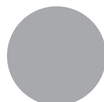

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THE FUNCTIONAL E1–E2 HETERODIMER HCV GLYCOPROTEINS

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ABSTRACT… Introduction: The two HCV envelope glycoproteins E1 and E2 are released from HCV polyprotein by signal peptidase cleavages. These glycoproteins are type I transmembrane proteins with a highly glycosylated N-terminal ectodomain and a C-terminal hydrophobic anchor. **Methods and pathways:** After their synthesis, HCV glycoproteins E1 and E2 associate as a non covalent heterodimer. The transmembrane domains of HCV envelope glycoproteins play a major role in E1–E2 heterodimer assembly and subcellular localization. The envelope glycoprotein complex E1–E2 has been proposed to be essential for HCV entry. **Results and conclusions:** However, for a long time, HCV entry studies have been limited by the lack of a robust cell culture system for HCV replication and viral particle production. Recently, a model mimicking the entry process of HCV lifecycle has been developed by pseudo typing retroviral particles with native HCV envelope glycoproteins, allowing the characterization of functional E1–E2 envelope glycoproteins., we review our understanding to date on the assembly of the functional HCV glycoprotein heterodimer.

Key words: HCV glycoproteins, Transmembrane domain, E1-E2 heterodimer

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INTRODUCTION

All viruses have evolved different ways of entering target cells to start replication and infection. The first step is the recognition of host cells through cell surface receptor(s). This engagement can mediate attachment and act as a primer for subsequent conformational alteration, leading to virus entry into host cell. In most cases, interaction with a receptor is important for defining the tropism of a virus for a particular organism, tissue or cell type. Enveloped viruses possess a lipid bilayer that surrounds their nucleocapsid. The glycoproteins present in their envelope are involved in the receptor-binding step. After attachment, the entry of these viruses into cells requires the fusion of the viral and a cellular membrane by a process that is also driven by the viral envelope glycoproteins. To fulfill these functions, viral envelope glycoproteins have to adopt different conformations during the virus lifecycle. so, these conformational changes have to occur at a precise time of the virus lifecycle, so that they have to be tightly modulated.

HCV encodes two envelope glycoproteins, named E1 and E2. Due to the lack of a cell culture system supporting efficient HCV replication and particle assembly has hampered the characterization of the envelope proteins present on the virion. Cell culture transient systems have allowed investigators to characterize the first steps in the biogenesis of HCV envelope glycoproteins. In addition, surrogate models have also been developed to study the entry steps of HCV lifecycle. However, it is only recently that a model mimicking the entry process of HCV lifecycle has been developed. This has been achieved by pseudo typing retroviral particles with native HCV envelope glycoproteins. This new tool and techniques allows the characterization of the assembly of functional HCV envelope glycoproteins.

SPECIAL BIOGENESIS ASPECTS OF HCV ENVELOPE GLYCOPROTEINS

Process of Cleavage of HCV Glycoproteins from the Viral Polyprotein

The genome of HCV encodes a single polyprotein. This ~3010 amino acid polyprotein is processed by cellular (signal peptidase and signal peptide peptidase) and viral proteases (NS2-3 and NS3-4A) to generate at least 10 polypeptides. The nonstructural proteins are released from the polyprotein after cleavage by HCV proteases NS2-3 and NS3-4A, the structural proteins are released by host endoplasmic reticulum (ER) signal peptidase(s)[1]. Processing mediated by a signal peptide peptidase also occurs at the C-terminus of the capsid protein . Cleavages in the polyprotein precursor proceed to completion during translation. Some cleavages occur at the E2/p7 and p7/NS2 sites, leading to the production of an uncleaved E2p7NS2[2] molecule. Most of NS2 is progressively cleaved from the E2p7NS2 precursor, cleavage between E2 and p7 does not change over time, for most HCV strains analyzed. This results in cleavage products consisting of E2, E2p7, p7, and NS2[3].

The N-terminal of HCV polyprotein. The arrows show host signal peptidase cleavages. Cleavages at E2/p7 and p7/NS2 sites is indicated by dotted arrows. Cleavage by the host cell signal peptide peptidase (SPP) is indicated.

