Chapter 2

Role of Host Proteins in HIV-1 Early Replication

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Abstract

After 33 years of the identification of HIV-1 infection, very little is known about the role of host cellular proteins. Till now considerable work has been done in the area of host-pathogen interactions facilitated by the viral proteins and host receptors. The role of the main receptor CD4 and co-receptors like CCR5, CXCR4 and their alternative receptors were well studied in disease progression. But the intracellular events during the host-pathogen interactions were poorly understood. Much data is available based on the global analysis of genome-wide RNA interference screens, yeast two-hybrid system and co-immunoprecipitation studies but their exact roles are not yet characterized. There are very few host proteins like APOBEC3G, LEDGF/p75, INI1, HMG I(Y), BAF which are well studied and characterized. Majority of the reported proteins are attributed to multiple functions. It will be useful to study such proteins to develop as future candidates in HIV-1 therapeutics.

Keywords: HIV, Reverse transcription, Host proteins, CD4, CCR5, CXCR4, Topoisomerase, PICs

1. Introduction

Host cell responses are key determinants of infection pertaining to infectious diseases. Different kind of host cell responses are exerted during the course of the infection, either a host defense response to restrict the invasion of pathogen or may promote the invasion. During the course of evolution, pathogens have acquired capability to protect themselves from host defense. This protection is mainly by modulating the key regulators of the host signal transduction mechanism. Unlike other pathogens, viruses are very small with a small genome which codes for the essential structural proteins and enzymes, a reason to consider them as primitive. These proteins are enough to takeover host cell and to control majority of the cellular processes. This takeover property may be due to its dependence on the host mechanisms to fulfill its

needs to establish itself and for replication in the host. For this they have to suppress certain mechanisms and promote others. It is very important to know the host factors involved the host-pathogen interactions, establishment of the infection and pathogenicity. In retrovirus especially in HIV infection, host proteins requirement starts from the beginning of attachment of the virus to its target host cell, reverse transcription, integration, transcription and translation of viral proteins, carrying of viral proteins to plasma membrane and release of viral particles. Overall knowledge on host proteins involvement in any infection would increase the possibilities of invention of the inhibitors for the host proteins which may be candidates for the future drug inventions.

2. Binding of virus to cell surface

Viruses have remarkable specificity for the host species and the cell types that they can infect. Similar to other viruses, human immunodeficiency virus (HIV) also has host and exhibits target cell specificity. This feature is based on the properties of a cell which can fulfill the virus catch and grab mechanism to complete its infection cycle (from attachment to the production of progeny viruses is considered as an infection cycle).

HIV as a single particle is called as virion. This virion consists of an outer envelope and inner capsid. Outer envelope is host derived plasma membrane with host cell surface molecules and viral transmembrane glycoprotein called gp41, which connects outer surface gp120 glycoprotein expressed from *env* gene of virus. Capsid is a cone shaped structure made up of a viral protein p24 (named based on its molecular weight 24 kDa) which is a processed poly-protein product of virus gene called *gag*. Envelope glycoprotein is translated as a 160 kDa polyprotein, later processed into two subunits of 120 kDa and another is 41 kDa by a cellular endo-protease [1]. One gp120 and one gp41 collectively form a unit and a trimer of this unit enmeshed in the viral envelope [2, 3]. The gp41 is the transmembrane portion and gp120 is the extracellular region which works as an anchor to grab the specific host cells. The primary/preliminary target for this gp120 is CD4 (receptor) and a secondary target is a chemokine receptor (co-receptor) of host cells.

2.1. Role of CD4

Leucocyte differentiation antigen, CD4 is a cellular receptor for HIV-1, HIV-2 and Simian immune deficiency virus (SIV). These viruses share CD4 as the common primary receptor and the binding sites on these viruses are highly conserved [4]. The CD4 receptor is found on CD4 T-cells (high expression) macrophages and dendritic cells (DCs; low expression). A CD4 binding site present on each monomer of gp120. Recruitment of one CD4 molecule on a single gp120 in the trimeric anchor can induce conformational changes in all three glycoprotein monomers of the trimer [5]. This binding of gp120 to CD4 can be blocked by the host antibodies produced against the gp120 of the virus which are called neutralizing antibodies. But due to variation in gp120 among virus population, there is a lag time in producing enough antibodies to block virus attachment. Furthermore, the conformational change occurs in the gp120, which

avoid recognition by the neutralizing antibodies, a process known as conformational masking. The conformational changes in gp120 allow it to bind to a second receptor on the CD4 cell surface [6].

The second docking area on the CD4+ cell surface is a chemokine receptor, a seven transmembrane (7TM) co-receptor namely C-C chemokine receptor type 5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4). The viral preference of using one co-receptor among others is called 'viral tropism'. The virus which can infect the macrophages predominantly uses CCR5 as their co-receptor. About 90% of all HIV infections involve the M-tropic HIV strain. CXCR4, also called fusin, which is a glycoprotein-linked chemokine receptor used by T-cell infecting (T-tropic) HIV to attach to the T cell. Indeed some HIV are reported to use co-receptors other than these two co-receptors like CCR1, CCR2b, CCR3, CCR8, CCR9, CXCR4, CX3CR1/V28, STRL-33/BONZO/CXCR6, GPR1, GPR15/BOB, APJ, ChemR23, RDC1, and Leukotriene B4 receptor though the mechanisms are unknown.

Once the HIV gp120 has attached to the CD4 molecule, it undergoes conformational changes which enables the binding of the gp120 to a co-receptor leads to the further structural rearrangements in the gp41 to fuse with the cell membrane and entry of the virions core into the cell's cytoplasm (Fig. 1). Once within a cell, virus is safe from neutralizing antibodies, but vulnerable to attack by CD8 cells (cytotoxic T-lymphocytes or CTLs).

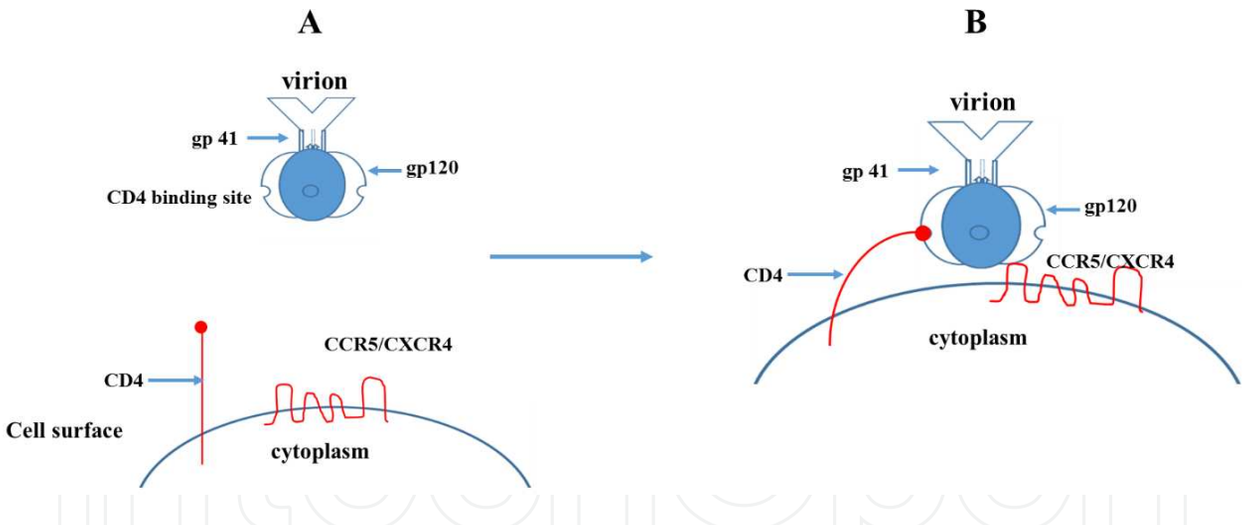

Figure 1. Virus and cell surface receptors interactions. In panel (A), schematic diagram represents virion particle and host cell surface receptors before attachment of the virion to the host. gp120, gp41 and CD4 binding region on gp120 of virus and CD4 and co-receptors (CCR5/CXCR4) were labeled. In panel (B), the attachment of CD4 in the CD4 binding site of gp120 and also binding of the co-receptor were shown.

