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Curr Opin Virol. Author manuscript; available in PMC 2013 February 1.

Published in final edited form as:
Curr Opin Virol. 2012 February ; 2(1): 14–19. doi:10.1016/j.coviro.2011.12.007.

Hepatitis C virus host cell entry

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Abstract

The hepatitis C virus (HCV) is a major medical problem with at least 130 million infected individuals worldwide. Over the last decade multiple host factors required for HCV cell entry have been identified, but a detailed understanding of their mechanistic interplay remains elusive. Nonetheless, recent advances in defining species-specific barriers of HCV transmission have allowed the identification of a minimal set of entry factors that are required for HCV infection of rodent cells and has culminated in an animal model that recapitulates HCV entry in vivo. A detailed understanding of the viral uptake pathway is imperative to define new drug targets allowing for more effective intervention against this devastating disease.

Introduction

HCV, the prototypical member of the *Hepacivirus* genus within the family *Flaviviridae,* is associated with the majority of newly diagnosed hepatocellular carcinomas in the United States [1,2] and is currently the leading cause of liver transplants worldwide [3]. An HCV vaccine is not available and current therapies are poorly effective [4,5] HCV-associated liver transplantation is only a palliative procedure due to universal infection of the graft after transplantation, often resulting in more rapid fibrosis progression and subsequent graft failure [6]. Even transient therapies inhibiting HCV cell entry could prevent graft infection and greatly improve the effectiveness of liver transplantation. Such therapeutic advances targeting this stage of the viral life cycle will require a much more solid understanding of HCV cell entry than is currently available. Here, we discuss recent advances in our understanding of molecules involved in viral uptake and their role in HCV tissue and species tropism, and we provide a putative mechanism of the HCV uptake pathway.

The HCV cell entry pathway

The HCV virion is comprised of a nucleocapsid core surrounded by a host-derived membrane bearing the E1 and E2 HCV glycoproteins, which mediate the majority of cell entry processes of the virion, including cell binding, endocytosis, and fusion in the low pH environment of early endosomes [7–15]. Both of these glycoproteins contain single transmembrane domains at their carboxy-termini that not only function to anchor them in

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membranes, but also contain ER-localization motifs[16,17]. The biogenesis of these proteins is closely linked and heavily influence by their coexpression (reviewed in [18]. The individual functions of these glycoproteins have not been completely delineated; while E2 has at least been shown to bind to some of the HCV cell surface entry factors, little is known about the roles of E1, mostly due to difficulties in purifying recombinant versions of this protein. Indeed, it has not been clearly defined which glycoprotein is responsible for mediating virion fusion.

The HCV entry process into human hepatocytes requires numerous host proteins including glycosaminoglycans (GAGs) [14,19], the low density lipoprotein receptor (LDL-R) [20–23], the high density lipoprotein receptor scavenger receptor class B type I (SR-BI, also known as CLA-1 and officially designated SCARB1) [24], tetraspanin CD81 [25], and two tight junction (TJ) proteins, claudin-1 (CLDN1) [26] and occludin (OCLN) [27,28] (Fig. 1). With so many cellular factors involved, it is clear the HCV does not follow the classic view of a virus that interacts with a single cellular receptor. It is likely that the incoming HCV virion uses the factors in a sequential manner to perform a range of functions along the cell entry process. GAGs may mediate initial, perhaps low affinity, interactions between the HCV virion and the cell surface [14,19]. LDLR may also be involved in cell binding, perhaps mediated by virion-associated apolipoproteins [23]. SR-BI antibody blocking studies indicate this protein plays a role in the binding of HCV to host cells, perhaps subsequent to GAG interactions, and is also involved in a post cell binding event [29–32]. The lipid uptake activity of SR-BI is also required for HCV cell entry, although this requirement may be indirect and reflect the involvement of cellular cholesterol levels to promote signalling events, HCV entry factor associations, and/or suitable target membranes for fusion (reviewed in [33]).

