Chapter 2

The HCV particle and its life cycle

The HCV particle

The hepatitis C virus (HCV) belongs to the *Hepacivirus* genus of the *Flaviviridae* family of viruses. HCV is an enveloped, positive-strand RNA virus that is spherical and has a diameter of between 40 and 80 nm in HCV-infected patients [1] and between 60 and 75 nm in cell culture systems [2]. It is composed of an envelope (derived from host cell membranes), two viral glycoproteins, envelope proteins (E)1 and E2, and an icosahedral capsid containing a positive-sense, single-stranded RNA genome (Figure 2.1). The HCV RNA genome is 9.6 kb in length [3], flanked by 5' and 3' untranslated regions (UTR), and contains two open reading frames (ORF). The large ORF encodes the entire HCV polyprotein and the alternative ORF produces a single protein, the F protein [4]. The role of the F protein is not well understood, although it has been suggested that it could be implicated in immune evasion [5]. The 5'UTR is highly conserved among different HCV isolates and the secondary structure contains four distinct stem-loops called internal ribosome entry sites (IRES) that are essential for the cap-independent translation of the genomic RNA [6]. The 3'UTR contains a variable region, followed by a poly-U/UC and 3'X region. Mechanisms underlying the functional roles of the 3'UTR region are unclear; nevertheless, a recent study has shown that the 3'UTR may enhance translation by transferring the host translation machinery components from the 3' to the 5' end of viral RNA [7].

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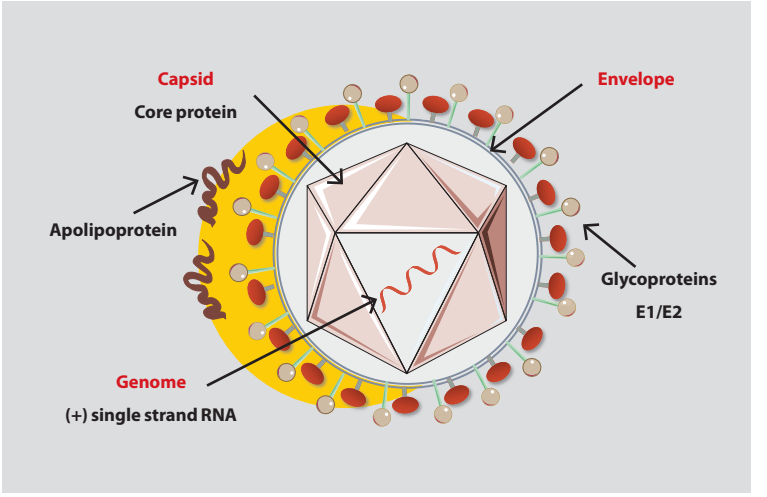

Figure 2.1 The structure of the hepatitis C virus lipo-viro-particle. E, envelope protein; RNA, ribonucleic acid. This figure was produced using Servier Medical Art, available from www.servier.com/Powerpoint-image-bank.

A unique aspect of HCV is that it is found in the blood in the form of lipo-viro-particles (LVP), which contain low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) components (eg, apolipoproteins E and B, and triglycerides) that surround the particle [8]. Although the precise role of LVP formation remains unclear, it seems that it plays a role in HCV entry (as HCV particles use receptors implicated in lipid uptake [see below section]) and in immune escape (as lipoproteins surrounding HCV particles potentially protect them from neutralizing antibody recognition) [8].

The HCV life cycle

The elucidation of the HCV life cycle has proven to have many important implications in the development of novel anti-HCV molecules, and therefore the following section will provide the most relevant information on the different steps of the viral life cycle (Figure 2.2).

Entry into the cell

The first step in the HCV life cycle is attachment to and entry into host cells. HCV has a restricted tropism, infecting predominantly hepatocytes,

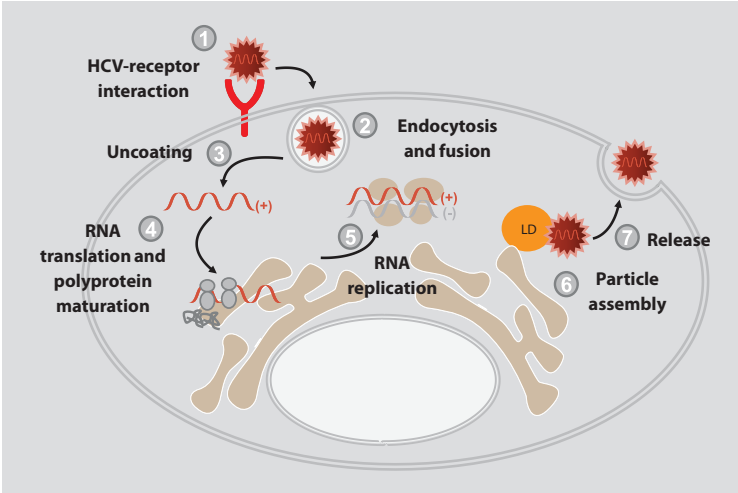

Figure 2.2 Schematic representation of the hepatitis C virus life cycle. The seven steps of the life cycle are depicted: 1, attachment; 2, entry by endocytosis and fusion; 3, uncoating; 4, RNA translation and polyprotein maturation; 5, RNA replication; 6, particle assembly on lipid droplets; and 7, release. HCV, hepatitis C virus; LD, lipid; RNA, ribonucleic acid. This figure was produced using Servier Medical Art, available from www.servier.com/Powerpoint-image-bank.

explaining the liver disorders induced by HCV infection. The process of HCV entry is meticulously orchestrated and involves many cellular receptors. First, the LVP binds to glycosaminoglycans, LDL receptor (LDLR) [9], and scavenger receptor class B type 1 (SR-B1) [10]. The interaction of HCV with SR-B1 then induces conformational changes of the viral envelope glycoprotein E2, leading to the binding of E2 to the tetraspanin CD81 [11]. CD81 and tight junction proteins (occludin and claudin-1) form a complex that triggers HCV to be internalized by clathrin-mediated endocytosis [12]. The low pH within the endosomal compartment induces major conformational changes of the E1 glycoprotein, leading to membrane fusion and capsid release into the cytoplasm.