In the absence of CD4, infection is inefficient and its significance *in vivo* is controversial. More than this binding and internalization, CD4 is involved in the signal transduction. Binding of gp120 to the CD4 induces rapid activation of the ERK/mitogen-activated protein (MAP) kinase pathway and stimulates expression of cytokine and chemokine genes by the binding of nuclear transcription factors (AP-1, NF-kB, and C/EBP) in both T-cell tropic and macrophage tropic strain. The activation of this signaling pathway requires functional CD4

receptors which is independent of binding to CXCR4 [7, 8]. Signal transduction by CD4 depends on its association with Lck, a src-family tyrosine kinase. Lck, interacts with CD4 with its unique NH2-terminal domain, and interacts with other intracellular signaling proteins with its SH2 and SH3 domains. Given the necessity of Lck kinase activity for T lymphocyte development and for mature T cell functions, perhaps Lck may function at different stages during T cell activation [9].

2.2. Role of chemokine receptors

Chemokine receptors are seven transmembrane (7TM) domain, G protein–coupled molecules that mediate the chemotaxis of T cells and phagocytic cells to the areas of inflammation [10]. Chemokine receptors have four domains exposed on the cell surface: the N terminus and three extracellular loops (E1, E2 and E3). Co-receptors take up different conformations on cell surface and on different cell types [11, 12], influencing their ability to support HIV infection. For X4 strains, E2 is critical. Deletion of the N terminus of CXCR4 affects some of the strains but not all [13], although, when present, participates in binding gp120 [14]. The gp120 of HIV-1 is structurally divided into five regions called Variable regions and represented with V1,V2 etc. The highly conserved region present between the variable region of gp120 functions as a co-receptor binding site [15]. *In vitro*, envelope glycoproteins in soluble form are even capable of co-receptor mediated signal transduction [16-18] which involves rapid phosphorylation of GPCRs at the carboxyl-terminal tail [19]. There are 21 potential phosphorylation sites in CXCR4 and only seven in CCR5. Chemokines, small low-molecular weight proteins, are the ligands that activate and signal through CCR5 and CXCR4 to mediate several cellular functions including development, leukocyte trafficking, angiogenesis, and immune response [20].

The extracellular loop2 (E2) which is present on the N terminus of the co-receptor, is responsible for the gp120 binding and HIV entry. After binding of the viral extracellular gp120 to the host cell surface, a cellular kinase called Focal adhesion kinase interacts with CCR5 [21]. The affinity of the gp120 and the co-receptors influences the strength of the signal transduction which can be correlated with the successes of post-fusion events of the virus [22]. This signal transduction from the co-receptor converts the host non permissive environment to the permissive environment for the virus establishment in the host. The binding capacity of the viral gp120 to the host co-receptor has been considered as the viral infectivity. Even though, the early and late events after co-receptor-gp120 interaction are not completely elucidated, yet these interactions have an important role in very early events of HIV-1 lifecycle.

2.3. CCR5

Virus using the CCR5 as co-receptor, infects the macrophages called M-tropic virus. This viral strain has ability to infect other cell types like dendritic cells and CD4 T-cells. Majority of the viral isolates utilize CCR5 co-receptors for their transmission. M-tropic HIV replicates in peripheral blood lymphocytes are less virulent and does not form syncytia. Syncytia are multinuclear cells which are result of cellular fusion.

The expression of CCR5 was observed on widely diverged cell types [23] and modulated by pro-inflammatory cytokines. A number of inflammatory CC-chemokines, like MIP-1, RANTES etc. [24] act as CCR5 agonists while, MCP-3 functions as antagonist. The cytosolic domain of the CCR5 bound with GPCRs (G-protein coupled receptors) transduces signals upon binding of ligands to CCR5. The signal transduction include different secondary messengers like cAMP, Ca2+, PI3-kinase, MAP kinases, as well as other tyrosine kinase cascades [7, 25-31]. The importance of the CCR5 mediated signaling [32] in HIV-1 infection was observed in CCR5 Δ 32 [33] mutant populations. Homozygous CCR5 Δ 32 hampers HIV's ability to infiltrate immune cells. Not only this, many other genetic mutations in CCR5 have effect on the progression and transmission of the HIV-1 infection. This mutation exerts resistance to many modes of HIV-1 infection [34].

Variation in the levels of chemokines was observed from person to person. In long-term non-progressors and seronegative individuals (people with repeated exposure to the virus but who do not become infected), unusually high levels of the CCR5 ligands were observed. These chemokines could function as natural competitive inhibitors to HIV-1 infection.[35].

2.4. CXCR4

T-tropic HIV uses CXCR4 as a co-receptor which belongs to the family of α-chemokine receptor. CXCR4 (known as fusin or X4) is also a GPCR with natural ligand CXCL12, known as Stromal Cell-Derived Factor 1 (SDF-1) [36, 37]. T-tropic virus can induce syncytium (SI) and are responsible for the rapid disease progression in HIV-positive individuals. During HIV-1 infections X4-tropic virus has the tendency of emergence and maintains higher viral loads and much lower CD4 cell counts in infected persons. Even though highly virulent, X4 infections are susceptible to antiretroviral therapy [7]. Mutational effect of CXCR4 in HIV-1 infection is not known due to its significant role in development and knockout mutant in mice for this gene is lethal at the embryonic stage [38].

CXCR4 also involved in cell death of $CD4^+$ T-cells which was induced by gp120 indicate an important *in vivo* role for CXCR4 mediated signaling. The interaction of gp120 with CXCR4 triggers a cell death pathway of Fas independent, mitochondrial dependent, cytochrome c mediated activation of caspase-9 and -3 [39]. Membrane fusion dependent $CD4^+$ T-cell death was observed in the virus strains of X4 and dual tropic (R5X4) [40].

However, majority of *in vivo* HIV-1 infection is mediated by M-tropic (R5) viruses could able to lyse their target cells and X4 viruses can kill $CXCR4^+$. It shows that, CD4 has no role in gp120 induced cell death. Moreover, cell lysis and syncytia formation were inhibited in the cells with high levels of CD4 expression. Interestingly, Glycol protein from non-infectious strains of X4 or R5X4 could not induce cell death [41].

Based on the capability to support infection of CD4+ cell lines, other than CCR5 and CXCR4 more than 14 potential co-receptors were identified *in vitro* [6] (Table 1). These receptors are members of (or closely related to) the chemokine receptor family. The significance of other co-receptors for HIV-1 replication *in vivo* and pathogenesis remains unclear.

Recently STRL-33 (CXCR6) functions as co-receptor for HIV-1 infection in primary T-cells [42], and in thymocytes, CCR8 were identified *in vitro* [43].

Co-receptors	Ligands	Role in viral replication	
		In vitro	*In vivo*
CCR1	MIP-1α, RANTES, MPIF-1, MCP-3	+	
CCR2b	MCP-1, MCP-2, MCP-3	+	
CCR3	Eotaxin, Eotaxin-2, MCP-3, MCP-4, RANTES	++	
CCR5	MIP-1α, MIP-1β, RANTES, MCP-2	++++	++++
CCR8	I-309	+	
CCR9	TECK	+	
CXCR4	SDF-1	+++	++
CX3CR1/V28	Fractalkine	+	
STRL-33/BONZO/CXCR6	CXCL16	+	
GPR1	?	+	
GPR15/BOB	?	+	
APJ	Apelin	+	
ChemR23	?	+	
RDC1	?	+	
Leukotriene B_4 receptor	Leukotriene B_4	+	

Table 1. HIV-1 receptors and cell tropism

3. CD4-independent infection

CD4 expression is not uniform in all hematopoietic cells. While, some cell types (T-cells) express high levels of CD4, others, including macrophages and dendritic cells (DCs), express barely detectable amounts. But T-cells as well as macrophages are susceptible to the HIV-1 infection *in vivo.* This susceptibility reveals the existence of alternative host cell surface receptors to which, HIV-1 may attach to cells by CD4-independent manner. Sugar groups present on the both virus and host cell surface (like mannose-specific macrophage endocytosis receptor) mediate the host pathogen interactions and helps in the HIV-1 infection [44]. Apart from the sugars, a cell surface protein (DC-SIGN) of dendritic cells [45, 46]. A closely related receptor to this (DC-SIGNR) on endothelial cells [47], Glycolipids namely galactocerebroside (GalC), galactosulfatide (sGalC) which express on neurons and glia in the brain, colon epithelial cell lines and, importantly, on macrophages [48-50] helps in HIV-1 infection. GalC supports suboptimal entry of particular HIV-1 strains without CD4, although infection requires a co-receptor [51]. Glycosaminoglycans like heparansulphate involved in the infection

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of HeLa cells [52]. Besides cell surface receptors, cell derived molecules incorporated onto virions such as integrin ICAM-1 (intercellular adhesion molecule-1) and LFA-1 (lymphocyte function-associated antigen-1) [53, 54] enhance the overall efficiency of virus entry.

Primary HIV-2 isolates generally infect $CD4^-$ co-receptor$^+$ cells more efficiently than HIV-1 [55, 56]. In these infections, CXCR4 plays a crucial role [56].

