doi:10.1017/S1431927611001395

Microsc. Microanal. 17 (Suppl 2), 2011
© Microscopy Society of America 2011

Structure of HIV Capsid and Interactions with Rhesus TRIM5α

G. Zhao[*], X. Meng[*], D. Ke[*], T. Vu[*], J. Ahn[*], V. B. Shah[**], R. Yang[**], C. Aiken[**], L. M. Charlton[*], A. M. Gronenborn[*] and P. Zhang[*]

[*]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260
[**]Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232

Mature HIV-1 particles contain a conical-shaped capsid that encloses the viral RNA genome and performs essential functions in the virus life cycle. HIV-1 capsid protein (CA) folds into two distinct domains connected by a flexible linker [1,2]. In mature virions, the assembled capsid structure is best described by a fullerene cone model that is made up from a hexameric lattice containing a variable number of CA pentamers [3]. Furthermore, CA protein assemblies exhibit structural polymorphism, with mature virions exhibiting predominantly conical morphology, while the immature viral particles are spherical assemblies. Therefore, readjustment at the different contact surfaces of CA is necessary in HIV-1 capsid formation. We present a cryoEM study of a tubular assembly of CA. In the tubular assembly, CA intermolecular interfaces vary slightly, accommodating the asymmetry present in tubes. This provides the necessary plasticity to allow for controlled virus capsid assembly. More importantly, in the cryoEM map, we identified a novel CTD-CTD interface at the local three-fold axis and confirmed the functional importance of this trimer interface by mutagenesis and chemical crosslinking (Fig 1) [4].

TRIM proteins play important roles in the innate immune defense against retroviral infection, including human immunodeficiency virus type-1 (HIV-1). Rhesus macaque TRIM5α (TRIM5α_{rh}) targets the HIV-1 capsid and blocks infection at an early post-entry stage, prior to reverse transcription [5]. TRIM5α is a tripartite motif protein, with RING, B-box, coiled-coil (CC) and C-terminal B30.2/SPRY domain [6]. Studies have shown that binding of TRIM5α to the assembled capsid is essential for restriction and requires the coiled-coil and B30.2/SPRY domains [7], but the molecular mechanism of restriction is not fully understood. Using cryoEM, combined with mutagenesis and chemical cross-linking, we investigated the direct interactions between HIV-1 CA assemblies and purified TRIM5α_{rh} containing coiled-coil and SPRY domains (CC-SPRY$_{rh}$). CC-SPRY$_{rh}$ binds to CA assemblies in a concentration-dependent manner, while under equivalent conditions the human protein did not bind. More importantly, CC-SPRY$_{rh}$, but not its human counterpart, disrupted CA tubes, releasing linear fragments of protofilaments consisting of CA hexamers (Fig. 1A&B). Furthermore, such structural disruption was prevented by inter-hexamer crosslinking at the CA trimer interface using P207C/T216C mutant CA, but not by intra-hexamer crosslinking via A14C/E45C at the NTD-NTD interface. The same disruption effect by TRIM5α_{rh} on the inter-hexamer interfaces also occurred with purified intact HIV-1 cores. These results provide insights concerning how TRIM5α disrupts the virion core (Fig. 1C) and demonstrate that structural damage of the viral capsid by TRIM5α is likely one of the important components of the mechanism of TRIM5α-mediated HIV-1 restriction.

https://doi.org/10.1017/S1431927611001395 Published online by Cambridge University Press

References
[1] C. Berthet-Colominas et al., *EMBO J.* 18 (1999) 1124.
[2] T. R. Gamble et al., *Science* 278 (1997) 849.
[3] B.K. Ganser, et al., *Science* 283 (1999) 80–83
[4] I. L. Byeon et al., *Cell* 139 (2009) 780–790.
[5] M. Stremlau, et al. *Proc Natl Acad Sci U S A* 103 (2006) 5514-5519.
[6] S. Nisole, et al. *Nature Reviews Microbiology* 3 (2005) 799-808.
[7] B.K. Ganser-Pornillos et al. *Proc Natl Acad Sci U S A*.108 (2011) 534-9
[8] This work was supported by NIH grant P50GM082251 and R01GM085043.

