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HIV-1 entry: Duels between Env and host antiviral transmembrane proteins on the surface of virus particles

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Abstract

Human Immunodeficiency Virus type-1 (HIV-1) is the causative agent of AIDS. Its entry step is mediated by the envelope glycoprotein (Env). During the entry process, Env vastly changes its conformation. While non-liganded Env tends to have a closed structure, receptor-binding of Env opens its conformation, which leads to virus-cell membrane fusion. Single-molecule fluorescence resonance energy transfer (smFRET) imaging allows observation of these conformational changes on the virion surface. Nascent HIV-1 particles incorporate multiple host transmembrane proteins, some of which inhibit the entry process. The Env structure or its dynamics may determine the effectiveness of these antiviral mechanisms. Here, we review recent findings about the Env conformation changes on virus particles and inhibition of Env activities by virion-incorporated host transmembrane proteins.

Introduction

Human Immunodeficiency Virus type-1 (HIV-1) is the causative agent of Acquired Immunodeficiency Syndrome (AIDS). Currently over 38 million people in the world are living with HIV-1 (2020 https://www.who.int/news-room/fact-sheets/detail/hiv-aids). Combination antiretroviral therapy has allowed the control of viral loads in infected individuals and thereby the steady decrease of new HIV-1 infection and mortality. However, in the absence of effective vaccines and cure regimens, the emergence of drug-resistant mutant viruses and long-term effects of the current anti-HIV-1 drugs remain as major challenges to human health and society. The HIV-1 entry, mediated by the viral envelope glycoprotein Env, is one of the promising targets for the development of new antiretrovirals due to its importance in HIV-1 replication cycle. Detailed understanding of the native structure and dynamics of Env, the sole viral protein exposed on the surface of infectious

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virus particles, is intensely pursued to help the efforts to develop vaccines and small compounds that block HIV-1 entry.

Overview of HIV-1 Entry

HIV-1 is a retrovirus that infects CD4$^+$ T cells [1], macrophages [2], and dendritic cells [3]. HIV-1 entry consists of attachment of a virus particle to target cells and subsequent fusion of viral and target cell membranes. Both virus attachment and fusion are driven by Env. Env is synthesized as a precursor protein, gp160 and processed into the surface glycoprotein gp120 and the transmembrane glycoprotein gp41. Heterodimers of gp120 and gp41 form the trimer, which is a functional unit on the surface of infectious virus particles [4]. Virus attachment is mediated by several cellular factors, but binding of gp120 to the host receptor CD4 is central to the attachment process on the surface of target cells [5]. The binding between gp120 and CD4 triggers conformational changes in Env that allow for a subsequent interaction of gp120 with coreceptors CCR5 or CXCR4 [6–8]. This interaction induces extension of gp41 and insertion of its N-terminal fusion peptide to the target cell membrane. Finally, gp41 refolds into a hairpin-like conformation in a six-helix bundle structure, leading to fusion of viral and host cell membranes [7–9].

