New HIV-1 Capsid Labeling System Does Not Support HIV-1 Uncoating During Nuclear Import

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BACKGROUND

HIV-1 uncoating is a prerequisite for viral DNA integration into the host genome and a promising target for antiviral therapy. However, the timing and cellular location of uncoating remain elusive, in part because current methods are unable to directly and accurately measure the amount of capsid protein (CA) loss from the infectious viral complexes. Bulk measurement of CA loss by biochemical methods or imaging of viral complexes may not reflect the behavior of infectious viral complexes, since only a small fraction of the viral complexes in the cell lead to infection. Quantification of CA by immunostaining with anti-CA antibody or live-cell imaging of viral complexes labeled with fluorescently-tagged cyclophilin A (CypA) is confounded by loss of epitope accessibility to the antibody or loss of interactions to CypA.

1.Efficient (>95%) labeling of virions with GFP-CA, ~2 fold loss of viral infectivity, and normal morphology at 1:10 phenotypic mixing with wild-type virus (GFP-CA 1:10).

2. The GFP-CA is incorporated into viral cores.

3. Intact (or mostly intact) viral cores are imported into the nucleus.

4. CPSF6 dissociates from viral cores <5 minutes of viral core uncoating.

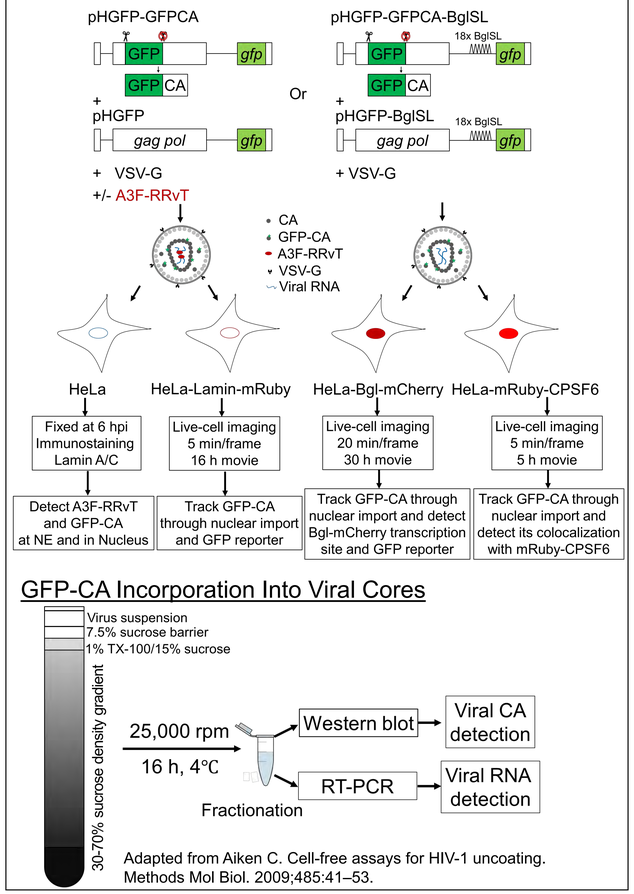
CONCLUSIONS

We developed a new HIV-1 capsid labeling system (GFP-CA) with >95% labeling efficiency of viral cores and only ~2 fold loss of viral infectivity. We validated that GFP-CA is incorporated into viral cores and can be used as a quantitative marker to measure the CA amounts associated with viral cores. Astonishingly, our results show that intact (or nearly intact) viral cores enter the nucleus through a mechanism involving interactions with host protein cleavage and polyadenylation specificity factor 6 (CPSF6), which dissociates from viral cores within 5 minutes of core uncoating.

Our findings fundamentally change our understanding of HIV-1 post-entry replication events.

METHODS

Direct Labeling of CA with GFP & Experimental Design

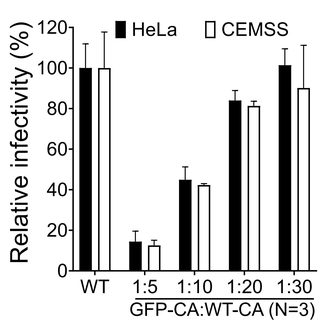


GFP-CA Incorporation Into Viral Cores

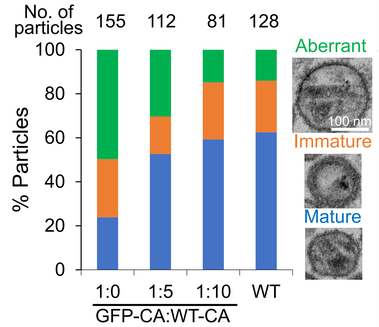
Adapted from Aiken C. Cell-free assays for HIV-1 uncoating. Methods Mol Biol. 2009;485:41–53.

