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Hepatitis C Virus Experimental Model Systems and Antiviral drug Research*

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Abstract: An estimated 130 million people worldwide are chronically infected with hepatitis C virus (HCV) making it a leading cause of liver disease worldwide. Because the currently available therapy of pegylated interferon-alpha and ribavirin is only effective in a subset of patients, the development of new HCV antivirals is a healthcare imperative. This review discusses the experimental models available for HCV antiviral drug research, recent advances in HCV antiviral drug development, as well as active research being pursued to facilitate development of new HCV-specific therapeutics.

Key words: Hepatitis C virus; Chronic liver disease; Experimental model systems; High throughput screening; Drug targets

Hepatitis C virus (HCV), a member of the Flaviviridae family of enveloped positive-strand RNA viruses, is a leading cause of liver disease worldwide. Although HCV infection is usually asymptomatic and 10-30% of infected individuals successfully clear the infection[2,145], ~70% of infections persist with the risk of progressive liver complications, such as fibrosis, cirrhosis, steatosis, insulin resistance, and/or hepatocellular carcinoma (HCC)[4,5,43,71,111,133,148,149], which can ultimately necessitate liver transplantation if HCV infection is not successfully treated (Fig. 1).

To date, combination pegylated interferon-alpha (pIFN-α) and ribavirin[52] is the licensed standard of care (SOC) treatment for HCV; however, several limitations restrict its use and efficacy. First, because viral, host, and environmental factors significantly affect the success of SOC treatment (reviewed in [162]), numerous contraindications limit the number of patients eligible for therapy. Of those who are treated, sustained virological response (SVR) is only achieved in ~80% of individuals infected with genotypes 2 or 3 and 40%-50% of individuals infected with genotypes 1 or 4[3]. In addition, therapy itself has a spectrum of toxic side effects and complications, which severely

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limit patient compliance and thus treatment efficacy [45]. Hence, with an estimated 130 million people worldwide chronically infected[4], and the number of HCV patients needing medical care is expected to increase dramatically over the next decade[171], the development of new more specific HCV antivirals is a healthcare imperative. This review will discuss the experimental models available for HCV antiviral drug research, the most recent advances in HCV antiviral drug development, as well as future research directions focused on developing new HCV-specific therapeutics.

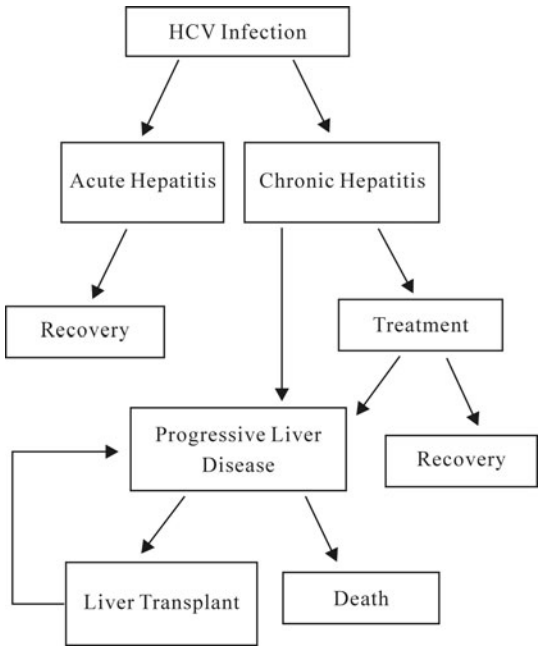

Fig. 1. Progression of HCV infection. HCV infection is successfully cleared by 10%-30% of individuals after a transient acute infection. However, at least 70% of infections result in long-term chronic infection. Chronic HCV infection can led to progressive liver disease such as fibrosis, cirrhosis, steatosis, insulin resistance, or hepatocellular carcinoma unless successfully treated. Ultimately, HCV-associated liver disease can necessitate liver transplantation. Unfortunately, for those who receive a liver transplant, allograft infection with HCV is universal with disease progression in the new liver significantly accelerated compared to pre-transplant rates.

HCV EXPERIMENTAL SYSTEMS AVAILABLE FOR ANTIVIRAL DRUG RESEARCH

Since it's discovery in 1989 as the causative agent on non-A non-B hepatitis[37], the HCV lifecycle and host-virus interactions that determine infection outcome have been difficult to study because experimental HCV cell culture infection systems and suitable small animal models have not been readily available. Consequently, the development of preventive vaccines and anti-HCV therapeutics has been severely hampered. Notably however, different model systems have now been developed and successfully used to study isolated aspects of the HCV lifecycle (Table 1) and three models in particular have significantly advanced our understanding of HCV and accelerated HCV antiviral development. These include the development of HCV replicons, HCV pseudotyped particles (HCVpp), and most recently infectious HCV cell culture systems, each of which is discussed in detail below.