Coreceptor usage determines HIV-1 cell tropism. In early studies, HIV-1 strains efficiently replicating in T cell lines and macrophages were called T cell-tropic and macrophage-tropic (M-tropic) strains, respectively. Later, it was found that the viruses that grow efficiently in T cell lines use CXCR4 as the coreceptor, whereas M-tropic viruses utilize CCR5. Thus, the classification of HIV-1 into two types, X4 T cell-tropic and R5 M-tropic viruses, had been commonly used. However, since CCR5-using viruses also grow in primary CD4$^+$ T cells, and not all CCR5-dependent virus isolates efficiently infect macrophages, the binary link between coreceptor usage (CXCR4 versus CCR5) and cell tropism (T cell versus macrophage) is no longer accepted. Currently, HIV-1 is classified into three types: (i) R5 T cell-tropic, (ii) X4 T cell-tropic, and (iii) R5 M-tropic [10]. Most of HIV-1 isolated at early stages of infection is R5 T-tropic virus [11]. This type of viruses requires high levels of CD4 on the surface of target cells for fusion. Therefore, the major target of these viruses is CD4$^+$ T cells but not macrophages, which express a low density of CD4 compared to CD4$^+$ T cells [12]. X4 T cell-tropic viruses emerge at late stages of HIV-1 infection in a proportion of HIV-1-infected patients. This coreceptor switch correlates with disease progression and immune activation as evident from the increase in HLA-DR$^+$ and HLA-DR$^+$CD38$^+$ populations in CD4$^+$ T cells [13–16]. R5 M-tropic viruses are mainly detected in brain tissues and the cerebrospinal fluid [17,18]. These viruses are able to enter cells expressing a low density of CD4 on the surface (i.e. macrophages) efficiently [10]. To enable efficient entry into CD4^{low} target cells, Env derived from these viruses binds to CD4 with a high affinity [19,20]. Thus, the adaptation to the low CD4 density determines the cell tropism of R5 viruses for macrophages versus primary CD4$^+$ T cells. As for the choice of coreceptors (i.e. R5 versus X4), the gp120 V3 loop, which is exposed upon Env-CD4 binding [21,22] and forms a part of the interface in Env-coreceptor binding [23], is a major molecular determinant [24]. Mechanistic aspects of Env-coreceptor binding had been hampered due to the lack of structural information, but recent studies finally revealed the details of the gp120-coreceptor interactions [21,23,25,26].

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HIV-1 fusion takes place at multiple sites [27]. Early studies suggested that HIV-1 entry takes place at the plasma membrane based on the observations, among others, that HIV-1 can fuse with target cells at neutral pH and that mutagenesis of the CD4 cytoplasmic tail that impairs ligand-induced internalization does not block HIV-1 infection [1,28,29]. However, more recent evidence obtained through inhibition of clathrin-mediated endocytosis, time-course analysis of surface accessibility of cell-associated viruses versus virus-cell fusion, and/or live cell imaging supports the contribution of virion endocytosis to HIV-1 infection [30–35]. Yet, whether endocytosis plays a role in productive HIV-1 entry into $CD4^+$ T cells is still debated due to the contradictory results obtained with this cell type [30,35–37]. The cell activation status also seems to affect the frequency of virus fusion at endosomes in primary CD4+ T cells [30]. Macropinocytosis, a form of clathrin-independent endocytosis, has been shown to contribute to HIV-1 entry into macrophages [38,39], but the details remain to be elucidated [27].

Considering the importance of the HIV-1 entry step in virus replication cycle and hence in development of therapeutic and preventive strategies, it is not surprising that a tremendous amount of research has been performed on many aspects of HIV-1 entry. For more comprehensive understanding on HIV-1 entry, Env structures, and strategies to block the entry process, readers are directed to recent excellent reviews [5,40–42]. In the subsequent sections, we will highlight recent findings in two aspects of the entry process, the conformational dynamics of Env and the effects of virus-incorporated host proteins.

Conformational dynamics of Env

The Env trimer on the surface of infectious native virions is structurally flexible. Pre-fusion Env trimers are thought to adopt a closed structure, which, along with sequence variations and heavy glycosylation, is likely to allow HIV-1 to evade the host immune system because key functional regions of Env are hidden [43]. Once Env binds to CD4, the closed structure transitions toward an open conformation to expose the co-receptor binding site [21,22,44–47] (also see above). However, unliganded Env trimers also spontaneously transition between closed, open, and intermediate conformations.

Single-molecule fluorescence resonance energy transfer (smFRET) imaging performed using the total internal reflection fluorescence microscopy has allowed real-time monitoring of this conformational change of Env on the surface of native virions occurring in the milliseconds-to-seconds range [48–51]. Non-liganded Env on the native virions shows three distinct conformations, low-FRET (State 1), intermediate-FRET (State 3), and high-FRET (State 2) conformations (Figure 1) [48,52,53]. State 1 likely represents the pre-triggered closed conformation. State 2 is an intermediate conformation, which is also observed for the non-CD4-bound Env protomer in an asymmetric Env trimer where other protomer(s) is bound to CD4. State 3 is observed with the Env protomer bound to CD4 and hence likely to be in an open conformation.