Sequences located N-terminally of E2/p7 and p7/NS2 cleavage sites can efficiently function as signal peptides. They are fused to a reporter protein, the signal peptides of p7 and NS2 are cleaved. The data indicate that inefficiency of cleavage at E2/p7 and p7/NS2 sites[4] is not due to the presence of suboptimal signal peptides. p7 polypeptide is a polytopic membrane protein containing two transmembrane domains with both its N- and C-termini oriented toward the ER lumen. The presence of the first transmembrane domain of p7 reduces the efficiency of p7/NS2 cleavage. Sequence analyses and mutagenesis studies have also identified structural determinants responsible for the cleavage at both E2/p7 and p7/NS2 sites . The short distance between the cleavage site of E2/p7 or p7/NS2 and the predicted transmembrane α-helix located downstream of the cleavage sites might impose structural constraints to these cleavage sites. Constraints in the processing of a polyprotein precursor are very essential for HCV to post-translationally regulate the kinetics and the level of expression of p7 as well as NS2 and E2 mature proteins[5].

Processing at the E2p7 site has been explored. It is being proved to be efficient in genotype 1b (strain BK) than in the genotype 1a (strain H77c).Sequence comparison of p7 signal peptides of these two viral strains has identified a difference of 3 amino acids and mutational analysis has shown that the V720L change in the H77c sequence increases the efficiency of processing at the E2/p7 site. When expressed alone, p7 protein has been shown to adopt a double membrane spanning topology with both extremities orientated luminally in the ER, the C-terminal part of E2p7 proteins has been found to be located in the cytosol. Data suggest that p7 can potentially adopt a dual transmembrane topology. It is to be shown whether an E2p7 with a cytosolic orientation of the C-terminus of p7 exists when this protein is expressed in the context of the polyprotein. p7 and NS2 are not essential for HCV genomic replication, they will likely to play their role in virion assembly; it is a process that is supposed to be regulated. It has been shown that p7 reconstituted into artificial lipid membranes homo-oligomerizes and behaves as an ion channel protein, when bound to E2, p7 cannot oligomerize and function as an ion channel, and the existence of E2p7 would reduce the amount of functional p7 molecules available. Production of precursors like E2p7NS2 and E2p7[6] might be a means to maintain p7 inactive during the phase of the accumulation of E2 molecules required for HCV envelope formation. The precursors might also control the temporal release of E2 and NS2.

The Glycosylation of HCV Envelope Glycoproteins

It may be noted that N-linked glycosylation is the most common types of protein modification, and occurs by the transfer of an oligosaccharide from a lipid intermediate to an Asn residue in the consensus sequence Asn-X-Thr/Ser of a nascent protein, where X is any amino acid except Pro .The addition of glycan is every time catalyzed by the

oligosaccharyltransferase, which is associated with the translocon through which peptidic chains emerge in the ER lumen. However, not every tripeptide sequence in a protein sequence is used for carbohydrate addition .In the secretary pathway, the glycans play a role in protein folding, quality control and certain sorting events. Viral envelope proteins usually contain N-linked glycans that can play a major role in their folding, in their entry functions or in modulating the immune response .The ectodomains of HCV envelope glycoproteins E1 and E2 are highly modified by N-linked glycans. E1 and E2 possess up to 6 and 11 potential glycosylation sites, respectively. Sequence analyses of glycoprotein E1 indicate that 5 potential N-glycosylation sites are strongly conserved among HCV genotypes. The presence of a proline residue immediately downstream the glycosylation site is unfavorable for glycosylation, and it has confirmed experimentally that this site is not glycosylated. The glycosylation site of E1 at position 250 is poorly conserved; this site is observed in genotypes 1b and 6[7]. Most E2 glycosylation sites are conserved. Global sequence analyses of potential glycosylation sites in E2 indicate that nine of the eleven sites are strongly conserved. The two remaining sites, N5 and N7, show the conservation levels of 75% and 89%.

Schematic representation of E1 and E2 features. The Position of N-linked glycans are indicated as an N followed by a number related to the relative position of the potential glycosylation site in glycoprotein.

Mutants of E1 and E2 has been produced to characterize the glycosylation of the proteins. In the context of the H strain, the 4 potential glycosylation sites of E1 was shown to be occupied by glycans. In the case of E2, study has shown that mutation of some glycosylation sites in the context of a truncated form of E2 alters the recognition by sera from HCV patient, the mutants was not characterized in terms of glycosylation and no clear conclusion can be drawn from this study. Glycosylation mutants have been produced in the context of truncated form of E2 ending at position 660. The E2 sequence of HCV isolate used in this study contains 10 instead of 11 potential glycosylation sites, the site N5 at position 476 being missing. The last two glycosylation sites, N10 and N11, were not occupied in E2 . However, one of the sites was occupied in the context of full-length E2. A recent mutagenesis study, in the context of an E2 glycoprotein containing 11 potential glycosylation sites, have shown that all the sites are occupied by glycans. E2 was expressed as a polyprotein containing full-length E1 and E2.