RNA genome translation and protein maturation

Upon decapsidation the HCV genome is released into the cytoplasm of the host cell, where it is considered by the cellular machinery as mRNA, and is therefore directly translated. The cellular ribosomes recognize the IRES at the 5'UTR and produce a polyprotein, which is then cleaved by

cellular host (signal peptidase and signal peptide peptidase) and viral proteases (non-structural [NS]2-NS3 and NS3-NS4) into ten distinct proteins: the structural and the NS proteins, the main functions of which are detailed in Table 2.1. Several host and viral (NS2, NS3, NS4A, NS4B) factors can modulate HCV translation. Among the host factors the human autoantigen, La, has been shown to favor ribosome assembly during initiation of translation [13] and the microRNA, miR-122, also play a major role in activating translation by targeting two adjacent sites upstream of the HCV IRES [14,15]. Translation and maturation of the viral proteins occur at the endoplasmic reticulum (ER) membrane of the host cell.

RNA genome replication

Replication occurs once the amount of viral protein is sufficient. The mechanisms implicated in the switch from translation to replication are poorly understood but it has been hypothesized that NS3 and autoantigen La are involved. One such hypothesis suggests that NS3 and autoantigen La, which have antagonist effects on translation (ie, NS3 inhibits translation while autoantigen La activates it), compete for binding to IRES and

Hepatitis C virus proteins	Main known functions
Structural proteins	
Core	Capsid protein
E1	Envelope glycoprotein — fusion of the viral with cellular membranes
E2	Envelope glycoprotein — attachment to the cell
Non-structural proteins	
p7	Formation of ion channel in endoplasmic reticulum membrane
NS2	Protease — cleavage at the NS2-3 site
NS3	Protease — cleavage at the NS3/4A, NS4A/B, NS4B/5A, and NS5A/B sites
NS4A	Helicase — role in the viral RNA replication process
NS4B	Cofactor of NS3
NS5A	Formation of membranous structures essential for viral replication
NS5B	Regulation of viral replication
	RNA-dependent-RNA polymerase — replication of the hepatitis C virus genome

Table 2.1 Hepatitis C virus proteins and their main functions. E, envelope protein; NS, non-structural; RNA, ribonucleic acid.

therefore could participate in this molecular switch from translation to replication [16].

The viral protein, NS5B, is the RNA-dependent-RNA polymerase responsible for RNA replication [17]. Replication takes place in a specific membrane structure — the membranous web — the formation of which is induced by the virus itself [18] and is considered as the HCV RNA factory. NS5B replicates the positive-sense strand into a negative-sense strand intermediate that serves as a template for the synthesis of the genomic strand. Several host and viral proteins are involved in the regulation of replication. Among these, cyclophilin A and B appear to have a critical role by regulating binding of the polymerase on the RNA template [19,20], and miR-122 also plays a role in HCV replication [14]. NS5B polymerase lacks proofreading activity and therefore HCV has high mutation rates (as is the case for most RNA viruses).

Assembly and release

Assembly and release of newly formed HCV particles are two events intimately linked to lipid metabolism. The first event in HCV assembly is the targeting of the core protein from the ER membrane, where it is translated, to particular cytoplasmic organelles called lipid droplets [21] (believed to be the platform of HCV assembly). The HCV RNA is relocated from the membranous web to lipid droplets in a mechanism that is dependent on the presence of NS5A on core coated-lipid droplets [22]. Trafficking of the core protein and NS5A seems to be partly regulated by one host protein, the diacylglycerol O-acyltransferase 1 (DGAT1) [23]. Other host proteins involved in lipid droplet morphogenesis, such as tail-interacting protein of 47 kDa (TIP47) (a lipid droplet-associated protein) [24] or seipin (involved in lipid droplet maturation) [25], seem to also play a role in assembly. Capsids subsequently migrate in the ER lumen and the viral envelope is acquired by budding of the ER membrane, where HCV glycoproteins E1 and E2 are anchored at the proximity of the assembly site. This final step of assembly is orchestrated by NS2 [26]. Finally, the nascent HCV particles undergo maturation through the VLDL secretory pathway during which they are associated with lipoproteins before being released by exocytosis as LVPs [27].

Key points

- The hepatitis C virus particle is a spherical (ø ~40–80 nm), enveloped virus (envelope contains viral glycoproteins E1 and E2), with a single-strand positive-sense RNA genome.
- The virus has an icosahedral capsid structure composed of core protein and circulates as lipo-viro-particles within the blood.
- The life cycle of HCV targets hepatocytes and consists of seven steps: attachment, entry, uncoating, RNA translation and polyprotein maturation, RNA replication, particle assembly on lipid droplets, and release.

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