Characterizing the structure of Hepatitis C virus pseudoparticles to inform vaccine design

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Hepatitis C virus (HCV) has chronically infected an estimated 58 million people around the globe, and it infects another 1.5 million every year. Despite effective therapeutics that have recently entered the market, cases are still on the rise in the United States, and no preventative treatment or vaccine is available for HCV. The viral envelope proteins E1 and E2 are the major targets for the immune system. However, E1 and E2 employ several mechanisms to evade the adaptive immune response including heavy glycosylation, epitope shielding, and several variable regions. Vaccine candidates using native HCV glycoprotein(s) as an immunogen have not been successful at eliciting an adequate protective immune response. The rational design of modified E1E2 vaccine candidates has been hindered by a lack of structural information. Here we study HCV E1E2 using lentiviral particles pseudotyped to express full-length HCV E1E2. These HCV pseudoparticles (HCVpp) were produced by transfecting a combination of packaging (HIV-GAG) and HCV E1E2 expressing plasmids into Expi293F cells. An optimized purification protocol was developed to extract HCVpp from the supernatant of transfected cells for structural studies. This process consists of polyethylene glycol (PEG) precipitation, sucrose cushion filtration, and separation with a discontinuous OptiPrep gradient. Purified particles were vitrified in liquid ethane, and cryo-electron microscopy revealed the presence of heterogenous HCVpp particles. This protocol is designed to accommodate large scale expression and attain high purity, enabling high-resolution cryo-electron microscopic and tomographic studies of native HCV E1E2 proteins. These studies will provide insight into the native conformation of E1E2, which is crucial for the design of a vaccine.