HHS Public Access

Author manuscript

Nat Rev Microbiol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Nat Rev Microbiol. 2015 August ; 13(8): 471–483. doi:10.1038/nrmicro3503.

HIV-1 Capsid: The Multifaceted Key Player in HIV-1 infection

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Abstract

In a mature, infectious HIV-1 virion, the viral genome is housed within a conical capsid core comprised of the viral capsid (CA) protein. The CA protein, and the structure into which it assembles, facilitate virtually every step of infection through a series of interactions with multiple host cell factors. This review describes our understanding of the interactions between the viral capsid core and several cellular factors that enable efficient HIV-1 genome replication, timely core disassembly, nuclear import and the integration of the viral genome into the genome of the target cell. We then discuss how elucidating these interactions can reveal new targets for therapeutic interactions against HIV-1.

HIV-1 is the virus responsible for acquired immune deficiency syndrome (AIDS). HIV-1 is a lentivirus which infects cells of the immune system, specifically $CD4^+$ T cells and macrophages, and the gradual depletion of infected T cells leads to the development of AIDS in individuals that are not on antiretroviral therapy. Following viral attachment to the surface of the infected cell and fusion between the viral and host cell membranes, the HIV-1 conical capsid core – which houses the viral RNA genome – is released into the cytoplasm of the infected cell. The capsid core then traffics towards the nucleus while the viral RNA genome is reverse transcribed into DNA. Notably, HIV-1 (and other primate lentiviruses, such as simian immunodeficiency virus (SIV)) have evolved the ability to traverse the nuclear envelope and enter the nucleus of non-dividing cells. Once in the nucleus, the viral genome becomes stably integrated into the genome of the target cell, where it directs the formation of progeny virions that are ultimately released from the cell to infect new target cells (Figure 1).

It is increasingly appreciated that the viral capsid protein (CA) plays a much more critical part in many of these steps than previously thought, including a critical role in the nuclear import of the viral genome. This review focuses on the events occurring during the early phase of the HIV-1 replication cycle – which includes the events from viral attachment to the host cell until viral integration into the host genome – focusing on the role of CA and the capsid core in mediating critical events during infection. We discuss models of viral uncoating, describe the cellular factors that are thought to interact with CA during HIV-1 infection, and discuss how elucidating these interactions can be used to design novel therapeutic strategies against HIV-1.

The HIV-1 capsid

Any discussion on the role of the capsid during the early events of the viral life cycle can quickly become confusing because the term ‘capsid’ can refer to both the capsid core and the protein (CA) that assembles to form the core. Here, we will use the term ‘CA’ to describe the protein and ‘capsid core’ to describe the conical structure which is a large assembly of CA and houses the viral genome.

Following release of an immature virion from an infected cell, the viral protease is activated and cleaves the approximately 5000 molecules of Gag precursor protein (Gag) present in the immature viral particle into its primary constituents: matrix protein (MA), CA, nucleocapsid protein (NC) and the p6 peptide [1]. At this time, CA spontaneously assembles into the characteristic fullerene cone observed in HIV-1 virions which houses the HIV genome, the viral replicative enzymes – integrase and reverse transcriptase – and some accessory proteins. This conical capsid core is comprised of approximately 1500 CA monomers [1], assembled predominantly into hexamers, with a handful of pentamers facilitating the curvature on the top and bottom of the core necessary to form a closed structure [2,3] (Figure 2). The CA protein itself is comprised of two domains, a ~150 amino acid N terminal domain (CA_{NTD}) and an ~80 amino acid C-terminal domain (CA_{CTD}). When assembled into pentamers and hexamers, the CA_{NTD} is located on the outer surface of the capsid core and the CA_{CTD} is oriented towards the interior of the structure (Figure 2). The CA_{NTD} contains three α-helices which stabilize the hexameric subunits of CA [4]. CA_{NTD}-CA_{CTD} contacts between adjacent CA monomers further stabilize hexameric or pentameric subunits [4–6]. This CA_{NTD}-CA_{CTD} interface also forms a binding pocket which interacts with numerous cellular factors that play a role in infection and is the target of two recently described antiviral compounds [7,8] (see below) (Figure 2).

Larger assemblies of hexamers and pentamers are held together by hydrophobic residues in the CA_{CTD}, which provide the basis of the mature conical capsid core [4–6]. Inside this core are two copies of the viral RNA genome, NC, RT, IN, and viral accessory proteins [9–11]. Therefore, the capsid core is thought to perform two primary functions. First, the capsid core may act to maintain RT and the genome in a closed environment. Given the numerous strand transfers necessary to convert the viral RNA genome to double stranded DNA, an intact core might ensure the efficient initiation of reverse transcription and effective elongation of the nascent viral DNA genome [12]. Second, the core acts to shield the viral genome from host factors, such as cGAS or TREX1, which can detect viral DNA and initiate an antiviral response capable of inhibiting infection [13–15], or degrade cytosolic viral DNA [16], respectively.

HIV-1 uncoating

Historically, HIV-1 uncoating has been considered from a structural perspective, defined as the process by which the capsid core dissociates from the rest of the reverse transcription complex (RTC) [17]. However, it is increasingly clear that CA, and the assembled capsid core, provide critical functionality at numerous steps of infection. For example, CA confers the ability to infect non-dividing cells, a property which separates lentiviruses such as HIV-1

from simple retroviruses [18,19]. Furthermore, some studies suggest that CA remains associated with the RTC following its translocation into the nucleus [20–24]. According to previous definitions of uncoating, the association of CA with the RTC complex in the nucleus would imply that uncoating is incomplete until the viral genome has entered the nucleus. However, studies examining the size constraints of cargos which traffic through the nuclear pore complexes (NPC) have found an upper limit of ~39nm [25,26], which is significantly smaller than the 50–60nm width of an intact fullerene cone [4]. Taken together, this suggests that the loss of CA from the RTC is unlikely to be a single, discrete event, thereby forcing some reconsideration of the definition of uncoating. Based on the putative function of CA and the capsid core during infection, the uncoating process can be thought of as occurring in at least two stages. The first stage is the loss of integrity of the intact core. This event seems to occur necessarily in the cytoplasm, as the capsid core is too large to remain intact as it traffics through the NPC. However, the recent observation that some CA remains associated with the RTC in the nucleus[23,24] suggests that whatever core destabilization that occurs prior to nuclear import, it does not remove all of the CA from the RTC.

Understanding the manner in which disassembly of the capsid core is coupled to critical aspects of infection is more than a problem of nomenclature. An intact capsid cone, housing the viral genome and viral proteins, may represent a fundamentally different therapeutic target than the small amount of CA that may remain associated with the RTC following entry into the nucleus, although both may represent viable therapeutic targets. Some host cell proteins, or antiviral drugs, which interact with CA may do so only in the context of assembled CA, or exhibit increased affinity for the assembled form of CA [7,8]. Therefore, it is important to determine to what extent assembled CA hexamers or pentamers continue to exist following partial disassembly of the original capsid core structure.

Much of our understanding of the stability of the capsid core is derived from studies of CA mutations which perturb this stability. In this regard, it is important to separate mutations which directly alter core stability from those that may indirectly affect core stability through altered interactions with certain host factors that facilitate uncoating (Box 1). For example, in vitro studies have identified CA mutations that increase or decrease the intrinsic stability of the core, and both types of mutations reduce or abrogate HIV-1 infectivity[12], suggesting that the stability of the capsid core is delicately balanced to promote infection. However, the identification of compensetory mutations that improve infectivity of hyperstable or unstable CA mutants without restoring the stability of the mutant capsid to levels similar to the wild type capsid[27] demonstrate the complex relationship between core stability and infection.

BOX 1

Methods of measuring uncoating

Several assays are available to measure uncoating of the HIV-1 capsid core.

