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Molecular Biology of Hepatitis C Virus: An Overview

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Abstract: The Hepatitis C virus (HCV) is an enveloped, positive strand RNA virus and has been classified as a separate genus Hepacivirus of the family Flaviviridae. The HCV genome is a linear molecule with a length of approximately 9.6 kb that is flanked by 5` and 3' untranslated regions (UTRs) and encodes to a nearly 3,010 amino acids polyprotein precursor. It is cleaved by combination of viral and host proteases into structural (C, P7, El, and E2) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B), essential for viral replication and viron formation in post-translational process. The 5` and 3' UTRs are highly conserved regions in HCV genome, crucial for molecular processes such as replication and translation. In this review, we summarize the current knowledge about HCV molecular and structural HCV biology. This knowledge may help to improve treatment strategies and development of vaccine against HCV.

Key words: HCV, replication, post-translational process, nonstructural proteins, Untranslated regions

Introduction

The HCV belongs to the flaviviriade family, the virus enveloped in a lipid bilayer enriched by envelope anchored protein (E). The envelope surrounds the nucleocapsid, composed of multiple copies of small basic protein (Core or C) as well as RNA genome (Chevaliez and Pawlotsky, 2006). Landford et al. (2001) reported that HCV is a positive sense, single stranded RNA molecule and has genome size of 9.6 kb. It consist of single open reading frame (ORF) that encodes to a polyprotein of 3,010 amino acids, the polyprotein is posttranslationally processed into structural (C, P7, El and E2) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins as shown in Figure 1(Beaulieu and Tsantrizos, 2004). The 5' end of ORF is flanked by 5` UTR of 341 nucleotides and 3` is flanked 3' UTR of 230 nucleotides. Both 5` and 3` UTRs are

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highly conserved regions in HCV genome, essential for molecular processes such as replication and translation (Lindenbach and Rice, 2001).

The advance knowledge of HCV about HCV molecular and structural HCV biology is necessary for treatment strategies and development of vaccine against HCV. With the objective to introduce the molecular mechanisms of HCV, the aim of this review to bring new material for this purpose.

Structural Organization of 5` and 3' UTR

The 5' UTR consists of 341 nucleotides and contains four domains, numbered as I to IV. A pseudoknot is present in domain III, and initiation codon for the ORF translation is present in domain IV. Domains II, III, and IV, collectively with the first 24 to 40 nucleotides of the core-encoding region, make up the internal ribosome entry site (IRES). The HCV polyprotein translation initiates at the IRES, a few nucleotides upstream of initiator codon (AUG) of polyprotein. The 40S subunit of ribosome comes in contact with the AUG codon by means of this IRES. Then eukaryotic initiation factor 3 (eIF3) binds to both the IRES and the 40S ribosomal subunit during translation, results in a complex formation that brings the codon AUG in contact with the Met-tRNA anticodon (Beales et al., 2001). It has also been reported that 5` UTR is highly conserved region in genome of HCV therefore it may be used for the detection of specific genotypes (Cantaloube, 2006).

The 3 UTR consists of 40 variable nucleotides, a poly polypyrimidine sequence and 98 highly conserved nucleotides that are also contain very stable stem-loop structures (Tanaka et al., 1996). The 3'UTR plays a vital function in initiating HCV genome replication (Penin et al., 2004).

Structural Proteins of HCV Genome

Core Protein

Among the structural proteins of HCV, core protein is expressed from the most conserved region of HCV genome. The core protein is a highly basic RNA-binding protein, forms the HCV capsid and is released as a precursor of 191 amino acids with molecular weight of 23 kDa (McLauchlan et al., 2002; Okamoto et al., 2004). The core protein includes three separate domains: a N-terminal hydrophilic domain (D1) of 120 amino acids, a C-terminal hydrophobic domain (D2) of 50 amino acids, and the last domain of 20 amino acids that serves as a signal peptide for an envelope protein (E1) located downstream. The D1 domain contains various positive charges and three nuclear localization signals (NLS) that are involved in RNA binding and nuclear localization (Suzuki et al., 2005). The D2 domain is important for association of core protein with membranes of endoplasmic reticulum (ER), mitochondria and lipid droplets (Schwer et al., 2004; Suzuki et al., 2005).

