The role of integrase in HIV-1 maturation

Čemeljić, Noa

UNIVERSITY OF RIJEKA
DEPARTMENT OF BIOTECHNOLOGY
Undergraduate Programme
Biotechnology and Drug Research

Noa Čemeljić

The Role of Integrase in HIV-1 Maturation

Bachelor's thesis

Rijeka, 2019.

UNIVERSITY OF RIJEKA
DEPARTMENT OF BIOTECHNOLOGY
Undergraduate Programme
Biotechnology and Drug Research

Noa Čemeljić

The Role of Integrase in HIV-1 Maturation

Bachelor's thesis

Rijeka, 2019.

Mentor of the thesis: Igor Jurak, PhD, Associate Professor

SVEUČILIŠTE U RIJECI
ODJEL ZA BIOTEHNOLOGIJU
Preddiplomski sveučilišni studij
Biotehnologija i istraživanje lijekova

Noa Čemeljić

Uloga integraze u sazrijevanju HIV-1

Završni rad

Rijeka, 2019.

Mentor rada: doc. dr. sc. Igor Jurak

Bachelor’s thesis is defended on June 26th, 2019, in front of the commission:

1. Ivana Ratkaj, PhD, Associate Professor
2. Željka Maglica, PhD, Associate Professor
3. Igor Jurak, PhD, Associate Professor (mentor)

The thesis has 23 pages, 11 figures, 0 tables and 26 literature references.

Summary

Integrase (IN) is one of the most important enzymes in the replication of the HIV-1 virus, as it integrates viral DNA (vDNA) into the genome of the host cells. However, recent studies have suggested a new surprising role of IN in HIV-1 maturation. If IN is inhibited either by mutations or by allosteric integrase inhibitors (ALLINIs), viral ribonucleoprotein complexes (vRNPs) are often eccentrically localized outside of the capsid (CA). Such viral particles are non-infectious and are blocked at an early stage of the reverse transcription in the target cells, as a result of premature degradation of IN and viral RNA (vRNA) genome, together with the spatial separation of reverse transcriptase (RT) from vRNA.

IN directly binds the vRNA genome in virions by its surface exposed lysine residues, exhibiting a distinct preference for selected vRNA structural elements. In this way, IN provides nucleation points by bridging separate RNA molecules, promoting the effective compaction of vRNPs. These interactions then ensure the proper packaging of vRNPs inside the CA.

Even though the mechanism of action of ALLINIs, an emerging class of anti-HIV drugs, is still not fully understood, it seems that they mostly act by blocking the IN-vRNA interaction, which results in aberrant non-infectious particles.

This non-catalytic role of IN enables the understanding of the HIV-1 maturation process in greater detail, which can be successfully used in the development of new drugs, such as ALLINIs. However, a lot of research still needs to be done, as there is a lot of detail in this IN-driven maturation process that still needs to be explained.

Keywords: HIV-1, integrase, maturation, vRNPs, vRNA packaging, IN-vRNA interactions, ALLINIs

Sažetak

Integraza (IN) je jedan od najvažnijih enzima u replikaciji HIV-1 virusa, budući da integrira virusnu DNA (vDNA) u genom stanice domaćina. Međutim, novije studije su predložile novu, iznenađujuću ulogu IN u sazrijevanju HIV-1. Ukoliko je IN inhibirana bilo mutacijama bilo alosteričkim inhibitorima integraze (ALLINI-ji), virusni ribonukleoproteinski kompleksi (vRNP-ovi) su često acentrično lokalizirani izvan kapside (CA). Ove čestice nisu infektivne i blokirane su u ranoj fazi reverzne transkripcije u ciljnim stanicama, kao rezultat preuranjene degradacije IN i virusnog RNA (vRNA) genoma, zajedno s prostornom odvojenošću reverzne transkriptaze (RT) od vRNA.

IN izravno veže vRNA u virionima putem svojih površinski izloženih lizinskih ostataka, pokazujući izrazite preference za određene vRNA strukturne elemente. Na ovaj način IN stvara nukleacijske točke, premošćujući odvojene RNA molekule i unaprjeđujući učinkovito zbijanje RNP-ova. Ove interakcije osiguravaju ispravno pakiranje vRNP-ova unutar CA.

Iako mehanizam djelovanja ALLINI-ja, skupine anti-HIV lijekova u nastajanju, još uvijek nije do kraja razjašnjen, čini se da oni većinski djeluju blokirajući IN-vRNA interakcije, što rezultira abnormalnim neinfektivnim česticama.

Ova nekatalitička uloga IN omogućava detaljnije shvaćanje procesa sazrijevanja HIV-1, što se može uspješno upotrijebiti u razvoju novih lijekova poput ALLINI-ja. Međutim, potrebno je provesti još mnogo istraživanja jer puno detalja u ovom IN potpomognutom procesu sazrijevanja još uvijek mora biti razjašnjeno.

Ključne riječi: HIV-1, integraza, sazrijevanje, vRNP-ovi, pakiranje vRNA, IN-vRNA interakcije, ALLINI-ji

Content

1. Introduction

The term "human immunodeficiency virus (HIV)" reffers to two species, HIV-1 and HIV-2, belonging to the group of retroviruses and subtype of lentiviruses, which can cause the acquired immunodeficiency syndrome – AIDS [1]. Although these two viruses are related, there are many biological differences including their genetic and structural elements, time of replication and progression of infection. HIV-1 is responsible for the majority of the AIDS related infections, by infecting macrophages, dendritic cells and especially CD4+ T-cells. Today, there are around 37 million people globally living with HIV-1, and every year almost 2 million people become newly infected, with approximately 1 million people dying from AIDS-related diseases [2]. As almost 40% of all people living with HIV-1 still does not have access to antiretroviral therapy [2], this epidemic poses one of the major world health problems.

HIV-1 is present only in certain body fluids, such as blood, semen, pre-seminal fluid, rectal fluids, vaginal fluids, and breast milk. For transmission to occur, these fluids must come in contact with mucous membranes, which are found inside the rectum, vagina, penis, and mouth, or with the damaged tissue. Therefore, the virus is primarily transmitted in three ways: sexually, vertically from a mother to a child (during pregnancy, birth or breastfeeding), and by direct blood contact with an infected persons' blood [3]. The HIV-1 diagnosis is mostly based on tests for HIV-1 antibodies, which are detected by using ELISA together with the PCR and Western blot techniques. These tests are today inexpensive and extremely accurate [4] [5].

The majority of the people will, without receiving antiretroviral therapy, develop AIDS symptoms in five to ten years after the infection, with the average lifespan being eleven years [6].

The first phase of HIV-1 infection, the acute phase, starts days to weeks upon the infection and lasts only 1-2 weeks. The patient experiences mononucleosis-like or flu-like symptoms, such as fever, headache,

lymphadenopathy, and rash. In this stage, HIV multiplies rapidly, destroying the CD4+ T-cells that try to fight off the infection. Because of the nonspecific nature of these symptoms, they are often misdiagnosed for the more common infectious diseases with the same or similar symptoms [5, 7].

The second phase is called the chronic HIV infection, but also known as asymptomatic HIV infection or clinical latency. During this phase, HIV continues to multiply in the body but at the very low levels, as a strong immune defense response reduces the number of viral particles in the bloodstream at the end of the acute phase. The duration of this stage is approximately 8 years, during which the infected person does not experience any symptoms, but can spread the virus [5, 7].

