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Molecular Mechanism of HIV-1 Entry

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Abstract

HIV-1 envelope glycoprotein [Env; trimeric $(gp160)_3$ cleaved to $(gp120/gp41)_3$] attaches the virion to a susceptible cell and induces fusion of viral and cell membranes to initiate infection. It interacts with the primary receptor CD4 and coreceptor (e.g., chemokine receptor CCR5 or CXCR4) to allow viral entry by triggering large structural rearrangements and unleashing the fusogenic potential of gp41 to induce membrane fusion. Recent advances in structural biology of HIV-1 Env and its complexes with the cellular receptors have revealed molecular details of HIV-1 entry and yielded new mechanistic insights. In this review, I summarize our latest understanding of the HIV-1 membrane fusion process and discuss possible pathways for productive viral entry.

Keywords

HIV-1; envelope glycoprotein; viral entry; membrane fusion

General Aspects of HIV-1 Entry

Viral membrane fusion is the first key step for enveloped viruses to enter host cells and establish infection. Although membrane fusion is an energetically favorable process, there are high kinetic barriers when two membranes approach each other, mainly due to repulsive hydration forces [1,2]. For viral membrane fusion, free energy to overcome these kinetic barriers comes from refolding of virus-encoded fusion proteins, which can transition (after 'priming', usually by proteolytic cleavage, either of the fusion protein or of an accompanying 'chaperone') from a high-energy, metastable prefusion conformational state to a low-energy, stable postfusion state when triggered by an appropriate ligand [3-5]. The fusion protein for HIV-1 is its envelope glycoprotein (Env; Figure 1A), which is also an important target for development of vaccines and therapeutics. The Env polypeptide chain is produced as a precursor, gp160, which trimerizes to $(gp160)_3$ and then undergoes cleavage by a host furin-like protease into two noncovalently associated fragments: the receptor-binding fragment gp120 and the fusion fragment gp41 [6]. Three copies of each fragment constitute the mature viral spike $(gp120/gp41)_3$. A picture of HIV-1 membrane fusion

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mechanism has emerged from extensive biochemical and structural studies, as depicted in Figure 1B. Sequential binding of gp120 to the primary receptor CD4 and a coreceptor (e.g., CCR5 or CXCR4) is believed to induce a cascade of refolding events in gp41 [7,8]. Gp41, with its C terminal transmembrane (TM) segment in the viral membrane, adopts a prefusion conformation within the precursor gp160. Cleavage between gp120 and gp41 makes the protein metastable with respect to the postfusion conformation. When triggered by gp120 binding to the receptors, the N terminal fusion peptide (FP) of gp41 translocates and inserts into the target cell membrane. Subsequent rearrangements involve refolding of gp41 into a hairpin conformation, creating a six-helix bundle known as the postfusion conformation, which places the FP and TM segments at the same end of the molecule and effectively brings the two membranes together. Formation of hemifusion stalk and subsequent fusion pore ensues membrane fusion and entry of the viral capsid into the target cell.

Viral membrane fusion has been reviewed extensively elsewhere [3,4,9,10]. Recent advances in structural biology of HIV-1 Env and its complexes with the cellular receptors have revealed additional molecular details of HIV-1 entry and provided new mechanistic insights. In this review, I summarize our latest understanding of HIV-1 Env-mediated membrane fusion and discuss possible pathways for productive viral entry.

Major Players in HIV-1 Entry

HIV-1 Env

HIV-1 Env is a type I membrane protein sitting in the viral membrane, with 50% of its mass as carbohydrate [11]. Env has long resisted high-resolution structural analysis, particularly, in the context of the membrane. Both HIV-1 and related SIV (simian immunodeficiency virus) Env spikes have been visualized on the surface of the virion by cryo-electron tomography (cryo-ET) (Figure 2A), but only at low resolutions (~20 Å) [12-14]. Earlier high-resolution structural studies focused on fragments of gp120 and gp41, truncated in important ways to facilitate protein crystallization and atomic-structure determination. The gp120 ‘core’ – stripped of variable regions, V1–V2 and V3, as well as the N- and C-terminal segments – was initially crystallized in two forms: in a deglycosylated, CD4-bound form (HIV-1) in complex with an antigen-binding fragment (Fab) recognizing a CD4-induced (CD4i) epitope [15,16], and in an unliganded and fully glycosylated state (SIV) [17]. Subsequently, crystal structures were reported of the HIV-1 gp120 core with an intact V3 loop or N- and C-terminal extensions, as well as in the unliganded form [18-20]. A soluble fragment of the gp41 ectodomain, which lacked the fusion peptide, the membrane-proximal external region (MPER), and the immunodominant C–C loop, was also studied by X-ray crystallography and NMR [7,8,21-23]. The structures showed gp41 as a trimer of helical hairpins (or a six-helix bundle) in its most stable, postfusion conformation. All of these structures were instrumental for the working model of HIV-1 entry outlined in Figure 1B.

Since 2013, a soluble form of the trimeric Env ectodomain, initially derived from the sequence of a clade A HIV-1 isolate BG505 and known as SOSIP.664 with modifications including a disulfide crosslink between gp120 and gp41, an Ile/Pro change at residue 559 and a truncation at residue 664 [24], has led to structure determinations by both cryo-EM and X-ray crystallography [25-27] (Figure 2A), although the SOSIP modifications abolish

viral infectivity [28]. The cryo-EM structure of a detergent-solubilized clade B HIV-1 JR-FL EnvΔCT construct without the cytoplasmic tail (CT) has also been described [29] (Figure 2A). These trimer structures, representing an important contribution to our understanding of Env molecular architecture, show that the conformation of gp41 in the prefusion state is distinct from the postfusion six-helix bundle [6,7] (Figure 2A). The V1–V2 loops from three gp120 protomers form a trimeric cluster at the apex of the Env trimer, partially protecting the V3 loop that tucks underneath the apex. Regions near the viral membrane, such as the MPER, transmembrane domain (TMD), and CT are either not present or invisible in these structures.