Antibody blocking studies reveal that CD81 plays a post-cell binding role in HCV cell entry [14]. Furthermore, although CD81 can interact with a soluble form of E2, it does not appear to be able to directly interact with mature HCV virions, suggesting that prior host cell interactions may alter the virion conformation to reveal CD81 binding sites. This hypothesis is supported by the isolation mutations within E1 and E2 that enhance the ability of HCV to utilize the normally poorly functional mouse CD81 ortholog [34]. These mutations appears to influence the glycoprotein conformation on the virion to promote a more open conformation than the wild type virion, as the mutant virus is more susceptible to E2 antibody neutralization, and displays higher affinity interactions with CD81 and reduced dependence on SR-BI and OCLN.

While HCV requires low pH for membrane fusion, the free virion is not inactivated by low pH treatment, which suggests that interactions between the virion and cellular factors primes the glycoproteins to respond to the low pH environment of the endosome. This hypothesis was supported by early experiments that showed that HCV can fuse directly at the cell surface when endosome acidification is blocked and cells are briefly washed with a low pH buffer. However this fusion event requires that cells with bound virions are incubated at 37°C prior to the low pH wash, suggesting that virus requires postbinding interactions with cellular factors to become fusogenic [10]. Recent studies suggest that CD81 may act to prime the fusogenic activity of the HCV glycoproteins [35]. The mouse CD81 adapted HCV mutant described above does not require incubation with cells at 37°C to fuse directly with the cell surface, suggesting it may already exhibit a more open or “pre-primed” conformation [34]. In addition, cells that lack CD81 do not support cell surface fusion, however this block can rescued by adding soluble recombinant CD81 protein [35]. Furthermore, simultaneous treatment of cell free virions with both a low pH and soluble CD81 impairs infectivity, likely by triggering irreversible release of the viral fusion peptide in the absence of a host membrane [35].

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CLDN1 likely plays a post-binding role in the HCV cell entry process, but exactly where and when this factor participates is not clear. Blocking experiments with antibodies directed against CLDN1 indicate this protein is used late in the entry process, around the time of virion internalization [26], or with kinetics of usage to overlap with those of CD81 [36,37]. A lack of reagents to directly block OCLN has prevented the examination of when this factor participates in the entry process.

Whether the HCV virion directs binds to either CLDN1 or OCLN has not been conclusively demonstrated, however if such an interaction is required for HCV cell entry it is not clear how such tight junction proteins become accessible to incoming virions, as the tight junction region is not accessible to HCV circulating in the bloodstream. The entry process of group B coxsackieviruses (CVB), which follows an elaborate route of polarized cell entry and has become a paradigm for this type of viral entry [38], may shed light on HCV cell entry. The primary CVB receptor, decay accelerating factor (DAF), is located on the luminal, or apical, surface of epithelial cells and directly available to incoming virus. A secondary entry factor, coxsackie-adenovirus-receptor (CAR), is TJ-associated and normally inaccessible to virions. However, CVB binding to DAF on the exposed cell surface stimulates intracellular c-Abl signaling that promotes the actin-dependent relocation of the virion/DAF complex to the tight junction region, where an interaction with CAR leads to virion endocytosis. Based on the expected localization of HCV entry factors, a similar stepwise entry cell entry pathway is conceivable for HCV, where virion interactions with basolaterally exposed factors mediate early binding events as well as post binding events that mediate the translocation of virions to CLDN1 and OCLN within normally inaccessible tight junctions. In support of this model, CD81 engagement, either by CD81 antibodies or soluble E2 protein, has been shown to activate Rho GTPases to mediate actin-dependant relocalization of such CD81 complexes to cell junction regions [39]. On the other hand, it remains possible that HCV does not even enter tight junction regions during entry. Indeed, evidence suggests that, in nonpolarized cells and partially polarized HepG2 cells, CLDN1 may interact with CD81 outside of cell junctions prior to infection and influence the cell entry process [40,41]. However, cell polarity can negatively regulate this interaction, as interactions between CLDN1 and both CD81 and the closely related tetraspanin CD9 was greatly reduced in highly polarized Caco-2 and MDCK cells [42]. Lupberger et al. recently reported that two receptor tyrosine kinases, epidermal growth factor receptor (EGFR) and ephrin receptor A2, are required for HCV cell entry, and possibly act by modulating the interaction between CD81 and CLDN1[43]. This scenario would be consistent with previous reports that EGFR activation influences CLDN1 localization and function [44].

