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The Molecular Structure of a Worm-Infecting Virus

Introduction

Discovered in 2011, Orsay (OV) remains the only known virus capable of infecting *Caenorhabditis elegans*, a valuable animal model for biological study[1]. The nematode shares many immune pathways with humans and has been used to characterize critical host-pathogen interactions responsible for disease[2]. With *C. elegans* as an established model organism, Orsay presents a promising opportunity to identify universal host factors exploited by gastrointestinal virus infection in animals. However, Orsay itself must be further characterized. Structural analysis of the virus and its encoded proteins would yield valuable information towards the mechanisms underlying its infection, providing foundational knowledge to develop Orsay's growing potential as a platform to characterize innate cellular mechanisms mediating antiviral defense and immunity.

Orsay contains two single-stranded RNA molecules encoding for capsid protein (CP) subunits, which self-assemble to form an outer protective protein coat; RNA-dependent RNA polymerase (RdRP), responsible for replicating the viral genome; and spike protein δ, which is required for infection and can either be free-floating or fused to CP (CP-δ) via a linker region[1,3] (Figure 1).

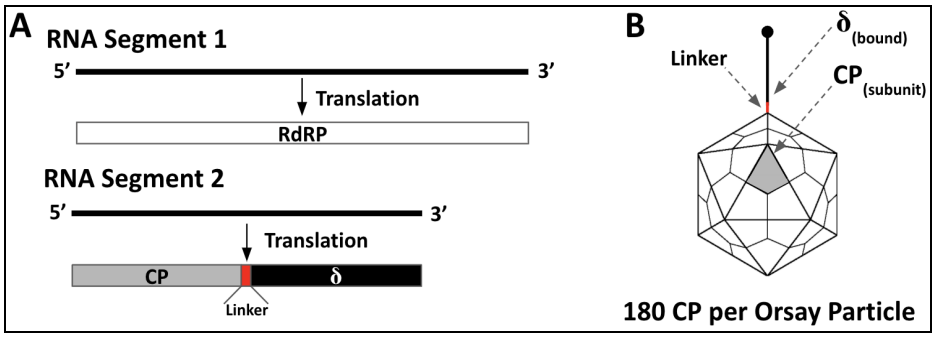

Figure 1. Structural and genomic outline of OV. (A) Schematic outlining the OV RNA genome and encoded viral proteins. (B) Schematic of an Orsay virus particle constructed from 180 CP (single subunit shaded in gray) and linked δ fiber protein (rounded protrusion).

Previous structural and functional analyses of Orsay have relied on the use of recombinant viral proteins rather than authentic virus particles[4,5]. As a result, the structure of a fully-assembled, infectious Orsay particle and presence of incorporated CP-δ remain unknown. Elucidating the structure of the viral complex would not only verify prior recombinant findings but also enable study of Orsay proteins within an infectious context. Thus, my initial work focused on the development of a culture and purification scheme capable of isolating native Orsay particles, allowing both high-resolution structural analysis and downstream experiments capable of identifying host factors required for viral entry.

Methods

I began by adapting prior work from Jiang *et al.* to derive a procedural basis for Orsay amplification[6]. Here, large-volume liquid cultures of *C. elegans* facilitate Orsay infection and replication. In the process, much of the virus sheds into the liquid culture media, providing a source of protein for purification with minimal contamination of internal cellular debris - thereby compensating for the lack of affinity tags.

I then hypothesized that, given the incredibly small size and mass of viruses, centrifugation alone should facilitate purification of Orsay from surrounding media. After first centrifuging at low speeds to remove macromolecular debris, the resulting supernatant is then subjected to ultracentrifugation that pellets its remaining contents - of which includes viral particles. The pellet can then be resuspended in a protein-stabilizing buffer and applied atop a density gradient solution for equilibrium sedimentation, which upon additional ultracentrifugation further separates proteins into distinct density-specific bands. Here, the compactness of viral complexes - denser than most other biomolecules - is utilized for purification (Figure 2).

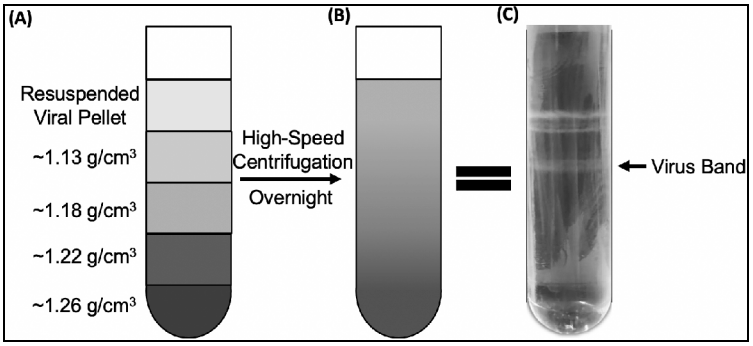

Figure 2. Equilibrium sedimentation purification of Orsay virus particles. (A) When layered atop a solution of differential density and spun at high speeds overnight, (B) the contents of the resuspended virus-containing pellet migrate and separate on the basis of density. (C) This results in the formation of distinct protein bands that can be individually harvested and analyzed.