***In vitro* core stability assay:** with this method, core stability is assessed by a centrifugation approach in which whole virions are spun through a detergent layer into a sucrose gradient [12,119]. This process removes the viral membrane and allows the

determination of relative stability of capsid cores harboring certain mutations or following pharmacological treatment [12,111]. This assay has been used to describe a "stability window", in which capsid cores that are either more stable or less stable than the capsid cores from wild type viruses exhibit severe infectivity defects [12]. However, this assay focus on bulk populations of cores, of which only a small percentage may be infectious, and core stability *in vitro* may not necessarily be reflective of uncoating *in vivo.*

Fate of capsid assay: this assay subjects lysates from infected cells to centrifugation through a sucrose cushion, through which intact particulate capsid cores pellet, while soluble capsid protein (CA) or virions which remain in the vesicular compartment do not [120]. Thus, the amount of intact, pelletable capsid cores following infection can be comparatively assayed. This assay was originally described as a method to measure core destabilization by the restriction factor TRIM5α [120], and has subsequently been utilized to assess the contribution of reverse transcription [51,121] and microtubule trafficking to capsid uncoating [39] and core stabilization by the restriction factor MX2 [122]. As the previous assay, this assay focus on bulk populations of cores, of which only a small percentage may be infectious.

In situ uncoating assays: Imaging based approaches have also been utilized to interrogate the amount of CA that remains associated with individual HIV-1 reverse transcription complexes (RTC) following infection. This assay relies on the fluorescent labelling of viral particles in the producer cell using the GFP-Vpr fusion protein which is incorporated into virions and remains associated with the viral RTC following fusion [35,12341]. Membrane labelling is also utilized to identify viral particles that have fused with the target cell, focusing the analysis on cytoplasmic capsid cores [39,42,124–126]. The level of CA associated with individual viral RTCs can be comparatively analyzed by immunofluorescence using capsid-specific antibodies. This assay was utilized to show that reverse transcription and microtubules facilitate HIV-1 uncoating [38,39]. As the previous methods, this method focus on bulk populations of capsid cores, of which only a fraction may be infectious [127].

Cyclosporin A (CsA) washout assay: This assay relies on our understanding of the TRIM-Cyp protein from owl monkeys, which utilizes a cyclophilin A (CypA) domain to bind to CA and potently inhibit infection by HIV-1 [128]. This restriction can effectively be turned off by addition of the drug cyclosporine A (CsA). Because CA is the target of TRIM-Cyp, insensitivity to CsA withdrawal suggests that the CA determinants of the viral core are no longer part of the viral RTC. Removal of CsA at different time points following infection can be used to determine what percentage of the infectious inoculum has become insensitive to TRIM-Cyp, allowing the "half-life" of uncoating to be calculated experimentally [38]. This assay is unique among the currently available assays of uncoating as it monitors the uncoating of virions that would otherwise go on to infect the cell, whereas the other available assays necessarily analyze the relative stability of all cytoplasmic viral complexes, many of which might not be infectious. This assay has been used to measure the contribuition of reverse transcription [38] or microtubules [39] to uncoating. However, conditions that substantially alter end point infectivity can make comparing relative uncoating half-lives open to many interpretations. Also, because

uncoating is being indirectly sensed by a CA-binding restriction factor, it is possible that host factors may compete with TRIM-Cyp for a limited number of CA binding sites [47].

An improved understanding of the links between CA, the stability of the capsid core, and HIV-1 infectivity is increasingly necessary given the growing evidence that assembled CA may provide a strong target for therapeutic intervention. Unlike HIV-1 enzymes, CA is genetically "fragile"[28], stretched to the limit genetically by the pressure to mature into a core of optimal stability [12] while maintaining the ability to interact with numerous host factors at critical steps of infection without allowing interactions with capsid targeting restriction factors designed to inhibit infection [29]. This means that CA does not have the genetic flexibility frequently utilized by viral proteins such as reverse transcriptase or protease to evade therapeutic strategies, as any mutation in CA is very likely to significantly impact the ability of CA to execute its critical functions[28]. Notably, CA assemblies from other retroviruses exhibit remarkable structural homology to those of CA from HIV-1[3031], despite a lack of sequence homology between these proteins. This suggests that the genetic fragility of HIV-1 CA is not due to an inability to adopt the basic structural elements required to generate a retroviral capsid core, but rather reflects the delicate balance of functional burdens placed on HIV-1 CA. It is also unclear what role, if any, unassembled CA species which remain associated with the viral RTC may have in infection. There are approximately 5000 CA monomers in the virions, but only approximately 1500 of these monomers are used to form the capsid core [1,4], so there is ample CA not associated with the core which may provide an additional function[30].

Finally, CA mutations that prevent the viral core from successfully navigating critical steps of infection also activate intrinsic host defense pathways that induce production of type I interferons (IFN) and the so-called "antiviral state" [14,15]. This implies that therapeutic interventions that target CA may be efficacious through a synergistic reduction of viral infectivity and the secondary induction of an antiviral immune response that makes cells more resistant to subsequent infection.

Models of HIV-1 uncoating

The recent demonstration that CA influences late events during HIV-1 infection, including nuclear import and genome integration [21,22,32–34], reveals that CA has functions beyond the simple encapsidation of the viral genome. Therefore, it is important to understand how the conical capsid core dissociates as the HIV genome progresses through reverse transcription and traffics to the nucleus for integration. Here, we describe the possible mechanisms by which core disassembly may occur, and highlight the data most supportive of, and discordant with, each potential model (Figure 3). Notably, these models may not be mutually exclusive, and disassembly of the capsid core may vary by cell type and the activation state of the infected cell at the time of infection.

Rapid core disassembly

Biochemical studies examining the content of viral RTCs suggested that CA and the conical capsid core are largely absent from RTCs isolated from cells soon after infection [35,36]. When

fractions containing the viral genome are interrogated for the presence of individual viral proteins, MA, reverse transcriptase and integrase are readily detectable in these fractions, while comparatively little CA is observed [35,36]. By comparison, CA from murine leukemia virus (MLV)remains much more stably associated with the viral RTC in identical assays [37]. These data support a model in which the HIV-1 capsid core dissociates almost immediately upon viral entry (Figure 3), and this model represented the prevailing view of uncoating for many years. However, a number of recent key observations have done irreparable harm to this model, including the ability of the viral capsid core to protect the genome from cytosolic DNA sensors [13–15] and to allow the infection of non-dividing cells [18,19]. Two very recent studies found that the factors which are thought to mediate the nuclear import of the RTC bind to a pocket only present on assembled CA [7,8], effectively closing the book on the idea that the core completely disassembles soon after viral entry.

Cytoplasmic Unocoating

A number of studies support the notion that a significant amount of CA is lost from the viral RTC as it undergoes reverse transcription and traffics towards the nucleus (Figure 3). Uncoating as measured by the ‘cyclosporin A (CsA) washout’ assay – which exploits the binding of the TRIM-Cyp restriction factor to CA (Box 1) – finds that HIV-1 gradually becomes insensitive to TRIM-Cyp binding in the 3–4 hours after infection, with a half-life of less than 1 hour [38–40]. While it is unclear how much CA needs to be lost in order for the capsid core to become insensitive to TRIM-Cyp restriction, the loss of sensitivity in this time window suggests that CA is gradually lost from the capsid core during this time.

The idea of significant core disassembly or remodeling in the first hours after infection is also supported by immunofluorescent interrogation of individual cytoplasmic viral complexes (Box 1).[Around two thirds of cytoplasmic RTCs, identified by the incorporation of fluorescent nucleotides, contain detectable amounts of CA at 4 hours post-infection [41]. A similar loss of CA from viral RTCs, identified by 5-ethynyl uridine-labelled RNA, was also observed 1–2 hours following infection [42]. Notably, these early measurements of uncoating precede the completion of reverse transcription, which normally peaks 8–12 hours after infection [38,39,43] and before the viral RTC has been observed within the nucleus [44]. These differences in uncoating kinetics suggest that some degree of uncoating occurs before the completion of reverse transcription, which is typically considered to be a cytoplasmic event, and before the arrival of the CA at the NPC.