The TMD of viral glycoproteins has usually been considered to be a passive membrane anchor and has been deleted for convenience in many previous studies of structure and biochemistry. Recent results on the TMD and its neighboring regions of HIV-1 Env in the context of lipid bilayer have painted a different picture. Several full-length HIV-1 Envs from difficult-to-neutralize HIV-1 primary isolates are conformationally homogeneous when expressed on the surface of human cells [30,31]. Their antigenicity correlates closely with antibody neutralization, with epitopes for most broadly neutralizing antibodies (bnAbs) exposed, and epitopes of all non-neutralizing antibodies concealed. Truncation of the CT from these homogeneous Envs has minimal impact on their fusogenic activity, but diminishes binding to trimer-specific bnAbs while exposing non-neutralizing epitopes on both gp120 and gp41 [30]. Other studies showed that the CT truncation had little effect on the Env antigenicity for certain HIV-1 isolates [32,33]. Nevertheless, these findings indicate that the CT has an unexpected role in constraining the conformational variability of the Env ectodomain, possibly through the TMD that connects them. The atomic structure of the TMD reconstituted in bicelles that mimic a lipid bilayer was then determined by NMR [34] (Figure 2A). The TMD forms a well-ordered trimer that protects three conserved arginines in the middle of the membrane. A set of mutations that disrupt the TMD trimer also alter antibody sensitivity of the ectodomain, confirming the critical role of the TMD to Env stability and antigenicity. These results may also explain why the antigenic properties of most recombinant, soluble Env preparations with the TMD deleted deviate from those of the native membrane-bound protein [24,31,35]. Furthermore, an NMR structure that contains both the TMD and MPER has recently been determined using the same bicelle system [36] (Figure 2A). Previous structural studies of a monomeric MPER peptide in detergent micelles suggested that the MPER might be buried in the viral membrane [37-39], but the MPER in the context of the TMD and lipid bilayer forms a trimeric assembly that is well ordered and not buried in the membrane, probably representing its prefusion conformation in the native Env. MPER mutations can also destabilize the Env ectodomain and influence its antigenic properties [36], consistent with the notion that the MPER is a control relay that modulates antigenic structure of the Env trimer.

A high-resolution structure of an intact HIV-1 Env, particularly in the context of membrane, is still needed for a full understanding of viral entry. Such a structure will also help to define the so-called native, prefusion state of Env, which has been an important but contentious issue in the HIV-1 vaccine field. Based on single-molecule fluorescence resonance energy transfer (smFRET) studies, it has been proposed that HIV-1 Env spontaneously transitions among three distinct conformations, corresponding to the prefusion state (State 1: low-

FRET), a default intermediate conformation (State 2: high-FRET), and the three CD4-bound conformation (State 3: intermediate-FRET), respectively [40-42]. The untriggered, prefusion state is probably the most appropriate vaccine candidate because it is recognized by most bnAbs and not by other strain-specific or non-neutralizing antibodies [24,30,35], although recent studies show that several bnAbs preferentially bind CD4-triggered Env [43]. Nevertheless, the smFRET data suggest that the soluble SOSIP trimer does not represent the native prefusion conformation [41,42]. This claim has been disputed by a double electron-electron resonance (DEER) spectroscopy study [44]. Moreover, the trimer-specific bnAbs (PG9, PG16, and PGT145) appear to stabilize the virion-associated Envs in the ‘state 1’ conformation [40,42]; the cryo-EM structures of SOSIP.664 Env in complex with these antibodies show no obvious differences from complexes with other antibodies [45]. A structure of the full-length Env in a membrane environment may be required to resolve this issue and hence to select the most relevant Env trimer immunogen for clinical studies.

Primary Receptor CD4

CD4 is a cell-surface glycoprotein containing four immunoglobulin domains (D1 to D4), and its ectodomain could project up to ~115 Å from the cell surface, with D1 at the membrane-distal end [46] (Figure 2B). CD4 plays an essential role in both T cell activation and HIV-1 infection. As a T cell coreceptor, CD4 interacts with major histocompatibility complex (MHC) class II molecules on the surface of antigen-presenting cells and helps to recruit a tyrosine kinase, $p56^{lck}$, to facilitate activation of helper T cells, thereby modulating adaptive immune responses [47,48]. As the primary receptor for HIV-1, CD4 mediates initial viral attachment and induces formation of the coreceptor binding site on gp120 [49,50]. Conformational changes in gp120, CD4, or both, are likely needed to bring the bound gp120 (or the virus) close to the coreceptor, a seven-transmembrane receptor embedded in the cell membrane.

Coreceptor

CCR5 and CXCR4 were first identified as the coreceptors for HIV-1 entry in 1996 [51-57]. They are chemokine receptors with seven transmembrane-spanning segments (7TMs) and belong to the family of G-protein-coupled receptors (GPCRs). Choice of coreceptor is the major determinant for viral tropism [58]. In general, viruses using CCR5 (R5 viruses) are responsible for viral transmission, and those using CXCR4 (X4 viruses) or both (dual-tropic; R5X4 viruses) emerge later during disease progression [59-62]. In addition to 7TM helices, both CCR5 and CXCR4 have an N-terminal segment and three extracellular loops (ECLs) exposed on the cell surface, as well as three intracellular loops (ICLs) and a cytoplasmic C-terminal tail. Crystal structures have been reported for a C-terminally truncated CXCR4 construct containing stabilizing mutations and a T4 lysozyme fusion, in complex with several different ligands, and for a similarly modified CCR5 construct containing a rubredoxin fusion in complex with either the anti-HIV drug maraviroc, or a modified chemokine, [5P7]CCL5 [63-66] (Figure 2C). These structures show the typical 7TM helical bundle topology seen for other GPCRs [67]. Consistent with the so-called two-site model [68], the N terminus of CXCR4 or CCR5 forms the chemokine-recognition site 1 (CRS1) to interact with the globular core domain of the chemokine, while their 7TM helices form a binding pocket (chemokine recognition site 2; CRS2) to accommodate the N terminus of the

chemokines. These structures yielded important information about the general architecture of CCR5 and CXCR4, as well as about their interactions with various ligands [63-66], but they fell short of explaining the molecular details of how they can function as HIV-1 coreceptors. In addition, other cell-surface receptors, including α4β7 integrin [69], the mannose-specific endocytosis receptor [70], DC-SIGN [71], glycolipid [72], and heparan sulfate [73], generally facilitate viral attachment to specific cell types or cell-to-cell spreading, but they do not actively promote membrane fusion.