Notably, non-liganded Env proteins of primary isolate strains JR-FL and BG505 display the signal for State 1 conformation more abundantly than Env of a lab-adapted HIV-1 strain NL4–3 [48,52]. Therefore, compared to the lab-adapted HIV-1 Env, Env trimers of the

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primary isolates likely spend a longer time in closed structures in the absence of ligands. The dynamic nature of Env structures has implications in development of vaccines aimed at eliciting broadly neutralizing antibodies (bNAbs). As noted in the tier classification of HIV-1 neutralization phenotypes [54], Env trimers that have open conformation as observed for lab adapted strains are readily blocked by non-bNAb antibodies (Tier 1A). In contrast, Env trimers of most circulating strains, which are the high priority target for bNAb vaccines, are associated with a predominantly closed conformation (Tier 2/3). Antibodies that neutralize Tier 1A viruses are often unable to neutralize Tier 2/3 viruses.

Transition of Env conformational states is likely to be mediated by various amino acid residues in gp120. Introducing an amino acid substitution in the V1/V2 loop or the β20-β21 region of JR-FL gp120 reduces State 1 conformation and increases State 2 and State 3 conformations [55,56]. These data in combination with the outcomes of other approaches indicate that the V1/V2 loops and the β20-β21 element, which are located at the trimer apex and near the CD4 contact site, respectively, play a role in maintaining the closed structure of Env. In addition, substitutions of specific amino acids in several regions of gp120 prevent transitioning of Env conformation from State 1 to State 2 and State 3 even when gp120 is bound to CD4 [57]. These and single particle cryoEM results identify the gp120 allosteric network that is involved in conformational changes of Env upon CD4 binding. The smFRET assay has also revealed the conformational states of Env that is bound to bNAbs or HIV-1 entry inhibitors [48,53]. Binding of Env with most of bNAbs stabilizes State 1 conformation despite the fact that the bNAbs recognize different epitopes. Similarly, BMS-626539, one of HIV-1 entry inhibitors, also increases State 1 conformation of Env. Therefore, stabilization of State 1 and/or inhibition of the transition of Env from State 1 to downstream conformations is a promising strategy for inhibition of HIV-1 entry.

Inhibition of HIV-1 entry by virion-incorporated host transmembrane proteins

In addition to Env, HIV-1 particles incorporate various host transmembrane proteins during the assembly at the plasma membrane. When incorporated into virions, some of the transmembrane proteins, such as ICAM-1, can promote HIV-1 entry through interactions with receptors on the surface of target cells [58]. HIV-1 also incorporates transmembrane proteins that inhibit the HIV-1 entry step. Earlier studies showed that tetraspanin proteins, which are efficiently incorporated into HIV-1 particles and inhibit cell-cell fusion [59,60], suppress post-virus attachment entry step(s) in a strain-specific manner [61]. Recent studies have identified an increasing number of virion-incorporated host transmembrane proteins that inhibit HIV-1 entry process (Figure 2), including serine incorporators (SERINCs) [62,63], interferon inducible transmembrane (IFITM) proteins [64–66], P-selectin glycoprotein ligand-1 (PSGL-1), and CD43 [67,68].

SERINCs.

SERINCs are multipass transmembrane proteins. It was originally reported that these proteins play a role in serine incorporation and promoting phosphatidylserine (PS) and sphingomyelin biosynthesis [69]. At least SERINC3 and 5 are known to prevent HIV-1

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infection, and SERINC5 has the stronger antiviral activity than SERINC3 [62,63]. SERINCs are counteracted by Nef, one of accessary proteins of HIV-1. In the absence of Nef, SERINCs are incorporated into progeny virions. The virion-incorporated SERINCs restrict HIV-1-cell fusion [62,63]. Although the exact molecular mechanism(s) by which SERINCs inhibit HIV-1 infection remains to be determined, SERINC5 reduces HIV-1 infectivity in a manner dependent on Env [62,63,70]. A correlation between the openness of Env trimer and the sensitivity to SERINCs (or Nef dependence) has been observed when Envs from different strains are compared [70–72]. Moreover, CD4, which induces open Env conformation (see above), was observed to sensitize a Tier 3 Env to SERINC5 [72]. Furthermore, the cytoplasmic tail (CT) of Env is required for the sensitivity to SERINC5 [73]. Upon deletion of the CT, which is known to alter Env conformation [74], HIV-1 is completely insensitive to SERINC5 [73]. Based on the sensitivity to neutralization by antibodies, the authors suggested that the CT-deleted Env may display a more closed conformation and thereby evade SERINC5-mediated inhibition [73]. However, the Env trimer propensity to adopt an open conformation as assessed by antibody neutralization does not appear to be the sole determinant for the SERINC sensitivity [75].