However, the perceived purpose of the viral capsid core during infection presents fundamental obstacles to this gradual model of uncoating. If the purpose of the core is to protect the genome from host DNA sensors or other restriction factors, or to provide a closed environment that facilitates reverse transcription, how can this be achieved in cores that begin to shed this protective coat in the first hours after fusion? The loss of core integrity might be expected to be coincident with the first detectable loss of CA from the capsid core, and such a loss of integrity would seemingly abrogate the protective function of the core. For example, it was recently reported that some CA mutations induce IFN responses during infection, potentially due to activation of cytosolic DNA sensors that recognize HIV-1 DNA [15]. Activation of the IFN response by these mutant viruses, but not by the wild type

virus, is interpreted to indicate that the wild type virus is able to keep the viral genome sequestered away from these sensors. This observation supports a protective role for the capsid core and is difficult to rationalize in a model where CA is gradually lost from the RTC. It is unclear how the CA assembly that forms the intact capsid core could tolerate the loss of a substantial amount of this assembly and still maintain these protective functions without becoming destabilized. It is certainly possible that other cellular factors are recruited to the RTC or Pre-Integration Complex (PIC), in a CA dependent fashion, and that these factors afford the protection that masks viral nucleic acids from antiviral sensors [45,46]. Validation of this gradual model of viral uncoating will require that these critical questions be addressed.

Cores at the pores

Other studies support a model where an intact capsid core arrives at the NPC, such that uncoating is functionally tethered to the nuclear import of the viral RTC [47] (Figure 3). The primary support for this model stems from the observation that viral cDNA generated during infection is not normally recognized by the antiviral DNA sensor cGAS [13,14]. CA mutations that inhibit the association between the RTC with certain cellular factors have also been reported to increase innate sensing and IFN production in infected macrophages (see below) [15]. The simplest mechanism by which CA might protect the viral genome from innate sensors such as cGAS is that the capsid core remains intact until disassembly occurs at the NPC, triggered by CA-associating factors. Electron micrographs of apparently intact cores at the NPC support the possibility that the separation of the viral genome from intact cores may occur at nuclear pores [44].

However, issues of timing and discordance with other studies require that this model be approached cautiously. For example, the intact cores observed at the NPC were found late in infection (12–48 hours), which is later than core disassembly, the completion of reverse transcription, and nuclear localization have been measured in other assay systems [38,39,41,42] (Box 1). Furthermore, correlative electron microscopy studies of HIV-1 RTCs in the cytoplasm which had incorporated fluorescent nucleotides did not reveal intact capsid cores [41]. This model is also difficult to rationalize with data suggesting a gradual model of uncoating [38,39,41,42]. Finally, this model is also inconsistent with the finding that CA mutations that increase core stability prevent reverse transcription [12], as these data suggest thatsome core remodeling must occur during reverse transcription in the cytoplasm. Therefore, validation of a model where intact cores dock at the NPC to avoid viral detection by cytosolic sensors should address these questions.

Viral and cellular determinants of uncoating

One fact that has made understanding the uncoating process difficult is that it is an aspect of the viral lifecycle that occurs concurrently, and is in many ways contingent on, other aspects of the viral lifecycle. As such, uncoating may not only be influenced by interactions between CA with specific host factors, but may be regulated in a spatiotemporal fashion by interactions driving other aspects of the viral lifecycle. Here, we describe the steps of the

viral lifecycle and cellular proteins which have been implicated in the uncoating process or other CA-dependent steps of infection.

Viral determinants of uncoating

Numerous lines of evidence now suggest that events or interactions within the viral capsid core can affect the uncoating process. For example, deletion or mutation of the viral integrase has been shown to have effects much earlier in the lifecycle than during the integration step itself [48,49]. Specifically, certain point or deletion mutations of integrase completely abrogate reverse transcription in the target cell, and these viruses generate capsid cores that are less stable than wild type cores, both *in vitro* and *in vivo* [48]. Taken together with the observation that integrase mutations can affect the morphology of HIV-1 cores [50], these data support a model whereby integrase provides stability to the core from inside the virion, which facilitates its association with cytoplasmic factors, (such as cyclophilin A (CypA)) [48] (see below) (Figure 4).

Two studies have also found that inhibition of reverse transcription delays uncoating. This observation was first made utilizing the CsA washout assay and *In situ* uncoating assays [38], and was subsequently corroborated using the 'fate of capsid' (FOC) assay, which measured an increase in the amount of intact capsid in the presence of reverse transcriptase inhibitors or when reverse transcriptase was genetically inactivated [51] (Box 1). These observations support a model in which the formation of nascent, polymerized viral DNA induces stress on the structure from within the core (Figure 4). It is known that CA mutations which form hyperstable cores exhibit a severe defect in reverse transcription [12], which is consistent with the hypothesis that the progression of reverse transcription requires, and normally induces, some degree of core remodeling necessary to accommodate the viral genome as it converts from relatively flexible single stranded RNA to comparatively rigid double stranded DNA. Alternatively, these data are also consistent with a model where interactions between reverse transcriptase and integrase cooperatively stabilize the core (Figure 4) [52]. In such a model, the onset of reverse transcription may dissociate the viral reverse transcriptase from these stabilizing interactions, which might explain the relevance of both reverse transcriptase and integrase to the uncoating process.

Cyclophilin A

CypA is a host peptidyl prolyl isomerase which has been known to interact with CA for more than 20 years [53–55]. Although CypA can be incorporated into virions, [53,55], the more biologically relevant interaction appears to occur in the cytoplasm of the target cell, where CypA can promote infection in some cell types [56,5758,59] through interaction with a conserved proline rich loop present on the HIV-1 CA [53,55,60]. CypA knockdown, genetic deletion, or inhibition with the immunosuppresive drug CsA (which inhibits CypA activity), all inhibit an early step in infection that alters normal reverse transcription [55,61–63]. However, CsA inhibition is incomplete and is cell type dependent [64,65]. CypA has been shown to catalyze the cis/trans isomerization of the Gly89-Pro90 peptide bond of CA [66], and this isomerization has been shown to induce conformational changes in residues in the CA_{NTD} that are distal to the CypA binding loop [66]. In the context of an assembled CA lattice, it is tempting to speculate that CypA-induced conformational changes in CA may

provide the mechanistic basic for disassembly of the capsid core (Figure 4). However, data supporting this hypothesis are contradictory, with one study observing *in vitro* destabilization of CA-NC complexes[67], whereas a different study found that CypA has the opposite effect, stabilizing viral cores *in vitro* [68].

The role of CypA during capsid uncoating is additionally complicated by the phenotype of mutants generated though continuous replication in the presence of CsA [61,69–71]. Mutations which allow for robust replication in the presence of CsA are also dependent on the presence of CsA for their replication in some cells [61,69,72], while in other cells these mutations confer CsA resistance but not dependence [56,57,61,64,65]. CsA resistant or dependent viruses still bind CypA [61,65], and expression levels of CypA can influence infection by these mutants [65,73,74]. Collectively, these observations make it difficult to assign a single, critical role to CypA in the uncoating process, although it seems clear that CypA plays a critical part in uncoating or some aspect of infection that is occurring concurrently with uncoating. In this regard, it is also important to note that most CsA resistant mutants are also unable to efficiently infect non-dividing cells [72,74], suggesting a previously unappreciated link between CsA resistance and the ability to infect non-dividing cells [64]. Understanding this phenotype may allow a better understanding of the role of CypA in a natural infection.

Cytoplasmic trafficking

Studies of fluorescently labelled HIV-1 viral particles have found that HIV-1 virions utilize dynein-dependent trafficking to move towards the nucleus during infection [41,75], and perturbing microtubule-mediated trafficking or depleting microtubule motor proteins inhibits HIV-1 infection [39,76,77]. Recently, two studies found that microtubule disruption and knockdown of microtubule motors delays uncoating of the viral capsid core (Figure 5). The first study utilized the CsA washout assay, the *in situ* uncoating assay and the FOC assay (Box 1) to demonstrate a delay in uncoating when microtubules were disrupted or when dynein heavy chain or kinesin 1 were depleted [39]. The second study[78] utilized the FOC assay to demonstrate that dynein depletion or inhibition delays uncoating. Therefore, it is possible that these observations reflect a ‘tug of war’ model of cytoplasmic uncoating of the viral core, in which uncoating is mediated by the opposing motor proteins dynein and kinesin 1 (Figure 5). However, it is worth noting that uncoating of adenovirus is also mediated by both dynein and kinesin 1, but occurs at the NPC, rather than in the cytoplasm [79]. Therefore, the data in these studies do not preclude a similar two-stage mechanism of HIV-1 core uncoating at the NPC, in which dynein is required for trafficking of the capsid core to the NPC and kinesin 1 is responsible for viral uncoating at the NPC (Figure 5). **However**, this model would seem to require the arrival of the capsid core at the NPC with faster kinetics than were reported in studies which observed intact cores at the NPC [44].

