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Cellular host factors involved in the translation of the HIV-1 genomic RNA

Paulina Rubilar Guzman

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THÈSE

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École Doctorale de Biologie Moléculaire, Intégrative et Cellulaire

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Cellular host factors involved in the translation of the HIV-1 genomic RNA

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Cellular host Factors involved in the translation of the HIV-1 genomic RNA

Abstract

Human Immunodeficiency virus type 1 (HIV-1) is a positive strand RNA virus belonging to the lentivirus genus of the *retroviridae* family, and it is the etiological agent of the pandemic acquired immunodeficiency disease syndrome (AIDS), which is a major health concern worldwide.

Throughout the HIV-1 replication cycle, the production of viral proteins depends exclusively on the cellular translational machinery. This is the reason why we have explored the role of some cellular factors that could control HIV-1 translation at different stages. We have focused our studies on the translation of the full length genomic RNA (gRNA), which serves both as genome for viral encapsidation and as a messenger for translation of Gag and Gag-Pol viral polyproteins.

1. The role of the RNA helicase DDX3 in HIV-1 translation Initiation

The fact that HIV-1 possesses a highly structured 5' untranslated region (5'UTR) prompted us to speculate that DDX3 may be involved in HIV-1 translation. We used a combination of in vitro and ex-vivo approaches to show that DDX3 was able to bind and form complexes with the 5'-UTR of HIV-1 to assist translation initiation. We also demonstrated that DDX3 can form a complex with initiation factors such as PABP, eIF4G and eIF4E.

2. Programmed Ribosomal Frameshift (PRF) in the genomic RNA of HIV-1

Translation of HIV-1 Gag-Pol polyprotein requires a -1 PRF. This mechanism allows the synthesis of Gag and Gag-Pol polyproteins, using the same mRNA template, at ratios of 95 and 5%, respectively. Keeping the -1PRF ratio is important as any change leads to reduction in virus infectivity.

By means of a dual reporter construct and full provirus replication system we were able to demonstrate that the stress granules associated protein TIAR, controls HIV-1 infectious progeny by regulating the ratio of the HIV-1 PRF.

Contrôle traductionnel de l'ARN génomique du VIH-1 par des facteurs cellulaires

Résumé

Le virus de l'immunodéficience humaine de type 1 (VIH-1) est un virus à simple brin positif qui appartient au genre *Lentivirus* dans la famille *retroviridae* et qui constitue l'agent étiologique du SIDA pandémique.

Pendant le cycle réplcatif du VIH-1, la traduction de protéines virales dépend exclusivement de la machinerie traductionnelle cellulaire. Pour cette raison, nous avons cherché à comprendre le rôle de quelques facteurs cellulaires qui pourraient contrôler la traduction du VIH-1 à différents niveaux. Nous avons centré nos recherches sur la traduction de l'ARN génomique (ARNg) du virus qui sert en même temps de génome pour être encapsidé et comme ARN messager pour la traduction des protéines virales Gag et Gag-Pol.

1. Le rôle de l'hélicase d'ARN DDX3 dans la traduction du VIH-1

L'ARNg du VIH-1 possède une région 5' non traduite très structurée, raison pour laquelle nous avons spéculé sur un possible rôle de DDX3 dans la traduction du VIH-1. Nous avons utilisé une combinaison de techniques in vitro et ex vivo afin de pouvoir démontrer que DDX3 était capable de lier et faire des complexes avec l'ARN de la région 5' non traduite pour promouvoir l'initiation de la traduction. Nous avons aussi pu démontrer que DDX3 formait des complexes avec les facteurs d'initiation de la traduction PABP, eIF4G et eIF4E.

2. Le changement programmé du cadre de lecture (PRF) dans l'ARN génomique du VIH-1

La traduction de la polyprotéine Gag-Pol du VIH-1 nécessite un décalage de phase de 1 nucléotide en arrière. Ce mécanisme permet la synthèse des protéines Gag et Gag-Pol avec des ratios de 95 et 5% respectivement à partir du même ARN. Cette proportion doit être conservée pour assurer la réplcation du virus.

Nous avons utilisé un système de double gène rapporteurs et un système de réplcation complète du provirus pour montrer que la protéine associée aux granules de stress TIAR pouvait contrôler la réplcation viral en régulant la proportion de ribosome qui assurent le décalage de phase.

Manuscript Overview

My thesis project involved studying how the cellular machinery is co-opted by HIV-1 for its own replication. Thus, this manuscript entails a wide range of topics including: the disease caused by HIV-1 and its current treatment, viral gene expression and replication, the host cell translational machinery, and a couple of host proteins involved in RNA metabolism such as helicases and cellular granules components.

In the next pages I will develop the most significant concepts on these thematic, subdivided in four chapters, and followed by an articles section containing the main results that we have obtained during my thesis. Three additional published articles, as a result of collaborative work on some topics related to viral protein translation and RNA silencing, are added as annexed articles.

Chapter I. HIV-1 Cycle and Therapy

I. HIV-1 Cycle and Therapy

This first chapter will serve to outline the HIV/AIDS pandemic and its origin. I will describe also, the main aspects of viral cycle and the different current therapy targets, in order to better visualize the significance of studying HIV-1.

I. a) Disease worldwide

The Human Immunodeficiency virus (HIV) infects immune cells and causes a gradual depletion of immune competence that progresses into the AIDS, which is the most advanced stage of infection.

According to the latest statistics from World Health Organization (WHO), at the end of 2013 there were about 35 million people living with HIV, with an average of 2.1 million of new infections for that year.

Deaths related to AIDS in 2013 reached approximately 1.5 million people (see Figure 1).

Global summary of the AIDS epidemic | 2013

Number of people living with HIV in 2013	Total	35.0 million [33.1 million – 37.2 million]
	Adults	31.8 million [30.1 million – 33.7 million]
	Women	16.0 million [15.2 million – 16.9 million]
	Children (<15 years)	3.2 million [2.9 million – 3.5 million]
<hr/>		
People newly infected with HIV in 2013	Total	2.1 million [1.9 million – 2.4 million]
	Adults	1.9 million [1.7 million – 2.1 million]
	Children (<15 years)	240 000 [210 000 – 280 000]
<hr/>		
AIDS deaths in 2013	Total	1.5 million [1.4 million – 1.7 million]
	Adults	1.3 million [1.2 million – 1.5 million]
	Children (<15 years)	190 000 [170 000 – 220 000]

WHO – HIV department | July 12, 2015



Figure 1. Latest global data on HIV/AIDS epidemic.

Data published by WHO during the year 2014. Deaths related to AIDS have diminished in the last years thanks to improved antiviral therapy.

The origin of HIV has been traced to zoonotic infections between simian immunodeficiency virus (SIV) from African primates and human bushmeat hunters. At the molecular level, several transmission events have

been identified that have given rise to several viruses that affect humans to a lesser extent (see Figure 2). This is the case of HIV type 2 (HIV-2), virus that was transmitted from sooty mangabeys and the HIV type 1 (HIV-1) from groups N and O transmitted independently from chimpanzees and the group P from gorillas. The group M (from Major) of HIV-1, was also transmitted from chimpanzees, and it has become the virus that spread with the greatest success and that is the responsible for the HIV pandemic (Sharp & Hahn B, 2011). Its origin has been localized to Kinshasa (now Democratic Republic of Congo) from where the virus spread was facilitated by advances in the transport network and by demographic changes (Faria et al., 2014)

Within the group M of HIV-1, the more abundant one, there are nine subtypes: A-D, F-H, J and K and an increasing number of circulating recombinant forms that may have different biological properties (Sharp & Hahn B, 2011; Taylor, Sobieszczyk, McCutchan, & Hammer, 2008). Globalization has allowed the spread of all subtypes around the globe. For instance the subtype B, which accounts for the great majority of HIV-1 infections in Europe and the Americas, arose from a single African strain that appears to have first spread to Haiti in the 1960s and then onward to the USA and other western countries.

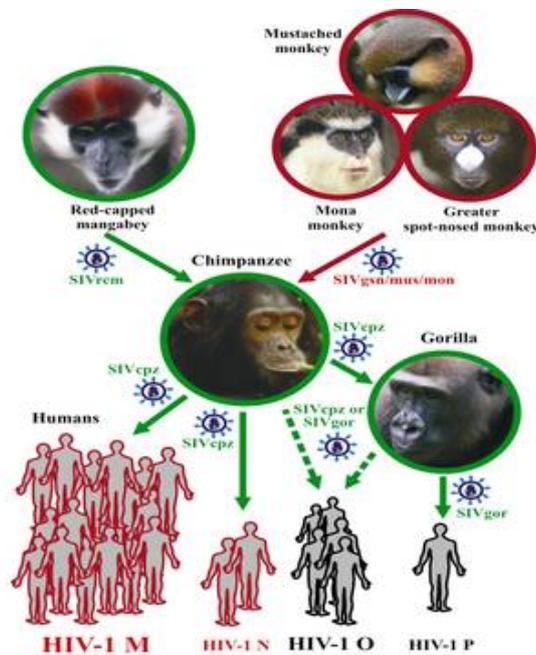


Figure 2. Zoonotic Transmission of Immunodeficiency virus.

According to phylogenetic studies HIV-1 was transmitted to humans in different zoonotic events from Simian Immunodeficiency Virus (SIV) infecting African primates. The group M is the more abundant worldwide.

Despite a previous documented outbreak in Africa in the 1970s, it was only in 1981 that concerns were raised from the Centers for Disease Control and Prevention (CDC) about an outbreak of opportunistic diseases in USA, like pneumonia and Kaposi's sarcoma, among young homosexual men. Isolation of the virus in 1983 (Barre-Sinoussi et al., 1983) classified the pathogen as an "HTLV-like retrovirus but different from previous isolates" (see Figure 3). Since the initial isolation and culture of the virus, major advances were quickly made towards a treatment and the first clinical trial took place at USA in 1986 to give rise to the first approved drug in 1987, which was the azidothymidine (AZT) (Fischl et al., 1987; Sepkowitz, 2001). At a clinical level, HIV infection encompasses a variety of symptoms ranging from strong T-cell depletion to autoimmunity, opportunistic infections, systemic inflammatory responses, energy deficit, dementia, and an increased incidence of cancers.

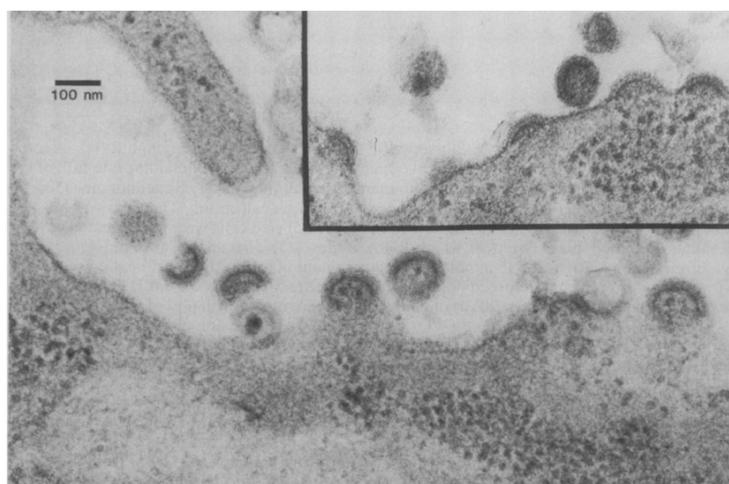


Figure 3. First electron microscopy image of HIV-1

(at that time described as a "HTLV-like retrovirus") replicating in cord lymphocytes. The inset shows various stages of particle budding at the cell surface. (Image published by (Barre-Sinoussi et al., 1983)).

HIV infection is now classified as a chronic disease which is still incurable. Three main phases may be distinguished during the progression of the disease (see Figure 4). Initially, within a few weeks after infection, a high viral replication occurs leading to an acute mononucleosis-like syndrome. This first acute phase is followed by a second and relatively latent period when a relative balance between constant virus replication and a regeneration of the CD4 T-cell reservoirs occurs. Finally, in the last phase, a higher viral replication together with a reduction of CD4 T-cell counts take place, leading to opportunistic infections, lymphomagenesis, and autoimmune phenomena from the final stages of disease (Perl & Banki, 2000).

The establishment and wide access to therapy had delayed the initiation of the later phase of disease, which in average takes 10 years without treatment. We will expose some of the current therapy targets later in this manuscript.

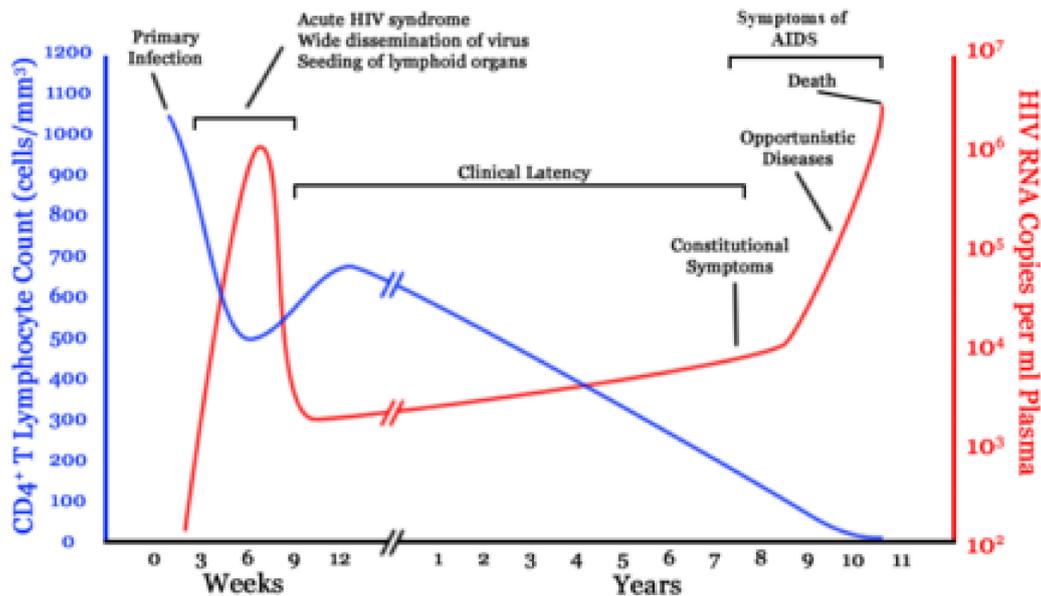


Figure 4. The average course of untreated HIV infection.

See the opposite relationship between HIV copies and CD4+ T cell counts. Currently, antiviral therapy extends the years of clinical latency shown here. (Taken from (Maartens, Celum, & Lewin, 2014))

I. b) Transmission and Prevention

Transmission of HIV remains mainly by sexual intercourse. An effective mother-to-child transmission may also occur during pregnancy, delivery or breastfeeding. In contrast, transmission via shared needles in drug users, only occurs in a minor percentage of cases. Infection initiates typically through the exposure of mucosal surfaces to the virus, which seems to be less efficient compared to the direct exposure into the blood, probably due to mucosal restriction factors (Rustagi & Gale, 2014).

There is no currently available vaccine, and the most promising vaccine trial to date has shown only a 31% of efficacy. (Rerks-Ngarm et al., 2009).

The condom remains the most effective protection against HIV-1 transmission via any sexual intercourse. In addition, male circumcision has been demonstrated to reduce the risk of infection in men by 60% (Auvert et al., 2005).

Higher viral load in the blood, is the main factor that increases the likelihood of infection by all transmission ways. Thus, it is believed that one log reduction of viremia reduce by two to three times the risk of transmission (Maartens et al., 2014). It is remarkable, however, that the relationship between viral load in the blood and in body fluids like semen or breast milk (the most common sources of infection) are not always correlated. Furthermore, prophylactic antiretroviral treatment during pregnancy of infected women is an immediate priority in order to avoid virus transmission into the foetus.

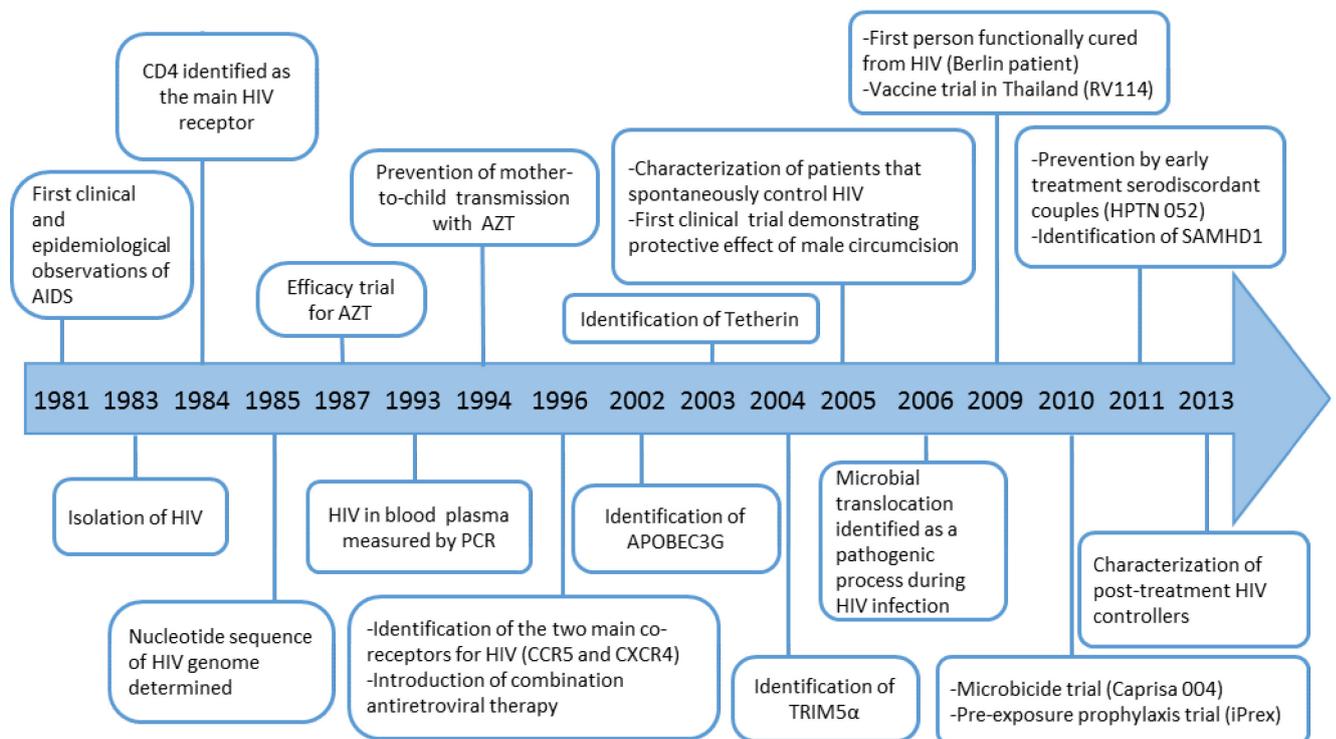


Figure 5. Timeline with main events on HIV-1 emerging and research.

Figure modified from (Barré-Sinoussi, Ross, & Delfraissy, 2013).

I. c) Viral life cycle

HIV-1 is an enveloped virus belonging to the *Lentivirus* genus from the *Retroviridae* family. Lentiviruses cause chronic persistent infections in various mammalian species, which are characterised by a slow development of disease symptoms or long clinical latency periods.

The HIV-1 genome consists of two copies of single stranded RNA with positive polarity of about 9kb in length. Briefly, its genomic organization consists of an arrangement of the genes *gag*, *pol* and *env* together with six extra accessory genes that are flanked by respective 5' and 3' untranslated regions. All these genes will be detailed later in this manuscript.

Once inside its host cell, this genomic RNA is reverse transcribed into a double-stranded DNA that is inserted in the host genome to give rise to the production of new spherical virions of approximately 120 nm in diameter, which are released at the plasma membrane (S.-K. Lee, Potempa, & Swanstrom, 2012). We shall dissect the viral life cycle herein by chronological events in the cell (see Figure 6).

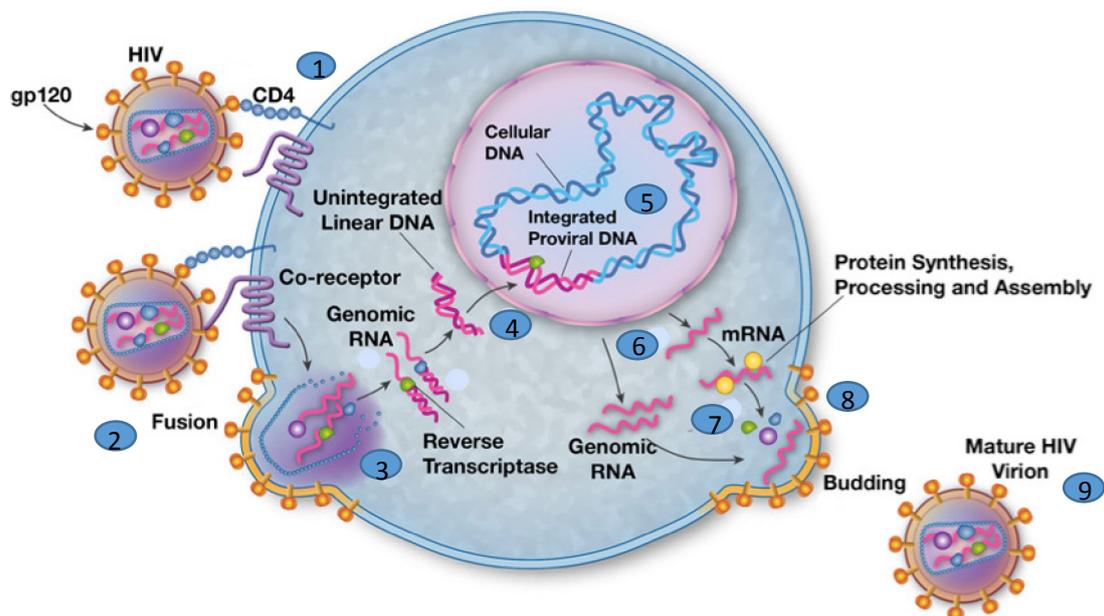


Figure 6. Representation of HIV-1 viral cycle.

Includes binding to the target cell (1), fusion (2), uncoating (3), reverse transcription (4), integration into host genome (5), mRNA export (6), viral protein translation (7), viral budding (8), and maturation once outside from the infected cell (9).

Viral Entry

The main target of the virus are activated CD4 T lymphocytes and, to a lesser extent, resting CD4 T lymphocytes, monocytes, macrophages and dendritic cells. These cells contain specific surface glycoproteins, including CD4 (cluster of differentiation 4) among others. HIV entry occurs via Interaction of the viral protein Env with the CD4 receptor and the chemokine coreceptors CCR5 or CXCR4 also present at the target cell surface. Alternatively, infection of CD4 negatives cells, such as astrocytes and renal epithelial cells, has been also documented via the mannose receptor or by direct cell-to-cell viral transfer from infected cells (Chen et al., 2011; Liu et al., 2004).

Sequentially, the entry of the virus into the cell start with the non-covalent interaction between the viral protein Env and non-specific adhesion molecules from the cell membrane including heparan sulfate and mannose-binding lectins such as DC-SIGN (Wilén, Tilton, & Doms, 2012). The Env protein is synthesized as a polyprotein that is later cleaved by the host furin-like proteases into the gp120 and gp41 subunits, which become inserted in the viral membrane (Haim, Salas, & Sodroski, 2012). Once bound to CD4, the viral gp120 subunit undergoes a conformational change that exposes the co-receptor binding site (see Figure 7). Binding of the coreceptor (CCR5 or CXCR4) induces the exposure of the hydrophobic gp41 peptide that inserts into the host cell membrane and bends back on itself to bring close enough the viral and host membranes to spontaneously triggers their fusion (Wilén et al., 2012).

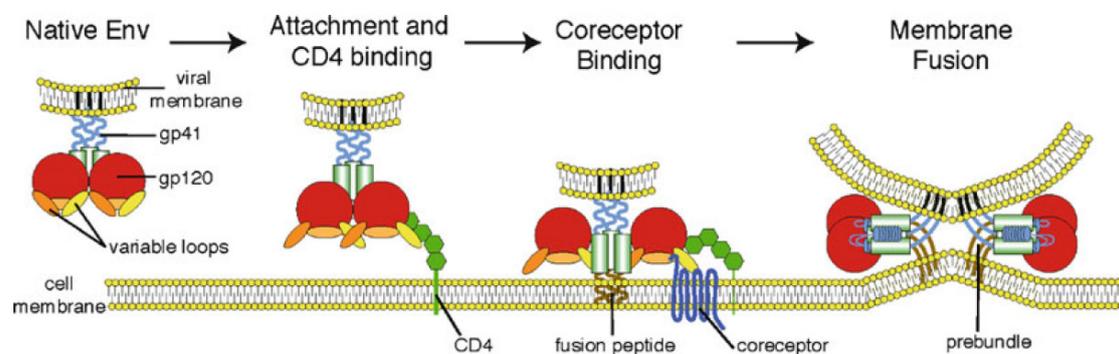


Figure 7. Schematic of HIV entry.