The third phase is known as AIDS, and it is the final stage of HIV- casused disease progression, when the immune system is already very weakened and not able to fight off opportunistic infections and cancers. AIDS is diagnosed when the concentration of CD4+ T-lymphocytes is lower than 200 cells per mm3 of blood, making the organism vulnerable to various opportunistic infections, such as pneumocystis pneumonia and candidal esophagitis, or cancers, such as Kaposi's sarcoma, Burkitt's lymphoma or cervical cancer. Without receiving antiretroviral therapy, people with AIDS usually survive about three years [5] [7].

HIV evolved from the simian immunodeficiency virus (SIV), which infects primates residing in the west and central Africa. As SIV is a relatively weak virus for humans and can be easily defeated by the human immune system, the most common explanation is that SIV evolutionary developed into HIV to successfully replicate in humans, and therefore secured its existence. The mass movement of people in the 20th century helped the virus to spread out of Africa, and it was first discovered and described in the mid-1980s by two independent research groups under the lead of Robert Gallo and Luc Montagnier [8].

The first HIV antiretroviral drug was zidovudine, which acts as a reverse transcriptase nucleoside inhibitor, approved by the US Federal Drug Agency (FDA) in 1987. However, only with the introduction of highly active

antiretroviral therapy (HAART) in 1996., which combined reverse transcriptase inhibitors with protease inhibitors, the AIDS-related death rates started to decline by 60-80 % [9]. Since then, HIV therapy became very efficient, causing HIV infection to become a chronic disease that progresses into AIDS very slowly. Today, HAART therapy is mostly administered in the fixed-dose combination, combining several antiretroviral drugs into one pill. These drugs are divided into several classes, depending on which viral enzyme or on which viral replication step they act: protease inhibitors, coreceptor antagonists, nucleoside and non-nucleoside reverse transcriptase inhibitors, fusion inhibitors and integrase inhibitors [10].

HIV infected patients can nowadays expect to achieve nearly normal life expectancy if taking antiretroviral therapy, which causes long viral suppression. However, they need to take lifelong medication, which has its side effects, such as causing higher rates of kidney, liver, cardiovascular or neurological diseases [11]. This has stimulated further research towards enhancing already existing therapy and finding new, even more efficient drug targets. One of such targets is the HIV-1 enzyme integrase (IN), for which the new research suggests that it is not only involved in the integration of HIV-1 genome inside the host cell genome, but also in the maturation of new viral particles. Even though it seemed that the replication and maturation cycle of HIV-1 is very well observed and known, recent discoveries, like in the case of IN, suggest there is still much to learn about these processes. This is calling for a need to revise our current knowledge about HIV-1 replication and maturation cycles introducing the new research data, and to think about the possible future applications of such discoveries, especially in the development of new drugs and in the treatment of patients.

2. Aim of the thesis

While the structure and catalytic function of IN are very well known, recent studies suggest that viral DNA integration is not the only role of IN, but that it is also involved in the viral particle maturation. Allosteric integrase inhibitors (ALLINIs), a new promising class of antiretroviral drugs, can block the integration steps, but can also block the interactions between the IN and viral RNA (vRNA) genome, causing the viral particles to be non-infectious. This strongly suggests that IN can be crucial for the proper HIV-1 maturation and its infectivity. The focus of this thesis is to discuss this newly found role of IN in virion maturation and how it can be used in the development of antiretroviral therapy of HIV-1 patients.

3. Discussion

3.1 Structure and genome of HIV-1

HIV-1 virion has spherical shape, with a diameter of approximately 100-150 nm (Figure 1). Two copies of positive single-stranded RNAs, which make the HIV-1 genome, are found inside the capsid (CA) made of viral p24 protein. The RNA molecules are tightly bound to the nucleocapsid (NC) p7 proteins. Two enzymes needed for the replication and development of the virion, reverse transcriptase (RT) and integrase (IN), are also inside the CA. The CA is surrounded by a matrix composed of p17 viral protein, which ensures the integrity of the virion. The third important enzyme in the replication, protease (PR), can be found between the CA and the matrix. The matrix is encircled by the viral envelope, mostly composed of host cell proteins and a lipid bilayer, as it is a part of the host cell membrane taken during the budding process of new viral particles. The envelope glycoprotein complex, which is essential for virus attachment to the recpetor on a target cell and entry of virus into the cell, consists of glycoprotein 120 (gp120) cap and a stem made of three glycoprotein 41 (gp41) molecules [5] [12].

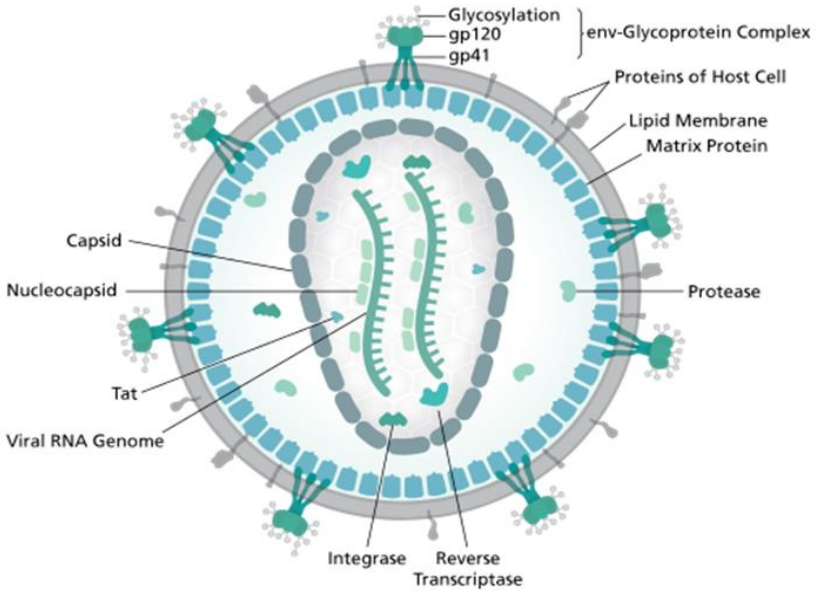

Figure 1: HIV-1 structure. Two RNA molecules bound to the nucleocapsid (NC) proteins are found inside the capsid (CA), which is surrounded by the matrix and the viral envelope containing glycoprotein complexes. The enzymes integrase (IN) and reverse transcriptase (RT) are found inside the CA, while protease (PR) is outside of the CA. The work of Thomas Splettstoesser (www.scistyle.com).

Two genomic RNA molecules are 9749 nucleotides long, containing the 5' CAP and 3' poly-A tail, together with the long terminal repeats (LTR) on 5' and 3' end (Figure 2). There are three main genes which code for main viral structural proteins and enzymes:

1) Gag gene encodes a polyprotein which is later processed by PR giving structural proteins p7 (nucleocapsid protein), p17 (matrix protein) and p24 (capsid protein)
2) Pol gene encodes viral enzymes PR, RT and IN
3) Env gene encodes the glycoproteins gp120 and gp41

There are two most important regulatory genes: Tat and Rev. Tat gene regulates the transcription of viral DNA in the host cell, while Rev regulates the gene expression of viral proteins, which will be later explained during the description of the HIV-1 replication cycle. The rest of the genes code for the

accessory regulatory proteins: vpr, vif, nef, and vpu, which assist in the viral replication [5].

