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Hepatitis C virus replication cycle

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Hepatitis C virus (HCV) is a positive strand RNA virus forming the genus Hepacivirus in the Flaviviridae family. Replication of HCV starts with the virus binding to hepatocytes, which are the primary, if not exclusive host cells. HCV entry is a multi-step and slow process (Panel 1). Interactions between HCV E1–E2 envelope glycoproteins and glycosaminoglycans (GAGs) contribute to primary binding of the virus particles to host cells. Owing to the association of HCV with (very) low-density-lipoproteins [(V)LDL] [1], the LDL receptor (LDLr) has also been proposed as a capture molecule. Upon this initial engagement, the scavenger receptor BI (SR-BI), the CD81 tetraspanin, together with the tight junction proteins Claudin-1 (CLDN-1) and Occludin (OCLN) synergistically contribute to HCV uptake in a clathrin-dependent manner [2]. Additional serum factors, e.g., high-density-lipoprotein, promote entry by increasing uptake kinetics through interaction with SR-BI [3]. HCV entry appears to occur in a pH-dependent manner, whereby the low endosomal pH induces conformational rearrangement of HCV glycoproteins, leading to fusion of the viral and endosomal membranes and subsequent uncoating.

Viral RNA released into the cytoplasm is translated via an internal ribosome entry site (IRES) located in the 5′ non-translated region of the genome [4] (Panel 2). RNA translation occurs at the rough endoplasmic reticulum (ER) giving rise to a ~3000 amino acid long polypeptide that is cleaved into 10 different products. The region from the core to the C-terminus of p7 is processed by host cell signal peptidases whereas the remainder is cleaved by two viral enzymes: (i) a cysteine protease residing in nonstructural protein 2 (NS2) that cleaves between NS2 and NS3, (ii) the NS3 serine protease that is activated by interaction with NS4A and that catalyzes processing at all other sites within the NS-region. An additional processing in the C-terminal region of the core protein is mediated by signal peptide peptidase, which is required to mobilize core protein for translocation to lipid droplets (LDs). In addition to the polypeptide, core protein derivatives (designated mini-cores and core+1 proteins) with unknown function are generated by alternative translation mechanisms.

All viral proteins are directly or indirectly (NS3 via NS4A) associated with ER-derived membranes (Panel 3) where they have to exert their function, including the NS3-mediated cleavage of cellular molecules required for signaling of innate immune responses (reviewed in [5]). Primarily by the action of NS4B, presumably in conjunction with NS5A, membranous replication vesicles (RVs) are induced and accumulate in the infected cell as distinct structures designated as the membranous web (Panel 4). This web in addition contains ER membranes and LDs. It is the site where viral RNA is amplified by the NS5B RNA-dependent RNA polymerase (RdRp) in conjunction with most, if not all other NS proteins and host cell factors such as cyclophilin A. By analogy to flaviviruses [6], RNA replication may occur in invaginations of the ER membrane in a semi-conservative and asymmetric manner (Panel 5). The positive strand genome is copied into a negative strand RNA via a replicative form (RF) that is used for the synthesis of excess amounts of positive strand RNAs via a replicative intermediate (RI). Initiation of RNA synthesis requires highly structured RNA elements in the 3′ NTR of the corresponding template strand (Panel 2). Newly synthesized RNA genomes are used for translation (production of new viral proteins), RNA replication, or formation of new infectious virions (assembly). The latter event place in close proximity of LDs where the core protein accumulates almost quantitatively (Panel 6). Viral RNA is thought to be delivered from replication sites to the core protein on LDs by the viral replicase [7]. Alternatively, NS5A, which has an intrinsic RNA binding capacity, may be released from the replicase complex to move onto the LD surface. Via a core–NS5A interaction, RNA could be delivered to the core protein thus triggering nucleocapsid formation. These capsids may bud into the ER lumen in a process that is tightly linked to VLDL synthesis [8]. Thus, HCV assembly very much depends on (V)LDL synthesis and requires several enzymes such a microsomal triglyceride transfer protein (MTP), but also apoE. This assembly pathway would explain the (V)LDL-like composition of HCV particles [1] and the incorporation of apoE (Panel 7). Whether apoB is required for HCV assembly is discussed controversially. It is not known whether HCV particles have a regular nucleocapsid or an unstructured core protein–RNA complex.

