

## **HIV-1 Reverse Transcriptase**

Human immunodeficiency virus (HIV) is the etiologic agent causing the Acquired Immunodeficiency Syndrome (AIDS), the world's most deadly infectious disease. According to the World Health Organization the total number of people living with the human immunodeficiency virus (HIV) rose in 2004 to reach its highest level ever: an estimated 39.4 million. In 2004 alone, approximately 4.9 million people acquired HIV, while the global AIDS epidemic killed 3.1 million people in the past year (6).

HIV is a member of the retrovirus family. An essential element in the life cycle of this family of viruses is the requirement for integrating a copy of its genetic material (genome) into the human host cell genome before virus replication can occur. HIV Reverse Transcriptase enzyme (RT) is responsible for producing the DNA copy of the viral RNA genome that will be integrated into the human DNA.

### **Structure and function of HIV-1 RT:**

HIV-1 RT is composed of an asymmetric heterodimer of two chains, a 51-kD subunit (p51) of 440 amino acids and a 66-kD subunit (p66) of 560 amino acids. The p66 subunit folds into two domains, an N-terminal polymerase domain (440 residues) and a C-terminal RNase H domain (120 residues). The polymerase domain is divided into four sub domains: the fingers (residues 1-85, 118-155), the palm (residues 86-117, 156-237), the thumb (residues 238-318), and the connection (residues 319-426), analogous to the human right hand. The p51 subunit has only the polymerase domain with the same fingers, thumb, palm and connection sub domains, however, their spatial arrangement differs markedly to those of the p66 subunit. (Fig.1)

A fascinating feature of the HIV-1 RT heterodimer is the structural asymmetry (1) which exists between the p66 and p51 subunits despite the fact that they are products of the same gene and exhibit identical amino acid sequences for the first 440 residues. The polymerase domain of p66 folds into an open extended structure containing a large active-site cleft while that of p51 is closed and compact (5).

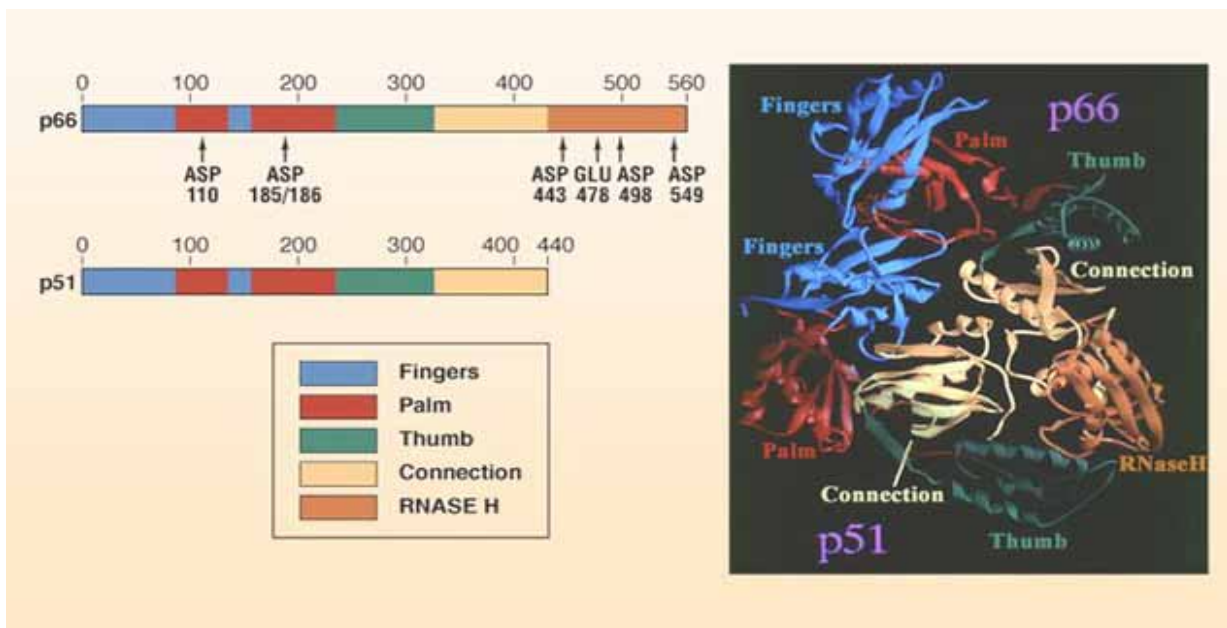


Fig: 1. Left: Illustration of the homologous sequence in the p66 and p51 subunits, and the residues included in each subdomain.