RESULTS

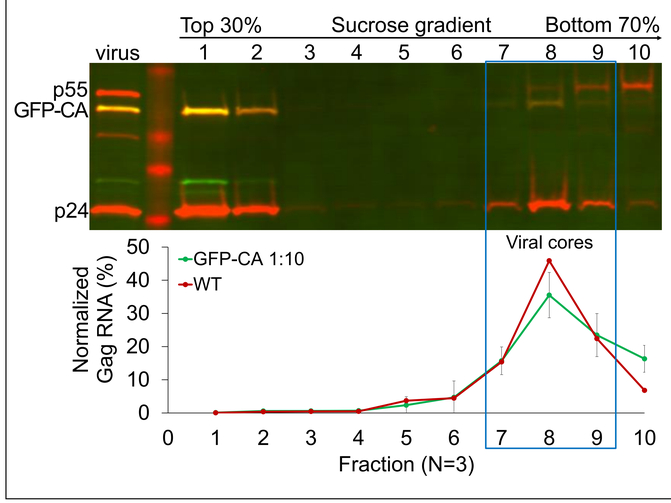
Infectivity in HeLa & CEM-SS



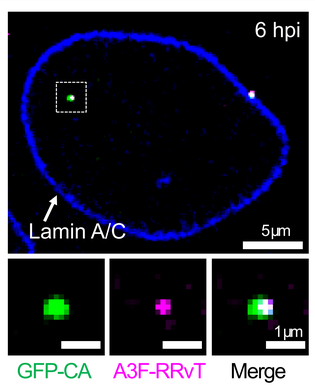
Virus Morphology



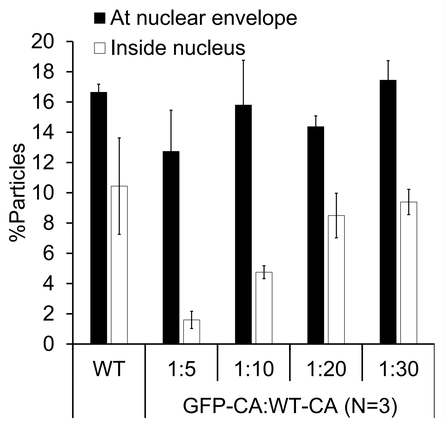
GFP-CA Incorporation into Viral Cores



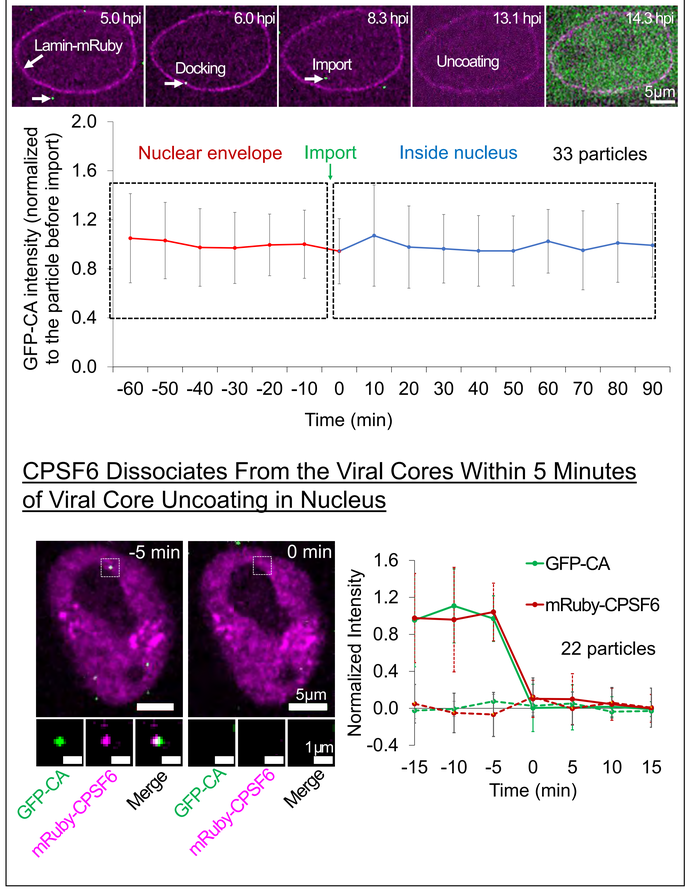
GFP-CA Cores Efficiently Associate with Nuclear Envelope and Enter Nucleus



70% (145/207) A3F-RRvT+ nuclear viral complexes were GFP-CA+



No Loss of GFP-CA in the Viral Cores During Nuclear Import



CPSF6 Dissociates From the Viral Cores Within 5 Minutes of Viral Core Uncoating in Nucleus