Virology 479-480 (2015) 221–233

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Advances in experimental systems to study hepatitis C virus *in vitro* and *in vivo*

Maria Teresa Catanese [a,*], Marcus Dorner [b,c,**]

[a] Division of Infectious Diseases, King's College London, London, United Kingdom
[b] Section of Virology, Imperial College London, London, United Kingdom
[c] Section of Hepatology, Imperial College London, London, United Kingdom

ARTICLE INFO

Article history:
Received 17 December 2014
Returned to author for revisions
4 February 2015
Accepted 3 March 2015
Available online 4 April 2015

Keywords:
Hepatitis C virus
Model systems
Replicon
Pseudoparticles
Soluble E2
Cell culture-derived HCV
Hepatocye-based models
Animal models

ABSTRACT

Hepatitis C virus (HCV) represents a global health concern affecting over 185 million people worldwide. Chronic HCV infection causes liver fibrosis and cirrhosis and is the leading indication for liver transplantation. Recent advances in the field of direct-acting antiviral drugs (DAAs) promise a cure for HCV in over 90% of cases that will get access to these expensive treatments. Nevertheless, the lack of a protective vaccine and likely emergence of drug-resistant viral variants call for further studies of HCV biology. With chimpanzees being for a long time the only non-human *in vivo* model of HCV infection, strong efforts were put into establishing *in vitro* experimental systems. The initial models only enabled to study specific aspects of the HCV life cycle, such as viral replication with the subgenomic replicon and entry using HCV pseudotyped particles (HCVpp). Subsequent development of protocols to grow infectious HCV particles in cell-culture (HCVcc) ignited investigations on the full cycle of HCV infection and the virus–host interactions required for virus propagation.

More recently, small animal models permissive to HCV were generated that allowed *in vivo* testing of novel antiviral therapies as well as vaccine candidates. This review provides an overview of the currently available *in vitro* and *in vivo* experimental systems to study HCV biology. Particular emphasis is given to how these model systems furthered our understanding of virus–host interactions, viral pathogenesis and immunological responses to HCV infection, as well as drug and vaccine development.

Contents

* Correspondence to: King's College London, School of Medicine. Department of Infectious Diseases, 2nd Floor, Borough Wing, Guy's Hospital, London SE1 9RT, United Kingdom. Tel.: +44 20 7188 3075.
** Correspondence to: Imperial College London, Department of Medicine, Section of Virology & Section of Hepatology, Medical School, Praed Street, London W2 1PG, United Kingdom. Tel.: +44 20 7594 5037.
E-mail addresses: maria.catanese@kcl.ac.uk (M.T. Catanese), m.dorner@imperial.ac.uk (M. Dorner).

http://dx.doi.org/10.1016/j.virol.2015.03.014
0042-6822/

Introduction

Hepatitis C virus (HCV) is a (+)-sense, single-stranded RNA virus of the *Flaviviridae* family that infects humans and chimpanzees via direct blood contact (e.g. intravenous drug use or contaminated blood supplies) and targets primarily hepatocytes. Current estimates indicate that more than 185 million people (approximately 3% of the world's population) have been infected with the virus (Mohd Hanafiah et al., 2013). HCV stains are classified into 7 genotypes based on phylogenetic and sequence analyses (>30% divergence at nucleotide level). Genotypes 1 and 3 are the most prevalent worldwide, accounting together for 137.7 million cases, while genotypes 4 and 5 are mostly distributed in lower-income countries (Messina et al., 2015). More than 70% of the individuals contracting HCV progresses on developing a chronic infection that often remains asymptomatic for decades. This suggests that the virus has evolved successful strategies to overcome antiviral cellular defences and coexist with its host. Ultimately, however, chronic HCV infection leads to the development of liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease.

For twenty years, HCV has been treated with interferon (IFN)-based regimens that present important side effects and are ineffective in at least 50% of cases (Manns et al., 2006). Exciting progress on the HCV therapy front were made in 2011, when the first direct-acting antivirals (DAAs) received approval from the US Food and Drug Administration. DAAs target the NS3/4A protease, the NS5A protein, or the NS5B polymerase and when used in appropriate combinations they achieve very high cure rates, with sustained virological responses (SVR) above 90% of cases (Aghemo and De Francesco, 2013).

The recent successes stem from two decades of efforts in building the necessary experimental systems that led to crucial breakthroughs on various aspects of HCV infection. The unique species and tissue tropism of HCV and the difficulty of generating suitable cell culture and small animal models has posed a great challenge to investigate HCV biology and the contribution of host responses to HCV persistence or clearance. On the 25th anniversary of the discovery of HCV, we can look back to assess the progress made and acknowledge that much has been learned about this pathogen. We can now study all the steps of its life cycle *in vitro* and in small animal models, dissect the virus–host interactions that mediate infection, and can begin to understand how the host contributes to establishment of chronicity, and progression of HCV-mediated disease.

HCV represents a peculiar and puzzling example of a virion, in that it circulates in the bloodstream of the infected individuals associated with host lipoproteins in a complex structure called the lipoviral particle (LVP) (Andre et al., 2002). HCV reaches its target organ, the liver, by crossing the fenestrated endothelium and interacting initially with attachment factors like heparan sulfate proteoglycans (HSPGs) and receptors such as the tetraspanin CD81 on the basolateral side of hepatocytes (Barth et al., 2003; Pileri et al., 1998). The interaction of HCV virions with endogenous lipoproteins represents an efficient mode of entry into the liver cells of a new host. In fact, the list of HCV receptors includes two lipoprotein receptors: the scavenger receptor class B, type I (SR-BI)(Scarselli et al., 2002a) and the low-density lipoprotein receptor (LDLR)(Agnello et al., 1999). The model of HCV entry has become increasingly complicated with six novel entry factors identified over the last eight years only: the tight junction molecules claudin-1 (CLDN1) (Evans et al., 2007) and occludin (OCLN) (Ploss et al., 2009), the epidermal growth factor receptor (EGFR) and the ephrin type-A receptor 2 (EphA2) (Lupberger et al., 2011), the cholesterol uptake molecule Niemann–Pick C1-like 1 (NPC1L1) (Sainz et al., 2012) and the transferrin receptor 1 (TFR1) (Martin and Uprichard, 2013). So far, direct proof of interaction with the virus exists for CD81 and SR-BI only, which were identified as candidate receptors precisely for their ability to bind the HCV envelope glycoprotein, E2 (Pileri et al., 1998; Scarselli et al., 2002a). Because of the spatial segregation of HCV receptors into different subcellular domains, active transport towards the apical side is thought to occur, bringing the virus in close proximity of additional entry factors, such as CLDN1 and OCLN. EGFR-mediated signaling via Ras, as well as Rho GTPase, have been proposed to induce lateral movement of CD81-bound virions to the cell-cell contact sites where CD81 would then engage CLDN1. HCV internalization then occurs by clathrin-mediated endocytosis. Finally, delivery of the virus to Rab5a-positive early endosomes should provide the acidic environment necessary to induce fusion (Zeisel et al., 2013). Fusion of the viral envelope with the endosome subsequently releases the viral capsid into the cytoplasm where the viral RNA genome is immediately translated to produce the HCV polyprotein. The concerted action of host- and viral-encoded proteases leads to the production of ten viral proteins. The first three proteins are structural components of the virion: Core, which forms the nucleocapsid, and the viral envelope glycoproteins, E1 and E2. The non-structural (NS) proteins include the ion channel p7, the auto-protease NS2, the protease/helicase NS3/4A, NS4B, NS5A and the viral RNA-dependent RNA polymerase NS5B. The replicase complexes assemble on the endoplasmatic reticulum (ER), forming invaginations of ER membranes called the “membranous web”. It is within these nuclease-resistant compartments that HCV RNA is transcribed. The resulting progeny HCV RNA is then packaged into viral capsids, decorated with viral glycoproteins and secreted from the cells via the secretory pathway. Although the exact mechanism of LVP formation is not fully elucidated, at some point during the maturation process, HCV virions acquire endogenous lipids and a thick shell of host-derived apolipoproteins coating the viral envelope that presumably aid both release and entry of the virus. In fact, the secretion of nascent virions from infected cells is tightly linked to the very-low density lipoprotein (VLDL) biosynthetic pathway, with the strongest evidence being that apoE is essential for particle release (Bartenschlager et al., 2011).

In addition to infecting hepatocytes from the bloodstream, an entry route that is termed “cell-free”, HCV particles can be directly transmitted between neighboring cells, so called “cell-to-cell” spread. The lateral movement of HCV without diffusion through the extracellular environment could facilitate viral dissemination, especially since two of its co-receptors, CLDN1 and OCLN, are recruited to the intercellular interface. The extent to which cell-free versus cell-to-cell transmission contribute to HCV persistence is unknown, but the latter route provides potential advantages in terms of infection efficiency and immune evasion and, as such, may be more relevant for maintaining infection over the course of the years.

In this review, we highlight the main *in vitro* and *in vivo* experimental systems that have enabled studies of HCV so far and provide an outlook on further developments that can improve our ability to understand and treat this virus.

In vitro systems

Since the discovery of HCV in 1989 (Choo et al., 1989), the lack of a cell culture system for the production of infectious HCV virions

represented a major obstacle to study this pathogen and to devise strategies for prophylactic and therapeutic interventions. Historically, patient-derived HCV isolates failed to initiate productive infection in cell culture. In 1997, the development of HCV molecular clones that were infectious in chimpanzees was an important initial breakthrough, however these viral genomes did not produce viral particles in cell culture (Kolykhalov et al., 1997; Yanagi et al., 1997). Soon after, different surrogate systems were developed to dissect individual steps of HCV life cycle. These include: the subgenomic replicon, the soluble E2 protein (sE2) and the HCV pseudo particles (HCVpp) (Fig. 1).

The HCV subgenomic replicon system

In 1999, Lohmann et al. defined the minimal set of HCV proteins required to initiate and maintain HCV replication in human cells (Lohmann et al., 1999). The HCV NS3/4A protease, NS4B, involved in forming the membranous web harboring the replicase complex (Konan et al., 2003), the NS5A protein as well as the RNA-dependent RNA polymerase NS5B are indispensable for HCV replication (Fig. 1b). The subgenomic replicon is based on a bicistronic RNA construct, which drives an antibiotic resistance gene from the HCV internal ribosomal entry site (IRES), while the HCV non-structural proteins (NS3-NS5B) are expressed from an encephalomyocarditis virus (EMCV) IRES. Transfection of *in vitro*-transcribed RNA into Huh-7 cells followed by antibiotic selection results in the production of cell lines stably replicating HCV at low levels. No infectious particles are released in this system, however, due to the lack of structural proteins. Several adaptive mutations are selected in the replicative process, ultimately leading to the emergence of variants with higher replicative fitness (Abe et al., 2007; Blight et al., 2000; Bukh et al., 2002; Ikeda et al., 2002; Krieger et al., 2001; Liang et al., 2005; Lohmann et al., 2001; Maekawa et al., 2004; Yi and Lemon, 2004). These mutations typically cluster in distinct regions of the N-terminus of the NS3 helicase (Krieger et al., 2001; Liefhebber et al., 2010), in NS4B (Welker et al., 2012) and in NS5A (Krieger et al., 2001; Shimakami et al., 2004; Tong and Malcolm, 2006). Subgenomic replicons have been described for HCV genotypes 1a (H77(Blight et al., 2003)), 1b (Con1 (Ikeda et al., 2002), HCV-N (Ikeda et al., 2002), HCV-BK (Graziani and Paonessa, 2004), HCV-J4 (Gates et al., 2004), 1B-2/HCV-O (Kato et al., 2003) and 1B-1/M1LE (Kato et al., 2003)), 2a (JFH1 (Targett-Adams and McLauchlan, 2005)), 3 (S52 (Saeed et al., 2013, 2012)), 4 (ED43 (Peng et al., 2012; Saeed et al., 2012)), 5a (Wose Kinge et al., 2014) and 6a (Yu et al., 2014) (Table 1) and have greatly contributed to the development of DAA currently licensed or in development. In addition to drug-selectable subgenomic replicon systems, several reporter-encoding versions have been described (King et al., 2002; Krieger et al., 2001), greatly simplifying the evaluation of antiviral drug potency. To date, the greatest advantage of the HCV subgenomic replicon over the HCVcc system is its non-restrictive nature in terms of HCV genotypes that can be studied.

