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Review

Multiple roles of the capsid protein in the early steps of HIV-1 infection

Ariberto Fassati*

The Wohl Virion Centre and MRC Centre for Medical & Molecular Virology, Division of Infection and Immunity, University College London, Cruciform Building, 90 Gower Street, London WC1E 6BT, UK

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ABSTRACT

The early steps of HIV-1 infection starting after virus entry into cells up to integration of its genome into host chromosomes are poorly understood. From seminal work showing that HIV-1 and oncoretroviruses follow different steps in the early stages post-entry, significant advances have been made in recent years and an important role for the HIV-1 capsid (CA) protein, the constituent of the viral core, has emerged. CA appears to orchestrate several events, such as virus uncoating, recognition by restriction factors and the innate immune system. It also plays a role in nuclear import and integration of HIV-1 and has become a novel target for antiretroviral drugs. Here we describe the different functions of CA and how they may be integrated into one or more coherent models that illuminate the early events in HIV-1 infection and their relations with the host cell.

Contents

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of AIDS or acquired immunodeficiency syndrome. It is a lentivirus uniquely adapted to replicate in human cells, in particular CD4+ cells such as helper T-lymphocytes, macrophages and microglial cells. HIV-1 tropism depends mainly on the expression of the cell receptor CD4 and co-receptors CCR5 and CXCR4. The seven-transmembrane domain chemokine receptor CCR5 is mainly present on the surface of memory CD4+ T-cells, macrophages and microglial cells, whereas the CXCR4 co-receptor is mainly expressed in naïve CD4+ T-cells (Freed and Martin, 2001).

The HIV-1 genome in its integrated DNA form is approximately 9.8 Kb in size and encodes for three polyproteins (Gag, Gag–Pol and Env) and six smaller accessory proteins (Vif, Vpr, Vpu, Tat, Rev and Nef). Env mRNA is spliced and translated into gp160, which is then cleaved by a furin-like cellular protease into a "surface" (SU also called gp120) and a "transmembrane" region (TR also called gp41). Gp120 is responsible for engaging with CD4 and the co-receptors, whereas gp41 induces fusion of the viral membrane with the cell membrane. Pol encodes for the viral enzymes protease (PR), reverse transcriptase (RT) and integrase (IN). Gag encodes for structural proteins matrix (p17 MA), capsid (p24 CA), nucleocapsid (p7 NC), p6 and spacer peptides Sp1 and Sp2 (Freed and Martin, 2001). Gag forms the capsid core and during or shortly after budding the viral protease cleaves it into the different

Abbreviations: CA, capsid protein; CsA, cyclosporine; CypA, cyclophilin A; CPSF6, cleavage and polyadenylation specificity factor subunit 6; Tnpo3, transportin 3.

* Tel.: +44 20 31082138; fax: +44 20 31082123.
E-mail address: a.fassati@ucl.ac.uk

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components inducing dramatic conformational rearrangements that have important functional consequences (Bharat et al., 2012).

In the unprocessed form, Gag polyproteins form a capsid core of approximately 100 nm diameter with a regular arrangement whereby p17 MA is at the outer region in direct contact with the viral membrane, followed by p24 CA and NC, which is central and contacts the viral RNA genome (Bharat et al., 2012; Briggs and Krausslich, 2011; Yeager et al., 1998). Following protease activation and Gag cleavage, the immature core undergoes a dramatic rearrangement resulting in a fullerene conical structure of 100–120 nm in length and 50–60 nm wide composed of approximately 250 CA hexameric rings organized in a lattice (Fig. 1) (Briggs et al., 2003; Ganser et al., 1999; Ganser-Pornillos et al., 2007). Importantly, the presence of 12 CA pentamers distributed at the edges of the cone, 7 at the wide end and 5 and the narrow end, allows the lattice to curve and assume the typical conical geometry (Pornillos et al., 2011). The CA subunit within the hexameric ring are stabilized by quite extensive N-terminal domain (NTD)–NTD intermolecular interactions and less extensive NTD–CTD intermolecular interactions, mainly between NTD helices 4 and 7 and CTD helices 8 and 11, whereas adjacent rings are linked together mainly by mobile C-terminal domain (CTD)–CTD intermolecular interactions (Pornillos et al., 2009) (Fig. 1). The mobility of the CTD–CTD interactions allows the formation of the curvature in the lattice (Pornillos et al., 2009). Some details of how the conical geometry is generated are still unknown because high resolution X-ray crystallography cannot be applied to an intact core.

CA forms the building block of the hemaxers in the lattice. It is a 24 kDa protein with independently folded NTD and a CTD that are flexibly linked (Berthet-Colominas et al., 1999; Gamble et al., 1997; Gitti et al., 1996; Momany et al., 1996). The structure is highly helical; the NTD contains 7 alpha helices and the CTD contains 4 (Gamble et al., 1997; Gitti et al., 1996). Furthermore CA has a large loop encompassing residues 85–93 between helices 4 and 5 that is exposed to the surface of the hexamer and binds cyclophilin A (CypA) (Gamble et al., 1996) (Fig. 2).