In glioma cell line (D-54 cells) binding of recombinant gp120 to the GalC or sGalC and a 180 kDa receptor activates signal transduction by a tyrosine-kinase which phosphorylates 130- and 115-kDa proteins [57]. It shows that not only main receptors but also the alternative receptors involved in signal transduction and host protein modifications in HIV-1 infections but the differences were observed in efficiency of infection and disease progression. It also conveys that HIV envelope glycoprotein can specifically bind to the different host cell surface receptors which can function as receptor or co-receptor and this feature provides flexibility to virus to infect wide variety of host cell types [56].

3.1. Fusion and internalization of viral particles

The HIV envelope glycoprotein is responsible not only for the virus attachment to the cell surface but also mediates viral entry. The two parts of the envelope; gp41 and gp120 trimer forms a functional unit, which under goes a series of structural changes (Fig. 2) upon binding to the CD4 and an appropriate chemokine receptors. These interactions promote conformational changes in gp120 and gp41, respectively. These changes exposes the fusion domain of the gp41 and allows to undergo fusion [58-60]. In the viral infection co-receptor has a crucial role in fusion. Fusion of viral particles in the absence of CD4 was observed but in the absence of co-receptor was not yet identified. Following attachment to the receptors, some virus particles enters into endosomes (Fig. 3) later the low pH of the endosomes promotes fusion [61]. However, HIV uses a co-receptor dependent and independent of pH [62].

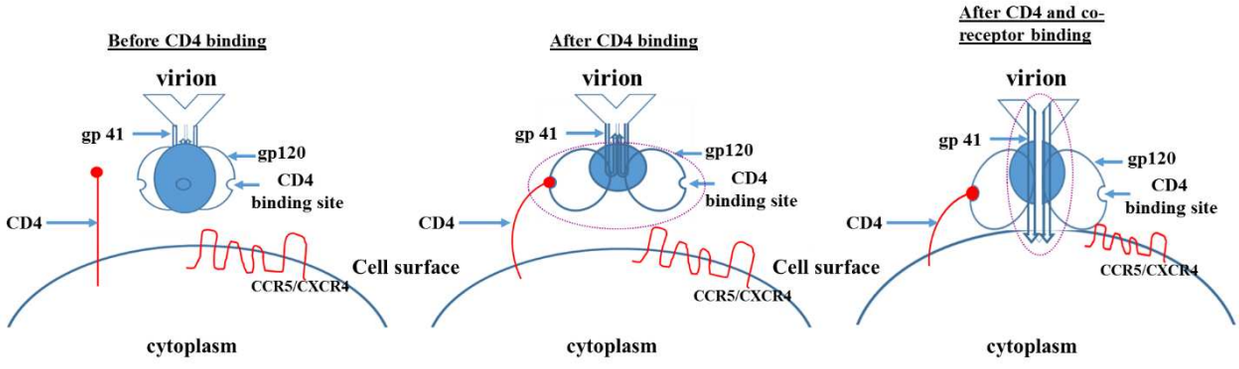

Figure 2. Schematic representation of structural changes in gp120 and gp41. Different conformational changes in gp120 and gp41 up on binding to the host cell surface receptors were represented schematically. From left, structures of the virion envelope gp120 before binding. Once gp120 bound to CD4 of host cell, structural change in the gp120 region in the pink dotted circle. The extreme right represents the changes in the gp120 and especially gp41 once receptor and co-receptor of the host cell interacts with gp120 of virus labeled in pink dotted circle.

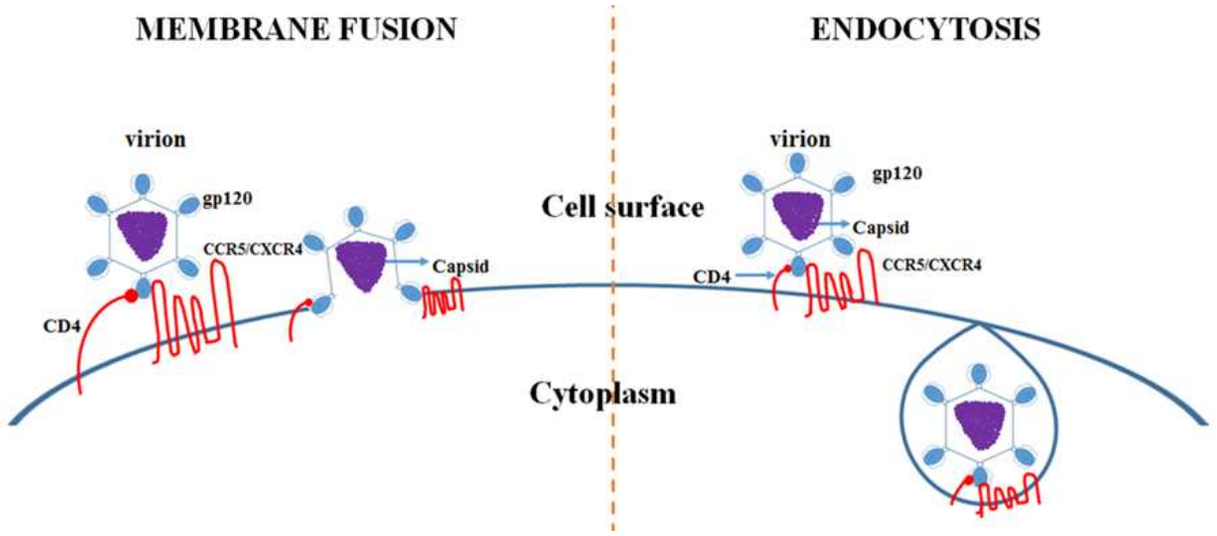

Figure 3. HIV-1 entry strategies. Two well reported strategies of HIV-1 entry into host cell. The direct membrane fusion upon binding to the CD4 and co-receptors like CCR5/CXCR4 and endocytosis mediated entry upon receptor and co-receptors binding were represented schematically.

CD4 is the primary receptor for HIVs, but virus penetration requires further interactions with chemokine receptor CCR5 or CXCR4. Earlier studies proposed that binding to co-receptor initiate fusion directly at the plasma membrane. The ability of formation of syncytia, and evidences of putative fusion events at the cell surface have supported the direct fusion at the plasma membrane [63]. However, recent evidence with endocytosis inhibitors and single-particle tracking revealed fusion and infection occur after endocytic uptake [64, 65]. Moreover, in macrophages HIV infection occurs through macropinocytosis [66, 67]. Based on the receptor density and mobility, the mode of viral entry to the host cell whether fusion on cell surface or after endocytosis will be determined [68].

3.2. Receptor-mediated endocytosis

Adhesion to the receptor initiates later events which enable viruses to enter the cytosol. The cortex is the potential barrier to prevent entry of large molecules [69]. Receptor-mediated signaling induced by envelope of the viruses allow viruses that undergo penetration at the cell surface and transit the cortex [70-72]. In HIV, Gαi (a heterotrimeric G protein subunit that inhibits the production of cAMP from ATP [73]) and CXCR4 on resting CD4+ve T cells activate cofilin which induces reorganization of the cortex that facilitates infection [71]. This evolvement of use of different mechanisms of viral invasion into host cell may have distinct advantages for virus, which provides broader range of cell types to infect or different ways to infect a same cell type [68] to bypass the host restriction mechanisms.

Immediately after its release into the cytoplasm, the viral core undergoes a partial and progressive disassembly, known as uncoating, that leads to the generation of sub viral particles called reverse-transcription complexes (RTCs) and pre-integration complexes (PICs). In HIV-1 the uncoating of capsid is coupled to the initiation of reverse transcription [74].

4. Reverse transcription of viral RNA

4.1. Uncoating of capsid

The events in retrovirus infection that occur between entry into the host cell and reverse transcription are less understood. Uncoating is the process of disintegration of the capsid and release of its components into the cytoplasm of the host cell. The uncoating of the HIV-1 capsid is thought to precede reverse transcription, whereas MLV capsid proteins remain associated with the reverse transcription and pre-integration complexes [75-77]. The uncoating of capsid is a temporarily regulated [78] and the host cell factors involved are poorly understood. There are few known host proteins function as restriction factors and block the HIV-1 infection. One of them is TRIM5α, which prevents retrovirus infection by disrupting an early, post entry events by associating with the retroviral capsid [79, 80]. In owl monkeys, RBCC domains of TRIM5 fused with cyclophilin A (CypA), a known capsid ligand, was identified. [81-87]. Mechanism of Cyp A in capsid uncoating or some other step in the post-entry phases of the HIV-1 life cycle not clearly understood [88]. TRIM5 has multiple roles in early infection. It can interfere with the uncoating process, intercept reverse transcription and viral genome transport to the nucleus by binding to the capsid proteins [89].