Soluble E2 ectodomain (sE2)

The first tool used to search for HCV receptors was a soluble form of the HCV glycoprotein E2 (sE2), which is devoid of its transmembrane region, leading to secretion of the ectodomain (Fig. 1c). Truncations are usually made at amino acid 661 (Michalak et al., 1997). The first two HCV entry receptors, the tetraspanin CD81 and the scavenger receptor SR-BI, were identified in screenings based on sE2 binding to human hepatoma cell lines (Pileri et al., 1998; Scarselli et al., 2002b). Although it is not produced in complex with E1, sE2 was shown to retain proper folding as indicated by its ability

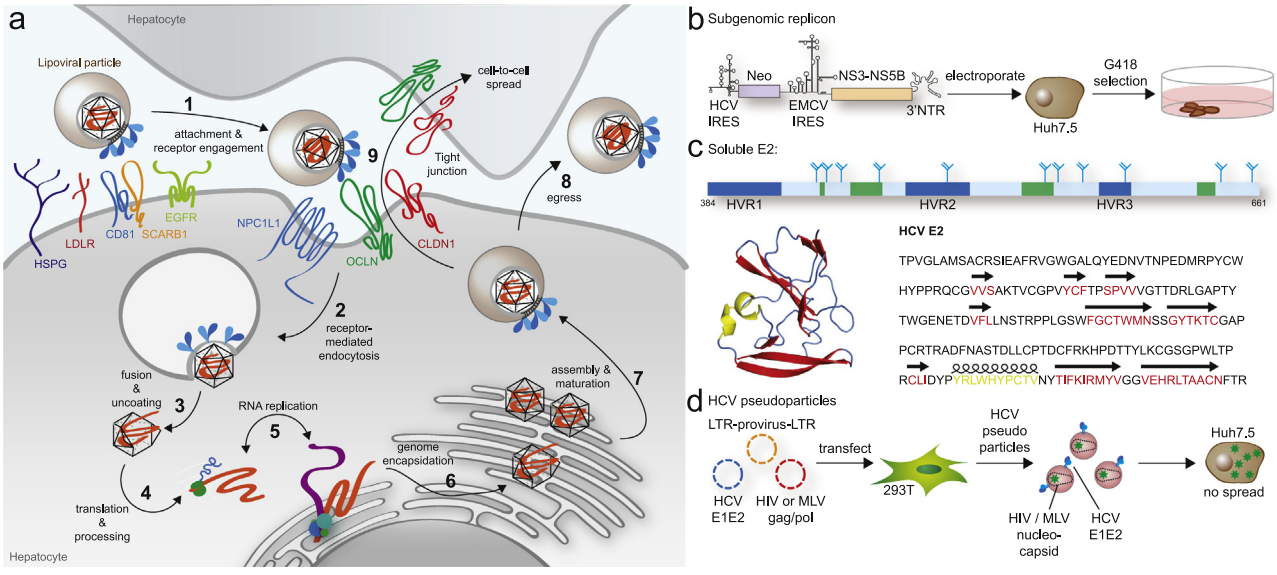


Fig. 1. HCV lifecycle and surrogate models systems to study HCV biology. **(a)** Overview of the HCV life cycle. The virus interacts with target cells by binding to attachment factors and receptors **(1)** that mediate its internalization through clathrin-mediated endocytosis **(2)**. Fusion of the viral and cellular membranes, triggered by the low pH of the endocytic compartment, leads to release of the positive-strand RNA genome (+) into the cytoplasm **(3)**. The HCV genome is translated by host ribosomes to generate a polyprotein precursor that is processed by both host and viral proteases to give rise to 10 viral proteins **(4)**. The non-structural (NS) proteins form a replication complex, which produces multiple copies of the HCV RNA genome via a minus-strand replicative intermediate **(5)**. Progeny RNA can either re-enter the lifecycle, to produce more HCV proteins or be packaged into nucleocapsids **(6)**. Budding of the nucleocapsid into the endoplasmic reticulum (ER) leads to the acquisition of the viral envelope as well as E1 and E2 glycoproteins. Along the secretory pathway, virions undergo maturation and associate with endogenous lipoproteins to form lipoviral particles **(7)**, which are released from the cell **(8)** or transmitted directly through tight junctions to infect neighboring cells by cell-to-cell spread **(9)**. **(b–d)** Surrogate model systems to study individual steps of HCV life cycle. **(b)** The HCV replicon encodes the selectable marker neomycin (neo) under control of the HCV IRES in the first cistron and the HCV replicase proteins (NS3-NS5B) under control of an heterologous IRES from EMCV in the second cistron, allowing for viral replication in hepatoma cells after HCV RNA delivery by electroporation. Replication of HCV RNA leads to the concomitant production of the selectable marker and enables selection of cells harboring replicating subgenomic HCV RNA by G418 treatment. **(c)** Schematic representation of the soluble ectodomain of the HCV glycoprotein E2 (sE2). The hypervariable regions (HVR) 1, 2 and 3 are indicated. The numbers correspond to the positions in the HCV polyprotein of the strain H77, genotype 1a. 3D structure of sE2 (courtesy of Prof J. Marcotrigiano and Dr M. Miller, Rutgers University). **(d)** HCV pseudoparticles (HCVpp) consist of unmodified HCV envelope glycoproteins assembled onto retroviral core particles and are produced by co-transfection of 293 T cells with expression vectors encoding (1) HCV E1/E2, (2) HIV Gag-Pol proteins and (3) a retroviral genome encoding a reporter gene such as luciferase or green fluorescent protein to allow quantitative measurement of HCV entry into target cells.

Table 1
Summary of subgenomic replicon and HCVcc systems for different HCV genotypes.

Strain	Genotype	Replicon	HCVcc
H77	1a	Blight et al. (2003), Grobler et al. (2003), Tscherne et al. (2006)	Pietschmann et al. (2006)[a], McMullan et al. (2007)[a], Li et al. (2015)
H77c	1a	Yi et al. (2004)	Russell et al. (2009), Yi et al. (2007), Scheel et al. (2009)[a], Yi et al. (2006)
HCV-1	1a	Lanford et al. (2006)	–
Con1	1b	Lohmann et al. (1999), Blight et al. (2000), Guo et al. (2001), Krieger et al. (2001), Lohmann et al. (2003)	Pietschmann et al. (2009)[a], Gottwein et al. (2009)[a], Kaul et al. (2007)[a], Pietschmann et al. (2006)[a]
HCV-N	1b	Guo et al. (2001), Ikeda et al. (2002), Yi et al. (2002)	–
HCV-BK	1b	Grobler et al. (2003)	–
HCV-J4	1b	Maekawa et al. (2004)	–
O	1b	Abe et al. (2007), Ikeda et al. (2005), Kato et al. (2003)	–
AH1	1b	Mori et al. (2008)	–
NC1	1b	Date et al. (2012)	Date et al. (2012)[a]
BHCV1	1b	–	Koutsoudakis et al. (2011)[a]
TNcc	1b	–	Li et al. (2012)
JFH1	2a	Kato et al. (2003)	Wakita et al. (2005), Zhong et al. (2005), Delagrange et al. (2007), Kang et al. (2009), Zhong et al. (2006) Kaul et al. (2007), Russel et al. (2008),
J6/JF-H1, Jc1	2a	–	Lindenbach et al. (2005)[a], Pietschmann et al. (2006)[a], Bungyoku et al. (2009)[a]
HCV-J8	2b	Saeed et al. (2012)	Gottwein et al. (2007)[a], Ramirez et al. (2014)
S52	3a	–	Gottwein et al. (2007)[a], Gottwein et al. (2009)[a]
452	3a	Saeed et al. (2012), Peng et al. (2012)	Kaul et al. (2007)[a], Pietschmann et al., (2006)[a]
ED43	4a	Kinge et al. (2014)	Scheel et al. (2008)[a]
SA1	5a	–	–
SA13	5a	Yu et al. (2014)	Gottwein et al. (2007)[a], Jensen et al. (2008)[a]
HK6a	6a	–	Gottwein et al. (2007)[a]
QC69	7a		Gottwein et al. (2007)[a]

[a] Chimeric constructs.

to interact with CD81, to block HCVcc entry and to bind antibodies from HCV infected patients (Whidby et al., 2009). Recently, the structure of E2 ectodomain in complex to two different Fabs was solved, revealing a globular shape with IgG-like folds and disordered flexible regions (Khan et al., 2014; Kong et al., 2013). Interestingly, this core region of E2 does not contain an obvious fusion peptide, nor does it undergo significant conformational rearrangements upon incubation at low pH, ruling out a direct involvement of E2 in the fusion process. These findings represent exciting insights into the mechanisms of HCV entry and will aid vaccine development.

HCV pseudo particles (HCVpp)

A major breakthrough for investigating the process of HCV entry was the development of pseudoparticles (HCVpp), which consist of defective retroviral particles expressing HCV envelope glycoproteins on their surface (Bartosch et al., 2003; Hsu et al., 2003). HCVpp are produced by co-transfecting 293T cells with three expression plasmids encoding: (1) the HCV glycoproteins E1 and E2, (2) Gag–Pol proteins of human immunodeficiency virus (HIV) or murine leukemia virus (MLV) and (3) a retroviral genome containing a reporter gene such as luciferase or GFP to allow quantitative measurement of HCV entry into target cells (Fig. 1d). This system was instrumental to study the role of E1 and E2 glycoproteins in HCV entry, to identify and validate candidate HCV attachment factors and receptors and to shed light on the mechanisms of virus internalization (Zeisel et al., 2013). Furthermore, the generation of HCVpp expressing patient-derived viral glycoprotein sequences enabled studies on the neutralizing properties of E1/E2-specific antibodies and led to the identification of cross-neutralizing epitopes (Tarr et al., 2006). HCVpp offer an opportunity for investigating the step of virus entry uncoupled from downstream events, facilitating for instance studies in cells that are not permissive to HCV replication. A caveat of this model is that it does not recapitulate the association of HCV with circulating lipoproteins. This is due to the fact that HCVpp are generated in a kidney cell line, which does not synthesize lipoproteins, and that these particles assemble similar to retroviruses. Nevertheless, the comparison between HCVpp and HCVcc can provide insights into key aspects of virion morphogenesis that impact on HCV entry and neutralization.

Model systems to study the complete HCV life cycle

Cell culture derived HCV (HCVcc)

HCV infection of cells with patient-derived isolates does not result in productive infection. The reasons for that still remain obscure. So far, the only exception is a genotype 2a HCV isolate from a Japanese patient with fulminant HCV-associated hepatitis (JFH1), which is able to initiate low-level infection in Huh-7 cells, paving the way to studying the complete HCV life cycle in cell culture (Lindenbach et al., 2005; Wakita et al., 2005; Zhong et al., 2005). The ability to propagate HCV *in vitro* has greatly benefitted our understanding of HCV biology. For example, the HCVcc system led to the identification of novel entry factors, such as NPC1L1 (Sainz et al., 2012), EGFR and EphA2 (Lupberger et al., 2011). This model system enabled for the first time studies of HCV assembly and egress (Hueging et al., 2014; Mancone et al., 2011; Nag et al., 2012), the characterization of biophysical and ultrastructural properties of the HCV virion (Catanese et al., 2013b; Gastaminza et al., 2010; Merz et al., 2011; Nielsen et al., 2006). Importantly, HCVcc was infectious in chimpanzees and human liver-chimeric mice (Lindenbach et al., 2006), facilitating the evaluation of vaccine candidates *in vivo* (de Jong et al., 2014; Law et al., 2008; Prentoe et al., 2011) and the development of genetically humanized mice (Dorner et al., 2013, 2011).

Combination of the original JFH1 NS3-NS5B region with the C-NS2 region from another genotype 2a isolate (J6) resulted in intragenotypic chimeras with enhanced replicative fitness (Lohmann

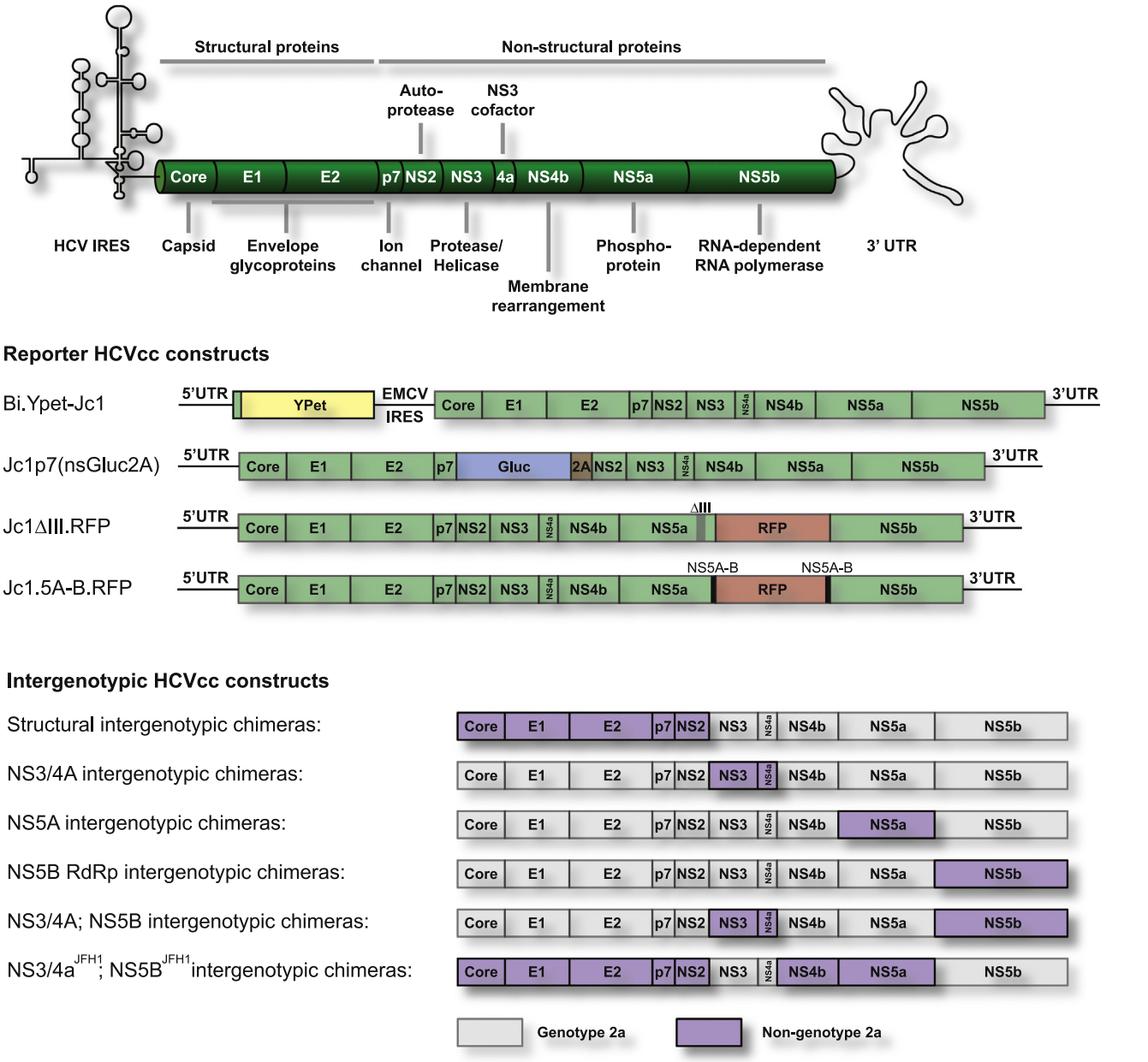
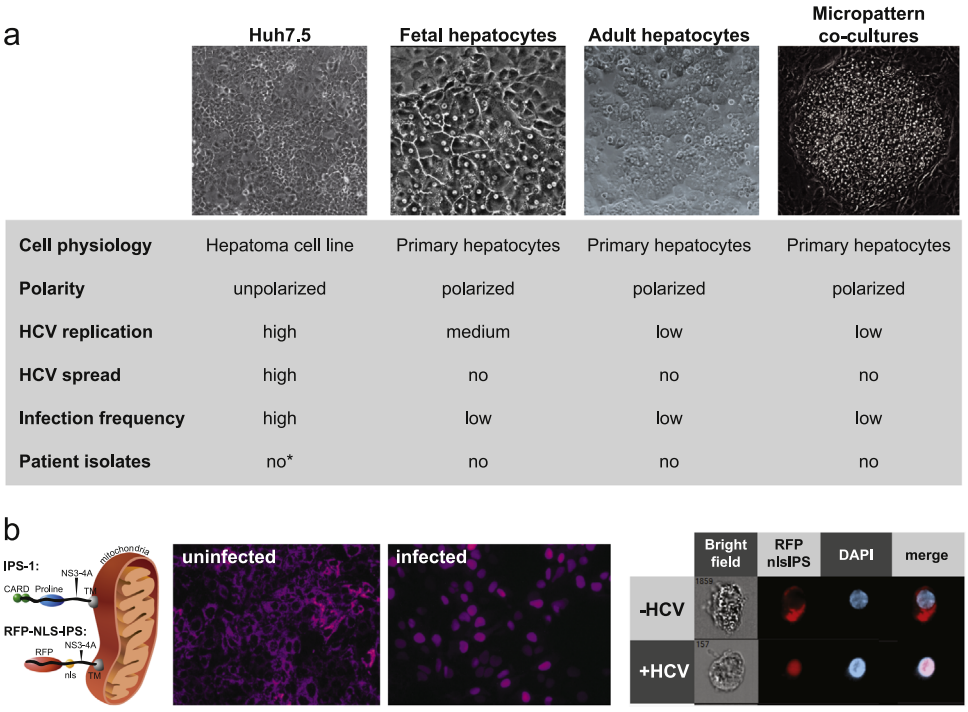