The addition of the glycan precursor is always catalyzed by the oligosaccharyltransferase. the enzyme is thought to have access only to nascent chalains as they always emerge from the ribosome at the luminal face of the rough ER. The glycosylation process of HCV envelope glycoprotein E1 has been analyzed in the context of a Man-P-Dol-deficient cell line (B3F7) and it has to occur post-translationally .indicating that the oligosaccharyltransferase has also access to the E1 glycoprotein for more than an hour after its translation. Characterization of HCV glycoprotein E1 has also shown that, in the absence of E2, different glycoforms of E1 are produced and the glycosylation of E1 is improved by co-expression of E2 in cis.

The Folding of HCV Envelope Glycoproteins

HCV envelope glycoproteins have been shown to assemble as a noncovalent E1E2 heterodimer. In heterologous expression systems, HCV envelope glycoproteins has a tendency to also form misfolded aggregates stabilized by disulfide bonds .The Analyses of HCV envelope glycoproteins with conformation-sensitive antibodies are therefore necessary to discriminate noncovalent heterodimers from misfolded complexes . Such discrimination can also be made by analyzing disulfide-bond formation by migrating HCV envelope glycoproteins on SDS-PAGE under non-reducing conditions.The Analyses of the formation of conformation-dependent epitopes and disulfide-bond formation indicate that folding of HCV envelope glycoproteins is a slow process. The folding of E1 has been shown to be dependent

on the co-expression of E2. In addition, it has also been shown that the folding of E2 is also dependent on the co-expression of E1. Altogether, these observations indicate that HCV envelope glycoproteins cooperate for the formation of a functional complex. These observations also indicate that, although some degree of folding can be observed in E2 expressed alone, both glycoproteins need to be co-expressed to analyze their functional properties.

During the process of folding, HCV envelope glycoproteins have been shown to interact with calnexin lectin-like ER chaperone, that shows an affinity for monoglucosylated N-linked oligosaccharides. Both E1 and E2 have been found to associate rapidly with calnexin and dissociate slowly, suggesting a role of this chaperone in the folding of HCV envelope glycoproteins). However, data suggest that only E1 interacts with calnexin .Differences in the cell lines used and/or in the levels of expression of the envelope glycoproteins might potentially explain these discrepancies. Further experiments in cell cultures infected with native HCV particles will be needed to confirm the involvement of calnexin in the folding E2.

The presence of glycans on HCV envelope glycoproteins can potentially affect their folding either directly or through interaction with calnexin. Site-directed mutagenesis studies have indeed shown that the absence of some glycans in E1 (N1 and N4) and E2 (N8 and N10) leads to misfolding of HCV envelope glycoproteins. This alteration in folding was not due to the lack of interaction of HCV envelope glycoproteins with calnexin, suggesting that the mutations would rather has a direct effect on protein folding. The presence of a large polar saccharide is known to affect the folding at least locally by orienting polypeptide segments toward the surface of protein domains.

TRANSMEMBRANE DOMAINS INVOLVEMENT IN THE BIOGENESIS OF E1E2 HETERODIMER

The Membrane Anchor and the Signal Sequence

Due to the resistance to alkaline or salt extraction, HCV envelope glycoproteins have been confirmed to be membrane associated proteins. Deletion of the C-terminal hydrophobic regions of these proteins leads to their secretion, indicating that these regions are involved in membrane anchoring .Sequence analysis of a large number of HCV isolates has shown that the C-termini of E1 and E2 contain hydrophobic sequences that are less than 30 amino acid residues long. The viruses of the Flaviviridae family, these regions are composed of two stretches of hydrophobic residues separated by a short segment containing at least one fully conserved positively charged residue. When fused to a reporter protein the second hydrophobic stretch functions as a signal sequence which is in agreement with the observation that HCV envelope glycoproteins are released from the polyprotein precursor after cleavage by host signal peptidase(s) It is worth noting that in the context of HCV polyprotein, only the sequence located at the C-terminus of the immature form of the capsid protein is a true signal peptide that will interact with the signal recognition particle sequences present at the C-terminus of E1 and E2 do not interact with the signal recognition particle, and they should be called signals of reinitiation of translocation. Deletion of these signals leads to the secretion of E1 and E2, indicating that these signals are involved in their membrane anchoring.

