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Review

Hepatitis C virus: life cycle in cells, infection and host response, and analysis of molecular markers influencing the outcome of infection and response to therapy

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ABSTRACT

Hepatitis C virus (HCV) is a major global health burden accounting for around 170 million chronic infections worldwide. Since its discovery, which dates back to about 30 years ago, many details of the viral genome organization and the astonishing genetic diversity have been unveiled but, owing to the difficulty of culturing HCV *in vitro* and obtaining fully susceptible yet immunocompetent *in vivo* models, we are still a long way from the full comprehension of viral life cycle, host cell pathways facilitating or counteracting infection, pathogenetic mechanisms *in vivo*, and host defences. Here, we illustrate the viral life cycle into cells, describe the interplay between immune and genetic host factors shaping the course of infection, and provide details of the molecular approaches currently used to genotype, monitor replication *in vivo*, and study the emergence of drug-resistant viral variants. **L.B. Dustin, CMI 2016;22:826**

Hepatitis C virus: a momentous virus for virology

The hepatitis C virus (HCV) was identified in 1989. This year marked a departure from traditional virology, based on isolation, cultivation, and biochemical studies, to modern virology that uses molecular biology and biotechnology to discover, characterize, and monitor viruses. HCV is indeed the first infectious agent discovered thanks to molecular biology techniques that, owing to the difficulty of replicating the virus *in vitro*, have been extensively used to define the molecular aspects of HCV biology.

Today, HCV is making history again. Recently developed direct-acting antivirals (DAAs) eliminate infection in over 90% of treated individuals and are changing the idea that antivirals, in general, can at most block viral replication and slow disease progression. Poorly tolerated interferon (IFN)-based therapeutic regimens are being rapidly replaced with IFN-free DAA regimens, and tissue damage in patients with advanced stages of disease is stabilized and possibly reversed [1]. Further, detailed study of HCV's dependence on host factors has permitted development of host-directed antiviral therapies. This review provides an overview of some key aspects of viral interaction at cellular and host levels and illustrates current methods for monitoring viral replication and genotyping with particular emphasis on fundamental and recent clinical findings important to determining susceptibility or resistance to DAAs.

HCV life cycle and host–cell interactions *in vitro*

HCV belongs the Flaviviridae, a large family of enveloped, single-stranded RNA viruses which is organized into the genera Hepacivirus, Flavivirus, Pestivirus and Pegivirus [2], and that includes many viruses transmitted by arthropods and is a growing matter of health concern [3]. The HCV life cycle is only partly understood; difficulties in establishing an *in vitro* model of replication and the complex network of cell surface molecules used to mediate viral entry have delayed comprehension of various molecular

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mechanisms [4,5]. Briefly, as shown in Fig. 1, the HCV virion circulates in the bloodstream either as a free particle or surrounded by host low-density lipoproteins [6], attaches onto the target cell membrane by sequential binding of various receptor molecules, and enters into the cell by a clathrin-mediated endocytosis process. Disruption of the viral capsid in the endocytic compartment releases the 9.6-kb single-stranded RNA genome of positive polarity into the cytoplasm. The RNA genome is then directly translated at the rough endoplasmic reticulum (ER) in a single polyprotein precursor of about 3000 amino acid residues that is eventually cleaved by cellular and viral proteases into ten mature products [7,8]. These proteins, enlisted in the order they are encoded, include the structural core and envelope glycoproteins E1 and E2, and the following nonstructural proteins: p7 viroporin and nonstructural protein 2 (NS2) that participate in virus assembly and release; NS3 and NS4A, the protease complex that is actively targeted by the protease inhibitor class of DAAs; NS4B, a membrane-associated protein that mediates virus–host interactions; NS5A, a zinc-binding and proline-rich hydrophilic phosphoprotein involved in HCV RNA replication and targeted by NS5A inhibitor DAAs; and NS5B, the RNA-dependent RNA polymerase targeted by nucleoside and non-nucleoside polymerase inhibitor DAAs. New virions are assembled in an ER-derived compartment and released by exocytosis following a Golgi-dependent secretory pathway. Along this process, the virus undergoes maturation and becomes surrounded by endogenous lipoproteins that, as described below, are believed to help immune escape [4,5]. Binding to host lipoproteins and envelopes without clearly discernible surface features confer to HCV virions low buoyant density and a broad size range (40–80 nm diameter) [5].

The lack of a reliable *in vitro* method to study HCV replication was due to the scarce adaptability of primary hepatic cells to *in vitro* propagation, no availability of viral isolates adapted to *in vitro* culture, and large use of cell-to-cell transmission to disseminate infection to neighbour cells. This mechanism has complicated identification of cellular receptors necessary for viral entry and is believed to facilitate immunological escape, virus persistence, and resistance to DAAs [9–12]. Also, receptor usage appears to depend upon cell type and infection via free-particle or cell-to-cell transmission [4,13].

