Testing for the Presence of HIV-1 Proteins Associated With vRNPs

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Background

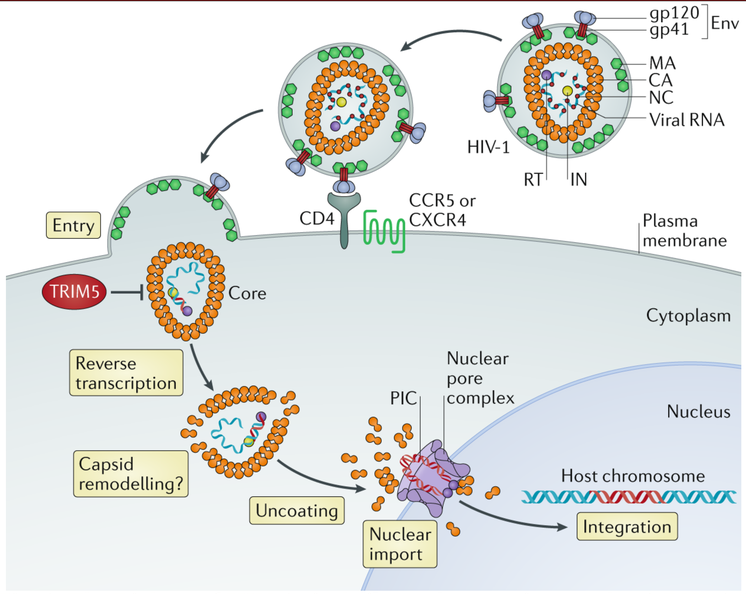

Fig. 1. The Biology of HIV-1 viral ribonucleoprotein complex (vRNPs). The Biology of HIV-1 viral ribonucleoprotein complex (vRNPs) during early steps of virus infection. The HIV-1 vRNP is made up of copy of viral RNA genome (vRNA) associated with enzymes reverse transcriptase (RT), integrase (IN) and the nucleocapsid (NC) protein. In infectious virions the vRNP is enclosed inside a conical capsid shell. Following cellular entry, the enclosing capsid transports the vRNPs into the nucleus. During this transport the vRNA genome in vRNPs is converted into a cDNA by RT through the process of reverse transcription. Once inside the nucleus, IN catalyzes the integration of the newly formed cDNA into the host-chromosomes.

Abstract

HIV-1 proteins are incorporated into virions as an initial precursor Gag/GagPol proteins. Following virus exit from the plasma membrane, a dimer of the protease (PR) domain of GagPol precursor cleaves itself from the rest of the proteins and then cleaves specific amino acid residues in the Gag/GagPol precursor proteins to release individual viral components, namely: matrix (MA), capsid (CA), SP1 and SP2 spacer peptides, the nucleocapsid (NC), p6 peptide, PR, reverse transcriptase (RT) and integrase (IN). This step of proteolytic processing is called virus maturation, as the vRNA is now condensed by NC, RT and IN to form the viral ribonucleoprotein complex (vRNPs). As the vRNPs are formed a conical capsid shell made up of 1600 CA proteins must polymerize in such a way that the vRNP is enclosed inside the cone shaped capsid. While vRNA, NC, RT and IN are known partners of the vRNP - whether other viral proteins also associate with the viral core remains unclear. Here we will incorporate fluorescently tagged HIV-1 proteins (SP1, SP2, NC, p6, PR) and incorporate these into virions and study their individual association with the vRNP complex. Our goal is to define the vRNP components and test for its presence, including function in target cells following HIV infection.

