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Review

# Intracellular nucleotide levels and the control of retroviral infections

Sarah M. Amie, Erin Noble, Baek Kim*

*Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, USA*

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ABSTRACT

Retroviruses consume cellular deoxynucleoside triphosphates (dNTPs) to convert their RNA genomes into proviral DNA through reverse transcription. While all retroviruses replicate in dividing cells, lentiviruses uniquely replicate in nondividing cells such as macrophages. Importantly, dNTP levels in nondividing cells are extremely low, compared to dividing cells. Indeed, a recently discovered anti-HIV/SIV restriction factor, SAMHD1, which is a dNTP triphosphohydrolase, is responsible for the limited dNTP pool of nondividing cells. Lentiviral reverse transcriptases (RT) uniquely stay functional even at the low dNTP concentrations in nondividing cells. Interestingly, Vpx of HIV-2/SIVsm proteosomally degrades SAMHD1, which elevates cellular dNTP pools and accelerates lentiviral replication in nondividing cells. These Vpx-encoding lentiviruses rapidly replicate in nondividing cells by encoding both highly functional RTs and Vpx. Here, we discuss a series of mechanistic and virological studies that have contributed to conceptually linking cellular dNTP levels and the adaptation of lentiviral replication in nondividing cells.

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## Reverse transcription and reverse transcriptases

All retroviruses, as well as retrotransposons, undergo a unique DNA synthesis process called reverse transcription, which converts single stranded RNA genomes into double stranded DNA. This process is catalyzed by virally encoded DNA polymerases, reverse transcriptases (RT) (reviewed in Hu and Hughes, 2012; Sarafianos et al., 2009). Unlike cellular DNA polymerases, which synthesize DNA from DNA templates, RTs can execute DNA polymerization from RNA templates as well. RTs also harbor RNaseH activity, which can degrade RNA templates annealed to the newly synthesized DNA. This activity enables RTs to undergo strand transfer and recombination (Berkhout et al., 1995; Levy et al., 2004; Negroni and Buc, 2000; Song et al., 2008; Zhang et al., 2000; Zhuang et al., 2002). RTs also use small RNAs (tRNAs and the polypurine tract RNAs) as primers to initiate first and second strand DNA synthesis, which are also removed by RNaseH activity (Huber and Richardson, 1990; Smith et al., 1999; Wohrl and Moelling, 1990; Wu et al., 1999). All RTs utilize dNTPs as substrates for DNA polymerization, and these dNTP substrates are provided by the infected cells. HIV-1 RT is the most well-characterized RT biochemically, structurally, and pharmacologically. This is due to it being one of the key viral proteins targeted by multiple pharmacological agents for the treatment of HIV-1 infected patients.

* Corresponding author. Fax: +1 585 473 9573.
*E-mail address:* baek_kim@urmc.rochester.edu (B. Kim).

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HIV-1 RT forms a heterodimer composed of the p66 subunit and the truncated p51 subunit. The p66 monomer harbors two enzymatically functional domains, a DNA polymerase domain at the N-terminus and an RNaseH domain at the C-terminal region (Huang et al., 1998). The DNA polymerase domain of the p66 monomer displays a molecular shape known as the right hand model that is commonly found in many DNA polymerases. The DNA polymerase active site is surrounded by three subdomains: the fingers, palm and thumb domains which interact with template, dNTP substrate and primer, respectively. Another well-characterized RT is from Murine Leukemia Virus (MuLV). Unlike HIV-1 RT, MuLV RT works as a monomer, but still maintains a right hand model for its DNA polymerase active site (Cote and Roth, 2008).

A structural study of the tertiary complex of HIV-1 RT (RT with bound template, primer, and dNTP) revealed specific residues near the binding pocket that interact with different chemical moieties of the dNTP (Huang et al., 1998). Mechanistic and structural studies of HIV-1 RT mutations that render viral resistance against nucleoside/nucleotide RT inhibitors provided the molecular architecture of the HIV-1 RT dNTP binding site. These RT inhibitors chemically mimic dNTP substrates, but have an altered sugar moiety for chain termination (reviewed in De Clercq, 2009). Three aspartic acid residues (D110, D185, and D186) form a triad with two metals coordinating with the three phosphates of the dNTP substrate during phosphodiester bond formation between the alpha-phosphate of the incoming dNTP and the 3′ OH of the DNA primer (Huang et al., 1998; Sarafianos et al., 1999, 2009). Other residues involved in interacting with the dNTP such as Y115, A114, Q151 and K65 were also studied for their functional roles in the enzymatic activities of HIV-1 RT (Boyer and Hughes, 2000; Ehteshami et al., 2008; Harris et al., 1998; McColl et al., 2008; Melikian et al., 2012; Weiss et al., 2002).