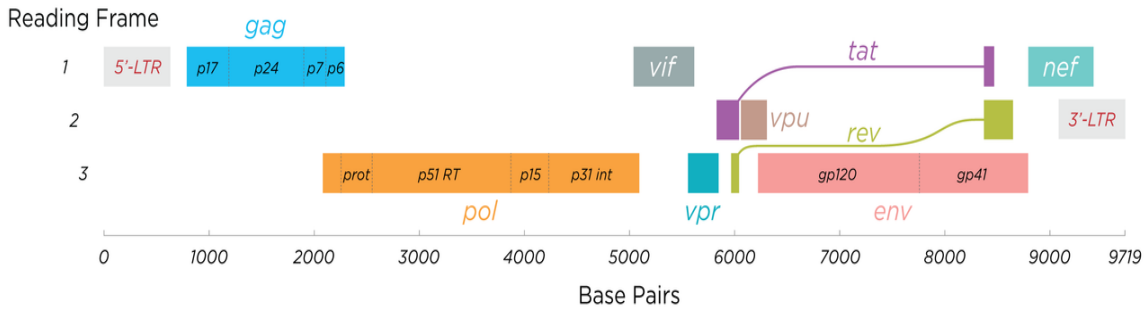

Figure 2: HIV-1 genome containing the structural genes (gag, pol, env), regulatory genes (tat and rev), and accessory regulatory genes (vpr, vif, nef, vpu). The work of Thomas Splettstoesser (www.scistyle.com).

3.2 HIV-1 replication cycle

The replication cycle of HIV-1 inside CD4+ T-lymphocytes can be divided into several steps (Figure 3):

In the first step, HIV-1 enters the cell by binding to the CD4 receptor on the T-cell surface, using the viral envelope glycoprotein gp120. This gp120 - CD4 interaction drives the conformational change of the viral envelope, enabling gp120 to bind to the T-cell coreceptors, CCR5 and CXCR4. In the next step, viral gp41 penetrates the cell membrane and brings the virus closer to the cell. When the viral envelope is found in the close proximity to the T-cell, it fuses with the cell membrane. Following the fusion of the envelope with the membrane, the CA containing two single-stranded vRNA and key viral enzymes (RT, IN and PR) is injected in the cell [5].

In the second step, the HIV-1 CA is uncoated and the RT copies the vRNA into the complementary two-stranded viral DNA molecule (vDNA). This process is extremely error-prone due to the poor proofreading potential of the RT, resulting in the great number of HIV-1 mutations. After the process of reverse transcription is completed, the RT degrades viral RNA molecules [5] [12].

In the third step, the IN binds to the vDNA, forming a complex known as intasome. The intasome is then transferred in the nucleus through a nuclear pore, where the IN integrates the vDNA in the host cell chromosome. This process will be described in greater detail later in the text.

In the fourth step, integrated vDNA is transcribed by host RNA II polymerase and various cell's transcription factors, such as NF-kB [13], to generate pre-messenger RNA (pre-mRNA), some of which undergo the splicing process to give mature mRNA. These mature mRNAs, which contain the Rev and Tat gene, are transported from the nucleus to the ribosomes for the translation. Produced Tat protein significantly enhances the transcription efficiency by recruting cell's transcription factors, while the Rev protein acts as an adaptor that returns to the nucleus and binds longer and larger mRNAs that do not undergo the splicing process and can not be alone exported from the nucleus, i.e. unspliced genomic RNA (gRNA). When Rev is bound to gRNAs, they are successfully exported in the cytoplasm. Some gRNA act as complete copies of the viral genome, while others contain genes from which new viral structural proteins and enzymes are synthesized [14]. The results of gRNA translation are long polypeptide chains containing several viral enzymes necessary for further viral development. These enzymes need to be cut from the polypeptide chain in the reaction catalyzed by protease to become functional [12].

In the fifth step, Gag proteins bind to the viral RNA copies and pack them into new viral particles that are transported to the cell membrane, where the particle binds to the internal side of the membrane. Env polyprotein gp160 is then processed by furin, an enzyme inside the Golgi Apparatus, giving rise to gp41 and gp120 glycoproteins. These glycoproteins are then also transported to the cell membrane, where gp41 acts as an anchor for gp120 inside the lipid bilayer. Viral particles then bud from the host cell, taking a part of the cell membrane as their viral envelope, thus destroying the infected T-lymphocyte. A newly formed virion is still immature and not infectious until all Gag polyproteins are processed by PR, forming the matrix, CA and NC proteins [12]. When this processing is done, a new

HIV-1 viral particle becomes infectious and can attack new cells, repeating the replication cycle.

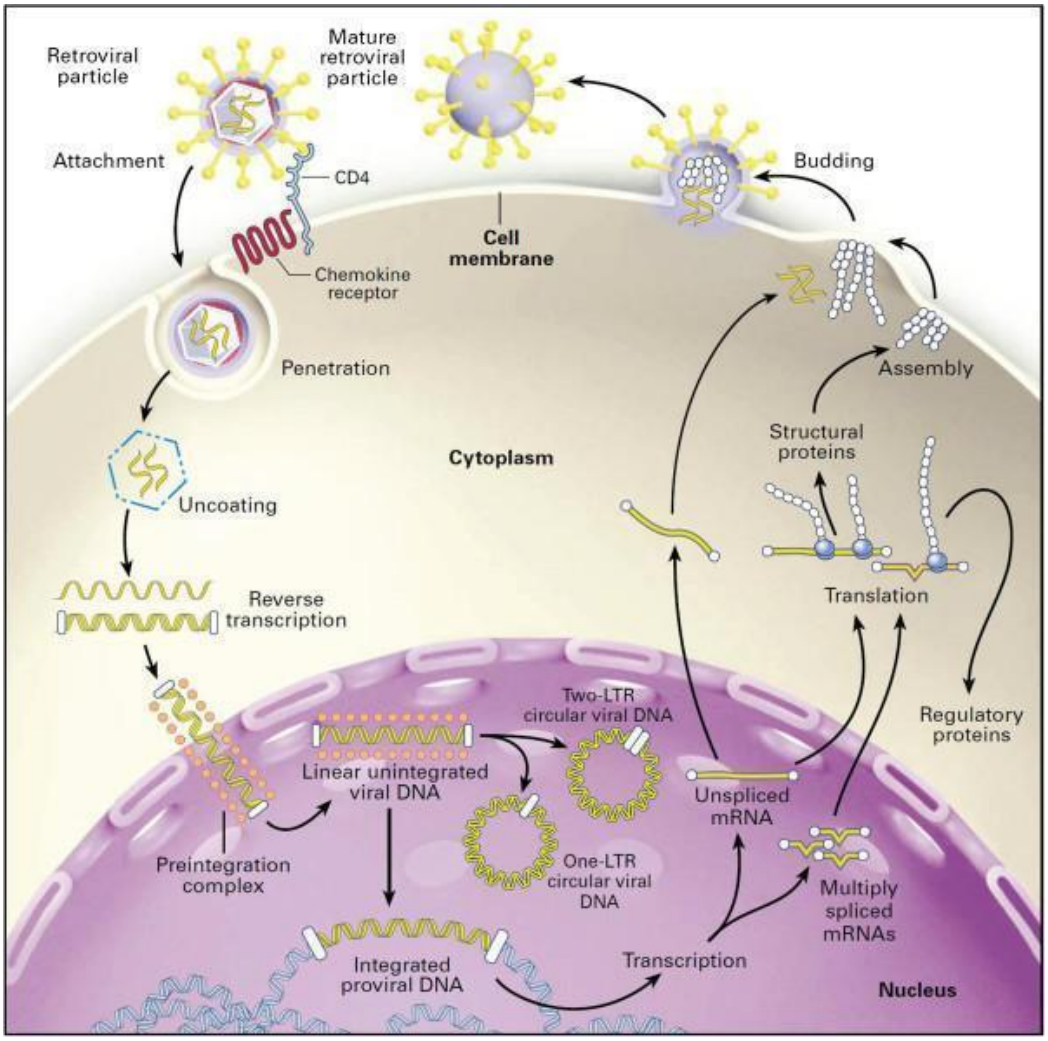

Figure 3: HIV-1 replication cycle. The cycle starts with the viral entry into the host cell, followed by the reverse transcription and integration of the viral cDNA into the host cell genome. Using the host cell's transcription factors, viral genes are transcribed and translated, forming new viral particles, which bud from the cell. Taken from Pasternak et al. 2013. [14].