The cell culture derived HCV (HCVcc) and the HCV trans-complemented particles (HCV_{TCP}) are among the most used methods to study replication of HCV replication *in vitro* [4]. HCVcc uses JFH1, a HCV genotype 2a strain isolated from a Japanese patient with fulminant hepatitis and replicates in Huh-7, a human cell line from hepatocellular carcinoma [14,15]. HCVcc generates infectious virus and, using either native or inter-genotype recombinant JFH1 variants, has allowed the identification of some HCV entry factors, defining virion structure and biochemical properties, and testing DAA potency. HCV_{TCP}, described in detail elsewhere [16,17], employs pseudotyped HCV virions generated in packaging cells transfected with viral proteins provided by different constructs. HCV_{TCP} can be theoretically obtained from any isolate but supports only single-round infection and is unable to spread.

HCVcc, HCV_{TCP}, and basically all *in vitro* methods use Huh-7 cells that, although permissive to HCV replication, differ from primary hepatocytes for different restriction mechanisms, diverse localization of HCV receptors, and absence of the cell polarity observed in hepatic tissue. As a result, viral entry, assembly, release, and cell-to-cell spread observed *in vivo* is not completely reproduced *in vitro* [5]. HepG2 cell clones and hepatoma cells derived from primary hepatocytes are permissive to HCV replication *in vitro* and should allow better understanding of virus–host cell interplay. In this regard, a growing body of evidence shows that host genetics impact

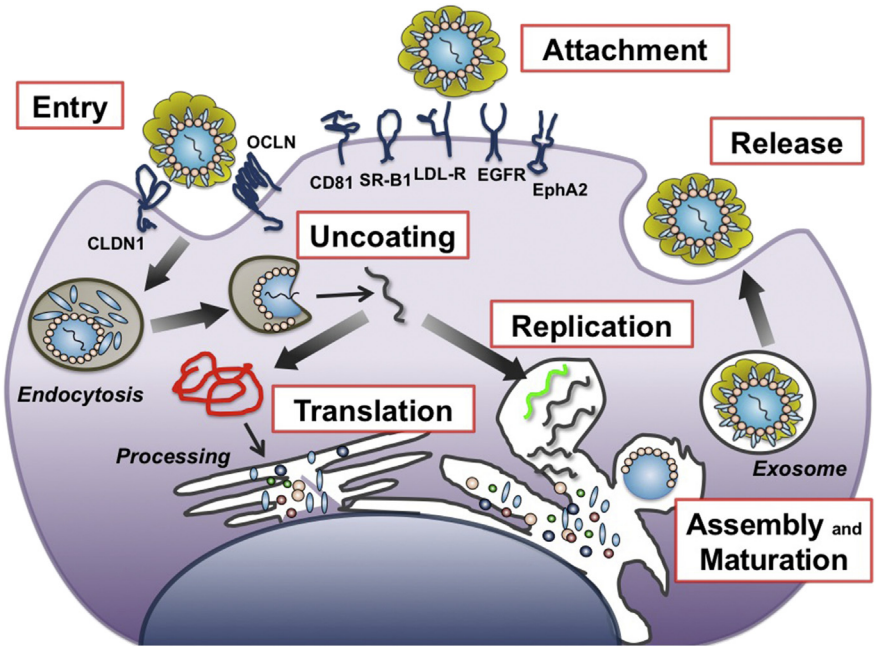

Fig. 1. The hepatitis C virus (HCV) replication cycle. The seven steps of the viral life cycle, indicated in the white boxes, are the following: attachment—the viral particle, surrounded with lipoproteins, binds the target cells by interacting with several receptors some, most of which are shown in figure, considered essential and others accessory; entry—following attachment, the virus enters through clathrin-mediated endocytosis; uncoating—the cellular and viral membranes fuse and the capsid is disorganized with a process triggered by the low pH of the endosome. After uncoating, the positive-strand RNA genome is released into the cytoplasm; translation—the genomic RNA is directly translated in a polyprotein precursor that is then cleaved into single proteins by both host and viral proteases; replication—the non-structural proteins and some host factors form a replication complex that synthesized multiple copies of the HCV RNA genome via a minus-strand replicative intermediate; assembly and maturation—packaging of viral progeny takes place in the endoplasmic reticulum from which the virion acquires the envelope with E1 and E2 glycoproteins. Maturation and association with endogenous lipoproteins to form lipoviral particles immediately follow; release—virions are released from the cells most likely by exocytosis or transmitted to other cells via a cell-free mechanism.