The ER Retention Function

The HCV envelope glycoproteins are retained in the ER and ER retention signals are present in the transmembrane domains of E1 and E2 .In addition, the charged residues of the transmembrane domains of E1 (Lys) and E2 (Asp and Arg) play a key role in the ER retention of these glycoproteins .It has been proposed that an additional ER retention signal might also be present in the ectodomain of E1 .Interestingly, in some conditions of overexpression a small fraction of HCV envelope glycoproteins has been shown to accumulate at the plasma membrane. Cell surface expression of E1 and E2 is likely due to the accumulation of small amounts of glycoproteins escaping the ER-retention machinery, due to saturation of this mechanism.

Role in Heterodimerization

The signal sequence and ER retention functions, the transmembrane domains of HCV envelope glycoproteins is playing a major role in the assembly of E1E2 heterodimer. Indeed, deletion of the transmembrane domain of E2 or its replacement by the anchor signal of another protein abolishes the formation of E1E2 heterodimer. The site-directed mutagenesis or alanine scanning insertion mutagenesis have confirmed that the transmembrane domains of E1 and E2 play a direct role in E1E2 assembly. Also, alanine scanning insertion mutagenesis allowed to identify two distinct segments in the transmembrane domain of E1 and one in the transmembrane domain of E2 that were specifically involved in E1E2 assembly. Single region located outside of the transmembrane domains has also been shown to be involved in heterodimerization.

The Topological Change in the Transmembrane Domain of HCV Glycoproteins

The topology of the transmembrane domain of HCV envelope glycoproteins has given rise to controversy. Indeed, the presence of a first hydrophobic stretch and a signal sequence function separated by charged residues in the transmembrane domains of E1 and E2 has suggested that they might be composed of two membrane spanning segments with the charged residues facing the cytosol. This type of organization has been observed in the C-terminal region of the envelope glycoprotein E2 of the alphaviruses as well as for the envelope proteins of the flaviviruses but sequence analysis and data of alanine scanning insertion mutagenesis were in favor of a single spanning topology of E1 and E2 transmembrane domain. The topology study of the transmembrane domains of HCV envelope proteins has been performed by determining the accessibility of their N- and C-termini in selectively permeabilized cells. Our work has shown that before signal sequence cleavage at their C-terminus, the transmembrane domains form a hairpin structure but after cleavage between E1 and E2 or between E2 and p7, the second C-terminal hydrophobic stretch is reoriented towards the cytosol, leading to the formation of a single membrane-spanning domain. Again, the charged residues located in the middle of the transmembrane domains were shown to play a crucial role in their structural dynamics.

The Role of HCV Envelope Glycoproteins in Virus Entry

For viruses, entry into the cytosol is a multistep process, during which the host cell assists the incoming virus. Viruses first attach themselves to components of the plasma membrane, which they use as non-specific attachment factors or as specific cell surface receptors. Viral attachment is mediated by the binding of a protein present at the surface of the virion to a molecule on the cell surface acting as a virus receptor. The envelope glycoprotein complex E1E2 is the viral component thought to be present at the surface of HCV particles and it is therefore the obvious candidate ligand for cellular receptors. Receptor binding can activate cellular endocytic pathways through which viruses are internalized in endosomes. When they reach the appropriate intracellular location, viruses are activated for penetration by cellular signals and make their way through the membrane of the endosome, or through the plasma membrane for those that do not enter by endocytosis. Enveloped viruses fuse their lipid envelope with the plasma membrane or the membrane of an endosome, resulting in the release of the nucleocapsid into the cytosol.

Specific Models to Study HCV Entry

In the absence of cell culture system to amplify HCV, manyl models have been developed to study HCV entry. The first approach, a soluble form of HCV glycoprotein E2 has been used to identify cell surface proteins potentially involved in HCV entry. Although this approach is potentially interesting in protein-protein interactions studies, it cannot be used to study the entire entry process. In addition, due to their cooperative role in folding, both glycoproteins need to be co-expressed to analyze their functional properties. To study the role of E1E2 envelope glycoproteins in HCV entry, several surrogate models of HCV particles

have therefore been developed. As an approach, virus-like particles have been produced in insect cells infected by a recombinant baculovirus containing the cDNA of HCV structural proteins. However these particles are not infectious and they are retained in an intracellular compartment. It is therefore difficult to evaluate how close these virus-like particles are to native virion. In addition, due to the absence of infectivity, these particles cannot be used to study the fusion process. Another approach to study HCV entry has been to produce virosomes by incorporating E1E2 heterodimers into liposomes .These virosomes can be used to study the interactions between E1E2 heterodimers and cell surface receptors. However, it has not been shown whether the envelope glycoproteins incorporated into these liposomes can induce fusion.