Viral and cellular determinants of HIV-1 nuclear import

As a lentivirus, HIV-1 possesses the ability to infect non-dividing cells, while most other retroviruses require cell division and the breakdown of the nuclear envelope to access target cell DNA and complete infection. Although previous studies implicated numerous viral

determinants as being responsible for the ability to infect non-dividing cells, more recent data provides compelling evidence that determinants in CA are responsible for the nuclear translocation of the viral genome [18,19,80]. This appreciation was rapidly followed by the identification of numerous cellular proteins, including components of the NPC, which interact with CA and are associated with trafficking from the cytoplasm to the nucleus [81–84]. These interactions drive critical steps of infection, and as such have clearly demonstrated that some CA, in its assembled form [7,8], remains associated with the RTC during this stage of infection. Here, we describe the cellular factors implicated in this process (reviewed in detail in reference [85]).

Capsid interaction with CPSF6

Cleavage and polyadenylation factor 6 (CPSF6) is an mRNA processing protein that shuttles between the nucleus and cytoplasm [23]. A Serine/Arginine (SR)-rich nuclear localization signal (NLS) on its C–terminus maintains a predominately nuclear steady state localization [23,86], although the protein shuttles dynamically between the nucleus and cytoplasm [87]. However, a study using a mouse cDNA expression screen found that a truncated form of CPSF6 (mCPSF6$_{1-358}$, which is missing its C-terminal SR rich domain and is therefore predominately cytoplasmic) potently inhibited infection by HIV-1 [83]. Inhibition of infection by mCPSF6$_{1-358}$ was observed against other primate lentiviruses, such as HIV-2 and SIV but not murine leukemia virus (MLV), a gammaretrovirus that cannot infect non-dividing cells [83], suggesting that CPSF6 plays a role in the ability of primate lentiviruses to infect non-dividing cells. In cells expressing mCPSF6$_{1-358}$, reverse transcription occurs normally, while the formation of 2-LTR circles is inhibited, and functional PICs accumulated in the cytoplasm of these cells, consistent with a defect in nuclear entry induced by CPSF6$_{1-358}$ [83]. A similar restriction was observed with a truncated form of human CPSF6, although notably, this human form inhibited infection at an earlier step, prior to reverse transcription [88]. At the level of the virus, CPSF6$_{1-358}$-mediated restriction clearly mapped to CA, as a CPSF6$_{1-358}$ resistant mutant virus was revealed to harbor a mutation in CA (N74D) [83] and CPSF6$_{1-358}$ was able to bind to *in vitro* assembled CA tubes [83,86,89]. The interaction between CPSF6$_{1-358}$ and CA was also supported by other studies which found that cytoplasmic CPSF6 stabilized viral cores *in vivo* [90] and CA-NC tubes *in vitro* [67]. The CA binding determinants in CPSF6 have been identified [91] and shown to interact with the binding pocket in assembled CA formed by intermolecular interactions between the CA$_{CTD}$ and CA$_{NTD}$ of neighboring CA molecules (Figure 2) [7,86]. Given the inhibitory effects mediated by CPSF6$_{1-358}$. which is generated by removal of the NLS from CPSF6, it seems likely that full-length CPSF6 has a role in mediating the nuclear import of the RTC, although this has yet to be formally demonstrated.

Capsid interaction with TNPO3

Three genome wide small interfering RNA (siRNA) screens for cellular factors required for HIV-1 infection identified Transportin-3 (TNPO3) as facilitating a late stage of HIV-1 infection [81,82,84]. TNPO3 is a member of the importin β family of proteins that governs the nuclear localization of S/R rich proteins [92]. A number of studies have revealed that TNPO3 can bind to the viral integrase [93–96]. Although this interaction appears robust and has been reproduced by a number of studies, this binding does not appear sufficient to explain the

dependence of HIV-1 infection on TNPO3 [94,95]. Instead, the dependence of HIV-1 infection on TNPO3 maps to determinants in CA [95]. Initial studies showed that TNPO3 depletion reduces the amount of viral integration, but does not reduce viral reverse transcription or 2-LTR circle formation [20,68,97,98]. However, this has been shown to be primarily due to the inability of the PCR primers typically used for the amplification of 2-LTR circles to distinguich between bona-fide 2-LTR circles and autointegration events [90]. This study demonstrated that TNPO3 depletion does indeed inhibit 2-LTR circle formation, which has also been reported by other studies [99,100]. Remarkably, a number of studies observed that although infectivity of wild type HIV-1 was potently inhibited by TNPO3 knockdown, infection by the N74D CA mutant[83] was unaffected by TNPO3 depletion [20,68,83,97,98]. Furthermore, TNPO3 also appears to bind to HIV-1 CA [20,98]. Collectivelly, these studies suggest that TNPO3 participates in the nuclear translocation of the viral RTC. The ability of TNPO3 to facilitate the nuclear translocation of the RTC might occur via one of two mechanisms. First, it may be that TNPO3 can also bind to CA. Given the resistance of the N74D mutant to TNPO3 depletion, this may occur at the same binding sight bound by CPSF6. Alternatively, TNPO3 may affect HIV-1 nuclear import indirectly by mediating the proper nuceloplasmic localization of other proteins relevant to the nuclear import of the RTC, such as CPSF6, as suggested by its ability to regulate the localization of S/R rich proteins [92].

Capsid interaction with NPC proteins

Nuclear pores are massive ~50 mDa complexes which regulate nucleocytoplasmic trafficking in all eukaryotic cells (reviewed in references [101,102]). Two components of NPCs, Nup358 (also known as RanBP2) and Nup153, were identified in genome wide screens for cellular factors required for HIV-1 infection [81,82,84]. Nup358 is a component of nuclear pore filaments which project outward towards the cytoplasm from the NPC and, like many other NPC channel proteins, possess phenylalanine/glycine (FG) repeats that form a hydrophobic meshwork that regulates traffic of molecules above ~40 kDa across the pore [101,102]. Nup358 knockdown attenuates HIV-1 infectivity, typically 3–8 fold [99,103,104], with most studies observing normal reverse transcription and reduced 2-LTR circle formation, suggesting that Nup358 knockdown prevents the efficient nuclear import of the RTC [82,99,100,103]. Notably, Nup358 also possesses a CypA homology domain, which has been shown to interact with the viral CA [99] and induce CA isomerization [105], suggesting that Nup358 mediated isomerization of CA induces core uncoating (Figure 3 and Figure 5). However, other studies have found that Nup358-mediated enhancement of HIV-1 infectivity is independent of the CypA homology domain [104], leaving the mechanism by which Nup358 engages HIV-1 unclear.

Nup153 is also present in the NPC and is conceptually similar to Nup358, although Nup153 is localized to the nuclear side of the NPC and its filaments containing FG repeats extend into the nucleoplasm, rather than the cytoplasm [101,102] (Figure 3 and Figure 5). The dependence of HIV-1 infection on Nup153 maps to the viral CA protein [21] and Nup153 has been shown to interact with CA [7,8,22,32], notably relying on the same binding pocket in CA that is required for many other interactions with nuclear import pathway factors. Notably, despite the localization of Nup153 to the nuclear side of the NPC, Nup153 knockdown

reduces the formation of 2-LTR circles [21,32,103]. As 2-LTR circles are considered a marker of PIC arrival to the nucleus, this result is counterintuitive, as Nup153 is generally considered a “nucleoplasmic” pore protein. Taken at face value, this would suggest that following arrival at the nucleoplasmic side of the NPC, a Nup153-dependent process performs some final step that is necessary to activate the integration activity of the PIC. Alternatively, it is worth considering that both Nup358 and Nup153 dynamically associate with the NPC [79,106], and Nup358 is known to be a critical adaptor of kinesin-1 [107]. Thus, it is possible that these Nup-dependent effects are driven by interactions between CA and NPC in the cytoplasm, which could be necessary for the subsequent nuclear translocation of the viral genome. Additional experiments are necessary to distinguish between these two possibilities.

