Novel versions of Cyclophilin A based markers to visualize HIV-1 capsids inside living cells

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ABSTRACT

This project aims to evaluate the amino acid composition in the host protein Cyclophilin A (CypA) domain required for its binding to the HIV-1 capsid. A chimeric version, CypA-DsRed (CDR), binds capsids with high avidity and serves as a marker for HIV-1 uncoating. A series of CypA modifications were evaluated in CDR for the ability to (1) bind capsids and incorporate into virus particles, (2) affect virus infectivity and (3) bind to native HIV-1 cores in vitro. HIV-1 particles were prepared by co-transfecting 293T cells with plasmid DNA encoding HIV-1 and CDR mutants, and their incorporation into virus particles were assessed. Confocal microscopy of virus supernatants showed that the truncated CDR versions were incorporated into HIV-1 virions, but less efficiently. The effects of CDR incorporation on virus infectivity and binding on glass were then evaluated. Analysis will collectively help pinpoint the amino acid residues in CypA domains that effectively bind to the HIV-1 capsid protein.

INTRODUCTION

Cyclophilin A is a key host-factor that binds HIV-1 capsids and facilitates virus infection of target cells. Prior work showed that CypA binds the G89 and P90 amino acid residues in capsids. However, the amino acids in CypA involved in this binding are not fully understood.

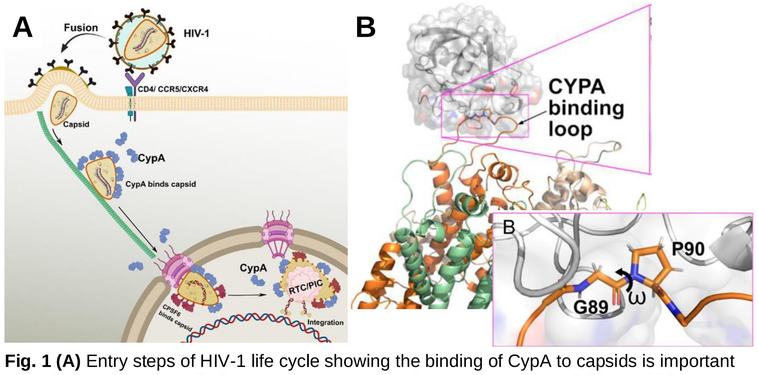


Fig. 1 (A) Entry steps of HIV-1 life cycle showing the binding of CypA to capsids is important until vDNA integration. **(B)** Structural data showing CypA binding to the G89 and P90 amino acid residues of capsid. Images were taken from (A) Padron and (B) Twizerimana

OBJECTIVE

Evaluate the incorporation of CDR recombinants with various deletions in CypA (Fig. 2) into virus particles

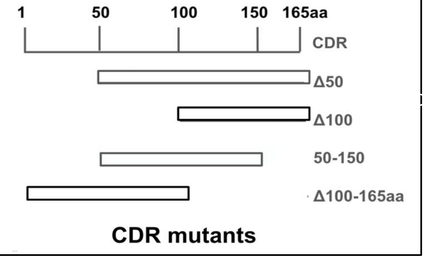
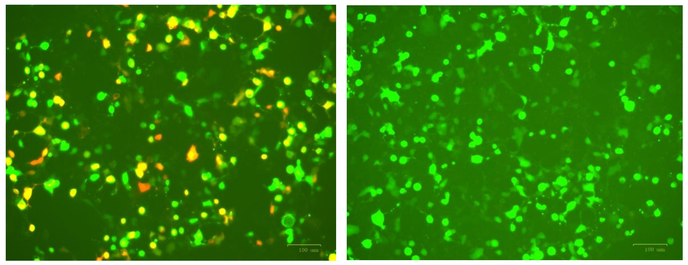


Fig. 2. Schematics showing CypA amino acid length and their deletions tested in this work

RESULTS

1. Transfection of CDR constructs in virus producer cells



pHIVeGFP and CDR | pHIVeGFP only (control)

Figure 3. Fluorescent microscopy shows production of CypA (tagged red) and virus particles (tagged green) in HEK 293T cells

2. CDR constructs do not affect virus infectivity

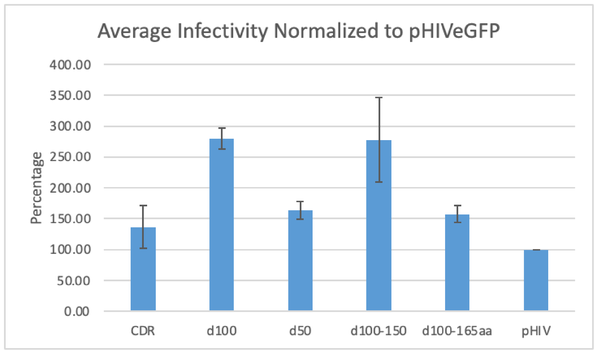


Figure 4. Luciferase reporter assays shows similar infective capacity of CDR-incorporated HIV-1 virions

3. A fraction of viruses incorporate CDR and its mutants

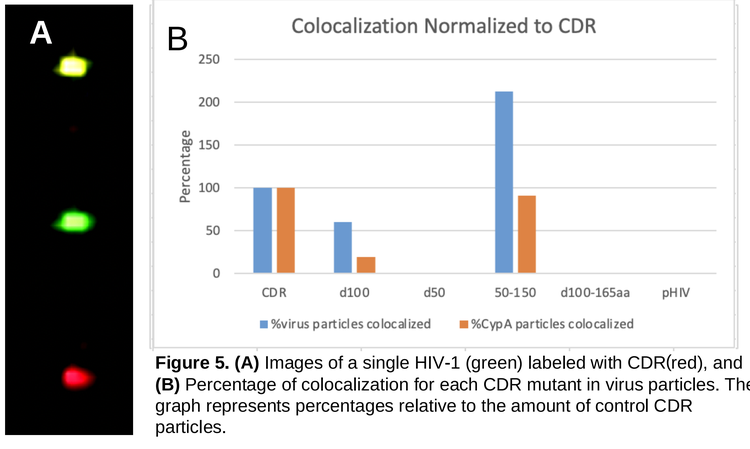


Figure 5. (A) Images of a single HIV-1 (green) labeled with CDR(red), and **(B)** Percentage of colocalization for each CDR mutant in virus particles. The graph represents percentages relative to the amount of control CDR particles.

DISCUSSION

SUMMARY

- Standard CDR, d100, d50, 50-150 bound to HIV-1 capsid protein and packaged into HIV-1 particles, albeit with different efficiency.
- The reason for different efficiencies is likely due to differences in plasmid vectors and/ or transfection results.
- Further work will evaluate if CDR and its truncations, when transfected equally into virus producing cells, will have altered binding to capsids.

Future Research

- Repeat transfections – control for cell production of proteins
- Control for CypA particles inside virions by lysis
- Smaller CypA domain truncations to narrow amino acids involved in capsid binding.

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