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Roles of HIV-1 capsid in viral replication and immune evasion

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ABSTRACT

The primary roles of the human immunodeficiency virus type 1 (HIV-1) capsid (CA) protein are to encapsidate and protect the viral RNA genome. It is becoming increasing apparent that HIV-1 CA is a multifunctional protein that acts early during infection to coordinate uncoating, reverse transcription, nuclear import of the pre-integration complex and integration of double stranded viral DNA into the host genome. Additionally, numerous recent studies indicate that CA is playing a crucial function in HIV-1 immune evasion. Here we summarize the current knowledge on HIV-1 CA and its interactions with the host cell to promote infection. The fact that CA engages in a number of different protein-protein interactions with the host makes it an interesting target for the development of new potent antiviral agents.

1. Introduction

The human immunodeficiency virus (HIV) type 1 is a member of the retrovirus family and is the causative agent of AIDS (acquired immunodeficiency syndrome). HIV-1 infection causes cell-mediated immune deficiency through the progressive loss and dysregulation of CD4+ T lymphocytes. In the mid-1990s, the advent of highly active antiretroviral therapy (HAART) significantly extended the lives of many patients (reviewed in Camacho and Teofilo, 2011; Shen and Siliciano, 2008) and although not a cure, the administration of different combinations of antiretroviral drugs has truly changed a universally fatal disease into a potentially chronic one.

The HIV-1 life cycle begins with attachment and fusion of the viral envelope to the cell membrane of a susceptible host (reviewed in Wilen et al., 2012), whereby the capsid disassembles to release the viral genome and accessory proteins into the cytoplasm. The HIV-1 single-stranded RNA (ssRNA) genome is converted into double-stranded DNA (dsDNA), which is transported into the nucleus and integrated into the host chromosome. The integrated provirus encodes the structural proteins Gag, Pol and Env, which are common to all retroviruses. Independent of any other viral protein, the polyprotein Gag is transported to the plasma membrane and assembles to produce particles that are morphologically indistinguishable from immature virions (Bell and Lever, 2013). The products of the *pol* gene include the viral protease (PR), reverse transcriptase (RT) and integrase (IN). The *env* gene produces a precursor for the envelope glycoproteins gp120 and gp41. Additionally, HIV-1 carries six regulatory and accessory genes being *tat*, *rev*, *nef*, *vif*, *vpr* and *vpu*, which are unique to HIV-1 and play important roles in viral replication and the development of AIDS. Late stages of virus assembly occur at the plasma membrane, where the virion acquires its host-derived lipid envelope leading to virus budding into the extracellular space (reviewed in Bieniasz, 2009; Meng and Lever, 2013; Sundquist and Krausslich, 2012).

The Gag polyprotein multimerizes on dimeric, positive-strand viral RNA genomes and directs assembly, budding and maturation (Bell and Lever, 2013). Gag is a modular protein which is proteolytically cleaved by PR into 6 domains that plays specific roles during the HIV-1 life cycle. Of these six, three are conserved folded domains being the N-terminus matrix (MA), capsid (CA) and nucleocapsid (NC). During the late phase of virus replication, the myristylated MA domain anchors Gag to specific membrane microdomains and promotes assembly at the plasma membrane (Bouamr et al., 2003). The highly basic stretch of residues in the NC domain is responsible for recruiting the HIV-1 genome and serves as a scaffold for Gag multimerization (Burniston et al., 1999; Cimarelli et al., 2000; Khorchid et al., 2002). Two small spacer regions, p2 and p1, flank the NC domain (Briggs et al., 2009). Finally, the C-terminal p6 domain of Gag recruits endosomal sorting complexes required for transport (ESCRT) – I and III machineries

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that facilitate budding and release of newly synthesized particles (reviewed in Meng and Lever, 2013; Morita and Sundquist, 2004). HIV-1 particle budding induces maturation, whereby Gag is cleaved by the viral PR, resulting in NC proteins that condense onto the viral ssRNA, the formation of a conical core around the viral ribonucleoprotein (RNP) by CA proteins, and MA proteins that remain attached to the host-derived lipid bilayer.

Approximately 1500 CA subunits (24 kDa) form the HIV-1 capsid (Ganser et al., 1999), which provides structural stability to the virion that protects the enclosed viral ssRNA genomes. The asymmetric architecture of the fullerene-shaped cone is the result of 12 pentamers and approximately 200 hexamers (Ganser et al., 1999; Pornillos et al., 2011). X-ray crystallography and multidimensional nuclear magnetic resonance (NMR) studies indicate that CA is composed of a C-terminal domain (CTD) comprising four α-helices (Du et al., 2011; Gamble et al., 1997) and a N-terminal domain (NTD) comprising seven α-helices and one β-hairpin (Gitti et al., 1996; Momany et al., 1996), which are connected by a flexible linker (Berthet-Colominas et al., 1999; Jiang et al., 2011). Several structures of isolated CTD and NTD exist (Byeon et al., 2009; Gamble et al., 1997; Gitti et al., 1996; Worthylake et al., 1999) and structural information regarding the full-length CA protein is limited due to large-scale interdomain motion between the two domains (Deshmukh et al., 2013; Du et al., 2011; Monaco-Malbet et al., 2000; Shin et al., 2011). Recently, Zhao et al., reported the complete atomic HIV-1 capsid model by cryo-electron tomography (Zhao et al., 2013). Individual CA monomers spontaneously self-assemble into sheets with a natural cylindrical (Byeon et al., 2009; Ganser et al., 1999; Gross et al., 1997; Li et al., 2000) or conical (Briggs et al., 2003; Ganser et al., 1999) curvature and are used to as a model to study the hexagonal lattice of the capsid core. Recent structural studies of these tubular assemblies define the NTD and CTD protein interfaces through which the CA subunits interact to form the HIV-1 capsid (Bayro et al., 2014; Byeon et al., 2009; Ganser-Pornillos et al., 2007; Li et al., 2000; Pornillos et al., 2009, 2011). These intermolecular interfaces include the hexameric ring formed by the NTD–NTD interface (Pornillos et al., 2009, 2011); the interface formed between CTDs, which connects adjacent hexameric rings (Byeon et al., 2009; Gamble et al., 1997; Ganser-Pornillos et al., 2007; Worthylake et al., 1999); and the hexamer stabilizing NTD–CTD intersubunit interface (Pornillos et al., 2009). Over the course of an infection, CA mutations that naturally arise at these interface sites have a negative impact on viral fitness with the most profound effects being at the NTD–NTD interface (Manocheewa et al., 2013).