Binding of cell receptor (CD4) is followed by a conformational change and co-receptor binding that allows fusion of membranes. Taken from (Wilén et al., 2012)

The efficiency of each of these ligand-receptors interactions is influenced by the lipid and protein composition of the membrane and by their organization at the surface of the target cell. For instance, the presence of unspecific attachment factors, such as heparan sulfate and DC-SIGN, accelerates recognition of the CD4 receptor (Baribaud, Pöhlmann, & Doms, 2001; Mondor, Ugolini, & Sattentau, 1998). In addition, a relative higher density of CD4 receptors present at the cell surface can facilitate viral entry (Wilen et al., 2012).

Evidence also suggests that HIV entry into the host may occur through endocytosis. This mechanism is believed to be relevant to viral internalization into macrophages, endothelial or epithelial cells where the interaction and binding would be dependent on the target cell lipid rafts (Dumas, Preira, & Salomé, 2014)

Viral Uncoating

Following entry, virions deliver the viral capsid to the cytoplasm. HIV-1 possesses a conical capsid composed of a polymer of capsid protein (CA) shielding the viral RNA genome associated to the viral proteins nucleocapsid (NC), reverse transcriptase (RT) and integrase (IN). The uncoating or dissociation of the genomic RNA from its capsid is not well understood, but evidence suggests that it must occur at, or near to the nuclear pore to allow the delivery of the reverse transcribed viral DNA through the cytoplasm avoiding its detection by the cell DNA sensors that could trigger an innate immune response (Arhel et al., 2007; Rasaiyaah et al., 2014). It has also been reported that perturbation of uncoating, such as premature uncoating, could have detrimental effects on downstream replication steps and may affect viral infectivity (Ambrose & Aiken, 2014).

Uncoating is highly dependent on cellular factors such as cyclophilin A (CypA), which interacts with viral CA to prevent premature uncoating and also seems to facilitate reverse transcription (Yamashita & Emerman, 2009). On the other hand, host factors such as the transportin 3 (TNPO3) protein, the cleavage and polyadenylation specificity factor 6 (CPSF6) and the nuclear pore proteins NUP358 and NUP153, can facilitate uncoating and transport through nuclear pores (Reviewed in (Ambrose & Aiken, 2014)).

Reverse Transcription

Reverse transcription of the viral genomic RNA (gRNA) into DNA takes place almost simultaneously with uncoating.

HIV-1 is known to package host tRNAs, specifically tRNA-Lys and tRNA-Ile within the viral particle. tRNA^{Lys} is used by HIV-1 as a primer to initiate reverse transcription (van Weringh et al., 2011) where it binds, by complementary base pairing, to the primer binding site (PBS) within the 5' end of the genomic RNA (gRNA) (see Figure 8). The viral RT starts DNA synthesis using cellular dNTPs and the gRNA as template. Due to its RNaseH activity and its ability to use RNA or DNA as template, the viral RT converts the gRNA into a linear double-stranded DNA (dsDNA) with identical sequences at both its 5' and 3' ends that form the long terminal repeats (LTRs) (Hu & Hughes, 2012). The linear dsDNA moves into the nucleus as a component of the pre-integration complex to integrate later into the host cell genome (see Figure 8).

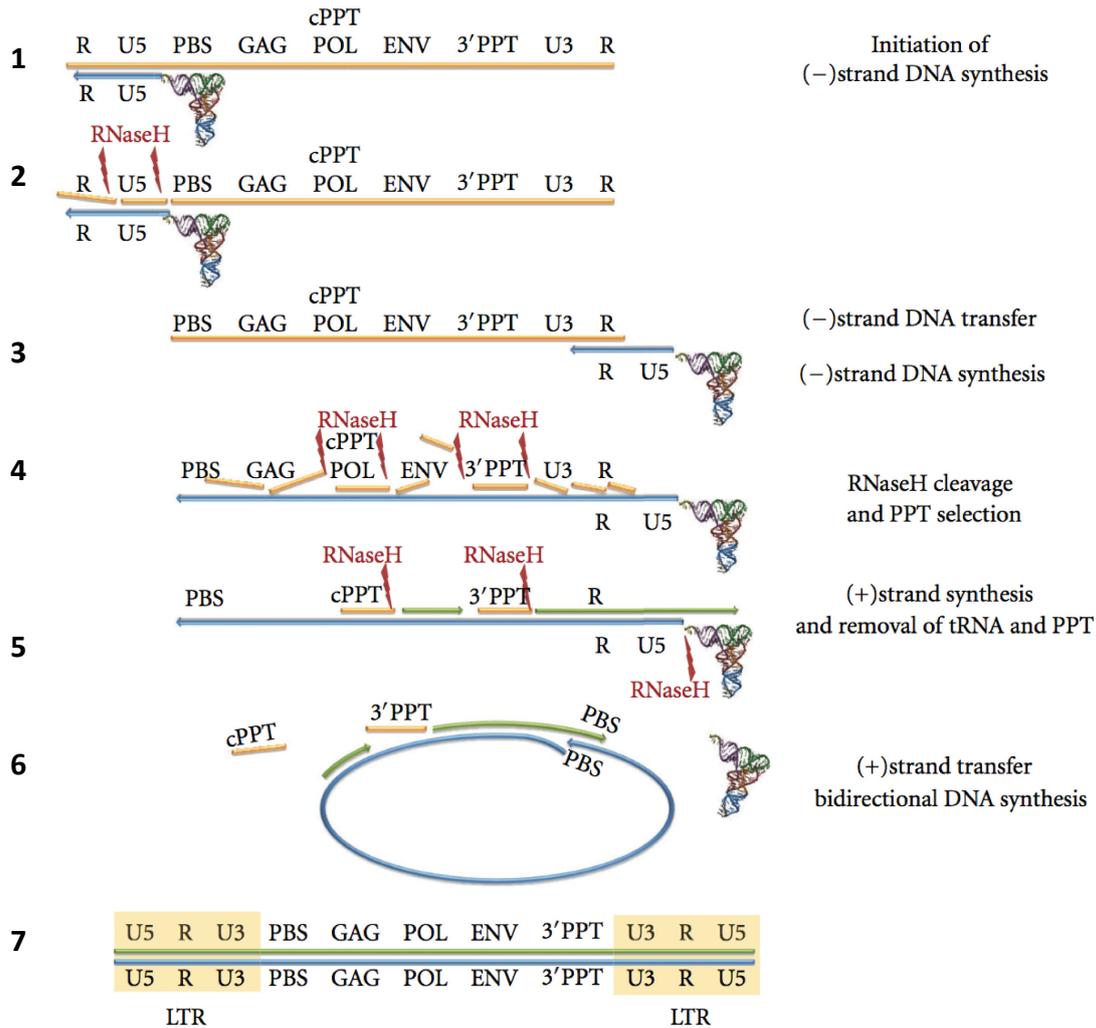


Figure 8. Schema of HIV-1 reverse transcription process.

1) Host cell tRNA-Lys3 hybridizes to the PBS as a primer for the starting of (-)strand DNA (blue) synthesis. Reverse transcription proceeds up to the 5'-end of the RNA genome. 2) RNA hydrolysis of R and U5, thanks to the RNase H activity of the RT. 3) Strand transfer of the (-)strand DNA by its hybridization with the R region in the 3' of the ssRNA genome. 4) DNA synthesis proceeds, and the RNase H function cleaves the RNA strand at numerous points leaving intact two specific sequences (cPPT, 3'PPT) that are resistant to the RNase H cleavage. 5) (-)strand DNA synthesis (green) initiation using PPTs as primers. The RNase H activity subsequently hydrolyzes the PPT segments and the junction of the tRNA:DNA hybrid, freeing the PBS sequence of the (+)strand DNA. 6) Strand transfer of the PBS sequence of the (+)strand DNA that anneals to the PBS on the (-)strand DNA. DNA synthesis then continues using the strand displacement activity of the RT. 7) The product is a linear dsDNA with long terminal repeats (LTRs) at both ends. Figure taken from (Esposito, Corona, & Tramontano, 2012).

DNA integration

Integration is mediated by the viral IN protein which is released into the nucleus along with viral uncoating.

Linear dsDNA stably interacts with IN and other associated cellular factors that are part of the pre-

integration complex (Craigie & Bushman, 2012; König et al., 2008). Integration occurs precisely at the termini of the viral DNA where both blunt extremities are 3'-processed by the same IN activity (see Figure 9). This 3'-processed linear DNA is now ready to be integrated, and this reaction can take place at many locations in the host genome. The preferred sites for integration is a matter of intense research, and it has been hypothesized that HIV preferentially integrates into active transcription units where a favourable environment for transcription is guaranteed (Lewinski et al., 2006).

On the other hand, the cellular factor LEDGF/p75 associates with IN to bring the viral dsDNA closer to chromatin for insertion (Ciuffi & Bushman, 2006). Other cellular factors are reported to be required for HIV-1 integration such as ANAPC2, MT1X, SNW1, IK, PRPF38A, and AQR, which are proteins involved in cellular transcription, cell signalling and splicing (Craigie & Bushman, 2012; König et al., 2008; Maartens et al., 2014). In addition, DNA repair proteins most probably support the final steps of integration in which DNA gaps at host-virus DNA junctions are processed and repaired (Craigie & Bushman, 2012) (see Figure 9).

Viral dsDNA can also undergo several circularization reactions that do not support subsequent replication of the virus. Self-circularization instead of integration into the host genome, seems to be mediated by cellular factors, and thus this phenomenon represents a growing research activity (Craigie & Bushman, 2012).

Integrase is a current target of viral therapy, and it will be described subsequently.

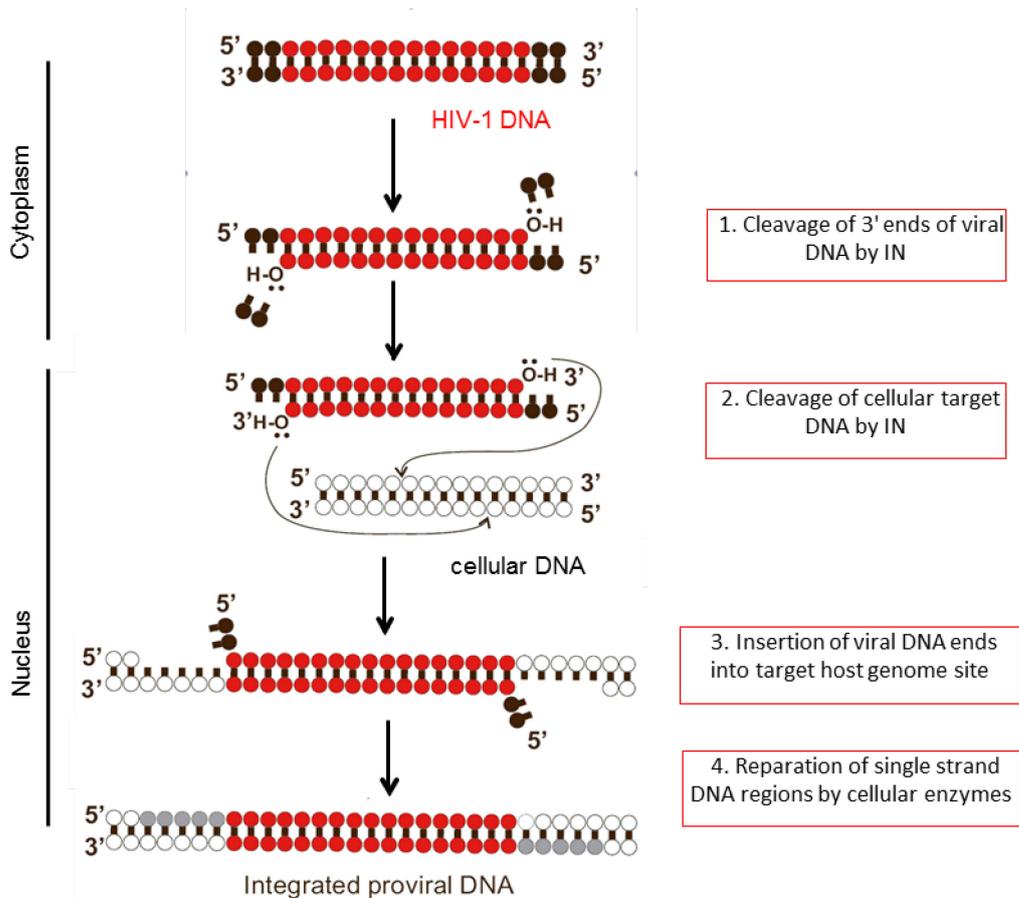


Figure 9. Schema of the sequence of events in HIV-1 integration.

1) integrase-catalyzed 3' processing of viral dsDNA; 2) integrase-catalyzed strand transfer into the cellular target DNA; 3) product of strand transfer inserted in the host genome; 4) DNA repair by cellular enzymes. Figure taken from (Savarino, 2007).

Transcription and splicing

Once integrated, the now called proviral DNA is replicated along with cellular DNA during cell division like any other host gene.

The HIV-1 LTR contains an extremely efficient promoter that includes three tandem SP1 binding sites, an efficient TATA element and a highly active initiator sequence. Additionally, an enhancer region, which contains two NF- κ B binding motifs, is essential to reactivate latent integrated provirus and to support virus replication in primary T-cells (Karn & Stoltzfus, 2012).

The proviral inserted HIV gives rise to more than 40 different mRNAs in the nucleus of infected cells by the use of a tightly controlled alternative splicing (see Figure 10). Splicing is regulated by the presence of suboptimal splice acceptors and by interactions of the transcript with host members of the hnRNP and SR

protein families (Bieniasz, 2012). Cellular splicing will not be detailed in this manuscript, for a recent review see (Ge & Porse, 2014).

Early after infection, proviral HIV-1 gives rise to fully spliced mRNAs that encode the viral regulatory proteins Tat and Rev. Later, during infection, transcription increases sharply together with the synthesis of incompletely spliced mRNAs and finally the full-length unspliced transcripts or genomic RNA (gRNA) is produced.

Early synthesis of the viral protein Tat is critical for infection as it activates viral transcription. Tat interacts with cyclin T1/CDK9 and binds to the RNA stem-loop structure at the beginning of the LTR called the transactivating response element (TAR). This complex promotes the hyperphosphorylation of RNA polymerase II to ensure efficient elongation of viral transcripts. The absence of Tat has been shown to lead to the accumulation of prematurely terminated transcripts (Karn & Stoltzfus, 2012; Kuzembayeva, Dilley, Sardo, & Hu, 2014).

Fully spliced viral mRNAs, encoding the early proteins Tat, Rev and Nef, can access the cytoplasm via the conventional cellular mRNA export pathways. For a review on RNA export see (Köhler & Hurt, 2007). On the other hand, the partially spliced (encoding Vif, Vpr, Vpx, Vpu, Env) and the unspliced gRNA require interaction with the viral protein Rev for export. In the nuclear pore, REV simultaneously binds to Crm1/Exportin1 and a structured RNA element called the Rev responsive element (RRE) that is present only on partially spliced and unspliced viral RNAs that allows them to be successfully exported into the cytoplasm (Bieniasz, 2012; Karn & Stoltzfus, 2012; Yedavalli, Neuveut, Chi, Kleiman, & Jeang, 2004).

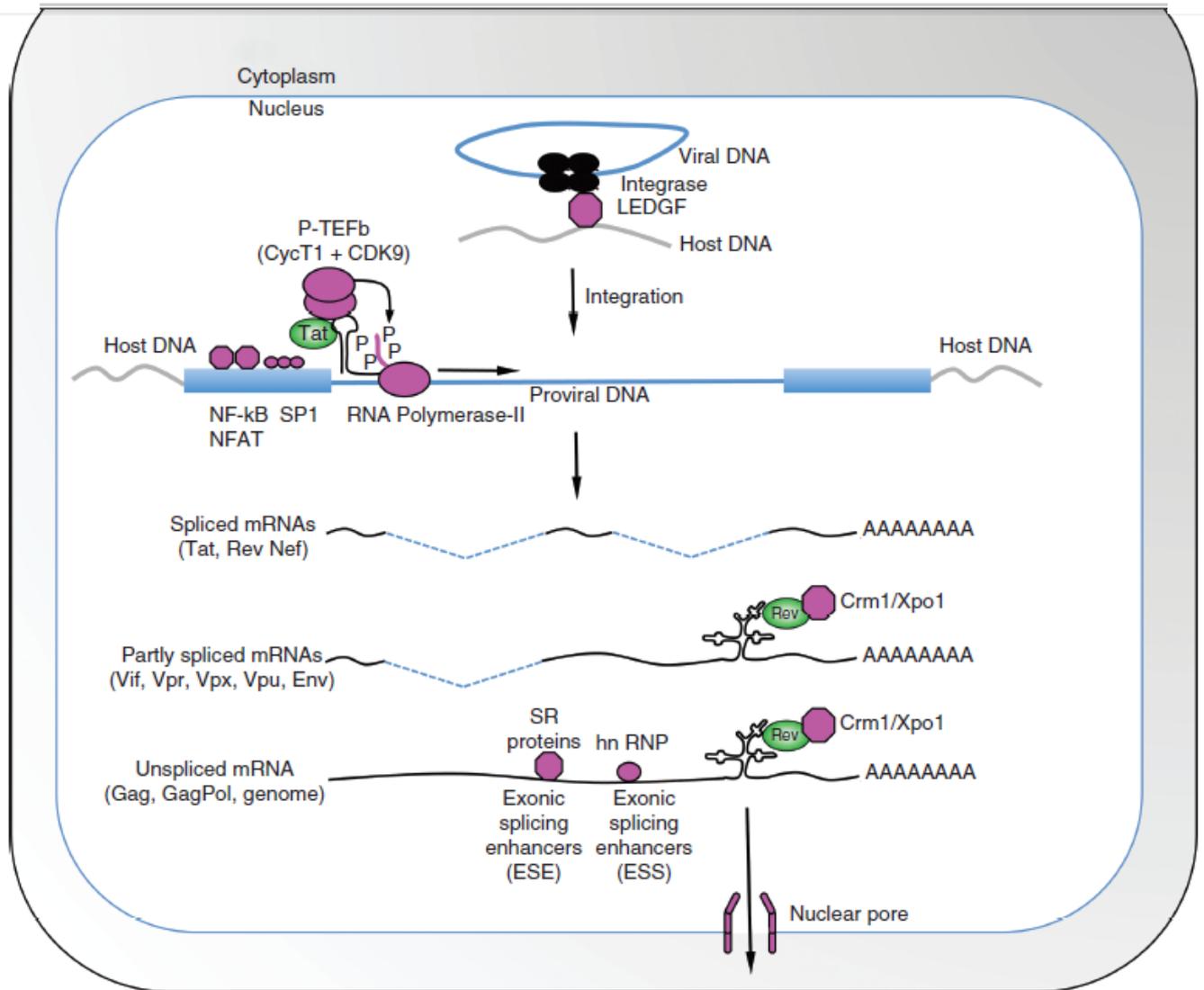


Figure 10. Schema of HIV-1 genome transcription.

Several different viral mRNAs are transcribed from the integrated proviral genome by the cellular RNAPol-II and associated factors. Messengers are fully- or partly-spliced and unspliced. Design taken from (Bieniasz, 2012)

Translation

Specific features of HIV-1 translation control will be discussed in detail later in this manuscript. Yet, different mechanisms such as IRESs, ribosomal frameshift, leaky scanning are used for proper synthesis of viral proteins and consequently for infectivity.

Briefly, once exported to the cytoplasm, viral proteins are synthesized from capped and polyadenylated viral RNAs.

The structural and enzymatic GAG and GAG-Pol polyproteins are produced from the gRNA and are then processed to give rise to the capsid (CA), matrix (MA), nucleocapsid (NC), protease (PR), reverse transcriptase (RT), integrase (IN) and p6 proteins. Production of Tat, Rev, Nef, Vif and Vpr proteins are initiated from alternatively spliced mRNAs and the Env and Vpu proteins are translated from the same mRNA via leaky scanning (see Figure 11).

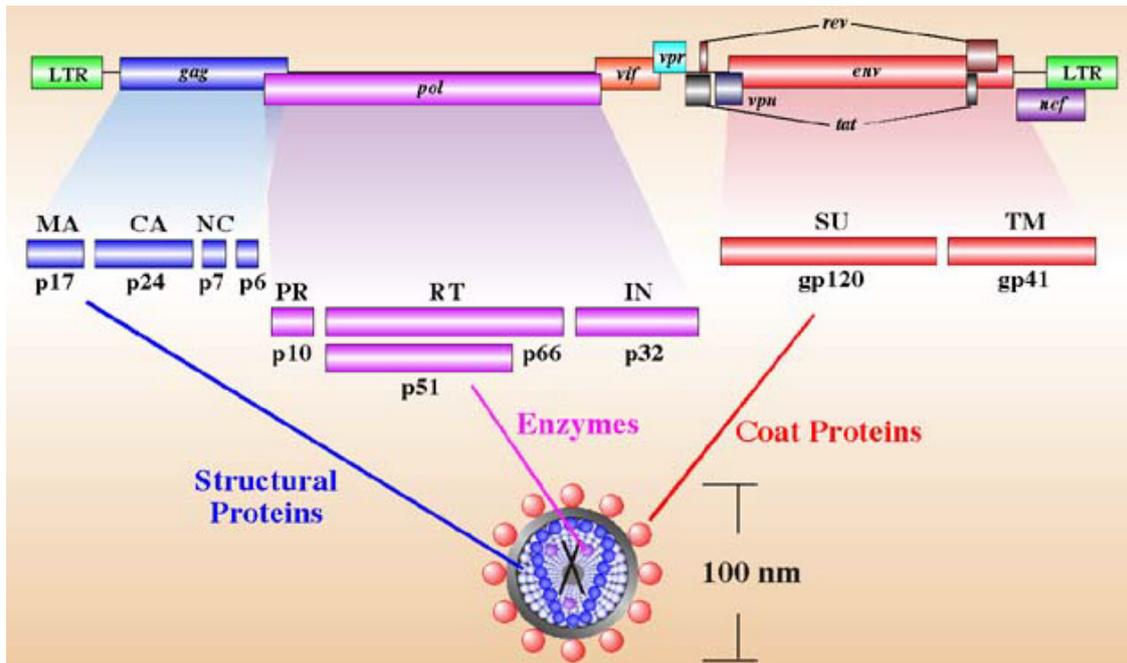


Figure 11. Representation of HIV genome organization.

Genome of 9.2 Kb length encoding 14 functional proteins and viral particle diameter of approximately 100-120 nm.

Viral assembly and exit

During the late stage of the viral life cycle, HIV-1 Gag assembles into a spherical immature capsid, and undergoes budding, release from the infected cell and final maturation.

The 55 kDa HIV-1 GAG protein is co-translationally myristoylated at its N-terminus on basic residues of the matrix (MA) protein domain, which is required for membrane targeting. A small number of Gag molecules transport dimers of the RNA genome to the plasma membrane, where additional Gag proteins are recruited through Gag-Gag interactions and nonspecific Gag-RNA interactions. Simultaneously, a few molecules of the entire Gag-Pol polyprotein are also recruited into the membrane for assembly (S.-K. Lee et al., 2012) (see Figure 12).

Assembly takes place at the plasma membrane of the cell to produce spherical membrane-bound virions of about 120 nm in diameter. Each virion contains approximately 2400 Gag molecules embedded in the membrane via the MA protein domain together with about 120 Gag-Pol molecules (Jacks, 1990). In order to become infectious, the PR domain must catalyze a series of cleavage events in a timely manner that is crucial for the formation of the mature viral core. Cleavage occurs simultaneous with, or just after, release of the immature virus. PR must dimerize and release itself from the Gag-Pol precursor in order to cleave the Gag and Gag-Pol polyproteins. From these cleaved products, the NC condenses and stabilizes the viral gRNA, whereas the processed CA protein constitutes the mature conical capsid structure around it. Modest changes in the local concentration of one of the viral enzymes can have deleterious effects on particle assembly, maturation, and infectivity. This is relevant to understand the effect of frameshift ratio changes that we will discuss later in this manuscript (S.-K. Lee et al., 2012; Lingappa, Reed, Tanaka, Chutiraka, & Robinson, 2014).

Additionally, some cellular factors are recruited by viral proteins to facilitate assembly and budding. For instance, specific interaction of the Gag protein with the ESCRT pathway components, a complex required for transport inside the cell, redirect this machinery to assembling virions. Similarly, ABCE1, a cellular ATPase, associates with intermediate Gag complexes during particle assembly and may assist in GAG multimerization. Finally, the HIV-1 Pol recruits clathrin that appears to be essential for viral budding and for regulation of the viral protease activity (Bieniasz, 2012).

It should be noted that during assembly, some cellular factors can eventually be encapsidated together with the viral gRNA, which may have a positive or deleterious effect on the infectivity of the viral particle.