Knowledge Gaps

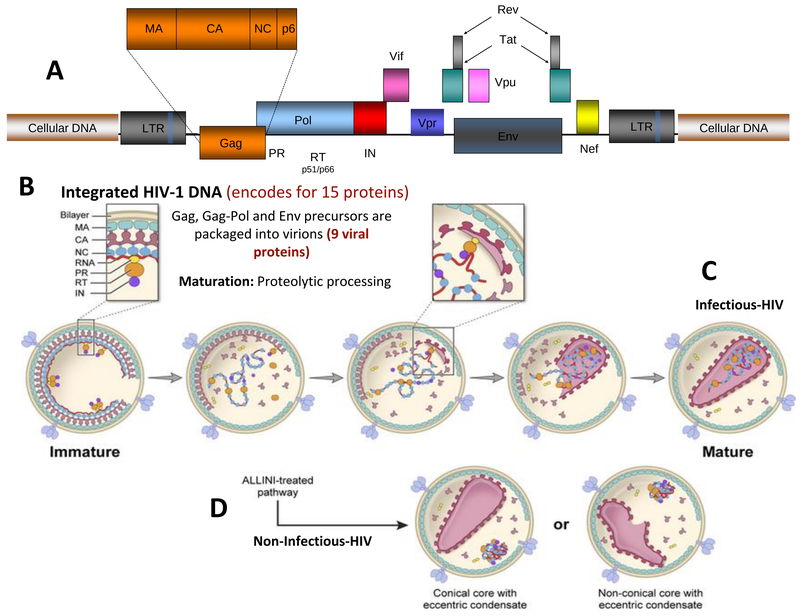

Fig. 2. Knowledge-Gaps. **(A)** The integrated vDNA encodes for 15 proteins, namely Matrix (MA), Capsid (CA), Nucleocapsid (NC), p6 from the Gag-gene, Enzymes protease (PR), reverse transcriptase (RT) and integrase (IN) from Pol-gene, the envelope (Env) and accessory proteins Vpr, Vpu, Vif, Tat, Rev and Nef. Only 9 of the 15 proteins are incorporated into virions in the form of long-precursors of Gag- , Gag-Pol and Env proteins, the accessory protein Vpr is also found in virions. **(B)** Virus assembly and exit takes place at the plasma-membrane as an immature HIV-1 particle. Following exit, the protease cleaves the precursors into individual components during the process of virion maturation. **(C)** Maturation leads to the formation of an infectious particle through the assembly of a conical capsid from individual CA-proteins. During this assembly, the vRNP is formed by condensation of vRNA performed by NC, RT and IN, **but how does the vRNP end-up inside the capsid shell remains unknown**. **(D)** Until recently, it was believed that stochastic processes drive vRNP encapsidation. Recent evidence showed that virions treated with an IN-multimerizing drug (ALLINI) changes the localization of vRNPs, outside capsids. Thus, arguing against stochastic vRNP encapsidation model. Moreover, the vRNP-localization of the other virus associated proteins remain unclear.

Expected Results & Future Directions

We expect that eGFP-fusion of the small p1, p2 and p6 proteins (product of Gag-cleavage) will package with vRNPs. We will verify the relevance of such packaging by deleting or modifying these 5-amino acids in the context of the full-length virus and test its role in virus maturation, vRNP encapsidation, and effect on infectivity by cellular, biochemical, and virological assays.

Experimental Approach & Methods Cont.

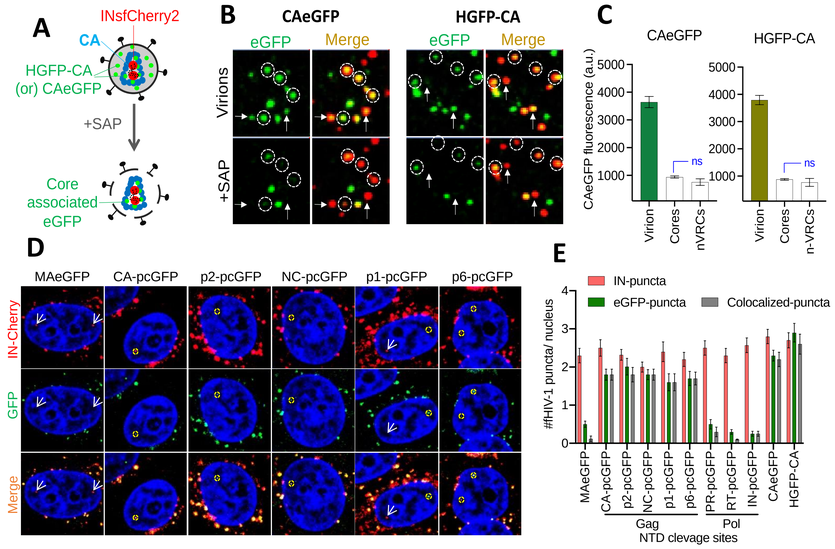

Fig. 4. Single virus imaging approach to localize GFP-fusion proteins in virions and inside the vRNPs.(A) Cartoon and (B) Images of dual labeled virus particles bound on glass. The HIV-1 particles are labeled with 2- colors a red-marker tagging the IN-protein of the vRNP and the green-marker tagged to CA-protein. The virus incorporated eGFP-(green)-tagged CA proteins re-distribute to the virus volume (free-floating), or a subset become associated with vRNPs. (C). Following infection of target cells with these viruses, the percolation of the coreassociated eGFP-signals into the nucleus identified vRNP association (compare signals of core-associated vs. nuclear replication complexes nVRCs, in C). The Francis lab has found that GFP-fusion proteins carrying the 5- aa's protease cleavage sites sequences of Gag-proteins (CA, p2, NC, p1 and p6) act as vRNP-targeting signals and remain associated with the viral replication complex inside the nucleus. While the 5-aa's protease cleavage sites of Pol proteins do not stick the HIV-complex inside the nucleus.
(D) Images, and (E) quantification of eGFPcolocalization with HIV-replication complexes in target cell nucleus. This observation provides a rationale that, the virus incorporated p1, p2, and p6 proteins may also participate in vRNP-formation and package with these complexes. We will test this hypothesis, by making eGFP-fusion of the said proteins and analysing their ability to associate with vRNPs and percolate into the nucleus of target cells during HIV infection.

References

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