Structural characterization of spumavirus capsid assemblies to understand conserved Ortervirales assembly mechanisms

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Introduction

The *Retroviridae* family consists of two subfamilies, the *Orthoretrovirinae* and the *Spumaretrovirinae*. Orthoretroviruses contain important human pathogens such as Human Immunodeficiency Virus 1 (HIV-1). This and other members of this family are also important model systems for understanding several aspects of the viral life cycle. During budding and egress, orthoretroviruses undergo a complex structural maturation process that is coordinated by proteolytic processing of the main structural polyprotein group specific antigen (Gag), conserved among all retroviruses (**Figure 1A**). Only once maturation has completed, are viruses infectious. Due to their importance, retrovirus assemblies have been studied extensively to identify relevant interaction surfaces that confer particle stability and morphology and play a role in maturation.

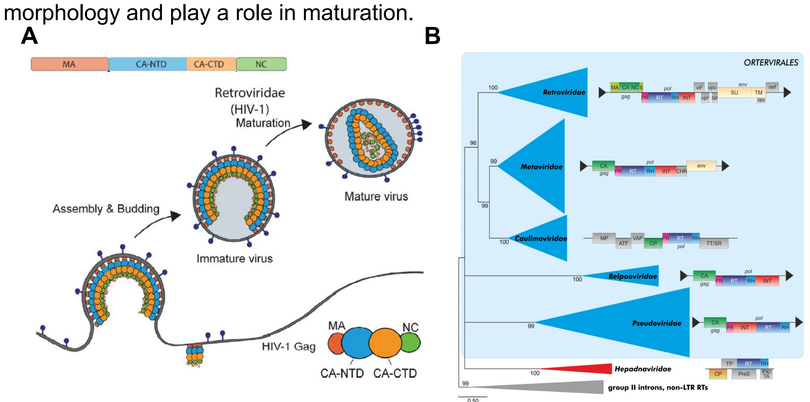


Figure 1- (A) Schematic representation of Retrovirus assembly and maturation (using HIV-1 as example); Gag domain architecture in retroviruses and proteolytic maturation process. (B) Maximum-likelihood phylogeny of viral reverse transcriptases, genomic organizations of selected representatives of reverse-transcribing viruses are shown next to the corresponding branches. Adapted from Obr & Schur, 2019 and Krupovic *et al* 2018

The ***Spumaretrovirinae*** subfamily is composed of the so-called spumaviruses or foamy viruses (FV), a slow evolving basal branch of the *Retroviridae* family that shares most of the common features of retroviruses and has several characteristics which make it suitable as a gene transfer vector, with significant advantages over gammaretroviral or lentiviral vectors. However, the best-studied member of the *Spumaretrovirinae*, **Prototypic Foamy virus (PFV)**, displays notable differences in its Gag protein. Mainly, unlike orthoretroviruses, PFV has one main proteolytic cleavage site, which is vital for viral morphology and infectivity. The function of three secondary sites that are essential for infectivity is still not understood.

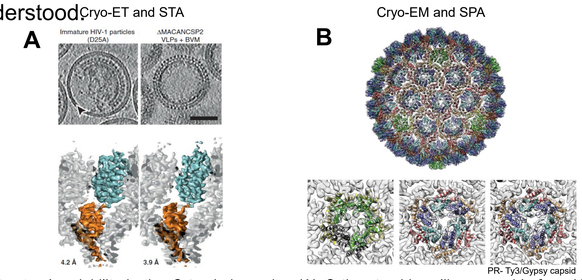


Figure 2- Structural variability in the *Ortervirales* order. (A) *Orthoretrovirinae*-like assembly from HIV-1. (B) Ty3/Gypsy icosahedral assembly. Cryo-EM methodologies used to obtain each structure are shown on top. Adapted from Schur *et al*, 2016 and Dodonova *et al* 2019

Another particularity of PFV assemblies is that they appear to lack a major structural maturation step, a trait they share with the retrotransposon Ty3/Gypsy. Concomitantly, Ty3/Gypsy has been reported to form icosahedral immature assemblies and PFV has an assembly morphology that is consistent with an icosahedron (**Figure 2**). The *Retroviridae* family along with other retro-transcribing viruses, including retrotransposons (such as Ty3 Gypsy) are members of the newly defined order named *Ortervirales* (**Figure 1B**). Currently, the evolutionary relationship within the order is not well resolved and a structural approach has been suggested to be more suited to answer this question.