Major efforts are undertaken to develop selective antiviral drugs [9]. Thus far, most compounds target the NS3 protease, the NS5B RdRp and NS5A, a RNA binding phosphoprotein required for RNA replication and assembly. Inhibition of the NS3 protease blocks polypeptide cleavage and thus formation of new replication sites, but probably does not affect already established replication complexes. In contrast, nucleosides and non-nucleosidic inhibitors, blocking the NS5B RdRp, most likely

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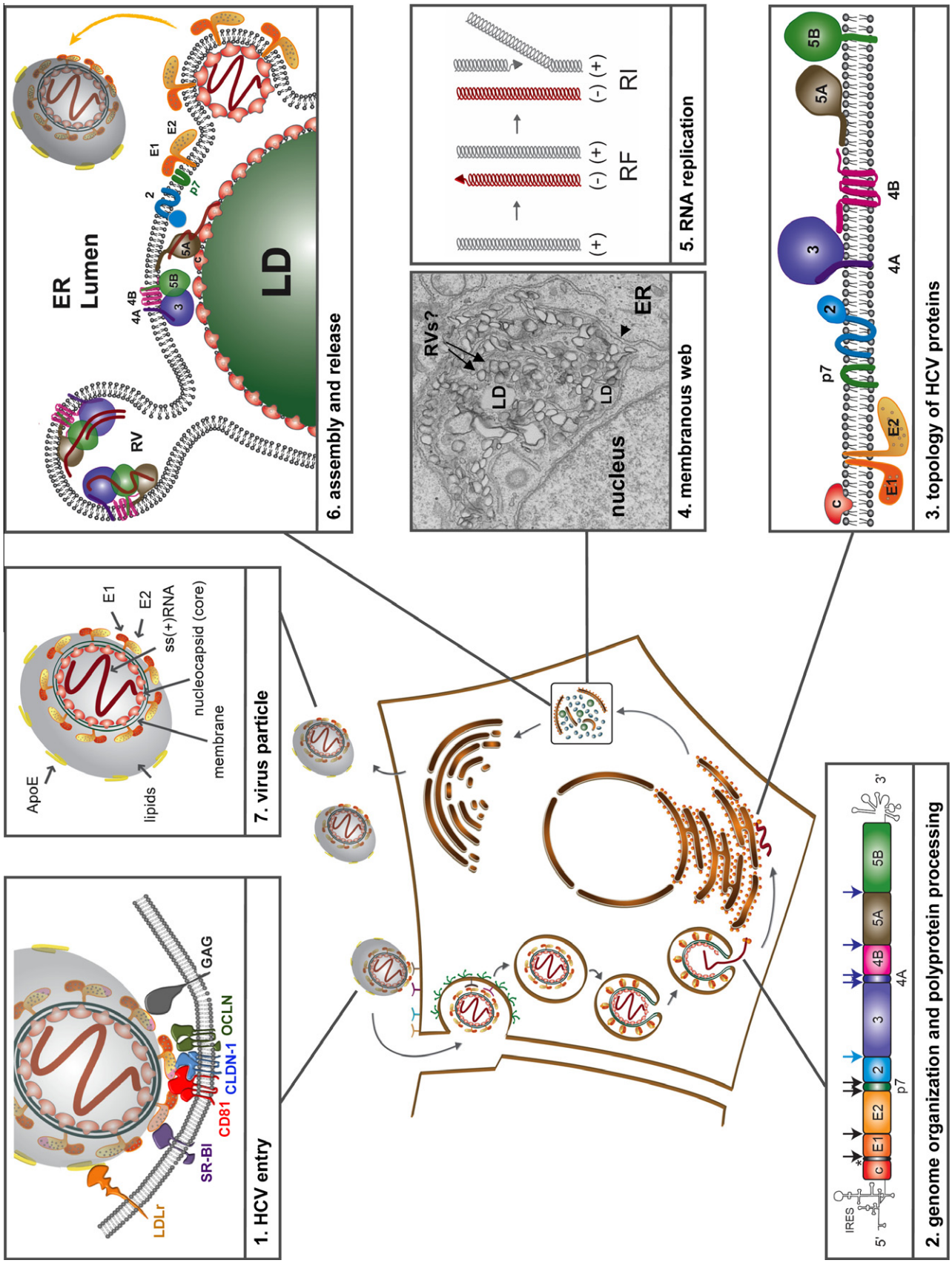
Hepatology Snapshot



affect activity of existing replication complexes. How compounds targeting NS5A interfere with HCV replication is unknown.

Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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