Fig. 2. Cell culture-derived HCV. **(a)** Structure of the HCV genome. Functions of the individual viral proteins are indicated. **(b)** HCVcc reporter viruses encode fluorescent (e.g. Ypet, RFP) or luminescent proteins (Gluc) to facilitate detection of HCV-infected cells. **(c)** Intergenotypic chimeras facilitating the study of individual HCV proteins (i.e. structural proteins C-NS2, NS3/4A, NS5A, NS5B or NS3/4A and NS5B) of different genotypes on the backbone of the replication-competent genotype 2a HCVcc system. Core to NS2 and NS5A intergenotypic chimeras are available for genotypes 1–7 whereas all other intergenotypic chimeras are restricted to individual genotypes. All intergenotypic viral genomes require *in vitro* selection and acquire adaptive mutations that increase viral replication.

et al., 2003). The resulting J6/JFH1 (Lindenbach et al., 2006) (genotype breakpoint between NS2 and NS3) or Jc1 (Pietschmann et al., 2006) (genotype breakpoint within NS2) were characterized by enhanced virus replication, spread and particle production (Fig. 2). In addition to various intergenotypic chimeras, HCVcc has also been serially passaged to select for viral clones with enhanced replication in hepatoma cells (Bungyoku et al., 2009; Catanese et al., 2013a; Date et al., 2012; Liu et al., 2012). The resulting titer-enhancing mutations are scattered throughout the HCV genome, suggesting that various mutations can promote viral propagation *in vitro*. Different mechanisms were identified to be responsible for this effect, ranging from enhanced entry, replication, to particle release and cell-to-cell spread

Several reporter genomes have been created, allowing convenient and dynamic measurements of viral replication using luminescent or fluorescent readouts. Reporter genes were either fused to viral proteins (i.e. NS5A) (Gottwein et al., 2011a, 2011b) or added as additional proteins (Andrus et al., 2011; Liu et al., 2012, 2014a; Chan et al., 2013; Horwitz et al., 2013), that are cleaved off the viral polyprotein by the NS3/4A protease (i.e. by duplicating the NS5A-B cleavage site) (Fig. 2). Since most DAAs have been developed using genotype 1 subgenomic replicon systems Bukh et al. developed several intergenotypic chimeras by replacing the C-NS2 region (Gottwein et al., 2007, 2010, 2009; Russell et al., 2009; Scheel et al., 2008; Jensen et al., 2008), the NS3/4A protease (Gottwein et al., 2013, 2011b), NS5A (Scheel et al., 2012, 2011; Gottwein et al., 2013; Galli et al., 2013; Li et al., 2014a) and/or NS5B (Herlihy et al., 2008) or all but NS3/4a and NS5b (Li et al., 2014a) of the J6/JFH1 genotype 2a backbone with other viral genotypes (1–7) to evaluate their potency against other HCV genotypes. This usually impairs viral fitness but



	Huh7.5	Fetal hepatocytes	Adult hepatocytes	Micropattern co-cultures
Cell physiology	Hepatoma cell line	Primary hepatocytes	Primary hepatocytes	Primary hepatocytes
Polarity	unpolarized	polarized	polarized	polarized
HCV replication	high	medium	low	low
HCV spread	high	no	no	no
Infection frequency	high	low	low	low
Patient isolates	no*	no	no	no

Fig. 3. Cell systems permissive to HCV infection. **(a)** Comparative analysis of cellular systems available for the study of HCV infection and their prperties. *A recent report (Yang et al., 2014) described the first human hepatoma cell line naturally permissive to HBV and HCV patient isolates. **(b)** The RFP-NLS-IPS cell based HCV reporter system and its application to identify HCV infection in living cells. The NS3/4A protease cleavage of IPS1 from the mitochondrial membrane is exploited to create a reporter by constructing a fusion protein between the IPS1 transmembrane domain, a nuclear localization signal (NLS) and a red fluorescence protein (RFP). This allows sensitive detection of infection in living cells even with low viral replication levels and can be exploited in fluorescence microscopy or image-based flow cytometry.

compensatory mutations can be selected, restoring wild-type replicative fitness. Recently, the first full-length genotype 1 and 2 HCV genomes were developed that recapitulate the complete virus life cycle *in vitro* (Li et al., 2012, 2014b; Ramirez et al., 2014).

Cell systems permissive to HCV infection

As expected for a human hepatotropic virus, HCV replicates primarily in human hepatocytes. Nevertheless, the development of cell systems to study HCV infection in the laboratory proved to be a big challenge, taking almost a decade from the discovery of the virus. This is likely a reflection of the fact that the cell models utilized do not fully mimic the liver physiology but also that HCV, being a chronic virus, may have not evolved to replicate in its target cells to very high levels.

To date, the cells most permissive to HCV replication *in vitro* are a human hepatoma cell line, Huh-7, and its derivatives (Fig. 3a). These cells vary significantly in their ability to support HCV replication, although the molecular mechanisms accounting for such phenomenon remain incompletely understood. This heterogeneity was exploited to select for cell clones that sustained higher replication levels. Among these, the most widely used are Huh-7.5 cells (Blight et al., 2002) thatcarry a loss-of-function missense mutation in Retinoic Acid-Inducible Gene I (RIG-I), resulting in defective antiviral signaling (Sumpter et al., 2005), and enablesignificantly higher production of infectious particles and robust spread in cell culture. A limitation of this system lies in the lack of cell polarity, which does not allow mimicking the compartmentalization of HCV receptors or the directionality of the secretory systems observed in polarized hepatocytes. As a result, certain aspects of HCV entry, assembly and interaction with lipoproteins or cell-to-cell spread may not be reproduced in Huh-7.5 cells (Steinmann and Pietschmann, 2013). In contrast, HepG2 cells are known to polarize in culture. Although normally refractory to HCV infection due to lack of CD81 expression, these cells can be rendered fully permissive to HCV upon ectopic expression of human CD81 and miR-122, to promote viral entry and replication, respectively (Flint et al., 2006; Mee et al., 2009; Narbus et al., 2011). The liver-specific microRNA miR-122, which binds to the 5'UTR of HCV and is thought to stabilize viral RNA (Jopling et al., 2006) furthermore is able to render non-hepatic cells permissive to HCV replication (Kambara et al., 2012; Vogt et al., 2013). Other approaches to study HCV in polarized human hepatoma cells included treatment with 1% dimethyl sulfoxide (Sainz and Chisari, 2006) and growth in 3D using matrigel or rotating wall vessel (Molina-Jimenez et al., 2012; Sainz et al., 2009).

While highly permissive hepatoma cell lines represented the obvious choice for initial studies of HCV biology, this culture system does not fully recapitulate all aspects of HCV replication in the liver and discrepancies may derive from the biased nature of this model. For instance, we are missing key information on the contribution that the host plays in the outcome of infection, be it viral clearance or persistence. As such, the ultimate goal in terms of cell system for HCV is to achieve reproducible and sustained infection in more physiologic cultures of primary human hepatocytes (PHH), which represent the natural host cell for HCV, from genetically diverse donors. However, a series of limitations have made this a significant challenge, including poor availability of these cells, high costs, loss of differentiation and short survival in cell culture. Even today, a major challenge remains, which limits HCV studies in physiological cell culture systems – the poor permissiveness of PHH to HCV infection.

Human fetal liver cells (HFLC) were shown to be long-lived and to support HCV replication to higher levels compared with cultured adult human hepatocytes (Iacovacci et al., 1997; Lazaro et al., 2007).

Although significantly less permissive than hepatoma cells, HFLC showed variable infection frequency depending on the donor cell preparations (Fig. 3a). In most cases, however, little to no viral spread was observed in these culturesunless interferon responses were antagonized by expressing paramyxovirus (PMV) V proteins

or by treating with a TBK-1 inhibitor that suppresses several pathogen-associated pattern recognition receptors, including RIG-I (Andrus et al., 2011; Catanese et al., 2013b).

In an effort to prolong the survival and differentiation state of PHH, co-culture models have been developed, based on the observation that feeder cells were fundamental to PHH viability and biological functions. Micropatterned co-cultures (MPCCs) consist of islands of PHH seeded on large collagen micropatterns surrounded by fibroblasts. PHH in this culture system are polarized and permissive to HCV, albeit the efficiency of infection remains very low due to innate antiviral immune responses (Ploss et al., 2010) (Fig. 3a). A combination of MPCCs with the strategies outlined above to inhibit IFN-mediated antiviral responses would improve both cell survival/physiology and susceptibility to HCV infection, thus facilitating studies in PHH.

Given the low replication levels and infection frequency in PHH cultures, conventional virological assays established for hepatoma cells, based on detection of viral antigens, were often not sensitive enough to ascertain HCV infection. Although the quantification of viral genomes by RT-qPCR represents a very sensitive technique, it suffers from high background due to contamination by viral inocula or input RNA used for transfection. To overcome this hurdle, cell reporter systems were developed that exploit the cleavage of the NS3/4A protease to monitor HCV infection. In one case, the recognition sequence of the viral protease was fused in frame with a secreted alkaline phosphatase (SEAP), enabling rapid and sensitive quantification of HCV infection and viral replication by measuring SEAP activity in the cell culture medium (Iro et al., 2009). The other version was based on the expression of a red fluorescent protein (RFP) and a nuclear localization signal (NLS) fused to residues 462–540 of human interferon-β promoter stimulator protein 1 (IPS-1, also called MAVS, Cardif, VISA), a mitochondrial protein targeted by HCV (Jones et al., 2010). This region of IPS-1 encompasses the NS3/4a protease cleavage site, therefore upon HCV infection, cleavage of IPS-1 results in relocalization of the fluorescent protein from the mitochondria to the nucleus (Fig. 3b). Importantly, cells expressing RFP-NLS-IPS can be used to track HCV infection in real time and at a single cell level. This cellular reporter system was successfully exploited for monitoring HCV cell-to-cell spread in hepatoma cells (Catanese et al., 2013a) and PHH (Andrus et al., 2011), for *ex vivo* studies of hepatocytes explanted from mouse livers (Fig. 3b) (Dorner et al., 2013) and for laser capture microdissection approaches that enabled transcriptional profiling of infected and uninfected PHH cells (Sheahan et al., 2014).

Recent studies using hepatocyte-like cells derived from induced pluripotent stem cells (iPSC) and human embryonic stem cells (hESC) have shown that these cells can be infected with HCVcc, offering an opportunity to study the contribution of host genetics to HCV pathogenesis (Roelandt et al., 2012; Schwartz et al., 2012; Wu et al., 2012)

In addition to hepatocytes, HCV RNA and proteins have been detected at low levels in various other tissues, including lymphatic system, brain and gut (Fletcher et al., 2012; Franks, 2012; Ito et al., 2010; Liu et al., 2014b; Pawlowski et al., 2014). However, it remains to be determined whether these truly represent extrahepatic reservoirs that contribute to HCV persistence and extrahepatic manifestations of chronic HCV infection (Cacoub et al., 2014). In this respect, testing sensitivity of these systems to antiviral compounds is key to distinguishing between HCV detection and productive infection (Dustin and Rice, 2011).