3.3 Integrase structure and catalytic function

HIV-1 integrase is a 32 kDa protein (288 amino acids) and a member of polynucleotidyl transferases family. The protein can be divided into three functionaly distinct domains (Figure 4): the N-terminal domain, the catalytic core domain, and the C-terminal domain [15].

The N-terminal domain (NTD) is a small dimer that is predominantly composed of hydrophobic residues, containing 50 amino acids (residues 1-50) and a zinc-binding HHCC (a pair of His and Cys residues) motif. Binding of zinc to this motif enhances the multimerization of IN into an active tetramer form [16], which is required for optimal enzymatic activity.

On the other hand, the catalytic core domain (CCD) is a spherical dimer composed of 162 amino acids (residues 51-212) which contains a highly conserved DD(35)E motif with the key residues of the IN active site: Asp64, Asp116, and Glu152. These three residues coordinate divalent metal ions of magnesium and manganese, which play a key role in the phosphodiester cleavage and bond formation reactions that are catalyzed during the integration process [15].

The C-terminal domain (CTD) is a dimer composed of 75 amino acids (residues 213-288) responsible for the non-specific binding of DNA during the integration process. It is considerably less conserved among retroviral INs than the two other domains [17].

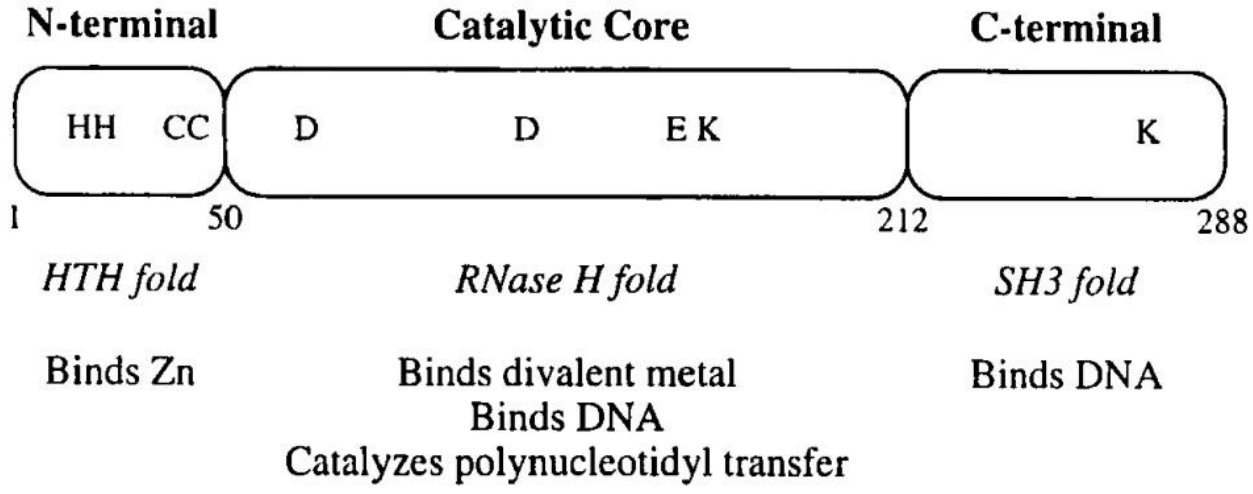

Figure 4: Schematic structure of HIV-1 IN, composed of N-terminal domain, catalytic core and C-terminal domain, each of them having a different role. Taken from Esposito and Craigie 1999. [17]

The role of IN in the vDNA integration is well described and documented. First, an IN tetramer binds both vDNA ends forming the intasome complex. The intasome then associates with the cellular protein

LEDGF/p75, which guides the intasome to the specific chromatin sites and enhances the efficiency of the integration process [18, 19].

The integration reaction occurs in three steps (Figure 5). First, in the step known as the 3' end processing, two nucleotides are removed from the vDNA 3' ends by a hydrolytic cleavage process, leaving two free 3'-OH groups. In the second step, known as the DNA strand transfer, these two 3'-OH groups are used to perform a nucleophilic attack on a pair of phosphodiester bonds separated by 5 bp in the target DNA, forming the integration intermediate where the vDNA 3' ends are covalently bound to the target DNA. In the final step, cellular enzymes repair the integration intermediate by removing the two unpaired nucleotides at each 5' end of the vDNA, followed by gap filling and ligation [17, 20].

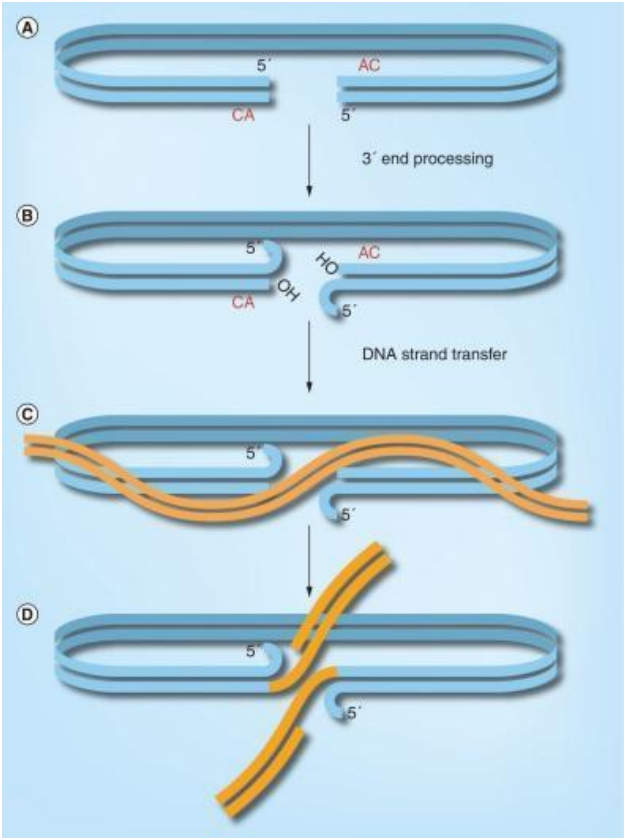

Figure 5: Steps of vDNA integration: 3’ end processing removes the two nucleotides from the vDNA 3’ ends and is followed by the DNA strand transfer, in which the two free OH groups attack the phosphodiester bond in the target DNA, resulting in the integration intermediate. Finally, cellular enzymes repair the intermediate, ensuring the integration of vDNA in the host cell’s genome. Taken from Craigie 2012. [20]

3.4 Integrase mutations

Surprisingly, IN mutagenesis screens have shown that various mutations in the IN coding region impair not only the integration but also other processes involved in HIV-1 replication. Based on the observed phenotypes, the IN mutations have been grouped into two general classes: class I mutations impair integration, while class II mutations affect reverse transcription, particle assembly, and maturation [21]. Some of these class II mutations result in severe morphological defects in HIV-1 virions, especially during virion maturation.