CA determinants dictate late events in infection

The current understanding of the role of the capsid core and CA in the steps culminating in the integration of the HIV genome into the genome of the host cell is confounded by multiple observations suggesting that the intact capsid core no longer exists in its initial, conical state in the first few hours after infection [38,39,41,42], yet determinants in CA can influence nuclear import pathways and integration site selection [32–34,83]. The idea that the capsid core must disassemble prior to nuclear import, while the binding pocket formed by assembled CA remains associated with the RTC in the nucleus, where it mediates interactions that dictate the final steps of infection, is a bit of a paradox that can be explained in several ways.

One possibility is that the recruitment of cellular factors to the viral core facilitates some degree of core disassembly while also influencing the subsequent engagement of other host factors which mediate the nuclear import and integration of the genome. For example, although Nup153-dependent steps are essential for HIV-1 infection, Nup153 dependence was lost when cells were infected in the presence of CsA or when CypA was knocked down [21]. Similarly, viral mutants carrying the CypA binding mutations G89V and P90A in CA are less sensitive than wild type virus to Nup153 knockdown [21,90,99]. These data suggest that early engagement of host factors in the cytoplasm can dictate the final CA-dependent steps of infection in the nucleus, although our understanding of how this is achieved remains unclear. It is possible that CypA engagement of the capsid core facilitates a minimal level of disassembly that is required for subsequent Nup153 interaction. Alternatively, CypA binding may protect the capsid core from binding other factors that would otherwise drive the virus down an alternative import pathway that is not reliant on Nup153. A similar possibility is supported by the observation that CA mutations associated with alternative nuclear import pathways and the inability to infect non-dividing cells exhibit a slower rate of uncoating compared to wild type HIV-1 (as measured with the CsA washout assay) [108]. Furthermore, the decreased rate of capsid uncoating can vary between the different CA mutations. For example, the Q63,67A CA mutation exhibits a long delay in uncoating, whereas E45A and N74D show less dramatic delays. However, the viral reverse transcriptase can polymerize nucleotides at rates up to 4 nucleotides per second, such that a 10 to 20 minute delay would present a very different structure as a more developed or mature RTC becomes accessible to cytoplasmic cellular factors that bind to the complex. In this way, determinants in CA could

influence the timing of uncoating and delayed uncoating could lead to association with a different series of factors involved in nuclear import pathway selection. This could be especially important in the infection of certain cell types that are know to have very low levels of the dNTPs necessary for reverse transcription, such as macrophages and resting T cells [109].

It also seems increasingly likely that a small amount of assembled CA remains associated with the RTC during nuclear translocation. Numerous studies now support the notion that many of the factors relevant to nuclear import, including CPSF6 and Nup153, bind to a conserved binding pocket formed by intermolecular CA_{NTD}-CA_{CTD} interactions in assembled CA [7,8,22,83,86]. CPSF6 binding to this pocket, for example, seemingly must include a nuclear phase, since mutations which render CPSF6 exclusively cytoplasmic generate an antiviral protein [91]. Consistent with this model, two imaging based studies have recently observed CA associated with nuclear HIV-1 complexes[23,24]. How this small amount of CA remains in the complex after uncoating remains a critical question. It is also notable that these two factors, the timing of cellular factor accessibility to the disassembled capsid core and the observation that small amounts of CA that remain associated with the RTC, are not mutually exclusive and could both influence late steps in infection such as nuclear import pathways and integration site selection.

One potential problem related to the small amount of CA that remains associated with the RTC is that it seems unlikely that there would be enough binding sites in CA for all of the CA-binding host factors such as CPSF6, NUPs, and TNP03. However, binding could be sequential, or some of the factors might function primarily to influence other host factors, such as the possibility that TNPO3 functions to regulate the localization of CPSF6, postulated above. Alternatively, enough biding sites may be present to allow the simultaneous binding to different sites. These possibilities appear intrinsically testable with the concerted utilization of the current tool box of uncoating assays (Box 1).

CA as the target of new antivirals

The potential value of understanding the uncoating process and the role of assembled CA during infection is underscored by the identification of two pharmacological inhibitors of HIV-1 infection that appear to modulate the stability of the capsid core and the ability of CA to interact with critical host fctors during infection. The Pfizer compound PF74 was originally identified in a high-throughout screen as a small molecule inhibitor of HIV-1 infection targeting the viral CA protein [110], and initial analysis suggested that PF74 could destabilize the capsid core in vitro [111]. Subsequent studies have observed a more nuanced mechanism of action of this drug, with core destabilization possibily occurring at higher drug concentrations, while potent inhibition is also observed at lower concentrations that, if anything, seem to stabilize the viral core [7,8,67]. Perhaps not surprisingly, this compound targets the same binding pocket in assembled CA required for binding to CPSF6 and Nup153 [7,8,22,86].

Another compound, identified by Boeringer Ingelheim, BI-2[112], targets similar regions of CA[8,112], with the interesting nuance that, unlike PF74, this compound destabilizes viral

cores in the low range of effective concentrations [113] and its binding pocket does not span the CA_{NTD}: CA_{CTD} interface [8]. Since BI-2 binding can inhibit CPSF6 binding [113], these differences between BI-2 and PF74 might provide tools with which to separate the uncoating process from the requirement to engage specific cellular factors, such as CPSF6 or Nup153, during infection. In agreement, one recent study has observed that HIV-1 remains susceptible to both PF74 and BI2 for hours after some degree of uncoating has occurred, as measured by the CsA washout assay [24].

Outlook

Although still incomplete, our developing understanding of the interactions occurring between host factors and CA during infection provides ample support for the notion that these interactions can provide therapeutic opportunities. In this regard, an understanding of the events driving the disassembly of the viral capsid core, and how these events are spatiotemporally regulated during infection may afford new or improved opportunities to inhibit infection.

Understanding these steps may have important therapeutic implications. Targeting CA therapeutically may afford the added benefit of inducing IFN-stimulated genes which activate the so called “antiviral state”, making cells more resistant to subsequent infection [14,15]. Recent studies suggest that that activation of these pathways affords better viral control in patients following interruption of ART [114] and in SIV models in rhesus macaques [115]. However, chronic activation of these pathways, and the inflammation this causes, are typically associated with the opposite effect in patients [115,116117]. Therefore, effective CA targeting strategies may activate IFNs in a way that is specific to the tissue in which HIV-1 is attempting to replicate in a temporally appropriate manner.

Alternatively, it is also important to consider that targeting the primary nuclear import pathway utilized during infection may increase the ability of the virus to utilize alternative import pathways[83], which in turn may have therapeutic consequences. Perturbing interactions with host factors that act during the late steps of the early phase of the viral replication cycle, such as TNPO3, Nup153 or Nup358, can alter the spectrum of integration sites utilized by the virus, driving integration away from transcriptionally active regions towards intergenic, transcriptionally silent regions of DNA [32–34]. Thus, infection may be made less efficient, but may lead to more integrations in transcriptionally silent regions, resulting in an increase in the reservoir of latently infected cells during the course of infection [118]. These are important considerations of CA targeted therapeutic opportunities moving forward, which seem likely to increase in number as our understanding of the basic science of CA during infection continues to develop.

Acknowledgments

The authors thank Owen Pornillos and Jeremy Luban for valuable discussions. TJH and EC were funded by NIH grant 5P50GM082545. EC was supported by NIH grant AI093258.