In vitro, uncoating of HIV-1 capsid required activated CD4+ lymphocytes. Two distinct cellular factors with molecular mass of approximately 60 and 160 kDa were found to be involved in capsid uncoating [90]. In contrary, cyclophilins mediated activation of T-cells by regulating the activity of calcineurin, a phosphatase necessary for T-cell activation was reported. [91]

4.2. Reverse transcription (Reverse transcriptase and cellular factors: regulators of HIV-1 reverse transcription)

Reverse transcription takes place in a complex organization called reverse transcription complex (RTC), is a nucleoprotein complex comprising viral RNA, a tRNA primer and newly synthesized DNA, along with these nucleic acids, viral factors and host factors present. Reverse transcription complex (RTC) is a composite organization of nucleoprotein complex comprising of viral RNA, tRNA primer and synthesized DNA where the reverse transcription takes place. RTC comprises of several other nucleic acids, host and viral factors.

The first host factors in association with reverse transcription which are packaged in virus particles is cellular $tRNA^{Lys3}$, which is bound to the aminoacyl-$tRNA^{Lys3}$, synthetase (LysRS) [92]. $tRNA^{Lys3}$ works as a primer by binding to the primer binding site (PBS) [93] of the HIV-1 genomic mRNA and is the first step in reverse transcription. Initiation of reverse transcription refers to the addition of the first five deoxynucleotides to the $tRNA^{Lys3}$ primer [94]. Several cellular factors which bound to Integrase (IN) and effects reverse transcription were identified [95] but their direct role in reverse transcription not yet illustrated. Some of the known cellular proteins are integrase interactor 1 (INI1, hSNF5) [96, 97], sin3A-associated protein (SAP18), histone deactylase 1 (HDAC1) [98] and survival motor neuron (SMN)-interacting protein 2 (Gemin2) [99].

INI1 is a component of the SWI/SNF chromatin remodeling complex of host cell [100] and is associated with virus. INI 1 Packaging into virus is specific for HIV-1 and is regulated by a direct interaction with the IN domain of the HIV-1 Gag-Pol protein [101]. INI1 associates with viral RTC/PIC [102] and stimulates IN [96] activity moreover involved in the regulation of reverse transcription.

Like INI1 of SWI/SNF complex, Sin3a-HDAC1 complex members Sin3a, Sap18, Sap30, and HDAC1 were found in virion [98]. HDAC1 is required for the initiation of reverse transcription and involved in a step between uncoating and reverse transcription which results in defective viral cDNA synthesis [98]. Another IN binding protein is Gemin2 is shown to be required for an early reverse transcription product (negative strand strong stop) or integration of viral DNA, suggesting that Gemin2 association with either the reverse transcription or pre-integration complexes [99]. It can recruit other cellular factors like DHX9 (RNA helicase A) [103], which intern associated with the SMN complex [104]. But the precise mechanism of how Gemin2 affects early replication remains to be determined.

Human antigen R (HuR) is a nuclear protein with nucleocytoplasmic shuttling capabilities [105]. It is a RNA binding protein with 3 RNA binding domains and exhibits high specificity and affinity for AU-rich elements (AREs) [106, 107]. HuR is required for optimal reverse transcription. While the mechanism behind this activity remains unclear, it appears to be due to an interaction with the RNase H domain of RT [108]. APOBEC3G (hA3G), is a host protein with negative effects on reverse transcription [109-112]. Furthermore, hA3G was a member of several ribonucleoproteins including DHX9 [103], hnRNP U [113], PABPC1 [114], YB-1 and SNRPA [115] which can affect HIV-1 replication including reverse transcription. A direct interaction between hA3G and HuR was reported [116]. By formation of protein complex, hA3G and HuR could regulate the functions of RTC in the cytoplasm.

A kinase anchor protein 1 (AKAP1) which bind the regulatory subunits of cAMP-dependent protein kinase A (PKA) and anchors them to various membranes throughout the cell [117]. Interaction between AKAP149 and HIV-1 RT was also reported [108]. Like HuR, it interacts with the RNase H region of RT but the mechanism of RT regulation was not yet clearly understood. DNA topoisomerases 1 (TOP1) is another host protein which interact with HIV-1 NC and participate in the initiation of the cDNA synthesis by enhancing the activity of HIV-1 RT [118, 119].

Several studies were conducted on the role of Topoisomerase II in HIV-1 infection. In eukaryotes two isoforms of topoisomerase II (Topo II) was identified. The smaller one is 170kDa topoisomerase II alpha (Topo IIα) and the bigger one is 180 kDa topoisomerase II beta (Topo IIβ) [120]. Recent evidence has proven their key role in viral infections [121, 122]. In response to HIV-1 infection, increased protein levels of Topo IIα and β were observed [123, 124]. Both of these isoforms were reported to undergo phosphorylation in HIV-1 infection [125-127]. These isoforms are phosphorylated by serine kinase present in the purified HIV-1 virion and are associated with pre-integration complexes (PICs) [126]. Topo II inhibitors abrogate HIV-1 replication cycle by interfering with the PICs formation [128]. Down regulation if these isoforms using siRNA resulting in impaired HIV-1 replication [124,129, 130] due to the incomplete reverse transcription. Co-localization studies reveled the associa-

tion of these isoforms with HIV-1 reverse transcriptase [124]. Similar to this, another host protein HMGI(Y) reported to be with involvement in covalent strand transfer in HIV-1 reverse transcription. [131].

APOBEC3G (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G, (A3G)) belong to a group of interferon-stimulated gene [132] and is an editing enzyme for nucleic acids. It blocks virus replication by deamination of viral minus-strand DNA, resulting in G-to-A hyper mutation. In addition to the deaminase activity, A3G has also been shown to directly inhibit HIV-1 reverse transcription by a non-editing mechanism [133, 134]. A3G may also reduce viral DNA synthesis and can inducing viral DNA degradation by interacting physically with HIV-1 reverse transcriptase [135]. Its action was blocked by the viral protein Vif and another host protein apoptosis signal-regulating kinase 1 (ASK1) by binding to Vif restores A3G function [136]. Cyclin-dependent kinase (CDK) 2 is a host protein which regulates the reverse transcriptase by phosphorylating on threonine which improves the increased efficiency and stability of reverse transcriptase and enhanced viral fitness. p21, a cell-intrinsic CDK inhibitor, counteracts the CDK2-dependent phosphorylation and significantly reduced the efficacy of viral reverse transcription [137].

4.3. Pre-integration complex formation

Immediately after disintegration of the capsid into the host cytosol, the viral +stand genomic RNA converted into double stranded DNA. This newly synthesized viral genomic DNA wrapped around the host and viral proteins in a protective manner and protected from nuclease degradation [138]. The viral single-stranded RNA genome is converted into a linear double-stranded DNA. The viral DNA intermediate then migrates to the cell nucleus and is covalently integrated into a host chromosome. The integration of reverse transcribed HIV-1 cDNA into a host cell chromosome is an essential step in the viral replication cycle [108, 109]. Retroviral integration *in vivo* is mediated by pre-integration complexes (PICs). PICs are be formed with viral and host cell proteins like high-mobility group protein A1 (HMGA1) and the barrier-to-auto-integration factor (BAF) which were well studied and identified their functions as cofactors for integration [139]. HMG I(Y) is another host protein required for the proper function of the PICs *in vitro* [131]. Other proteins like XRCC6, TFRC and HSP70 were identified as in association with viral DNA [140]. Topoisomerase IIα and β isoforms were also identified as nucleoprotein components of PICs, which suggesting their significant role in HIV-1 replication [128].

5. Integration of viral DNA into host genome

Integration of the viral DNA is mediated by PICs formation. These PICs are capable of performing integration *in vitro*. Even though specific (HMG I(Y)) and non-specific (bovine RNase A) could able to restore the activity, but only BAF can restore the native structure of the HIV-1 protein–DNA intasome from salt stripped PICs [141]. HIV-1 integration in host genome is not a random event. In majority of cases, it takes place in AT rich, euchromatin

region. Even though PIC formation can protect the DNA and helps in the nuclear transport, it cannot guide the integration in a proper location in the host genome. A host protein called cellular lens epithelium-derived growth factor (LEDGF/p75) which binds both chromosomal DNA and HIV integrase [142], directs the integration to a location where active transcription takes place under its control [143]. The interaction between the integrase and INI1 stimulates the DNA-joining activity of the integrase and helps to target the viral DNA towards active genes [96, 102, 144]. Presence of a Topo II cleavage site in the HIV-1 promoter and also at 180 bp upstream of the HIV-1 integration site [145, 146] and association of these Topo II isoforms with HIV-1 PICs [128]. Based on the available information, it can be derived that, IN alone can carry out the integration reaction but for the selection of the proper location in the host genome for the integration and success full HIV-1 gene transcription, Integrase required the support of the host factors.