Right: Structure of RT and orientation of the subdomains in the p66 and p51 subunits

HIV-RT replicates the RNA using three main activities:

- 1) 5'-->3' RNA directed DNA polymerization -synthesizes DNA on the RNA template
- 2) RNase -degrades RNA in the RNA: DNA hybrid
- 3) 5'-->3' DNA directed DNA polymerization -synthesizes DNA on the DNA template

In addition to requiring DNA elongation and RNA degradation activities of the enzyme, complete reverse transcription of genome requires two initiation events one using tRNA<sup>Lys-3</sup> as a primer for minus-strand DNA synthesis and one using RNase H-resistant polypurine tract RNA as a primer for positive-strand DNA synthesis (4).

#### **Polymerase active site:**

The polymerase active site contains three conserved aspartic acid residues (Asp110, Asp185, Asp186) located in the p66 palm and lies at the base of the DNA-binding cleft.

The DNA binding cleft is formed by the fingers palm and thumb subdomains of the p66. The connection subdomains of both p66 and p51 form the floor of the DNA-binding cleft. The Asp 185 and Asp 186 form part of the YMDD motif (Tyr-Met-Asp-Asp) of HIV-1 RT, corresponding to the more general YXDD motif ( X = met (HIV), Val, Leu or Ala), which is highly conserved in retroviral RTs (Fig. 2). The Asp185 forms a H-bond with the 3'-OH group of Pri1 (primer). Nearly all protein-DNA interactions involve atoms of the sugar-phosphate backbone of the nucleic acid. However the phenoxyl side-chain of Tyr 183, which is part of the conserved YMDD motif, has H-bonding interactions with the nucleotide bases in the minor groove of the template-primer (2).

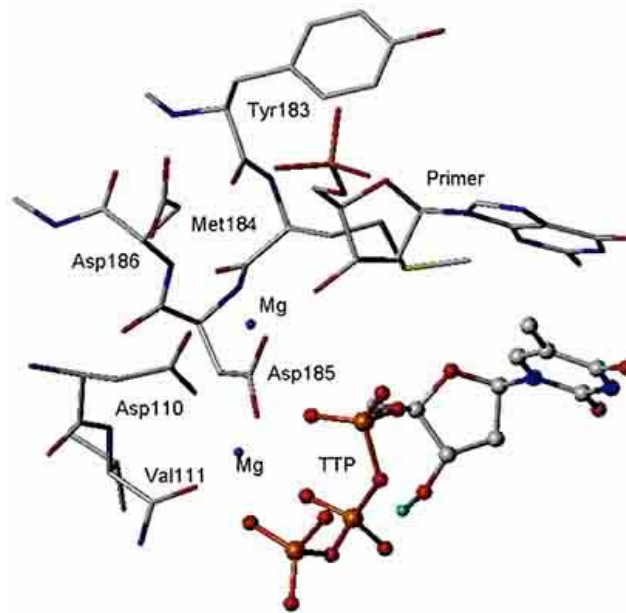


Fig: 2. Illustration of the active site of polymerase domain with the YMDD motif and  $Mg^{+2}$  ions

Within the palm domain there are also two conserved  $Mg^{+2}$  ions which assist polymerization of the incoming dNTP. During polymerization, the  $Mg^{+2}$  ions bind tightly and stabilize the pentavalent transition state on the alpha phosphate. The 2-metal ion mechanism provides a cooperative stabilization of the incoming dNTP and is the basis of the polymerization reaction.

### **dsDNA bound to the RT:**

The dsDNA bound to the RT (2HMI) has a hybrid structure. The five base-pairs near the polymerase active site have a conformation similar to A-form DNA, while the nine base-pairs towards the RNase active site have a conformation similar to B-form DNA. There is a significant bend involving the four base-pairs that join the A-form and B-form DNA portions (2). The interesting feature of the structure is that the helical axis of A-form and B-form make an angle of 41°.

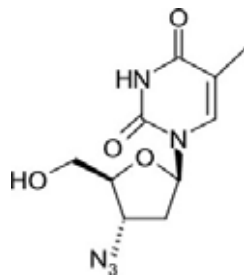
#### **RNase active site:**

There are few important residues which ensure proper positioning of the DNA-RNA template within RT. Five of the amino acids in the p66 subunit which compose the RNase H Primer Grip, hydrogen bond directly with the oxygens of the alpha phosphate within the DNA template backbone. These are: Ala360, His361, Tyr501, Lys476, and Thr473. Additionally, Gly359 and Ile505 add van der Waals interactions to stabilize the phosphate backbone while Glu 475 and Thr473 hydrogen bond with the ribose moiety of a dNTP.