The HIV-1 virion maturation starts when PR cuts the long Gag-encoded polyproteins. The cleaved NC proteins from Gag polyprotein then bind with the vRNA inside the conical CA, thus forming the viral ribonucleoprotein complexes (vRNPs). The majority of the proposed virion maturation models have therefore been focused on the proteolytic cleavage processes. However, in particles with the class II IN mutations, the vRNPs are eccentrically localized outside of the CA, leaving it empty. Such particles are noninfectious, and are blocked at an early stage of reverse transcription in the cells they attack [22]. These findings first revealed that IN has an active role in the encapsidation of the vRNA inside the mature conical CA.

3.5 Allosteric integrase inhibitors (ALLINIs)

The early class of IN inhibitors, IN strand transfer inhibitors (INSTIs), inhibit DNA strand transfer activity, as they bind to the intasome at the IN active site and remove the terminal deoxyadenosine of vDNA, which would otherwise be used by IN to cut specific chromatin sites in the target cell [23]. A new class of IN inhibitors which has emerged only recently, allosteric IN inhibitors (ALLINIs), function differently. Their ability to inhibit the interaction between the IN and the cellular integration cofactor LEDGF/p75 was long considered to be the main mode of their action.

However, it was only recently discovered that these compounds also affect the late stage of HIV-1 replication, independently of LEDGF/p75

inhibition [24]. Similar to some class II IN mutants, viral particles produced during ALLINI treatment show very similar morphological defects of vRNPs mislocalization outside of the CA (Figure 6). A big fraction of virions that were formed under the ALLINI treatment has malformed and non-conical CA, while the fraction with the normal closed conical CA is markedly reduced. In the majority of these virions, even if the conical CA was assembled, the vRNPs were not incorporated inside it, but remained outside, between the CA and the viral envelope. IN also tends to be mislocalized, found outside of the CA or loosely attached to it. Furthermore, when IN is supplied to such virions, some of the malformed CA are replaced with the conical ones [25].

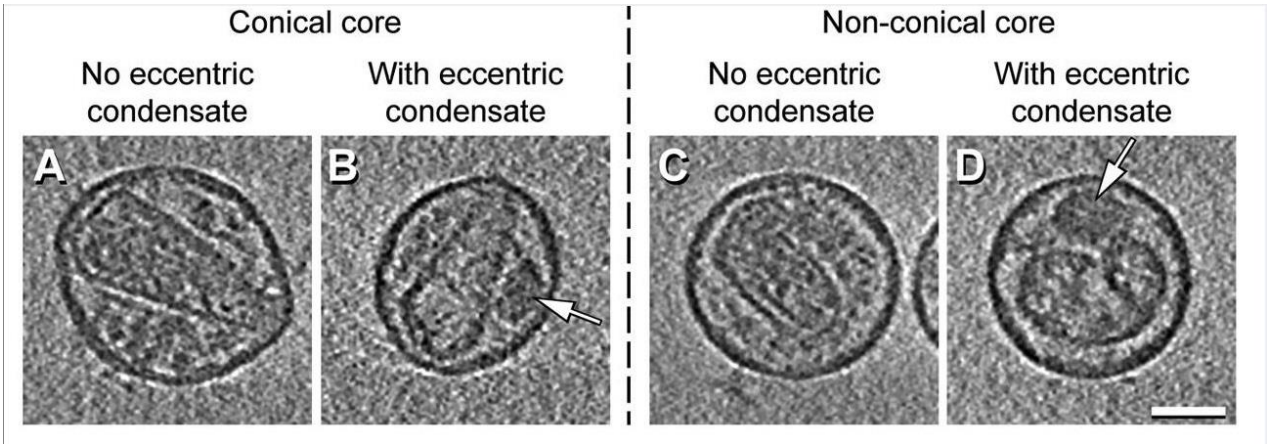

Figure 6: Comparison of viral particles with and without eccentric condensate and conical core. Taken from Fontana et al. 2015. [25]

ALLINIs require both the IN and vRNA to induce the formation of vRNP eccentric condensate, as viral constructs that cannot express the vRNA did not yield a big fraction of eccentric aggregates when ALLINI-treated, compared with the normal virions that contain the vRNA [25]. This suggests that the disruption of IN-vRNA interaction causes the improper incorporation of vRNPs into the CA during the HIV-1 core morphogenesis, which may be the mechanism by which ALLINIs adversely affect virion maturation.

3.6 Reverse transcription blockade

As already mentioned, the particles containing eccentric condensate are blocked at an early stage of reverse transcription in the target cells, even though they have all the components necessary for this process (vRNA genome, functional RT and normal NC-RNA levels). In the HIV-1 wild type, vRNA and IN cannot be degraded by the host cell metabolism and defense pathways because they are found inside the protective CA.
Since this is not the case in the aberrant eccentric particles, vRNA and IN found outside of the CA are prematurely degraded upon the infection of the target cells, while RT remains active inside the CA lattice. This premature degradation of the vRNA genome, together with the spatial separation of RT from the eccentrically localized vRNPs, can explain this transcription defect of eccentric particles [26].

3.7 Integrase - vRNA interactions

Using the CLIP-seq techniques, it was determined that IN is directly bound to vRNA in virions (Figure 7) and that this binding is not uniform across the viral genome, as IN shows a strong preference for certain vRNA elements (Figure 8). For example, IN strongly binds the trans-activation response (TAR) element on vRNA, especially the TAR loop and 3-nt bulge of TAR [22].

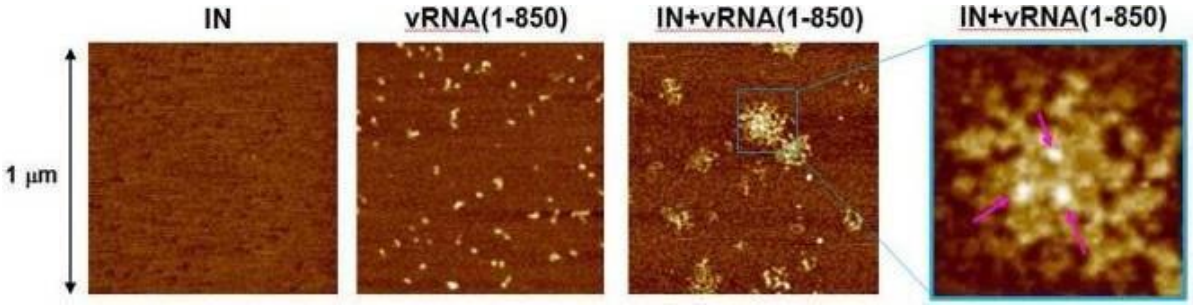

Figure 7: AFM images of purified samples of IN alone, vRNA and IN+vRNA. The zoomed far-right image contains arrows pointing to the bright spots corresponding to the IN bound to vRNA. Taken from Kessel et al. 2016. [22].