5.1. Transport of PICs into nucleus

Transportation of the PICs to the nucleus can be divided in two parts. One is transportation to the nuclear periphery and second is from periphery to inside the nucleus. After completion of the successful cell surface attachment, capsid internalization and degradation in cytoplasm which leads to the reverse transcription of the viral genome RNA to DNA and the formation of the pre-integration complex to protect the DNA from the host nucleases takes place. This nucleoprotein PICs should travel from the cytosol to the nucleus to form provirus which is an integrated viral DNA in host chromatin and can produce progeny virus [147, 148].

The PICs in the cytoplasm translocate to the perinuclear compartment by the cytoplasmic movements by the cytoskeleton. Actin and microtubule [149] selectively plays a role in this transport with the help of Myosin VI and Dynein [148]. The dynein complex proteins such as dynein light chain1 (DYNLL1), Tctex1 and Dynactin have been shown to be involved in this process. But very little is known about how HIV-1 targets cytoskeleton. Many of the viral elements found in association with the PIC have been proposed to be important for HIV-1 nuclear import.

Studies on the HIV-1 infection in dividing and non-dividing cells provided enough evidences to believe that PICs enter the nucleoplasm by crossing the nuclear envelope through nuclear pore complexes (NPCs), which form stable channels through the nuclear envelope and gate-keep the trafficking of molecules between the nucleus and cytoplasm [150]. The RTC/PIC with the size of~100-250nm [149, 151, 152] cannot cross the nuclear pore. So, only few important components of the PIC may enter into the nucleus [149, 152].

For nuclear transport, KPNs and NUPs functions as carrier proteins by binding to the integrase. KPN α adaptor proteins importin α1 (Rch1) [153] and importin α3 (KPNA4) [154], to which KPN β1 proteins bound additionally. Importin 7 [155, 156] and transportin 3 [157, 158] are recruited by the nuclear localization signal (NLS) present on IN. In both dividing and non-dividing cells Impα3 which interacts with IN is found to be essential nuclear import and replication [154]. In addition to these, IN can directly interact with the KPN β1, NUP153 [159], Pom121 [160] or hCG1 [161] who has possible interactions with IN and Vpr and can facilitate nuclear import [162].

6. Summary

After 33 years of the identification of HIV-1 infection, very little is known about the role of host cellular proteins. Till now considerable work has been done in the area of host–pathogen interactions facilitated by the viral proteins and host receptors. The role of the main receptor like CD4 and co-receptors like CCR5, CXCR4 and their alternative receptors were well studied with the role of their signaling in disease progression. But the intracellular events of the host–pathogen interactions were poorly understood. Much data is available based on the global wide analysis of genome-wide RNA interference screens, yeast two-hybrid system and co-immunoprecipitation studies but their exact roles were not yet characterized. There are very few host proteins like APOBEC3G, LEDGF/p75, INI1, HMG I(Y) and BAF, which were well studied and characterized. Majority of the reported proteins were attributed with multiple functions. It is very useful to study such proteins to develop as future candidates to HIV-1 therapy.

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References

[1] Moulard, M. and E. Decroly, *Maturation of HIV envelope glycoprotein precursors by cellular endoproteases.* Biochim Biophys Acta, 2000. 1469(3): p. 121-32.

[2] Weiss, C.D., J.A. Levy, and J.M. White, *Oligomeric organization of gp120 on infectious human immunodeficiency virus type 1 particles.* J Virol, 1990. 64(11): p. 5674-7.

[3] Earl, P.L., R.W. Doms, and B. Moss, *Oligomeric structure of the human immunodeficiency virus type 1 envelope glycoprotein.* Proc Natl Acad Sci U S A, 1990. 87(2): p. 648-52.

[4] Sattentau, Q.J., et al., *The human and simian immunodeficiency viruses HIV-1, HIV-2 and SIV interact with similar epitopes on their cellular receptor, the CD4 molecule.* AIDS, 1988. 2(2): p. 101-5.

[5] Salzwedel, K. and E.A. Berger, *Cooperative subunit interactions within the oligomeric envelope glycoprotein of HIV-1: functional complementation of specific defects in gp120 and gp41.* Proc Natl Acad Sci U S A, 2000. 97(23): p. 12794-9.

[6] Clapham, P.R. and A. McKnight, *HIV-1 receptors and cell tropism.* Br Med Bull, 2001. 58: p. 43-59.

[7] Popik, W., J.E. Hesselgesser, and P.M. Pitha, *Binding of human immunodeficiency virus type 1 to CD4 and CXCR4 receptors differentially regulates expression of inflammatory genes and activates the MEK/ERK signaling pathway.* J Virol, 1998. 72(8): p. 6406-13.

[8] Popik, W. and P.M. Pitha, *Binding of human immunodeficiency virus type 1 to CD4 induces association of Lck and Raf-1 and activates Raf-1 by a Ras-independent pathway.* Mol Cell Biol, 1996. 16(11): p. 6532-41.

[9] Ravichandran, K.S., T.L. Collins, and S.J. Burakoff, *CD4 and signal transduction.* Curr Top Microbiol Immunol, 1996. 205: p. 47-62.

[10] Power, C.A. and T.N. Wells, *Cloning and characterization of human chemokine receptors.* Trends Pharmacol Sci, 1996. 17(6): p. 209-13.

[11] Baribaud, F., et al., *Antigenically distinct conformations of CXCR4.* J Virol, 2001. 75(19): p. 8957-67.

[12] Lee, B., et al., *Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and coreceptor function.* J Biol Chem, 1999. 274(14): p. 9617-26.

[13] Picard, L., et al., *Role of the amino-terminal extracellular domain of CXCR-4 in human immunodeficiency virus type 1 entry.* Virology, 1997. 231(1): p. 105-11.

[14] Doranz, B.J., et al., *Identification of CXCR4 domains that support coreceptor and chemokine receptor functions.* J Virol, 1999. 73(4): p. 2752-61.

[15] Kwong, P.D., et al., *Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody.* Nature, 1998. 393(6686): p. 648-59.

[16] Davis, C.B., et al., *Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5.* J Exp Med, 1997. 186(10): p. 1793-8.

[17] Hesselgesser, J., et al., *CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons.* Curr Biol, 1997. 7(2): p. 112-21.

[18] Weissman, D., et al., *Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor.* Nature, 1997. 389(6654): p. 981-5.

[19] Marchese, A., et al., *G protein-coupled receptor sorting to endosomes and lysosomes.* Annu Rev Pharmacol Toxicol, 2008. 48: p. 601-29.

[20] Viola, A. and A.D. Luster, *Chemokines and their receptors: drug targets in immunity and inflammation.* Annu Rev Pharmacol Toxicol, 2008. 48: p. 171-97.

[21] Cicala, C., et al., *Induction of phosphorylation and intracellular association of CC chemokine receptor 5 and focal adhesion kinase in primary human CD4+ T cells by macrophage-tropic HIV envelope.* J Immunol, 1999. 163(1): p. 420-6.

[22] Arthos, J., et al., *CCR5 signal transduction in macrophages by human immunodeficiency virus and simian immunodeficiency virus envelopes.* J Virol, 2000. 74(14): p. 6418-24.

[23] Rottman, J.B., et al., *Cellular localization of the chemokine receptor CCR5. Correlation to cellular targets of HIV-1 infection.* Am J Pathol, 1997. 151(5): p. 1341-51.

[24] Blanpain, C., et al., *CCR5 and HIV infection.* Receptors Channels, 2002. 8(1): p. 19-31.

[25] Furler, R.L. and C.H. Uittenbogaart, *Signaling through the P38 and ERK pathways: a common link between HIV replication and the immune response.* Immunol Res, 2010. 48(1-3): p. 99-109.

[26] Cohen, P.S., et al., *The critical role of p38 MAP kinase in T cell HIV-1 replication.* Mol Med, 1997. 3(5): p. 339-46.

[27] Francois, F. and M.E. Klotman, *Phosphatidylinositol 3-kinase regulates human immunodeficiency virus type 1 replication following viral entry in primary CD4+ T lymphocytes and macrophages.* J Virol, 2003. 77(4): p. 2539-49.

[28] Del Corno, M., et al., *HIV-1 gp120 and chemokine activation of Pyk2 and mitogen-activated protein kinases in primary macrophages mediated by calcium-dependent, pertussis toxin-insensitive chemokine receptor signaling.* Blood, 2001. 98(10): p. 2909-16.