## Cellular dNTP levels

While ribonucleoside triphosphates (rNTPs) act as substrates for RNA polymerization, as well as functioning as energy carriers and substrates of numerous cellular kinases, the sole utility of cellular dNTPs is for DNA synthesis. The steady-state level of cellular dNTPs is tightly regulated primarily by the cell cycle, and both biosynthesis and consumption of cellular dNTPs occurs during G1/S and S phase (Bjursell and Skoog, 1980; Cohen et al., 1983). Expression of enzymes involved in dNTP biosynthesis such as ribonucleotide reductase (RNR) (Bjorklund et al., 1990; Engstrom et al., 1985; Mann et al., 1988) and thymidine kinase (TK) (Stewart et al., 1987) are elevated prior and during cellular replication. Thus, rapidly dividing cells such as cancer cells and transformed cell lines harbor much higher levels of cellular dNTPs than normal cells due to a larger proportion of the cell population going through S phase (Angus et al., 2002; Jackson et al., 1980; Skoog and Bjursell, 1974). Indeed, an elevated cellular dNTP level is considered a biochemical marker of transformed/cancerous cells (Angus et al., 2002; Jackson et al., 1980; Skoog and Bjursell, 1974; Traut, 1994) (Fig. 1). It had also been postulated that dividing cells contain higher cellular dNTP pools than nondividing cells. However, unlike primary human dividing cell types, the actual dNTP concentrations of primary human terminally differentiated/nondividing cells had not been measured, mainly due to the lack of a sensitive dNTP assay. In 2004, the dNTP concentrations of human terminally differentiated/nondividing monocyte-derived macrophages were measured using a highly sensitive HIV-1 reverse transcriptase (RT) based dNTP assay (Diamond et al., 2004). Indeed, the dNTP concentration of human macrophages (20–40 nM) was 22–320-fold lower than that of activated human peripheral blood mononuclear cells (PBMCs) primarily composed of $CD4^{+}$ T cells (2–5 μM) (Diamond et al., 2004; Kennedy et al., 2010) (Fig. 1). While the dNTP concentration discrepancy between these two HIV-1 target cell types was expected, the magnitude of the dNTP concentration discrepancy raised a fundamental question: How can HIV-1 synthesize proviral DNA in macrophages harboring such a limited dNTP substrate pool? One practical reference linking dNTP concentrations and DNA polymerization is polymerase chain reaction (PCR). Indeed, the dNTP concentration normally included for PCR is 250 μM. Thus, the dNTP concentrations found in human macrophages are at least 5000 times lower than the dNTP concentrations used in PCRs. It was unclear how HIV-1 RT was able to synthesize proviral DNA in macrophages containing such low dNTP substrate concentrations.

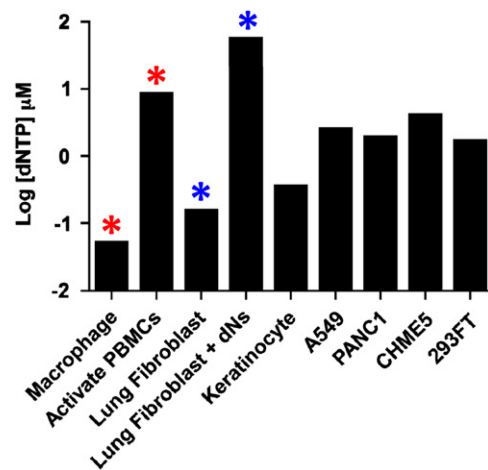


**Fig. 1.** Cellular dNTP concentrations of human primary cells, human cancer cells and transformed cell lines: Red asterisks: The dNTP concentration difference between primary human macrophages and activated PBMCs. Blue asterisks: Effect of dN treatment on cellular dNTP concentrations in primary human lung fibroblasts. A549: human adenocarcinoma alveolar basal epithelial cell line, PANC1: human pancreas epithelioid carcinoma cell line, CHME5: transformed human microglia cell line, 293FT: transformed human embryonic kidney cell line. This figure was prepared by modifying the data previously presented (Jackson et al., 1980).