Approaches based on antibody binding also revealed that SERINC5 modifies the conformation of SERINC-sensitive Env on the surface of virions [76,77]. The changes in Env conformation include ones in gp41, perhaps leading to suppression of the fusion pore formation between viruses and target cells as well as spontaneous inactivation of Env [78]. Suppression of fusion pore formation by SERINCs is further examined in detail using the cryo-electron tomography of giant plasma membrane vesicles, which enabled observation of each step in the HIV-1 fusion process. This approach demonstrated that virion-incorporated SERINC3 and 5 stall HIV-1 fusion at the hemifusion and abnormal early fusion steps and inhibit opening of fusion pores [79]. Another line of investigation suggests a role for interactions between Env trimers; a 3D superresolution microscopy technique showed that SERINC5 blocks the formation of Env clusters on the surface of virus particles, which promotes efficient HIV-1 entry [80], without affecting Env incorporation [81]. The deletion of Env CT alters the distribution pattern of Env clusters [80], and Env clustering requires its interactions with cholesterol on the virus particles through the CT [82]. Therefore, considering that the CT-deleted Env is insensitive to SERINC5 [73], SERINC5 might affect the interactions of the Env CT with cholesterol directly or indirectly via changes in other lipids that interact with cholesterol. Nevertheless, the two restriction mechanisms, that is, the modification of Env conformation and the inhibition of Env clustering, are unlikely to require altering lipid composition of virus particles because lipid mass spectrometry revealed that SERINC5 does not affect steady-state lipid composition of virus-producing cells and virions [83]. However, SERINCs may still affect lipid clustering or inter-leaflet distribution in the viral envelop membrane. In addition, it is currently unknown whether these mechanisms postulated for the antiviral function of SERINC5 are mutually exclusive or not.

IFITMs.

IFITM proteins are small transmembrane proteins consisting of a transmembrane domain, a hydrophobic intra-membrane-associated domain, and an intervening highly conserved

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intracellular loop [84,85]. Among IFITM proteins, IFITM1, 2, and 3 inhibit HIV-1 infection [84–86]. Earlier studies suggested that these IFITM proteins modulate fluidity or rigidity of target cell membranes, resulting in inhibition of virus-cell fusion [87–90]. Notably, it has been reported that CXCR4- and CCR5-dependent HIV-1 strains show different sensitivity to IFITM1 versus IFITM2/3; CXCR4-dependent viruses are more sensitive to IFITM2/3, whereas CCR5-requiring viruses are more susceptible to IFITM1 [91]. Since IFITM proteins show different subcellular localization patterns [91–93], it was suggested that the site of coreceptor-triggered HIV-1 fusion might correlate with the sensitivity to each IFITM protein [91]. Of note, endocytosis inhibitors diminish the inhibitory effect of IFITM2 and 3 on infection of primary isolates, suggesting that the viruses utilize endocytosis for entry and that IFITM2 and IFITM3 could inhibit fusion to the endosomal membrane [91]. Transmitted/founder (T/F) viruses, which are isolated from recently infected patients and CCR5-dependent, are shown to be resistant to all IFITM proteins expressed in target cells [91,94,95]. However, the sensitivity of T/F viruses to restriction by IFITM proteins is a matter of debate; a more recent study showed that IFITM proteins in target cells inhibit infection of T/F viruses [96].