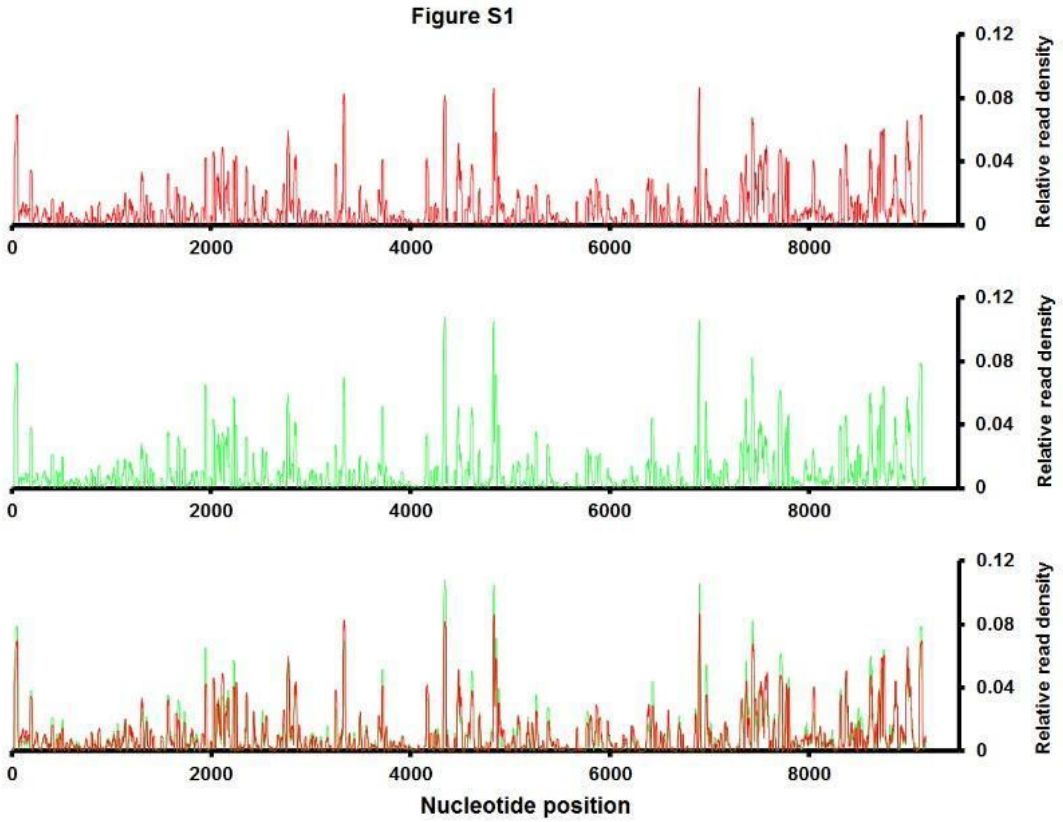

Figure 8: CLIP-Seq Analysis of IN binding sites on the vRNA genome from two independent experiments (the highest and middle graph), showing a nearly perfect overlap between IN binding sites (lowest graph). Taken from Kessel et al. 2016. [22].

The surface exposed lysine residues (K264, K266, K269, K273) in the IN C-terminal domain (CTD) are believed to be the major sites of IN-vRNA interaction, as substitutions of these residues result in IN failing to bind vRNA. These mutations did not detectably affect other known IN functions nor processing of HIV-1 polyproteins by the protease, packaging of genomic RNA or particle release. However, the particles in which IN-vRNA interactions did not occur contained eccentric vRNP condensates and were noninfectious, as they fail to undergo the reverse transcription process in the target cells [22].

It appears that IN provides nucleation points by bridging separate vRNA molecules. It does not coat the vRNA, even when it is added in large excess - around four subunits of IN bind per one vRNA molecule [22]. In this way, IN effectively compacts the two copies of the viral genome, significantly

impacting the vRNP architecture, as well as ensuring the incorporation of vRNPs within the protective CA [22].

Because of these multiple IN-vRNA binding sites, IN multimers can efficiently bridge different segments of vRNA, promoting the vRNP compaction. Furthermore, the redundancy of structural elements along the vRNA on which the IN can bind allows the virus not to depend only on a single IN binding to a particular element on vRNA [22].

When ALLINIs bind to the IN CCD domain, they promote multiple protein-protein contacts and induce aberrant IN multimerization (Figure 9). The vRNA interacting residues are then shielded by these multimers and unable to productively engage vRNA [22]. On the other hand, ALLINI-induced aberrant IN multimerization does not affect the NC binding on vRNA, which means that vRNP condensation takes place idependently, before the core formation [26].

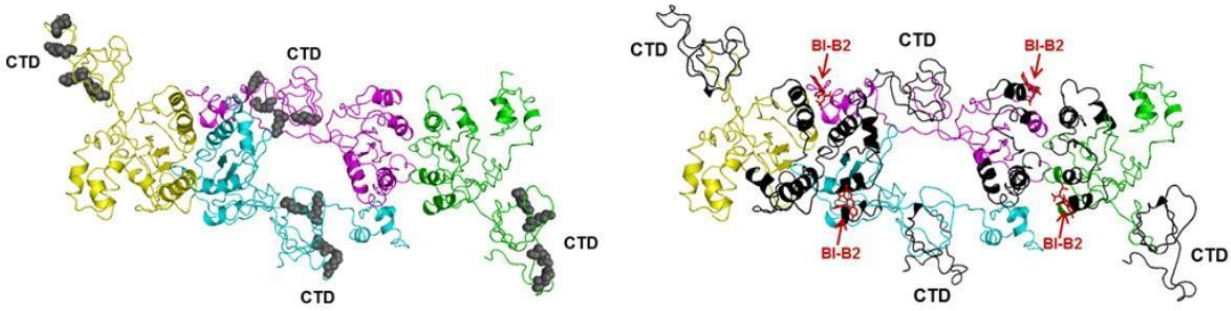

Figure 9: A molecular model of the IN tetramer with vRNA and ALLINI binding sites (K264, K266, K269, K273) shown as black spheres (left), and with the bound BI-B2 ALLINIs (right). Taken from Kessel et al. 2016. [22].

3.8 HIV-1 maturation model

These discoveries showed that IN initiates vRNP incorporation into the CA, followed by the complete CA formation around them. When the maturation starts with the Gag-polyprotein processing by the protease [25], IN binds to the vRNA and ensures the packaging of the vRNA-NC complex inside the conical CA. ALLINIs, as well as some class II IN mutations, induce

aberrant IN multimerization which disrupts the binding of this enzyme to the vRNA. This leads to vRNPs being localized outside of the CA and to the formation of the noninfectious eccentric particles (Figure 10).