## Kinetic differences in dNTP utilization between reverse transcriptases from lentiviruses and other retroviruses

RTs consume cellular dNTPs during reverse transcription, and thus the rate of proviral DNA synthesis is kinetically dependent on the cellular dNTP concentrations. It was predicted that RTs of lentiviruses may have evolved to efficiently synthesize DNA even at low dNTP concentrations in order to complete proviral DNA synthesis in nondividing cells. RT proteins of other retroviruses, which replicate exclusively in dividing cells with high dNTP concentrations, would not have this evolutionary pressure. Indeed, lentiviral RTs such as HIV-1, SIV and FIV can efficiently synthesize DNA at the low dNTP concentrations found in macrophages (Banapour et al., 1991; Rogers et al., 2002; Skasko et al., 2005), whereas oncoretroviral RTs such as MuLV, AMV, FV and FeLV efficiently synthesize DNA only at the high dNTP concentrations found in dividing cells (Diamond et al., 2004; Santos-Velazquez and Kim, 2008; Skasko et al., 2005). Fig. 2 presents the Km value difference between HIV-1 RT and MuLV RT with respect to the concentration of human macrophages and activated PBMCs which were determined by two independent dNTP assays (primer extension based assay (15) and quantitative LC-MS/MS (33)). These observations support the idea that lentiviruses may have evolved RTs with specific biochemical properties (low *Km* values close to the

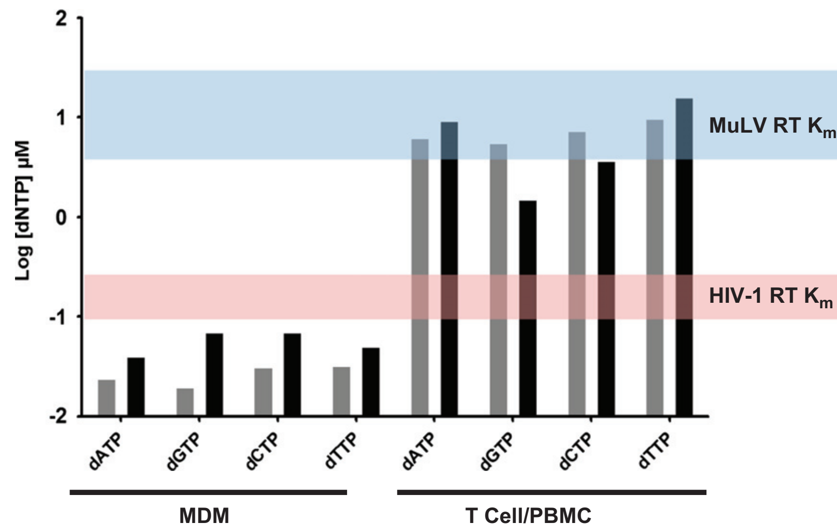


**Fig. 2.** Comparison of the Km values of HIV-1 RT and MuLV RT with respect to dNTP concentrations in primary human macrophages and T cell/PBMCs. The dNTP concentrations of human macrophages (MDM) and activated T cells/PBMCs were determined by the primer-extension based assay (grey bars, (Diamond et al., 2004)) and quantitative LC–MS/MS assay (black bars, (Kennedy et al., 2010)). The ranges of the RT Km values were marked by two longitudinal bars (red for HIV-1 RT and blue for MuLV RT). This figure was prepared by modifying the data presented previously (Diamond et al., 2004; Kennedy et al., 2010; Skasko et al., 2005). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cellular dNTP concentration of macrophages) for efficient replication in this target cell type.

## Mechanistic features of HIV-1 RT that contribute to efficient dNTP utilization

There are two possible mechanistic scenarios to improve dNTP utilization: (1) tight dNTP binding affinity and (2) rapid enzymatic catalysis. These potential enzymatic features were tested for HIV-1 RT and MuLV RT by determining pre-steady state kinetic values, $K_d$ and $k_{pol}$ (Skasko et al., 2005). While both RTs displayed very similar kpol values, HIV-1 RT showed 10–100 times tighter dNTP binding affinity than MuLV RT (Skasko et al., 2005). This suggests that HIV-1 may have evolved to tightly bind dNTP substrates in order to execute efficient proviral DNA synthesis in the poor dNTP pools found in macrophages. In contrast, MuLV may not need an RT with tight dNTP binding affinity as this virus exclusively infects dividing cells containing abundant dNTPs.