Other models have been based on pseudotyping of viral vectors. The first model that has been developed was based on vesicular stomatitis virus (VSV) pseudotyped with modified E1 and/or E2 glycoproteins . In the particles, the transmembrane domains of HCV envelope glycoproteins have been replaced by the transmembrane domain and cytoplasmic tail of the VSV envelope glycoprotein G. This allows the export of HCV envelope glycoproteins to the cell surface but some doubts have been raised on the infectivity of such VSV pseudotyped particles. In addition, replacement of HCV envelope glycoproteins has been shown to alter their entry function.

Presently, retrovirus have also been used to produce pseudotyped particles containing HCV envelope glycoproteins . Murine leukemia virus (MLV) or human immunodeficiency virus (HIV) vectors were used. Retroviruses are indeed well known to be able to incorporate in their envelope a variety of cellular and viral glycoproteins , they can also easily package and integrate genetic markers into host cell DNA .All these properties were exploited to produce viral pseudoparticles expressing E1E2 at their surface and packaging a reporter gene that allows to monitor viral infection of the target cell. HCV pseudoparticles (HCVpp) are produced by transfecting 293T cells with three expression vectors encoding the E1E2 polyprotein, the retroviral core proteins and a packaging-competent retrovirus-derived genome containing a marker gene. Because MLV and HIV are supposed to assemble at the plasma membrane and HCV glycoproteins are retained in the ER, a first approach has been to modify the transmembrane domains of E1 and E2 to re-address them at the plasma membrane but the pseudoparticles bearing such modified HCV envelope glycoproteins were not infectious. Intrestingly, in the absence of any modification of HCV envelope glycoproteins, infectious pseudoparticles were produced .Interestingly, due to saturation of the ER retention machinery, the cells used to produce HCVpp were shown to express a small fraction of HCV envelope glycoproteins at the plasma membrane . This accumulation at the plasma membrane might therefore be sufficient to incorporate native HCV envelope glycoproteins into retroviral pseudotyped particles.

The Production of HCV pseudoparticules (HCVpp). For the production of HCVpp, human embryo kidney cells 293T are transfected with three expression vectors. The first vector encodes retroviral Gag and Pol proteins. Gag proteins are responsible for particle.

The data thus accumulated on these pseudoparticles strongly suggest that they mimic the early steps of HCV infection. Indeed, they exhibit a preferential tropism for hepatic cells and they are specifically neutralized by anti-E2 monoclonal antibodies as well as sera from HCV-infected patients The HCVpp therefore represent the best tool available to study functional HCV envelope glycoproteins. An analysis of the glycoproteins associated with HCVpp has shown the heterogeneous nature of E1 and E2 incorporated into HCVpp .This highlights the difficulty in identifying forms of the HCV glycoproteins that initiate infection. However, characterization of HCVpp envelope glycoproteins with conformation-sensitive neutralizing monoclonal antibodies has shown that the functional unit is a noncovalent E1E2

heterodimer .In addition, coexpression of both envelope glycoproteins has been shown to be necessary to produce infectious pseudo particles, confirming that only the E1E2 heterodimer is functional.

The HCV Receptors

As a primary approach to identify potential HCV receptor(s), a soluble form of HCV glycoprotein E2 has been used. This allowed to identify the CD81 tetraspanin as a putative receptor for HCV. An approach identified the scavenger receptor class B type I (SR-BI) .a high-density lipoprotein (HDL)-binding molecule, and the mannose binding lectins DC-SIGN and L-SIGN as additional candidate receptors for HCV. Heparan sulfate has also been shown to interact with HCV glycoprotein E2, suggesting that this type of molecule can play a role in HCV entr). An approach using virus-like particles produced in insect cells has led to the identification of the asialoglycoprotein receptor as another candidate receptor for HCV .Finally, because of the physical association of HCV with low- or very-low-density lipoproteins (LDL or VLDL) in serum, the LDL receptor has also been proposed as another candidate receptor for HCV.