Membrane

The HIV-1 membrane contains high levels of sphingolipids and cholesterol, similar to detergent-resistant membrane microdomains but different from typical host cell membranes [74], presumably reflecting the lipid composition of the highly selective virus-budding sites. Blocking biosynthesis of either cholesterol or sphingolipid in virus-producing cells, or removal of cholesterol from viral or target cell membranes, substantially reduces virus production and/or infectivity [74-78]. Although the underlying mechanism for the requirement of these lipids remains unknown, cholesterol depletion from the cell membrane has been shown to abolish gp120-induced association between CD4 and CCR5 [79], suggesting that cholesterol may be required for the formation of the CD4–Env–CCR5 complex. In addition, HIV-1 is generally believed to enter host cells by direct fusion with the plasma membrane, but there is also evidence suggesting that productive entry requires endocytosis [80].

Interactions among the Key Players

Productive viral entry requires concerted interactions among all the major players in the membrane fusion process. Recent advances in structural biology have revealed additional molecular details of these interactions.

Env–CD4

Our first view of the Env–CD4 interactions came from the crystal structure of the gp120 core in complex with 2D CD4 and a CD4-induced antibody 17b [16] (Figure 3A). The gp120 core contains two associated domains, named ‘inner’ and ‘outer’, which are stabilized in their mutual orientation by a four-strand β-sheet, the ‘bridging sheet’, formed by two β hairpins, the stem (β2–β3) of the V1–V2 loop from the inner domain, and a hairpin (β20-β21) that projects from the outer domain. D1 of CD4 interacts, primarily through its C” edge, with both the inner and outer domains, as well as with the bridging sheet [16]. A 3D reconstruction of the intact Env spike on the virion surface in complex with 2D CD4 and 17b by cryo-ET was also reported at ~20 Å resolution [14] (Figure 3B). The two domains of CD4 assume an orientation almost perpendicular to the threefold axis of Env trimer (probably parallel to the membrane plane). When compared to the unliganded Env trimer (Figure 2A), CD4 binding appears to induce major structural rearrangements, visible even at this resolution, leading to an outward rotation and displacement of gp120 protomers, accompanied by a rearrangement of the gp41 region along the threefold axis.

Using the SOSIP design, more recent studies have shown that the Env trimer adopts an open conformation when triggered by CD4 [81,82]. From the structure of the subtype B B41 SOSIP Env trimer in complex with CD4 and the CD4i 17b Fab at 3.7 Å resolution (Figure 3C), CD4-induced conformational changes include V1–V2 flip and displacement, V3 exposure, bridging sheet formation, repositioning of the fusion peptide and other gp41 rearrangements. The exposed V3 loop and the newly formed bridging sheet are likely to make up the binding site for the coreceptor [83,84]. It is noteworthy that the V1–V2 stem, perhaps together with the V1–V2 loop, would need to flip by 180° to match its configuration in the CD4-induced bridging sheet. A cryo-EM reconstruction was also generated at 5.2 Å to test the effect of CD4 alone on Env conformational changes [82]. Within the limits of the resolution, no major differences were observed in the absence or presence of the 17b Fab, suggesting that this antibody did not induce further conformational changes.

When the SOSIP Env trimer is further stabilized by another disulfide bond between mutated residues 201C and 433C (DS; [85]) to block CD4-induced changes and trapped by PGT145, only a single CD4 can bind to the trimer. In that complex, CD4 has an orientation (almost parallel to the trimer axis) very different from that of three CD4s bound to the Env trimer [86] (Figure 3D). It has been suggested that this complex may represent the initial contact between Env and CD4, leading to definition of a second CD4-binding site (CD4-BS2) on the inner domain of a neighboring gp120 protomer. There are no obvious differences, however, between the SOSIP trimer structure in this complex and the unliganded form. By the smFRET approach, distinct conformational states (State 2 and State 3) have been assigned to the gp120 conformation of an asymmetric trimer with one CD4 bound and the three-CD4-bound Env trimer, respectively [41]. Binding of a gp120–gp41 interface-specific antibody 8ANC195 to the CD4-bound SOSIP trimer leads to a partially open conformation [87,88], but whether this conformation represents a true intermediate in the opening of a native Env has not yet been determined.

Env–Coreceptor

The interaction of the membrane-embedded coreceptor with Env has eluded efforts to crystallize a complex. That barrier has finally been overcome by contemporary cryo-EM, as described in the next paragraph [89]. Various approaches had previously indicated that the footprint of a coreceptor on gp120 would include the V3 loop and the bridging sheet [83,84,90], and that gp120 would contact the coreceptor by the N terminus and ECL2 of CCR5; and by the N terminus, ECL2, and ECL3 of CXCR4 [91,92]. It has been suggested that the N terminus of the coreceptor makes direct contacts with the bridging sheet in gp120 and that the tip of the V3 loop inserts into the CRS2 of the coreceptor formed by the 7TM helices [84]. The interactions of the V3 loop with CCR5 or CXCR4 have also been modeled by molecular dynamics simulation and free energy calculation [64,66,93,94]. Tyrosine sulfation at the N terminus can only enhance HIV-1 entry for CCR5, but not for CXCR4 [95,96]. The C-terminal tail, containing palmitoylation motifs and several phosphorylation sites, is involved in cell signaling, but it seems not to be required for its HIV-1 coreceptor function [97]. Chemokines such as MIP-1α, MIP-1β, CCL5/RANTES, and CXCL12/SDF-1 all can efficiently block gp120 binding and prevent viral infection [98-100], consistent with an overlap of the two binding sites on a coreceptor.