## Structural features of HIV-1 RT contributing to the uniquely tight dNTP binding affinity

To determine the structural features of HIV-1 RT that contribute to the high binding affinity, the dNTP binding pocket of the HIV-1 RT ternary complex structure was compared with that of other DNA polymerases (Huang et al., 1998). Interestingly, the Q151 residue lies near the 3′ OH of the incoming dNTP. This structural model suggested that the side chain of the Q151 residue is able to form a hydrogen bond with the 3′ OH (O3) of the incoming dNTP substrate (green in Fig. 3) (Weiss et al., 2002). It is likely that this residue may be structurally involved in the tight dNTP binding of HIV-1 RT. This was further examined by constructing a Q151N mutant which harbors a side chain that is one methylene group shorter than the wild type Q151 RT and thus cannot form a hydrogen bond with the 3′ OH of the incoming dNTP substrate (orange in Fig. 3). It was predicted that Q151N would have a reduced dNTP binding affinity ($K_d$), but would maintain a catalysis rate ($k_{pol}$) similar to wild type RT. This was confirmed by pre-steady state kinetic analysis

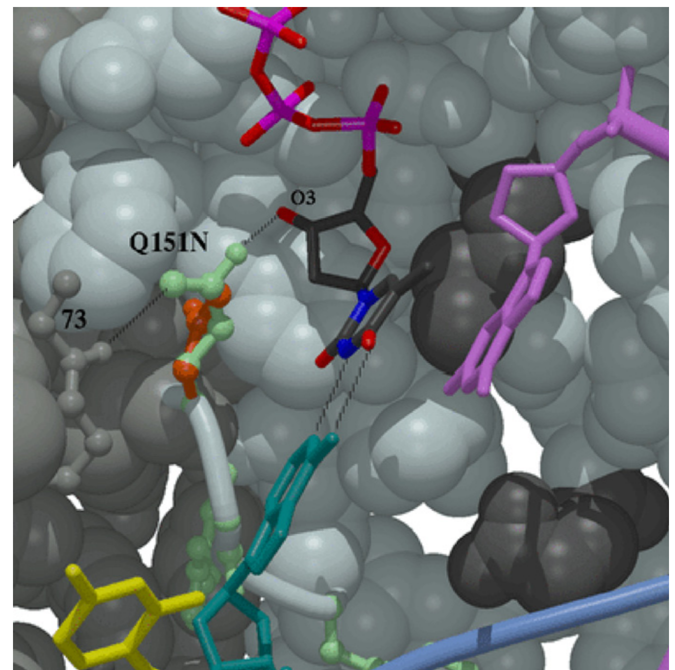


**Fig. 3.** Models for the direct interaction between the side chain of HIV-1 RT Q151 residue and 3′–OH of incoming dNTPs. This structural view of the active site of the HIV-1 RT complex with dNTP and template/primer was adapted from a 1D RT-based model previously reported (Weiss et al., 2002). This figure shows the interaction between wild type residue Q151 (light green) or mutant residue N151 (orange) and the incoming dTTP (black for base and sugar and red for triphosphate), base-paring with the template nucleotide (dark blue). Template (blue line), primer (purple), and incoming dNTP are shown and the nearby R73 (gray) residue is also marked. While the Q151 side chain makes a hydrogen bond with the 3′–OH (O3) of the incoming dTTP, the Q151N mutant residue has a shortened R side chain and cannot interact with the sugar on the incoming dTTP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

demonstrating that the Q151N mutant showed a 120-fold higher $K_d$ value compared to wild type RT, but with the same wild type $k_{pol}$ value (Weiss et al., 2002). Another dNTP binding mutant, V148I which lies in close proximity to the Q151 residue, also reduced the

dNTP binding affinity of HIV-1 RT, but less significantly than the Q151N mutant (Operario et al., 2006). Both Q151 and V148 residues are conserved among all lentiviral RT sequences. More importantly, these two HIV-1 RT mutants with reduced dNTP binding affinity failed to synthesize DNA at the low dNTP concentrations found in macrophages, but were still fully active at the dNTP concentrations found in activated T cells (Operario et al., 2006), suggesting that these two HIV-1 RT mutants kinetically mimic MuLV RT. Interestingly, MuLV RT also encodes a Q190 residue, which appears to be equivalent to the Q151 of HIV-1 RT. However, mutations in the Q190 residue of MuLV RT more severely reduce the biochemical activity of RT and viral infectivity than mutations in the Q151 residue of HIV-1 RT. Collectively, these biochemical studies support the idea that HIV-1 RT may have evolved to gain a unique interaction with the 3′ OH of the incoming dNTP in order to execute efficient proviral DNA synthesis in macrophages harboring limited dNTP pools.