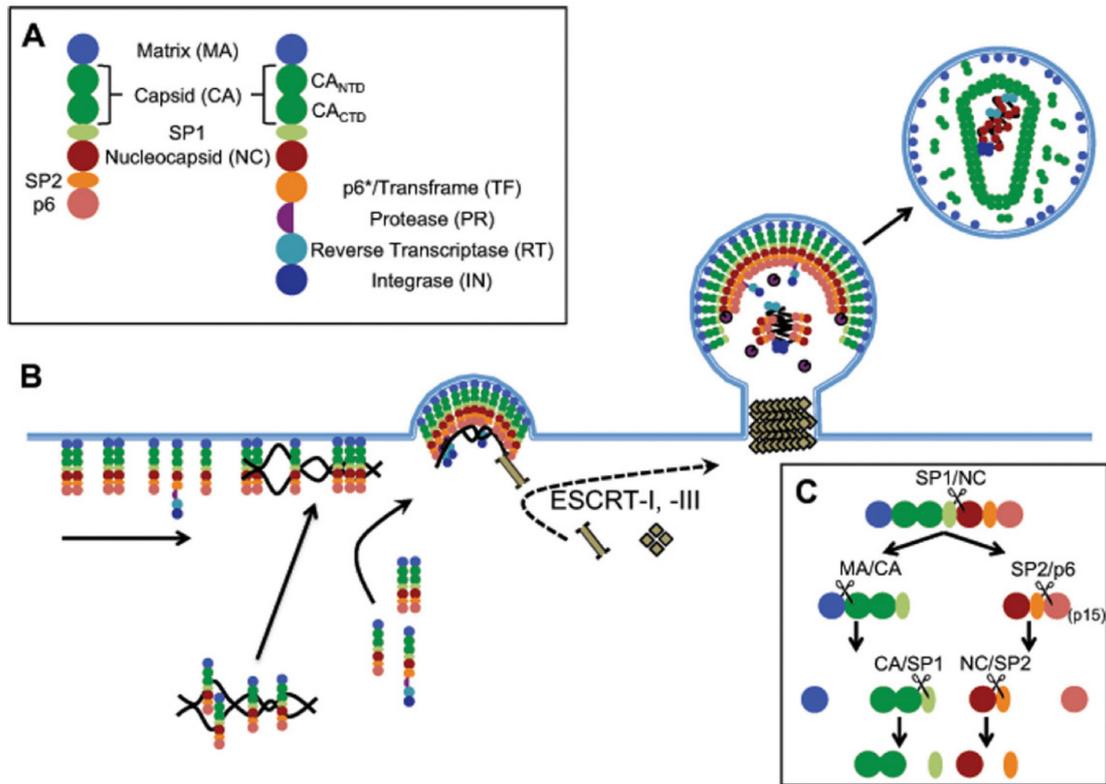


Figure 12. Schema of HIV-1 Assembly.

A) Gag and Gag-Pol polyproteins. B) Schema of the HIV-1 budding process at the plasma membrane, starting by the recruitment of viral gRNA and followed by GAG conformational change. C) Sequential proteolytic processing of HIV-1 Gag polyprotein. SP: spacer or site for protease cleavage. Design taken from (S.-K. Lee et al., 2012)

I. d) Restriction factors

Restriction cellular factors are responsible for impairing the transmission of immunodeficiency viruses among close host species as well as avoiding viral replication within different cell types. Viral strains able to overwhelm restriction factors have adapted themselves to spread through one or more host species; whereas other strains have been restrained to a unique host or even disappeared. Several HIV-1 Restriction Factors have been reported to act at different stage of viral cycle. We will only mention those that have been well characterized (see Figure 13).

Tetherin is an interferon-alpha induced factor present in the membrane which tethers nascent HIV-1 virions to the cellular plasma membrane, via homo-dimerization. Thus, it inhibits virion release and leads to particles degradation (Perez-Caballero, Hatziioannou, Martin-Serrano, & Bieniasz, 2004). Tetherin exerts

its antiviral activity on various enveloped viruses and is antagonized by the viral protein Vpu which favours the release of progeny virions, with the exception for the group O of HIV-1 in which vpu is susceptible to tetherin (Sauter et al., 2009).

SamHD1, is a deoxynucleotide triphosphohydrolase that inhibits HIV replication by reducing the levels of the cellular pool of deoxynucleotides that are required for viral reverse transcription. SamHD1 restriction can be alleviated by the HIV-1 accessory proteins Vpx that causes the degradation of SamHD1 by recruiting it to a ubiquitin ligase complex (Barré-Sinoussi et al., 2013; Bieniasz, 2012; Maartens et al., 2014; Rustagi & Gale, 2014).

TRIM5 α , is an ubiquitin ligase antiviral protein targeting viral CA that leads to premature HIV-1 uncoating, which seems to be associated also with the inhibition of reverse transcription. TRIM5 α has been reported to be partially responsible for the inefficiency of HIV-1 replication in many nonhuman primates cells (Ambrose & Aiken, 2014; Bieniasz, 2012; Maartens et al., 2014)

The **APOBEC3** family of proteins are cytidine deaminases. They are incorporated within viral particles where they inhibit reverse transcription. APOBEC3 acts by deaminating numerous cytidines residues in the nascent negative strand of viral DNA. This generates a G-to-A hypermutation of the coding strand, which renders the virus incapable of further propagation. APOBEC3 proteins are normally antagonized by the viral protein Vif, which induces its ubiquitination and proteasome-mediated degradation (Bieniasz, 2012; Maartens et al., 2014; Rustagi & Gale, 2014).

Other restriction factors, such as members of the **IFITM** protein family, which inhibit viral entry by blocking viral membrane fusion with plasma membrane, or **schlafen 11**, which depletes the cytosolic tRNA pool, have no viral protein antagonists reported yet (Rustagi & Gale, 2014).

I. e) Current therapy targets

Despite the fact that none of the current available antiretroviral treatments are able to clear the virus from the body, treatments have significantly increased the life expectancy of patients by delaying progression of the disease. Typically, after initiation of antiretroviral therapy, the plasma viral load decreases to

concentrations below the lower limit of detection in most of people. However, the recovery of CD4 T cells in individuals on antiretroviral therapy is variable (Maartens et al., 2014).

More than 25 licensed drugs that block HIV replication at many steps of the virus lifecycle are available and the evolution of antiretroviral therapy, since the first drug was available in 1987, is evidenced by regimens less toxic and more effective. Currently, highly active antiretroviral therapy (HAART) may include inhibitors of viral enzymes (RT, PR and IN) and/or an entry inhibitors targeting the cellular co-receptor CCR5 (Barré-Sinoussi et al., 2013; Maartens et al., 2014) (see Figure 13).

RT inhibitors: These are the most common drugs used against the virus. RT was the very first target of therapy with the development of AZT (or Zidovudine) almost 30 years ago. Currently, RT inhibitors are split into two different types: the nucleoside reverse transcriptase inhibitors (NRTIs) and the non-nucleoside reverse transcriptase inhibitors (NNRTIs). As NRTIs need to be phosphorylated by the host cell machinery to become substrates of RT, they are considered as “prodrugs”. NRTIs are then incorporated into viral DNA during the RT process and they block elongation as they lack a 3' hydroxyl group required for the incorporation of the subsequent nucleotide. In contrast, NNRTIs directly bind to the RT to impair its activity but they are not incorporated into the nascent DNA (Hu & Hughes, 2012).

Protease Inhibitors: These are analogues that bind the enzyme with much higher affinity compared to the original substrates. This results in the impairment of protease activity on GAG/GAG-Pol processing and viral maturation which leads to the decrease of infectivity (S.-K. Lee et al., 2012).

Integrase inhibitors: These are drugs that inhibit the strand transfer reaction during the integration process. Once bound to IN, they block the access of target host DNA into active site and they displace the 3' end of the viral DNA from the active site which disrupts catalysis (Craigie & Bushman, 2012).

Entry Inhibitors: These represent the first therapeutic approaches to target a host cellular factor, instead of viral protein. The first representative drug from this group, Maraviroc, targets the co-receptor CCR5 (Perry, 2010). The use of the coreceptors CCR5 or CXCR4 defines the viral tropism of the HIV strain that infects the cell.

However, all antiretroviral therapies taken in the presence of continuous viral replication will result in the selection of sub-populations of mutated HIV virus that develop resistance to these drugs. Patients who develop antiretroviral resistance can transmit resistant virus to others (see Figure 14). This is why genetic analysis of viral populations from each patient (sequencing of key regions from viral genome) allows doctors to adapt the therapy in case of resistance. However, this kind of diagnostic is less available in low income countries.

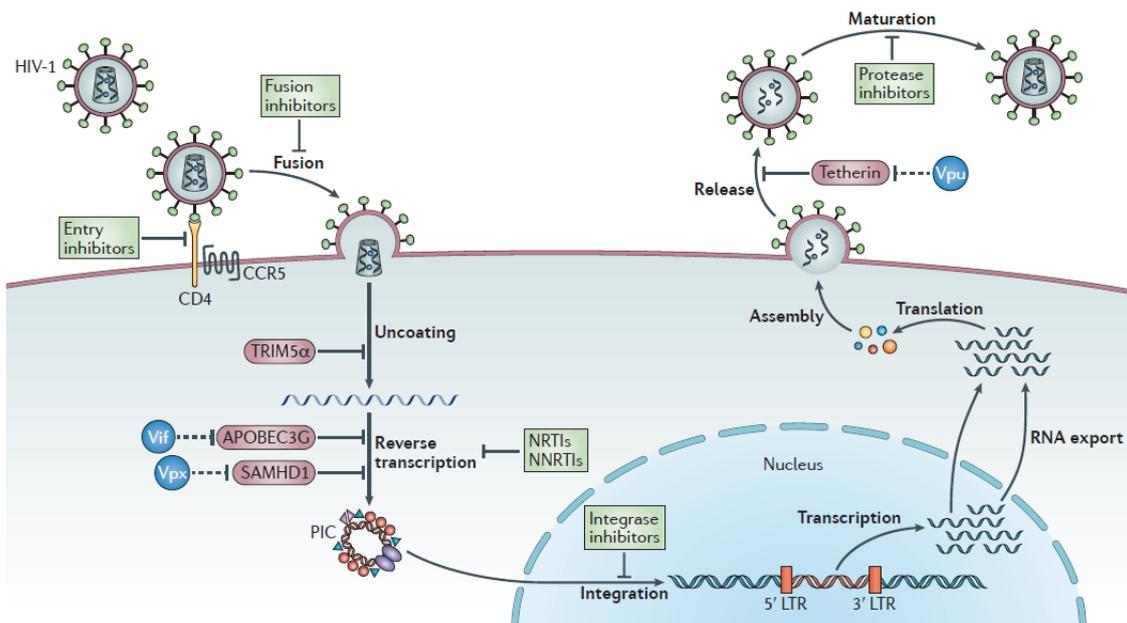


Figure 13. Schema of current therapy targets and cell restriction factors along HIV-1 replication cycle.

Taken from (Barré-Sinoussi et al., 2013).

I. f) Hope for transmission prevention and new Therapy strategies

Vaccines development is limited by variations or shielding of immunodominant epitopes within the viral envelope glycoprotein (Rustagi & Gale, 2014). Nevertheless, new strategies aim at enhancing the 31% of protection reached by the most promising vaccine trial until now (Rerks-Ngarm et al., 2009).

Up to now, the only medical report of a cured HIV patient comes from the “Berlin patient”, a man with HIV on antiretroviral therapy who had acute myeloid leukaemia and received two bone marrow transplants from a donor with a homozygous defect in CCR5. The patient, who is without antiretroviral therapy, has little or not detected virus in the plasma or tissue for more than 6 years. There has recently been another

interesting reported case in a child, that was treated with an aggressive combination of antiretroviral drugs. However, it appears that viral rebound became detectable after some months without treatment (Maartens et al., 2014).

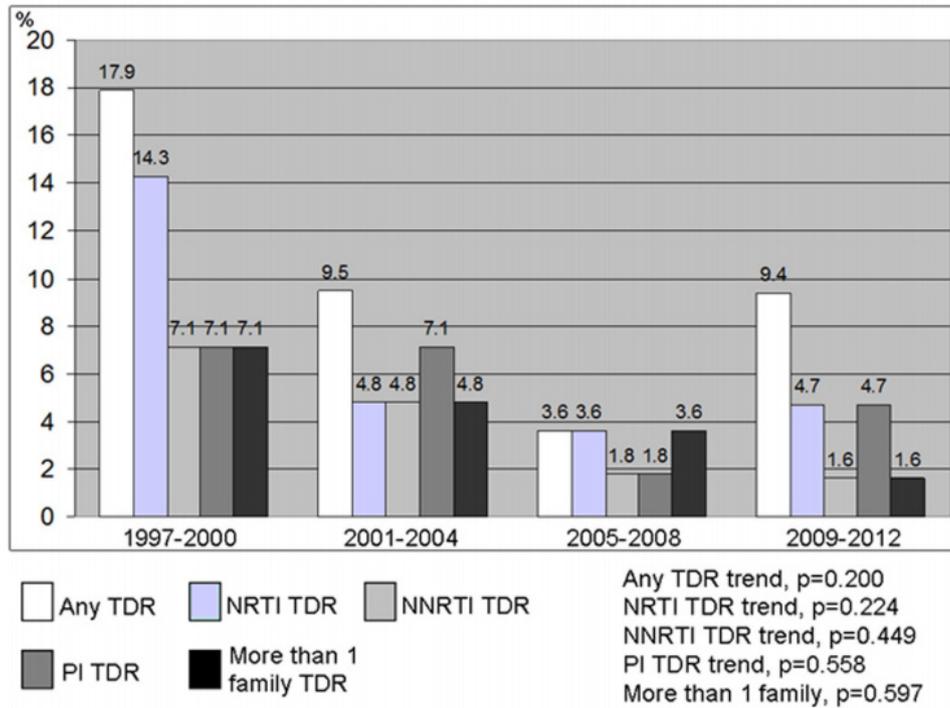


Figure 14. Data on drug resistance transmission.

Patients with acute or recent HIV-1 infection in Barcelona within 16 Years (1997-2012). If the general drug resistance has decreased from the beginning of therapy in the late 90's; prevalence remains around a 9%. (Data Published by (Ambrosioni et al., 2015)).

Chapter II. Translation in Eukaryotes

II. Translation in Eukaryotes

Translation allows quick temporal changes in the cell to face different needs or environmental changes. This process takes place on the mRNA and is driven by the ribosome, which is able to decode the genetic information into an amino acid chain. At a structural level, the eukaryotic ribosome is composed of 4 ribosomal RNAs (18S, 25S, 5.8S, and 5S) and 79 well conserved “core proteins” (Gilbert, 2011). In addition, a different group of proteins, called translation machinery associated (TMA) proteins are bound to a subset of ribosomes under specific conditions.

Eukaryotic mRNA is modified and harbors at its 5' end a 7-methyl guanosine cap, and a poly(A) repeat at its 3' end. These modifications have several roles during translation, including enhanced translatability and the stability of the mRNA (Costello et al., 2015).

Translation can be divided in three main steps: initiation, elongation and termination/recycling. I will describe each of these steps below.

II. a) Initiation

Whereas translation initiation in prokaryotes occurs by direct recruitment of the ribosome at the Shine-Dalgarno sequence, upstream of the initiation codon, eukaryotic ribosomes are unable to bind directly to the mRNA, and they need the assistance of eukaryotic initiation factors (eIFs). eIFs allow ribosome landing and assembly on the mRNA as well as unwinding any secondary structure that might otherwise impair ribosome movement along it. At least 17 eIFs have been described to date, and the number of eIFs required changes according to the mechanism of initiation employed (Richard J Jackson, Hellen, & Pestova, 2010).

There are two different mechanisms of translation initiation in eukaryotes. The most frequently used is the so-called **Cap-dependent** translation initiation (see Figure 15). This begins with the binding of the eukaryotic initiation factor 4F (eIF4F) complex to the cap structure present at the 5' end of the mRNA. This eIF4F complex comprises the cap-binding protein eIF4E, the RNA helicase eIF4A and the scaffold protein eIF4G. Then, the small 40S ribosomal subunit, which is associated with the translation initiation factors eIF3, eIF1, eIF1A, the ternary complex (that includes eIF2, GTP and the initiator met-tRNA_i) and probably

eIF5, are recruited to the mRNA by the interaction between eIF3 and eIF4G. This constitutes the 43S ribosomal pre-initiation complex (PIC) that scans along the 5' untranslated region (5'UTR) of the messenger until it reaches the initiation codon in an optimal sequence context. At that point, the 48S initiation complex is formed and hydrolysis of the eIF2-bound GTP occurs. The consensus for this optimal context sequence was first described by M. Kozak and corresponds to [A/G]xx**AUGG** in mammals with a purine (A/G) at position -3 and a G at position +4 from the initiation AUG codon that are critical for start codon recognition (Kozak, 1986; Zur & Tuller, 2013). For a detailed review of start codon selection, see (Asano, 2014; Zur & Tuller, 2013). At the initiation codon, the 60S large ribosomal subunit associates with the PIC to form the 80S competent ribosome for elongation (Richard J Jackson et al., 2010). Additionally, the poly(A)-binding protein (PABP) circularizes the mRNA by interacting with the poly(A) tail and eIF4G, thus enhancing the rate of translation initiation probably by allowing faster recycling of post-termination ribosomal complexes (Gray, Coller, Dickson, & Wickens, 2000; Richard J Jackson et al., 2010).

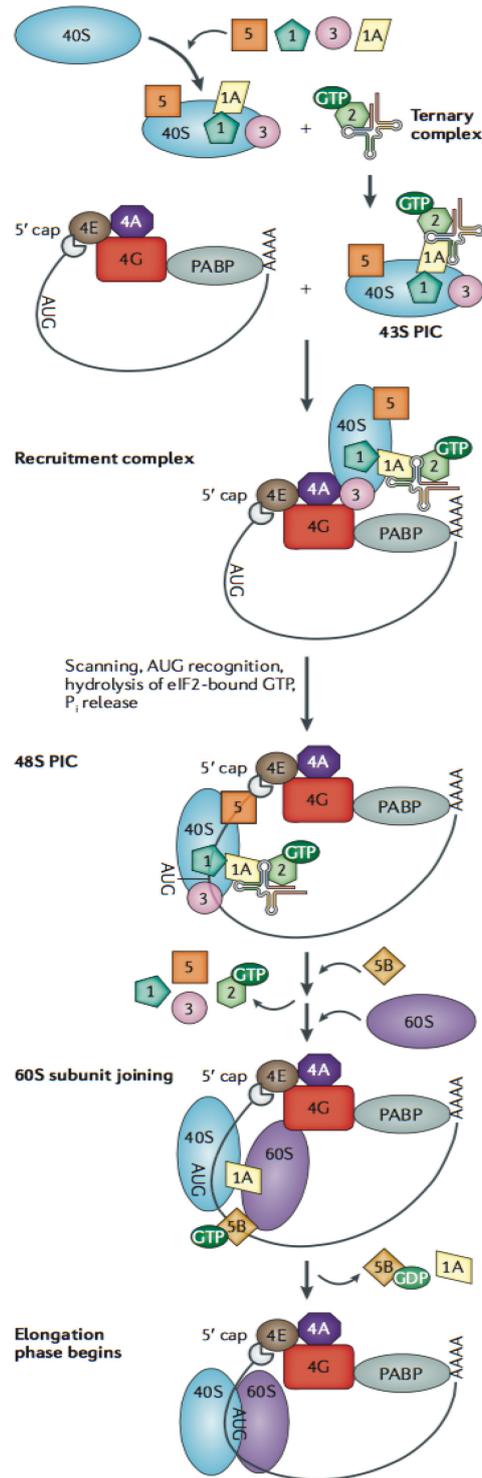


Figure 15. Schema of cap-dependent translation initiation.

The small ribosomal subunit (40S) associates with eukaryotic translation initiation factors (eIFs) 1, 1A, 3 and 5 and with the ternary complex (initiator Met-tRNA_i, eIF2 and GTP), to form the 43S pre-initiation complex (PIC). In parallel the cap structure in the mRNA is recognized by the complex eIF4F (4G, 4A, 4E) that recruits of the 43S PIC. The complex scans from 5' to 3' until an initiation codon is found (48S initiation complex). Next, the large ribosomal subunit (60S) is recruited to the complex together eIF5B at the time that other eIFs are released. After subunit joining, eIF1A and eIF5B-GDP are released and translation elongation begins. Design taken from (Kong & Lasko, 2012).

A second mechanism of translation initiation also exists in eukaryotes called **cap-independent** translation initiation (see Figure 16). This starts with the recruitment of ribosomes to a highly structured RNA element known as internal ribosome entry site (IRES). Although the utilization of eIFs may differ among IRES elements, it is widely accepted that cap-independent translation does not require the cap-binding protein eIF4E (Fitzgerald & Semler, 2009). In addition, non-canonical eIFs known as IRES trans-acting factors (ITAFs) are usually required for proper IRES activity. The specific function of ITAFs remains unclear (Komar & Hatzoglou, 2011) but it has been suggested that they may act as RNA chaperones to maintain the appropriate IRES structure to recruit the 40S ribosomal subunit (Roberts & Holcik, 2009). IRES structures were first described in the naturally uncapped mRNAs from picornavirus (Jang et al., 1988; Pelletier, Kaplan, Racaniello, & Sonenberg, 1988), but a growing list of viral and cellular IRES-containing mRNAs has been reported (Fitzgerald & Semler, 2009; Hellen & Sarnow, 2001; López-Lastra, Rivas, & Barría, 2005). Similar than in the previous mechanism, once recruited at the IRES structure, the PIC may scan (or not, according to the distance from IRES) to find the initiation codons. For instance, on the Hepatitis C virus (HCV) IRES, the PIC lands directly close to the AUG codon whereas in entero-rhinoviruses, like Poliovirus (PV), the PIC is recruited at the IRES and then scans towards a downstream initiation codon located about 150 nucleotides away from the entry site. Aphthoviruses, like Foot and Mouth disease virus (FMDV), represent the intermediate situation with initiation taking place at two different AUG codons; one is located close to the entry site and a second one is reached by ribosomal scanning (R J Jackson & Kaminski, 1995; Prévôt et al., 2003).

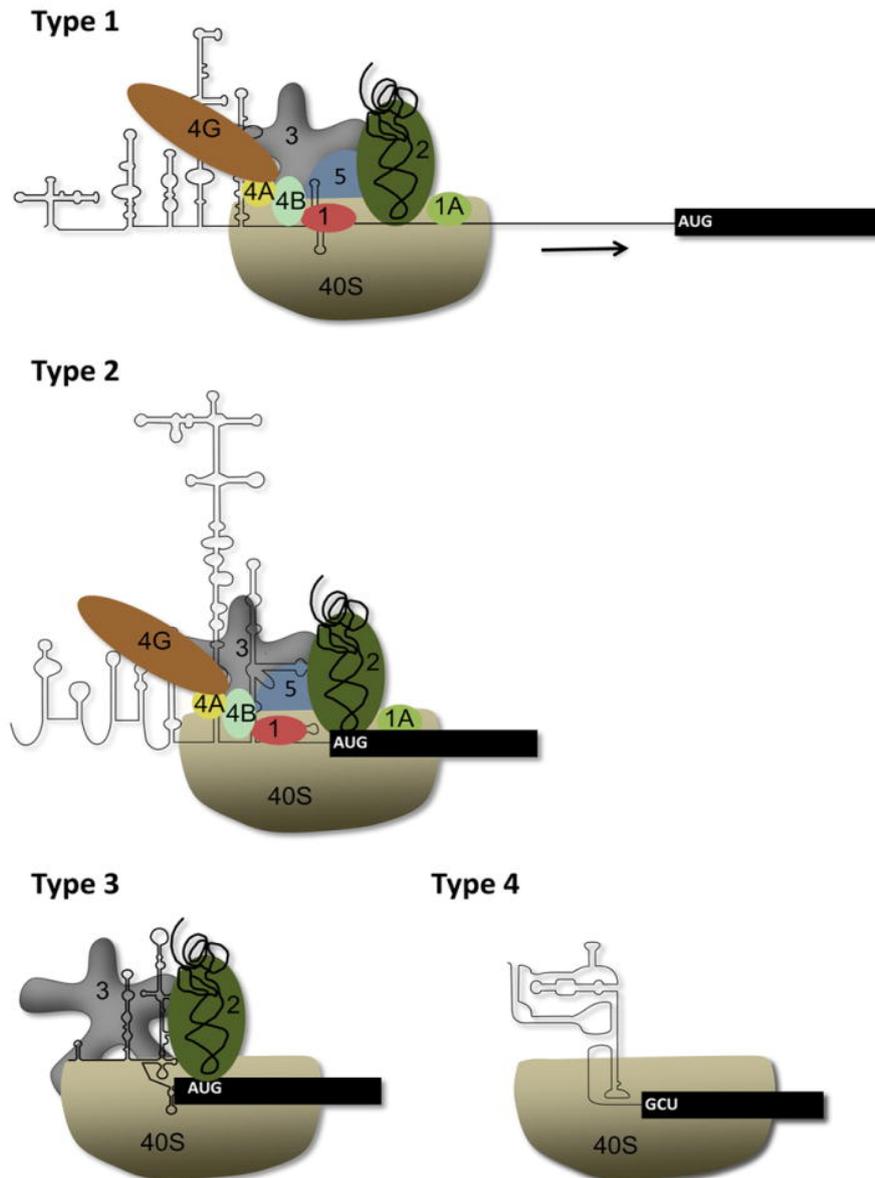


Figure 16. Schema of cap-independent translation initiation.

Internal ribosome entry sites (IRESs) of types 1-4 are illustrated here. The Type 1 and 2 IRESs such as the PV (top) and (EMCV) IRES (middle), respectively use the central domain of eIF4G and eIF4A to recruit the 43S pre-initiation complex (eIF1, eIF1A, eIF3, eIF2/GTP/Met-tRNA^{iMet}) subunit upstream of the AUG start codon. This may be followed by PIC scanning down in a 5' to 3' direction to the initiation codon, in the cases where they are not adjacent. Type 3 IRESs, represented by the hepatitis C virus (HCV) IRES, can recruit the PIC directly to the AUG, in the absence of any initiation factors. They require, however, eIF3 and one of the factors able to bring in the initiator Met-tRNA, such as eIF2-GTP, eIF2D (ligatin), or eIF2A. Type 4 IRESs, such as the intergenic region (IGR) IRES, do not require any initiation factors to initiate translation. As they initiate translation from the A-site they do not require initiator tRNA-Met. Some IRESs require ITAFs (IRES transacting factors) as HIV IRES, which are not represented in this figure. Design taken from (Thompson, 2012).