[29] Lee, C., et al., *Macrophage activation through CCR5- and CXCR4-mediated gp120-elicited signaling pathways.* J Leukoc Biol, 2003. 74(5): p. 676-82.

[30] Popik, W. and P.M. Pitha, *Early activation of mitogen-activated protein kinase kinase, extracellular signal-regulated kinase, p38 mitogen-activated protein kinase, and c-Jun N-terminal kinase in response to binding of simian immunodeficiency virus to Jurkat T cells expressing CCR5 receptor.* Virology, 1998. 252(1): p. 210-7.

[31] Lopez-Ilasaca, M., *Signaling from G-protein-coupled receptors to mitogen-activated protein (MAP)-kinase cascades.* Biochem Pharmacol, 1998. 56(3): p. 269-77.

[32] Shen, R. and P.D. Smith, *Mucosal correlates of protection in HIV-1-exposed sero-negative persons.* Am J Reprod Immunol, 2014. 72(2): p. 219-27.

[33] Samson, M., et al., *Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene.* Nature, 1996. 382(6593): p. 722-5.

[34] Carrington, M., et al., *Genetics of HIV-1 infection: chemokine receptor CCR5 polymorphism and its consequences.* Hum Mol Genet, 1999. 8(10): p. 1939-45.

[35] De Clercq, E. and D. Schols, *Inhibition of HIV infection by CXCR4 and CCR5 chemokine receptor antagonists.* Antivir Chem Chemother, 2001. 12 Suppl 1: p. 19-31.

[36] Murphy, P.M., et al., *International union of pharmacology. XXII. Nomenclature for chemokine receptors.* Pharmacol Rev, 2000. 52(1): p. 145-76.

[37] Fredriksson, R., et al., *The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints.* Mol Pharmacol, 2003. 63(6): p. 1256-72.

[38] Marechal, V., et al., *Opposite effects of SDF-1 on human immunodeficiency virus type 1 replication.* J Virol, 1999. 73(5): p. 3608-15.

[39] Roggero, R., et al., *Binding of human immunodeficiency virus type 1 gp120 to CXCR4 induces mitochondrial transmembrane depolarization and cytochrome c-mediated apoptosis independently of Fas signaling.* J Virol, 2001. 75(16): p. 7637-50.

[40] Lin, G., et al., *Identification of gp120 binding sites on CXCR4 by using CD4-independent human immunodeficiency virus type 2 Env proteins.* J Virol, 2003. 77(2): p. 931-42.

[41] LaBonte, J.A., N. Madani, and J. Sodroski, *Cytolysis by CCR5-using human immunodeficiency virus type 1 envelope glycoproteins is dependent on membrane fusion and can be inhibited by high levels of CD4 expression.* J Virol, 2003. 77(12): p. 6645-59.

[42] Sharron, M., et al., *Expression and coreceptor activity of STRL33/Bonzo on primary peripheral blood lymphocytes.* Blood, 2000. 96(1): p. 41-9.

[43] Lee, S., et al., *CCR8 on human thymocytes functions as a human immunodeficiency virus type 1 coreceptor.* J Virol, 2000. 74(15): p. 6946-52.

[44] Larkin, M., et al., *Oligosaccharide-mediated interactions of the envelope glycoprotein gp120 of HIV-1 that are independent of CD4 recognition.* AIDS, 1989. 3(12): p. 793-8.

[45] Curtis, B.M., S. Scharnowske, and A.J. Watson, *Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120.* Proc Natl Acad Sci U S A, 1992. 89(17): p. 8356-60.

[46] Geijtenbeek, T.B., et al., *DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells.* Cell, 2000. 100(5): p. 587-97.

[47] Pohlmann, S., et al., *DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans.* Proc Natl Acad Sci U S A, 2001. 98(5): p. 2670-5.

[48] Fantini, J., et al., *Infection of colonic epithelial cell lines by type 1 human immunodeficiency virus is associated with cell surface expression of galactosylceramide, a potential alternative gp120 receptor.* Proc Natl Acad Sci U S A, 1993. 90(7): p. 2700-4.

[49] Harouse, J.M., et al., *Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide.* Science, 1991. 253(5017): p. 320-3.

[50] Seddiki, N., et al., *A monoclonal antibody directed to sulfatide inhibits the binding of human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein to macrophages but not their infection by the virus.* Biochim Biophys Acta, 1994. 1225(3): p. 289-96.

[51] Delezay, O., et al., *Co-expression of CXCR4/fusin and galactosylceramide in the human intestinal epithelial cell line HT-29.* AIDS, 1997. 11(11): p. 1311-8.

[52] Mondor, I., S. Ugolini, and Q.J. Sattentau, *Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans.* J Virol, 1998. 72(5): p. 3623-34.

[53] Paquette, J.S., et al., *Level of ICAM-1 surface expression on virus producer cells influences both the amount of virion-bound host ICAM-1 and human immunodeficiency virus type 1 infectivity.* J Virol, 1998. 72(11): p. 9329-36.

[54] Fortin, J.F., et al., *Role of the leukocyte function antigen-1 conformational state in the process of human immunodeficiency virus type 1-mediated syncytium formation and virus infection.* Virology, 1999. 257(1): p. 228-38.

[55] Reeves, J.D., et al., *Primary human immunodeficiency virus type 2 (HIV-2) isolates infect CD4-negative cells via CCR5 and CXCR4: comparison with HIV-1 and simian immunodeficiency virus and relevance to cell tropism in vivo.* J Virol, 1999. 73(9): p. 7795-804.

[56] Endres, M.J., et al., *CD4-independent infection by HIV-2 is mediated by fusin/CXCR4.* Cell, 1996. 87(4): p. 745-56.

[57] Schneider-Schaulies, J., et al., *HIV-1 gp120 receptor on CD4-negative brain cells activates a tyrosine kinase.* Virology, 1992. 191(2): p. 765-72.

[58] Chan, D.C., et al., *Core structure of gp41 from the HIV envelope glycoprotein.* Cell, 1997. 89(2): p. 263-73.

[59] Weissenhorn, W., et al., *Atomic structure of the ectodomain from HIV-1 gp41.* Nature, 1997. 387(6631): p. 426-30.

[60] Binley, J.M., et al., *Redox-triggered infection by disulfide-shackled human immunodeficiency virus type 1 pseudovirions.* J Virol, 2003. 77(10): p. 5678-84.

[61] Skehel, J.J., et al., *Membrane fusion by influenza hemagglutinin.* Cold Spring Harb Symp Quant Biol, 1995. 60: p. 573-80.

[62] Clapham, P.R. and A. McKnight, *Cell surface receptors, virus entry and tropism of primate lentiviruses.* J Gen Virol, 2002. 83(Pt 8): p. 1809-29.

[63] Stein, B.S., et al., *pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane.* Cell, 1987. 49(5): p. 659-68.

[64] Daecke, J., et al., *Involvement of clathrin-mediated endocytosis in human immunodeficiency virus type 1 entry.* J Virol, 2005. 79(3): p. 1581-94.

[65] Miyauchi, K., et al., *HIV enters cells via endocytosis and dynamin-dependent fusion with endosomes.* Cell, 2009. 137(3): p. 433-44.

[66] Marechal, V., et al., *Human immunodeficiency virus type 1 entry into macrophages mediated by macropinocytosis.* J Virol, 2001. 75(22): p. 11166-77.

[67] Carter, G.C., et al., *HIV-1 infects macrophages by exploiting an endocytic route dependent on dynamin, Rac1 and Pak1.* Virology, 2011. 409(2): p. 234-50.

[68] Grove, J. and M. Marsh, *The cell biology of receptor-mediated virus entry.* J Cell Biol, 2011. 195(7): p. 1071-82.

[69] Marsh, M. and R. Bron, *SFV infection in CHO cells: cell-type specific restrictions to productive virus entry at the cell surface.* J Cell Sci, 1997. 110 (Pt 1): p. 95-103.

[70] Wang, X., et al., *Integrin alphavbeta3 is a coreceptor for human cytomegalovirus.* Nat Med, 2005. 11(5): p. 515-21.

[71] Yoder, A., et al., *HIV envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells.* Cell, 2008. 134(5): p. 782-92.

[72] Taylor, M.P., O.O. Koyuncu, and L.W. Enquist, *Subversion of the actin cytoskeleton during viral infection.* Nat Rev Microbiol, 2011. 9(6): p. 427-39.

[73] Birnbaumer, L., *Expansion of signal transduction by G proteins. The second 15 years or so: from 3 to 16 alpha subunits plus betagamma dimers.* Biochim Biophys Acta, 2007. 1768(4): p. 772-93.