In addition to the role of IFITM proteins in target cells, IFITM proteins in virus-producing cells also attenuate HIV-1 fusion through inhibition of Env processing or when these proteins are incorporated into virions [64–66,97]. Therefore, IFITM proteins suppress HIV-1 fusion in either target cells or virus-producing cells. Similar to SERINC5 [70], Env, in particular the V3 loop, determines the sensitivity to IFITM3 [94]. However, it appears that IFITM3 and SERINC5 target Envs that may differ in sampling of conformations. When a panel of Env isolates is compared for sensitivities to a CD4 blocking antibody and soluble CD4 (sCD4), which inform about the Env-CD4 binding affinity, SERINC5-sensitive Envs tend to show stronger CD4 binding, whereas no such correlation is observed between the IFITM3 sensitivity and CD4 binding [98]. Moreover, upon binding to a CD4 mimetic that stabilizes the open conformation [99], an Env isolate resistant to both SERINC5 and IFITM3 is sensitized markedly to SERINC5 but only modestly, if any, to IFITM3 [98]. The mechanism(s) by which the presence of IFITM proteins in virus-producing cells and/or progeny virus particles inhibits entry of progeny virions remains to be determined. While a reduction in virion-associated Env levels upon IFITM3 expression in virus-producing cells has been observed in some studies [94,97,100,101], others did not detect a difference in Env incorporation using different experimental systems [64,66,91,102]. Furthermore, even in the study where reduced Env incorporation is observed, the impact of IFITM3 on virion infectivity cannot be fully explained by the Env quantity in virions [100,101]. Whether virion-incorporated IFITM proteins affect viral membrane rigidity, as is shown to occur in target cells [89,90,101] or whether they alter other properties of the envelope membrane is unknown. However, virion incorporation of IFITM3 increases the sensitivity of Env to several neutralizing antibodies [102], suggesting that IFITM3 directly or indirectly affects the Env conformation on the virus particle surface.

PSGL-1 and CD43.

PSGL-1 is a mucin-like type I transmembrane glycoprotein, and CD43 is a sialomucin type I transmembrane glycoprotein [103–105]. These proteins are primarily expressed on

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the surface of lymphocytes, mediating cell tethering and rolling through interactions with selectin family proteins to promote cell migration into inflamed tissue [106]. Both PSGL-1 and CD43 are also known to prevent cell-cell interactions via its extended extracellular domains estimated to be 45–50 nm long [107–110]. We previously showed that PSGL-1 and CD43 associate with HIV-1 structural protein Gag at the plasma membrane of virus-producing cells and get incorporated into nascent virions [111–113]. Later, both PSGL-1 and CD43 were identified as antiviral factors that reduce infectivity of progeny virions when it is expressed by virus-producing cells [114,115]. These proteins share some of characteristics of restriction factors, such as a signature of positive selection during primate evolution [114,115] and downregulation upon HIV-1 infection [67,68,115–117]. Therefore, PSGL-1 and CD43 could potentially be classified as restriction factors. More recently, Fu et al. and our group discovered that virion-incorporated PSGL-1 and CD43 inhibit the HIV-1 entry step [67,68]. These studies revealed that unlike SERINCs and IFITM proteins in virus particles, PSGL-1 and CD43 suppress virus entry at the step of virus attachment to target cells and that they inhibit the attachment regardless of molecules mediating virus-cell binding. This inhibition requires the intact extended extracellular domain at least for PSGL-1. Since these extracellular domains are longer than combined length of receptor and ligand pairs mediating HIV-1-cell binding, these results suggest that virion-incorporated PSGL-1 and CD43 create a physical barrier that sterically prevents HIV-1 from binding to target cells or inserting its fusion peptide into the target cell membrane. Indeed, the cryo electron tomography in the presence of fusion inhibitors revealed that the distance between a target cell and cell-attached HIV-1 by extended pre-hairpin intermediate Env is 15.6±2.8 nm [118], that is, ~3 fold shorter than the lengths of PSGL-1 and CD43. Extracellular domains of PSGL-1 and CD43 are highly *O*-glycosylated [104,105,119], and the *O*-glycosylation is thought to contribute to the maintenance of the extended structure [120]. Other mucins and mucin-like proteins, such as CD164, PODXL1, PODXL2, CD34, TMEM123, and MUC1, have a similar structure, that is, highly *O*-glycosylated extended extracellular domains. These proteins, collectively termed SHREK proteins, are also incorporated into progeny HIV-1 and reduce HIV-1 infectivity at least in overexpression experiments [121]. These observations support the possibility that virion-incorporated transmembrane proteins that have the elongated extracellular domains can sterically hinder virus attachment to target cells.