The above evidence suggests the following hypothetical HCV cell entry model (Fig. 1). The HCV virion exists in a closed conformation prior to interaction with the host cell. Glycans and apoliproteins shield the majority of the HCV glycoproteins from immune surveillance, leaving only minimal GAG and SR-BI interacting domains exposed. These interactions result in exposure of the CD81 binding site, and interaction with this protein in turn primes the low-pH fusion activity of the virion. However, according to this model, fusogenic virus must still translocate to the tight junctions in order to be endocytosed. This could occur through an association between CD81 and CLDN1, either pre-existing or stimulated by virion-mediated activation of receptor tyrosine kinase signalling, and subsequent actin-dependant transport of the virion into the tight junction region, where endocytosis may be mediated by interactions with OCLN. While this may be an attractive model, fully defining this process and how cell polarity influences the HCV cell entry process will likely require additional cell systems that are more polarized than those currently available and that support robust infection with cell culture grown HCV.

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HCV entry contributes to tissue and species tropism

Several clinical observations have fostered speculations on the existence of putative extrahepatic sites of HCV infection. For example, HCV infected patients frequently show encephalopathy and neuropsychiatric disorders suggesting an involvement of the central nervous system [45]. Indeed, it has been shown that HCV can enter human peripheral neuro-blastoma and -epithelioma cells in vitro [46,47] and HCV RNA has been detected brain tissue from HCV infected individuals [48]. However, analysis of post mortem collected specimens is challenging because of the high propensity of sample contamination and direct evidence of active HCV RNA replication in these tissues has not been provided. In addition, chronic HCV carriers frequently exhibit signs of various lymphoproliferative disorders. HCV RNA has indeed been observed in association with hematopoietically derived cells including B and T lymphocytes, monocytes, and dendritic cells. This could be interpreted as the reflection of a productive entry event. However, it has been demonstrated that at least cell-culture produced HCV cannot enter any subset of peripheral blood mononuclear cells [49], suggesting that HCV particles and/or RNA may adhere to these cells but not efficiently enter. HCV protein has also been found in epithelial cells of intestinal specimens collected from HCV infected individuals [50]. In cell culture, the colorectal adenocarcinoma Caco-2 cell line supports viral uptake [51]. While these data are intriguing it remains to be unambigously demonstrated that HCV can enter non-parenchymal cells in vivo and, more importantly, that these putative extrahepatic sites have any clinical relevance.

Determinants governing HCV tissue tropism are poorly understood. While all stages of the HCV life cycle appear to be specifically attuned to the hepatocyte environment, host cell entry appears to play an important, and at least the earliest, role in governing the HCV hepatotropism. Although the minimal set of HCV cell entry factors are present in various nonliver mammalian tissues, suboptimal expression levels and/or an inadequate subcellular localization may limit their ability to promote HCV cell entry. CD81 is ubiquitously expressed in almost all nucleated cells. OCLN is present in all tight junction complexes present in polarized cell layers. However, the OCLN transcript is subject to post-transcriptional splicing creating considerable mRNA diversity. These alternatively spliced forms are differentially abundant across tissues and have different capacities to support viral entry [52], which might contribute to HCV tissue tropism and possibly modify the outcome of HCV infection in humans. CLDN1 and SR-BI are present in multiple tissues but the combination of both at high levels is only present in the liver, which has led to the hypothesis that these two entry factors primarily control HCV tissue specificity at the entry level.