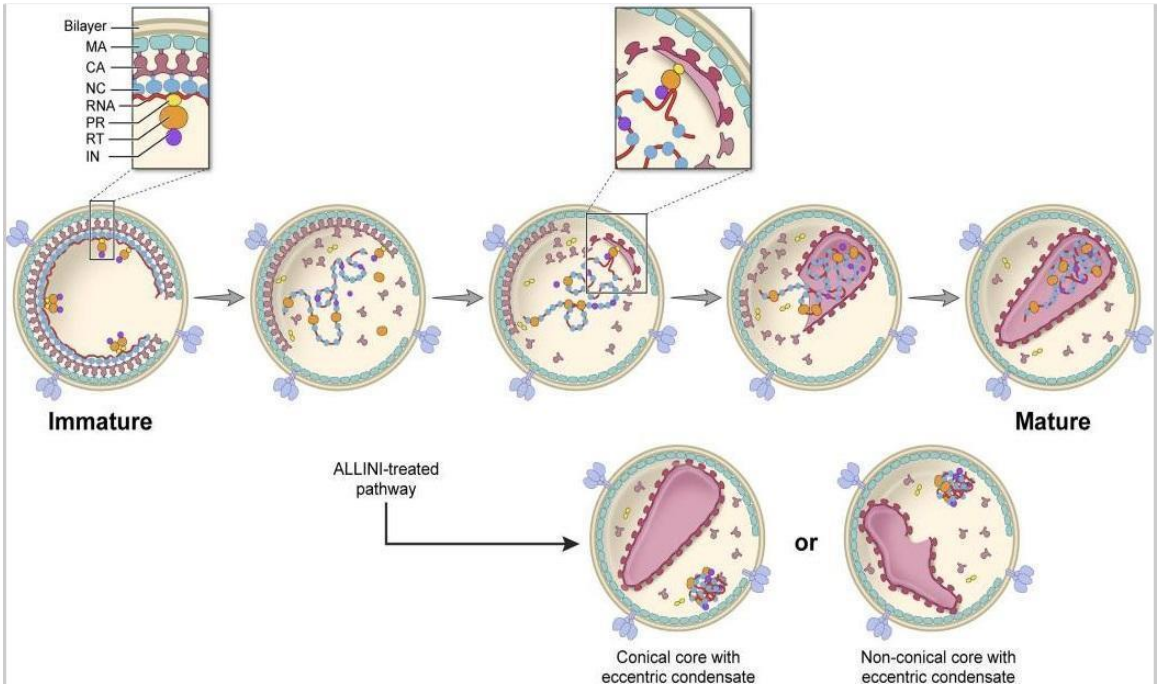

Figure 10: Model for the role of IN in coordinating HIV-1 CA assembly and vRNP incorporation into the mature core. Taken from Fontana et al. 2015. [25].

3.9 ALLINI's mechanism of action

When the previously described results of ALLINI treatment of viral particles are taken into account, it is clear that ALLINIs are very promising new class of IN inhibitors, that could significantly enrich the already existing antiretroviral therapy. Even though there is a general idea on how ALLINIs work, there is still not a definite proposed mechanism model, like for the ALLINI precursors, INSTIs (Figure 11). When INSTIs are bound in the IN active site, their chelating moiety interacts with the two magnesium ions by moving the 3'-OH end of vDNA away from the active site by approximately 6 A. The newly formed complex is then stabilized by the halobenzyl moiety, which is able to fit within a tight pocket and make the pi-stacking interaction with the C16 atom. These interactions lead to the removal of the 3'-OH

group from the active site, so it can not attack the target DNA [15]. This mechanism of action is shared by the two best known IN inhibitors, Raltegravir and Elvitegravir.

Such a model still cannot be established for ALLINIs, for example BI-D and BIB-2 (Figure 12). The mechanism is most likely multimodal, combining their previously known ability to disrupt the IN-LEDGF/p75 interaction, together with their newly discovered ability to induce the aberrant IN multimerization. While this extends our understanding of virion maturation and how ALLINIs work, there is still a lot of research needed to be done to fully understand these processes.

Elvitegravir **Raltegravir**

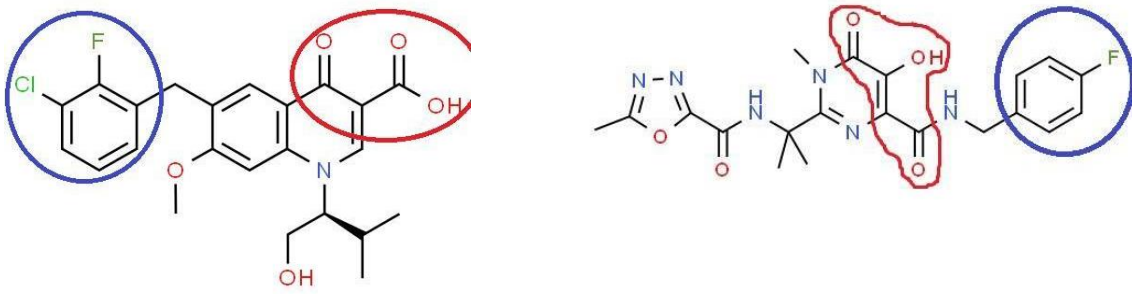

Figure 11: Structure and activity of Elvitegravir and Raltegravir. A halobenzyl group in a hydrophobic pocket (blue circle) and a triad that will chelate the two Mg2+ ions (red circle) are visible. Taken and adapted from ChemSpider.

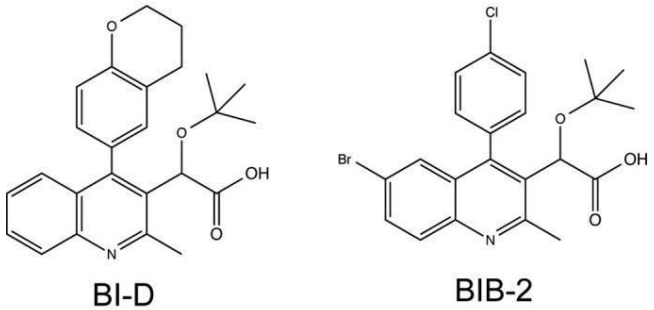

Figure 12: Chemical structures of two ALLINIs: BI-D and BIB-2. Taken from Fontana et al. 2015. [25]

4. Conclusion

The aforementioned evidence presented from several research groups (Kessl et al., Madison et al., Jurado et al., Fontana et al.) strongly suggests that HIV-1 IN plays an important role during virion maturation. The possibility that IN ensures the proper packaging of vRNPs inside the conical CA comes as a very suprising and unexpected discovery, which enables the greater understanding of HIV-1 replication and maturation cycles, and demands an update of the current information regarding these processes.

These discoveries also shed a light upon the mechanism of action of ALLINIs, which are already being used in clinical trials. These drugs seem to act mostly by blocking the IN-vRNA interaction, which is necessary for the proper vRNP incorporation inside the CA, causing their eccentric localization between the CA and the viral envelope. ALLINIs therefore prove themselves as a promising new type of IN inhibitors that could significantly enrich the HIV-1 antiretroviral therapy.

These discoveries have a great perspective to be used not only in the development of ALLINIs, but also in the development of many other HIV-1 antiretroviral drugs, as a great number of them may follow a similar mechanism of action as ALLINIs. This constant development of antiretroviral therapy against HIV is extremely important due to a great number of viral mutations that arise during the virus replication cycle, which help it to acquire resistance against existing drugs.

Even though this non-catalytic role of IN has a great potential to be used in the development of new types of IN inhibitors, a lot of details still need to be explained and clarified in the proposed new model of HIV-1 maturation. Therefore, a lot of further research is required in order to fully understand HIV-1 maturation and replication processes and efficiently use this knowledge in the development of new anti-HIV drugs.

5. Literature

1. Weiss, R.A., *How does HIV cause AIDS?* Science, 1993. **260**(5112): p. 1273-9.
2. UNAIDS.org, *https://www.unaids.org/en/resources/fact-sheet*.
3. CDC.gov, *https://www.cdc.gov/hiv/basics/transmission.html*.
4. Fearon, M., *The laboratory diagnosis of HIV infections.* Can J Infect Dis Med Microbiol, 2005. **16**(1): p. 26-30.
5. Whitley, R.J. and B. Roizman, *Clinical Virology, 4th ed.* 4 ed. 2017: ASM Press.
6. UNAIDS; WHO., *2007 AIDS epidemic update (http://data.unaids.org/pub/epislides/2007/2007_epiupdate_en.pdf).*
7. U.S. Department of Health and Human Services, *The stages of HIV infection (https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/19/46/the-stages-of-hiv-infection)*.
8. Schmid, S., *The discovery of HIV-1.* Nature, 2018.
9. Moore, R.D. and R.E. Chaisson, *Natural history of HIV infection in the era of combination antiretroviral therapy.* AIDS, 1999. **13**(14): p. 1933-42.
10. Arts, E.J. and D.J. Hazuda, *HIV-1 antiretroviral drug therapy.* Cold Spring Harb Perspect Med, 2012. **2**(4): p. a007161.
11. Passaes, C.P. and A. Saez-Cirion, *HIV cure research: advances and prospects.* Virology, 2014. **454-455**: p. 340-52.
12. Roizman, B., D.M. Knipe, and R.J. Whitley, *Fields virology*. 6 ed. HIV-1: Pathogenesis, Clinical Manifestations, and Treatment. 2013, Philadelphia, PA: Lippincott Williams & Wilkins.
13. Hiscott, J., H. Kwon, and P. Genin, *Hostile takeovers: viral appropriation of the NF-kappaB pathway.* J Clin Invest, 2001. **107**(2): p. 143-51.