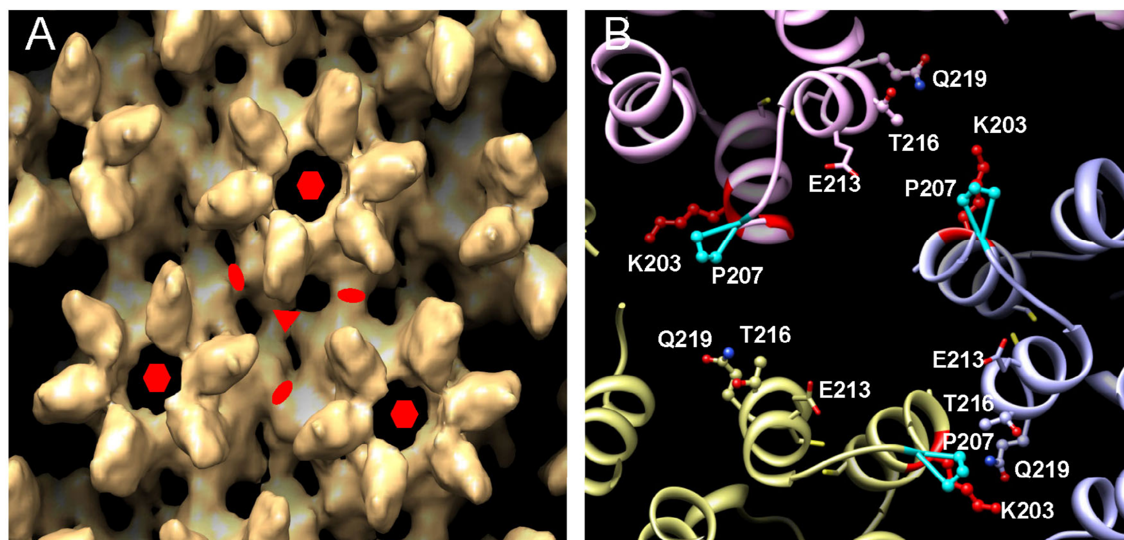

FIG 1. Inter-hexamer interactions at the local three-fold axis (A) Surface rendering of the density map of CA assembly displaying three neighboring hexamers. (B) Detailed view of the three-fold axis illustrating the interactions at the interface.

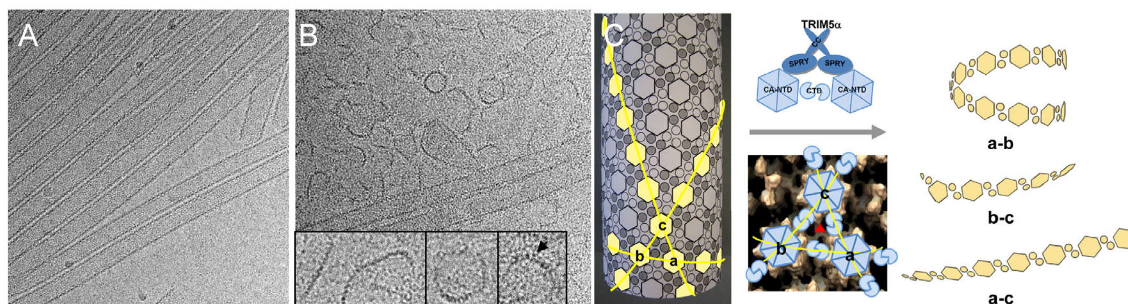

FIG 2 CryoEM analysis of the TRIM5α CC-SPRY interaction with wild-type CA assemblies. Low-dose projection images of CA assemblies incubated with human (A), or rhesus (B) TRIM5α CC-SPRY. (C) Model of TRIM5αrh CC-SPRY in HIV-1 CA restriction. Binding of rhesus TRIM5α CC-SPRY to assembled HIV-1 CA weakens the CTD trimer interface, thereby causing disruption of the lattice and releasing fragmented protofilaments (right panel).

https://doi.org/10.1017/S1431927611001395 Published online by Cambridge University Press