Glossary terms

2-LTR circles	The completely reverse transcribed HIV-1 genome is flanked on either side by Long Terminal Repeats (LTRs) which ultimately define the genomic boundaries of the provirus following successful integration. At a low frequency, the cellular non-homologous end joining (NHEJ) repair pathway joins the LTRs, resulting in 2-LTR circles. As the NHEJ pathway is only active in the nucleus, 2-LTR circles are a surrogate for nuclear entry of the PIC.
Antiretroviral Therapy	The combination of pharmacological inhibitors of viral enzymes, including reverse transcriptase, protease, and more recently integrase, that potently suppresses viral replication, viral load and prevents the development of AIDS in patients.
Antiviral state	Generalized description of the state induced following induction of Interferon Stimulated Genes, or ISGs [133], which collectively act to reduce infection by a broad range of viruses.
cGAS	Cyclic GMP-AMP synthase (cGAS) is an intrinsic sensor of cytosolic DNA that, when activated, initatiates the expression of interferon dependent genes associated with the antiviral state [13,14]
Dynein	A microtubule motor protein in cells which couples ATP hydrolysis to mechanical movement of cellular cargoes. Dynein transports cargoes towards the minus-end of the microtubule, which are typically at the microtubule organizing center (MTOC) adjacent to the nucleus.
Fullerene Cone	A closed conical structure comprised primarily of linked hexagonal rings. This term was originally used to describe hollow carbon structures which assume spherical or elliptical shapes. This shape is also assumed by the hexamers and pentamers of CA protein which form the viral core [3]
Integrase	All retroviruses express an integrase enzyme (IN) which is responsible for inserting the double stranded DNA genome generated by RT into the host cell DNA.
Kinesin	Kinesin motor proteins couple ATP hydrolysis to mechanical movement of cargoes, as does dynein. However, unlike dynein, there are many types of kinesins[107], and these motors typically traffic cargoes towards the plus-end of microtubules, away from the nucleus.
Lentivirus	The genera of retroviruses which includes HIV-1 and related primate immunodeficiency viruses. Lentiviruses are distinguished by the expression of specific regulatory proteins and the ability to infect non-dividing cells.
Microtubule	Microtubules are a component of the cytoskeleton which are formed from polymerized tubulin. Interactions with dynein and kinesin

	motores, they provide the framework necessary to facilitate the transport of numerous cargos, including viruses, which are otherwise too large to diffuse through the protein dense cytoplasm [134].
Nuclear Pore Complex (NPC)	Large (~50 mDa) multiprotein assemblies which govern transport across the nuclear envelope. NPCs are comprised of approximately 30 different proteins, termed nucleoporins (Nups) [102]
Preintegration complex (PIC)	Following the completion of reverse transcription, integrase mediated endonuclease priming of the 5′ and 3′ ends of the genome generates a replicative intermediate capable of integrating into target DNA. We use the term PIC when the ability to integrate into surrogate DNA has been demonstrated in specific studies.
Protease	All retroviruses express an aspartyl protease (PR) which cleave immature polyproteins incorporated into virions, including Gag and less abundant Gag-Pro and Gag-Pro-Pol polyproteins. PR is a critical target of antiretroviral therapy, as polyprotein cleavage is absolutely necessary for viral infectivity.
Restriction factor	A protein with antiviral activity when expressed in cells. Generally, such antiviral proteins exhibit signs of positive selective pressure and clear evidence of viral adaptation designed to mitigate the antiviral activity of the protein.
Reverse Transcriptase	All retroviruses express a reverse transcriptase (RT) enzyme, a DNA polymerase which copies the viral genomic RNA in the process of reverse transcription. During this process, RT uses both RNA and DNA templates to generate a linear, double stranded DNA genome. RT is a critical target of antiretroviral therapy.
Reverse Transcription Complex (RTC)	Once the viral ribonucleoprotein enters the target cell and begins reverse transcription of its RNA genome, it is refered to as an RTC. As reverse transcription is thought to intiate rapidly following fusion, we utilize this term to generically describe the infectious viral complex following fusion.
Simple Retrovirus	A retrovirus, such as murine leukemia virus, encoding only *gag*, which encodes for viral structural proteins, such as matrix and capsid, *pro,* which encodes the viral protease, *pol,* which encodes RT and integrase proteins, and *env,* which encodes the viral envelope.
TREX1	Cytosolic exonuclease which degrades HIV-1 DNA which accumulates in target cells. Despite this seemingly antiviral function, TREX1 mediated degradation of viral DNA products correlates with an inhibition of innate immune sensors leading to type I interferon activation [16]

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Biographies

Ed Campbell, Ph.D.:

Dr. Campbell is an Associate Professor at the Stritch School of Medicine at Loyola University Chicago in the Department of Microbiology and Immunology. He completed both his undergraduate and graduate studies at the University of Illinois, at the Champaign-Urbana and Chicago campuses, respectively. With his Ph.D. in microbiology and immunology, Dr. Campbell undertook postdoctoral research and training at Northwestern University in the Fienberg School of Medicine, in the lab of Dr. Tom Hope, before joining the faculty of Loyola University Stritch School of Medicine in Chicago in 2008. His research focus is on the mechanisms by which cellular proteins positively or negatively regulate viral infection and the development of quantitative fluorescent imaging techniques designed to monitor the fate of the HIV-1 viral complex following fusion.

Tom Hope Ph.D.

Dr. Hope holds multiple appointments as a Professor in the Departments of Cell and Molecular Biology, Obstetrics and Gynecology, and Biomedical Engineering at Northwestern University. He received his Ph.D. from UC Berkeley in Immunology and did his postdoctoral training at UC San Francisco where he began to study HIV. To study the cell biology of HIV, the Hope lab has developed a series of techniques and tools that allow the fluorescent labeling of HIV particles or viral proteins and the identification of infected cells. This technology now allows the ability to follow virions and infected cells from the tissue culture dish to whole animal models.

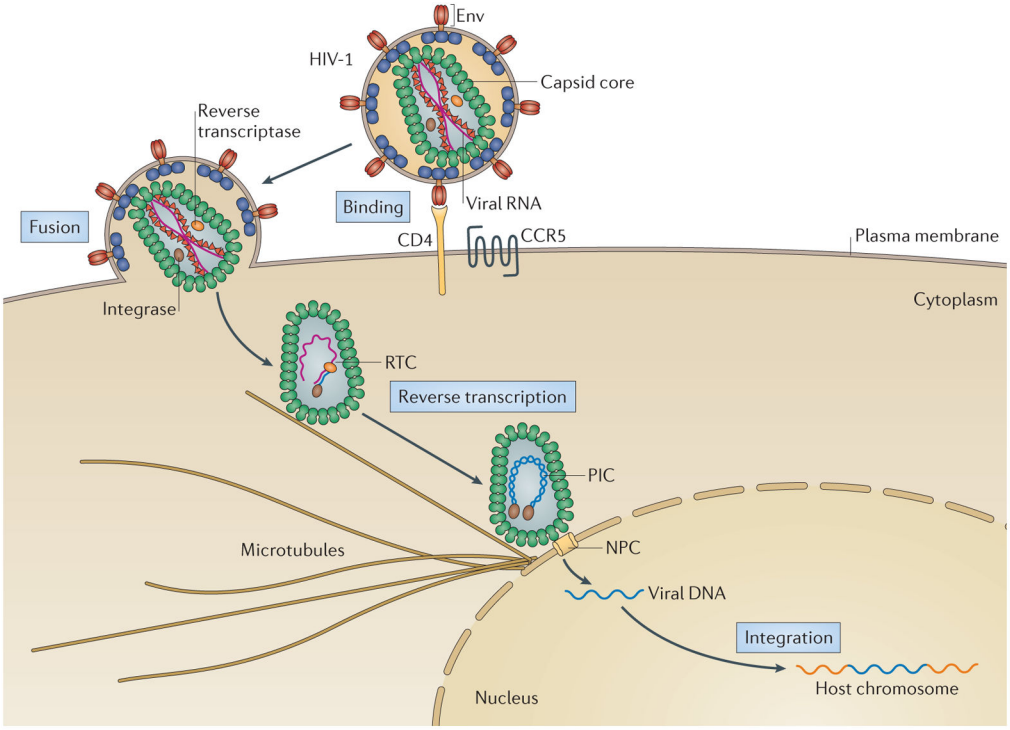

Figure 1. The early phase of the HIV-1 replication cycle

HIV-1 infection is initiated by the binding of the viral envelope (Env) glycoproteinand the CD4 receptor and the chemokine coreceptors, CCR5 or CXCR4, on the cell surface. This interaction results in fusion of the viral and cellular membranes and release of the viral capsid core into the cytoplasm. At this point, the virus begins reverse transcription, whereby it converts its RNA genome into the double stranded DNA genome that ultimately integrates into the host cell chromosome. At this point, the viral complex is referred to as the reverse transcription complex (RTC) and. During this time, the viral capsid core utilizes the microtubule network of the host cell to traffic towards the nucleus. Following arrival at the nucleus, the pre-integration complex (PIC) that contains the viral genome traffics through the nuclear pore complex (NPC) in a process that is dependent of the viral capsid protein (CA), although the precise interactions mediating this step, and the state of the viral capsid core during this step, are unclear. Following nuclear import, the completely transcribed viral genome is inserted into the host cell chromosome. This integrated provirus is then responsible for the expression of viral proteins necessary for the generation of progeny virions from the infected cell.

