b disputes the previously seen binding to the HIV-1 LTR (Cismasiu et al., 2008). However, Bcl11b could be binding upstream of the region for the primer set in this study, and there could be cell line-specific challenges when comparing our studies in HEK293T cells to the more relevant T lymphocyte derived cell lines.

**Figure 11:** ChIP of HEK293T cells transfected with Bcl11b-HA or FLAG-NELF-B. ChIP was performed using the targeted antibodies listed along the horizontal axis. The primer set used was -45 bp to +72 bp of the HIV-1 LTR. The vertical axis indicates the relative amount of measured transcript.
DISCUSSION

The AIDS pandemic continues to take the lives of millions of individuals every year; however, the increasing efficiency and availability of ART is decreasing the annual death rate. Current therapeutic strategies have changed a fatal diagnosis into a manageable, chronic disease. Unfortunately, the battle is not won, as ART merely decreases viral loads to clinically undetectable levels, leaving patients dependent on costly and highly frequent medications. Furthermore, upon cessation of these treatments, viral load rebounds and AIDS symptoms return. The advent of ART was a huge step in the global fight against HIV infection, but it is now clear that targeting the latent cell reservoirs will be necessary to find a cure.

A significant hurdle to activating transcription of HIV in latent reservoirs is overcoming promoter-proximal pausing of the RNA polymerase II complex. This form of transcriptional silencing is maintained by either a presence or absence of several host cell nuclear transcription factors. Of particular interest are Bcl11b and NELF, which have already been associated with transcriptional repression. Bcl11b, primarily known as a T-cell commitment factor that limits the expression of a variety of genes to maintain a T-cell identity during development, is known to associate with other HIV repressive factors such as NuRD and histone remodeling enzymes. Preliminary research from our lab has indicated an
interaction between Bcl11b and NELF, leading to this current study on the role of Bcl11b in HIV transcription.

Initial transfections of HEK293T cells confirm the repressive activity of Bcl11b, as well as NELF-B. Together, Bcl11b and NELF-B significantly reduce HIV to an even greater extent (Figure 5), further suggesting a possible relationship in their mechanism of action at the HIV transcription start site. However, Western blot analysis shows lower levels of Bcl11b expression when expressed with NELF-B. Since NELF-B is a general repressor of transcription, it is thought that it is inhibiting expression of Bcl11b transcription which prompted qPCR analysis of the mRNA expression. Unfortunately, our results of this analysis (Figure 6) are flawed and do not uncover the problem.

We suggest that there is a connection between the repressive mechanisms of Bcl11b and NELF. However, our studies of the possible interaction of Bcl11b and NELF do not reveal a direct physical interaction. Following discovery of a successful immunoprecipitation strategy for these particular transcription factors, our results indicate that there is no direct interaction between the factors. This finding suggests that if these factors share a common pathway for HIV repression, there may be a yet to be identified intermediate factor coordinating the interaction. We suggest that this factor could be NCoR1 or HDAC3, which would be consistent with our previous mass spectrophotometry data, as well as with the current literature. Furthermore immunoprecipitations need to be performed in Jurkat cells overexpressing
Bcl11b to fully rule out a direct physical interaction with NELF. Stable Bcl11b overexpression and knockdown lines were successfully established in Jurkat cells. Future studies will examine the outcomes of HIV infection in these stable cells lines.

Inhibition of HIV transcription is associated with a nucleosome positioned at the transcriptional start site. Reversal of the repression occurs with chromatin modifications by histone deacetylases and histone methyl transferases. Bcl11b has been shown to interact with both nucleosome remodeling complexes (NuRD) and histone remodeling factors, and we suggest that recruitment of these factors is the mechanism behind the repression of HIV transcription seen in our studies. Further experiments are required to highlight the pathway of recruitment of these factors by Bcl11b in CD4+ T cells.

Previous studies performed by Cismasiiu et al., 2008 suggest that Bcl11b binds directly to the HIV-1 LTR and is involved in recruitment of the NuRD complex, and it is through this promoter proximal binding and nucleosome remodeling that transcription is blocked. In order to further investigate this mechanism, future experiments should include a ChIP targeting Bcl11b and NuRD, to measure HIV-1 transcripts further upstream from the transcriptional start site. Also, as previously mentioned, Bcl11b is involved in the NF-κB signaling pathway, an important mechanism behind T-cell activation. Future experiments will include HIV-LUC constructs that have a mutated NF-κB binding site in order to explore the connection in signaling pathways. This would expand
our current understanding of Bcl11b's mechanism of repression. Furthermore, it is important for the context of HIV-1 latency to expand our current experiments into establish latent cell lines, such as ACH2 cells. Comparing overexpression, or repression, of Bcl11b expression in a latent cell line to primary CD4+ T cells will elaborate upon Bcl11b’s impact to maintaining cellular latency.