## Interplay between dNTP binding of HIV-1 RT and viral cell tropism

Virological implications of the unique biochemical features of HIV-1 RT were investigated by employing the recent knowledge about the large dNTP concentration discrepancy between the two HIV-1 target cell types, activated $CD4^+$ T cells and macrophages. One central prediction is that, unlike wild type RT, the reduced dNTP binding mutant RTs should fail to support proviral DNA synthesis in macrophages due to limited dNTP pools, but should be able to replicate in activated $CD4^+$ T cells as well as any dividing cells containing abundant dNTP pools. Indeed, while the HIV-1 variant vectors containing Q151N or V148I mutant RTs efficiently supported proviral DNA synthesis and transduction in various dividing cell lines and primary human activated $CD4^+$ T cells, both completely failed to transduce primary human macrophages (Diamond et al., 2004; Jamburuthugoda et al., 2006). Basically, the HIV-1 variants harboring the reduced dNTP binding RT mutants behave like MuLV or other retroviruses that exclusively replicate in dividing cells. This virological finding is also consistent with the biochemical findings discussed above (Skasko et al., 2005; Weiss et al., 2002). Collectively, in addition to the Env protein (Hwang et al., 1991) and several viral accessory proteins such as Vpr and Vpx (reviewed in Ayinde et al., 2010), the mechanistic and structural features of reverse transcriptase also contribute to retroviral cell tropism by supporting optimal proviral DNA synthesis kinetics through the proper utilization of the available cellular dNTPs. Furthermore, based on these biochemical and virological findings (Diamond et al., 2004; Jamburuthugoda et al., 2006; Skasko et al., 2005; Weiss et al., 2002), it was hypothesized that other viruses containing DNA polymerase mutants with reduced dNTP binding affinity may replicate exclusively in cell types harboring elevated dNTP pools such as cancer cells. This was confirmed by a series of adenovirus polymerase variants that replicate preferentially in cancer cells and also function as oncolytic viruses (Capella et al., 2012).

## Mechanistic interactions between SAMHD1, Vpx, and cellular dNTP levels and the effects on retroviral replication

Unlike HIV-1, HIV-2 and various SIV strains encode an accessory protein, Vpx, which was known to facilitate viral infection in macrophages (Goujon et al., 2007; Sharova et al., 2008). A series of recent studies revealed that Vpx counteracts a protein, SAM domain and HD domain-containing protein 1 (SAMHD1), which serves as an HIV restriction factor (Hrecka et al., 2011; Laguette et al., 2011). Both enzymatic and structural studies reported that the HD domain of SAMHD1 harbors dNTP triphosphohydrolase activity, which is allosterically activated by dGTP, and acts to directly decrease dNTP levels by hydrolyzing dNTPs into deoxynucleosides (dNs) and triphosphates (Goldstone et al., 2011; Powell et al., 2011). Indeed, a recent work revealed that SAMHD1 is responsible for the limited cellular dNTP pools in macrophages. The elevation of cellular dNTPs in macrophages, which was induced by either SAMHD1 degradation mediated by Vpx (red arrow in Fig. 4) or treatment with dNs, the precursors of dNTPs, accelerates proviral DNA synthesis and viral infectivity in macrophages (Lahouassa et al., 2012). A follow-up study also reported a highly coordinated and sequential interplay between SAMHD1 degradation, dNTP level elevation, and accelerated HIV-1 proviral DNA synthesis kinetics, upon the treatment of macrophages with Vpx (Kim et al., 2012). In addition, another recent study reported that Vpx also enhances HIV-1 infection of resting $CD4^+$ T cells by degradation of SAMHD1 followed by an increase in dNTP levels (Baldauf et al., 2012). Collectively, these findings support that the extremely low cellular dNTP pool serves as a myeloid cell-specific biochemical anti-lentiviral restriction factor, which is maintained by SAMHD1 dNTP triphosphohydrolase activity. Interestingly, genetic alterations in SAMHD1 result in a rare human genetic developmental disorder, Aicardi–Goutières syndrome (AGS), which mimics viral infection and immune activation (Leshinsky-Silver et al., 2011; Rice et al., 2009; Thiele et al., 2010). A recent study reported that $CD14^+$ monocytes from AGS patients are highly susceptible to HIV-1 infection, which is consistent with the idea that SAMHD1 restricts HIV replication in myeloid cell types (Berger et al., 2011).