#### **Inhibitors and therapy:**

Therapies involving three-drug combinations are now the standard treatment for HIV-1 infections. The approved drugs inhibit two viral enzymes: reverse transcriptase (RT) and protease. There are two types of RT inhibitors, Nucleotide analogs or Nucleoside/Nucleotide Reverse Transcriptase Inhibitor (NRTIs) and Nonnucleoside Inhibitors (NNRTIs) (Fig.3). The Nucleoside analogs lack a 3'-OH group and, when incorporated into viral DNA by HIV-1 RT, they act as chain terminators, blocking viral DNA synthesis (Fig.4). NNRTIs are noncompetitive inhibitors, which bind to a hydrophobic pocket in RT near the polymerase active site and prevent RT from carrying out the polymerization reaction. NNRTIs do not interfere with substrate binding (3).

Structure of AZT a NRTI:



3'-Azido-3'-deoxythymidine

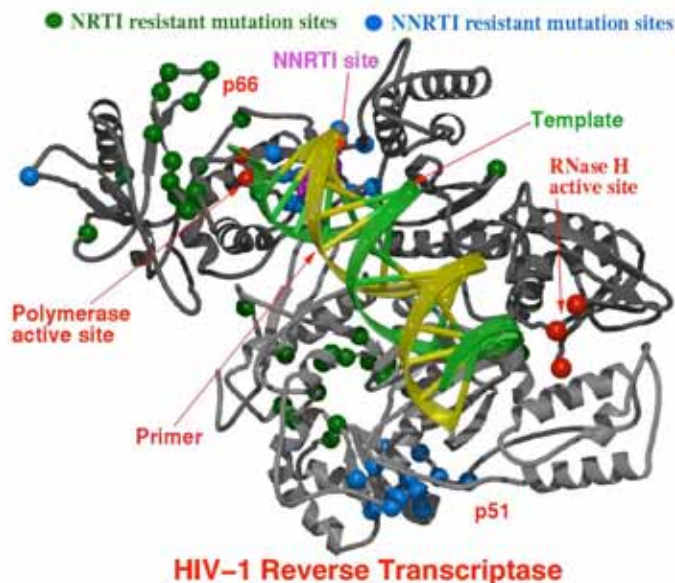


Fig: 3. RT with the NNRTI and NRTI bound to it

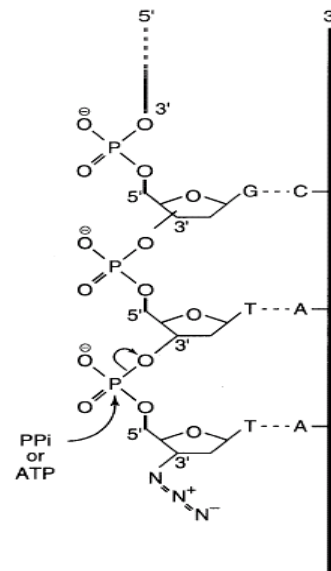


Fig: 4. The RNA template with the terminating DNA chain due to addition of AZT (NRTI)

Despite the successes with such treatments, there are still considerable problems with drug toxicity and the development of viral resistance.

### References:

- 1) Cremer, n., Tachedjian, G., (2002), Modulation of the oligomeric structure of HIV-1 Retroviral Enzymes by Synthetic Peptides and Small Molecules, *Eur. J. Biochem.* **269**, 5103-5111.
- 2) Ding, J., Das, K., Hsiou, Y., Sarafianos, S., Clark, A., Molina, J., Tantillo, C., Hughes, S., Arnold, E., (1998). Structure and Functional Implications of the Polymerase Active Site Region in a Complex of HIV-1 RT with a Double-stranded DNA Template-primer and Antibody Fab Fragment at 2.8 Å Resolution. *J. Mol. Biol.* **284**, 1095-1111.

3) Huges, S., (2001) Molecular Matching: NNRTIs can Enhance the Dimerization of HIV type 1 Reverse Transcriptase, *Proc. Natl. Acad. Sci. USA*, **98**, 6991-6992.

4) Pata, J., Stirtan, W., Goldstein, S., Steitz, T., (2004), , Structure of HIV-1 Reverse Transcriptase Bound to an Inhibitor Active against mutant Reverse Transcriptases resistant to tother Nonnucleoside inhibitors, *Proc. Natl. Acad. Sci. USA*, **101**,10548-10553.

5)Wang, J., Smerdon, S., Jager, J., Kohlstaedt, A., Rice, A., Friedman, J.,Steitz, T., (1994) Structural Basis of Asymmetry in The Human Immunodeficiency virus type 1 Reverse Transcriptase Heterodimer, *Proc. Natl. Acad. Sci. USA*, **91**, 7242-7246.

6)[www.biochemistry.ucla.edu/biochem/Faculty/Feigon/153bh/2005/Justin\\_Miyamoto/hiv-mg.html](http://www.biochemistry.ucla.edu/biochem/Faculty/Feigon/153bh/2005/Justin_Miyamoto/hiv-mg.html)