Thanks to recent advances in cryo-EM, the long-awaited structure of an intact and fully glycosylated gp120 in complex with 4D CD4 and an unmodified human CCR5 has finally been reported [89]. As postulated previously [63,64], there are indeed two major contacting interfaces between gp120 and CCR5 (Figure 4). First, the V3 loop of gp120 inserts into the CRS2 of CCR5 and makes direct contacts with all the 7TM helices. The conserved ^{310}GPGR(Q)^{313} motif at the tip of the V3 loop penetrates by approximately 1/3 of the thickness of the lipid bilayer into the CRS2 pocket, with the residue Pro311 reaching most deeply (Figure 4). A segment near the V3 tip adopts a conformation very similar to that of the N terminus of [5P7]CCL5 [66]. Both the structures present a proline residue (Pro311 of gp120 and Pro3 of [5P7]CCL5) to reach the bottom of the CRS2 (Figure 4). The ECL2 of CCR5 forms an almost semicircular grip as it wraps around the V3 loop, making direct contacts with residues from both the V3 stem and crown [18]. The second major interface between Env and its coreceptor is formed by the N terminus of CCR5 and the bridging sheet of gp120. The N terminus of CCR5 adopts an extended conformation and makes several sharp turns, latching on to the surface of the bridging sheet (Figure 4). Three tyrosine residues, Tyr10, Tyr14, and Tyr15 of CCR5 can all be sulfated [95], and make the most intimate contacts with gp120. Tyr10 and Tyr14, but not Tyr15, appear to be sulfated. These two residues mimic the interactions of two sulfated tyrosines, Tyr100 and Tyr100c, in the antibody 412d with gp120 [84]. There is also an O-linked glycan at Ser7, a glycosylation site identified previously [101], and this carbohydrate may also help to maintain the configuration of the N terminus of CCR5. Thus, it appears that the extensive interfaces between gp120 and CCR5 ensue the high-affinity interaction of Env with its coreceptor.

CD4–Coreceptor

Whether there is any physical association between CD4 and the coreceptor remains controversial, even though such association may in principle provide synergy between the two receptors and benefit efficient HIV-1 entry. Constitutive cell surface association between CD4 and CCR5 in the absence of Env was reported based on coimmunoprecipitation data [102], but such a conclusion was not supported by immunomicroscopy data [103,104]. In the CD4–gp120–CCR5 structure, the long axis of CD4 lies almost perpendicular to the vertical axis of the TM helices of CCR5 and probably parallel to the membrane plane, leaving the TM domain of CD4 up to 100 Å away from CCR5, even if it bends back towards CCR5, and making it difficult for the two to have direct contacts at least when both are bound to gp120.

A Detailed Model for Early Steps of HIV-1 Entry

Despite the tremendous progress in structural biology of HIV-1 Env and its complexes with the cellular receptors, proposing a coherent molecular model for HIV-1 entry remains challenging, mainly because of the limitations of the new structures (e.g., artificial modifications in the Env constructs; extra ligands required for stability; and absence of membrane). Nevertheless, with these caveats in mind, we can consider the following model for the early steps of HIV-1 entry, depicted in Figure 5.

First, binding of CD4 to gp120 allows initial attachment of the virus to the surface of the target cell. The complex of a single CD4 bound to the SOSIP trimer shows a plausible

orientation for CD4 to make an initial contact with the Env, but no significant structural rearrangements in the Env are observed in this complex, presumably because of the presence of PGT145 and the DS modification, which can prevent the opening of the trimer – a hallmark of CD4-induced conformational changes. We do not know how stable the complex of a single CD4 and Env trimer is if the structure indeed represents a physiologically relevant intermediate state. Likewise, we also do not know whether a single CD4 is sufficient to trigger the conformational changes of Env in the absence of PGT145 and the DS mutation. smFRET data seem to suggest that a single CD4 can indeed trigger large changes, but these data also dispute that the SOSIP trimer represents the native, untriggered prefusion conformation [41]. Thus, there are uncertainties even for our understanding of the CD4-mediated attachment step. To further complicate matters, we still cannot explain why a CD4-specific antibody, ibalizumab, an FDA-approved drug targeting an epitope near the D1–D2 junction on the opposite side of the gp120 binding site on CD4, is a potent inhibitory antibody of HIV-1 entry [105,106], suggesting that there are additional roles of CD4 in HIV-1 entry yet to be uncovered.

Using advanced microscopy and spectroscopy imaging techniques, a recent study suggests an intriguing stoichiometry among Env trimer, CD4, and coreceptor during membrane fusion, which involves possible oligomerization of both receptors and also varies between different viruses [107]. Early studies showed that the Env trimer remains fully functional even with one or more gp120 protomer(s) defective in CD4 binding or coreceptor binding [108], suggesting that single CD4/coreceptor binding to an Env trimer is sufficient to promote HIV-1 entry. In principle, it may be a plausible scenario since there are only ~14 Env spikes per virion [12] and the probability for an Env trimer to engage three CD4 and three coreceptors at the same time would not be high.

If a single CD4 can induce opening of an Env trimer and formation of the coreceptor binding site, does the coreceptor trigger additional changes in gp120 that unleash the fusogenic potential of gp41? Unexpectedly, the new CD4–gp120–CCR5 structure shows no obvious allosteric changes in gp120 that can propagate from the CCR5 binding site to gp41, as comparison of the CD4- and CCR5-bound gp120 and the CD4-bound gp120 showed no major differences in the gp120 core region ([89]; Figure 5). The only significant changes are reconfiguration of the V3 loop and flipping back of the N and C termini of gp120 near its interface with gp41.

Based on our current understanding of the structure of the HIV-1 Env trimer [25-27,29,82], the prefusion gp41, which forms a so-called ‘4-helix collar’ with its four helices in (α6, α7, α8, and α9; Figure 5) [27] and wraps around the N and C termini of gp120, would be unstable and likely to enter an irreversible refolding process if gp120 dissociates. Thus, partial or complete gp120 dissociation may be the crucial ‘trigger’ that initiates a series of structural rearrangements of gp41 that promote membrane fusion. CD4 binding leads to a large shift of the C terminus of the helix α6 away from the gp120 termini, creating a pocket, filled by the fusion peptide [82], which packs against the gp120 N terminus (Figure 5). When the intrinsic conformational dynamics cause the fusion peptide to dissociate from the pocket, it will open up one side of the gp41 grip on the gp120 termini, and the N-terminal segment of gp120 can then bend back to adopt the conformation observed in the CCR5-

bound structure (Figure 5). The structural changes in the gp120 termini can prevent the fusion peptide from reoccupying the pocket and effectively weaken the gp120–gp41 interactions, possibly leading to complete dissociation. Spontaneous or CD4-induced gp120 shedding from the HIV-1 Env trimers is well documented for many HIV-1 isolates [109,110], indicating that gp120 is prone to dissociation from gp41 even in the absence of a coreceptor. The effect of the membranes and Env trimer organization will require further investigation.