Summarized in Fig. 4 are the effects of Vpx on the cellular dNTP concentration in macrophages and the *Km* values of HIV-1/MuLV RTs. The *Km* values of the lentiviral RTs are significantly lower than RTs of the retroviruses that replicate only in dividing cells. However, the dNTP concentrations found in macrophages are still lower than the *Km* values of the lentiviral RTs, indicating that, although the low *Km* values of RTs allows lentiviruses to be able to synthesize proviral DNA at low dNTP concentrations (blue arrow in Fig. 4), HIV-1 RT alone is still not sufficient for achieving maximal proviral DNA synthesis kinetics in macrophages. However, in the case of the lentiviruses encoding Vpx, the dNTP concentration is elevated above the *Km* value of lentiviral RTs (red arrow in Fig. 4) leading to accelerated reverse transcription kinetics and more efficient completion of proviral DNA synthesis in macrophages (Kim et al., 2012). Therefore, Vpx and the uniquely low *Km* values of RTs synergistically enhance the replication efficiency of these lentiviruses in macrophages Fig. 5.

## Unexpected frequent incorporation of rNMPs during HIV-1 proviral DNA synthesis in macrophages

Unlike dNTPs, which are exclusively used for DNA synthesis, cellular rNTPs are widely used for various key molecular and biochemical functions in the cell. rNTPs serve as substrates of cellular RNA polymerases and numerous cell signaling kinases. They also act as energy carriers in the cell. Due to the versatility of rNTPs, it was expected that even macrophages would harbor high cellular rNTP concentrations. Indeed, LC–MS/MS analysis confirmed that human primary macrophages have high levels of rNTPs (mM range), which are very similar to the levels found in activated $CD4^+$ T cells (Kennedy et al., 2010). This high rNTP concentration in macrophages is consistent with the observation that SAMHD1, which is responsible for the low dNTP pools in macrophages, does not hydrolyze rNTPs (Goldstone et al., 2011).

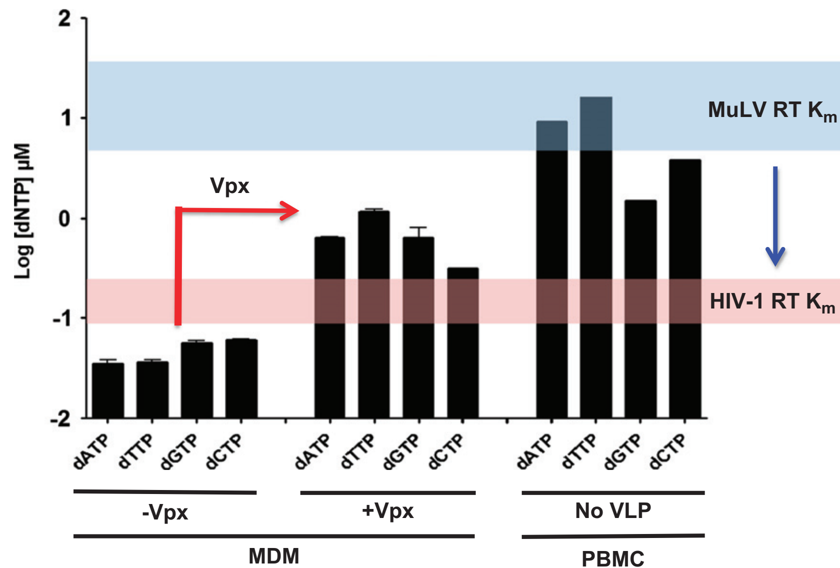


**Fig. 4.** Effect of Vpx on cellular dNTP concentrations with respect to the Km values of HIV-1 RT and MuLV RT. The concentrations of four dNTPs in primary human macrophages treated with the virus like particles with or without Vpx protein as well as the dNTP concentrations of human primary activated PBMCs are presented (Lahouassa et al., 2012). Blue arrow indicates the Km difference between HIV-1 RT and MuLV RT, while the red arrow indicates the elevation of dNTP concentration induced by the Vpx protein. The Km value of HIV-1 RT (red bar) stays above the dNTP concentration of macrophages. However, the Vpx treatment increases the dNTP concentration above the Km value of HIV-1 RT in macrophages. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