The mature core protein interacts with the 5′ and 3′ UTRs consequently prevents function of IRES, and initiates dimerization of genome (Cristofari et al., 2004). Thus for accurate HCV particle morphogenesis, the interaction of core protein with cell membranes or lipids droplets and envelope glycoproteins is necessary. In addition, HCV core protein may affect cell proliferation, cell signaling, cell death, gene transcription, lipid metabolism and host immune responses (Lai Ware, 2000; Kato, 2001; McLauchlan et al., 2007).

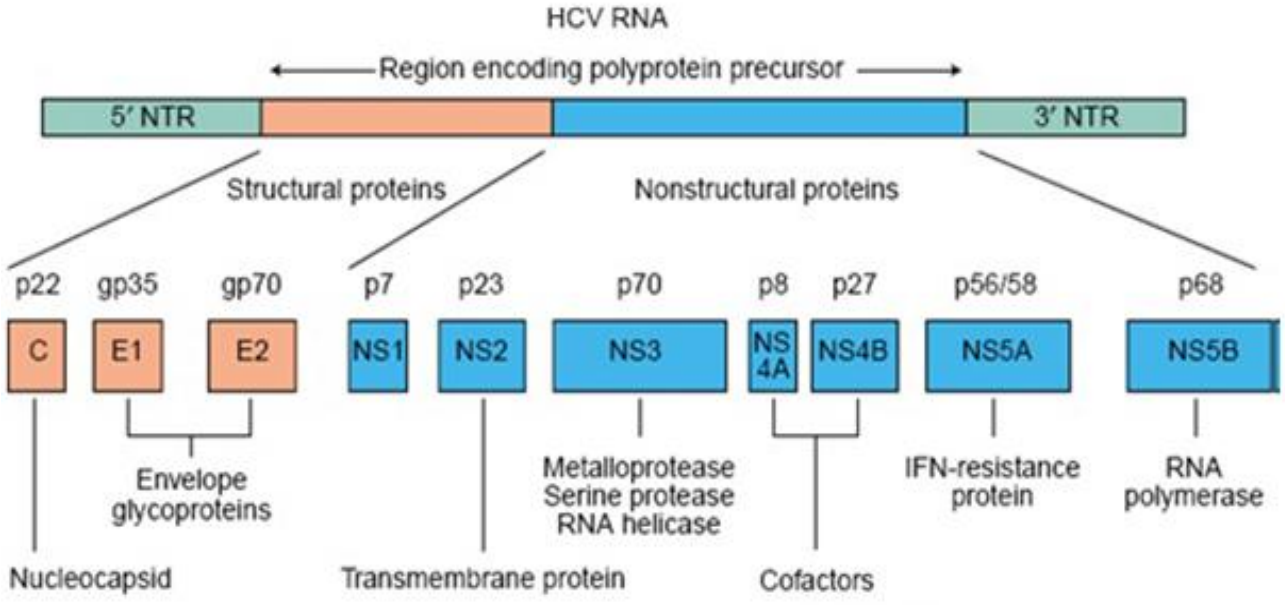

Figure 1: HCV Genome structure and proteins expression (Beaulieu and Tsantrizos, 2004).

E1 and E2 Envelope Glycoproteins

The E1 and E2 are two envelope glycoproteins that are necessary elements of the HCV virion envelope and essential for HCV entry and attachment, carrying molecular weight 33-35 kDa and 70-72 kDa respectively(Lavillette et al., 2006; Lindenbach et al., 2007).

E1 and E2 are classified as type I transmembrane glycoproteins and approximately 30 amino acids constitute C-terminal domain. However the N-terminal ectodomains of these envelope glycoproteins are of 160 and 334 amino acids respectively (Cocquerel et al., 2000). The transmembrane domains of E1 and E2 are made up of two segments of hydrophobic amino acids that are separated by a region containing highly conserved charged residues. They perform various functions, including, ER localization, membrane anchoring and heterodimer assembly (Meyer et al 2004). Both envelope glycoproteins are highly glycosylated as E1 contains approximately 5 glycosylation sites whereas E2 contains about 11 glycosylation site (Goffard et al 2005). Moreover, E2 contains hypervariable regions and amino acids sequences present in these regions vary approximately 80% between HCV genotypes (Ashfaq et al., 2011).

p7 protein

The p7 protein of HCV is an integral membrane protein of 63 hydrophobic amino acids, with two α-helices, two TM1 and TM2 transmembrane domains and a basic loop that is located in the cytoplasm (Carrere-Kremer et al., 2002). It has been reported that when recombinant expression plasmids were used and they revealed that p7 is detected in the plasma membrane, mitochondrial membranes and endoplasmic reticulum (Carrere-Kremer *et al.*, 2002; Griffin et al., 2005). The p7 protein is vital for HCV assembly, in vitro release and in vivo infection (Sakai et al., 2003; Steinmann et al., 2007).