In vivo models

Chimpanzee

Chimpanzees (*Pan troglodytes*) are naturally susceptible to HCV infection and their use in HCV research has greatly advanced our understanding of HCV biology. With over 98% genetic identity to humans it is not surprising that HCV can utilize chimpanzee host factors with comparable efficiency to human orthologs. Ranging from early studies aiding in the identification of HCV as the cause of non-A non-B hepatitis (Weiner et al., 1990), the creation of an infectious cDNA clone for HCV (Kolykhalov et al., 1997; Yanagi et al., 1997; Yanagi et al., 1998) to the study of immune responses (Bigger et al., 2001; Grakoui et al., 2003; Major et al., 2002; Mizukoshi et al., 2002; Nascimbeni et al., 2003) and the evaluation of vaccine candidates (Elmowalid et al., 2007; Forns et al., 2000; Fuller et al., 2013; Meunier et al., 2011; Mikkelsen et al., 2011) the chimpanzee was the most important *in vivo* model for HCV until 2013 when chimpanzee research was banned in most countries due to ethical concerns .

Tupaia

Besides chimpanzees, the treeshrew (*Tupaia belangeri*) was shown to be susceptible to HCV infection (Xie et al., 1998). HCV can utilize the tupaia orthologs of CD81, SR-BI, CLDN1 and OCLN for gaining entry into tupaia hepatocytes. (Tong et al., 2011; Jia et al., 2008; Xu et al., 2007; Barth et al., 2005) Infection leads to transient HCV viraemia, which results in mild hepatitis during the acute phase of infection (Xu et al., 2007). While encouraging, the inability to recapitulate the chronic phase of HCV, the unavailability of tools to study host responses as well as the difficulties in breeding treeshrews in captivity limits their widespread use in HCV research.

Genetically humanized mouse models

Mice are naturally resistant to HCV infection. Both virus entry and replication are limited in murine cells. The second extracellular loops of murine CD81 and OCLN have been identified as responsible for restricting species tropism (Ploss et al., 2009). Mice expressing human CD81 and OCLN, either via adenoviral gene delivery or by transgenic expression, were shown to support HCV entry (Dorner et al., 2011) making this model suitable to evaluate vaccine candidates (Giang et al., 2012), neutralizing antibodies and cellular pathways important for virus entry (Fig. 4a). However, there is no intracellular accumulation of viral RNA, indicating that viral RNA replication is insufficiently recapitulated in murine cells (Dorner et al., 2011). While the HCV protease is able to impair IFN signaling in murine cells by cleaving IPS-1 (Vogt et al., 2013), additional pathways may be responsible for inhibiting viral replication. Studies in murine embryonic fibroblasts have demonstrated that PKR- (Chang et al., 2006) and IRF3-mediated immune responses (Lin et al., 2010) are the main roadblock for HCV replication in murine cells. Combining expression of human HCV entry factors with blunting of the mouse innate immune responses (i.e. by using STAT1$^{-/-}$ mice) was recently shown to enable the recapitulation of the entire HCV life cycle, albeit at low levels (Dorner et al., 2013)(Fig. 4b). In addition to delayed B and T cell responses to HCV infection, liver infiltration of IFN-producing NK cells coincided with decline of HCV replication. A recent report compared different murine strains and described striking differences in the ability of these models to maintain chronic HCV infection (Chen et al., 2014). These data confirm that the host genetic background plays an important role in HCV infection. Additionally, the presence of a fully developed immune system in Imprinting Control Region (ICR) mice expressing human CD81 and OCLN was associated with reduced levels of pre-albumin, hepatic inflammation, leukocyte infiltration and fibrosis development, suggesting that innate immune activation is an essential driver of immune-mediated HCV pathogenesis (Chen et al., 2014).

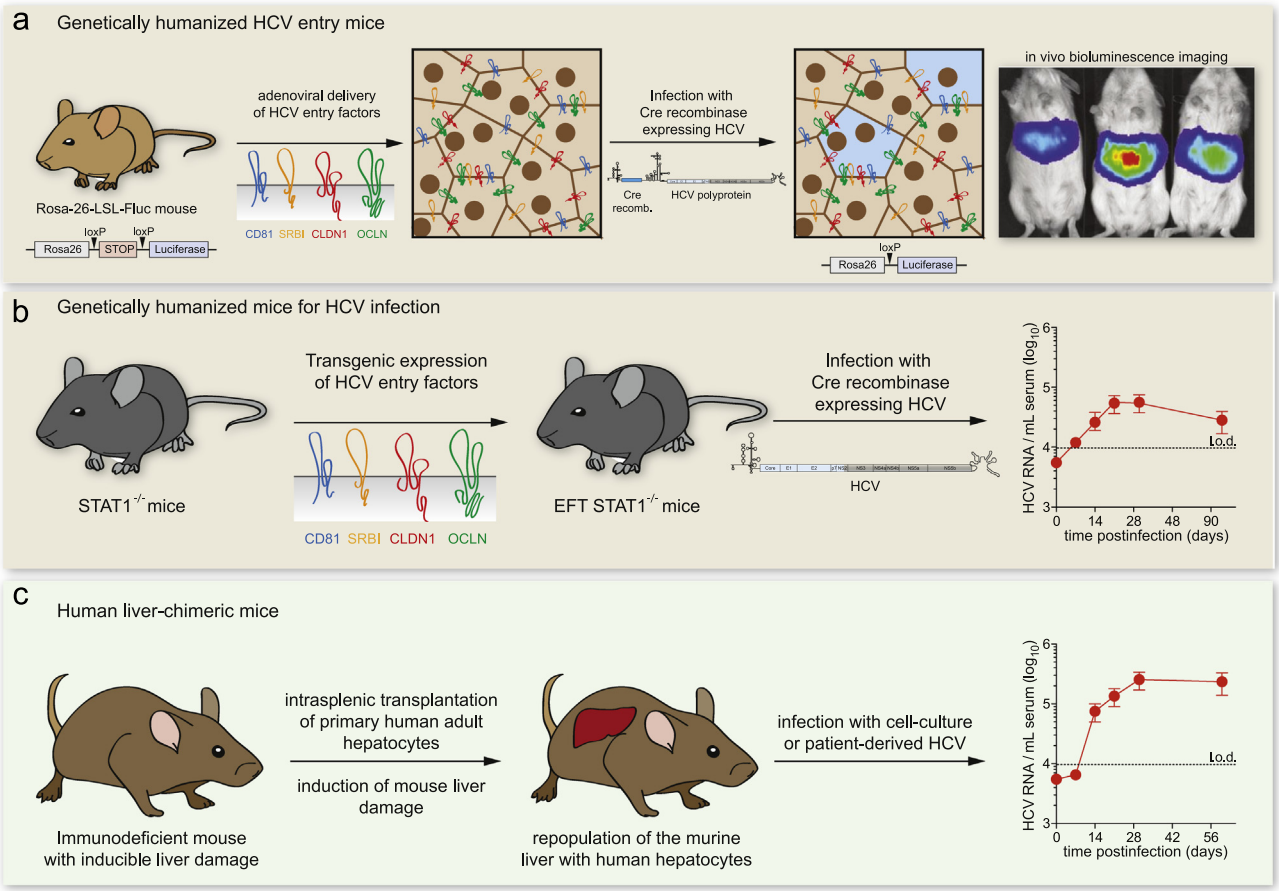


Fig. 4. HCV small-animal models. (a) Immunocompetent Rosa26Fluc luciferase reporter mice are rendered susceptible to HCV entry by adenoviral or transgenic expression of the human orthologs of HCV receptors (CD81, OCLN, SR-BI and CLDN1). Infection with a Cre recombinase-encoding HCV genome triggers Cre/loxP-based excision of a transcriptional stop cassette, activating luciferase expression, which is quantified by *in vivo* bioluminescence imaging. (b) Genetically humanized mice recapitulating all steps of the HCV life cycle are based on expression of human HCV receptors in STAT1-deficient animals. This facilitates persistent HCV infection, which can be detected for up to 90 days. (c) Human liver-chimeric mice are based on the ability of human hepatocytes to proliferate and repopulate a murine liver with genetically encoded liver injury. The immunodeficient background enables repopulation of the liver with up to 90% human hepatocytes, which in turn renders these mice permissive to patient-derived HCV.

Human liver chimeric mouse models

Direct humanization of the murine liver via xenotransplantation offers an attractive model system for studying HCV interactions with PHH *in vivo*. A common feature of all recipient strains is the requirement for extensive immunosuppression in order to facilitate human graft maintenance(Fig. 4c). This is usually accomplished by using SCID/Bg, Rag2$^{-/-}$IL2γR^{null} or NOD.Rag2$^{-/-}$IL2γR^{null}-based recipients, which lack B cell, T cell as well as NK cell responses(Boermans et al., 1992; Le-Barillec et al., 2005; Pearson et al., 2008). The liver is usually a quiescent organ and proliferation of hepatocytes requires regenerative signals. In human liver-chimeric mouse models, induction of liver injury to the murine hepatocytes is commonly exploited for the delivery of proliferative cues to human hepatocytes. Four models for the induction of murine liver injury have been described. The initial and most widely used model is based on transgenic overexpression of the urokinase Plasminogen activator (uPA) in the liver of mice (Dandri et al., 2001; Mercer et al., 2001; Rhim et al., 1995). Homozygous animals are characterized by progressive liver injury and, upon intrasplenic injection of primary human hepatocytes will engraft to high levels. More recent models have, in addition to the induction of liver injury added the inducibility of liver insults to increase viability of recipient animals. Among those, the fumaryl acetoacetate hydrolase (*Fah*)-deficient strain is most widely used(Azuma et al., 2007). Knock-out of the *Fah* gene, which is responsible for metabolizing fumarylacetoacetate to acetoacetate and fumarate results in accumulation of succinylacetate and succinylacetone, which are toxic metabolites and induce liver and renal damage. Supplementing these mice with 2-[2-nitro-4-(trifluoromethyl)benzoyl]cyclohexane-1,3-dione (NTBC) inhibits an upstream component in the tyrosine breakdown pathway, thus protecting the animals from accumulation of toxic metabolites. Other modes of inducible liver injury include expression of HSV thymidine kinase (TK) (Hasegawa et al., 2011) and expression of a fusion protein of the FK506 binding protein (FKBP) and caspase 8 (AFC8) (Washburn et al., 2011). Liver injury is induced in these models by providing either an FKBP dimerizer to activate caspase 8 signaling or gancyclovir to activate the HSV thymidine kinase. The advantage of using inducible liver injury models over uPA-expressing mice is that the murine liver injury is reversible, thus enabling longer life spans of reconstituted animals by cycling liver injury. Reconstitution levels with human hepatocytes can be conveniently monitored using human-specific serum albumin Elisa assays and studies revealed that 1 mg/mL of human albumin, which is the minimum level for infection studies with HCV correlates to 10% of the liver being engrafted with human hepatocytes (Kawahara et al., 2010). Strikingly, not only HCVcc can be used to infect human liver-chimeric mice but also patient isolates of different genotypes have successfully be used to launch infection (Mercer et al., 2001). Even though HCV infection can be maintained for extensive periods of time there are no reports of pathogenesis (i.e. fibrosis, cirrhosis or HCC) induced by HCV. Nevertheless, human liver-chimeric mice have significantly contributed to a better understanding of HCV biology, ranging from the evaluation of HCV neutralizing (Akazawa

et al., 2013; Broering et al., 2009; de Jong et al., 2014; Fafi-Kremer et al., 2010; Law et al., 2008; Meuleman et al., 2011) or receptor-blocking (Ji et al., 2014; Meuleman et al., 2008) (Lacek et al., 2012; Vercauteren et al., 2014) antibodies, the validation of the role of EGFR, EphA2 (Lupberger et al., 2011) and NPC1L1(Sainz et al., 2012) in HCV entry as well as the study of drug candidates targeting various aspects of the HCV life cycle (Bhat et al., 2012; Gebert et al., 2014; Gu et al., 2013; Jopling et al., 2006; Ray et al., 2013; Vanwolleghem et al., 2007; Watanabe et al., 2013; Yang et al., 2010).

Recently, models harboring both a human liver and a human immune system have been reported (Bility et al., 2014, 2012; Washburn et al., 2011; Wilson et al., 2014). While the maintenance of human hepatocytes is sufficiently achieved using mildly immunodeficient strains, engraftment and maintenance of a human immune system following human hematopoietic stem cell injection is only efficient in mice on the NOD background. Both, AFC8 mice and *Fah*-deficient NOD.Rag2$^{-/-}$IL2gR^{null} (FRGN) mice were shown to engraft successfully with human liver and immune system components, facilitating the study of human immune responses to hepatotropic infectious diseases. AFC8 mice reconstituted with human liver and immune system have been shown to develop liver fibrosis in response to HCV infection even though detection of liver engraftment in this model requires qPCR amplification of albumin rather than measuring human serum albumin levels. Despite the challenges in maintenance, engraftment and the not fully functional immune system these models clearly constitute the most advanced model to study HCV in its natural host environment.

Conclusions and future perspectives

The range of available model systems to study HCV has massively increased since the construction of the first infectious cDNA clone. An advantage of some of the early *in vitro* systems was that they provided the opportunity to selectively study individual aspects of the HCV life cycle. With the development of the HCVcc system, the initial findings could be integrated into a more complex model, advancing our understanding of virus assembly, release and cell-to-cell spread. Several inter-genotypic chimeras have been created, allowing, in combination with animal models, evaluation of HCV antiviral agents and vaccine candidates. However, there is still urgent need for *in vitro* systems permissive to patient-derived HCV isolates. A recent report shows promising first steps towards this goal (Yang et al., 2014). Moreover, protocols to differentiate iPSC and hESC cells into hepatocyte-like cells and to maintain PHH in physiological systems will offer the opportunity to unveil novel virus–host interactions as well as to understand the contribution of the host to disease progression. With the introduction of highly effective genome editing tools, it now becomes feasible to identify important host factors using the same genetic background in a virtually inexhaustible source of iPSC-derived hepatocytes.