Consequently, ribosomal scanning is a key issue in translation initiation both in cap-dependent or cap-independent initiation mechanisms. Scanning process usually requires unwinding of RNA secondary

structures present within the 5'UTR and it is mediated by RNA helicases that allow ribosomal movement along it. The RNA helicase eIF4A (which is part of the eIF4F complex) is the best characterized helicase required for translation initiation, and it is in part responsible for unwinding of secondary structures that otherwise would interfere with ribosome attachment and scanning (Parsyan et al., 2011). We will discuss, once again, about the activity of this helicase, later in this manuscript.

Other RNA helicases, have been implicated in translation initiation, including DHX29 and DHX9 that promote translation of specific mRNAs containing highly structured 5'-UTR (Parsyan et al., 2011; Pisareva, Pisarev, Komar, Hellen, & Pestova, 2008; Tettweiler & Lasko, 2006). An alternative to the scanning mechanism, has been called RNA looping, where the initiation codon would be non-covalently bridged to the ribosome recruitment site by a double-stranded RNA structure; thus the pre-initiation complex could be transferred directly to the initiation codon and bypass part of the 5'UTR (Paek, Park, Hong, & Jang, 2012; Young et al., 2014).

II. b) Elongation

During elongation, the 80S ribosome synthesizes a new polypeptide chain by the addition of consecutive amino acids while moving towards the 3' end of the mRNA, one triplet after another, by using the energy from GTP hydrolysis. Ribosome continues until it reaches a stop codon, upon the new synthesized polypeptide is released and the 80S ribosome disassembled into the 60S and the 40S subunits that are recycled for a new round of translation.

In eukaryotes, 64 codons encode 20 different amino acids and three termination signals (Plant et al., 2007). Aminoacyl tRNAs (aa-tRNAs) that form standard Watson-Crick interactions with the first two bases in a codon and form either canonical or non-Watson-Crick pairs at the third or "wobble" position are called cognate-tRNAs. The remaining aa-tRNAs are considered near- and non-cognate tRNAs, which could be rejected by the ribosome. Utilization of the later ones, is called misreading or a missense error and occurs at low frequencies (10^{-3} and 10^{-4} per codon) (Plant et al., 2007; Wohlgemuth, Pohl, & Rodnina, 2010). Another kind of error that occurs at a still lower frequency (3×10^{-5}) is ribosomal frameshifting (Hansen,

Reihani, Oddershede, & Sørensen, 2007; Jelenc & Kurland, 1979). We will describe frameshifting in detail later in this manuscript as it takes place in a programmed fashion during HIV-1 gRNA translation.

Elongation in eukaryotes requires only a few eukaryotic elongation factors (eEFs): the eEF1 (composed of eEF1A and eEF1B) and the eEF2.

The eEF1A is organized in three different domains. Domain I binds GTP/GDP whereas domains II and III bind the aa-tRNA. Domains I and II interact with the eEF1B complex and domain III constitutes also an actin bundling domain that allows it to interact with actin filaments (Eltschinger, Greganova, Heller, Bütikofer, & Altmann, 2012; Li, Wei, Abbott, & Harrich, 2013; Murray, Edmonds, Liu, & Condeelis, 1996).

In a sequential order, the complex eEF1A-GTP-aa-tRNA enters to the A site of the 80S ribosome, where codon-anticodon interactions occur (see Figure 17). Correct pairing (by a cognate aa-tRNA), induces ribosome catalyzed peptide-bond formation on the recently entered aa-tRNA and GTP hydrolysis by eEF1A. Subsequently, eEF2 mediates 80S ribosome translocation through its GTPase activity, displacing the peptidyl tRNA to the P site, and leaving a vacant A site for the next aminoacyl-tRNA to enter for the next round of amino acid incorporation. The deacylated tRNA is shifted from the P to the E site (Dang et al., 2011; Knight et al., 2015)

The rate of elongation can be modulated by the phosphorylation of eEF2 on Thr56. This phosphorylation inhibits eEF2 activity by physically blocking its entry into the A ribosomal site, therefore reducing the rate of translocation (Knight et al., 2015).

During translation elongation, ribosomes are thought to occasionally stall, when for example they encounter strong secondary structures within the mRNA or run into long stretches of rare codons. Stalling is recognized by an intricate surveillance mechanism that causes release of the ribosome on the one hand, and cleavage of the mRNA on the other. (Leppek, Schott, & Stoecklin, 2011).

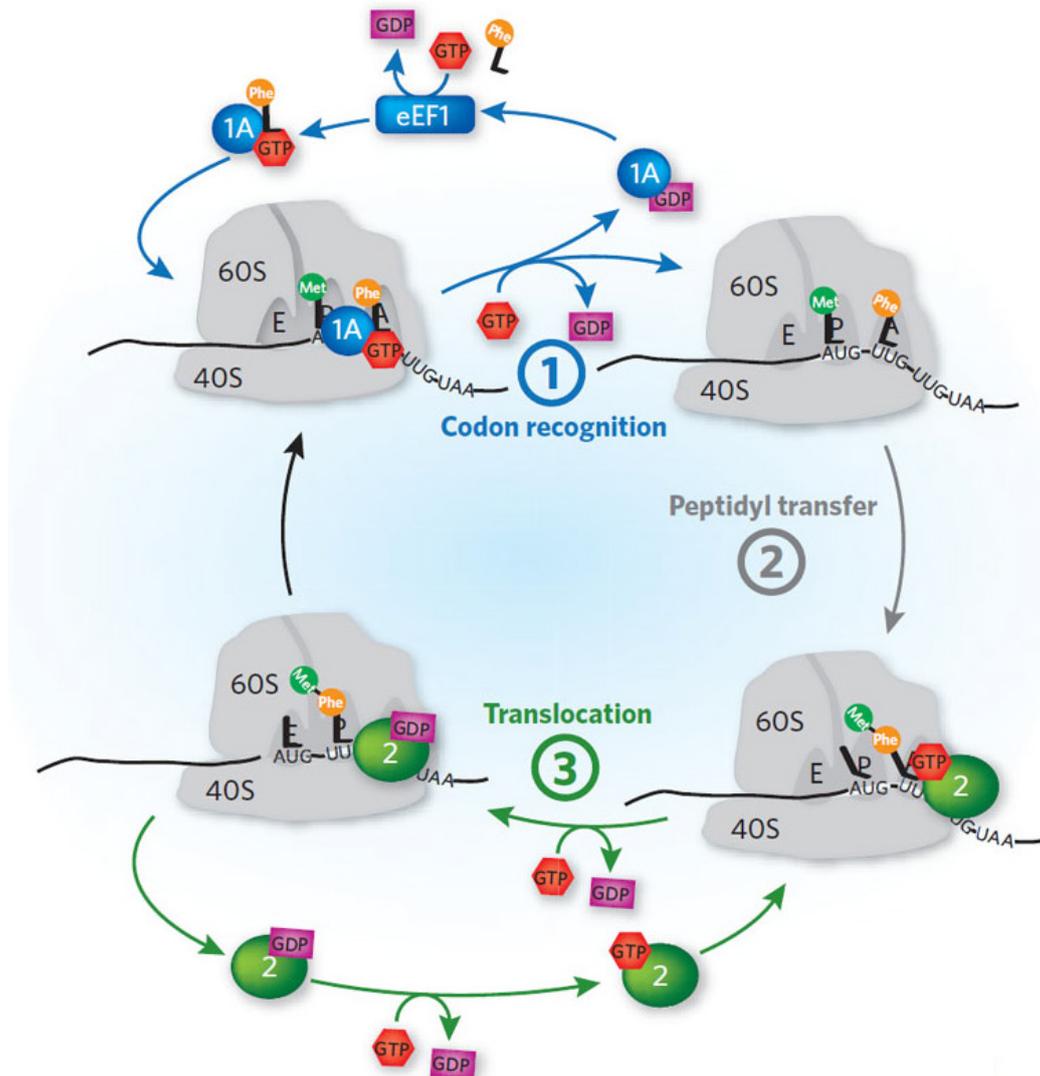


Figure 17. Schema of Translation elongation.

After Met-tRNA is placed in the P-site of the ribosome, elongation factor eEF1A delivers aminoacyl-tRNA into the A-site and catalyzes peptide bond formation which result in the transfer of the nascent peptide onto the A-site tRNA. The translocase eEF2 pushes the peptidyl-tRNA into the P-site and the deacylated tRNA into the E-site, freeing the A-site for the next round of elongation. Design adapted from (Schneider-Poetsch, Usui, Kaida, & Yoshida, 2010)

There are several mechanisms that control translation fidelity during the course of translation elongation. These include the availability of aa-tRNAs (Kramer & Farabaugh, 2007; Wohlgemuth et al., 2010), the proper structure and function of ribosomal proteins (Meskauskas & Dinman, 2007; Plant et al., 2007), the presence of antibiotics (Dang et al., 2011) as well as the geometry of base pairing at the ribosomal decoding center (Ogle, Murphy, Tarry, & Ramakrishnan, 2002). It has been recently shown, that several viruses target eukaryotic elongation factors in order to enhance their replication (reviewed by Li et al., 2013)). In the

specific case of HIV-1, several direct associations between EFs and MA, NC, IN, RT or Nef viral proteins have been reported (Li et al., 2013), while a precise mechanism and role for these interactions remains to be elucidated. On the other hand, it has been reported a direct binding of eEF1A and Gag polyprotein (Cimarelli & Luban, 1999). eEF1A is encapsidated within viral particles and it would contribute to the incorporation of tRNAs into HIV-1 particle.

II. c) Termination

The presence of a stop codon (UAA, UAG or UGA) triggers the translation termination step. This process is mediated by the eukaryotic translation termination factors eRF1 and eRF3 (see Figure 18). The eRF1 factor mediates stop codon recognition, while eRF3 potently stimulates peptide release. After protein release, eRF1 remains bound to the post-termination complex (post-TC), and in conjunction with the ATP-binding cassette protein ABCE1, it dissociates the post-TC into the 60S subunit, and the tRNA- and mRNA-bound 40S subunit. For a detailed review see (Richard J Jackson, Hellen, & Pestova, 2012).

Translation termination is also linked to several mechanisms of control and mRNA decay that will not be described here. For a review see (Celik, Kervestin, & Jacobson, 2014; Inada, 2013; Kervestin & Jacobson, 2012)

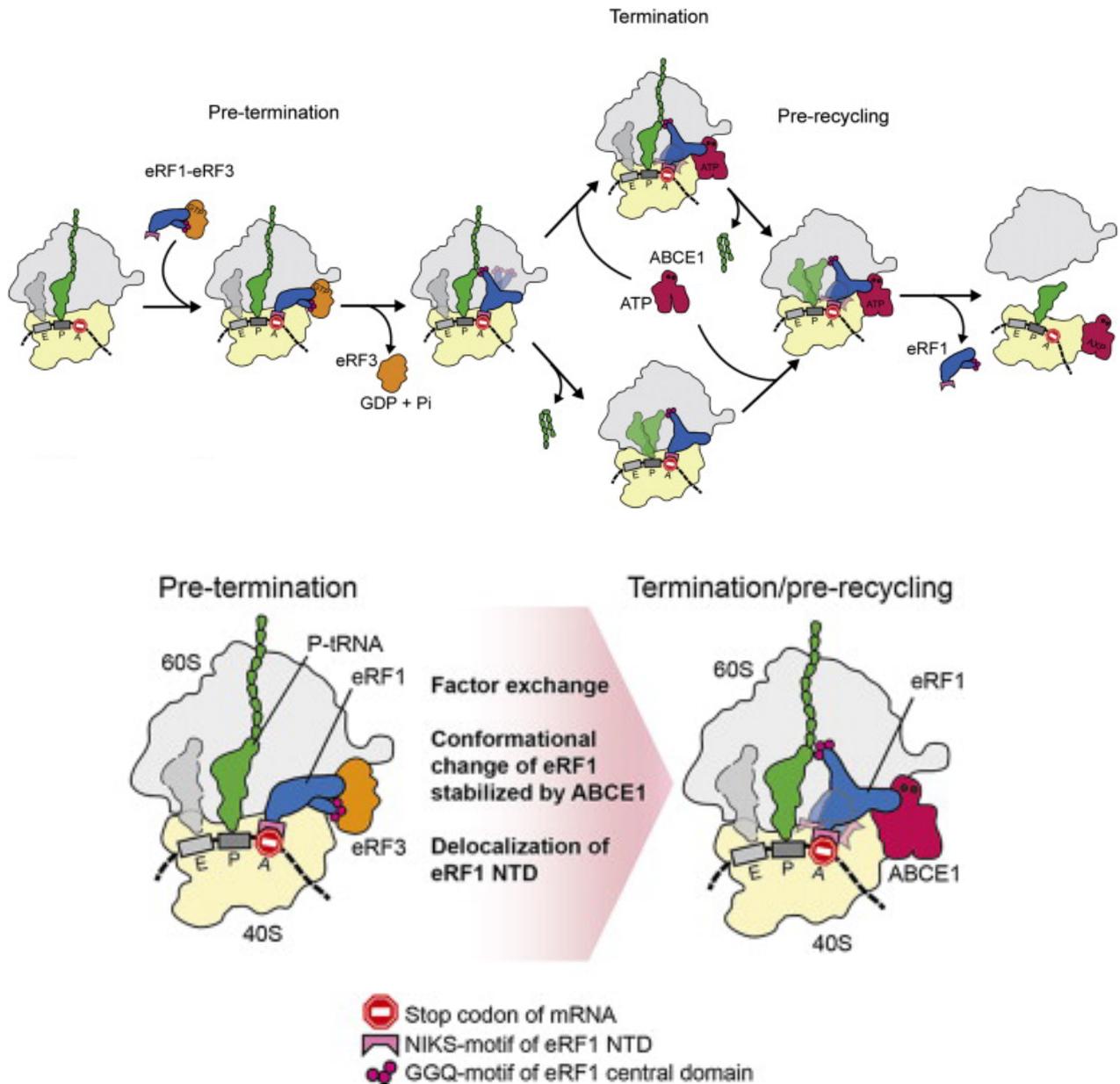


Figure 18. Schema of translation termination and recycling.

The eRF1-eRF3-GTP ternary complex recognizes a stop codon in the A site. GTP hydrolysis allows dissociation of eRF3 and correct positioning of eRF1 into the PTC. It may promote direct peptide release (ribosome a-below), or alternatively, eRF1 may be stabilized by the ABCE1 protein (ribosome a-above), which stimulates peptide release and ribosome dissociation. Designs taken from (Preis et al., 2014).

II. d) Translation of HIV-1 proteins

Viruses are intracellular parasites that rely exclusively on the host translational machinery to synthesize their proteins. Consequently, viruses have evolved many different strategies to exploit the cellular translational machinery for their own benefit (Roberts & Holcik, 2009).

A classic example is the shut-off of host cell mRNA translation during Poliovirus infection. Poliovirus encodes the viral protease 2A responsible for viral maturation. This protease also cleaves the initiation factor eIF4G and thereby inhibits cap-dependent translation, while viral mRNAs are selectively translated through cap-independent mechanism mediated by the use of an IRES in their 5'UTR (Balvay, Rifo, Ricci, Decimo, & Ohlmann, 2009).

We will describe some findings regarding the translation of the HIV-1 gRNA, both at the initiation and elongation steps. Translation of HIV-1 has been the subject of intensive research in the last few years. HIV-1 is integrated into the host cell genome, and it is transcribed like cellular genes by the RNA polymerase II to produce capped and polyadenylated mRNAs (Frankel & Young, 1998). As such, the HIV-1 gRNA is a good template for cap-dependent translation (Berkhout, Arts, & Abbink, 2011; Miele, Mouland, Harrison, Cohen, & Lever, 1996). However, it has been described that functional IRES elements are present within the HIV-1 5'UTR and the coding region (see Figure 19), which are able to drive translation during the G2/M phase of the cell cycle or during cellular stress. These are situations in which cap-dependent translation is usually inhibited (Brasey et al., 2003; Gendron et al., 2008; Monette, Ajamian, López-Lastra, & Mouland, 2009; Vallejos et al., 2011).

Interestingly, it has also been reported that several host proteins modulate the activity of the HIV-1 gRNA translation. Namely, it has been demonstrated that cellular proteins like the lupus autoantigen (La), Staufen1 or TAR RNA binding protein (TRBP) can enhance translation (Dugré-Brisson et al., 2005; Svitkin et al., 1994). We also reported that the TAR motif at the beginning of the 5'UTR was a key regulator of HIV-1 translation (Soto-Rifo, Limousin, et al., 2012). This motif can bind cellular proteins that are required for optimal translation of HIV-1 (Soto-Rifo, Limousin, et al., 2012). The factors mentioned above and the specific roles of factors involved in G2/M on HIV-1 IRES activity, rise the interesting question as to the

coexistence of cap-dependent and cap-independent initiation in HIV-1 and how this virus is able to switch between these two mechanisms or whether key cellular factors are involved.

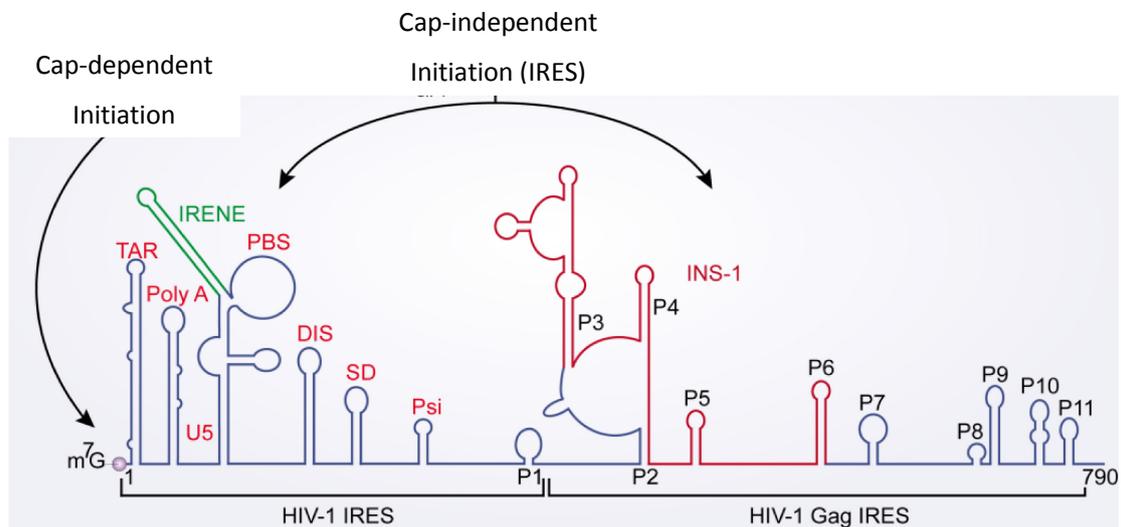


Figure 19. Schema of the 5'UTR and the beginning of the coding region from HIV-1.

According to published data, HIV-1 could initiate translation by a cap-dependent mechanism or by means of one of the two IRESs present within the 5'UTR and the beginning of the coding region. Data taken from (Al-Hashimi, 2009).

It has also been reported that the 5'UTR of HIV is naturally structured (Berkhout 2000) (see Figure 20), which is a property that should be largely detrimental to ribosomal scanning and translation (Parkin 1988, Geballe 1992, Miele 1996). However, the HIV-1 gRNA gets very actively translated in cells (Bolinger 2009) or in the reticulocyte lysate supplemented with HeLa extracts (Soto-Rifo, Limousin, et al., 2012). This paradoxical effect is explained by the presence of RNA structures, such as TAR, which have shown to facilitate HIV-1 translation in cells by its association with cellular RNA binding proteins.

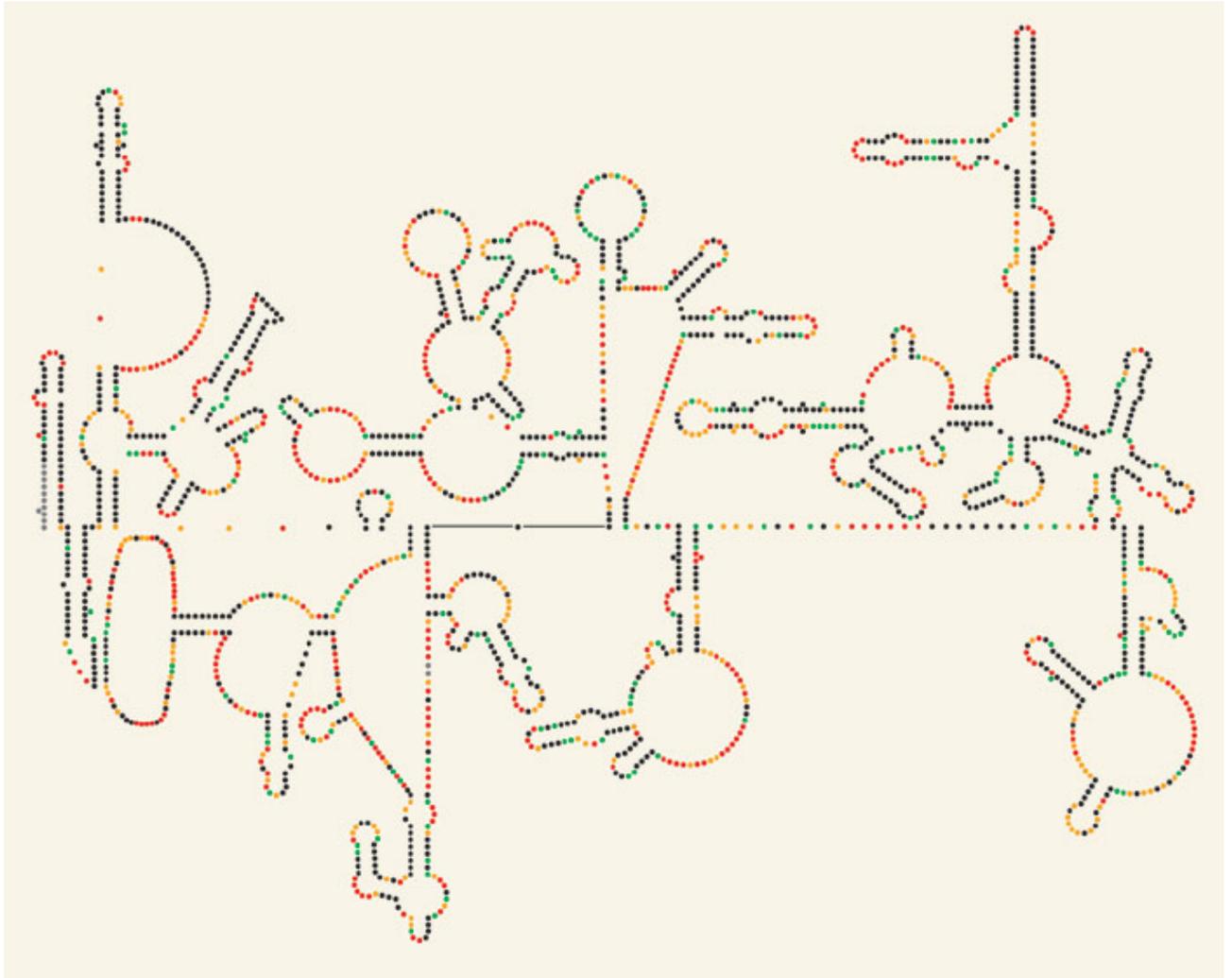


Figure 20. Proposed RNA secondary structure of a section of the HIV-1 RNA,

(RNA section includes 5'UTR and a part of the coding region). This highly structured RNA, would constitute, in theory, a complex obstacle for messenger translation. Data taken from (Al-Hashimi, 2009).

Cellular RNA helicases have been implicated in translation initiation. In particular, DHX29 and DHX9 are thought to promote translation of specific mRNAs containing highly structured 5'-UTR (Jesús De la Cruz, Kressler, & Linder, 1999; Pisareva et al., 2008). These helicases would promote RNP rearrangements to prepare the 5'UTR for efficient ribosomal scanning (Bolinger, Sharma, Singh, Yu, & Boris-Lawrie, 2010; Parsyan et al., 2011; Tettweiler & Lasko, 2006). My work has focused on the RNA helicase DDX3, and it involved showing that it is required for efficient translation of the HIV-1 gRNA and other highly structured mRNAs. The binding of DDX3 on TAR mediates the assembly of a preinitiation complex that allows

ribosomal binding (Soto-Rifo, Rubilar, & Ohlmann, 2013; Soto-Rifo, Rubilar, et al., 2012). This is described in greater details in the section containing the articles.

HIV-1 translation is also tightly controlled during the elongation phase of protein synthesis by programmed ribosomal frameshift (PRF).