A number of cell-surface molecules bind viral envelope glycoproteins without mediating entry, and validation of a viral receptor or co-receptor requires proof that the putative receptor is necessary for infection. This is not easy for HCV due to the absence of a robust cell culture system to amplify this virus. The recent development of HCVpp has allowed to further investigate the role of candidate receptors in virus entry. Among all the candidate receptors, only CD81 and SR-BI have been shown to play a direct role in HCVpp entry. Indeed, antibodies directed against CD81 or SR-BI as well as siRNA targeting these receptors reduce HCVpp infectivity. A soluble domain of CD81 is also able to compete with HCVpp infectivity. In addition, HDL, the natural ligands of SR-BI, are able to markedly enhance HCVpp entry. This HDL-mediated enhancement of HCVpp entry involves a complex interplay between SR-BI, HDL and HCV envelope glycoproteins. Interestingly, the involvement of CD81 and SR-BI in HCVpp entry seems to be conserved among all the HCV genotypes.

Interactions between viral envelope glycoproteins and potential receptors can have other consequences than virus entry. It has been shown that intracellular interaction between HCV envelope glycoproteins and CD81 can lead to secretion of exosomes containing E1 and E2 glycoproteins .Interestingly, a soluble form of E2 is also able to bind CD81 at the surface of natural killer cells, and this interaction inhibits cytotoxicity and cytokine production by these cells .Binding of a soluble form of E2 can also provide a co-stimulatory signal for T cells and up-regulate matrix metalloproteinase-2 in human hepatic stellate cells . It remains however to be determined whether HCV glycoprotein expressed in the context of native particles will have the same effects on cell functions.

HCVpp have also been used to investigate the role of other candidate receptors in HCV entry. HCVpp as well as native HCV particles have been shown to bind to cells expressing L-SIGN and DC-SIGN .Although these molecules are not expressed on hepatocytes, HCV interactions with L-SIGN and DC-SIGN may contribute to establishment or persistence of infection both by the capture and delivery of virus to the liver and by modulating dendritic cell functions as recently suggested .lastly, there is no clear evidence that the LDL receptor is a major receptor for HCVpp.

Interestingly, all the cells permissive to HCVpp co-express CD81 and SR-BI and are of liver origin .However, there are some other cell lines coexpressing CD81 and SR-BI that are non-permissive to infection and which are of non-hepatic origin. These results suggest that additional molecule(s), expressed in hepatic cells only, are necessary for HCV entry. Further investigations with HCVpp should allow identifying such molecule(s).

Specific Functional Regions of HCV Envelope Glycoproteins.

HCVpp has been used to investigate the functional role of some regions of HCV envelope glycoproteins in virus entry. Mutagenesis studies of the transmembrane domains of HCV envelope glycoproteins have shown that some mutations can affect the entry function of HCVpp without alteration in the biogenesis of E1E2 heterodimer and their incorporation into HCVpp (Ciczora Y, Callens N, Montpellier C, Bartosch B, Cosset FL, Op De Beeck A, Dubuisson J,[9]. This suggests that in addition to their role in E1E2 heterodimerization, the transmembrane domains of HCV glycoproteins might play a role in coordinating protein reorganization for the fusion process to occur[13].

Some Studies of E2-CD81 interactions and identification of epitopes recognized by antibodies that inhibit these interactions suggest that the CD81-binding region consists of discrete segments of E2 that are rearranged within the same domain during E2 folding .Besides this putative binding region, the hypervariable region 1 (HVR1) a 27-amino acid long segment found at the N-terminus of E2, has also been suggested to play a role in cell attachment. This region evolves rapidly in infected individuals, suggesting that it is under strong immune pressure. Although an HCV clone lacking HVR1 was shown to be infectious in chimpanzee, this mutant virus was attenuated, suggesting that HVR1 plays a facilitating role in HCV infectivity .In addition, deletion of HVR1 reduces HCVpp infectivity and abolishes HDL-mediated enhancement of HCVpp infectivity. Despite strong amino acid sequence variability related to strong pressure towards change, the chemicophysical properties and conformation of HVR1 are highly conserved, and HVR1 is a globally basic stretch, with basic residues located at specific sequence positions. Functional studies of HCVpp containing mutations in HVR1 indicate that infectivity increases with the number of basic residues in HVR1 (Callens N, Ciczora Y, Bartosch B, Vu-Dac N, Cosset FL, Pawlotsky JM, Penin F, Dubuisson J,[9]. an addition, a shift in position of some charged residues modulates infectivity. These data suggest that HVR1 is a region involved in interaction with a host molecule involved in HCV entry. However, it remains to be determined whether SR-BI or another putative receptor is involved in this interaction.