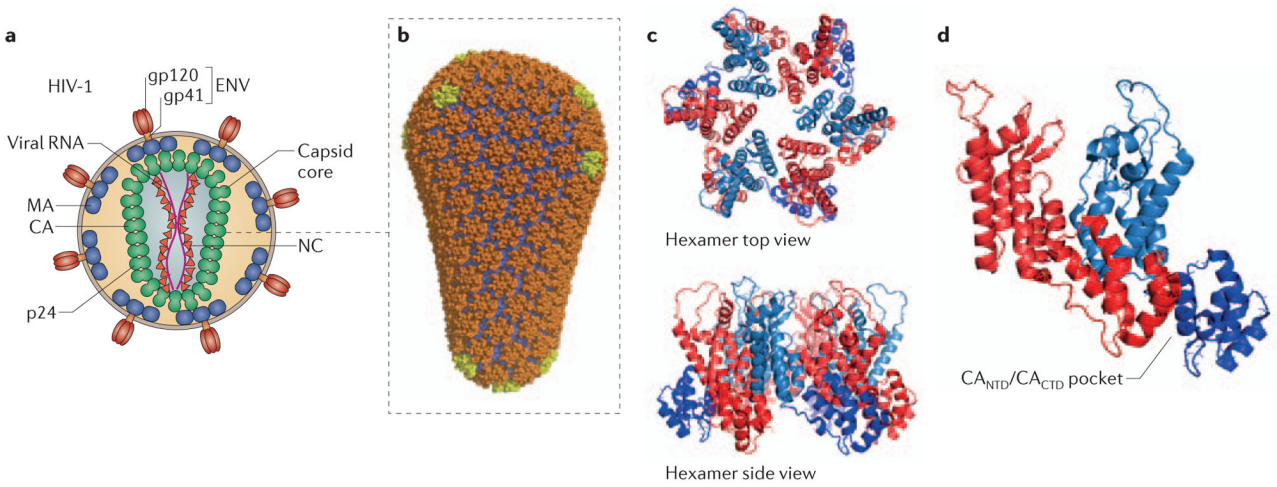

Figure 2. Capsid structure and function

a. Schematic representation of the HIV-1 mature virion, showing the viral proteins envelope (Env, which is made up by gp120 subunits and gp41 subunits), and the Gag polypeptide-derived proteins matrix (MA), capsid (CA) and nucleocapsid (NC). The conical viral capsid core is assembled from CA hexamers and pentamers. The capsid core harbours the viral RNA genome, which is associated with NC. B. The conical capsid core assembles into a fullerene cone, containing hexameric (orange) and pentameric (yellow) CA subunits. C. Top view and side view of the hexameric subunits that form the primary capsid core of HIV-1. The CA N-terminal domain (CA_{NTD}) (blue) and the CA C-terminal domain (CA_{CTD}) (red) that stabilize the assembled hexamer. Structure of two CA monomers of a hexamer illustrating the CA_{NTD}-CA_{CTD} pocket that mediates interactions between CA and host cell proteins. The conical capsid structure was modified with permission from reference [129].

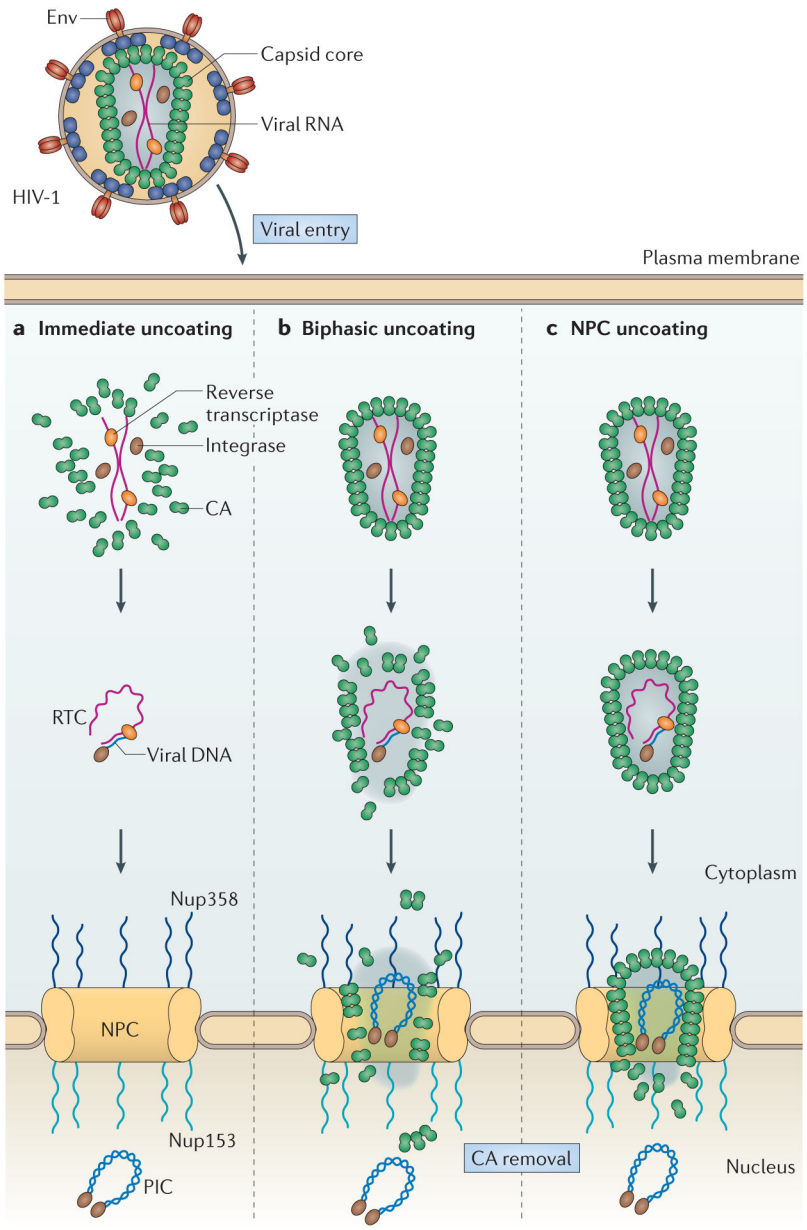

Figure 3. Models of viral uncoating

Experimental evidence suggests three potential mechanisms by which uncoating of the viral capsid core of HIV-1 might occur. Early biochemical studies suggested that core disassembly occurred rapidly and relatively completely soon after HIV-1 fusion to the plasma membrane (Immediate uncoating, part a.) [26,27]. Other studies, including a number of imaging based approaches, support a model where some core disassembly occurs in the cytoplasm, while a measurable amount of the viral capsid protein (CA) remains associated with the reverse transcription complex (RTC) that mediates the association with critical host factors and nuclear import (Gradual uncoating, part b)[38,42]. Alternatively, other studies support the notion that the core remains intact until it arrives at the nuclear pore complex (NPC), allowing it to protect its replicating genome from cytosolic DNA sensors (NPC

uncoating, part c)[14,15]. Both the biphasic uncoating and NPC uncoating models can support the finding that some level of CA remains associated with the pre-integration complex (PIC) in the nucleus [20,22,23]. Env, viral envelope glycoproteins.