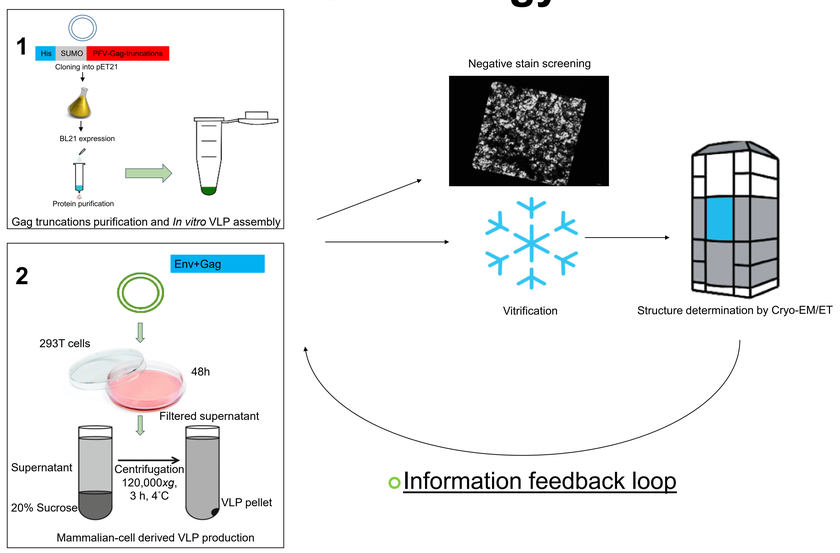
Open questions

- FV structure
  - How does **FV capsid assemble** to form a virus particle?
  - Does the assembly phenotype **resemble retrotransposons** like Ty3/Gypsy or other retroviruses?
  - Is there a conformational change in the assembly due to **PFV-Gag maturation**? Can this be attributed to **Gag cleavage sites**?
- *Ortervirales* structural conservation
  - What is the **evolutionary relationship** between FV and the rest of the *Retroviridae* and the *Ortervirales*? Where do they fit?
  - What are the important **assembly interaction interfaces** within the capsid? How can this information explain **conserved or divergent assembly mechanisms**?

Impact and Innovation

- First high resolution structure of PFV assembly
- Basal and slow evolving retrovirus structural features might underscore basic structural components for capsid assembly and morphology
- Resolution of Ortervirales evolutionary relationships might shed light on viral origins
- From a biotechnological point of view:
  - Development of more efficient viral vectors
  - Vectorization of Gag

Methodology



The experimental work is divided into four components, Firstly the production of VLPs to be used to be used in structural studies. VLPs will be obtained in one of two ways: (**1**) Gag truncation proteins will be expressed and purified in *E.coli* and used to optimize an in vitro assembly system by modifying buffer conditions. (**2**) Mammalian cell-derived VLPs can be produced by co-transfecting cells with plasmids coding for Env, as well as p68 (a truncated version of Gag) and purified by ultracentrifugation. Samples will be screened using TEM and negative stain. The second component is the use of cryo-EM and cryo-ET to generate the desired structures. As a third component, after high resolution datasets are acquired, the analysis and interpretation of the generated assembly structures will be performed. Lastly, the information obtained in the previous components will inform further experiments, such as the generation of relevant Gag mutants to validate residues required for virus assembly.

Preliminary results and outlook

Different truncation variants of the PFV-Gag were readily purified and parameters for *in vitro* assembly conditions were optimized for two of those truncation variants (CA300-477 and CANC300-621). These high efficiency assemblies were vitrified and used to acquire high resolution data-sets on a Thermo Scientific™ Titan Krios™ Cryo-TEM at 300 kV Figure (**Figure 3A** and **B**).
For the next steps, we will use this dataset to reconstruct the structure of the assembly via subtomogram averaging. We will aim to achieve a resolution of at least 8 Å, which will enable the rigid body fitting of existing NMR structures of CA300-477. This will allow us to interpret the structure and identify the relevant residues and interactions within it that confer stability and morphology to the assemblies.

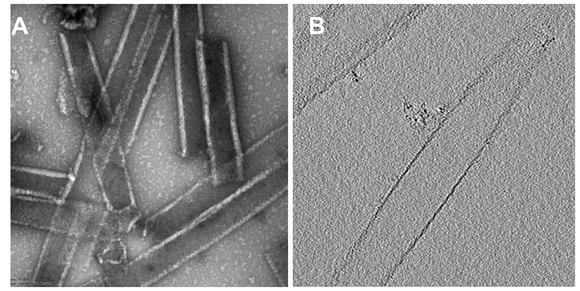


Figure 3- Prototypic Foamy Virus (PFV) virus like particles (VLPs). (A) Transmission electron microscope images of CA300-477 in vitro assembled tubes, samples were stained with 2% Uranyl Acetate. (B) Slice of a Cryo-EM tomogram acquired of plunge frozen CA300-477 *in vitro* assembled tubes.

As an alternative option to obtain a PFV lattice, 293T cells were transfected with a dual expression plasmid coding for the p68 truncation protein of PFV-Gag as well as the PFV Env protein. This combination of structural proteins has been reported before to be all that is necessary for the production of enveloped VLPs in the supernatant of transfected cells. The presence of structures similar to enveloped VLPs was observed budding into intracellular compartments in the cytoplasm of transfected cells (but not in the controls), through plastic embedding and ultrathin section experiments (**Figure 4**).
The next steps in the project are to finalize the production and purification in the mammalian cell system. After obtaining sufficiently high-quality samples, these will be used for plunge freezing and structural determination. Furthermore, we will compare the obtained structures (from in vitro and cell derived systems) to previously described structures of the Ortervirales order to identify the conserved features that provide stability and morphology to PFV assemblies, as well as better resolving evolutionary relationships within the order .

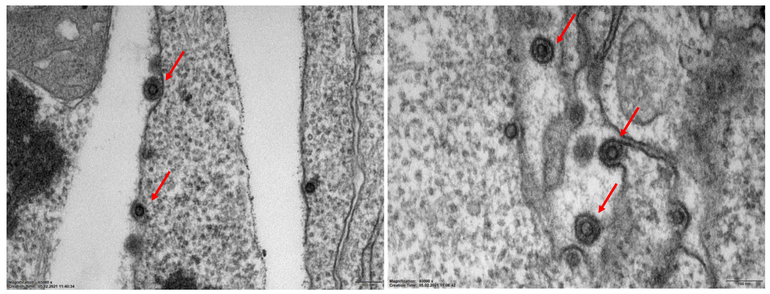


Figure 4- Prototypic Foamy Virus (PFV) virus like particles (VLPs). Transmission electron microscope images of PFV-VLPs containing p68 and Env after plastic embedding and ultra-thin sectioning of transfected 293T cells. Red arrows point to released PFV-VLPs as well as budding PFV-VLPs. Plastic embedding and ultrathin sectioning performed by V. Zheden.

References

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