Nonstructural proteins of HCV

NS2

The NS2 protein is a transmembrane glycocylated protein of 21-23 kDa and participates in proteolytic cleavage of NS2-NS3 junction of the polyprotein of HCV (Yamaga, et al., 2002; Lorenz et al., 2006). Jirasko and coworkers (2008) have revealed that two amino acids histidine 143 and cysteine 184 are crucial for proteolytic cleavage of NS2. Moreover, Noorali et al. (2011) reported that NS2, together with the amino- terminal domain of the NS3 protein (NS2-3 protease) comprises a zinc-dependent metallo-protease that performs cleavage of NS2-NS3 junction.

NS3

The NS3 is a multifunctional protein of 70 kDa, contains serine protease domain of 180 amino acids at N-terminal and RNA helicase/NTPase domain of 450 amino acids at C-terminal that plays an important role in unwinding double-stranded DNA (Lindenbach et al., 2007; Shiryaev et al., 2012). Similarly, NS3 inhibits functions of host protein kinases (Iwai, et al., 2011). Similarly, NS3 protease is the principal candidate for the development of anti-viral drug due to its essential role in HCV genome replication. (Gupte and Arankalle, 2012).

NS4A

The NS4A is 8 kDa multifunctional protein, important in HCV life cycle. About 21–30 amino acids in the central region of NS4A act as a cofactor of NS3 protease (Yi-Hen, et al., 2007). NS3-NS4A protease interacts with host cell proteins and pathways that are important in the lifecycle and pathogenesis of HCV infection (Noorali et al., 2011). NS3-NS4A protease performs cleavage at the NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/ NS5B junctions of HCV polyprotein (Chevaliez and Pawlotsky, 2006). Moreover, the NS4A may interact with NS4B and NS5A and consequently uncleaved NS4B-5A complex supports NS5A hyperphosphorylation (Lindenbach et al., 2007).

NS4B

The NS4B is an integral membrane protein of 27 kDa, detected in ER or ER-derived membrane localization (Lundin et al., 2003). The NS4B contains an amphipathic helix at N-terminal and four transmembrane domains that are responsible for membrane association (Elazar et al., 2004). In replication, the NS4B acts as a membrane anchor for the replication complex (Elazar et al., 2004; Gretton et al., 2005). NS4B inhibits activities of RNA dependent RNA polymerase (RdRp) and cellular syntheses (Kato et al., 2002; Piccininni et al., 2002), and affects induction of interleukin 8 (IL-8) and transformation of NIH3T3 cell lines (Park et al., 2000; Kadoya et al., 2005). In addition, NS4B has a GTPase activity that is critical for HCV RNA replication (Einav et al., 2004).

NS5A

The NS5A is a phosphorylated zinc-metalloprotein of 56-58 kDa. It plays an important role in HCV RNA replication as well as in regulation of cellular pathways (Masaki, et al., 2008). The NS5A contains an amphipathic α-helix of 1-30 amino acids at N-terminal that is crucial for assembly of the replication complex and membrane localization in perinuclear membranes (Penin and his coworkers (2004). Downstream to amphipathic α-helix, the NS5A protein contains three more domains and first domain contains a zinc binding motif in which four cysteine residues are conserved (Elazar et al., 2003; Penin et al., 2004). Recently, a study proposed that HCV RNA replication is inhibited by

mutation in NS5A sequence (Nakamoto et al., 2014). Moreover, this protein modulates HCV RNA replication and host processes by direct and indirect interaction with a variety of host regulatory factors (Shimakami, et al., 2004).