As an additional mechanism, PSGL-1-mediated inhibition of Env incorporation into nascent virions at the plasma membrane has been proposed based on the experiments in which 293T or Jurkat cells were used as virus-producing cells [67,122]. Consistent with this possibility, in virus-producing Jurkat cells, PSGL-1 interacts with Env gp41 and alters the localization of gp41. However, in another study in which HIV-1 is produced from PBMCs, amounts of virion-incorporated PSGL-1 do not inversely correlate with amounts of Env in the virions [123]. Therefore, whether PSGL-1 affects Env incorporation into virions in primary $CD4^+$ T cells warrants future investigation. Likewise, another proposed antiviral mechanism in which PSGL-1 inhibits actin depolymerization in the virions, thereby reducing virion infectivity [122], awaits validation using primary $CD4^+$ T cells. Finally, PSGL-1 in target cells was suggested restrict HIV-1 reverse transcription through inhibition of actin disassembly [115,122]. However, another group observed that PSGL-1 expression in target cells did not

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block any early HIV-1 replication step including reverse transcription [67]. Therefore, it remains to be determined which aspect of experimental conditions caused this discrepancy and to what extent PSGL-1 expressed in target cells inhibits reverse transcription under physiological conditions.

Concluding remarks

Understanding of conformational dynamics of Env on the virions is important not only for the development of neutralizing antibodies and antiretrovirals but also for elucidating the action of host-encoded antiviral proteins. The smFRET approach visualized real-time conformational dynamics of non-liganded and CD4-bound, antibody-bound, or small compound-bound Env trimer on the surface of native virions and defined the predominant conformation of the Env trimer in the milliseconds-to-seconds time scale. This technique identified the previously uncharacterized asymmetric intermediate state, key Env residues regulating the conformational transitioning of Env, and the conformational state of bNAb-bound or entry inhibitor-bound Env. The presence of asymmetric intermediate State 2 suggests that Env trimers more intricately rearrange the conformation upon receptor and coreceptor binding during HIV-1 entry than we previously imagined. Further understanding of detailed mechanism(s) regulating conformational changes within an Env protomer and between protomers, the latter using different approaches [124], will potentially allow us to develop new entry inhibitors and vaccine strategies. It is also important to determine the high-resolution structure of State 1 Env, since it may explain the mechanisms of inhibition by bNAbs and an entry inhibitor, which stabilize this state. Notably, high-resolution structures of gp120 obtained thus far are largely based on a soluble Env trimer engineered for stabilization (SOSIP) [21,22,44,125–131], which was found to be in the State 2 conformation [53].

Various virion-incorporated host transmembrane proteins that inhibit HIV-1 entry were recently identified. However, the molecular mechanism(s) by which these proteins suppress HIV-1 entry remain to be determined. For example, how SERINCs affect Env conformation and clustering is unclear. Whether IFITM proteins act on the behavior of Env trimers on the virus surface and whether they target Env in a specific conformation remain to be determined. Whether PSGL-1 and CD43 inhibit HIV-1 binding to target cells due to lengths or chemical properties of these proteins, for example, increased negative charge or heavy glycosylation, needs to be addressed. In addition, how these proteins get incorporated into infectious virus particles warrants future investigation. Elucidation of these mechanisms could offer insights into new therapeutic strategies.