While the abundance of entry factors in different tissues likely affects viral tropism, negative regulators of entry expressed in non-hepatic cells may also contribute to restricting HCV tissue tropism. Immunoglobulin superfamily member 8 (Igsf8), also known as EWI-2int or CD316, binds to CD81 and has recently been demonstrated to function as a dominant negative inhibitor of HCV entry in vitro [53]. Igsf8 is not expressed in hepatocytes, but is present in several non-hepatic cell lines and is abundant in B and T cells as well as the brain. Ectopic expression of Igsf8 in a hepatoma cell line (Huh-7) blocks HCV entry by inhibiting the interaction between CD81 and the viral glycoproteins. This finding suggests that, in addition to the presence of specific uptake factors in hepatocytes, the lack of a specific inhibitor may contribute to the hepatotropism of HCV. It is also conceivable that HCV can potentially enter other non-hepatic cell types, but is unable to efficiently establish RNA replication due to the lack of liver specific host factors that are required for HCV replication, such as microRNA-122 [54].

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HCV has a narrow host range infecting only humans and chimpanzees. Although the basis of this highly restricted species tropism is incompletely understood, analyzing the viral life cycle in mouse cells and in mice has helped to shed some light on species-specific barriers of HCV transmission. Although all four proteins are required for efficient HCV cellentry into mouse cells, the murine orthologs of SR-BI and CLDN1 function as efficiently as the human verions [27]. Conversely, human CD81 and OCLN define the species barrier during the viral uptake process. Several residues within the second extracellular loop of CD81 have been demonstrated to be involved in binding of HCV E2, and they are not or only partially conserved across species [55,56]. The regions in OCLN conferring species tropism have been identified in the second extracellular loop of this protein [27,57]. It was recently demonstrated that mice can indeed be engineered to take up HCV in a viral glycoprotein-dependent fashion by simply expressing human CD81 and OCLN [58]. While this new genetically model holds great promise to dissect HCV entry in vivo it remains to be determined whether mice can be coaxed to support the entire viral life cycle. Downstream of viral entry, HCV RNA replication is extremely inefficient in mouse cells, thereby creating an additional barrier in species tropism. However, using a transcomplementation-based system, mouse hepatoma cell lines have been shown to be capable of supporting assembly and release of infectious HCV particles [59]. This result provides hope that, if the block to HCV RNA replication in mouse cells can be overcome, the ambitious goal of generating a tractable HCV mouse model can indeed be achieved. Such a mouse model would open up unprecedented opportunities to dissect viral pathogenesis and to evaluate drug and vaccine candidates [60].

Conclusions

HCV entry is a highly complex process that has yet has to be fully characterized. Multiple cellular and viral factors have been implicated in the viral uptake process, but their exact role and the kinetics of their engagement remain incompletely understood. HCV entry appears to contribute to both tissue and species tropism. Dissection of viral uptake has not only been instrumental to engineering new animal models for HCV infection, but will continue to shed light on new and possibly more suitable antiviral drug targets.

Acknowledgments

We wish to thank Carina Storrs for careful editing of this manuscript. This work was supported in part by award number RC1DK087193 (A.P.) from the National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases, Infectious Disease Society of America Astella Young Investigator Award (A.P.), award number R00AI077800 from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (M.J.E.), and the Pew Charitable Funds (M.J.E.).

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Highlights

- HCV utilizes a complex set of host factors to facilitate uptake into its host cell
- Detailed mechanism of HCV entry remains incompletely understood
- Human CD81 and OCLN are required for HCV infection of mouse cells and mice
- Blocking HCV entry constitutes an attractive therapeutic approach in particular to prevent universal graft re-infection after liver transplantation