Another challenge we face is that most HCV research relies on two dimensional cell cultures with poor polarization, while cell polarity is a crucial aspect of HCV entry into hepatocytes and is also likely to impact virus release and spread. Furthermore, the complexity of the liver is poorly recapitulated so far. In fact, none of the available experimental systems utilizes non-parenchymal cells (i.e. Kupffer cells, stellate cells), leaving their role in HCV disease progression entirely unexplored.

Major advances were made in the ability to study HCV *in vivo*, ranging from the identification of the species tropism-defining HCV receptors CD81 and OCLN to the importance of innate immunity in controlling HCV replication. This in turn led to the development of immunocompetent models facilitating the study of vaccine candidates, the ability to delineate important pathways required for productive infection and the ability to evaluate drug candidates. Recent advances furthermore include the generation of a pigtail macaque (*Macaca nemestrina*) model permissive to HCV infection (Date et al., 2012). Nevertheless, improvements to these models can still be made. For instance, HCV infection can only be established in these models with high titer inocula.

Human liver-chimeric mice on the other hand are permissive to patient-derived HCV, thus allowing the study of relevant viral genotypes. The recent combination of human liver-chimeric mice with human immune system mice enabled to recapitulate for the first time the entire HCV life cycle in human hepatocytes in the presence of a human immune system. However, improvements to this model are still needed prior to its applicability to study vaccine candidates. B cell responses, which are required for antibody production, are only rudimentary in human immune system-containing mice as well as myeloid cells, which are underrepresented in most model systems. Furthermore, there is no evidence for the presence of non-parenchymal cells other than hepatocytes in human liver-chimeric mice, urging the need for the development of models harboring human Kupffer cells, stellate cells or liver sinusoidal endothelial cells, thus facilitating the dissection of their impact on HCV pathogenesis.

In conclusion, the *in vitro* and *in vivo* model systems developed for HCV so far have greatly contributed to our understanding of the intricate viral life cycle, host-pathogen interactions, as well as immunology. Despite the utilization of these models for the development of DAAs promising over 90% SVR, significant challenges still remain. The inability to determine the optimal treatment regimen on a personalized medicine level, using patient-derived HCV will likely lead to the emergence of resistance mutations. Even though patients with successful SVR are considered cured these drug-resistant viral strains might be propagated through de novo infections, thus making DAAs less effective. Additionally, even if all infected individuals obtained treatment, at anticipated SVR rates of 90%, over 17 million patients will likely fail therapy, even with multi-DAA combinations. Combining these numbers with the large number of people who is not aware of having contracted HCV or cannot afford DAA treatment mandates further research into developing therapeutic as well as prophylactic strategies applicable to all people infected.

Acknowledgments

The authors thank Dr Raffaele de Francesco (INGM) and Dr Philip Meuleman (Ghent University) for critical reading of the manuscript, Prof Joseph Marcotrigiano and Dr Matthew Miller (Rutgers University) for providing the 3D model of sE2 and Prof Lynn Dustin (University of Oxford) for insightful discussions.

References

Abe, K., Ikeda, M., Dansako, H., Naka, K., Kato, N., 2007. Cell culture-adaptive NS3 mutations required for the robust replication of genome-length hepatitis C virus RNA. Virus Res. 125, 88–97.

Aghemo, A., De Francesco, R., 2013. New horizons in hepatitis C antiviral therapy with direct-acting antivirals. Hepatology 58, 428–438.

Agnello, V., Abel, G., Elfahal, M., Knight, G.B., Zhang, Q.X., 1999. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. Proc. Natl. Acad. Sci. USA 96, 12766–12771.

Akazawa, D., Moriyama, M., Yokokawa, H., Omi, N., Watanabe, N., Date, T., Morikawa, K., Aizaki, H., Ishii, K., Kato, T., Mochizuki, H., Nakamura, N., Wakita, T., 2013. Neutralizing antibodies induced by cell culture-derived hepatitis C virus protect against infection in mice. Gastroenterology 145 (447–455), e441–e444.

Andre, P., Komurian-Pradel, F., Deforges, S., Perret, M., Berland, J.L., Sodoyer, M., Pol, S., Brechot, C., Paranhos-Baccala, G., Lotteau, V., 2002. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol. 76, 6919–6928.

Andrus, L., Marukian, S., Jones, C.T., Catanese, M.T., Sheahan, T.P., Schoggins, J.W., Barry, W.T., Dustin, L.B., Trehan, K., Ploss, A., Bhatia, S.N., Rice, C.M., 2011. Expression of paramyxovirus V proteins promotes replication and spread of

hepatitis C virus in cultures of primary human fetal liver cells. Hepatology 54, 1901–1912.
Azuma, H., Paulk, N., Ranade, A., Dorrell, C., Al-Dhalimy, M., Ellis, E., Strom, S., Kay, M.A., Finegold, M., Grompe, M., 2007. Robust expansion of human hepatocytes in Fah-/-/Rag2-/-/Il2rg-/- mice. Nat. Biotechnol. 25, 903–910.
Bartenschlager, R., Penin, F., Lohmann, V., Andre, P., 2011. Assembly of infectious hepatitis C virus particles. Trends Microbiol. 19, 95–103.
Barth, H., Cerino, R., Arcuri, M., Hoffmann, M., Schurmann, P., Adah, M.I., Gissler, B., Zhao, X., Ghisetti, V., Lavezzo, B., Blum, H.E., von Weizsacker, F., Vitelli, A., Scarselli, E., Baumert, T.F., 2005. Scavenger receptor class B type I and hepatitis C virus infection of primary tupaia hepatocytes. J. Virol. 79, 5774–5785.
Barth, H., Schafer, C., Adah, M.I., Zhang, F., Linhardt, R.J., Toyoda, H., Kinoshita-Toyoda, A., Toida, T., Van Kuppevelt, T.H., Depla, E., Von Weizsacker, F., Blum, H.E., Baumert, T.F., 2003. Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. J. Biol. Chem. 278, 41003–41012.
Bartosch, B., Dubuisson, J., Cosset, F.L., 2003. Infectious hepatitis C virus pseudo-particles containing functional E1-E2 envelope protein complexes. J. Exp. Med. 197, 633–642.
Bhat, P., Gnanasundram, S.V., Mani, P., Ray, P.S., Sarkar, D.P., Das, S., 2012. Targeting ribosome assembly on the HCV RNA using a small RNA molecule. RNA Biol. 9, 1110–1119.
Bigger, C.B., Brasky, K.M., Lanford, R.E., 2001. DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection. J. Virol. 75, 7059–7066.
Bility, M.T., Curtis, A., Su, L., 2014. A chimeric mouse model to study immunopathogenesis of HCV infection. Methods Mol. Biol. 1213, 379–388.
Bility, M.T., Zhang, L., Washburn, M.L., Curtis, T.A., Kovalev, G.I., Su, L., 2012. Generation of a humanized mouse model with both human immune system and liver cells to model hepatitis C virus infection and liver immunopathogenesis. Nat. Protoc. 7, 1608–1617.
Blight, K.J., Kolykhalov, A.A., Rice, C.M., 2000. Efficient initiation of HCV RNA replication in cell culture. Science 290, 1972–1974.
Blight, K.J., McKeating, J.A., Marcotrigiano, J., Rice, C.M., 2003. Efficient replication of hepatitis C virus genotype 1a RNAs in cell culture. J. Virol. 77, 3181–3190.
Blight, K.J., McKeating, J.A., Rice, C.M., 2002. Highly permissive cell lines for subgenomic and genomic hepatitis C virus RNA replication. J. Virol. 76, 13001–13014.
Boermans, H.J., Percy, D.H., Stirtzinger, T., Croy, B.A., 1992. Engraftment of severe combined immune deficient/beige mice with bovine foetal lymphoid tissues. Vet. Immunol. Immunopathol. 34, 273–289.
Broering, T.J., Garrity, K.A., Boatright, N.K., Sloan, S.E., Sandor, F., Thomas Jr., W.D., Szabo, G., Finberg, R.W., Ambrosino, D.M., Babcock, G.J., 2009. Identification and characterization of broadly neutralizing human monoclonal antibodies directed against the E2 envelope glycoprotein of hepatitis C virus. J. Virol. 83, 12473–12482.
Bukh, J., Pietschmann, T., Lohmann, V., Krieger, N., Faulk, K., Engle, R.E., Govindarajan, S., Shapiro St, M., Claire, M., Bartenschlager, R., 2002. Mutations that permit efficient replication of hepatitis C virus RNA in Huh-7 cells prevent productive replication in chimpanzees. Proc. Natl. Acad. Sci. USA 99, 14416–14421.
Bungyoku, Y., Shoji, I., Makine, T., Adachi, T., Hayashida, K., Nagano-Fujii, M., Ide, Y.H., Deng, L., Hotta, H., 2009. Efficient production of infectious hepatitis C virus with adaptive mutations in cultured hepatoma cells. J. Gen. Virol. 90, 1681–1691.
Cacoub, P., Gragnani, L., Comarmond, C., Zignego, A.L., 2014. Extrahepatic manifestations of chronic hepatitis C virus infection. Dig. Liver Dis..
Catanese, M.T., Loureiro, J., Jones, C.T., Dorner, M., von Hahn, T., Rice, C.M., 2013a. Different requirements for scavenger receptor class B type I in hepatitis C virus cell-free versus cell-to-cell transmission. J. Virol. 87, 8282–8293.
Catanese, M.T., Uryu, K., Kopp, M., Edwards, T.J., Andrus, L., Rice, W.J., Silvestry, M., Kuhn, R.J., Rice, C.M., 2013b. Ultrastructural analysis of hepatitis C virus particles. Proc. Natl. Acad. Sci. USA 110, 9505–9510.
Chan, K., Robinson, M., Yang, H., Cornew, S., Delaney Iv, W.E., 2013. Development of a robust luciferase reporter 1b/2a hepatitis C virus (HCV) for characterization of early stage HCV life cycle inhibitors. Antiviral Res. 98, 85–92.
Chang, K.S., Cai, Z., Zhang, C., Sen, G.C., Williams, B.R., Luo, G., 2006. Replication of hepatitis C virus (HCV) RNA in mouse embryonic fibroblasts: protein kinase R (PKR)-dependent and PKR-independent mechanisms for controlling HCV RNA replication and mediating interferon activities. J. Virol. 80, 7364–7374.
Chen, J., Zhao, Y., Zhang, C., Chen, H., Feng, J., Chi, X., Pan, Y., Du, J., Guo, M., Cao, H., Chen, H., Wang, Z., Pei, R., Wang, Q., Pan, L., Niu, J., Chen, X., Tang, H., 2014. Persistent hepatitis C virus infections and hepatopathological manifestations in immune-competent humanized mice. Cell Res. 24, 1050–1066.
Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W., Houghton, M., 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244, 359–362.
Dandri, M., Burda, M.R., Torok, E., Pollok, J.M., Iwanska, A., Sommer, G., Rogiers, X., Rogler, C.E., Gupta, S., Will, H., Greten, H., Petersen, J., 2001. Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology 33, 981–988.
Date, T., Kato, T., Kato, J., Takahashi, H., Morikawa, K., Akazawa, D., Murayama, A., Tanaka-Kaneko, K., Sata, T., Tanaka, Y., Mizokami, M., Wakita, T., 2012. Novel cell culture-adapted genotype 2a hepatitis C virus infectious clone. J. Virol. 86, 10805–10820.
de Jong, Y.P., Dorner, M., Mommersteeg, M.C., Xiao, J.W., Balazs, A.B., Robbins, J.B., Winer, B.Y., Gerges, S., Vega, K., Labitt, R.N., Donovan, B.M., Giang, E., Krishnan, A., Chiriboga, L., Charlton, M.R., Burton, D.R., Baltimore, D., Law, M., Rice, C.M., Ploss, A., 2014. Broadly neutralizing antibodies abrogate established hepatitis C virus infection. Science Transl. Med. 6, 254ra129.
Dorner, M., Horwitz, J.A., Donovan, B.M., Labitt, R.N., Budell, W.C., Friling, T., Vogt, A., Catanese, M.T., Satoh, T., Kawai, T., Akira, S., Law, M., Rice, C.M., Ploss, A., 2013. Completion of the entire hepatitis C virus life cycle in genetically humanized mice. Nature 501, 237–241.
Dorner, M., Horwitz, J.A., Robbins, J.B., Barry, W.T., Feng, Q., Mu, K., Jones, C.T., Schoggins, J.W., Catanese, M.T., Burton, D.R., Law, M., Rice, C.M., Ploss, A., 2011. A genetically humanized mouse model for hepatitis C virus infection. Nature 474, 208–211.
Dustin, L.B., Rice, C.M., 2011. HCV and Blood Cells: How Can We Distinguish Infection from Association? HCV Infection and Cryoglobulinemia. Springer-Verlag, Italia.
Elmowalid, G.A., Qiao, M., Jeong, S.H., Borg, B.B., Baumert, T.F., Sapp, R.K., Hu, Z., Murthy, K., Liang, T.J., 2007. Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. Proc Natl. Acad. Sci. USA 104, 8427–8432.
Evans, M.J., von Hahn, T., Tscherne, D.M., Syder, A.J., Panis, M., Wolk, B., Hatziioannou, T., McKeating, J.A., Bieniasz, P.D., Rice, C.M., 2007. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. Nature 446, 801–805.
Fafi-Kremer, S., Fofana, I., Soulier, E., Carolla, P., Meuleman, P., Leroux-Roels, G., Patel, A.H., Cosset, F.L., Pessaux, P., Doffoel, M., Wolf, P., Stoll-Keller, F., Baumert, T.F., 2010. Viral entry and escape from antibody-mediated neutralization influence hepatitis C virus reinfection in liver transplantation. J. Exp. Med. 207, 2019–2031.
Fletcher, N.F., Wilson, G.K., Murray, J., Hu, K., Lewis, A., Reynolds, G.M., Stamataki, Z., Meredith, L.W., Rowe, I.A., Luo, G., Lopez-Ramirez, M.A., Baumert, T.F., Weksler, B., Couraud, P.O., Kim, K.S., Romero, I.A., Jopling, C., Morgello, S., Balfe, P., McKeating, J.A., 2012. Hepatitis C virus infects the endothelial cells of the blood–brain barrier. Gastroenterology 142 (634-643), e636.
Flint, M., von Hahn, T., Zhang, J., Farquhar, M., Jones, C.T., Balfe, P., Rice, C.M., McKeating, J.A., 2006. Diverse CD81 proteins support hepatitis C virus infection. J. Virol. 80, 11331–11342.
Forns, X., Payette, P.J., Ma, X., Satterfield, W., Eder, G., Mushahwar, I.K., Govindarajan, S., Davis, H.L., Emerson, S.U., Purcell, R.H., Bukh, J., 2000. Vaccination of chimpanzees with plasmid DNA encoding the hepatitis C virus (HCV) envelope E2 protein modified the infection after challenge with homologous monoclonal HCV. Hepatology 32, 618–625.
Franks, I., 2012. Hepatitis: brain endothelial cells support HCV entry and replication. Nat. Rev. Gastroenterol. Hepatol. 9, 65.
Fuller, M.J., Callendret, B., Zhu, B., Freeman, G.J., Hasselschwert, D.L., Satterfield, W., Sharpe, A.H., Dustin, L.B., Rice, C.M., Grakoui, A., Ahmed, R., Walker, C.M., 2013. Immunotherapy of chronic hepatitis C virus infection with antibodies against programmed cell death-1 (PD-1). Proc Natl. Acad. Sci. USA 110, 15001–15006.
Galli, A., Scheel, T.K., Prentoe, J.C., Mikkelsen, L.S., Gottwein, J.M., Bukh, J., 2013. Analysis of hepatitis C virus core/NS5A protein co-localization using novel cell culture systems expressing core-NS2 and NS5A of genotypes 1–7. J. Gen. Virol. 94, 2221–2235.
Gastaminza, P., Dryden, K.A., Boyd, B., Wood, M.R., Law, M., Yeager, M., Chisari, F.V., 2010. Ultrastructural and biophysical characterization of hepatitis C virus particles produced in cell culture. J. Virol. 84, 10999–11009.
Gates, A.T., Sarisky, R.T., Gu, B., 2004. Sequence requirements for the development of a chimeric HCV replicon system. Virus Res. 100, 213–222.
Gebert, L.F., Rebhan, M.A., Crivelli, S.E., Denzler, R., Stoffel, M., Hall, J., 2014. Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. Nucleic Acids Res. 42, 609–621.
Giang, E., Dorner, M., Prentoe, J.C., Dreux, M., Evans, M.J., Bukh, J., Rice, C.M., Ploss, A., Burton, D.R., Law, M., 2012. Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. Proc Natl. Acad. Sci. USA 109, 6205–6210.
Gottwein, J.M., Jensen, S.B., Serre, S.B., Ghanem, L., Scheel, T.K., Jensen, T.B., Krarup, H., Uzcategui, N., Mikkelsen, L.S., Bukh, J., 2013. Adapted J6/JFH1-based Hepatitis C virus recombinants with genotype-specific NS4A show similar efficacies against lead protease inhibitors, alpha interferon, and a putative NS4A inhibitor. Antimicrob. Agents Chemother. 57, 6034–6049.
Gottwein, J.M., Jensen, T.B., Mathiesen, C.K., Meuleman, P., Serre, S.B., Lademann, J.B., Ghanem, L., Scheel, T.K., Leroux-Roels, G., Bukh, J., 2011a. Development and application of hepatitis C reporter viruses with genotype 1 to 7 core-nonstructural protein 2 (NS2) expressing fluorescent proteins or luciferase in modified JFH1 NS5A. J. Virol. 85, 8913–8928.
Gottwein, J.M., Scheel, T.K., Callendret, B., Li, Y.P., Eccleston, H.B., Engle, R.E., Govindarajan, S., Satterfield, W., Purcell, R.H., Walker, C.M., Bukh, J., 2010. Novel infectious cDNA clones of hepatitis C virus genotype 3a (strain S52) and 4a (strain ED43): genetic analyses and in vivo pathogenesis studies. J. Virol. 84, 5277–5293.
Gottwein, J.M., Scheel, T.K., Hoegh, A.M., Lademann, J.B., Eugen-Olsen, J., Lisby, G., Bukh, J., 2007. Robust hepatitis C genotype 3a cell culture releasing adapted intergenotypic 3a/2a (S52/JFH1) viruses. Gastroenterology 133, 1614–1626.
Gottwein, J.M., Scheel, T.K., Jensen, T.B., Ghanem, L., Bukh, J., 2011b. Differential efficacy of protease inhibitors against HCV genotypes 2a, 3a, 5a, and 6a NS3/4A protease recombinant viruses. Gastroenterology 141, 1067–1079.
Gottwein, J.M., Scheel, T.K., Jensen, T.B., Lademann, J.B., Prentoe, J.C., Knudsen, M.L., Hoegh, A.M., Bukh, J., 2009. Development and characterization of hepatitis C virus genotype 1–7 cell culture systems: role of CD81 and scavenger receptor class B type I and effect of antiviral drugs. Hepatology 49, 364–377.