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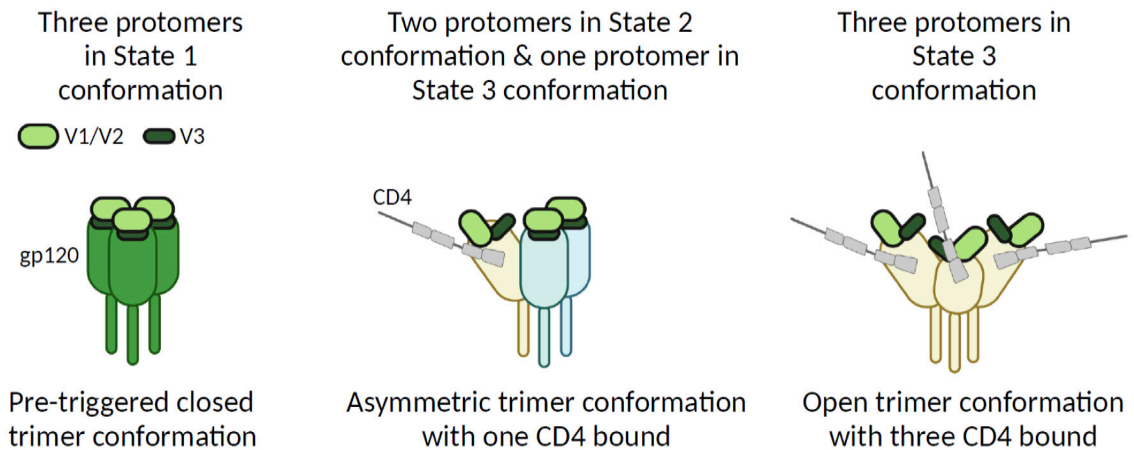

Figure 1. Conformational dynamics of the Env trimer.

smFRET imaging revealed three conformational states of Env protomers (State 1, State 2, and State 3). State 1 Env is likely to represent a non-liganded conformation in pre-triggered trimers that have a closed structure. State 2 represents an intermediate conformation, which is observed for the non-CD4-bound Env protomer in an asymmetric Env trimer where other protomer(s) is bound to CD4. State 3 Env is observed with the Env protomer bound to CD4, which has an open conformation. In non-liganded Env, the V1/V2 loop masks the V3 loop that binds to coreceptors, CCR5 and CXCR4. Once CD4 binds to Env, the V3 loop is exposed. High-resolution structure of State 1 Env has not been determined. Created with BioRender.com.

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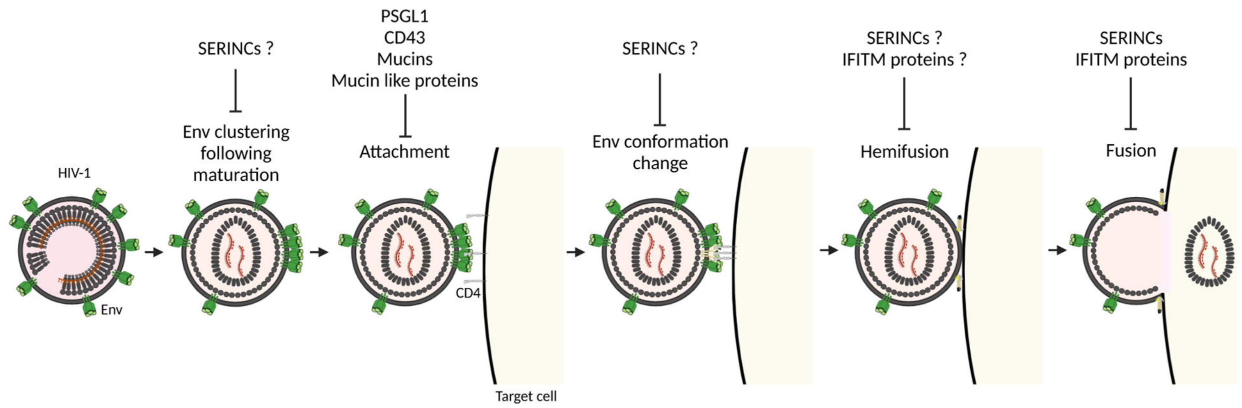

Figure 2. Inhibitory effect of antiviral proteins to HIV-1 entry steps.

SERINCs and IFITM proteins prevent fusion, and PSGL-1, CD43, mucins, and mucin-like proteins inhibit virus attachment to target cells. For clarity, coreceptors are not shown. Created with BioRender.com.