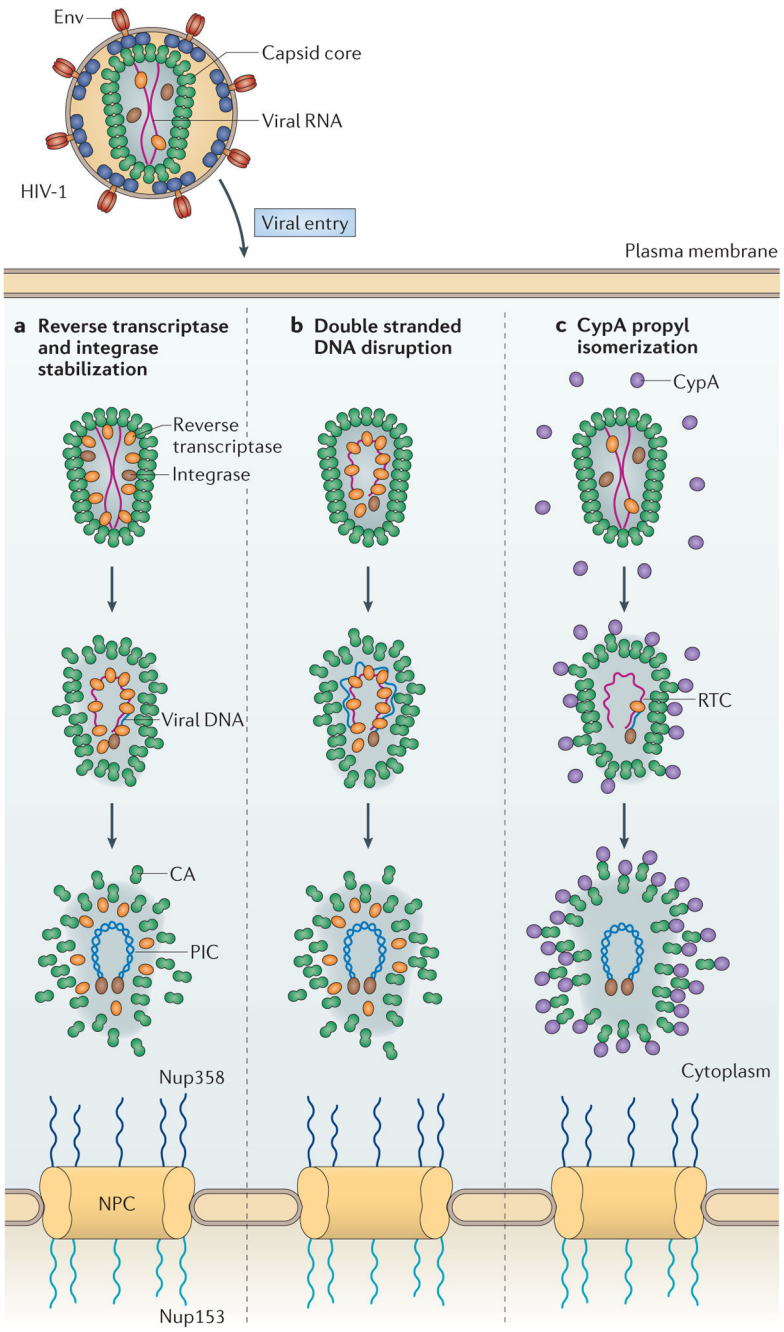

Figure 4. Viral and cellular factors involved in HIV-1 uncoating

Experimental evidence suggests that different viral and cellular factors are involved in uncoating of the viral capsid core of HIV-1. **A.** Interactions between the viral enzymes reverse transcriptase and integrase inside the capsid core [52] may provide structural support to the core, which is lost upon assembly of the reverse transcription complex (RTC) and initiation of reverse transcription **B.** Facilitation of uncoating by reverse transcription[38,51] may occur as the development of double stranded DNA during reverse transcription induces destabilizing activity from within the core. **C.** Following binding of cyclophilin A (CypA) to

CypA binding loops exposed on the surface of the viral capsid protein (CA), CypA may mediate uncoating through isomerization of the proline in the CypA binding loop of CA[66]. Simultaneously, or perhaps alternatively, CypA binding may prevent the interaction between the capsid core and a cryptic restriction factor [57,130,131]. Env, viral envelope glycoproteins; PIC, pre-integration complex; NPC, nuclear pore complex.

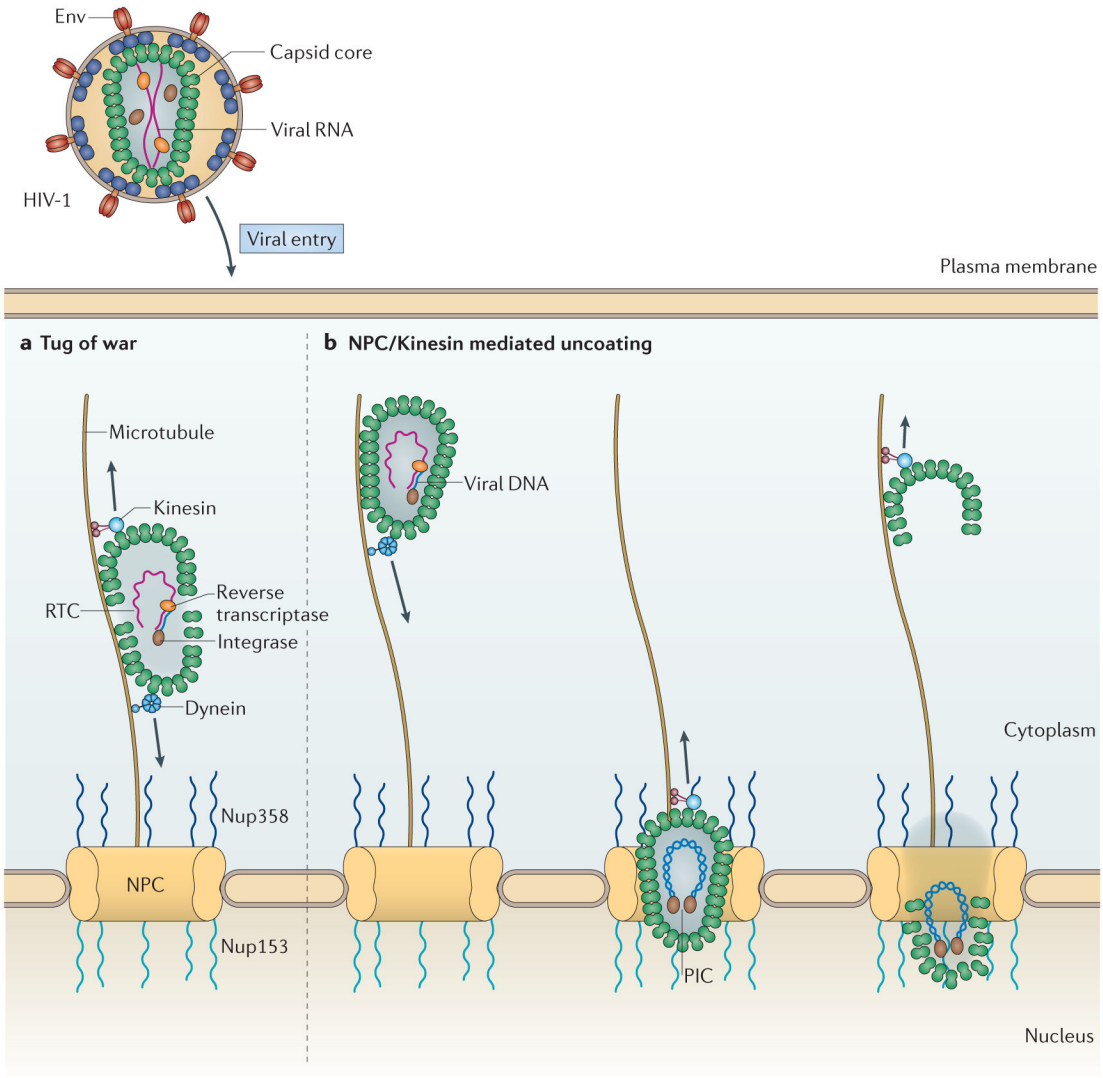

Figure 5. Mechanisms of microtubule mediated HIV-1 uncoating

The dependence of microtubule motors on the process of uncoating of the HIV-1 capsid core [39,78] may occur via a number of mechanisms. A. Uncoating may occur through a bi-directional "tug-of-war" mediated by opposing dynein and kinesin motor proteins [132]. B. Alternatively, dynein medited trafficking may be necessary to facilitate the interaction between the capsid core and components of the nuclear pore complex (NPC). Engagement of NPC components, such as Nup358, might directly induce uncoating [99,105]. Alternatively, these factors may act to anchor the core at the NPC while the engagement of the core by kinesin motor proteins provides the force necessary for core destabilization [39]. Env, viral envelope glycoproteins; PIC, pre-integration complex; RTC, reverse transcription complex.