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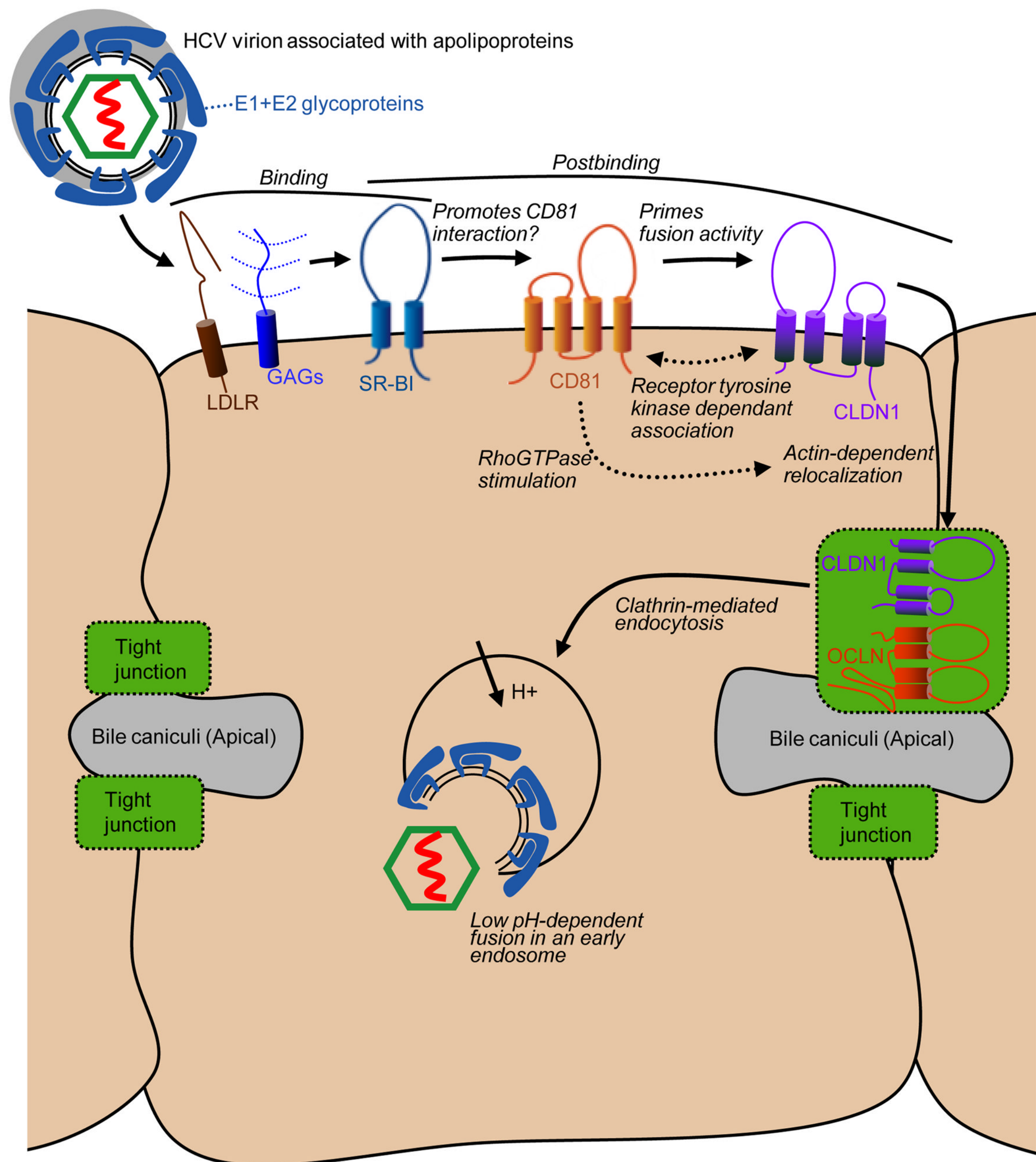

Figure 1.

Potential route of HCV entry into polarized hepatocytes.

E1 and E2 = HCV envelope glycoproteins 1 and 2, LDLR = low density lipoprotein receptor, GAGs = glycosaminoglycans, SR-BI = scavenger receptor type B class 1, CLDN1 = claudin 1, and OCLN = occludin.