Grakoui, A., Shoukry, N.H., Woollard, D.J., Han, J.H., Hanson, H.L., Ghrayeb, J., Murthy, K.K., Rice, C.M., Walker, C.M., 2003. HCV persistence and immune evasion in the absence of memory T cell help. Science 302, 659–662.
Graziani, R., Paonessa, G., 2004. Dominant negative effect of wild-type NS5A on NS5A-adapted subgenomic hepatitis C virus RNA replicon. J. Gen. Virol. 85, 1867–1875.
Gu, Z., Graci, J.D., Lahser, F.C., Breslin, J.J., Jung, S.P., Crona, J.H., McMonagle, P., Xia, E., Liu, S., Karp, G., Zhu, J., Huang, S., Nomeir, A., Weetall, M., Almstead, N.G., Peltz, S.W., Tong, X., Ralston, R., Colacino, J.M., 2013. Identification of PTC725, an orally bioavailable small molecule that selectively targets the hepatitis C Virus NS4B protein. Antimicrob. Agents Chemother. 57, 3250–3261.
Hasegawa, M., Kawai, K., Mitsui, T., Taniguchi, K., Monnai, M., Wakui, M., Ito, M., Suematsu, M., Peltz, G., Nakamura, M., Suemizu, H., 2011. The reconstituted 'humanized liver' in TK-NOG mice is mature and functional. Biochem. Biophys. Res. Commun. 405, 405–410.
Herlihy, K.J., Graham, J.P., Kumpf, R., Patick, A.K., Duggal, R., Shi, S.T., 2008. Development of intergenotypic chimeric replicons to determine the broad-spectrum antiviral activities of hepatitis C virus polymerase inhibitors. Antimicrob. Agents Chemother. 52, 3523–3531.
Horwitz, J.A., Dorner, M., Friling, T., Donovan, B.M., Vogt, A., Loureiro, J., Oh, T., Rice, C.M., Ploss, A., 2013. Expression of heterologous proteins flanked by NS3-4A cleavage sites within the hepatitis C virus polyprotein. Virology 439, 23–33.
Hsu, M., Zhang, J., Flint, M., Logvinoff, C., Cheng-Mayer, C., Rice, C.M., McKeating, J.A., 2003. Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. Proc Natl. Acad. Sci. USA 100, 7271–7276.
Hueging, K., Doepke, M., Vieyres, G., Bankwitz, D., Frentzen, A., Doerrbecker, J., Gumz, F., Haid, S., Wolk, B., Kaderali, L., Pietschmann, T., 2014. Apolipoprotein E codetermines tissue tropism of hepatitis C virus and is crucial for viral cell-to-cell transmission by contributing to a postenvelopment step of assembly. J. Virol. 88, 1433–1446.
Iacovacci, S., Manzin, A., Barca, S., Sargiacomo, M., Serafino, A., Valli, M.B., Macioce, G., Hassan, H.J., Ponzetto, A., Clementi, M., Peschle, C., Carloni, G., 1997. Molecular characterization and dynamics of hepatitis C virus replication in human fetal hepatocytes infected in vitro. Hepatology 26, 1328–1337.
Ikeda, M., Yi, M., Li, K., Lemon, S.M., 2002. Selectable subgenomic and genome-length dicistronic RNAs derived from an infectious molecular clone of the HCV-N strain of hepatitis C virus replicate efficiently in cultured Huh7 cells. J. Virol. 76, 2997–3006.
Iro, M., Witteveldt, J., Angus, A.G., Woerz, I., Kaul, A., Bartenschlager, R., Patel, A.H., 2009. A reporter cell line for rapid and sensitive evaluation of hepatitis C virus infectivity and replication. Antiviral Res. 83, 148–155.
Ito, M., Masumi, A., Mochida, K., Kukihara, H., Moriishi, K., Matsuura, Y., Yamaguchi, K., Mizuochi, T., 2010. Peripheral B cells may serve as a reservoir for persistent hepatitis C virus infection. J. Innate Immun. 2, 607–617.
Jensen, T.B., Gottwein, J.M., Scheel, T.K., Hoegh, A.M., Eugen-Olsen, J., Bukh, J., 2008. Highly efficient JFH1-based cell-culture system for hepatitis C virus genotype 5a: failure of homologous neutralizing-antibody treatment to control infection. J. Infect. Dis. 198, 1756–1765.
Ji, C., Liu, Y., Pamulapati, C., Bohini, S., Fertig, G., Schraeml, M., Rubas, W., Brandt, M., Ries, S., Ma, H., Klumpp, K., 2014. Prevention of hepatitis C virus infection and spread in human liver chimeric mice by an anti-CD81 monoclonal antibody. Hepatology.
Jia, Z.S., Du, D.W., Lei, Y.F., Wei, X., Yin, W., Ma, L., Lian, J.Q., Wang, P.Z., Li, D., Zhou, Y.X., 2008. Scavenger receptor class B type I mediates cell entry of hepatitis C virus. J. Int. Med. Res. 36, 1319–1325.
Jones, C.T., Catanese, M.T., Law, L.M., Khetani, S.R., Syder, A.J., Ploss, A., Oh, T.S., Schoggins, J.W., MacDonald, M.R., Bhatia, S.N., Rice, C.M., 2010. Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. Nat. Biotechnol. 28, 167–171.
Jopling, C.L., Norman, K.L., Sarnow, P., 2006. Positive and negative modulation of viral and cellular mRNAs by liver-specific microRNA miR-122. Cold Spring Harbor Symposia Quant. Biol. 71, 369–376.
Kambara, H., Fukuhara, T., Shiokawa, M., Ono, C., Ohara, Y., Kamitani, W., Matsuura, Y., 2012. Establishment of a novel permissive cell line for the propagation of hepatitis C virus by expression of microRNA miR122. J. Virol. 86, 1382–1393.
Kato, N., Sugiyama, K., Namba, K., Dansako, H., Nakamura, T., Takami, M., Naka, K., Nozaki, A., Shimotohno, K., 2003. Establishment of a hepatitis C virus sub-genomic replicon derived from human hepatocytes infected in vitro. Biochem. Biophys. Res. Commun. 306, 756–766.
Kawahara, T., Toso, C., Douglas, D.N., Nourbakhsh, M., Lewis, J.T., Tyrrell, D.L., Lund, G.A., Churchill, T.A., Kneteman, N.M., 2010. Factors affecting hepatocyte isolation, engraftment, and replication in an in vivo model. Liver Transpl. 16, 974–982.
Khan, A.G., Whidby, J., Miller, M.T., Scarborough, H., Zatorski, A.V., Cygan, A., Price, A.A., Yost, S.A., Bohannon, C.D., Jacob, J., Grakoui, A., Marcotrigiano, J., 2014. Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2. Nature 509, 381–384.
King, R.W., Zecher, M., Jeffries, M.W., Carroll, D.R., Parisi, J.M., Pasquinelli, C., 2002. A cell-based model of HCV-negative-strand RNA replication utilizing a chimeric hepatitis C virus/reporter RNA template. Antivir. Chem. Chemother. 13, 353–362.
Kolykhalov, A.A., Agapov, E.V., Blight, K.J., Mihalik, K., Feinstone, S.M., Rice, C.M., 1997. Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA. Science 277, 570–574.
Konan, K.V., Giddings Jr., T.H., Ikeda, M., Li, K., Lemon, S.M., Kirkegaard, K., 2003. Nonstructural protein precursor NS4A/B from hepatitis C virus alters function and ultrastructure of host secretory apparatus. J. Virol. 77, 7843–7855.
Kong, L., Giang, E., Nieusma, T., Kadam, R.U., Cogburn, K.E., Hua, Y., Dai, X., Stanfield, R.L., Burton, D.R., Ward, A.B., Wilson, I.A., Law, M., 2013. Hepatitis C virus E2 envelope glycoprotein core structure. Science 342, 1090–1094.
Krieger, N., Lohmann, V., Bartenschlager, R., 2001. Enhancement of hepatitis C virus RNA replication by cell culture-adaptive mutations. J. Virol. 75, 4614–4624.
Lacek, K., Vercauteren, K., Grzyb, K., Naddeo, M., Verhoye, L., Slowikowski, M.P., Fafi-Kremer, S., Patel, A.H., Baumert, T.F., Folgori, A., Leroux-Roels, G., Cortese, R., Meuleman, P., Nicosia, A., 2012. Novel human SR-BI antibodies prevent infection and dissemination of HCV in vitro and in humanized mice. J. Hepatol. 57, 17–23.
Law, M., Maruyama, T., Lewis, J., Giang, E., Tarr, A.W., Stamataki, Z., Gastaminza, P., Chisari, F.V., Jones, I.M., Fox, R.I., Ball, J.K., McKeating, J.A., Kneteman, N.M., Burton, D.R., 2008. Broadly neutralizing antibodies protect against hepatitis C virus quasispecies challenge. Nat. Med. 14, 25–27.
Lazaro, C.A., Chang, M., Tang, W., Campbell, J., Sullivan, D.G., Gretch, D.R., Corey, L., Coombs, R.W., Fausto, N., 2007. Hepatitis C virus replication in transfected and serum-infected cultured human fetal hepatocytes. Am. J.Pathol. 170, 478–489.
Le-Barillec, K., Magalhaes, J.G., Corcuff, E., Thuizat, A., Sansonetti, P.J., Phalipon, A., Di Santo, J.P., 2005. Roles for T and NK cells in the innate immune response to Shigella flexneri. J. Immunol. 175, 1735–1740.
Li, Y.P., Ramirez, S., Humes, D., Jensen, S.B., Gottwein, J.M., Bukh, J., 2014a. Differential sensitivity of 5′UTR-NS5A recombinants of hepatitis C virus genotypes 1–6 to protease and NS5A inhibitors. Gastroenterology 146 (812-821), e814.
Li, Y.P., Ramirez, S., Jensen, S.B., Purcell, R.H., Gottwein, J.M., Bukh, J., 2012. Highly efficient full-length hepatitis C virus genotype 1 (strain TN) infectious culture system. Proc. Natl. Acad. Sci. USA 109, 19757–19762.
Li, Y.P., Ramirez, S., Mikkelsen, L., Bukh, J., 2014b. Efficient infectious cell culture systems of the hepatitis C virus prototype strains HCV-1 and H77. J. Virol..
Liang, C., Rieder, E., Hahm, B., Jang, S.K., Paul, A., Wimmer, E., 2005. Replication of a novel subgenomic HCV genotype 1a replicon expressing a puromycin resistance gene in Huh-7 cells. Virology 333, 41–53.
Liefhebber, J.M., Hensbergen, P.J., Deelder, A.M., Spaan, W.J., van Leeuwen, H.C., 2010. Characterization of hepatitis C virus NS3 modifications in the context of replication. J. Gen. Virol. 91, 1013–1018.
Lin, L.T., Noyce, R.S., Pham, T.N., Wilson, J.A., Sisson, G.R., Michalak, T.I., Mossman, K.L., Richardson, C.D., 2010. Replication of subgenomic hepatitis C virus replicons in mouse fibroblasts is facilitated by deletion of interferon regulatory factor 3 and expression of liver-specific microRNA 122. J. Virol. 84, 9170–9180.
Lindenbach, B.D., Evans, M.J., Syder, A.J., Wolk, B., Tellinghuisen, T.L., Liu, C.C., Maruyama, T., Hynes, R.O., Burton, D.R., McKeating, J.A., Rice, C.M., 2005. Complete replication of hepatitis C virus in cell culture. Science 309, 623–626.
Lindenbach, B.D., Meuleman, P., Ploss, A., Vanwolleghem, T., Syder, A.J., McKeating, J.A., Lanford, R.E., Feinstone, S.M., Major, M.E., Leroux-Roels, G., Rice, C.M., 2006. Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro. Proc. Natl. Acad. Sci. USA 103, 3805–3809.
Liu, S., Chen, R., Hagedorn, C.H., 2014a. Direct visualization of hepatitis C virus-infected Huh7.5 cells with a high titre of infectious chimeric JFH1-EGFP reporter virus in three-dimensional Matrigel cell cultures. J. Gen. Virol. 95, 423–433.
Liu, S., Xiao, L., Nelson, C., Hagedorn, C.H., 2012. A cell culture adapted HCV JFH1 variant that increases viral titers and permits the production of high titer infectious chimeric reporter viruses. PLoS One 7, e44965.
Liu, Z., Zhao, F., He, J.J., 2014b. Hepatitis C virus (HCV) interaction with astrocytes: nonproductive infection and induction of IL-18. J. Neurovirol. 20, 278–293.
Lohmann, V., Hoffmann, S., Herian, U., Penin, F., Bartenschlager, R., 2003. Viral and cellular determinants of hepatitis C virus RNA replication in cell culture. J. Virol. 77, 3007–3019.
Lohmann, V., Korner, F., Dobierzewska, A., Bartenschlager, R., 2001. Mutations in hepatitis C virus RNAs conferring cell culture adaptation. J. Virol. 75, 1437–1449.
Lohmann, V., Korner, F., Koch, J., Herian, U., Theilmann, L., Bartenschlager, R., 1999. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285, 110–113.
Lupberger, J., Zeisel, M.B., Xiao, F., Thumann, C., Fofana, I., Zona, L., Davis, C., Mee, C.J., Turek, M., Gorke, S., Royer, C., Fischer, B., Zahid, M.N., Lavillette, D., Fresquet, J., Cosset, F.L., Rothenberg, S.M., Pietschmann, T., Patel, A.H., Pessaux, P., Doffoel, M., Raffelsberger, W., Poch, O., McKeating, J.A., Brino, L., Baumert, T.F., 2011. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. Nat. Med. 17, 589–595.
Maekawa, S., Enomoto, N., Sakamoto, N., Kurosaki, M., Ueda, E., Kohashi, T., Watanabe, H., Chen, C.H., Yamashiro, T., Tanabe, Y., Kanazawa, N., Nakagawa, M., Sato, C., Watanabe, M., 2004. Introduction of NS5A mutations enables subgenomic HCV replicon derived from chimpanzee-infectious HC-J4 isolate to replicate efficiently in Huh-7 cells. J. Viral. Hepat. 11, 394–403.
Major, M.E., Mihalik, K., Puig, M., Rehermann, B., Nascimbeni, M., Rice, C.M., Feinstone, S.M., 2002. Previously infected and recovered chimpanzees exhibit rapid responses that control hepatitis C virus replication upon rechallenge. J. Virol. 76, 6586–6595.
Mancone, C., Steindler, C., Santangelo, L., Simonte, G., Vlassi, C., Longo, M.A., D'Offizi, G., Di Giacomo, C., Pucillo, L.P., Amicone, L., Tripodi, M., Alonzi, T., 2011. Hepatitis C virus production requires apolipoprotein A-I and affects its association with nascent low-density lipoproteins. Gut 60, 378–386.
Manns, M.P., Wedemeyer, H., Cornberg, M., 2006. Treating viral hepatitis C: efficacy, side effects, and complications. Gut 55, 1350–1359.
Martin, D.N., Uprichard, S.L., 2013. Identification of transferrin receptor 1 as a hepatitis C virus entry factor. Proc. Natl. Acad. Sci. USA 110, 10777–10782.

