

Role of NS4B protein in the formation of the Replication Complex in Langat Virus

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Flaviviruses are positive-sense RNA viruses whose intracellular replication is marked by endoplasmic reticulum (ER) membrane remodeling. The genome replication step is one of the least understood processes in the viral life cycle. Upon flavivirus infection, viral genome is translated as a single polyprotein comprising both capsid and non-capsid (“non-structural”, NS) proteins. The polyprotein upon protease cleavage forms inverted buds on the ER, called replication organelles (ROs). ROs are the site of viral genome replication. Inside the membrane bud, the NS proteins co-localize with the viral genomic RNA and bring about its replication. But, how the non-enzymatic NS proteins (NS2A, NS2B, NS4A, NS4B) initiate the formation of ROs or partake in genome replication is yet to be deciphered. Recently, using cryo-ET we have studied the RCs in situ in Langat virus, a BSL2 model organism for TBEV. The tomograms highlighted the occurrence of macromolecular densities at the membrane neck of ROs, which on segmentation yielded a molecular mass of approximate 500 kDa. In this study, we investigate the probable role of NS4B in the formation of this ‘neck’. To investigate this, NS4B was successfully expressed in mammalian and insect cell expression systems and affinity purified using a Flag tag in the presence of detergent. Size exclusion chromatography (SEC) revealed the presence of two distinct oligomeric states, which was further confirmed by negative stain analysis. Preliminary single-particle cryo-EM analysis of the lower oligomeric state fraction has yielded a reconstruction at approximately 6Å resolution, consistent with a ring-like oligomeric architecture. These initial results provide a structural framework for understanding how NS4B may contribute to RO neck formation.

Future work will focus on improving the resolution of the cryo-EM reconstruction, determining the precise oligomeric state and stoichiometry of the complex, establishing the role of this assembly in RO biogenesis in situ using cryo-ET, and investigating how NS4B coordinates with other NS proteins to drive ER membrane remodeling during flavivirus replication.