

Optimizing Semliki Forest Virus Replication Organelle Purification for Cryo-electron Tomography of the Neck Complex

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Alphaviruses are mosquito-borne, enveloped viruses that are small and spherical in shape, with a positive-sense single stranded RNA genome of approximately 11-12 kb. Semliki Forest virus (SFV) is closely related to the re-emerging Chikungunya virus (CHIKV). SFV can infect humans, causing fever, severe headaches, and muscle and joint pain. During infection, SFV remodels host cell membranes to form replication organelles (RO), known as “spherules”, which serve as microenvironments for viral RNA replication⁽¹⁾. The spherule neck complex comprising viral non-structural proteins (nsP1–4) along with unidentified host factors. Previous studies reveal that a macromolecular complex (~1.2-1.4 MDa) is located at the spherule neck. This complex is bipartite, comprising a membrane bound “base” (nsP1, nsP2 and nsP4), and a barrel-shaped, three-ringed “crown” on the cytoplasmic side, composed of nsP3 and host-factors⁽²⁻⁴⁾. This project aims to purify intact and active SFV spherules from infected mammalian cells using native fractionation methods⁽⁵⁾, determine the high-resolution architecture of the neck complex using cryo-electron microscopy, and perform proteomic profiling of membrane fractions to identify host proteins interacting within the ROs. Ultimately, these studies will elucidate the complete viral replication complex and provide a framework for the rational design of novel antiviral therapeutics.

References:

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