Mee, C.J., Harris, H.J., Farquhar, M.J., Wilson, G., Reynolds, G., Davis, C., van, I.S.C., Balfe, P., McKeating, J.A., 2009. Polarization restricts hepatitis C virus entry into HepG2 hepatoma cells. J. Virol. 83, 6211–6221.
Mercer, D.F., Schiller, D.E., Elliott, J.F., Douglas, D.N., Hao, C., Rinfret, A., Addison, W. R., Fischer, K.P., Churchill, T.A., Lakey, J.R., Tyrrell, D.L., Kneteman, N.M., 2001. Hepatitis C virus replication in mice with chimeric human livers. Nat. Med. 7, 927–933.
Merz, A., Long, G., Hiet, M.S., Brugger, B., Chlanda, P., Andre, P., Wieland, F., Krijnse-Locker, J., Bartenschlager, R., 2011. Biochemical and morphological properties of hepatitis C virus particles and determination of their lipidome. J. Biol. Chem. 286, 3018–3032.
Messina, J.P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G.S., Pybus, O.G., Barnes, E., 2015. Global Distribution and Prevalence of Hepatitis C Virus Genotypes. Hepatology 61, 77.
Meuleman, P., Bukh, J., Verhoye, L., Farhoudi, A., Vanwolleghem, T., Wang, R.Y., Desombere, I., Alter, H., Purcell, R.H., Leroux-Roels, G., 2011. In vivo evaluation of the cross-genotype neutralizing activity of polyclonal antibodies against hepatitis C virus. Hepatology 53, 755–762.
Meuleman, P., Hesselgesser, J., Paulson, M., Vanwolleghem, T., Desombere, I., Reiser, H., Leroux-Roels, G., 2008. Anti-CD81 antibodies can prevent a hepatitis C virus infection in vivo. Hepatology 48, 1761–1768.
Meunier, J.C., Gottwein, J.M., Houghton, M., Russell, R.S., Emerson, S.U., Bukh, J., Purcell, R.H., 2011. Vaccine-induced cross-genotype reactive neutralizing antibodies against hepatitis C virus. J. Infect. Dis. 204, 1186–1190.
Michalak, J.P., Wychowski, C., Choukhi, A., Meunier, J.C., Ung, S., Rice, C.M., Dubuisson, J., 1997. Characterization of truncated forms of hepatitis C virus glycoproteins. J. Gen. Virol. 78 (Pt 9), 2299–2306.
Mikkelsen, M., Holst, P.J., Bukh, J., Thomsen, A.R., Christensen, J.P., 2011. Enhanced and sustained CD8+ T cell responses with an adenoviral vector-based hepatitis C virus vaccine encoding NS3 linked to the MHC class II chaperone protein invariant chain. J. Immunol. 186, 2355–2364.
Mizukoshi, E., Nascimbeni, M., Blaustein, J.B., Mihalik, K., Rice, C.M., Liang, T.J., Feinstone, S.M., Rehermann, B., 2002. Molecular and immunological significance of chimpanzee major histocompatibility complex haplotypes for hepatitis C virus immune response and vaccination studies. J. Virol. 76, 6093–6103.
Mohd Hanafiah, K., Groeger, J., Flaxman, A.D., Wiersma, S.T., 2013. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 57, 1333–1342.
Molina-Jimenez, F., Benedicto, I., Dao Thi, V.L., Gondar, V., Lavillette, D., Marin, J.J., Briz, O., Moreno-Otero, R., Aldabe, R., Baumert, T.F., Cosset, F.L., Lopez-Cabrera, M., Majano, P.L., 2012. Matrigel-embedded 3D culture of Huh-7 cells as a hepatocyte-like polarized system to study hepatitis C virus cycle. Virology 425, 31–39.
Nag, A., Robotham, J.M., Tang, H., 2012. Suppression of viral RNA binding and the assembly of infectious hepatitis C virus particles in vitro by cyclophilin inhibitors. J. Virol. 86, 12616–12624.
Narbus, C.M., Israelow, B., Sourisseau, M., Michta, M.L., Hopcraft, S.E., Zeiner, G.M., Evans, M.J., 2011. HepG2 cells expressing microRNA miR-122 support the entire hepatitis C virus life cycle. J. Virol. 85, 12087–12092.
Nascimbeni, M., Mizukoshi, E., Bosmann, M., Major, M.E., Mihalik, K., Rice, C.M., Feinstone, S.M., Rehermann, B., 2003. Kinetics of CD4+ and CD8+ memory T-cell responses during hepatitis C virus rechallenge of previously recovered chimpanzees. J. Virol. 77, 4781–4793.
Nielsen, S.U., Bassendine, M.F., Burt, A.D., Martin, C., Pumeechockchai, W., Toms, G.L., 2006. Association between hepatitis C virus and very-low-density lipoprotein (VLDL)/LDL analyzed in iodixanol density gradients. J. Virol. 80, 2418–2428.
Pawlowski, T., Radkowski, M., Malyszczak, K., Inglot, M., Zalewska, M., Jablonska, J., Laskus, T., 2014. Depression and neuroticism in patients with chronic hepatitis C: correlation with peripheral blood mononuclear cells activation. J. Clin. Virol. 60, 105–111.
Pearson, T., Shultz, L.D., Miller, D., King, M., Laning, J., Fodor, W., Cuthbert, A., Burzenski, L., Gott, B., Lyons, B., Foreman, O., Rossini, A.A., Greiner, D.L., 2008. Non-obese diabetic-recombination activating gene-1 (NOD-Rag1 null) interleukin (IL)-2 receptor common gamma chain (IL2r gamma null) null mice: a radioresistant model for human lymphohaematopoietic engraftment. Clin. Exp. Immunol. 154, 270–284.
Peng, H.K., Lin, C.K., Yang, S.Y., Tseng, C.K., Tzeng, C.C., Lee, J.C., Yang, S.C., 2012. Synthesis and anti-HCV activity evaluation of anilinoquinoline derivatives. Bioorg. Med. Chem. Lett. 22, 1107–1110.
Pietschmann, T., Kaul, A., Koutsoudakis, G., Shavinskaya, A., Kallis, S., Steinmann, E., Abid, K., Negro, F., Dreux, M., Cosset, F.L., Bartenschlager, R., 2006. Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. Proc. Natl.ional Acad. Sci. USA 103, 7408–7413.
Pileri, P., Uematsu, Y., Campagnoli, S., Galli, G., Falugi, F., Petracca, R., Weiner, A.J., Houghton, M., Rosa, D., Grandi, G., Abrignani, S., 1998. Binding of hepatitis C virus to CD81. Science 282, 938–941.
Ploss, A., Evans, M.J., Gaysinskaya, V.A., Panis, M., You, H., de Jong, Y.P., Rice, C.M., 2009. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. Nature 457, 882–886.
Ploss, A., Khetani, S.R., Jones, C.T., Syder, A.J., Trehan, K., Gaysinskaya, V.A., Mu, K., Ritola, K., Rice, C.M., Bhatia, S.N., 2010. Persistent hepatitis C virus infection in microscale primary human hepatocyte cultures. Proc. Natl. Acad. Sci. USA 107, 3141–3145.
Prentoe, J., Jensen, T.B., Meuleman, P., Serre, S.B., Scheel, T.K., Leroux-Roels, G., Gottwein, J.M., Bukh, J., 2011. Hypervariable region 1 differentially impacts viability of hepatitis C virus strains of genotypes 1 to 6 and impairs virus neutralization. J. Virol. 85, 2224–2234.
Ramirez, S., Li, Y.P., Jensen, S.B., Pedersen, J., Gottwein, J.M., Bukh, J., 2014. Highly efficient infectious cell culture of three hepatitis C virus genotype 2b strains and sensitivity to lead protease, nonstructural protein 5A, and polymerase inhibitors. Hepatology 59, 395–407.
Ray, U., Roy, C.L., Kumar, A., Mani, P., Joseph, A.P., Sudha, G., Sarkar, D.P., Srinivasan, N., Das, S., 2013. Inhibition of the interaction between NS3 protease and HCV IRES with a small peptide: a novel therapeutic strategy. Mol. Ther. 21, 57–67.
Rhim, J.A., Sandgren, E.P., Palmiter, R.D., Brinster, R.L., 1995. Complete reconstitution of mouse liver with xenogeneic hepatocytes. Proc. Natl. Acad. Sci. USA 92, 4942–4946.
Roelandt, P., Obeid, S., Paeshuyse, J., Vanhove, J., Van Lommel, A., Nahmias, Y., Nevens, F., Neyts, J., Verfaillie, C.M., 2012. Human pluripotent stem cell-derived hepatocytes support complete replication of hepatitis C virus. J. Hepatol. 57, 246–251.
Russell, R.S., Kawaguchi, K., Meunier, J.C., Takikawa, S., Faulk, K., Bukh, J., Purcell, R.H., Emerson, S.U., 2009. Mutational analysis of the hepatitis C virus E1 glycoprotein in retroviral pseudoparticles and cell-culture-derived H77/JFH1 chimeric infectious virus particles. J. Viral. Hepat. 16, 621–632.
Saeed, M., Gondeau, C., Hmwe, S., Yokokawa, H., Date, T., Suzuki, T., Kato, T., Maurel, P., Wakita, T., 2013. Replication of hepatitis C virus genotype 3a in cultured cells. Gastroenterology 144 (56-58), e57.
Saeed, M., Scheel, T.K., Gottwein, J.M., Marukian, S., Dustin, L.B., Bukh, J., Rice, C.M., 2012. Efficient replication of genotype 3a and 4a hepatitis C virus replicons in human hepatoma cells. Antimicrob. Agents Chemother. 56, 5365–5373.
Sainz Jr., B., Barretto, N., Martin, D.N., Hiraga, N., Imamura, M., Hussain, S., Marsh, K.A., Yu, X., Chayama, K., Alrefai, W.A., Uprichard, S.L., 2012. Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. Nat. Med. 18, 281–285.
Sainz Jr., B., Chisari, F.V., 2006. Production of infectious hepatitis C virus by well-differentiated, growth-arrested human hepatoma-derived cells. J. Virol. 80, 10253–10257.
Sainz Jr., B., TenCate, V., Uprichard, S.L., 2009. Three-dimensional Huh7 cell culture system for the study of Hepatitis C virus infection. J. Virol. 6, 103.
Scarselli, E., Ansuini, H., Cerino, R., Roccasecca, R.M., Acali, S., Filocamo, G., Traboni, C., Nicosia, A., Cortese, R., Vitelli, A., 2002a. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. EMBO J. 21, 5017–5025.
Scarselli, E., Ansuini, H., Cerino, R., Roccasecca, R.M., Acali, S., Filocamo, G., Traboni, C., Nicosia, A., Cortese, R., Vitelli, A., 2002b. The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. EMBO J. 21, 5017–5025.
Scheel, T.K., Gottwein, J.M., Jensen, T.B., Prentoe, J.C., Hoegh, A.M., Alter, H.J., Eugen-Olsen, J., Bukh, J., 2008. Development of JFH1-based cell culture systems for hepatitis C virus genotype 4a and evidence for cross-genotype neutralization. Proc. Natl. Acad. Sci. USA 105, 997–1002.
Scheel, T.K., Gottwein, J.M., Mikkelsen, L.S., Jensen, T.B., Bukh, J., 2011. Recombinant HCV variants with NS5A from genotypes 1–7 have different sensitivities to an NS5A inhibitor but not interferon-alpha. Gastroenterology 140, 1032–1042.
Scheel, T.K., Prentoe, J., Carlsen, T.H., Mikkelsen, L.S., Gottwein, J.M., Bukh, J., 2012. Analysis of functional differences between hepatitis C virus NS5A of genotypes 1–7 in infectious cell culture systems. PLoS Pathog. 8, e1002696.
Schwartz, R.E., Trehan, K., Andrus, L., Sheahan, T.P., Ploss, A., Duncan, S.A., Rice, C.M., Bhatia, S.N., 2012. Modeling hepatitis C virus infection using human induced pluripotent stem cells. Proc. Natl. Acad. Sci. USA 109, 2544–2548.
Sheahan, T., Imanaka, N., Marukian, S., Dorner, M., Liu, P., Ploss, A., Rice, C.M., 2014. Interferon lambda alleles predict innate antiviral immune responses and hepatitis C virus permissiveness. Cell Host Microbe 15, 190–202.
Shimakami, T., Hijikata, M., Luo, H., Ma, Y.Y., Kaneko, S., Shimotohno, K., Murakami, S., 2004. Effect of interaction between hepatitis C virus NS5A and NS5B on hepatitis C virus RNA replication with the hepatitis C virus replicon. J. Virol. 78, 2738–2748.
Steinmann, E., Pietschmann, T., 2013. Cell culture systems for hepatitis C virus. Curr. Topics Microbiol. Immunol. 369, 17–48.
Sumpter Jr., R., Loo, Y.M., Foy, E., Li, K., Yoneyama, M., Fujita, T., Lemon, S.M., Gale Jr., M., 2005. Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase RIG-I. J. Virol. 79, 2689–2699.
Targett-Adams, P., McLauchlan, J., 2005. Development and characterization of a transient-replication assay for the genotype 2a hepatitis C virus subgenomic replicon. J. Gen. Virol. 86, 3075–3080.
Tarr, A.W., Owsianka, A.M., Timms, J.M., McClure, C.P., Brown, R.J., Hickling, T.P., Pietschmann, T., Bartenschlager, R., Patel, A.H., Ball, J.K., 2006. Characterization of the hepatitis C virus E2 epitope defined by the broadly neutralizing monoclonal antibody AP33. Hepatology 43, 592–601.
Tong, X., Malcolm, B.A., 2006. Trans-complementation of HCV replication by non-structural protein 5A. Virus Res. 115, 122–130.
Tong, Y., Zhu, Y., Xia, X., Liu, Y., Feng, Y., Hua, X., Chen, Z., Ding, H., Gao, L., Wang, Y., Feitelson, M.A., Zhao, P., Qi, Z.T., 2011. Tupaia CD81, SR-BI, claudin-1, and occludin support hepatitis C virus infection. J. Virol. 85, 2793–2802.
Vanwolleghem, T., Meuleman, P., Libbrecht, L., Roskams, T., De Vos, R., Leroux-Roels, G., 2007. Ultra-rapid cardiotoxicity of the hepatitis C virus protease inhibitor BILN 2061 in the urokinase-type plasminogen activator mouse. Gastroenterology 133, 1144–1155.
Vercauteren, K., Van Den Eede, N., Mesalam, A.A., Belouzard, S., Catanese, M.T., Bankwitz, D., Wong-Staal, F., Cortese, R., Dubuisson, J., Rice, C.M., Pietschmann, T., Leroux-Roels, G., Nicosia, A., Meuleman, P., 2014. Successful anti-scavenger receptor class B type I (SR-BI) monoclonal antibody therapy in humanized mice after