[74] Zhang, H., et al., *Morphologic changes in human immunodeficiency virus type 1 virions secondary to intravirion reverse transcription: evidence indicating that reverse transcription may not take place within the intact viral core.* J Hum Virol, 2000. 3(3): p. 165-72.

[75] Fassati, A. and S.P. Goff, *Characterization of intracellular reverse transcription complexes of human immunodeficiency virus type 1.* J Virol, 2001. 75(8): p. 3626-35.

[76] Fassati, A. and S.P. Goff, *Characterization of intracellular reverse transcription complexes of Moloney murine leukemia virus.* J Virol, 1999. 73(11): p. 8919-25.

[77] Miller, M.D., C.M. Farnet, and F.D. Bushman, *Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition.* J Virol, 1997. 71(7): p. 5382-90.

[78] Forshey, B.M., et al., *Formation of a human immunodeficiency virus type 1 core of optimal stability is crucial for viral replication.* J Virol, 2002. 76(11): p. 5667-77.

[79] Stremlau, M., et al., *The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys.* Nature, 2004. 427(6977): p. 848-53.

[80] Perron, M.J., et al., *TRIM5alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells.* Proc Natl Acad Sci U S A, 2004. 101(32): p. 11827-32.

[81] Cowan, S., et al., *Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism.* Proc Natl Acad Sci U S A, 2002. 99(18): p. 11914-9.

[82] Besnier, C., Y. Takeuchi, and G. Towers, *Restriction of lentivirus in monkeys.* Proc Natl Acad Sci U S A, 2002. 99(18): p. 11920-5.

[83] Dodding, M.P., et al., *Capsid processing requirements for abrogation of Fv1 and Ref1 restriction.* J Virol, 2005. 79(16): p. 10571-7.

[84] Luban, J., et al., *Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B.* Cell, 1993. 73(6): p. 1067-78.

[85] Nisole, S., et al., *A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1.* Proc Natl Acad Sci U S A, 2004. 101(36): p. 13324-8.

[86] Yap, M.W., et al., *Trim5alpha protein restricts both HIV-1 and murine leukemia virus.* Proc Natl Acad Sci U S A, 2004. 101(29): p. 10786-91.

[87] Thali, M., et al., *Functional association of cyclophilin A with HIV-1 virions.* Nature, 1994. 372(6504): p. 363-5.

[88] Grattinger, M., et al., *In vitro assembly properties of wild-type and cyclophilin-binding defective human immunodeficiency virus capsid proteins in the presence and absence of cyclophilin A.* Virology, 1999. 257(1): p. 247-60.

[89] Stremlau, M., et al., *Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor.* Proc Natl Acad Sci U S A, 2006. 103(14): p. 5514-9.

[90] Auewarakul, P., et al., *Uncoating of HIV-1 requires cellular activation.* Virology, 2005. 337(1): p. 93-101.

[91] Schreiber, S.L. and G.R. Crabtree, *The mechanism of action of cyclosporin A and FK506.* Immunol Today, 1992. 13(4): p. 136-42.

[92] Cen, S., et al., *Incorporation of lysyl-tRNA synthetase into human immunodeficiency virus type 1.* J Virol, 2001. 75(11): p. 5043-8.

[93] Saadatmand, J., et al., *Interactions of reverse transcriptase sequences in Pol with Gag and LysRS in the HIV-1 tRNALys3 packaging/annealing complex.* Virology, 2008. 380(1): p. 109-17.

[94] Dobard, C.W., M.S. Briones, and S.A. Chow, *Molecular mechanisms by which human immunodeficiency virus type 1 integrase stimulates the early steps of reverse transcription.* J Virol, 2007. 81(18): p. 10037-46.

[95] Luban, J. and S.P. Goff, *The yeast two-hybrid system for studying protein-protein interactions.* Curr Opin Biotechnol, 1995. 6(1): p. 59-64.

[96] Kalpana, G.V., et al., *Binding and stimulation of HIV-1 integrase by a human homolog of yeast transcription factor SNF5.* Science, 1994. 266(5193): p. 2002-6.

[97] Rain, J.C., et al., *Yeast two-hybrid detection of integrase-host factor interactions.* Methods, 2009. 47(4): p. 291-7.

[98] Sorin, M., et al., *Recruitment of a SAP18-HDAC1 complex into HIV-1 virions and its requirement for viral replication.* PLoS Pathog, 2009. 5(6): p. e1000463.

[99] Hamamoto, S., et al., *Identification of a novel human immunodeficiency virus type 1 integrase interactor, Gemin2, that facilitates efficient viral cDNA synthesis in vivo.* J Virol, 2006. 80(12): p. 5670-7.

[100] Wang, W., et al., *Diversity and specialization of mammalian SWI/SNF complexes.* Genes Dev, 1996. 10(17): p. 2117-30.

[101] Yung, E., et al., *Specificity of interaction of INI1/hSNF5 with retroviral integrases and its functional significance.* J Virol, 2004. 78(5): p. 2222-31.

[102] Turelli, P., et al., *Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication.* Mol Cell, 2001. 7(6): p. 1245-54.

[103] Roy, B.B., et al., *Association of RNA helicase a with human immunodeficiency virus type 1 particles.* J Biol Chem, 2006. 281(18): p. 12625-35.

[104] Pellizzoni, L., et al., *A functional interaction between the survival motor neuron complex and RNA polymerase II.* J Cell Biol, 2001. 152(1): p. 75-85.

[105] Fan, X.C. and J.A. Steitz, *Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs.* EMBO J, 1998. 17(12): p. 3448-60.

[106] Myer, V.E., X.C. Fan, and J.A. Steitz, *Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay.* EMBO J, 1997. 16(8): p. 2130-9.

[107] Vakalopoulou, E., J. Schaack, and T. Shenk, *A 32-kilodalton protein binds to AU-rich domains in the 3' untranslated regions of rapidly degraded mRNAs.* Mol Cell Biol, 1991. 11(6): p. 3355-64.

[108] Lemay, J., et al., *HuR interacts with human immunodeficiency virus type 1 reverse transcriptase, and modulates reverse transcription in infected cells.* Retrovirology, 2008. 5: p. 47.

[109] Bishop, K.N., et al., *APOBEC3G inhibits elongation of HIV-1 reverse transcripts.* PLoS Pathog, 2008. 4(12): p. e1000231.

[110] Guo, F., et al., *Inhibition of tRNA(3)(Lys)-primed reverse transcription by human APOBEC3G during human immunodeficiency virus type 1 replication.* J Virol, 2006. 80(23): p. 11710-22.

[111] Guo, F., et al., *The interaction of APOBEC3G with human immunodeficiency virus type 1 nucleocapsid inhibits tRNA3Lys annealing to viral RNA.* J Virol, 2007. 81(20): p. 11322-31.

[112] Li, X.Y., et al., *APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription.* J Biol Chem, 2007. 282(44): p. 32065-74.

[113] Valente, S.T. and S.P. Goff, *Inhibition of HIV-1 gene expression by a fragment of hnRNP U.* Mol Cell, 2006. 23(4): p. 597-605.

[114] Afonina, E., M. Neumann, and G.N. Pavlakis, *Preferential binding of poly(A)-binding protein 1 to an inhibitory RNA element in the human immunodeficiency virus type 1 gag mRNA.* J Biol Chem, 1997. 272(4): p. 2307-11.

[115] Konig, R., et al., *Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication.* Cell, 2008. 135(1): p. 49-60.

[116] Gallois-Montbrun, S., et al., *Antiviral protein APOBEC3G localizes to ribonucleoprotein complexes found in P bodies and stress granules.* J Virol, 2007. 81(5): p. 2165-78.

[117] Feliciello, A., M.E. Gottesman, and E.V. Avvedimento, *The biological functions of A-kinase anchor proteins.* J Mol Biol, 2001. 308(2): p. 99-114.

[118] Takahashi, H., et al., *Human immunodeficiency virus type 1 reverse transcriptase: enhancement of activity by interaction with cellular topoisomerase I.* Proc Natl Acad Sci U S A, 1995. 92(12): p. 5694-8.

[119] Shoya, Y., et al., *Human topoisomerase I promotes HIV-1 proviral DNA synthesis: implications for the species specificity and cellular tropism of HIV-1 infection.* Proc Natl Acad Sci U S A, 2003. 100(14): p. 8442-7.

[120] Drake, F.H., et al., *Purification of topoisomerase II from amsacrine-resistant P388 leukemia cells. Evidence for two forms of the enzyme.* J Biol Chem, 1987. 262(34): p. 16739-47.