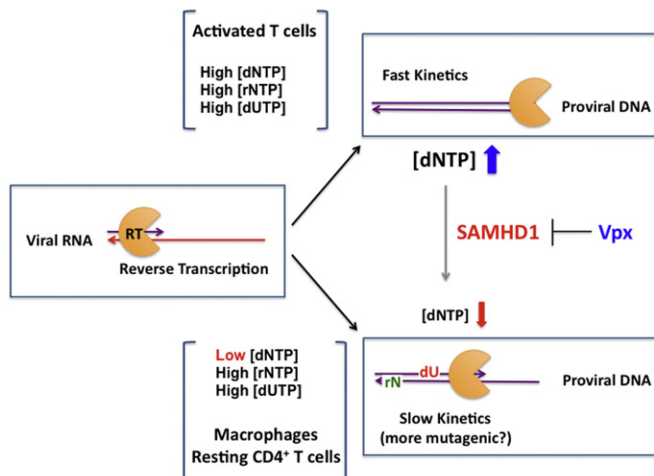


**Fig. 5.** Model for the mechanistic interplay among cellular dNTPs, SAMHD1, proviral DNA synthesis, and potential mutagenic consequences during HIV reverse transcription in macrophages. RT enzyme kinetics is controlled by the availability of cellular dNTPs, which significantly varies between dividing CD4$^+$ T cells and macrophages. These kinetic disparities appears to be tightly regulated by cellular (SAMHD1) and viral (Vpx) players. The extremely low abundance of canonical dNTPs in macrophages can force RT to incorporate mutagenic non-canonical cellular nucleotides such as rNTPs and dUTP, generating a potentially mutagenic landscape in macrophages.

Importantly, due to limited dNTP pools in macrophages, the high levels of rNTPs generate a much larger discrepancy between dNTP and rNTP concentrations in macrophages, compared to activated CD4$^+$ T cells (Kennedy et al., 2010). This large concentration disparity and the abundant rNTP pool may force HIV-1 RT to incorporate ribonucleoside monophosphates (rNMPs) during proviral DNA synthesis in macrophages, but not in activated CD4$^+$ T cells. Indeed, a series of biochemical simulations validated that HIV-1 RT can incorporate rNMPs during DNA synthesis, but only using macrophage dNTP/rNTP concentrations (Kennedy et al., 2010). This was further confirmed by the use of rN chain terminators (rNCTs) which are ribonucleosides lacking a 3′ OH. rNCTs differ from dN chain terminators, such as AZT, in that rNCTs have a 2′ OH. Predictably, if HIV-1 incorporates rNMPs during reverse transcription, rNCTs should inhibit HIV replication. Indeed, treatment with rNCTs (i.e., rACT) efficiently inhibited HIV-1 replication in macrophages, but not in activated CD4$^+$ T cells (Kennedy et al., 2010). This virological data supports that HIV-1 incorporates rNMPs during reverse transcription in macrophages. A recent follow-up study reported that HIV-1 RT incorporates rNMPs at a rate of 1 in 150 nucleotides in macrophages, which is approximately 40 times more frequent than the enzymatic incorporation of an incorrect dNTP by HIV-1 RT (Kennedy et al., 2012).

However, the virological consequences of rNMP incorporation during reverse transcription remain to be tested. One clue for this comes from long standing research about rNMP incorporation by cellular DNA polymerases. It has been well established that rNMPs embedded in DNA induce DNA polymerase pausing and mutation synthesis (Ji et al., 1994; Nick McElhinny et al., 2010; Shen et al., 2012). Interestingly, most organisms contain repair systems to specifically remove rNMPs embedded in dsDNA. RNaseH2 is a key enzyme that initiates the removal of rNMPs from dsDNA by making 5′ end nicks at rNMP sites (Rychlik et al., 2010). More interestingly, RNaseH2 is another gene that contributes to AGS when it is mutated (Crow et al., 2006; Rice et al., 2007). Also, a recent study from siRNA screening reported that RNaseH2 is involved in the HIV-1 life-cycle, though its mechanism remains unclear (Genovesio et al., 2011). Indeed, it was recently revealed that rNMPs embedded in HIV-1 proviral DNA likely remain unrepaired in macrophages, because of lower expression levels of RNaseH2 in macrophages and delayed gap repair activity found in macrophages, compared to activated CD4$^+$ T cells (Kennedy et al., 2012).