challenge with HCV variants with in vitro resistance to SR-BI-targeting agents. Hepatology 60, 1508–1518.
Vogt, A., Scull, M.A., Friling, T., Horwitz, J.A., Donovan, B.M., Dorner, M., Gerold, G., Labitt, R.N., Rice, C.M., Ploss, A., 2013. Recapitulation of the hepatitis C virus life-cycle in engineered murine cell lines. Virology 444, 1–11.
Wakita, T., Pietschmann, T., Kato, T., Date, T., Miyamoto, M., Zhao, Z., Murthy, K., Habermann, A., Krausslich, H.G., Mizokami, M., Bartenschlager, R., Liang, T.J., 2005. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat. Med. 11, 791–796.
Washburn, M.L., Bility, M.T., Zhang, L., Kovalev, G.I., Buntzman, A., Frelinger, J.A., Barry, W., Ploss, A., Rice, C.M., Su, L., 2011. A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. Gastroenterology 140, 1334–1344.
Watanabe, T., Sugauchi, F., Tanaka, Y., Matsuura, K., Yatsuhashi, H., Murakami, S., Iijima, S., Iio, E., Sugiyama, M., Shimada, T., Kakuni, M., Kohara, M., Mizokami, M., 2013. Hepatitis C virus kinetics by administration of pegylated interferon-alpha in human and chimeric mice carrying human hepatocytes with variants of the IL28B gene. Gut 62, 1340–1346.
Weiner, A.J., Kuo, G., Bradley, D.W., Bonino, F., Saracco, G., Lee, C., Rosenblatt, J., Choo, Q.L., Houghton, M., 1990. Detection of hepatitis C viral sequences in non-A, non-B hepatitis. Lancet 335, 1–3.
Welker, M.W., Susser, S., Welsch, C., Perner, D., Fuller, C., Kronenberger, B., Herrmann, E., Zeuzem, S., Sarrazin, C., 2012. Modulation of replication efficacy of the hepatitis C virus replicon Con1 by site-directed mutagenesis of an NS4B aminoterminal basic leucine zipper. J. Viral. Hepat. 19, 775–783.
Whidby, J., Mateu, G., Scarborough, H., Demeler, B., Grakoui, A., Marcotrigiano, J., 2009. Blocking hepatitis C virus infection with recombinant form of envelope protein 2 ectodomain. J. Virol. 83, 11078–11089.
Wilson, E.M., Bial, J., Bial, G., Jensen, B., Greiner, D.L., Brehm, M.A., Grompe, M., 2014. Extensive double humanization of both liver and hematopoiesis in FRGN mice. Stem Cell. Res. 13, 404–412.
Wose Kinge, C.N., Espiritu, C., Prabdial-Sing, N., Sithebe, N.P., Saeed, M., Rice, C.M., 2014. Hepatitis C virus genotype 5a subgenomic replicons for evaluation of direct-acting antiviral agents. Antimicrob. Agents Chemother. 58, 5386–5394.
Wu, X., Robotham, J.M., Lee, E., Dalton, S., Kneteman, N.M., Gilbert, D.M., Tang, H., 2012. Productive hepatitis C virus infection of stem cell-derived hepatocytes reveals a critical transition to viral permissiveness during differentiation. PLoS Pathog. 8, e1002617.
Xie, Z.C., Riezu-Boj, J.I., Lasarte, J.J., Guillen, J., Su, J.H., Civeira, M.P., Prieto, J., 1998. Transmission of hepatitis C virus infection to tree shrews. Virology 244, 513–520.
Xu, X., Chen, H., Cao, X., Ben, K., 2007. Efficient infection of tree shrew (Tupaia belangeri) with hepatitis C virus grown in cell culture or from patient plasma. J. Gen. Virol. 88, 2504–2512.
Yanagi, M., Purcell, R.H., Emerson, S.U., Bukh, J., 1997. Transcripts from a single full-length cDNA clone of hepatitis C virus are infectious when directly transfected into the liver of a chimpanzee. Proc. Natl. Acad. Sci. USA 94, 8738–8743.
Yanagi St, M., Claire, M., Shapiro, M., Emerson, S.U., Purcell, R.H., Bukh, J., 1998. Transcripts of a chimeric cDNA clone of hepatitis C virus genotype 1b are infectious in vivo. Virology 244, 161–172.
Yang, D., Zuo, C., Wang, X., Meng, X., Xue, B., Liu, N., Yu, R., Qin, Y., Gao, Y., Wang, Q., Hu, J., Wang, L., Zhou, Z., Liu, B., Tan, D., Guan, Y., Zhu, H., 2014. Complete replication of hepatitis B virus and hepatitis C virus in a newly developed hepatoma cell line. Proc. Natl. Acad. Sci. USA 111, E1264–1273.
Yang, X., Haurigot, V., Zhou, S., Luo, G., Couto, L.B., 2010. Inhibition of hepatitis C virus replication using adeno-associated virus vector delivery of an exogenous anti-hepatitis C virus microRNA cluster. Hepatology 52, 1877–1887.
Yi, M., Lemon, S.M., 2004. Adaptive mutations producing efficient replication of genotype 1a hepatitis C virus RNA in normal Huh7 cells. J. Virol. 78, 7904–7915.
Yu, M., Peng, B., Chan, K., Gong, R., Yang, H., Delaney, W.t., Cheng, G., 2014. Robust and persistent replication of the genotype 6a hepatitis C virus replicon in cell culture. Antimicrob. Agents Chemother. 58, 2638–2646.
Zeisel, M.B., Felmlee, D.J., Baumert, T.F., 2013. Hepatitis C virus entry. Curr. Top.. Microbiol. Immunol. 369, 87–112.
Zhong, J., Gastaminza, P., Cheng, G., Kapadia, S., Kato, T., Burton, D.R., Wieland, S.F., Uprichard, S.L., Wakita, T., Chisari, F.V., 2005. Robust hepatitis C virus infection in vitro. Proc. Natl. Acad. Sci. USA 102, 9294–9299.