Indeed production of the GAG-POL polyprotein, from the HIV-1 gRNA, occurs by means of a -1 frameshift. This mechanism allows a regulated percentage of ribosomes to shift back one nucleotide at the end of the gag reading frame in order to produce a fusion with the pol reading frame that otherwise would not be translated. The frequency of spontaneous frameshift errors in the cell has been estimated to less than 3×10^{-5} (Hansen et al., 2007); while in the case of HIV GAG-POL, PRF frequency borders 1000 times more (5×10^{-2}).

The GAG-POL to GAG ratio has been shown to be critical for viral infectivity, as it impacts on viral particle assembly and gRNA dimerization (Hung, Patel, Davis, & Simon, 1998; Shehu-xhilaga, Crowe, & Johnson, 2001). If the HIV-1 genome is modified (by the addition of 1 nucleotide at the frameshift site), so that gag and pol are in frame and Gag-Pol is produced 100% of the time, the resulting mutant viruses are unable to produce viral particles (Shehu-xhilaga et al., 2001).

However, the correlation between magnitude of changes in the PRF ratio and its impact on infectious progeny remains controversial. This could be explained by using different frameshift signals as PRF models, each one of them with its specific PRF ratio employing eventually different mechanisms of control (Biswas, Jiang, Pacchia, Dougherty, & Peltz, 2004; Dulude, Berchiche, Gendron, Brakier-Gingras, & Heveker, 2006; Hung et al., 1998).

Programmed ribosomal frame shift requires, at least, two basic RNA elements: a slippery site (specific sequence where the ribosome makes PRF) and the frameshift stimulatory signal (a segment of RNA with secondary structure that forces the ribosome to pause). In the case of HIV-1, the slippery sequence consists of the 7 nucleotides U UUU UUG (codons separated by spaces) and the frameshift stimulatory signal is a two-stem structure of about 50nt in length located at the immediate 3' from the slippery site (Dulude, Baril, & Brakier-Gingras, 2002). Disruption of any of these elements has a deleterious effect on viral

replication (see Figure 21). Interestingly, it has been found after sequencing about 1,000 HIV-1 slippery sequences, that all of them conserved the identical UUUUUUA heptamer, which indicates a negative selection for viruses that contain mutations in this region. Moreover, substituting this site with UUUAAAA, another equivalently efficient slippery sequence, reduced viral titre more than a 1,000-fold (Biswas et al., 2004).

Ribosomal pausing, generated by the frameshift stimulatory signal, seems to be an important step for an efficient PRF. Since the diameter of the mRNA entry tunnel in the ribosome is too small to accommodate double-stranded RNA (stem or pseudoknots), any RNA secondary structures must be disrupted before being decoded by the ribosome. The ribosome has been shown to possess an important intrinsic mRNA helicase activity for resolving duplex structures during translation *in vitro* (Chang, 2012). Moreover, there is some evidence of a direct relationship between the strength of the stimulatory signal and the efficiency of PRF. This evidence has shown that *in vitro*, an increase in the stability of a pseudoknot structure augmented the PRF ratio (Hansen et al., 2007). However, in the case of HIV-1, the presence of a two-stem structure (requiring less energy for unwinding) seems to be sufficient to ensure correct PRF efficiency.

Several molecules have been shown to also influence PRF efficiency. This is the case of the peptidyl transferase inhibitor, Anisomycin, which specifically affects the accommodation step during elongation by impairing binding of the aa-tRNA into the A site of the peptidyl transferase center (Domínguez, Gómez-Lorenzo, & Martín, 1999). As such, Anisomycin is often used as a positive control of PRF as it increases its ratio in a dose-dependent manner (J. D. Dinman & Wickner, 1994).

Some cellular factors that control PRF in different models have been reported. For instance, Annexin A2 (ANXA2), has been shown to bind to the pseudoknot of avian coronavirus infectious bronchitis virus (IBV) and reduce the viral replication (Kwak, Park, & Jeong, 2011). Also depletion of eRF1 increases HIV-1 PRF and reduces HIV-1 viral progeny (Kobayashi, Zhuang, Peltz, & Dougherty, 2010). The exact mechanism by which this factor exert its role is unknown, however, it was recently shown that RF1 depletion produced defective particles containing 10-15 times more gRNA (Chamanian et al., 2013; Kobayashi et al., 2010). Previously, a mutation of eRF1 in yeast was reported to promote stop codon readthrough and to increase

the frequency of frameshift suppression (Drugeon et al., 1997). However the role of this translation termination factor during elongation of the mRNA is not clear.

In the section containing the articles, I present in detail our results related to the effect of the cellular protein TIAR in the HIV-1 PRF.

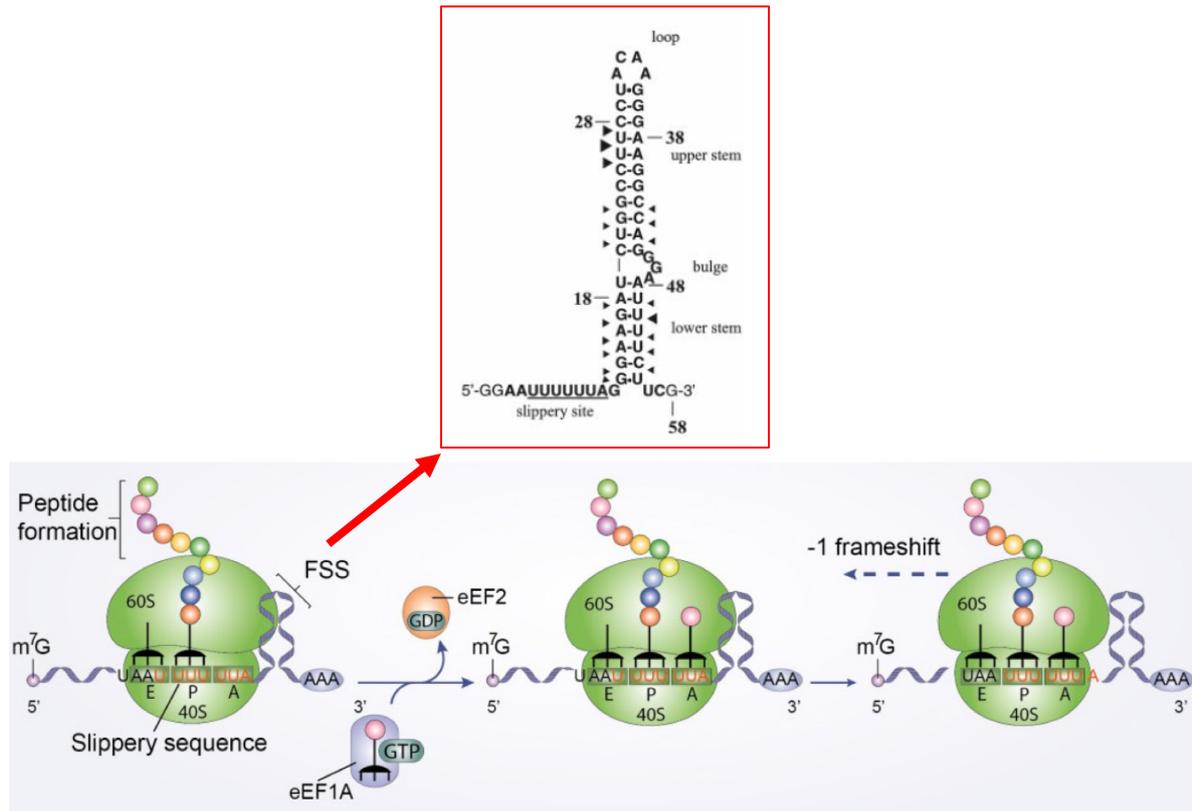


Figure 21. Simplified schema of ribosomal frameshift in HIV-1 gRNA.

Top panel: The two-stem structure proposed by (Dulude et al., 2002). Bottom panel: the ribosome pauses at slippery heptanucleotide sequence (UUUUUA) because of the frameshift stimulatory signal (FSS); a secondary structure. At this place ribosomes can go backward 1 nucleotide (-1 frameshift) or resume translation in the original frame. Design published by (Guerrero et al., 2015)

Chapter III. RNA Helicases and DDX3

III. RNA helicases and DDX3

During the first part of my thesis, I was interested in the role of the RNA helicase DDX3 in translation initiation. In order to better understand the functions of DDX3, I will first introduce the general properties of helicases during RNA lifecycle and then come back to the roles described for DDX3.

III. a) General description

Helicases are generally described as enzymes that use energy derived from NTP hydrolysis (usually ATP) to unwind double-stranded nucleic acids or to remodel RNA-protein complexes. These enzymes are ubiquitous through animalia, plantae, fungi and bacteria kingdoms and they are found in some viruses (Gustafson & Wessel, 2010; Linder & Owttrim, 2009; Panepinto et al., 2005; Perumala, Raneyb, & Benkovic, 2012; Schütz et al., 2010). Helicases share several conserved motifs and although the enzymatic mechanisms across helicases differ between super families (SF) and subfamilies, they all contain a conserved RecA-like core with NTPase activity. RNA Helicases belonging to SF1 and SF2, which are the best characterized, enclose in their primary sequence two core regions involved in ATP-binding/hydrolysis and RNA binding (Gustafson & Wessel, 2010) whereas helicases belonging to SF3 to SF5 contain only one core region. DEAD- and DExH-box helicases belonging to SF2 the most common helicases; therefore, they will be mentioned thereafter to illustrate most of the functions described for RNA helicases. More specifically we will refer to the functions described for DDX3, as it makes part of the research accomplished during my thesis.

III. b) RNA helicase activity

Names and classification of helicases are complicated as usually names are previous to full characterization of the proteins (for an overview of similitudes and differences within groups see (Berger, 2008)). Helicases activities are diverse and different mechanisms are proposed to be used by these proteins that could differ even within the same group. As paradigms of SF2 helicases, with the two core regions in charge of ATP-binding/hydrolysis and RNA binding activities, DExD- and DExH-box proteins conserve within their core regions the aminoacids Asp-Glu-(any)-Asp or Asp-Glu-(any)-His respectively. Crystal structures from these

core regions have given some insights in understanding how these proteins execute their function. Thus, it has been proposed that ATP binding primes DEAD helicases for RNA substrate binding by means of a conformational change that create a high affinity RNA binding site. DExH helicases, on the other hand, stay bound to the RNA after each unwinding step (Schütz et al., 2010). Some DEAD-box helicases bind ATP and RNA in a cooperative way, and they are considered not to be very processives; whereas DExH-box helicases, such as the hepatitis C virus helicase NS3, seems able to bind RNA in the absence of ATP (Schültz 2010). Some processives viral RNA helicases like the nucleoside triphosphate (NTP) phosphohydrolase II (NPH-II) and NS3 display a canonical duplex unwinding activity with translocation that requires directionality; whereas DEAD helicases unwind duplexes by local strand separation without any directionality (Jankowsky 2010). Moreover, in the case of the yeast DEAD helicase Ded1, it has been shown that the cooperative binding of ATP and RNA ligands, subsequently promotes the ATPase activity of the protein, thus, classifying this helicase as an ATP-dependent RNA-binding protein and a RNA-dependent ATPase (Senissar et al., 2014).

The core regions of these helicases are flanked by very divergent N- and C-terminal regions that usually contain accessory domains. These domains seem to interact with RNA substrates as well as being hot-spots for posttranslational modifications (generally phosphorylation) and protein–protein interactions (Fairman-Williams, Guenther, & Jankowsky, 2010; Gustafson & Wessel, 2010). These accessory domains are also believed to participate in the control of the helicase activity. Some examples of these interactions are detailed thereafter; nevertheless it is interesting to mention that the overexpression of accessory domains alone (often N-terminal domains) leads to dominant negatives phenotypes, which suggests they sequester cofactors or bind to their substrates in an unspecific fashion (Banroques, Cordin, Doère, Linder, & Tanner, 2011; Tanner & Linder, 2001).

As highlighted above, not all helicases unwind nucleic acids and some of them utilize instead the energy from NTP hydrolysis to “translocate” (move along the double or single strand) to alter ribonucleoprotein (RNP) conformations. This translocation concept is relevant in order to understand the multiple critical processes by which RNA helicases are involved as RNA chaperones, ATP-dependent RNA helicases and

unwindases, co-activators and co-repressors of transcription or RNAPases by mediating RNA-protein association and dissociation (Fairman-Williams et al., 2010; Leitão, Costa, & Enguita, 2015).

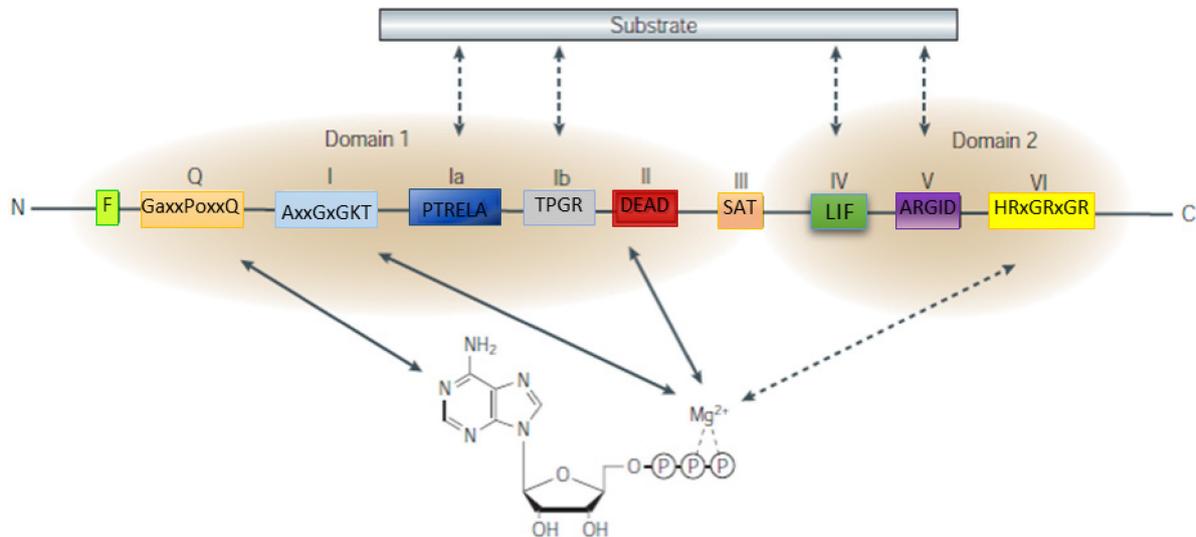


Figure 22. Simplified schema of a DEAD Helicase.

The schema represents the conserved motifs and interaction with its substrates. Figure modified from (Rocak & Linder, 2004).

Helicases throughout RNA metabolism

Most helicases are involved in different steps of RNA metabolism. Therefore, we will mention some of the best described examples in order to understand the critical role of these enzymes throughout the RNA lifecycle.

During transcription, helicases belonging to SF2, such as the yeast SWI/SNF complex, are involved in transcriptional regulation through chromatin remodelling and with the assembly of the transcription-initiation complex (Havas et al., 2000; Tanner & Linder, 2001). Other helicases, namely the human RNA helicases DDX5 and DDX17, are believed to play roles in transcriptional regulation. The DEAD-box core region of these helicases interacts with the RNA polymerase II and act as coactivator, to regulate its transcriptional activity (Gustafson & Wessel, 2010)

Growing evidence associates the unwinding activity of RNA helicases with proper RNA folding (Jankowsky, Guenther, & Jankowsky, 2011) which is important for their biological activity. For instance, DEAD-box RNA helicases have been shown to assist RNA folding *in vivo* (Lieber, Gu, Lu, Shimazaki, & Tsai, 2010) while mitochondrial RNA helicases Mss116p and CYT-19 would act as RNA chaperones to guide the native RNAs conformation through a series of folding steps or by remodelling of the RNA molecule (Rocak & Linder, 2004).

Furthermore, during splicing, it has been suggested that unwinding activity of RNA helicases may ensure the directionality and the efficiency of the catalytic reactions by preventing RNA re-association and thereby allowing different RNA-RNA or RNA- protein interactions (Tanner & Linder, 2001). For instance, the human RNA helicases DDX5 and DDX17 have been documented to be necessary for splicing (Gustafson & Wessel, 2010). Other reports revealed that DDX3 has been found to also play a role during splicing (Ariumi, 2014; Schröder, 2010).

The role of RNA helicases in translation has been widely documented. Indeed, RNA helicases are often found within large ribonucleoprotein complexes (RNP) where translation takes place. For instance, the RNA helicase DDX3 has been immunoprecipitated together with at least 10 subunits of the translation initiation factor, eIF3 (Lee 2008). As mentioned previously, the RNA helicase eIF4A, also known as DDX2, is a well conserved eukaryotic initiation factor that unwinds the mRNA secondary structures during scanning of the ribosome. eIF4A, is the prototype member of the DEAD-box helicase and its ATPase activity is stimulated by the association with either the eIF4B or eIF4H initiation factors, thus demonstrating the relevance of the control exerted by the association of helicases with accessory factors. Another example of the role of helicases in translation is the Vasa protein. The helicase activity of the latter was shown to be required for translational control of germline-specific mRNAs in *Drosophila*. However, the DBY human homologue of the Vasa-like gene appears to be restricted to determine male fertility (Gustafson & Wessel, 2010; Rocak & Linder, 2004).

RNA helicases are also required during the programmed destruction of mRNAs containing premature stop codons by nonsense-mediated RNA decay (NMD). Namely, Upf1p is an RNA helicase belonging to the SF1 and its deletion was shown to be associated with an increase of stop codons read-through, thereby leading

to the stabilization of nonsense codon-containing mRNAs (Tanner & Linder, 2001). Also, the DExH-box helicases Ski2 and Dob1 assist the eukaryotic exosome in RNA degradation, while the DEAD-box Helicase Dhh1 is required for mRNA decapping (Rocak & Linder, 2004).

Finally, several studies have shown additional roles for RNA helicases. For instance, DDX4 has been associated to both the small interfering RNA and micro-RNA processing pathways (Nagamori, Cruickshank, & Sassone-Corsi, 2011); while depletion of DDX3 has been reported to alter cell cycle (Lai, Chang, Shieh, & Tarn, 2010).

III. c) RNA helicases in viral infections

Helicases were also shown to be involved at different levels during some viral infection. We will only mention some of the best characterized examples.

First, it is noteworthy that some viruses encode their own RNA/DNA helicases, as it is the case of NPH-II from vaccinia virus and NS3 from flaviviruses like Hepatitis C, Dengue, Yellow Fever, West Nile or Japanese encephalitis virus (all belong to the DExH-box family of helicases) (Cordin, Banroques, Tanner, & Linder, 2006; Leitão et al., 2015). Because of their relevant function, these helicases have been intensively studied as possible antiviral therapy targets. As such, nucleoside analogues, have been successfully used as NTPase/helicase inhibitors of West Nile virus (Kwong, Rao, & Jeang, 2005) and a few inhibitors of NS3 helicase from HCV have progressed into clinical trials although most of the compounds which inhibit HCV RNA replication in cells have also shown high cytotoxicity (Franca, Belfiore, Spadari, & Maga, 2008; Leitão et al., 2015)

On the other hand, the innate immune systems in eukaryotes exhibits several strategies as viral defense, including RNA interference (RNAi)-based strategies and the pattern recognition receptors (PRRs) leading to activation of interferon (IFN) response. RNA helicases are closely related to these antiviral defenses. For instance, the RNAi enzyme Dicer 1, which catalyzes the generation of small double-stranded RNAs, is a DExD/H helicase (Deddouche et al., 2008). In the case of the activation of the interferon response, vertebrates have evolved a complex system of receptors that can detect the presence of foreign nucleic

acids (Ulvila, Hultmark, & Rämét, 2010). Thus, in addition to Toll-Like Receptors (TLR), there is a set of cytoplasmic PRRs belonging to the DExD/H-box and DEAD-box helicases which sense viral nucleic acids to trigger the innate antiviral response (Deddouche et al., 2008). For instance, RIG-I and MDA-5 helicases, recognize dsRNA and induce INF type I signalling via their CARD domain; whereas LGP2 helicase, lacking a CARD domain, but similarly recognizing dsRNA has been proposed to act as a negative-feedback regulation of IFN signalling (Rothenfusser et al., 2005). Some negative ssRNA genome viruses, such as influenza, naturally activate RIG-I helicase and thus trigger the IFN response during infection (Rehwinkel 2010). However, paramyxoviruses, which also possess ssRNA genome, have the ability to interfere with this signalling pathway by inhibiting the cellular activities of the RIG-I and melanoma differentiation-associated protein 5 (MDA5) as well as downstream components in the type I IFN induction cascades (Goodbourn & Randall, 2009).

In the case of HIV-1, DDX1 has been shown to enhance HIV-1 replication whereas DDX24 is required during packaging of the virus (Ma et al., 2008). In contrast overexpression of the RNA helicase DHX30 disturb HIV-1 genome packaging (Zhou et al., 2008). On the other hand, recent studies associate others helicases with viral infections, such as human Cytomegalovirus (HCMV), which takes advantage of the helicase UAP56 for efficient replication (Zielke et al., 2012). Similarly, during HCV infection, a complex containing the RNA helicase DDX6, the viral RNA and the core protein is formed which is essential to promote HCV replication (Jangra, Yi, & Lemon, 2010). Finally, a last example is the avian Infectious Bronchitis virus (IBV) which enhance its replication by interacting with the DDX1 helicase (Xu et al., 2010).

III. d) DDX3 and HIV-1

DDX3 is an RNA helicase belonging to the DEAD-box family. It shuttles between the nucleus and the cytoplasm and has been reported to participate in several mRNA biogenesis steps, including transcription, RNA splicing, mRNA migration, cell cycle and recently in translational control (Shih et al., 2012; Soto-Rifo et al., 2013; Soto-Rifo, Rubilar, et al., 2012). Characterization of the DDX3 enzymatic activities remain incomplete. However, it has been reported that It can use different NTPs and it has a relaxed substrate

specificity. Remarkable, binding of nucleic acids increased the apparent rate of hydrolysis and the catalytic efficiency of the ATPase activity of DDX3 (Franca et al., 2008).

DDX3 was previously associated with HIV-1 to be required for efficient transport of intron-containing viral RNAs into the cytoplasm (Yedavalli et al., 2004).

The yeast homologue of DDX3, Ded1, is essential for cell viability and required for translation initiation (Berthelot, Muldoon, Rajkowitsch, Hughes, & McCarthy, 2004; Chuang, Weaver, Liu, & Chang, 1997; J de la Cruz, Iost, Kressler, & Linder, 1997) and it was recently found to interact with the cap-binding complex in yeast (Senissar et al., 2014). Similarly, human DDX3 interacts with several translation initiation factors as eIF3 and eIF4E, eIF4A, eIF2 α and the Poly-A binding protein (PABP) (C. S. Lee et al., 2008; Schröder, 2010). In addition, DDX3 is a component of stress granules (Shih et al., 2012).

DDX3 has been shown to play a role during replication of several viruses. For instance it was involved in direct RNA sensing, due to its ability to bind poly I:C and viral RNA in solution (Mulhern & Bowie, 2010); whereas it was related to the inhibition of Hepatitis B virus (HBV) replication via interaction with the viral Pol and inhibition of IFN induction (Ariumi, 2014). Although the Hepatitis C virus (HCV) was reported to require DDX3 for replication, some other work describes an interaction of the HCV core protein with the C-terminal region of DDX3 that seems to inhibit replication of a specific HCV genotype 1b (Ariumi, 2014).

The K7 protein from Poxvirus, interacts with DDX3 in its N-terminal region avoiding in this way the IFN β promoter activation (Schröder, 2010). As mentioned before, during HIV-1 infection, DDX3 is involved in the export of unspliced viral RNAs into the cytoplasm via interaction with the nuclear export protein CRM1 (Yedavalli et al., 2004). Finally, DDX3 was shown to play a role in the lifecycle West Nile virus (WNV), Japanese encephalitis virus, norovirus, pestivirus, vaccinia virus and cytomegalovirus (Ariumi, 2014).

Several lines of evidence prompted us to hypothesize about a putative role of DDX3 in HIV-1 translation. First of all, DDX3 was identified in a genetic screen as an essential co-factor for replication (Bushman et al., 2009). Secondly, the fact that HIV-1 possesses a highly structured 5' untranslated region where helicases, such as DDX3, may exert their function, led us to think that the latter may be required either to unwind

these structures or to allow the remodelling of the viral leader. Thirdly, other RNA helicases have been involved in several steps of the HIV-1 cycle (Feng et al., 2004; Jeang & Yedavalli, 2006; Zhou et al., 2008).

Previous reports showing an involvement of DDX3 in translation were controversial. Whether these associations enhanced or impaired general translation remained unclear. For example, association of DDX3 with eIF4E was shown to inhibit translation by interfering with the recruitment of eIF4G into eIF4E and consequently would have an effect on the translation of most of the cellular mRNAs (Schröder, 2010). However, another report had shown that DDX3 was required for efficient translation of mRNAs that contain a long or structured 5' UTR and it could be dispensable for general mRNA translation (Lai et al., 2010).

Therefore, all these controversies prompted us to revisit the role of DDX3 on HIV-1 translation and this has allowed us to provide an original molecular mechanism for its function. (see articles 1 and 2).