Results

Upon visualizing the virus-containing band with electron microscopy, I noticed the presence of two contaminants: membranous aggregates of cell-derived lipids in addition to smaller, ring-like particles (Figure 3).

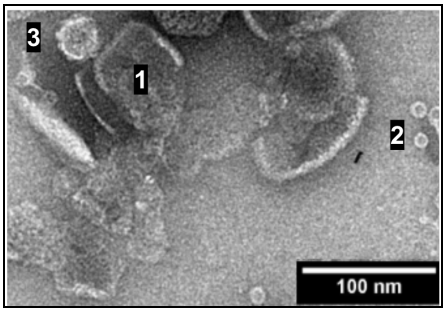

Figure 3. Initial purification runs resulted in OV samples of substandard purity. Analysis of initial virus band samples with transmission electron microscopy revealed considerable contamination in the form of (1) membranous aggregates and (2) small, ring-like particles in addition to the (3) expected viral capsids.

In an attempt to eliminate the membranous aggregates, I hypothesized the addition of detergent to the virus pellet resuspension buffer would be capable of solubilizing and breaking apart these complexes - thereby reducing their mass and preventing co-elution during equilibrium sedimentation. This proved effective, as subsequent experiments in the presence of detergent yielded cleaner samples. However, the ring-like contaminant persisted, imparting a faint yellow color to the solution.

Owing to the color and approximate size of the ring-like particles, I speculated the contaminant was ferritin, an Fe^{2+} storage protein highly expressed in *C. elegans*. Based on ferritin's physiological function, I theorized the contaminant should possess similar affinity for other divalent metal cations such as Ni^{2+}. Microbeads embedded with Ni^{2+} are a common tool in protein purification used to bind and separate recombinant, histidine-tagged proteins. Thus, the putative-ferritin contaminant should respond similarly: binding to the beads as the remaining virus-containing sample flows through. Analysis of nickel-treated samples indicated the procedure was successful, resulting in a substantial yield of Orsay virions with ≥95% purity (Figure 4A).

Both the purity and yield of the OV sample suggested it should serve as a promising candidate for structural analysis via cryogenic electron microscopy. With help from our collaborators, initial screenings have already produced a preliminary model of the composite Orsay virion (Figure 4B), which after additional refinement clearly displays electron density corresponding to a capsid-linked CP-δ fiber (Figure 4C and 4D).

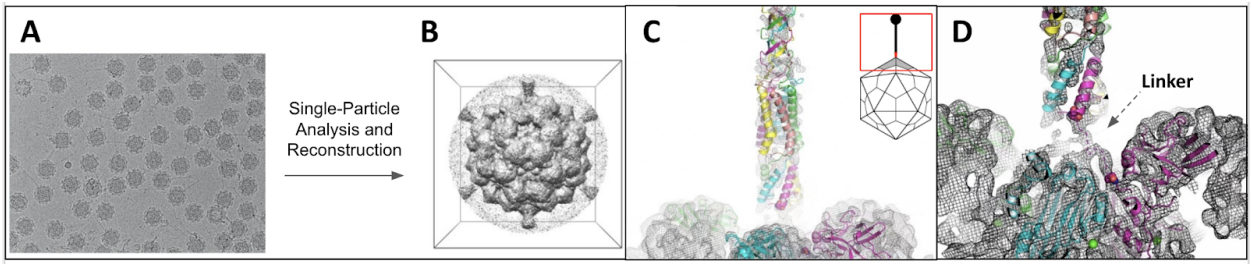

Figure 4. Preliminary cryo-EM modeling of Orsay. (A) A representative cryo-electron micrograph and (B) initial electron density map of the Orsay virion following single-particle analysis. (C) Following refinement and additional data collection, CP-δ can be clearly distinguished and mapped using prior solved, recombinant Orsay structures (D) in addition to the elusive linker region connecting the spike and capsid.

Conclusions and Future Work

The preliminary model alone provides novel insights into the native structure of Orsay, namely by confirming the existence of a covalently linked CP-δ fiber incorporated on the five-fold axis of symmetry within the viral particle. In addition, the linker region connecting the spike and capsid proteins is clearly visible - but unable to be modeled.

Thus, work remains to produce a higher-resolution model via further data collection and reconstruction. This model will also determine if any unique structural features lie within the fiber-containing vertex that contributes to the role of δ in enabling entry and infection. Finally, preliminary work with *C. elegans* gene knockdowns has led to the identification of a putative host receptor required for Orsay infection, presumably through interactions mediated by CP-δ. Further assessment for interaction between the candidate and CP-δ is required to verify this hypothesis, of which a means to purify native, CP-δ containing OV will prove invaluable. Overall, my research will deepen our understanding of this novel virus - in addition to providing a technical foundation in the advanced protein biochemistry and biophysical techniques required for a career in structural biology, complementing my future graduate studies and research interests.

References

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