## Unusually high dUTP concentration in macrophages acts as an anti-parasitic cellular defense

Non-canonical dNTPs such as dUTP have been known as an anti-parasitic defense that can induce lethal mutagenesis for

infecting pathogens and parasites that utilize dNTPs during their DNA genome replication. Considering the extremely poor dNTP pools in macrophages, it was reasoned that dUTP, which competes against canonical cellular dTTP during DNA synthesis, can serve as an effective weapon against pathogens in macrophages. More importantly, LC–MS/MS analysis revealed an unusually high concentration of dUTP in macrophages, compared to the cellular dTTP concentration (Kennedy et al., 2011). In addition, a biochemical simulation demonstrated that HIV-1 RT fails to distinguish between dTTP and dUTP during reverse transcription and thus efficiently incorporated dUMP. Indeed dideoxyuridine (ddU), which is a chain terminator of deoxyuridine (dU), effectively inhibits HIV-1 replication in macrophages, but not in activated $CD4^+$ T cells (Kennedy et al., 2011). This study provides evidence that HIV-1 may encounter much stronger antiviral pressure in macrophages from the unusually high dUTP concentration, compared to activated $CD4^+$ T cells. Indeed, HIV-1 has evolved a mechanism to counteract the potential viral genome error catastrophe induced by dUMP incorporation during reverse transcription. Incorporated dUMPs are removed by cellular UNG2 packaged into the virion (Mansky et al., 2000; Priet et al., 2005). Furthermore, cellular cytidine deaminases such as APOBEC3G are capable of creating dUMP sites in proviral DNA, which can also contribute to viral mutagenesis, though this effect is counteracted by another viral accessory protein, Vif (Harris et al., 2003; Mangeat et al., 2003; Marin et al., 2003; Yu et al., 2004; Zhang et al., 2003). Throughout evolution, retroviruses encountered a series of cellular pressures that can lead to viral genomic mutations. The perfect balance of mutation rate and viral fitness must have been achieved to provide for viral escape without leading to error catastrophe.

## Summary and perspectives

HIV reverse transcription and RT biochemistry are currently being revisited, particularly due to the recent discovery of SAMHD1, which regulates dNTP levels. Is it clear that while the cell receptor and co-receptor recognition by retroviral envelope proteins is a primary determinant of cell tropism (Hwang et al., 1991), viruses must have evolved to overcome other cell type specific metabolic discrepancies. Indeed, as illustrated in Fig. 1, the scarcity of cellular dNTPs in macrophages, which is engineered by the enzymatic activity of SAMHD1, is a clear example of a metabolic bottleneck that HIV has to counteract during viral infection (Goldstone et al., 2011; Hrecka et al., 2011; Laguette et al., 2011; Lahouassa et al., 2012; Powell et al., 2011). In addition, the poor dNTP availability in macrophages generates a unique metabolic environment that promotes viral mutagenesis induced by frequent rNMP and non-canonical dUMP incorporation (Kennedy et al., 2010, 2011, 2012). These observations caused us to predict that macrophages may serve as a viral reservoir that contributes to the unique viral genomic hypermutability of HIV-1. Finally, unlike retroviruses, other large DNA viruses such as herpes viruses and cytomegaloviruses also infect macrophages and thus will encounter low dNTP pools during replication. However, these DNA viruses are equipped with their own dNTP biosynthesis machinery that consists of several enzymes such as ribonucleotide reductase and thymidine kinase (Leiden et al., 1980; Lembo and Brune, 2009). Possibly, these large DNA viruses gained their own dNTP biosynthesis machineries throughout their evolution to counteract the SAMHD1-induced low dNTP availability in nondividing target cell types. Collectively, SAMHD1 may be a primitive cellular defense tool that was developed to effectively control the replication of dNTP-utilizing pathogens, particularly in nondividing cell types that do not require cellular dNTPs due to lack of cellular chromosomal DNA replication.

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