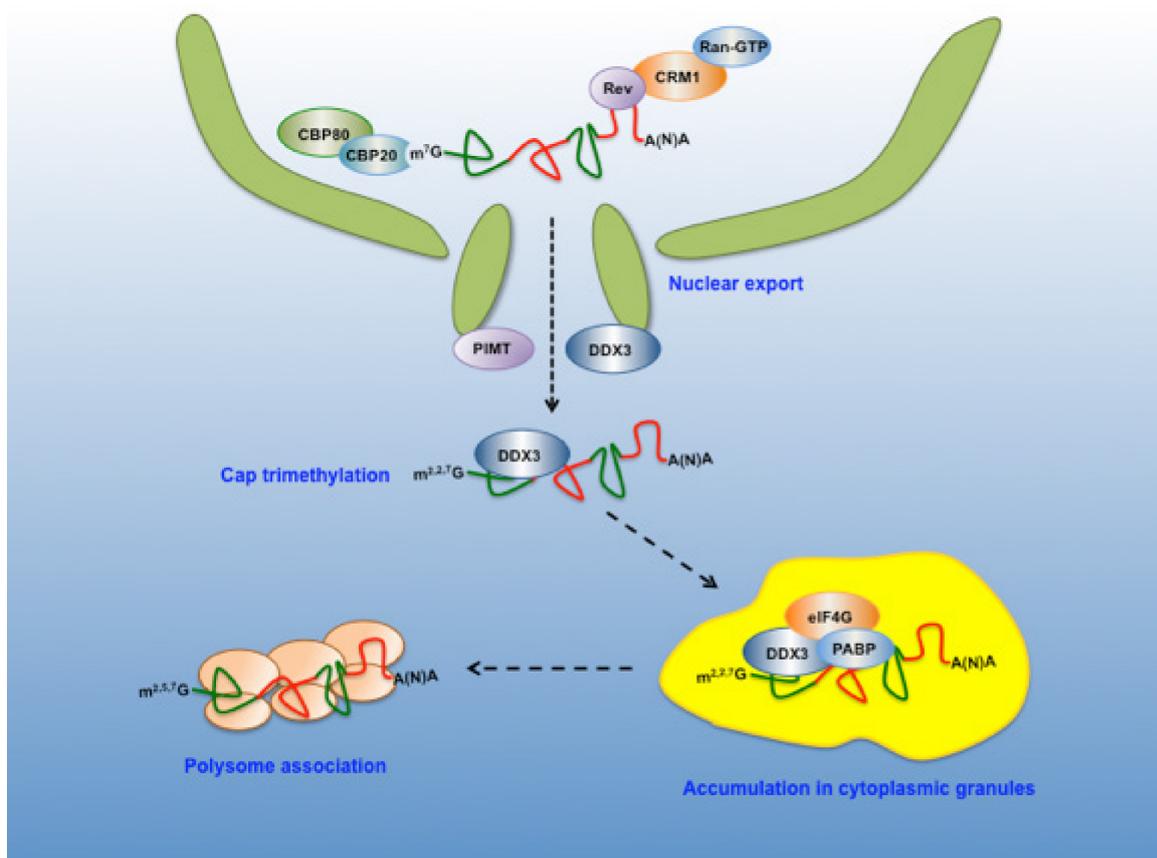


Figure 23. Design of DDX3 function during HIV-1 replication.

DDX3 helicase helps in the exporting of the unspliced HIV-1 gRNA out from the nucleus. Once in the cytoplasm, it forms a complex with the cap-binding initiation factors to favour translation initiation and also has been associated to the protection of the viral gRNA during stress granules formation.

Chapter IV. Stress Granules and TIA-1 Related Protein (TIAR)

IV. Stress granules and TIAR

During the second part of my thesis, we have focalised on the function of TIAR during translation elongation of HIV-1 gRNA. TIAR is a key protein involved in the assembly of Stress Granules (SG). To better understand its functions, we will first introduce the cellular stress granules in general terms and then describe the structure and functions of TIAR and related SG proteins to finish with the discussion of reported roles of TIAR during viral infections.

IV. a) Cellular Stress granules

Several different types of cytoplasmic granules have been described: processing bodies (PB), stress granules, neuronal transport granules, sponge bodies and exosome granules (for a review see (Adjibade & Mazroui, 2014; Buchan, 2014)). They are classified according to the cellular context in which they are found, their putative roles and the presence of protein markers; nevertheless, they all correspond to cytoplasmic RNP foci grouped without a limiting membrane and they all share some components. Cellular granules are highly dynamic and it seems more and more likely that they interact with each other. Indeed it has been described a PB-SG docking, fusion and apparent maturation (Buchan, Muhlrad, & Parker, 2008; Hoyle, Castelli, Campbell, Holmes, & Ashe, 2007; N. Kedersha et al., 2005) and it is also known that SG components are mobile and can traffic in and out from granules (Bhattacharyya, Habermacher, Martine, Closs, & Filipowicz, 2006; Bley et al., 2014; Buchan & Parker, 2009). We will now only focus on SG as they are more relevant to the subject of this manuscript.

The precise function of SG is controversial; however, their assembly under cellular stress conditions and the analysis of their components have given some insights into possible roles. SG contain non-translating mRNAs together with initiation factors, such as eIF4E, eIF4G, eIF3 and PABP-1 and the small ribosomal subunit (Anderson & Kedersha, 2002) together with several RNA binding proteins. Therefore, it appears that one of the main function of SG is to protect mRNAs that are translationally arrested in the cell. This is consistent with the fact that increasing the pool of non-translating mRNAs stimulates SG (and PB) assembly, as observed upon inhibition of translation initiation or inhibition of mRNA decay factors, expression of

decay resistant mRNA, or drugs that promote ribosome–mRNA dissociation (N. L. Kedersha, Gupta, Li, Miller, & Anderson, 1999; N. Kedersha et al., 2000; Teixeira, Sheth, Valencia-Sanchez, Brengues, & Parker, 2005). Similarly, all the mechanisms affecting translation initiation, such as increasing phosphorylation of eIF2 α (which reduces availability of tertiary initiation complex), inhibition of eIF4A activity, and sequestration of eIF4E, are directly associated with an increase in SG assembly. Unexpectedly, translation inhibition is not always correlated with the presence of visible PB or SG (Kato et al., 2012; Kwon, 2007)(Kato 2012; Kwon 2007).

The presence of RNA induced silencing complex (RISC) components, together with proteins that play a role in RNA decay such as G3BP, TTP, Roquin and UPFs (Athanasopoulos et al., 2010; Gardner, 2008; Stoecklin et al., 2004; Tourrière et al., 2003) within SG, point out to a possible role of SG in mRNA translation repression and turnover. However decay factors such as deadenylases, de-capping proteins and exoribonucleases are generally excluded from SG (Anderson & Kedersha, 2008) and are rather found within PB.

On the other hand, proteins associated with RNA stability such as HuR, FMRP, as well as TIA proteins have been also shown to localize within SG (N. L. Kedersha et al., 1999; Mazroui et al., 2002). Similarly, it has been shown that mRNAs stocked in SG would be capable of re-initiate translation once the stress period is over (Yamasaki & Anderson, 2008). In some cases, the exit of the mRNAs from the SGs is assisted by proteins such as STAUFEN, which stimulate translation and disassemble SG (Thomas, Martinez Tosar, Desbats, Leishman, & Boccaccio, 2009). Altogether, these evidences point out a role of SG in the temporal protection of the mRNAs from decay and thus, allowing eventually a sorting of mRNAs into different mRNPs (Anderson & Kedersha, 2008).

Finally, it has also been suggested that SG, as well as other granules, might facilitate the physical separation of connected processes like translation versus translation-repression and decay.

Assembly of SGs occurs in response to environmental signals such as heat shock, starvation, viral infection, etc., but it can be also induced artificially by chemical compounds such as sodium arsenite, which increases the phosphorylation of eIF2 α via activation of HRI kinase. Chemical induction of SG, independent of

phosphorylation of eIF2 α , could also be achieved by Patemine A and Hippuristanol which both lead to SG assembly acting as inhibitors of the eIF4A helicase. On the other hand, cycloheximide blocks translation during elongation leading to SG granule disassembly via stabilization of polysomes, and it serves as a control of *bona fide* SG.

The assembly of SG is rendered possible by specific RNA-protein, protein-protein and prion like motifs that are present within some key SG proteins such as in the TIA family proteins and RasGAP-associated endoribonuclease (G3BP). Remarkably, the TIA and G3BP families of proteins together with eIFs like PABP or eIF3 constitute *bona fide* SG markers. For a review of drugs used in SG research as well as *bona fide* protein markers see (Panas, Kedersha, & McInerney, 2015). In addition, assembly and disassembly of SG seems to be assisted by the motor proteins dynein and kinesin which can be localized also within SG (Loschi, 2009; Ivanov, 2003).

IV. b) TIAR description

TIAR belongs to the T cell restricted intracellular antigen (TIA) family of proteins which are key players in SG assembly in response to changes in the environmental condition like heat shock, starvation, oxidative stress or viral infection. It participates in translation arrest and protects cellular mRNAs from degradation during the stress interval (Waris, Wilce, & Wilce, 2014). TIA proteins also participates in splicing regulation and translation repression (Carrascoso, Sánchez-Jiménez, & Izquierdo, 2014; Del Gatto-Konczak et al., 2000; Piecyk et al., 2000).

At a structural level, TIAR is a protein of about 375 amino acids that harbors three RNA recognition motifs (RRM) that are necessary for RNA binding and recruitment into stress granules and a prion related domain (PRD) which is similar to the aggregation domains found in prion proteins (see Figure 24). There are two isoforms of TIAR that are generated by alternative splicing: a shorter one lacking 17 amino acids within the first RRM domain is called TIARb while the longer isoform is called TIARa (Izquierdo & Valcárcel, 2007).

Each of the RRM motifs seems to contribute differentially to the RNA/DNA recognition (Dember, Kim, Liu, & Anderson, 1996; Kim et al., 2013) whereas the PRD domain is critical for mRNP assembly into stress

granules, but it does not seem to have a role in RNA binding or translational repression (Damgaard & Lykkeandersen, 2011).

The PRD domain allows spontaneous protein self-assembly, probably leading to SG nucleation (Furukawa 2009). This mechanism seems to be somehow similar to the pathogenic amyloid formation and this is why SG proteins, like TIAR, have been proposed to be involved in neurodegenerative disease (Wolozin, 2012). For a review abroad the association with general diseases see (Buchan, 2014).

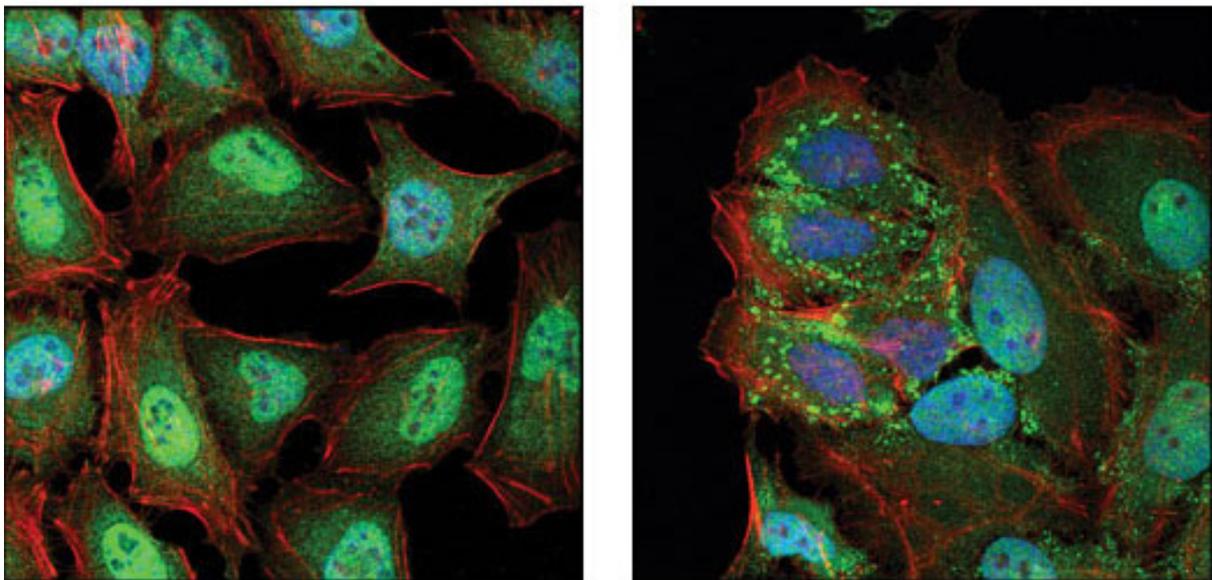
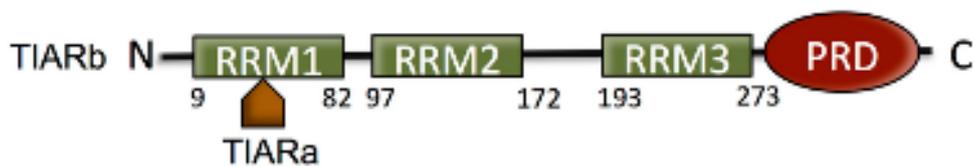


Figure 24. Schema of TIAR motifs and image of TIAR within stress granules.

a) Simplified schema of TIAR isoforms and protein domains. The two isoforms of TIAR proteins result from alternative splicing. (The isoform containing 17 amino acids within RRM1 is referred as isoform TIARa and the other TIARb). TIAR proteins contain three RNA recognition motifs (RRMs) that provide nucleotide binding specificity and a Q-rich prion-related domain (PRD), involved in stress granule (SG) assembly. Schema taken from (Waris et al., 2014). b) Confocal immunofluorescent analysis of HeLa cells, untreated (left) or UV-treated (right). Under normal condition TIAR (green) shuttles between the nucleus and the cytoplasm, while it accumulates in cytoplasmic granules after stress inducing stimulus. Image taken from cell signalling antibody datasheet. <http://media.cellsignal.com/pdf/5137.pdf>

As mentioned before, TIAR mediates translational repression of some mRNAs. This has been shown to be exerted by at least two different mechanisms. First, by the recognition of adenine/uridine-rich elements (AREs) that are present in the 3'-untranslated region of mRNAs of some inflammatory mediators. By binding to these elements, TIAR can sequester them into SGs that accumulate in the cytoplasm throughout the stress period (Mazan-mamczarz, Lal, Martindale, Kawai, & Gorospe, 2006). A second mechanism has been shown to occur by direct binding and inhibition of translation initiation of mRNAs that contain a terminal 5'-oligopyrimidine (TOP) elements found specifically in some ribosomal proteins and translation factors (Damgaard & Lykke-andersen, 2011).

IV. c) TIAR and viral infection

The involvement of TIAR in viral infection was first observed with the Sendai virus (Iseni et al., 2002), where the protein was shown to bind an A/U-rich sequence of some viral transcript intermediates and to regulate apoptosis induced by the virus. After this initial report, several viral infections have been also reported to induce the formation of SGs whereas some other viruses can disrupt their assembly. A few examples are given below but for a comprehensive review, please refer to (Onomoto, Yoneyama, Fung, Kato, & Fujita, 2014; Valiente-Echeverría, Melnychuk, & Moulard, 2012).

Among viral infections that promote SG assembly for their benefit are: HCV which takes advantage of G3BP and TIAR for successful replication and egress (Garaigorta, Heim, Boyd, Wieland, & Chisari, 2012) and *Chikungunya virus* that uses G3BP proteins for efficient replication (Scholte et al., 2015).

Other viruses have elaborated strategies to impair SG assembly. This is the case of West Nile and Dengue viruses which both recruit TIA/TIAR into replication complex, and this results in the impairment of SG assembly (Emara & Brinton, 2007). Poliovirus 3C protease cleave G3BP, to stimulate the disassembly of SG during infection (White, Cardenas, Marissen, & Lloyd, 2007).

In the case of lentiviruses, the situation is even more complex. Indeed, HIV-1 was shown to impair SG in the course of viral infection, assisted by STAU 1 (Abrahamyan et al., 2010); whereas the closely related HIV-2 utilises the TIAR protein to mediate the recruitment of its gRNA into SG (Soto-Rifo et al., 2014).

Finally, it is noteworthy that SG composition seems to be altered during viral infection. Namely, the inclusion of specific host factors or the absence of *bona fide* SG markers have been reported. These “SG-like” have been documented, for instance, through *Vesicular Stomatitis virus* replication (Dinh et al., 2013) and HIV-2 (Soto-Rifo et al., 2014).

Articles

1. DEAD-box protein DDX3 associates with eIF4F to promote translation of selected mRNAs.

Soto-Rifo R, **Rubilar PS**, Limousin T, de Breyne S, Décimo D, Ohlmann T.
EMBO J. 2012 Sep 12;31(18):3745-56.

doi: [10.1038/emboj.2012.220](https://doi.org/10.1038/emboj.2012.220)

Article summary

Translation of highly structured mRNAs has been a matter of research for the last past years. As a general rule, long and highly structured 5'untranslated regions (5'UTR) are translated less efficiently compared to shorter and less structured ones. However, the long and structured 5'UTR, from the HIV-1 genomic RNA, is translated with high efficiency in the course of *in vitro* infection which contradicts the rules mentioned above. As protein synthesis relies on several translation initiation factors, it has been widely accepted that, both canonical and non-canonical translation factors might be needed to overcome the structural barrier imposed by these complex viral RNA motifs. Previous studies on RNA helicases have already demonstrated their role for the unwinding of mRNA secondary structures, in order to facilitate translation. Here, we describe the requirement of the RNA helicase DDX3 for translation initiation of a specific group of mRNAs containing complex 5'UTRs. By using HIV-1 as a model we could demonstrate that DDX3 binds to the TAR RNA structure that is immediately at the 5' end of the genomic RNA. Once bound, DDX3 can remodel the RNA secondary structure or RNA-protein complexes to favour eukaryotic Initiation factor complex 4F (eIF4F) binding to the m⁷GTP cap to promote entry of the 43S ribosomal subunit. By using *in vitro* and *ex vivo* assays we have shown that the role of DDX3 in translation initiation of these complex mRNAs occurs before ribosomal scanning and that DDX3 is incorporated within the complex eIF4F.

2. The DEAD-box helicase DDX3 substitutes for the cap-binding protein eIF4E to promote compartmentalized translation initiation of the HIV-1 genomic RNA.

Soto-Rifo R, **Rubilar PS**, Ohlmann T.
Nucleic Acids Res. 2013 Jul;41(12):6286-99

doi: [10.1093/nar/gkt306](https://doi.org/10.1093/nar/gkt306)

Article summary

Here we describe the mechanism by which DDX3 promotes HIV-1 genomic RNA translation initiation. Using *in vitro* approaches combined with confocal microscopy, we were able to demonstrate that DDX3 is able to substitute for the eukaryotic initiation factor 4E (eIF4E) to promote the formation of an initiation complex with the poly-A binding protein (PABP) and the eukaryotic initiation factor 4G (eIF4G). Formation of this gRNA/DDX3/PABP/eIF4G complex was associated with the detection of large cytoplasmic granules reminiscent to stress granules. These granules do not contain the eIF4E cap binding protein and their assembly relies on the ATPase activity of DDX3.

3. TIA-1 related protein (TIAR) alters HIV-1 frameshift ratio and regulates viral progeny (DRAFT)

Rubilar PS, Soto-Rifo R, Mure F, Ohlmann T.

Article summary

Viruses need to exploit the host cellular machinery to translate their genomes and HIV-1 is a clear example of this concept. One of the mechanisms used by this virus to synthesize its proteins is the so-called programmed ribosomal frameshift (PRF). It takes place along translation of the viral gRNA, and it is a key mechanism to ensure the production of an accurate ratio between the structural viral proteins coded by gag and the viral enzymes produced from pol. Any alterations in this PRF ratio leads to a drastic reduction of viral progeny. We have hypothesized that some cellular factors may be involved in the control of the PRF ratio. Our experimental strategy consisted of using reporter constructions containing the frameshift signal from HIV-1 and a single round HIV-1 provirus replication system. We could demonstrate that the stress granules associated protein TIAR was involved in the regulation of HIV-1 PRF and that changes in the cellular expression of this protein had drastic consequences on HIV-1 infectious progeny.

Introduction

The Human Immunodeficiency Virus (HIV) is the etiological agent of the Acquired Immunodeficiency Disease Syndrome (AIDS). According to statistics from WHO at the end of 2013 there are about 35million people living with HIV and it remains one of the biggest health threats worldwide. Nevertheless, there is some hope with the venue of Highly Active Antiretroviral Therapy (HAART) which currently combines an association of viral enzymes inhibitors (Reverse Transcriptase (RT), Protease (PR) and Integrase (IN)) and/or an entry inhibitor targeting the cellular co-receptor CCR5 (Barré-Sinoussi et al., 2013; Maartens et al., 2014). Notwithstanding the success of HAART over the last decade, the search for new drugs targeting host cellular factors has risen as the hope to control the increasing viral resistance against drugs targeting viral proteins.

During the HIV infectious cycle, several host factors are exploited for a successful viral infection and progeny release and a lot of efforts has been made to search and characterize some of the essential factors required for viral infection (Brass et al., 2008).

Transcription, splicing and export of the proviral HIV-1 DNA inserted in the host genome, generate several mRNAs that code for 6 regulatory and auxiliary proteins: Tat, Rev, Vpu, Vpr, Nef and Vif (Freed, 2001). In addition, the genomic RNA (gRNA) is an unspliced single strand RNA of positive polarity which codes for the Gag and Gag-Pol polyproteins that are processed later by the viral protease to give rise to the capsid (CA), matrix (MA), nucleocapsid (NC), PR, RT, IN and p6 proteins (Karn & Stoltzfus, 2012).

Interestingly, synthesis of the Gag-Pol polyprotein occurs by means of an error of translation that is called Programmed Ribosomal Frameshift (PRF) and allows a regulated percentage of ribosomes to shift one nucleotide back at the end of the *gag* reading frame to keep translating along the *pol* frame. The frequency of spontaneous frameshift errors in the cell has been

estimated to less than 3×10^{-5} (Hansen et al., 2007); while in the case of HIV Gag-Pol, PRF frequency is about 1000 times more (5×10^{-2}).

The Gag-Pol to Gag ratio has been demonstrated to be critical for viral infectivity, apparently by regulating viral particle assembly and gRNA dimerization (Hung et al., 1998; Shehu-xhilaga et al., 2001). Thus, PRF has been suggested to become a possible target for HIV-1 therapy but a full understanding of the mechanisms that regulate PRF is still missing.

In this work, we have searched for cellular factors that could control HIV-1 replication at the level of translation and we have found that both overexpression and silencing of the cellular factor TIAR (TIA-1 Related Protein) could impact HIV-1 replication by specifically altering the PRF ratio.

TIAR is a key protein driving Stress Granules (SG) assembly in response to environmental condition like heat, starvation, oxidative stress or viral infection. It participates in translation arrest and protects cellular mRNAs from degradation during the stress interval (Waris et al., 2014). TIAR harbors three RNA recognition motifs (RRM) that are required for RNA binding and recruitment into stress granules and a Prion Related Domain (PRD) which is similar to the aggregation domains found in prion proteins. This PRD domain is critical for mRNP assembly into stress granules but does not seem to have a role in RNA binding or translational repression (Damgaard & Lykke-andersen, 2011).

TIAR mediated translational repression has been shown to be exerted by, at least, two different mechanisms. The first of them is by recognition of the Adenine/Uridine rich elements (ARES) that are present in the 3'-untranslated region of mRNAs coding for some inflammatory mediators (Mazan-mamczarz et al., 2006). In this case, TIAR binds to these messengers in order to sequester them into SG that will then accumulate in the cytoplasm throughout the period of stress. The second mechanism has been shown to occur by direct binding and

inhibition of translation initiation in mRNAs that contain a terminal 5'-oligopyrimidine (TOP) elements such as those coding for ribosomal proteins and translation initiation factors (Damgaard & Lykke-andersen, 2011; Waris et al., 2014).

Many viral infections induce the formation of SGs in the cytoplasm (for a review, see (Valiente-Echeverría et al., 2012)). In the case of HIV-1, the situation is somehow particular as this virus was shown to counteract the formation of SGs in the course of viral infection (Abrahamyan et al., 2010) whereas during infection of the closely related HIV-2, TIAR protein mediated the recruitment of its gRNA into SG (Soto-Rifo et al., 2014).

In the data presented herein, we have investigated the functional relationship between the TIAR protein and the replication of HIV-1. We have been able to establish for the first time a correlation between the intracellular concentration of TIAR and its effects on HIV-1 expression, notably at the level of translation. These data show that variations in the intracytoplasmic TIAR concentration misregulates the production of Gag-Pol by impacting the efficiency of PRF.

Results

Both silencing and overexpression of TIAR reduce viral progeny and alter PRF ratio

In order to investigate the effect of TIAR on HIV-1 replication, we have co-transfected in HeLa cells a plasmid coding for TIAR and the full length HIV-1 provirus (pNL4.3). After 48 hours, supernatant containing virus was collected and exogenous RT activity was measured. A 30% decrease in viral progeny was observed (Fig 1A left panel) whereas, at the same time, the level of cytoplasmic HIV-1 gRNA was slightly increased (Fig 1A right panel). This prompted us to perform a complementary experiment that consisted in depleting TIAR from HeLa cells. For this, we have used a previously described (Mazan-mamczarz et al., 2006; Soto-Rifo et al., 2014) siRNA duplex against TIAR that was transfected 48h before addition of the HIV-1 provirus. Silencing of TIAR was found to be total as controlled by western blot at the end of the experiment (96h post si-RNA transfection) (Data not shown). Under these experimental conditions, the measure of the RT activity revealed an 80% reduction in viral progeny (Fig. 1B left panel) without significant changes in the cytoplasmic gRNA (Fig 1B, right panel).