HCV envelope glycoproteins are highly glycosylated and some maturation of these glycans has been observed on HCV envelope glycoproteins associated with HCVpp; Mutation of some glycosylation sites in HCV envelope glycoproteins can reduce or abolish HCVpp infectivity without apparently affecting folding and incorporation of the glycoproteins into the particles N-linked glycans at position N2 and N4 of E2 have indeed been shown to be essential for the entry functions of HCV envelope glycoproteins. In addition, some other glycans (N2 of E1 and N5, N6 and N11 of E2) can also modulate HCVpp entry. Further studies will be necessary to determine whether these mutations affect receptor binding or the fusion properties of HCV envelope glycoproteins.

The Mechanisms of HCV Entry

Virus attachment to receptors initiates a series of events that lead to virus entry. For enveloped viruses, the entry process is controlled by viral surface glycoproteins that undergo triggered conformational changes from a metastable state to a lower energy state. This structural change leads to the exposure of a buried functional element, named the fusion peptide and is believed to provide the energy required for the merging of the lipid bilayers, So far, viral fusion proteins have been shown to fall into two different structural classes designated as class I and II Class I fusion proteins possess N-terminal or N-proximal fusion peptides, and they are synthesized as a precursor that is cleaved into two subunits by host cell proteases. In some cases (e.g., influenza HA[14], the two subunits remain associated through a disulfide bond, whereas in others (e.g. HIV Env) the two subunits remain associated through noncovalent interactions. The proteolytic processing event creates the metastable state of the fusion protein. In their native metastable conformation, class I fusion proteins form trimeric spikes at the surface of the virions with the fusion subunit being highly helical. Upon a fusion trigger

event (receptor binding at the cell surface or low pH in endosomes), the trimeric proteins transiently form an extended conformation allowing the hydrophobic fusion peptide to insert into the target membrane. Protein refolding leads then to the formation of very stable trimeric structures in which both the N-proximal fusion peptide and the C-proximal membrane anchor are juxtaposed at the same end to allow virus and cell membrane connection and hemifusion.

Class II viral fusion proteins have a completely different structure. They are predominantly non-helical, instead having a beta-sheet type structure; they are not cleaved during biosynthesis; and they possess an internal fusion peptide with a loop conformation. The proteins are oriented parallel to the membrane, and they have a three-domain architecture with domain I beginning at the N-terminus, domain II containing the internal fusion loop, and domain III being at the C-terminus. In addition, class II fusion proteins are synthesized as a complex with a second membrane glycoprotein (prM for flaviviruses; pE2 for alphaviruses). Newly synthesized E and prM proteins of the tick borne encephalitis virus associate to form noncovalent heterodimers that are incorporated into immature virions by budding into the ER lumen. These particles are then transported through the secretory pathway and shortly before release from the cell, the activation of the fusogenic potential occurs by the cleavage of the accessory protein prM by a cellular furin protease in the trans-Golgi network. After prM cleavage, the E protein exists as a metastable homodimers at the virion surface. The ectodomains of the dimers are orientated antiparallel to one another .The architecture of the alphavirus Semliki Forest virus spike is similar to that of tick borne encephalitis virus E, but in this case, the metastable oligomer is a heterodimer of the fusion protein E1 and the companion protein E2 with an associated small protein E3 .In addition, contrary to flaviviruses, alphaviruses have been shown to bud from the plasma membrane.

Comparison of flavivirus and hepacivirus envelope proteins. In the Flaviviridae family, class II fusion proteins (depicted in light grey) have been described in the flaviviruses (E protein of tick born encephalitis and dengue viruses).

Both alphaviruses and flaviviruses enter target cells by receptor-mediated endocytosis. The receptor recognition function is carried by the fusion protein itself for the flaviviruses (E) and by the companion protein (E2) for the alphaviruses. Exposure to the acidic pH of the endosomes triggers a major conformational change of the envelope involving dissociation of the native homodimer (for flaviviruses) or heterodimer (for alphaviruses) and the irreversible formation of homotrimers of the fusion proteins .

Based on its classification in the Flaviviridae family, HCV envelope has been proposed to contain a class II fusion protein .As found in the case of alphaviruses and flaviruses, HCVpp entry is pH dependent. These observations indicate that HCV may enter the cells through endocytosis[18]. The cell surface receptor(s) recognized by HCV should therefore traffic cell-bound virions to endosomal compartments. However, characterization of the route of HCV entry needs further investigations. Contrary to what is observed for other class II envelope proteins, there is no evidence that HCV envelope glycoproteins are matured by a cellular endoprotease during their transport through the secretory pathway .In addition, HCV envelope glycoproteins are highly glycosylated, whereas other describsed class II envelope proteins contain a very low number of glycans[12]. Interestingly, some of the glycans present on HCV envelope glycoproteins seem to be involved in controlling HCV entry.

