attenuated in comparison to HCV-Jc1.

By live cell microscopy a spatio temporal organization of HCV structural protein expression and movement within living Huh7.5 cells was revealed. At early time points the E1-protein is mainly found in the ER and in proximity to lipid droplets and colocalizes with NS5A replication complexes. In contrast, E1-containing vesicles emerge during later phases of infection. Moreover proximity ligation assay (PLA) shows increasing interaction with E2 over time. The putative particle accumulations in the nuclear-distant places can by bleached by FRAP and are not reappearing in comparison to ER near protein aggregations, indicating transport compartments disconnected from protein translation.

In sum, our experiments demonstrate that HCV-Jc1 with mCherry tagged E1 is a valuable tool to study HCV assembly and release in living cells. Furthermore we can track transport-compartments for further elucidation of the cellular pathways involved in HCV release.

Structure and Assembly

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An aptamer against the matrix binding domain on the hepatitis B virus capsid inhibits nucleocapsid envelopment

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By a genetic approach, a small domain (matrix domain, MD) in the large hepatitis B virus (HBV) surface protein L and a narrow region (matrix binding domain, MBD) including the amino acid residue I126 on the capsid surface were mapped in which point mutations like core-I126A specifically blocked nucleocapsid envelopment. Possibly, both domains directly interact with each other during virion morphogenesis. By the SELEX method with counter selection we evolved DNA aptamers from an oligonucleotide library (25 random nucleotides flanked by 15 constant nucleotides on each side) binding to purified recombinant capsids (positive selection) but not binding to the corresponding I126A mutant capsid (negative selection). Aptamers bound to capsids were separated from unbound molecules by filtration. After 13 rounds of consecutive positive and negative selections and amplifications 12 different aptamers were found among 73 clones. The four most frequent aptamers represented more than 50 % of the clones. The main aptamer AO-01 (13 clones, 18 %) showed the lowest dissociation constant (K_d) of 180 +/- 82 nM for capsid binding among the four molecules. Its K_d value for the I126A capsid was 1306 +/- 503 nM. Cotransfection of Huh7 cells with AO-01 and an HBV genomic construct resulted in 47 % inhibition of virion production 3 days post transfection relative to an aptamer with random sequence. The half-life of AO-01 in transfected cells was around 2 hours which might explain the incomplete inhibition. This result, however, supports the importance of the MBD for nucleocapsid envelopment. Inhibiting the MD-MBD interaction by a low molecular weight substance might represent a new approach for an antiviral therapy.

Structure and Assembly

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Some like it special - the membrane anchor of the pestivirus Erns RNase

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E^{rns} is an essential virion glycoprotein of pestiviruses with a RNase activity that suppresses host cellular innate immune responses upon being partially secreted from the infected cells. E^{rns} lacks a typical surface protein membrane anchor. Instead, it contains a long C-terminal amphipathic helix. This unusual C-terminus plays multiple roles, as it serves as membrane anchor, as signal peptidase cleavage site and as retention/secretion signal. To gain better understanding of the mechanisms underlying these diverse functions we analyzed the structure and membrane binding properties of this sequence.

As a first step we performed CD spectroscopy experiments in different setups which revealed a strong helical folding of the E^{rns} anchor in membrane simulating environments like micelles, vesicle and bicelles. Furthermore, we analyzed the orientation of the helix by OCD spectroscopic. These experiments showed that the helix is slightly inclined with respect to the membrane surface. Monte Carlo and molecular dynamics simulations confirmed the helical folding determined by CD spectroscopy and showed that the helix is accommodated in the amphiphilic region of the lipid bilayer with a slight tilt rather than lying parallel to the surface which also verifies the OCD spectroscopy data. The structural model of a long amphipathic helix with a slightly tilted orientation was further confirmed by NMR spectroscopy. These analyses identified a central stretch of 15 residues within the helix which is fully shielded from the aqueous layer. Additionally, the NMR data as well as the Monte Carlo simulations showed a putative hairpin like structure of the direct C-terminal end. This C-terminal region was found to influence the number of water shielded amino acids in the helical core area and thereby stabilizes E^{rns} membrane association.

These findings explain the strong membrane binding of the protein despite of the lack of a standard membrane anchor and provide clues to understanding establishment of E^{rns} membrane contact, processing and secretion.