ABSTRACT

Alphaviruses, a genus of vector-borne viruses belonging to the family *Togaviridae*, encode a small ion-channel-forming protein, 6K, and its transframe variant (TF) during infections. Although 6K/TF have vital roles in glycoprotein transport, virus assembly, and budding, there is no mechanistic explanation for these functions. We investigated the distinct biochemical functionalities of 6K and TF from the mosquito-borne alphavirus, Chikungunya Virus. We show that like 6K, TF is also capable of forming ion channels in bilayer membranes. The assemblies formed by 6K in membranes are structurally more complex and potentially more ion-restrictive than those formed by TF. Both 6K and TF show strong affinity toward the ER membranes, indicating that the localization of these components at the plasma membrane, as previously reported, is either linked to post-translational modification or mediated through interaction with binding partners. Currently, there is no experimental structural information available for either CHIKV 6K or TF. Even in their proposed hexameric state, the anticipated molecular masses of 6K and TF assemblies are approximately 40-50 kDa, thereby making them difficult targets for single-particle Cryo-EM. Different approaches, including detergents, membrane mimetics, and multiple tagging strategies were evaluated to improve the Cryo-EM analysis of these small membrane proteins. These studies identified several experimental limitations associated with Cryo-EM analysis of small viroporins and established strategies that may enhance future structural investigations of CHIKV 6K, TF, and other small membrane proteins. Together, these findings provide structural and functional insights into the distinct roles of 6K and TF in the alphavirus life cycle.

In parallel, structural characterization of Flock House Virus (FHV) was performed using cryo-EM to obtain a high resolution structure of the virus and to understand the organization of viral RNA within the intact capsids. The icosahedral reconstruction was used to define the mature capsid architecture, whereas the asymmetric reconstruction was utilized to understand the organization of the encapsulated genome and other asymmetric structural features in the wild-type mature particle. The asymmetric reconstruction further revealed two concentric layers of internal genome density within the virion, providing structural insights into the organization of the encapsulated bipartite RNA genome.

Together, these studies expand our understanding of alphavirus viroporin biology and genome organization in non-enveloped RNA viruses, providing new structural and functional insights into the molecular mechanisms governing viral assembly, genome organization, and infectivity.