This effect of TIAR on viral replication prompted us to search for a possible role of this protein in the control of Programmed Ribosomal Frameshift (PRF) that occurs between *gag* and *pol* ORFs in the HIV-1 gRNA. In order to monitor this, we have used a frameshift reporter construct (validated in (Jacobs & Dinman, 2004)) that expresses the *Renilla luciferase* gene and the *Firefly luciferase* under the control of the HIV-1 frameshift signal (*Firefly luciferase* in a -1 frame compared to *Renilla* frame) (See Supplementary Figure 1A). The Firefly/*Renilla* ratio is normalised with a control construction containing a single extra nucleotide that places *Firefly* in frame compared to *Renilla luciferase* and allows translation of the fusion protein (See supplementary Figure 1B). The PRF efficiency is calculated as described in Supplementary Figure 1C and was measured under normal conditions to be around 5% in HeLa

cells. In order to have a comparison with drugs that are known to target the efficiency of PRF, we have first tested the effect of *Anisomycin* which is a peptidyl transferase inhibitor known to increase PRF efficiency (Jacobs & Dinman, 2004). Using our reporter construct, the addition of *Anisomycin* resulted in a moderate increase of the -1 PRF (Fig. 1C, left panel). However, this relative small change in the PRF ratio was correlated with a very strong reduction of viral progeny as quantified by the level of exogenous RT activity when *Anisomycin* was used after transfection of HIV-1 provirus (Fig 1C, right panel).

Therefore, we next investigated the effects of the overexpression (Figure 1 D, left panel) and the knock down (Figure 1 D, right panel) of the endogenous TIAR protein on the efficiency of PRF by using the constructs described above. We found that changes in the ratio were moderate similarly to what was obtained with *Anisomycin*. However, when we looked at the consequences of these changes at the level of viral replication, we were surprised to find that the silencing of TIAR resulted in quite a dramatic decrease of viral infectivity as measured, this time, by the amount of infectious viral particles detected in HeLa-p4 cells (Figure 1F). As a control, different concentrations of *Anisomycin* were added and found to have a similar impact on viral replication (Figure 1E).

Rescue of viral progeny requires a tight regulation of TIAR expression

In order to verify whether the effects observed upon transfection of the siRNAs against TIAR were specifically due to the depletion of the endogenous protein and not caused by any side effects, we have constructed a si-resistant form of TIAR (siR-TIAR) protein with three silent point mutations in the region of siRNA alignment (see Supplementary Figure 1 D for details). Please note that, in order to avoid to disrupt cell metabolism, only partial silencing of TIAR was targeted (Figure 2D) by transfecting the HIV-1 provirus only 24 hours after siRNA duplex addition. Equal concentration of provirus was transfected in all the assays together with

different concentrations of siR-TIAR. 48h post-transfection, supernatant containing virus was collected and transferred to HeLa-P4 cells in order to quantify infectious viruses (Fig. 2A and see Materials and Methods). Under these experimental conditions we found that the lowest amount of siR-TIAR was sufficient to restore infectivity to control levels while the highest amount of the resistant protein was not as efficient (Fig 2A). The reasons for that remained undetermined. The quantification by qRT-PCR of the concentration of HIV-1 genomic RNA found either in supernatants or in the cytoplasmic fraction, did not reveal any significant changes that could account for these effects on infectious progeny (Fig 2B and 2C). It is noteworthy that exogenous RT activity was also quantified and correlated with data presented in Figure 2A (data not shown).

Over-expression of TIAR RRM or PRD domains does not recapitulate the effects of the full length protein.

The TIAR protein is a 375 amino acids long protein that contains three RNA Recognition Motifs (RRMs) in its amino terminal part and a Prion Related Domain (PRD) in its carboxyl-terminal end (see cartoon on Fig. 3A). To investigate which of these domains is involved in the modulation of frameshifting we have individually expressed each of them in HeLa cells together with the frameshift reporter construct (Fig. 3B). As shown previously, the full length protein was found to enhance the frameshift ratio (see TIAR), this was not the case for either RRM or PRD when expressed individually. Essentially similar results were obtained on provirus replication with no significant variation on viral progeny quantification upon overexpression of each domain of TIAR (Fig 3C). Therefore, we next looked at the effects of TIAR and each of these domains on the translational activity of the HIV-1 genomic RNA. For this, we have used a modified provirus clone in which a *Renilla luciferase* gene was inserted in frame (see cartoon) with the *gag* gene as described previously (Soto-Rifo, Limousin, et al., 2012). Upon transfection of this plasmid into cells, the Renilla activity is measured together

with the quantification of the cytoplasmic concentration of the genomic HIV-1 RNA. As shown on Figure 3D, the overexpression of the full length TIAR protein, or any of its domains, did not affect significantly the translation of the HIV-1 genomic RNA suggesting that the effect of TIAR is restricted at the level of frameshifting

TIAR regulates PRF ratio in vitro

We next wanted to investigate the effects of TIAR on PRF by using an *in vitro* approach. For this, we have used an *in vitro* transcribed frameshift reporter construct in which the RNA signal that controls the HIV-1 or SARS frameshifting was inserted in the intercistronic spacer (see Figure 4A). This plasmid was transcribed *in vitro* with a cap at its 5' extremity and was translated in the Rabbit Reticulocyte Lysate (RRL) system. We have first tested the effect of adding Anisomycin on both the HIV-1- and the SARS-containing constructs and we found that translation of the Renilla was significantly impaired for both in a dose dependent manner (left panels from Fig 4B and 4C). Interestingly, when we looked at the effect of Anisomycin, we found that the PRF ratio was drastically increased in the case of the HIV-1 containing RNA whereas the effect was only moderate, and not statistically significant, for the SARS containing RNA (compare right panels from Fig 4B and 4C). We then went on to test the effect of TIAR on *in vitro* PRF. Unfortunately, we have been unable to obtain the purified recombinant protein. Therefore, we have used another strategy that consisted of adding HeLa cytoplasmic cell extracts in which a plasmid coding for TIAR or GFP as control was overexpressed before (see Materials and Methods). These extracts were then used to complement the RRL in which the HIV-1 and SARS-containing RNAs were translated. For both of them, this showed that TIAR decreased the translation of the first capped gene with an effect more pronounced on the HIV-1 construct (Figure 4D left panel). Interestingly, we also observed an increase in the efficiency of PRF of about 25% for the same construct (Figure 4 D right panel) whereas the efficiency

of PRF for the SARS construct did not change significantly under the same experimental conditions (Fig 4 E right panel) confirming a specific effect of TIAR on the HIV-1 PRF.

Discussion

In this work, we have shown that any change in the intracellular concentration of TIAR by either overexpression or silencing, was able to reduce HIV-1 progeny in a provirus single round replication model. This effect could be explained by a putative role of TIAR in the regulation of HIV-1 programmed frameshift in the genomic RNA. As it is widely accepted that both an increase and a decrease in the PRF ratio can seriously disrupt viral progeny (Biswas et al., 2004; Brierley & Dos Ramos, 2006; Shehu-xhilaga et al., 2001), we searched for any changes in the PRF ratio controlled by HIV-1 signal, under TIAR silencing or overexpression. This resulted in moderate variations of the PRF ratio controlled by the HIV-1 frameshift signal but to a comparable magnitude of those induced by the addition of the peptidyl transferase inhibitor Anisomycin. Interesting, by using infection of HeLa-p4 cells we could demonstrate that these relatively modest changes in PRF ratio resulted in a drastic decrease in the synthesis of newly formed infectious viruses. Although the correlation between the magnitude of changes in the PRF ratio and its consequences on infectious progeny are still a matter of debate (Biswas et al., 2004; Dulude et al., 2006; Hung et al., 1998), our data confirm that moderate changes in PRF can cause a dramatic impact on viral infectivity. Interestingly, the modulation that we observe in the PRF values are quite similar to those already published; likewise, similar differences between *in vitro* and *ex-vivo* systems have been already documented (Jacobs & Dinman, 2004). The most dramatic change in HIV-1 infectivity was obtained with the silencing of TIAR by RNA interference and this could not be attributed to an off-target effect as we were able to restore infectivity by transfecting a si-RNA resistant version of TIAR. However, this rescue

was at its best with the lowest amount of the protein, confirming that its intracellular concentration must be finely tuned to control the PRF ratio and viral replication.

Given the fact that TIAR is an RNA-binding protein, we initially expected the RRM motifs that are contained in its N-terminal part, to be both necessary and sufficient for its function on HIV-1. These RNA recognition motifs (RRM) were previously described to be necessary for both RNA attachment and the recruitment in the stress granules of the ribonucleoprotein complex (RNP) composed of TIAR and the target mRNA. However, this was not the case as the RRM domain alone did not exert any significant effect on HIV-1 replication. Nevertheless, we have not further investigated the possibility that this minimal fragment could still bind the RNA. The C-terminal part of TIAR exhibits an aggregation domain (PRD), which is also critical for mRNP assembly into stress granules but does not seem to have any role in RNA binding or translational repression (Damgaard & Lykke-andersen, 2011). Once again, expression of the PRD domain alone failed to recapitulate the effect of the full length protein on viral replication. Moreover, expression of any of these domains did not affect significantly translation of the HIV-1 genomic RNA as determined by using the Renilla-provirus. Interestingly, it has recently been shown that stress granules formation does not occur during HIV-1 infection as expression of the genomic RNA has the ability to prevent granule formation, even in the presence of Arsenite which is a potent chemical inducer of these cytoplasmic foci (Abrahamyan et al., 2010). This constitutes a major difference with the closely related HIV-2 virus which activates and is incorporated in stress granule in the absence of active viral translation (Soto-Rifo et al., 2014). Interestingly, recruitment of the HIV-2 genomic RNA into SGs seems to require the TIAR protein (Soto-Rifo et al., 2014). When the two human retroviruses are co-expressed, replication of the HIV-1 retains the ability to block granule formation even in the presence of the HIV-2 genomic RNA (Soto-Rifo et al., 2014). Therefore, this may suggest that the need for TIAR protein for HIV-1 replication is not dependent on the

formation of SGs in the cytoplasm of the host cell. However, this needs to be investigated by cellular imaging. Finally, in order to confirm a direct role of TIAR in the general mechanism of PRF, we have used reporter RNAs controlled by HIV-1 or SARS-coV frameshifting signals that were translated in the Rabbit Reticulocytes Lysate (RRL) system, supplemented with cell extracts from HeLa overexpressing TIAR (or GFP as control). Addition of these TIAR containing cell extracts increased PRF controlled by HIV-1 whereas they have the opposite effect on the SARS-coV controlled reporter mRNA, suggesting a specific effect for TIAR on HIV-1. It is noteworthy that changes in the PRF ratio for both reporters were associated to translation repression as measured by the decrease of the Renilla activity for both constructs.

It has been postulated that the efficiency PRF could be inversely correlated with the overall efficiency of protein synthesis, e.g. a high translation rate is associated to a poor ratio of PRF (Gendron et al., 2008). In our hands, this does not seem to be always the case. Indeed, when using *in vitro* reporter translation with Anisomycin, we obtained a similar decrease of translation for both HIV-1 and SARS-coV controlled reporters but the ratio of PRF increased strongly for the HIV-1 containing construct but not for the SARS one. Similar when using TIAR overexpressing extracts, translation repression was rather associated to a decrease of SARS-coV controlled PRF ratio. It could be hypothesized that different mechanisms of slippage already described between SARS-coV and HIV-1 (Brierley & Dos Ramos, 2006) could influence the effect of TIAR on PRF; this awaits further investigation. Only a few studies have demonstrated a role of trans-acting factors in PRF. For instance, mutations in the gene coding for Elongation Factor 1 alpha (EF1 α) in *S. cerevisiae* were related to changes in PRF of the L-A virus and a decrease in virus copy number of M1 satellite yeast virus (J D Dinman & Kinzy, 1997). Infectious Bronchitis Virus PRF was shown to be controlled by the cellular factor Annexin A2 but no functional effect on the virus has ever been reported (Kwak et al., 2011). In the specific case of HIV-1, depletion of eukaryotic Release Factor 1 (eRF1) was

associated with an increase in the HIV-1 PRF ratio together with the production of defective viral particles (Kobayashi et al., 2010), whereas Large Ribosomal Protein 4 (RPL4) overexpression increased HIV-1 PRF (Green, Houck-loomis, Yueh, & Goff, 2012). However, none of the above mentioned cases have shown a functional effect on viral progeny under both increase and decrease of expression, as we show herein with the TIAR protein. In conclusion, we believe that the intracellular concentration of TIAR may be a potent sensor to control the activity of the HIV-1 PRF and can have dramatic effects on the production of viral progeny. Such a novel role for this protein will merit further investigations in the future as it may be used as a therapeutic target.

Materials and Methods

Cell culture

HeLa cells were grown using DMEM (GIBCO, Invitrogen) supplemented with 10% Fetal Bovine Serum and 100U/100ug Penicillin-Streptomycin. HeLa-p4 cells were cultured similarly.

DNA transfection

HIV-1 molecular clone NL4-3, TIAR-GFP or TIAR-HA vectors are described previously (Soto-Rifo et al., 2014). p2-Luc vectors were kindly donated by Dr. I. Brierley and siR-TIAR was made from TIAR-HA vector by adding 3 punctual mutations (see supplementary Fig D).

All DNA transfection were achieved using JetPEI® (PolyPlus Transfection) according manufacturing instructions. Cells were recovered at 48h post transfection and used for Renilla/Firefly activity assessment, RNA extraction and RT-qPCR, viral progeny recovery, cell extracts or western blot as indicated.

siRNA transfection

siTIAR duplex has been previously described (Mazan-mamczarz et al., 2006). TIAR silencing was accomplished either at 24h or 48h post transfection using INTERFERin® (Polyplus Transfection).

PRF assessment

Cells transfected with p2-luc vectors were washed twice with PBS and lysated using Renilla Lysis buffer (Promega Ma USA). Translation activity assessments from each enzyme were accomplished separately using 20ul of lysate and 50uL of Renilla Luciferase or Firefly luciferase substrates (Promega Ma USA). All quantification were made using Mithras LB 940 Multimode Microplate Reader (Berthold Technologies). PRF ratio equation is detailed in Supplementary Fig C.

RNA isolation and RT-qPCR

Cytoplasmic RNA extraction and RT-qPCR were accomplished as previously described (Soto-Rifo et al., 2014). Except for virus from cell supernants that were isolated using NucleoSpin® RNA Virus Isolation Kit (MACHEREY-NAGEL) adding a synthetic Renilla luciferase mRNA into the lysis buffer for use as qPCR internal control.

Viral quantification

In order to calculate functional HIV-1 progeny, cell supernants were ultracentrifuged 2hrs at 75000 rpm in a Beckman TL-100. Viral pellets were resuspended in 100µL of PBS.

To assess RT activity, 10 µL aliquots were mixed with 15µL of RT activity buffer (60 mM Tris-HCl (pH 8.0), 180 mM KCl, 6 mM MgCl₂, 6 mM dithiothreitol, 0.6 mM EGTA, 0.12% Triton X-100) for virus inactivation and then mixed with 25µL of "HOT" RT activity buffer (RT buffer supplemented with 10 µCi/mL of dTTP α³²P, oligo dT and polyrA) and incubated 1 hr. at 37°C. 10µL were spotted in duplicate in a Whatman paper and quantified using phosphoimager.

At least 3 different dilutions from each viral sample were incubated 3hrs. into semi-confluent HeLa-p4 cell and then washed and fresh culture medium added to stop infection after 72hrs.

Cells were washed, fixed and then incubated in X-gal substrate 1hr at 37°C. Blue spots were counted under light microscope.

Western Blot

Rabbit anti-TIAR #5137 (Cell signalling Technology®) and mouse anti-p24 (NIH) were used as primary antibodies. According secondary antibodies were detected using SuperSignal West Pico Chemiluminiscent substrate (Thermo Scientific-Pierce IL USA).

HeLa lysate preparation

Cell pellets were resuspended with 1 volume of hypotonic buffer (10mM Hepes-KOH pH=7,6; 10mM KOAc; 0,5mM MgOAc; 1mM DTT and protease inhibitor cocktail) on ice and passed through 23G needle 20 times for efficient lysis. Lysated cells were centrifuged 10min at 14.000 xg at 4°C. Recovered supernants were treated with 0,15U/ul of nuclease (Roche, Germany) and CaCl₂ 1mM final by 15min at room temperature. Nuclease was stopped with 10mM final of EGTA. Samples were then centrifuged 5min at 14.000 xg at 4°C and supernants (or extracts) recovered for protein quantification by Bradford assay alicuoted and saved at -80°C

In vitro transcription and translation experiments

Transcription of linearized p2Luc vectors was achieved as described previously (Soto-Rifo, Rubilar, et al., 2012) using T7 RNA polymerase and Cap-analog.

Capped, non-polyadenilated RNAs were translated 30min at 30°C in a final volume of 10uL, using 75mM KCl; 0,5mM MgCl₂; aminoacids 20mM each, 0,5ul of RNase out and 50% of Rabbit lysate (Promega). When complementing with HeLa extracts, every RNA (100ng) was pre-incubated 5min at 37°C with 15ug of HeLa extract before adding the rest of reagents mix.

PRF was assessed as above after direct addition 50ul of Renilla Lysis buffer.

Statistics

One way analysis of variance or repeated measures ANOVA followed by Dunnet's multicomparison tests, or paired T-test (1 or 2 tailed) were accomplished using Prism GraphPad software. * corresponds to a P-value <0.05, ** corresponds to a P-value <0.01 and *** to a P-value of <0.001.

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Figure legends

Fig. 1 *Changes in TIAR expression leads to a decrease in viral replication and a change in the PRF ratio*

(A) pNL4.3 and TIAR-EGFP vectors were co-transfected into HeLa cells. After 48h, exogenous RT activity was assessed from cells supernants (left panel) viral genomic RNA was quantified in the cytoplasm (A right panel). Results are presented as mean +/- SD of 3 independent experiments (one-tailed paired *t*-test).

(B) HeLa cells were treated with the siTIAR duplex 48 h before transfection of the pNL4.3 provirus. Supernants were collected after 48h for RT activity (left panel). Results are presented as mean +/- SD of 3 independent experiments (one-tailed paired *t*-test).

(C) HeLa cells were transfected with p2lucHIV-1 (left panel) or pNL4.3 vector (right panel) and Anisomycin was added to the culture medium at the different concentrations indicated on the figure. 48h after, cells were lysed for PRF ratio quantification (left panel) or supernants collected for assessment of viral exogenous RT activity (right panel). Results are presented as mean +/- SD of 3 independent experiments (Repeated measures ANOVA and Dunnett's multicomparison test).

(D) HeLa cells were treated for 48h with a siTIAR duplex. Then, the p2Luc-HIV-1 vector was transfected and PRF ratio was calculated 48h post-transfection. Results are presented as mean +/- SD of 2 independent experiments.

(E) Virus containing supernants isolated from HeLa cells treated with different concentrations of Anisomycin were used to infect HeLa-p4 reporter target cells to determine the infectivity of the viral progeny. Results are presented as mean +/- SD of 3 independent experiments (ANOVA one way analysis of variance).

(F) Virus containing supernants isolated from HeLa cells treated with a siTIAR duplex were used to infect HeLa-p4 reporter target cells to determine the infectivity of the viral progeny. Results are presented as mean +/- SD of 3 independent experiments (Paired T-test).

Fig. 2 *si-Resistant TIAR Rescues viral replication in a dose dependent manner*

HeLa cells were treated for 24h with a siTIAR duplex before transfection of the pNL4.3 provirus. Then, different ratios of a si-resistant HA-TIAR mixed with a GFP control plasmid were then transfected for 48 hours as indicated at the bottom of each panel. (A) Supernatant containing virus was collected to determine the infectivity of the viral progeny or (B) to determine the concentration of genomic RNA in the extracellular supernatant. (C) Intracytoplasmic HIV-1 gRNA concentration was also determined. (D) Western blotting performed on HeLa cell extracts treated as described above and revealed with antibodies recognizing the HA-TIAR resistant protein, the TIAR endogenous protein and the viral protein p24. Results are presented as mean \pm SD of 4 independent experiments (Repeated measures ANOVA or paired T-test).

Fig. 3 *Full length TIAR controls viral replication*

(A) Schematic cartoon of the TIAR protein and its RRM and PRD domains. (B) Full length TIAR or individual domains (RRM/PRD) were co-transfected in HeLa cells together with the p2LucHIV-1 vector as indicated at the bottom of the figure. 48 hours post transfection, the PRF ratio was evaluated as described in supplementary Fig C. (C) Full length TIAR or individual domains (RRM/PRD) were co-transfected in HeLa cells together with the pNL4.3 provirus as indicated at the bottom of the figure. 48 hours post transfection, the exogenous RT activity was evaluated and cytoplasmic gRNA quantified by qRT-PCR. (D) Full length TIAR or individual domains (RRM/PRD) were co-transfected in HeLa cells together with the Renilla-pNL4.3 vector as indicated at the bottom of the figure. 48 hours post transfection, the Renilla activity was assessed and Renilla mRNA quantified by qRT-PCR

Results are presented as mean \pm SD of 4 independent experiments (One way analysis of Variance or Repeated measures ANOVA and Dunnett's multicomparison test).

Fig. 4 *TIAR controls the HIV-1 PRF in vitro*

(A) Schematic representation of the construct used in this study. A cap structure was inserted at the 5' end of the reporter during in vitro transcription. The intercistronic spacer contains either the HIV-1 or the SARS-coV frameshift RNA signal. (B) Different concentrations of Anisomycin (as indicated at the bottom of each panel) were added to the RRL translational mixture with the p2LucHIV-1 mRNA at 30°C for 30 minutes. Renilla activity (first gene) was measured to determine translation activity (left panel) whereas the ratio of PRF was calculated as described in supplementary Fig. C. (C) Different concentrations of Anisomycin (as indicated) were added to the RRL translational mixture with the p2LucSARS mRNA, Translation and PRF ratio were calculated as in B. (D) and (E) A blend of protein extracts from HeLa cells (15ug total) was prepared by mixing extracts from HeLa cells that were previously transfected either with the TIAR expression plasmid or a GFP control as indicated at the bottom of the panel. These extracts were then utilised to complement the RRL system programmed with either (D) p2LucHIV-1 mRNA or (E) the p2LucSARS-CoV mRNA. Translation was then accomplished for 30 minutes at 30°C and translation and PRF ratio were calculated as above.

Results are presented as mean \pm SD of at least 3 independent experiments (Repeated measures ANOVA and Dunnett's multicomparison test).