If the gp120 dissociation to activate gp41 does not depend on CCR5 binding, then why would a coreceptor still be needed? First, gp120 dissociation in the absence of a coreceptor may be nonproductive because, when a virion attaches to the target cell surfaces with the Env trimer forming a complex only with CD4, the distance between the fusion peptide gp41 and the cell membrane surface can be ~160 Å (Figure 5). If gp120 dissociates prematurely, the fusion peptide is too far away to insert itself into the target membrane. Binding of gp120 to CCR5 will bring the fusion peptide within a 70 Å range (Figure 5), consistent with the proposed distance that the fusion peptide needs to translocate during gp41 refolding to reach the target membrane [27]. Second, gp120–CD4 association, measured by single-molecule force spectroscopy with infectious virions and live host cells, is unstable and is rapidly reversible unless CCR5 binding immediately follows [111,112]. CCR5 therefore stabilizes the CD4-induced conformational changes, which are already competent for promoting membrane fusion. Third, fusion pore formation likely requires more than one Env trimer [113], as shown for other viral fusion proteins [114]. A stable Env–receptor complex would be beneficial for synchronizing several Env trimers to undergo the same conformational changes. A coreceptor probably functions by stabilizing and anchoring the CD4-induced conformation of the Env trimer near the cell membrane to facilitate productive membrane fusion. Thus, according to this model, fortuitous naming of the HIV-1 primary receptor and coreceptor could not be more accurate even in a mechanistic sense.

If three CD4 molecules and subsequently three coreceptors are needed to activate one Env trimer, binding to three copies of CD4 and CCR5 simultaneously by one Env trimer is indeed possible, as modeled in Figure 5. Considering the orientation of the CD4 long axis, the question arises as to whether three bound CD4s would bring the Env trimer sufficiently close to the cell membrane for productive fusion, even in the absence of a coreceptor. Experimental data do not seem to support this scenario since, while CD4-independent entry has been described [115], no coreceptor-independent entry has ever been observed. Moreover, the dissociated gp120–CCR5 complexes would have to diffuse away quickly enough, otherwise they could conceivably clash with the three incoming fusion peptides, which are supposed to insert into the target membrane. Finally, such a stoichiometry would also make HIV-1 entry a very inefficient process if multiple triggered Env trimers need to come together to induce fusion pore formation in concert. Thus, while possible, a required full occupancy of Env trimer by three CD4s and three coreceptors appears to be a tall order for HIV-1 to fill for the first critical step in its life cycle.

Another interesting aspect regarding the long axis of CD4 in the CCR5 complex, which lies almost perpendicular to the vertical axis of the TM helices of CCR5 and probably parallel to the plane of lipid bilayer, is its potential impact on the membrane. If the ectodomain of CD4

is sufficiently rigid, and cannot bend too much, the formation of the CD4–gp120–CCR5 complex may induce a local bend in the membrane. Whether this possible membrane bending can explain why depletion of cholesterol, which often promotes membrane curvature, can block gp120-induced colocalization of CD4 and CCR5 on cell surfaces [79], or whether there are other roles that phospholipids may play during HIV-1 entry, will require further investigation.

Concluding Remarks and Future Directions

HIV-1 entry is undoubtedly one of the most fascinating yet challenging problems in molecular virology. A full understanding of this process not only can guide development of vaccines and therapeutics, but can also have important implications for viral entry of other enveloped viruses as well as many cellular events involving membrane fusion. Despite tremendous progress in the field, discussed above, many critical questions regarding molecular details of HIV-1 entry remain to be answered (see Outstanding Questions). Further investigations on a full-length Env in the context of membrane will likely lead to a more complete and accurate picture than what we currently have of its various conformations that are relevant to membrane fusion, as well as its interactions with the cellular receptors and HIV-1 matrix protein. Rapid technological advances in cryo-EM as well as other live-cell-imaging methods have shown great promises in tackling these otherwise difficult problems and may soon allow us to replace the model in Figure 1A with a real molecular movie.

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Highlights

HIV-1 entry is the first critical step in the viral life cycle.

Structural studies of the HIV-1 envelope spike and its cellular receptors have revealed important mechanistic insights.

Many critical questions on HIV-1 entry remain to be answered in future investigations.

Outstanding Questions

- What is the nature of the State 1 of Env observed by smFRET, and is it really different from the SOSIP trimer structure?
- Do we have a complete and accurate picture of CD4-induced conformational changes of Env trimer?
- What is the stoichiometry of Env, CD4, and coreceptor for productive membrane fusion?
- What is the structure of the prehairpin intermediate conformation of gp41?
- How do the MPER, TMD, and CT regions rearrange during the fusion process?
- How many triggered Env trimers are needed for fusion pore formation?
- Does the HIV-1 matrix protein, which is believed to interact with the CT of Env, have any impact on viral entry?