There are approximately 1500 CA monomers in a mature HIV-1 core but there appears to be an excess of Gag molecules in the immature core (up to 5000) (Briggs et al., 2004). Upon Gag cleavage, some CA molecules are not incorporated into the mature core but the function of the excess CA is not known (Briggs et al., 2004). The mature retroviral core is less stable than the immature form and it has been difficult to isolate intact HIV-1 cores to perform biochemical and genetic studies. However it is possible to purify small amounts of mature HIV-1 cores by sedimentation in sucrose gradients through a thin layer of mild detergent, which strips the envelope and matrix (Aiken, 2009). Alternatively, many aspects of the core structure and function can be studied in vitro upon formation of the so-called “tubes”. These structures are generated by incubating purified recombinant CA or CA–NC with short RNAs or single stranded DNA oligonucleotides in the appropriate salt conditions (Ganser et al., 1999; Gross et al., 1998). The introduction of the point mutation R18A in CA helps generating lattices of different geometry, including spheres, cones and cylinders, which recapitulate the bonafide core structure (Ganser-Pornillos et al., 2007).

2. CA, core uncoating and reverse transcription

Following cell-receptor mediated entry, HIV-1 starts reverse transcribing its RNA genome. An early function of HIV-1 CA is to provide a suitable environment for reverse transcription within the so-called reverse transcription complex (RTC). The virus must also shed its core or “uncoat” to progress through the various steps of the life cycle. We have an incomplete understanding of these early steps, however some progress has been made in recent years. Biochemical fractionation in sucrose gradients of the cytosol of acutely infected cells indicated that uncoating happens quite early post-infection, probably within 1 h in the case of HIV-1 but only after nuclear entry following mitosis in the case of murine leukemia virus (MLV) (Fassati and Goff, 1999, 2001; Karageorgos et al., 1993). Notably, the results of the biochemical fractionation used to characterize MLV RTCs have been recently confirmed using a combination of elegant imaging and genetic approaches (Prizan-Ravid et al., 2010). Because the same fractionation procedure was used to characterize both viruses, the simplest explanation for the different time of uncoating of HIV-1 and MLV is that the MLV core is more stable than the HIV-1 core in the cytoplasm of infected cells. This also explains, at least in part, why MLV cannot infect non dividing cells, given that large amounts of CA are still associated with its RTC and pre-integration complex (PIC), presumably making it too bulky to go across nuclear pores (NPCs) (Bowerman et al., 1989; Fassati and Goff, 1999). The instability of the HIV-1 core as determined in biochemical assays may be less pronounced in the cytoplasm of infected cells, where host factors could stabilize it. However recent genetic evidence supports the idea that HIV-1 uncoating happens as early as 30–45 min post-infection (Hulme et al., 2011; Perez-Caballero et al., 2005). In fact the time of uncoating seems to be cell-type dependent, faster in HeLa cells, slower in CD4+ T-lymphocytes, suggesting that host cells factors may play a role as well (Arfi et al., 2009). There is evidence supporting the idea that significant uncoating happens before reverse transcription is completed. APOBEC3G is a restriction factor for HIV-1, counteracted by the viral accessory protein Vif, that promotes G to A mutations by deaminating C to U on the negative sense, single stranded viral DNA generated during reverse transcription (Malim, 2009). Recently, it has been shown that APOBEC3G in the target cells can attack the incoming virus in macrophages, suggesting that the protein has access to the viral genome before the completion of reverse transcription (Koning et al., 2011). LEDGF/p75 is a host co-factor that binds to HIV-1 IN and stimulates targeted integration into the host genome (Engelman and Cherepanov, 2008). A dominant form of LEDGF/p75 that localizes exclusively in the cytoplasm can target IN and perturb integration, suggesting that IN is accessible within the RTC/PIC (Meehan et al., 2011). Several host cell factors have been implicated in promoting reverse transcription, including nucleic acids binding proteins, DNA repair and splicing factors (Konig et al., 2008) and it is not unreasonable to assume that at least some of them act in the target cells on the incoming RTC, which must therefore be accessible. More recent data demonstrates that uncoating and reverse transcription proceed in parallel and may influence each other (Arfi et al., 2009; Hulme et al., 2011). It also not clear how much CA is shed from the core in the cytoplasm. Biochemical studies found that most CA is shed from the HIV-1 RTC and PIC (Bukrinsky et al., 1993; Farnet and Haseltine, 1991; Fassati and Goff, 2001; Karageorgos et al., 1993; Miller et al., 1997), yet it is quite possible that host factors compensate in part for the intrinsic instability of the HIV-1 core inside cells and that in fact more CA remains associated with the RTC than previously thought.

If uncoating happens too quickly however, reverse transcription does not take place. This is supported by two lines of evidence. TRIM5α is a restriction factor that blocks HIV-1 infection by targeting the incoming capsid core and inducing its proteasomal degradation (Malim and Bieniasz, 2012). TRIM5α acts very early post-infection (<1 h) and causes premature uncoating, leading to aborted reverse transcription (Malim and Bieniasz, 2012; Roa et al., 2012; Stremlau et al., 2006). Second, mutations in CA that make the HIV-1 core unstable also cause premature uncoating and aborted reverse transcription (Forshey et al., 2002). In fact there is elegant work based on specific mutations in CA and their functional characterization showing that optimal stability of the HIV-1 core is important for reverse transcription and for later