NS5B

The NS5B is an endoplasmic-reticulum-membrane-associated protein of 68 kDa with RNA-dependent polymerase activity. Replication of HCV RNA proceeds in two steps: synthesis of a complementary minus-strand RNA using genome as template, and subsequent synthesis of genomic plus-strand RNA from this minus-strand RNA template. The key enzyme in both steps is the NS5B (Mosley et al., 2012). Mutations in the NS5B gene affect its membrane association with ER and consequently RNA replication. However, oligomerization and intramolecular interactions of NS5B enhance RNA synthesis (Lindenbach et al., 2007).

HCV RNA Translation, Post-Translational Processing and Replication

The HCV is attached to its receptor complex, usually the CD81 molecules at the surface of various human cells, act as a post-attachment entry co-receptor with other cellular factors and initiate HCV binding and entry into hepatocytes (Cormier et al., 2004). When attached, HCV is fused with cell membrane and then nucleocapsid of enveloped HCV is released into the cell cytoplasm. Fusion is performed by HCV envelope glycoproteins and directly takes place at the cell membrane or after HCV internalization into endosomes (Lescar et al., 2001; Lindenbach and Rice, 2001).

Decapsidation of HCV nucleocapsid releases free genomic as a positive-strand RNA into the cytoplasm of cell. In cytoplasm this positive-strand RNA and newly synthesized RNAs serve as messenger RNAs for synthesis of the HCV polyprotein. HCV genome translation is controlled by domains II to IV of IRES in the 5′ UTR and few nucleotides of the core coding region. IRES domain I plays an important role in initiating IRES-dependent translation (Luo et al., 2003). The IRES initiates cap-independent internal translation of HCV polyprotein by using both cellular proteins such as eukaryotic initiation factors (eIF2 and eIF3) and HCV proteins (Ji et al., 2004; Otto and Puglisi, 2004).

Translation of HCV genome produces a large precursor polyprotein, which is targeted to the membrane of ER for translocation of the E1 ectodomain into the ER lumen by the internal signal sequence located between the core and E1 sequences. When the signal sequence is cleaved by the host signals peptidase, an immature form of the core protein is produced, which is further processed by a host signal peptide protease to finally produce the mature core protein (McLauchlan et al., 2002; Penin et al., 2004a). The host signal peptidase also performs cleavages at the E1–E2 junction, the C-terminal end of E2 and between p7 and NS2 to release p7 protein. However, an incomplete cleavage may produce non-cleaved E2-p7 protein, the function of which is not known. E1 and E2 envelope glycoproteins subsequently undergo several post-translational modification steps, including N-glycosylation, conformation and assembly of E1E2 heterodimers (Penin et al., 2004b).

The zinc-dependent NS2- NS3 protease performs cleavage at NS3-NS2 junction. NS3 along with its cofactor NS4A performs cleavage at the NS3-NS4A, NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B junctions. The cleavage sites are recognized by the NS3-NS4A protease due to presence of a common sequence (Asp/GluXXXXCys/Thr-Ser/Ala), (Bartenschlager and Lohmann, 2000; Lindenbach and Rice, 2005).

Viral infection causes rearrangements of intracellular membranes of host, which are prerequisite for the formation of a replication complex due to association of nascent RNA strands, viral proteins and cellular factors. The HCV NS4B protein here is sufficient to initiate the formation of a membranous web (Egger et al., 2002; Gretton et al., 2005). The membranous web is derived from ER membranes, made up of the non-structural HCV proteins and host cell replication complex, which are located closely in perinuclear membranes (Bartenschlager et al., 2004). This web is full of cholesterol and fatty acids, their degree of saturation affects HCV replication (Kapadia and Chisari, 2005).

The replication also takes place in membranous web and seems to be semi conservative. This process occurs in two steps, which are performed by the NS5B RdRp. In first step, the positive-strand genome RNA serves as a template for the synthesis of a complementary negative-strand. In the second step, negative-strand RNA serves as a template to produce many new strands of positive- strand RNA that are subsequently used for HCV polyprotein translation and synthesis of new intermediates of replication (Bartenschlager et al., 2004). Genome encapsidation occurs in the endoplasmic reticulum and nucleocapsids are enveloped and matured into the Golgi apparatus. Then newly produced HCV virions are released in the pericellular space by exocytosis (Penin et al., 2004).

Conclusion:

As advance knowledge about molecular and structural HCV biology is required to improve treatment strategies and development of vaccine against HCV. Therefore, in this review current knowledge about HCV genome structure and regulation of molecular mechanisms by expressed viral proteins was summarized to bring new material for this purpose.

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