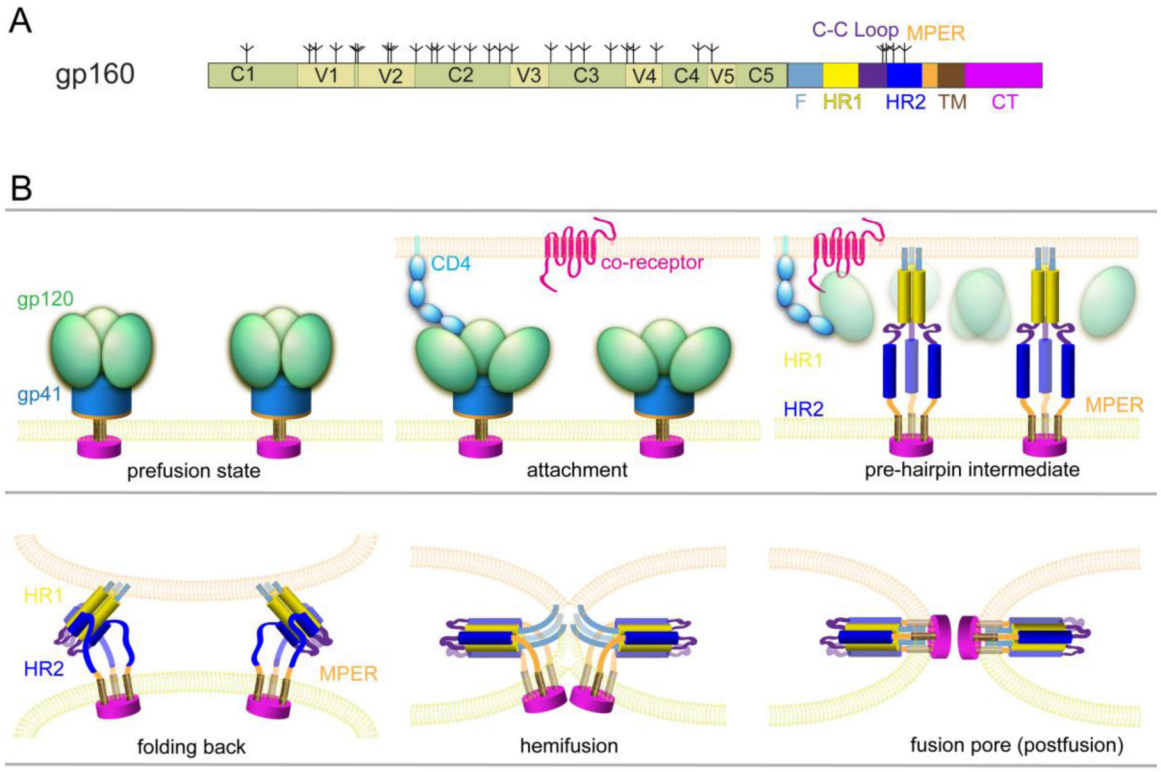

Figure 1.

HIV-1 Env and Viral Entry. (A) The full-length HIV-1 Env, gp160. Segments of gp120 and gp41 include: C1–C5, conserved regions 1–5; V1–V5, variable regions 1–5; F, fusion peptide; HR1, heptad repeat 1; C–C loop, the immunodominant loop with a conserved disulfide; HR2, heptad repeat 2; MPER, membrane-proximal external region; TM, transmembrane anchor; CT, cytoplasmic tail; tree-like symbols, glycans. (B) Membrane fusion likely proceeds stepwise as follows. (i) Binding of gp120 to CD4, and a coreceptor allows viral attachment and triggers structural changes in Env. (ii) Dissociation of gp120 and insertion of the fusion peptide of gp41 into the target cell membrane leads to the prehairpin intermediate [116]. (iii) HR2 folds back onto the inner core of HR1 and brings the two membranes together. (iv) A hemifusion stalk forms and resolves into a fusion pore [117]. The number of Env trimers required for fusion pore formation remains speculative.

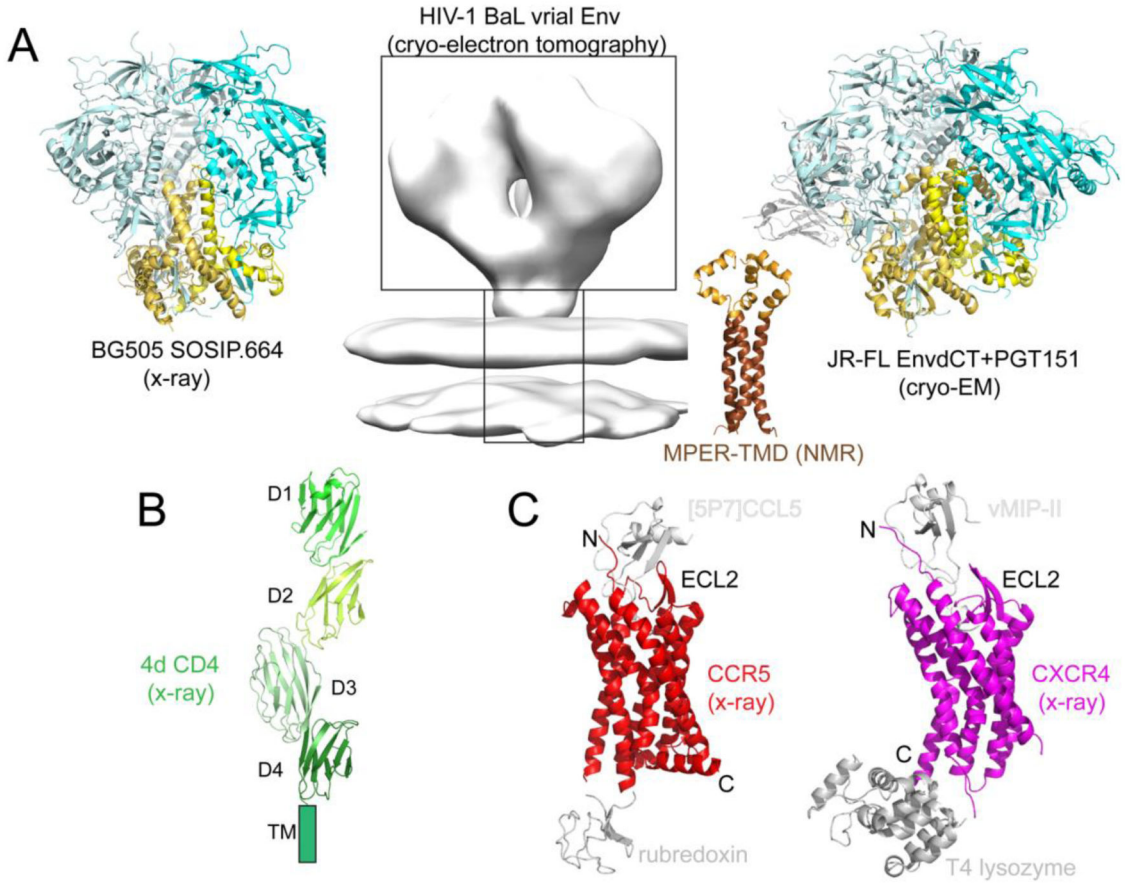

Figure 2.