In this review, we attempt to present a unifying picture of CA throughout HIV-1 replication. We delve into the emerging roles that HIV-1 CA plays throughout the virus life cycle and examine the various host cell co-factors that interact with HIV-1 CA to inhibit downstream processes and initiate innate immunity or to promote HIV-1 infectivity.

2. The role of CA in post-entry events

Once the viral core enters the cytoplasm it undergoes a controlled disassembly process from the viral RNP complex, termed uncoating. This restructuring process results in the formation the reverse transcription complex (RTC) and initiates reverse transcription. The CA subunit interactions confer an intrinsic stability to the HIV-1 capsid as demonstrated by mutagenesis studies at the NTD–NTD and CTD–CTD intersubunit interfaces (Byeon et al., 2009; Forshey et al., 2002; Wacharapornin et al., 2007; Yang and Aiken, 2007; Yufenyuy and Aiken, 2013). For example, the CA mutations E128A/R132A strengthen the capsid core and reduce the efficiency of reverse transcription (Forshey et al., 2002).

Different target cells demonstrate variable kinetics of CA protein loss and reverse transcription (Arfi et al., 2009), however, genetic evidence indicates that HIV-1 uncoating can occur as early as 30 min post-infection (Hulme et al., 2011; Perez-Caballero et al., 2005). This timing is consistent with an elegant experiment in which "click" chemistry (Jao and Salic, 2008) of incoming HIV-1 RNA and fluorescent confocal microscopy demonstrate a rapid and progressive loss of CA from the core post-infection and diffusion of RTC components throughout the cytoplasm (Xu et al., 2013). Although the precise timing of uncoating and reverse transcription remains elusive, it appears that the two processes impact on each other and may be occurring in parallel (Arfi et al., 2009; Hulme et al., 2011). Clearly uncoating is not a spontaneous process and many recent studies highlighting host cellular factors that assist in disassembly of the CA core to facilitate optimal HIV-1 reverse transcription (Fig. 1) (Ambrose and Aiken, 2014; Auewarakul et al., 2005; Fassati, 2012; Hilditch and Towers, 2014; Warrilow et al., 2008).

2.1. Pin1

Post-translational modifications such as phosphorylation often play important roles in modulating protein activity and protein-protein interactions. Several reports provide evidence that the CA protein is predominantly phosphorylated at serine residues (Mervis et al., 1988; Veronese et al., 1988). Pin1 is a peptidyl-prolyl isomerase that specifically binds phosphorylated serine/threonine-proline motifs and catalyzes the *cis/trans* isomerization of the peptide bond. Pin1 knockdown by RNA interference (RNAi) or mutation at CA residues Ser16A/Pro17A impairs HIV-1 infectivity at the reverse transcription step and results in an accumulation of CA cores in the cytoplasm (Misumi et al., 2010). Moreover, Pin1 interacts with the CA core through the phosphorylated Ser16-Pro17 residues (Cartier et al., 1999; Misumi et al., 2010), which are modified specifically by virion-associated extracellular signal-regulated kinase 2 (ERK2) (Dochi et al., 2014). Suppression of ERK2 packaging results in reduced phosphorylation of CA residue Ser16, which translates to a blockade in reverse transcription and an attenuation of HIV-1 replication (Dochi et al., 2014). The authors hypothesize that CA phosphorylation acts a molecular switch to promote Pin1-dependent uncoating (Fig. 1) (Dochi et al., 2014; Misumi et al., 2010).

2.2. PDZD8

The PDZ-domain-containing protein 8 (PDZD8) is a newly identified host factor contributing to HIV-1 post-entry events (Guth and Sodroski, 2014). This host protein was identified using *in vitro* assembled HIV-1 CA-NC complexes, which produce cylindrical tubes with a hexameric lattice that mimics the viral capsid (Ganser et al., 1999; Li et al., 2000). The CA-NC complexes act as a substrate for cytoplasmic lysate components and reveal PDZD8 as possessing capsid-stabilizing properties (Fig. 1) (Guth and Sodroski, 2014). The cellular role of PDZD8 is poorly defined but has been proposed to be involved in regulating microtubule stability (Henning et al., 2011) and previously identified as an HIV-1 co-factor of unknown function (Henning et al., 2010). Depletion of PDZD8 adversely affects HIV-1 capsids by accelerating disassembly and blocking HIV-1 infection at a step before reverse transcription, suggesting that PDZD8 is exerting an effect on HIV-1 uncoating (Guth and Sodroski, 2014; Henning et al., 2010). Initial results indicate that the capsid stabilizing effect of PDZD8 may extend to a broad range of species, including other retroviruses (murine leukaemia virus, simian immunodeficiency virus) (Guth and Sodroski, 2014).