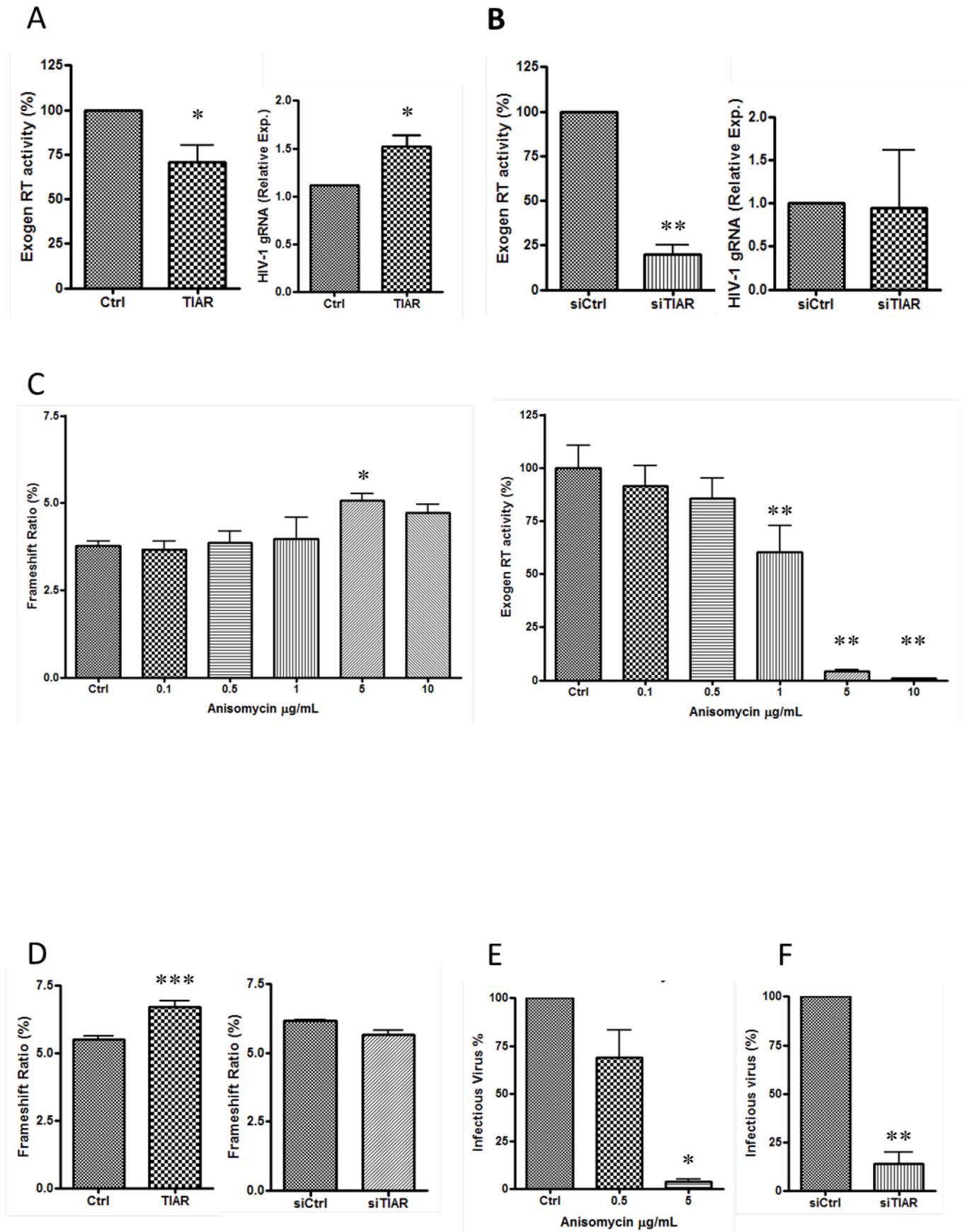


Fig. 1

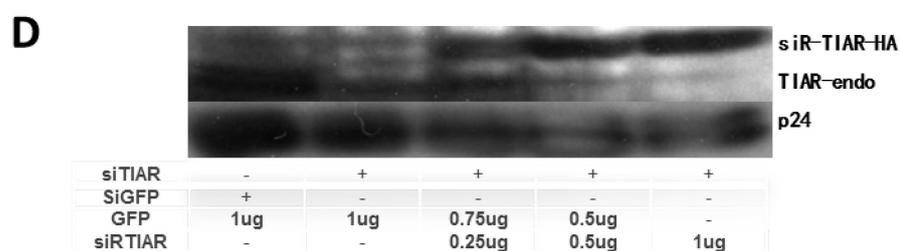
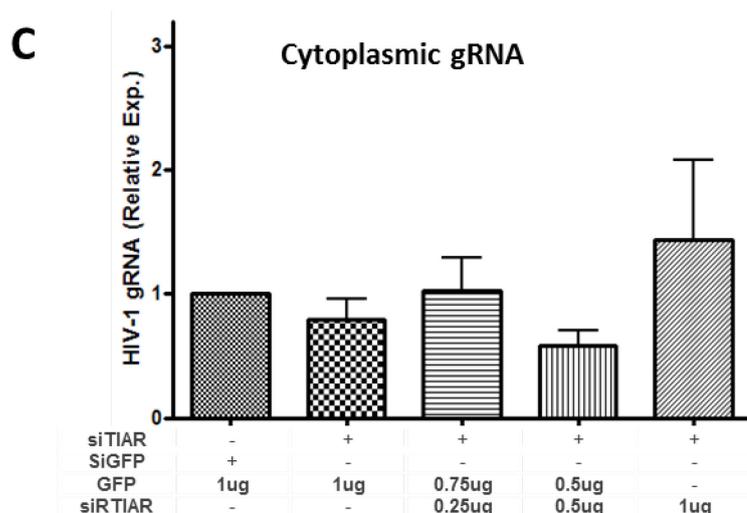
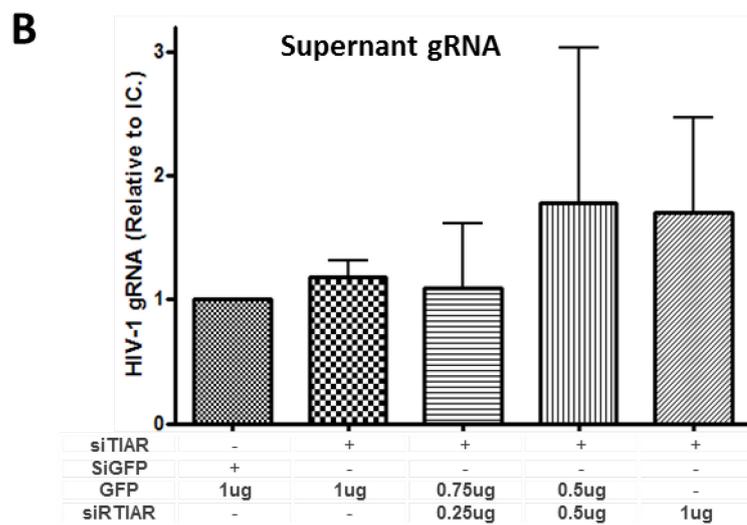
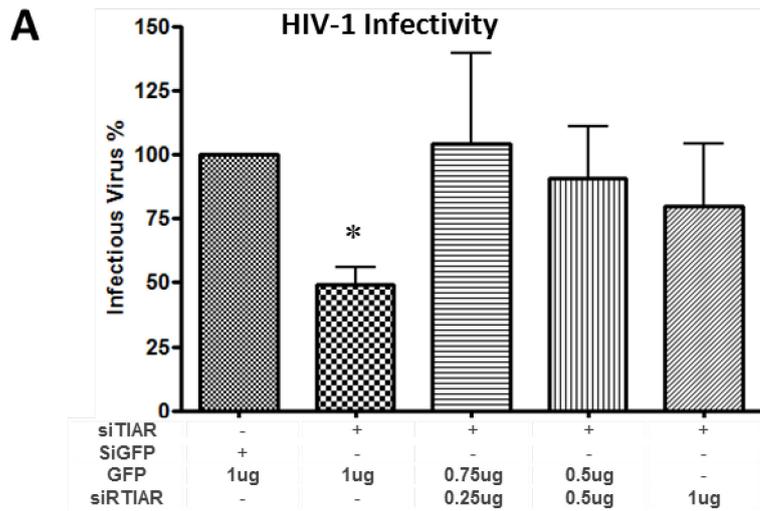


Fig. 2

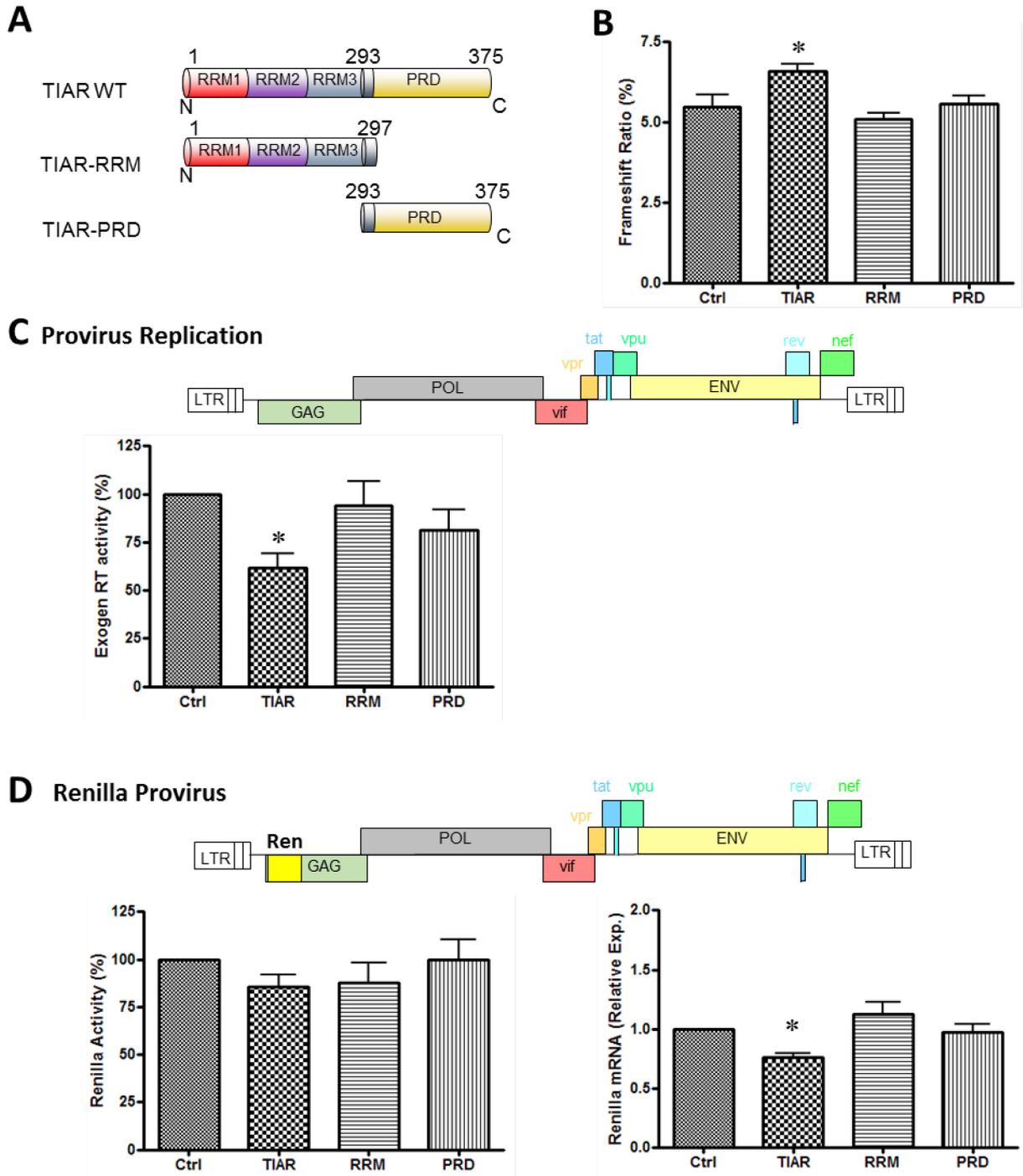


Fig. 3

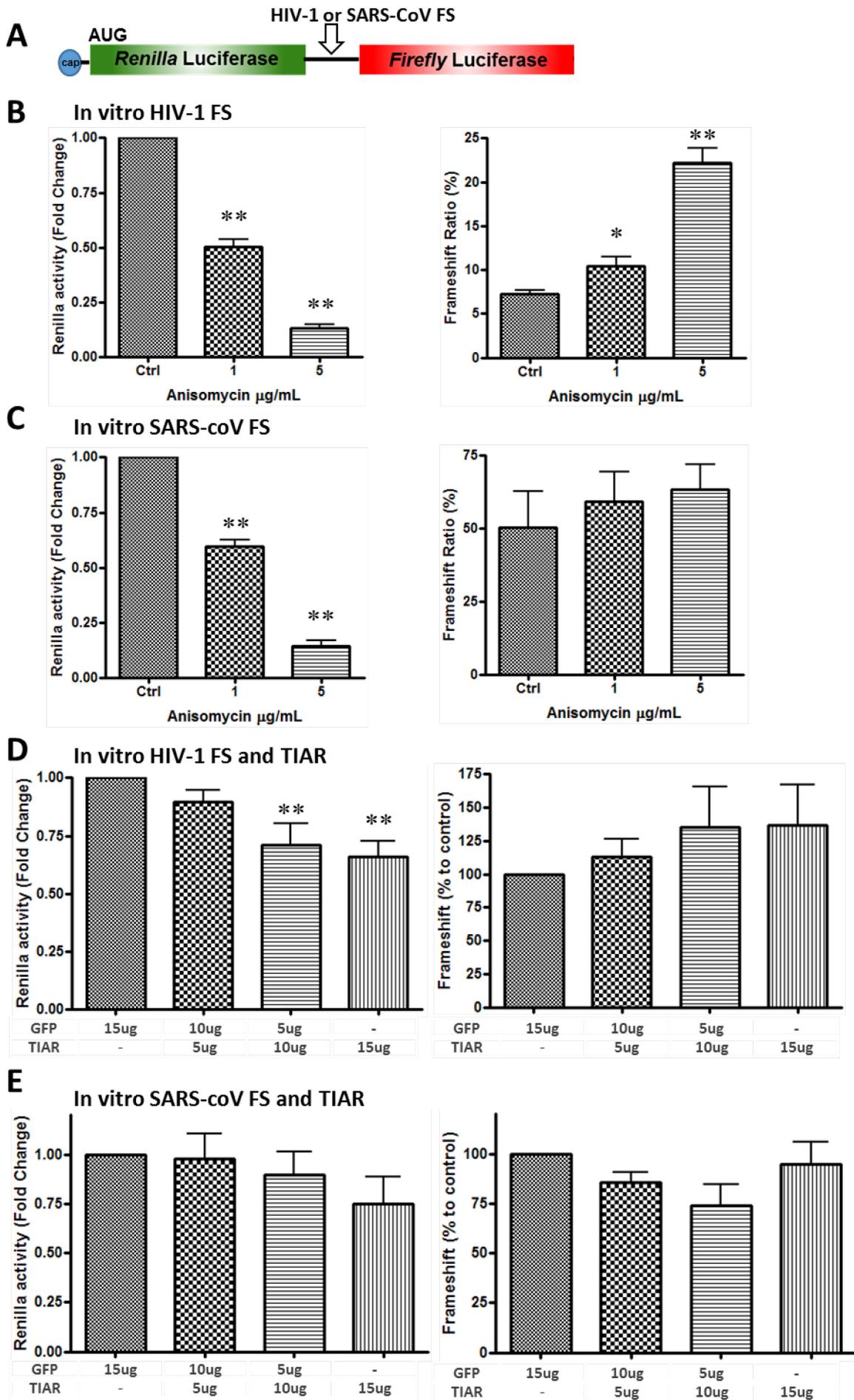
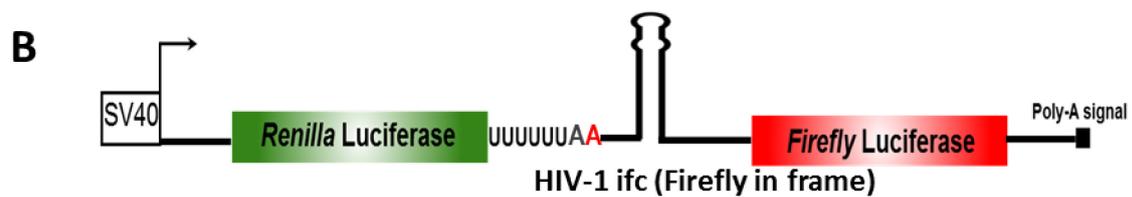
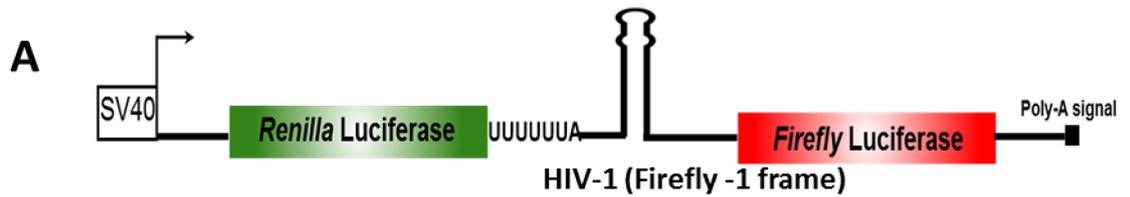


Fig. 4

Supplementary Figures



C

$$PRF = \frac{(firefly_{-1frame}/renilla_{-1frame})}{(firefly_{ifc}/renilla_{ifc})} * 100$$

D

Amino-acid	K - G - Y - S - P - V - A
siTIAR	AAG GGC TAT TCA TTT GTC AGA
Sequence siR-TIAR	---AAG GGC TAC TCT TTC GTC AGA-----

Conclusions and Perspectives

Conclusions and Perspectives

It is commonly accepted that RNA viruses have often evolved ways to compact their genomes which often results in the use of shared RNA regions for different purposes. In the case of HIV- the genomic RNA serves both as a messenger for viral production and as a genome for generation of new infectious virions. In the lab we are focused on the multiple mechanisms that regulate translation of the full length HIV-1 genomic RNA. First of all, the RNA structure of the messenger itself, has been shown to be critical for replication. For instance, the presence of IRES structure both within the 5'UTR and the coding region of HIV-1, were demonstrated to be necessary to drive production of the viral proteins when cellular translation is slowed down in the course of infection. In addition, HIV-1 also uses the leaky scanning mechanism to regulate the production of both the Env and Vpu proteins, by means of alternative initiation codon usage from the same subgenomic RNA.

However, viral protein synthesis during HIV-1 replication is not solely supported by the architecture of the mRNAs but also relies on the presence of multiple cellular proteins that assist in the binding of the ribosome and its progression all along the messenger. Amongst these proteins, one finds the canonical translation factors but also the host cellular proteins that are required.

During my thesis I have investigated the role of some of these cellular factors that could control HIV-1 replication at the translational level and I have tried to solve the possible molecular mechanism involved in this regulation. In this context, we have identified the DDX3 RNA helicase as an essential factor to promote efficient translation initiation of the HIV-1 gRNA. DDX3 is not the first RNA helicase described to play a role in HIV-1 translation, as RHA was identified previously (Tettweiler & Lasko, 2006). Nevertheless the role of DDX3 is unique and cannot be replaced by any other RNA helicases such as eIF4A or Ded1. Moreover, DDX3 acts upstream of RHA, in the very early steps of translation initiation even before ribosome binding has taken place. Interestingly, DDX3 was also shown to play a role in several other virus life cycles (Ariumi, 2014) and appears to be a possible wide spectrum antiviral molecule. Nevertheless, our work has opened novel questions concerning, for instance, a possible association of this helicase with other cellular or viral proteins that remain to be identified. Therefore, it would be noteworthy to use high

throughput proteomic approaches to analyse all DDX3 partners under physiological conditions as well as during the viral replication cycle. Another interesting prospect concerns the involvement of DDX3 in the mechanism used by HIV-1 to initiate translation. Indeed, ribosome entry can take place either at the 5' end of the gRNA or directly onto the IRES sequences in the 5'UTR or the coding region. So far, we have only investigated the role of DDX3 on the cap-dependent mechanism but its remodelling activity could well also be involved in IRES-driven ribosome entry. A differential evaluation of DDX3 function under cap- or IRES-driven translation initiation mechanism of HIV-1 would be a very informative, but remains technically challenging. Finally, on a broader prospect, functional virus quantification after DDX3 silencing and rescue would evaluate the significance of this protein during the whole viral replication cycle, serving also as a reliable method for the testing of helicase inhibitor molecules as antiviral drugs.

During the last few months of my thesis, I was also interested in an unusual phenomenon occurring along translation elongation of the HIV-1 gRNA, which implies a very controlled change of the open reading frame. A change of reading frame by the ribosome is normally considered an error leading to premature termination or the production of an aberrant protein. However, in the context of the HIV-1 genomic RNA, it becomes an interesting strategy used to control the ratio of expression between structural and enzymatic proteins. Despite the fact that many other viruses use the same mechanism, the phenomenon occurs in ratios that seem to be unique for each of them and has probably evolved to ensure the best replication yield. As frameshift can occur efficiently in *in vitro* systems, it certainly does not need any viral proteins to be active. Nonetheless, the ratio of PRF between *in vitro* and *ex-vivo* situations can vary greatly (as well as it does between different cell types) indicating that some proteins may modulate this activity. This is the reason why, we have done a small screening to search for cellular factors involved in PRF. We found TIAR, a protein which is found in cytoplasmic stress granules, as a first candidate and we could demonstrate a tight relationship between TIAR expression and functional viral progeny, and, above all between TIAR expression concentration and the frameshift ratio. These changes in the frameshift ratio were very discrete compared to other viral models. Nevertheless, these small effects on PRF had huge impact on viral progeny. This was particularly obvious with the use of Anisomycin as positive control of frameshift. For instance, a high dose of Anisomycin increased by 25% the HIV-1 PRF ratio, and was correlated with about a 90%

reduction in HIV-1 progeny; whereas a lower dose of Anisomycin that increased only by 5% the HIV-1 PRF ratio (not significant), was yet related to a significant 40% reduction of HIV-1 progeny. Similarly, induced changes in TIAR expression reduced viral progeny by 40 to 85% correlated with PRF ratios ranging from not significant to a 25% increase. This data supports the idea that the biological relevance of PRF changes can only be measured by functional virus replication assessment.

Our work also raises questions about the control of translational fidelity. For instance, are the cellular co-factors associated to the elongating ribosome or do they only play a role at the level of mRNA binding? And do ribosomes change their composition, in terms of associated proteins, in order to accomplish programmed frameshift? Along the same line, we would very much like to know whether TIAR could bind directly into the HIV-1 frameshift signal and at which level does TIAR interact with the pausing ribosome? In addition, comparative experiments with SARS-CoV (another virus using PRF) or MuLV (a virus using translational read-through), would be instrumental to determine whether TIAR would be an exclusive HIV-1/frameshift factor or could be a general translation fidelity related protein used by other viruses. Furthermore, considering that HIV-1 replication impairs stress granules formation, it would be interesting to determine the cellular localization of the viral RNA when TIAR is knockdown or overexpressed. Finally, in order to investigate a possible exploitation of this data for therapeutic purposes, it would be necessary to assess any eventual deleterious effect of changing the intracellular concentration of TIAR especially since the latter has been suggested to play a role in neurodegenerative diseases and to be needed in other viral infections (Onomoto et al., 2014).

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Articles Annexes

1. Different effects of the TAR structure on HIV-1 and HIV-2 genomic RNA translation

Soto-Rifo R, Limousin T, **Rubilar PS**, Ricci EP, Décimo D, Moncorgé O, Trabaud MA, André P, Cimarelli A, Ohlmann T.

Nucleic Acids Res. 2012 Mar;40(6):2653-67.

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2. HIV-2 genomic RNA accumulates in stress granules in the absence of active translation

Soto-Rifo R, Valiente-Echeverría F, Rubilar PS, Garcia-de-Gracia F, Ricci E, Limusin T, Decimo D, Mouland A, Ohlmann T. *Nucleic Acids Res.* 2014 Nov 10;42(20):12861-75

doi: [10.1093/nar/gku1017](https://doi.org/10.1093/nar/gku1017)

3. miRNA repression of translation in vitro takes place during 43S ribosomal scanning.

Ricci EP, Limousin T, Soto-Rifo R, Rubilar PS, Decimo D, Ohlmann T.

Nucleic Acids Res. 2013 Jan 7;41(1):586-98

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Abbreviations

5'UTR: 5' Untranslated region

ABCE1: ATP binding cassette protein E1

AIDS: Acquired Immunodeficiency Syndrome

ANXA2: Annexin A

APOBEC3: apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like-3

AZT: Azidothymidine

CA: capsid protein

CARD: Caspase activation and recruitment domains

CCR5: C-C chemokine receptor-5

CDC: Centers for disease control and prevention

CPSF6: cleavage polyadenylation specificity factor 65

CXCR4: CXC chemokine receptor-5

CypA: cyclophilin A

dNTPs: Deoxynucleotid triphosphate

dsDNA: double-strand DNA

eEFs: eukaryotic elongation factors

eIF4E: eukaryotic Initiation factor 4E

eIF4F: eukaryotic Initiation factor 4 F

eIF4G: eukaryotic Initiation factor 4G

eIFs: eukaryotic initiation factor

ESCRT-I: endosomal sorting complex required for transport-I

FMDV: Foot and mouth disease virus

G3BP: Ras-GAP SH3 domain-binding protein

gRNA: genomic viral RNA

HAART: Highly activity antiretroviral therapy

HBV: Hepatitis B Virus

HCMV: Human cytomegalovirus

HCV: Hepatitis C virus

HIV-1: human immunodeficiency virus type 1

HIV-2: human immunodeficiency virus type 2

hnRNP: heterogeneous ribonuclear protein family

IBV: infectious bronchitis virus

IFITM: Interferon-induced transmembrane protein

IFN: interferon

IN: Integrase

IRESs: Internal ribosome binding site

ITAFs: IRES trans-acting factors

LEDGF/p75: lens epithelium-derived growth factor

LTRs: long terminal repeats

MA: matrix protein

MDA-5: melanoma differentiation-associated gene 5

miRNA: micro RNA

mRNA: Messenger RNA

NC: nucleocapsid

NF- κ B: nuclear factor k-light-chain-enhancer of activated B cells

NNRTIs: non-nucleoside reverse transcriptase inhibitors

NPH II: nucleoside triphosphate (NTP)-phospho hydrolase II

NRTIs: nucleoside reverse transcriptase inhibitors

PABP: poly-A binding protein

PB: processing bodies

PBS: Primer binding site

PIC: pre-initiation complex

post-TC: post termination complex

PR: protease protein

PRD: Prion related domain

PRF: Programmed ribosomal Frameshift

PRRs: pattern recognition receptors

PV: poliovirus

qRT-PCR: Quantitative reverse transcriptase polymerase chain Reaction

RIG-I: retinoic acid-inducible gene 1

RISC: RNA induced silencing complex

RNA: ribonucleic Acid

RNAi: RNA interference

RRE: rev responsive element

RRL: rabbit reticulocytes lysate

RRM: RNA recognition motif

RT: reverse transcriptase

SamHD1: sterile alpha motif domain and hydrolase domain-1

SARS-coV: Severe acute respiratory syndrome- coronavirus

SG: stress granules

siRNA: small interferent RNA

siR-TIAR: si-Resistant

SIV: Simian immunodeficiency virus

ssRNA: single-strand RNA

TAR: Transactivation region

TIAR: TIA-1 related protein

TMA: translation machinery associated

TNPO3: transportin 3

TOP: terminal 5' oligo-pyrimidine

TRBP: Tar RNA binding protein

TRIM5 α : tripartite motif-5 α

tRNAs: transfer RNA

WHO: World Health Organization

WNV: West Nile virus