Structures of the Key Players in HIV-1 Entry. (A) Structures of HIV-1 Env. The crystal structure of the unliganded HIV-1 BG505 SOSIP.664 Env trimer (pdb ID: 4ZMJ; [85]) that lacks the membrane-proximal external region (MPER), transmembrane domain (TMD), and cytoplasmic tail (CT) is shown in ribbon diagram with gp120 in cyan and gp41 in yellow. The EM density in gray is a 3D reconstruction of the unliganded HIV-1 BaL Env spike on the surface of the virion by cryo-electron tomography (EMDB ID: EMD-5019 (Env portion); EMDB ID: EMD-5022 (membrane portion)). The structure of the MPER-TMD reconstituted in bicelles that mimic a lipid bilayer was determined by nuclear magnetic resonance (NMR) (pdb ID: 6E8W; [36]). The MPER is in orange, and the TMD is in brown. The single particle cryo-EM structure of the detergent-solubilized clade B HIV-1 JR-FL EnvΔCT construct without the CT in complex with bnAb PGT151 (pdb ID: 5FUU; [29]) is shown with gp120 in cyan, gp41 in yellow, and PGT-151 Fab in gray. (B) Crystal structure of soluble 4 domain CD4 (pdb ID: 1WIO; [46]). D1–D4 and the location of the transmembrane segment (TM) are indicated. (C) Crystal structure of a modified CCR5 in complex with a modified chemokine [5P7]CCL5 (an antagonist; pdb ID: 5UIW; [66]). CCR5 is shown in ribbon diagram in red, the internally fused rubredoxin and the ligand in gray. N terminus (N), C terminus (C) and the second extracellular loop (ECL2) are indicated. Crystal structure of an engineered CXCR4 in complex with a viral chemokine antagonist vMIP-II (pdb ID: 4RWS; [65]). CXCR4 is shown in magenta, the fused T4 lysozyme in and the ligand in gray.

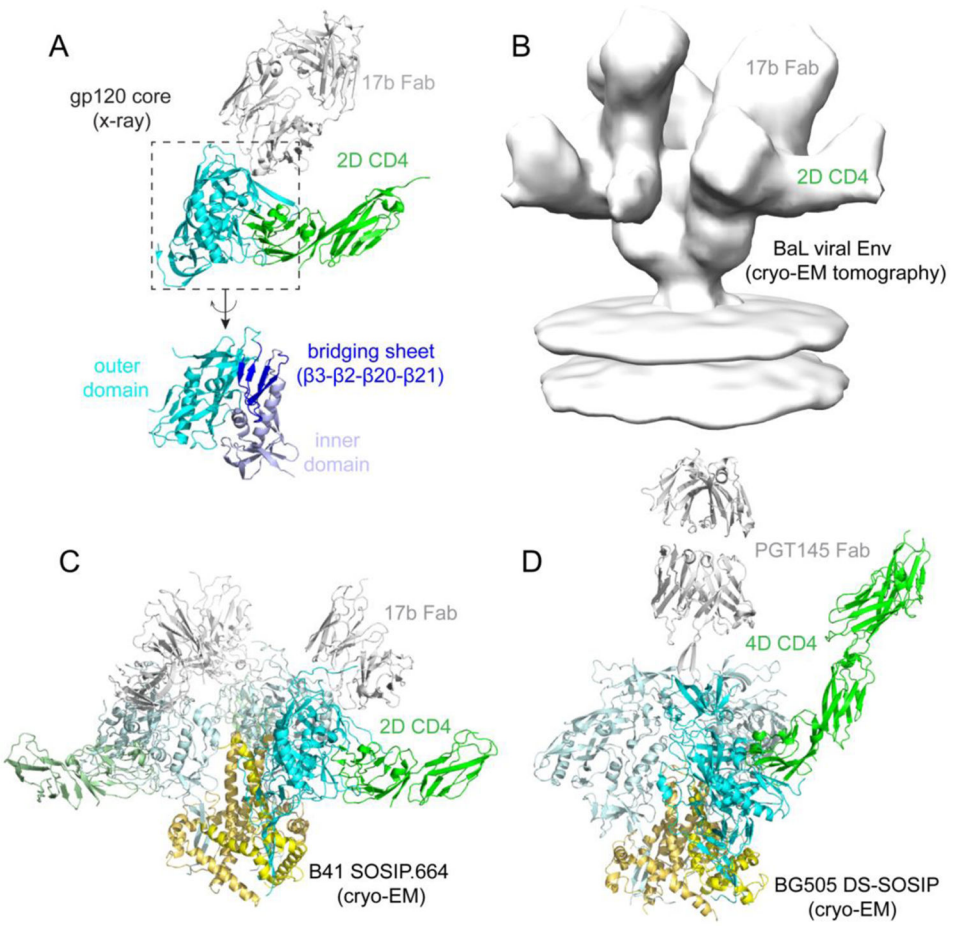

Figure 3.

Env–CD4 Interaction. (A) The crystal structure of the first gp120 core in complex with 2D CD4 and 17b Fab (pdb ID: 1G9M; [16]). the gp120 core is in cyan, CD4 in green, and 17b in gray. This structure led to a definition of the inner domain, outer domain, and the bridging sheet as indicated. (B) 3D reconstruction of the HIV-1 BaL Env spike on the surface of virion in complex with 2D CD4 and 17b Fab determined by cryo-electron tomography (EMDB ID: EMD-5020 (Env portion); EMDB ID: EMD-5023 (membrane portion)). (C) The single-particle cryo-EM structure of B41 SOSIP.664 Env trimer in complex with 2D CD4 and 17b (pdb ID: 5VN3; [82]) is shown with gp120 in cyan, gp41 in yellow, CD4 in green, and 17b Fab in gray. (D) The cryo-EM structure BG505 DS-SOSIP.664 Env trimer in complex with 4D CD4 and PGT145 Fab (pdb ID: 5U1F; [86]) is shown with gp120 in cyan, gp41 in yellow, CD4 in green, and PGT145 Fab in gray.