[121] Rainwater, R. and K. Mann, *Differential increase in topoisomerase II in simian virus 40-infected cells.* J Virol, 1990. 64(2): p. 918-21.

[122] Benson, J.D. and E.S. Huang, *Two specific topoisomerase II inhibitors prevent replication of human cytomegalovirus DNA: an implied role in replication of the viral genome.* J Virol, 1988. 62(12): p. 4797-800.

[123] Mandraju, R.K. and A.K. Kondapi, *Regulation of topoisomerase II alpha and beta in HIV-1 infected and uninfected neuroblastoma and astrocytoma cells: involvement of distinct nordihydroguaretic acid sensitive inflammatory pathways.* Arch Biochem Biophys, 2007. 461(1): p. 40-9.

[124] Lokeswara Balakrishna, S., N. Satyanarayana, and A.K. Kondapi, *Involvement of human topoisomerase II isoforms in HIV-1 reverse transcription.* Arch Biochem Biophys, 2013. 532(2): p. 91-102.

[125] Muller, W.E., et al., *Effect of nonviable preparations from human immunodeficiency virus type 1 on nuclear matrix-associated DNA polymerase alpha and DNA topoisomerase II activities.* J Acquir Immune Defic Syndr, 1990. 3(1): p. 1-10.

[126] Kondapi, A.K., et al., *A biochemical analysis of topoisomerase II alpha and beta kinase activity found in HIV-1 infected cells and virus.* Arch Biochem Biophys, 2005. 441(1): p. 41-55.

[127] Ponraj, K., et al., *HIV-1 associated Topoisomerase IIbeta kinase: a potential pharmacological target for viral replication.* Curr Pharm Des, 2013. 19(26): p. 4776-86.

[128] Kondapi, A.K., N. Satyanarayana, and A.D. Saikrishna, *A study of the topoisomerase II activity in HIV-1 replication using the ferrocene derivatives as probes.* Arch Biochem Biophys, 2006. 450(2): p. 123-32.

[129] Filion, L.G., et al., *Inhibition of HIV-1 replication by daunorubicin.* Clin Invest Med, 1993. 16(5): p. 339-47.

[130] Bouille, P., et al., *Antisense-mediated repression of DNA topoisomerase II expression leads to an impairment of HIV-1 replicative cycle.* J Mol Biol, 1999. 285(3): p. 945-54.

[131] Farnet, C.M. and F.D. Bushman, *HIV-1 cDNA integration: requirement of HMG I(Y) protein for function of preintegration complexes in vitro.* Cell, 1997. 88(4): p. 483-92.

[132] Strebel, K., J. Luban, and K.T. Jeang, *Human cellular restriction factors that target HIV-1 replication.* BMC Med, 2009. 7: p. 48.

[133] Malim, M.H., *APOBEC proteins and intrinsic resistance to HIV-1 infection.* Philos Trans R Soc Lond B Biol Sci, 2009. 364(1517): p. 675-87.

[134] Luo, K., et al., *Cytidine deaminases APOBEC3G and APOBEC3F interact with human immunodeficiency virus type 1 integrase and inhibit proviral DNA formation.* J Virol, 2007. 81(13): p. 7238-48.

[135] Wang, X., et al., *The cellular antiviral protein APOBEC3G interacts with HIV-1 reverse transcriptase and inhibits its function during viral replication.* J Virol, 2012. 86(7): p. 3777-86.

[136] Miyakawa, K., et al., *ASK1 restores the antiviral activity of APOBEC3G by disrupting HIV-1 Vif-mediated counteraction.* Nat Commun, 2015. 6: p. 6945.

[137] Leng, J., et al., *A cell-intrinsic inhibitor of HIV-1 reverse transcription in CD4(+) T cells from elite controllers.* Cell Host Microbe, 2014. 15(6): p. 717-28.

[138] Khiytani, D.K. and N.J. Dimmock, *Characterization of a human immunodeficiency virus type 1 pre-integration complex in which the majority of the cDNA is resistant to DNase I digestion.* J Gen Virol, 2002. 83(Pt 10): p. 2523-32.

[139] Lin, C.W. and A. Engelman, *The barrier-to-autointegration factor is a component of functional human immunodeficiency virus type 1 preintegration complexes.* J Virol, 2003. 77(8): p. 5030-6.

[140] Schweitzer, C.J., et al., *Proteomic analysis of early HIV-1 nucleoprotein complexes.* J Proteome Res, 2013. 12(2): p. 559-72.

[141] Chen, H. and A. Engelman, *The barrier-to-autointegration protein is a host factor for HIV type 1 integration.* Proc Natl Acad Sci U S A, 1998. 95(26): p. 15270-4.

[142] Llano, M., et al., *An essential role for LEDGF/p75 in HIV integration.* Science, 2006. 314(5798): p. 461-4.

[143] Ciuffi, A., et al., *A role for LEDGF/p75 in targeting HIV DNA integration.* Nat Med, 2005. 11(12): p. 1287-9.

[144] Wang, W., et al., *Purification and biochemical heterogeneity of the mammalian SWI-SNF complex.* EMBO J, 1996. 15(19): p. 5370-82.

[145] Howard, M.T. and J.D. Griffith, *A cluster of strong topoisomerase II cleavage sites is located near an integrated human immunodeficiency virus.* J Mol Biol, 1993. 232(4): p. 1060-8.

[146] Pommier, Y., et al., *DNA topoisomerases I & II cleavage sites in the type 1 human immunodeficiency virus (HIV-1) DNA promoter region.* Biochem Biophys Res Commun, 1994. 205(3): p. 1601-9.

[147] De Rijck, J., et al., *Lentiviral nuclear import: a complex interplay between virus and host.* Bioessays, 2007. 29(5): p. 441-51.

[148] Jayappa, K.D., Z. Ao, and X. Yao, *The HIV-1 passage from cytoplasm to nucleus: the process involving a complex exchange between the components of HIV-1 and cellular machinery to access nucleus and successful integration.* Int J Biochem Mol Biol, 2012. 3(1): p. 70-85.

[149] McDonald, D., et al., *Visualization of the intracellular behavior of HIV in living cells.* J Cell Biol, 2002. 159(3): p. 441-52.

[150] Hoelz, A., E.W. Debler, and G. Blobel, *The structure of the nuclear pore complex.* Annu Rev Biochem, 2011. 80: p. 613-43.

[151] Pereira, C.F., et al., *HIV taken by STORM: super-resolution fluorescence microscopy of a viral infection.* Virol J, 2012. 9: p. 84.

[152] Lelek, M., et al., *Superresolution imaging of HIV in infected cells with FlAsH-PALM.* Proc Natl Acad Sci U S A, 2012. 109(22): p. 8564-9.

[153] Gallay, P., et al., *HIV-1 infection of nondividing cells through the recognition of integrase by the importin/karyopherin pathway.* Proc Natl Acad Sci U S A, 1997. 94(18): p. 9825-30.

[154] Ao, Z., et al., *Importin alpha3 interacts with HIV-1 integrase and contributes to HIV-1 nuclear import and replication.* J Virol, 2010. 84(17): p. 8650-63.

[155] Fassati, A., et al., *Nuclear import of HIV-1 intracellular reverse transcription complexes is mediated by importin 7.* EMBO J, 2003. 22(14): p. 3675-85.

[156] Ao, Z., et al., *Interaction of human immunodeficiency virus type 1 integrase with cellular nuclear import receptor importin 7 and its impact on viral replication.* J Biol Chem, 2007. 282(18): p. 13456-67.

[157] Christ, F., et al., *Transportin-SR2 imports HIV into the nucleus.* Curr Biol, 2008. 18(16): p. 1192-202.

[158] Larue, R., et al., *Interaction of the HIV-1 intasome with transportin 3 protein (TNPO3 or TRN-SR2).* J Biol Chem, 2012. 287(41): p. 34044-58.

[159] Woodward, C.L., et al., *Integrase interacts with nucleoporin NUP153 to mediate the nuclear import of human immunodeficiency virus type 1.* J Virol, 2009. 83(13): p. 6522-33.

[160] Fouchier, R.A., et al., *Interaction of the human immunodeficiency virus type 1 Vpr protein with the nuclear pore complex.* J Virol, 1998. 72(7): p. 6004-13.

[161] Le Rouzic, E., et al., *Docking of HIV-1 Vpr to the nuclear envelope is mediated by the interaction with the nucleoporin hCG1.* J Biol Chem, 2002. 277(47): p. 45091-8.

[162] Matreyek, K.A. and A. Engelman, *Viral and cellular requirements for the nuclear entry of retroviral preintegration nucleoprotein complexes.* Viruses, 2013. 5(10): p. 2483-511.