The intraviral protein interaction network of hepatitis C virus.

Abstract:

Hepatitis C virus (HCV) is a global health problem and one of the main reasons for chronic liver diseases such as cirrhosis and hepatocellular carcinoma. The HCV genome is translated into a polyprotein which is proteolytically processed into 10 viral proteins. The interactome of the HCV proteins with the host cell has been worked out; however, it remains unclear how viral proteins interact with each other. We aimed to generate the interaction network of these 10 HCV proteins using a flow-cytometry-based FRET assay established in our laboratory (Banning, C., Votteler, J., Hoffmann, D., Koppensteiner, H., Warmer, M., Reimer, R., Kirchhoff, F., Schubert, U., Hauber, J., and Schindler, M. (2010) A flow cytometry-based FRET assay to identify and analyse protein-protein interactions in living cells. PLoS One 5, e9344). HCV proteins were constructed as fusions with the chromophores CFP and YFP. All HCV fusions were expressed and localized to specific subcellular compartments, indicating that they were functional. FACS-FRET measurements identified a total of 20 interactions; 13 of these were previously described and have now been confirmed in living cells via our method. Among the seven novel protein binding pairs, HCV p7 plays a pivotal role. It binds to the HCV capsid protein Core and the two glycoproteins E1 and E2. These interplays were further demonstrated in the relevant context of Huh7.5 liver cells expressing infectious HCV. Our work
demonstrates the feasibility of rapidly generating small interaction networks via FACS-FRET and defines the network of intra-HCV protein interactions. Furthermore, our data support an important role of p7 in HCV assembly.