14. Pasternak, A.O., V.V. Lukashov, and B. Berkhout, *Cell-associated HIV RNA: a dynamic biomarker of viral persistence.* Retrovirology, 2013. **10**: p. 41.
15. Hajimahdi, Z. and A. Zarghi, *Progress in HIV-1 Integrase Inhibitors: A Review of their Chemical Structure Diversity.* Iran J Pharm Res, 2016. **15**(4): p. 595-628.
16. Zheng, R., T.M. Jenkins, and R. Craigie, *Zinc folds the N-terminal domain of HIV-1 integrase, promotes multimerization, and enhances catalytic activity.* Proc Natl Acad Sci U S A, 1996. **93**(24): p. 13659-64.
17. Esposito, D. and R. Craigie, *HIV integrase structure and function.* Adv Virus Res, 1999. **52**: p. 319-33.
18. Debyser, Z., et al., *Host factors for retroviral integration site selection.* Trends Biochem Sci, 2015. **40**(2): p. 108-16.
19. Engelman, A. and P. Cherepanov, *The lentiviral integrase binding protein LEDGF/p75 and HIV-1 replication.* PLoS Pathog, 2008. **4**(3): p. e1000046.
20. Craigie, R., *The molecular biology of HIV integrase.* Future Virol, 2012. **7**(7): p. 679-686.
21. Engelman, A., *In vivo analysis of retroviral integrase structure and function.* Adv Virus Res, 1999. **52**: p. 411-26.
22. Kessl, J.J., et al., *HIV-1 Integrase Binds the Viral RNA Genome and Is Essential during Virion Morphogenesis.* Cell, 2016. **166**(5): p. 1257-1268 e12.
23. Hare, S., et al., *Retroviral intasome assembly and inhibition of DNA strand transfer.* Nature, 2010. **464**(7286): p. 232-6.
24. Jurado, K.A., et al., *Allosteric integrase inhibitor potency is determined through the inhibition of HIV-1 particle maturation.* Proc Natl Acad Sci U S A, 2013. **110**(21): p. 8690-5.
25. Fontana, J., et al., *Distribution and Redistribution of HIV-1 Nucleocapsid Protein in Immature, Mature, and Integrase-Inhibited*

Virions: a Role for Integrase in Maturation. J Virol, 2015. **89**(19): p. 9765-80.

26. Madison, M.K., et al., *Allosteric HIV-1 Integrase Inhibitors Lead to Premature Degradation of the Viral RNA Genome and Integrase in Target Cells.* J Virol, 2017. **91**(17).

Acknowledgements

I would like to give a huge thanks to my mentor, Associate Professor Igor Jurak, PhD, on advice and support during the writing of my thesis. Big thanks also go to my parents for providing me with a huge support throughout all of my studies. Finally, I would like to thank all my friends and colleagues from the Department of Biotechnology for showing interest in this topic.

europass Curriculum vitae

PERSONAL INFORMATION

Čemeljić Noa

Pomerio 21, 51000 Rijeka (Croatia)

+385953988104

noacem@gmail.com

WORK EXPERIENCE

05/2019–06/2019

Student traineeship

Jadran Galenski Laboratorij; Nastavni zavod za javno zdravstvo PGŽ-a, Rijeka (Croatia)

04/2015–Present

Member

European Youth Parliament Croatia

06/2018–Present

Vice - president

European Youth Parliament Croatia

09/2017–06/2019

Lobby attendant

HNK Ivana pl. Zajca, Rijeka (Croatia)

EDUCATION AND TRAINING

09/2012–06/2016

EQF level 4

Prva riječka hrvatska gimnazija, Rijeka (Croatia)

10/2016–Present

EQF level 6

Department of Biotechnology, University of Rijeka, Rijeka (Croatia)

PERSONAL SKILLS

Mother tongue(s): Croatian

Foreign language(s)

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	C1	C1	C1	C1	C1
	IELTS Academic Test - OBS 7.5, CEFR level C1				
German	A1	A1	A1	A1	A1
Italian	A2	A2	A1	A1	A1

Levels: A1 and A2: Basic user - B1 and B2: Independent user - C1 and C2: Proficient user
Common European Framework of Reference for Languages

Communication skills

Excellent communication skills gained through active membership in the European Youth Parliament (EYP) and work in the Croatian National Theatre "Ivan pl. Zajc".

Great presentation skills gained through university seminars and through attending more than 30 EYP conferences in the whole of Europe.

Significant knowledge on the functioning of interpersonal relationships gained through chairing various committees on EYP conferences.

Organisational / managerial skills

Excellent organizational skills gained by organizing numerous conferences of European Youth Parliament all across Europe, including the position of the core Human Resources organizer of International EYP Forum held in Luxembourg.

Great leadership skills, acquired as a Vice-President of European Youth Parliament Croatia, the main focus being Human Resources and Regional Development.

Excellent team-leading skills gained as the President and Vice-president of several EYP conferences, being responsible for the academic quality of the conference.

Job-related skills

Student practice in Jadran Galenski laboratory and Public Health Department of Primorsko - Goranska county.

Understanding and using basic laboratory techniques in the fields of chemistry (general, analytical, bioinorganic, organic, physical) and molecular biology.

Digital skills

SELF-ASSESSMENT				
Information processing	Communication	Content creation	Safety	Problem-solving
Proficient user	Proficient user	Proficient user	Proficient user	Proficient user

Digital skills - Self-assessment grid

Good command of the Office Suite (Microsoft Word, Microsoft Excel, Microsoft Power Point).

Basic knowledge of programming in C++.

Basic command of Avogadro, Chimera and VMD.

Other skills

Debating

Reading: a member of the Rijeka City Library Book Club

Piano player

Driving licence

B

ADDITIONAL INFORMATION

Honours and awards

City of Rijeka internship for excellent students in the academic year 2018/19

Conferences

More than 30 European Youth Parliament (EYP) Conferences, including:

- EYP International Forum, Luxembourg City, Luxembourg, 2018 - position: **Core Organiser** for Human Resources
- 83rd EYP International Conference, Laax, Switzerland, 2016 - position: **delegate**
- 45th EYP National Conference, Milano, Italy, 2019 - position: **jury member**
- 10th EYP National Conference, Belgrade, Serbia, 2017 - position: **organizer**
- 3rd EYP National Session, Ljubljana, Slovenia, 2017 - position: **organizer**
- 24th EYP Regional Forum, Mostar, Bosnia and Herzegovina, 2018 - position: **Vice-President**
- 28th EYP National Conference, Zagreb, Croatia, 2018 - position: **chairperson**
- 26th EYP National Conference, Rijeka, Croatia, 2015 - position: **delegate**
- 1st EYP National Session, Sarajevo, Bosnia and Herzegovina, 2015 - position: **delegate**

...