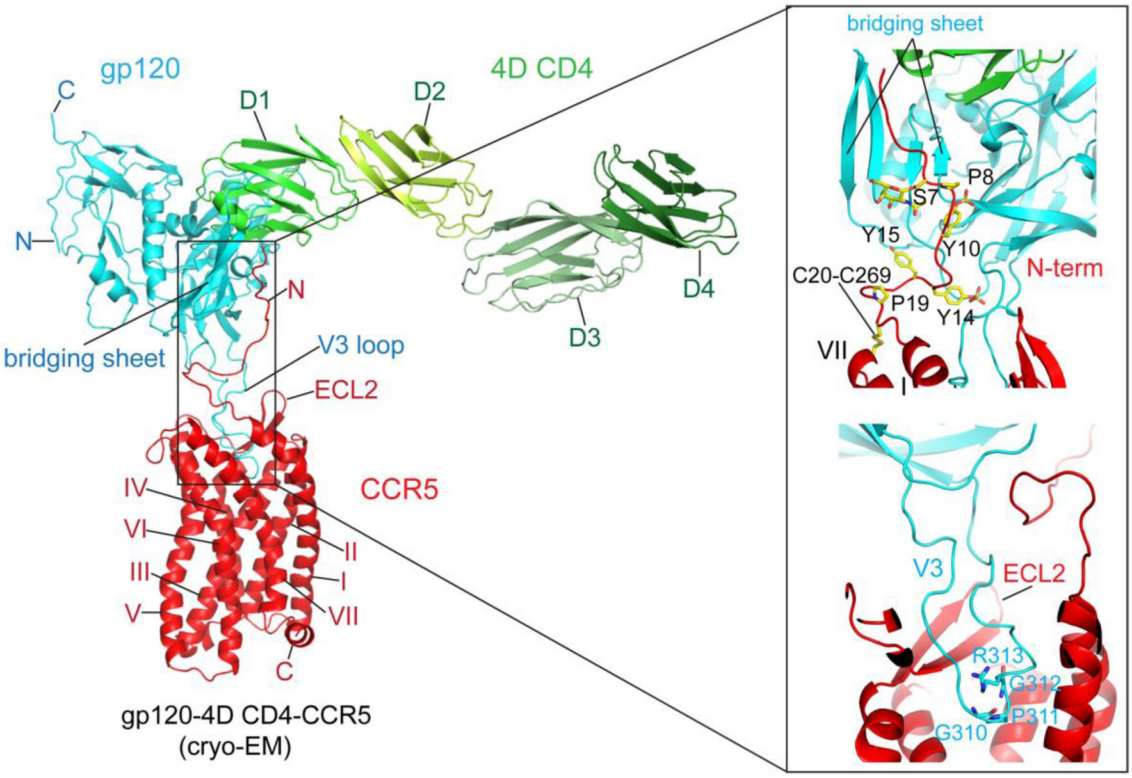

Figure 4.

Env–CCR5 Interaction. *Left.* Overall structure of the 4D CD4–gp120–CCR5 complex (pdb ID: 6MET; [89]) shown in ribbon diagram. N, N terminus; C, C terminus; ECL2, extracellular loop 2; I, II, III, IV, V, VI, VII for transmembrane helices (TM) 1–7. V3 loop and the bridging sheet of gp120 are also indicated. *Right.* Close-up views of the interfaces between gp120 and CCR5. First, the N terminus of CCR5 is attaching to the surface of the four-stranded bridging β sheet formed by the V1V2 stem and β21–β22 of gp120. Residues Ser7, Pro 8, sulfated Tyr 10, sulfated Tyr14, Tyr15, and Pro19, as well as the disulfide between Cys20 and Cys269 of CCR5, are highlighted in stick model. The O-linked glycan at Ser7 is also shown. Second, V3 is inserting into the CRS2. The conserved GPGR motif of V3 is highlighted in stick model, and ECL2 of CCR5 is indicated.

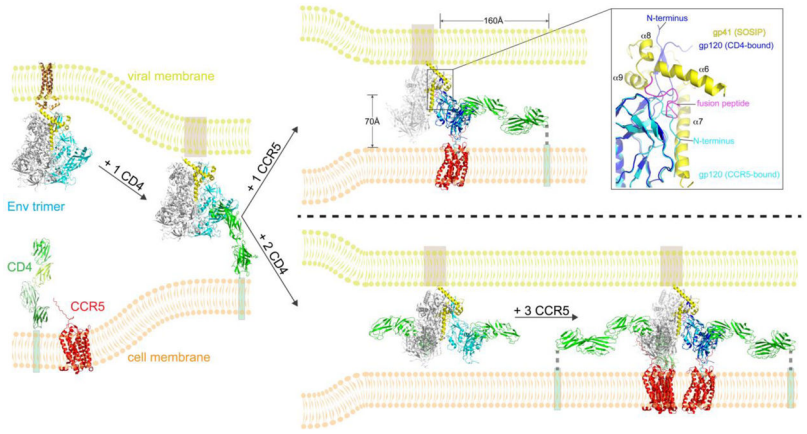

Figure 5.

Molecular Model of Early Steps of HIV-1 Entry. From left to right, the structures of unliganded Env trimer ectodomain and MPER–TMD in the prefusion conformation sitting in the viral membrane (light green) and the structures of CD4 and CCR5 in the target cell membrane (light orange). The virus attaches to the target cell by the binding of a single CD4 (green) to one gp120 (cyan) in the Env trimer. The stoichiometry among Env trimer, CD4, and coreceptor during the fusion process is unknown. If single CD4/coreceptor binding is sufficient to induce productive membrane fusion, immediate binding by CCR5 (red) prevents rapid dissociation between gp120 and CD4, stabilizes the CD4-induced conformational changes within the Env trimer, and also brings the trimer close to the cell membrane. The fusion peptide (magenta) flips out due to intrinsic conformational dynamics, allowing bending back of the N and C termini of gp120, which blocks the fusion peptide from resuming its original position in the trimer. The movements of the fusion peptide and gp120 termini effectively weaken the noncovalent association between the two subunits and may lead to partial or complete dissociation of gp120 and a series of refolding events in gp41 to adopt the prehairpin intermediate conformation with the fusion peptides inserting into the target cell membrane. On the right side, a close-up view of the gp120 N- and C-terminal region. Four helices (α6, α7, α8, and α9) of gp41 forming the 4-helix collar together with the fusion peptide that grips the N and C termini of gp120 in the CD4-bound trimer are indicated. The N terminus of the CCR5-bound gp120 overlaps with the fusion peptide in the CD4-bound trimer. If three CD4 molecules, and subsequently three coreceptors, are required to activate one Env trimer, a full occupancy of an Env trimer by three copies of each CD4 and CCR5 simultaneously is indeed possible, but such a stoichiometry may be difficult for the virus to fulfill.