There remains some controversy on the identity of HCV fusion protein. It has been proposed that E1 might be a good candidate because sequence analyses suggest that it might contain a putative fusion peptide in its ectodomain it can be said that potential structural homology with other class II fusion proteins suggests that E2 could be the fusion protein .Mutagenesis studies in the putative fusion peptides of the envelope glycoproteins associated with HCVpp as described for the

flavivirus envelope protein E should be helpful for further characterization of HCV fusion protein. In addition, a high-resolution structure of HCV envelope glycoproteins help understanding the fusion mechanism of the virus.

The Inhibition of HCV Envelope Glycoprotein Functions by Neutralizing Antibodies

As they are exposed at the surface of the virion, the envelope proteins are targets of neutralizing antibodies. These antibodies block a viral infection by inhibiting virion binding or membrane fusion. Understanding the mechanisms of neutralization needs therefore a good knowledge of the mechanism of entry. The role of neutralizing antibodies in HCV infection and disease progression remained unclear for a long time, largely because of the lack of assays to measure and quantify their activity. The old experiments showed that serum from a chronically infected patient could neutralize HCV infectivity in a chimpanzee model, giving evidence for antibody-mediated neutralization of HCV Neutralizing antibodies can be identified by their ability to prevent HCV replication in a lymphoid cell line.

Themost recent development of HCVpp offers the possibility to study HCV neutralization with defined sequences of HCV envelope glycoprotein so the use of HCVpp in neutralization studies is being validated .As determined with HCVpp, it seemed that the majority of chronically infected patients have cross-reactive neutralizing antibodies. In contrast, neutralizing antibodies have not been detected in several cases of acute resolving infection, and the detection of neutralizing antibodies in acutely infected individuals did not seem to be associated with viral clearance. However, in some patients a progressive emergence of a relatively strong neutralizing response in correlation with a decrease in viremia investigations on a large number of acutely infected patients will be necessary to determine the role of neutralizing antibodies in controlling HCV infections. Interestingly, it has been observed that HCVpp infectivity is enhanced by human sera, and this enhancement of infectivity can partly mask the presence of neutralizing antibodies also, HDL have been identified as the component responsible for serum-mediated enhancement of infectivity.

For a quite long time, the HVR1 sequence of E2 has been proposed to be a major target for neutralizing antibodies, data obtained with the HCVpp model indicate that neutralizing epitopes located outside of HVR1 also exist. Surprisingly, characterization of HCVpp with monoclonal antibodies has allowed to identify conformation-dependent and -independent neutralizing epitopes outside of HVR1. Conformation-dependent human monoclonal antibodies have also allowed to identify three immunogenic domains in E2 with neutralizing antibodies being restricted to two of these domains. Whether E2 domains identified with these monoclonal antibodies are similar to the antigenic structural and functional domains of the envelope protein E of the flaviviruses remains to be determined.

CONCLUSIONS

The Studies of the biogenesis of HCV envelope glycoproteins have shown the pivotal role of the transmembrane domains in the assembly of a noncovalent E1E2 heterodimer in the ER[5]. More recently, the development of the HCVpp model has allowed investigating the role of E1E2 heterodimer in virus entry. Functional regions in HCV envelope glycoproteins can now be identified and potential receptors can be validated. Entry is an essential step in the life cycle of a virus, which can potentially be blocked by neutralizing antibodies or antiviral drugs that target the envelope proteins of the virus. The Understanding of viral and cellular components involved in HCV invasion into the host cell, combined with a comprehension of the mechanisms that govern this process, should be open the possibility of developing new therapeutic approaches.

The development for Future Trends

The development of the HCVpp model has allowed to initiate the characterization of the entry function of HCV envelope glycoproteins. The use of HCVpp will continue to provide information on the role of HCV envelope glycoproteins in viral

entry. The recent development of a full-length clone that is infectious in cell culture provides new opportunities to study the functions of HCV envelope glycoproteins. A comparison of the properties of HCV envelope glycoproteins produced in HCVpp and in this infectious clone will be very useful to validate the data that have been generated during the past three years. Moreover, this infectious clone will allow for the first time to decipher the role of HCV envelope glycoproteins in virion assembly. Finally, obtaining a high-resolution structure of HCV envelope glycoproteins will also be necessary to understand the fusion mechanism of this virus.

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Patience is not about how long someone can wait.

It's about how well they behave while they wait.

Unknown