Overall, these studies have confirmed Bcl11b having a repressive role in HIV-1 transcription. Early transfection experiments continue to indicate a synergistic role of Bcl11b and NELF-B in inhibiting HIV-1 transcription, but their physical interaction remains veiled behind a possible intermediate factor that we have yet to associate with this complex. Further studies including other associated transcription factors, such as NCoR1 and NuRD, need to be performed to help further illustrate the mechanisms behind promoter-proximal RNA polymerase II pausing in HIV-1 latent cell populations. As a critical T-cell commitment factor, Bcl11b may also be a significant factor in maintaining latent HIV-1 provirus reservoirs. A greater understanding of the intricate mechanisms behind HIV-1 transcriptional silencing, and premature termination are critical to uncover future therapeutic strategies targeted at purging HIV-1 latent cell reservoirs.
REFERENCES


Plasticity. *Journal of Immunology Research, 2012.*
doi:10.1155/2012/678705

doi:10.1042/BJ20080925

doi:10.1182/blood-2006-05-021790


Han, Y., Lin, Y. B., An, W., Xu, J., Yang, H.-C., O’Connell, K., ... Siliciano, R. F. (2008). Orientation-dependent regulation of integrated HIV-1 expression
by host gene transcriptional readthrough. *Cell Host & Microbe, 4*(2), 134–146. doi:10.1016/j.chom.2008.06.008


Shibata, H., Nawaz, Z., Tsai, S. Y., O'Malley, B. W., & Tsai, M. J. (1997). Gene silencing by chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is mediated by transcriptional corepressors, nuclear receptor-corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). *Molecular Endocrinology (Baltimore, Md.), 11*(6), 714–724. doi:10.1210/mend.11.6.0002


CURRICULUM VITAE

ANDREW WOERNER

Born 1989
(916) 768 0741
ajwoerner@gmail.com

EDUCATION
Bachelor of Science, Biology                        August 2007 - December 2011
San Diego State University

Master of Science, Medical Sciences, in progress    August 2012 – May 2014
Boston University School of Medicine

WORK EXPERIENCE
Teaching Assistant                                  Fall 2013
Boston University School of Medicine
Boston, MA
• Facilitate discussion on topics of biochemistry, and cell and molecular biology
• Provide further assistance to students struggling in the course

National Staff Member                               Fall 2011 – Summer 2012
Music is Medicine
San Diego, CA
• Involved in the staggering growth of the youth-based non-profit organization
• Founded a chapter at San Diego State University, and assisting in the formation of chapters at other regional colleges, including University of San Diego and UC San Diego
• Provide assistance to the marketing of the national organization, via media relations and other avenue

Retail Salesman/Assistant Manager                   Spring 2010 – Fall 2011
Pet Kingdom/Sports Arena Tropicals
San Diego, CA
• Using a broad knowledge of the husbandry of a vast array of exotic species, sold new pets to customers after being fully educated on the care requirements
• Provide complete care for the store’s large amount of animal stock
• Maintain organization of the daily chores of employees in the department
RESEARCH EXPERIENCE

Graduate Research Assistant  
Spring 2013 – Summer 2014  
Boston University Medical Center Section of Infectious Diseases  
Boston, MA  
Principal Investigator: Andrew J. Henderson, Ph.D.  
Thesis Title: Bcl11b, a T-cell commitment factor, and its role in Human Immunodeficiency Virus-1 transcription.  
  • Cell culture and transfection  
  • Western blot analysis  
  • Protein and chromatin immunoprecipitations

Research Assistant  
Spring 2011 – Summer 2012  
San Diego State University  
Biology Department  
Principal Investigator: Rebecca Lewison, Ph.D.  
San Diego, CA  
  • Studied ecology of local green sea turtles in San Diego Bay using sonic/radio telemetry

San Diego State University  
Biology Department  
Principal Investigator: Rulon Clark, Ph.D.  
San Diego, CA  
  • Used stable isotope analysis to analyze the lifetime feeding habits of local rattlesnake species through blood, scale, and prey samples

San Diego State University  
Biology Department  
Principal Investigator: Brian T. Hentschel, Ph.D.  
San Diego, CA  
  • Analyzed feeding parameters for the metamorphosis requirements of the marine invertebrate larvae of Diaulula sandiegensis

VOLUNTEER EXPERIENCE

Academic Mentor  
Fall 2013 – Present  
Minds Matter  
Boston, MA  
  • Provide academic guidance to high-achieving low-socioeconomic status high school students

Pediatrics Department Volunteer  
Winter 2013 – Winter 2014  
bWell Center of Boston Medical Center  
Boston, MA
• Provide resources and influence the families of BMC Pediatrics for a healthy lifestyle
• Organize activities for children that meets the center’s goal for healthy living

**Academic Mentor**  
Winter 2013 – Summer 2013  
Boston Partners in Education  
Boston, MA  
• Provide academic assistance to high school students in Math and Science  
• Mentor students on responsible academic principles

**Health Clinic Coordinator**  
Summer 2011 – Summer 2012  
Flying Samaritans, SDSU Chapter  
San Diego, CA  
• Contributed to the founding of the SDSU chapter, and the organization of a new clinic in Ejido Matamoros, Mexico  
• Provide free health care to 60 local residents on a monthly basis  
• Coordinate medical supply inventory, pharmacy inventory, and work as clinic manager during operation

**Emergency Department Volunteer**  
Summer 2011 – Spring 2012  
Sharp Metropolitan Hospital  
San Diego, CA  
• Provide assistance to the health care staff on a weekly basis  
• Maintain the sterility of patient rooms  
• Transport patients and patients’ families around the premises

**PRESENTATIONS**


**HONORS/AWARDS**

Trustee Scholar  
Fall 2007 – Spring 2008  
• Academic scholarship, University of San Diego

**CLUBS/ORGANIZATIONS**

President  
Summer 2011 – Summer 2012  
Music is Medicine, San Diego State University Chapter  
The Flying Samaritans, San Diego State University