HCV replicons

Shortly after the 1989 cloning of the HCV genome [37], full length HCV RNA was synthesized and shown to be infectious in chimpanzees after intrahepatic

Table 1. HCV Experimental Models

Experimental Models
HCV *in vitro* models
• HCV molecular clones [9,66,185]
• Expression systems [38,112,130,146]
• Subgenomic and full length replicons [21,22,75,84,118,167]
• Pseudotype particles (HCVpp) [10,72]
• Infectious cell culture-propagated HCV (HCVcc) [113,167,191]
HCV *in vivo* models
• Tree shrews, marmosets/tamarins [44,83,169,175,190]
• Chimpanzees [37,94]
• Immunotolerized rat model [173]
• Transgenic mouse model [69,86,92,107,127,136]
• Heterotopic liver graft mouse model [49,76,119]
• Hepatic xenorepopulation mouse model [19,89,90,143]
Surrogate systems
• GB virus B or bovine viral diarrhea virus [12,28]

inoculation[13,70,94,176,177,179] providing insights into salient aspects of HCV infection and pathogenesis [93,178] (reviewed in [29] and [100]); however, these consensus clones were found to be replication defective in cell culture, limiting in vitro HCV model development. This changed in 1999, when Lohman *et al* overcame the in vitro HCV replication barrier by devising a genotype 1b HCV replicon system[118] based upon previous autonomous replication models of other flaviviruses[14,88]. Specifically, selectable subgenomic HCV RNAs were engineered in which the region encoding the HCV structural genes, with or without NS2, was replaced by the selectable antibiotic resistant marker, neomycin phosphotransferase (Neo). Upon transfection into mammalian cells, Neo is translated via the HCV internal ribosome entry site (IRES), while the viral nonstructural (NS) proteins required to replicate the input template RNA are synthesized from the encephalomyocarditis virus (EMCV) IRES (Fig. 2). Antibiotic-resistant cell clones, harboring autonomously replicating HCV replicon RNA can then be selectively expanded.

Although it was discovered that robust HCV replicon replication required numerous cell culture adaptive mutations[21,98,116,117] which when cloned back into a full length construct resulted in an HCV genome incapable of producing infectious virus[8] or causing infection in chimpanzees following intrahepatic inoculation[30,139], HCV replicons have proven invaluable for the in vitro study of HCV replication and subgenomic replicons of several genotypes (e.g. 1a, 1b and 2a) as well as full-length replicons having been developed[21,22,75,84,167]. In addition to the identification of RNA elements and proteins involved in the viral replication process, HCV replicons have provided the means to characterize the viral replication complex at the biochemical and ultrastructural level with fluorescently-tagged viral proteins, such as a NS5A-GFP fusion, having allowed for visualization and tracking HCV replication complexes in living cells [155,172]. Most important to anti-HCV drug discovery and the study of HCV drug resistance, numerous replicon constructs with exogenous reporters, such as luciferase, secreted alkaline phosphatase, chloramphenicol transferase, beta-lactamase, or beta-galactasidase have facilitated the development of reporter-based HCV replication screening assays[53,67,98,129,182,183].

HCV pseudotyped particles (HCVpp)

To allow for the study of HCV entry, researchers created infectious pseudoparticles incorporating the HCV E1-E2 glycoproteins on murine leukemia virus (MLV) or HIV-1 retroviral core particles[10,72]. Although HCVpp only resemble HCV at the level of surface glycoprotein expression, their ability to mimic HCV entry has helped dissect the HCV entry process identifying numerous cellular entry factors and neutralizing epitopes on the viral glycoproteins [63,87,166,187]. With pseudotype particles of all six HCV genotypes now available encoding for various reporter proteins (e.g. GFP, β-galactosidase, or luciferase) the HCVpp system represents a focused tool for studying HCV entry across many HCV genotypes and thus will undoubtedly continue to facilitate the development of viral entry inhibitors, as illustrated by a 2009 report describing a luciferase-based HCVpp high throughput screening approach for the identification of HCV entry inhibitors[180].

HCV cell culture infection systems

The ability to recapitulate the entire viral lifecycle in vitro was finally achieved in 2005 when several groups

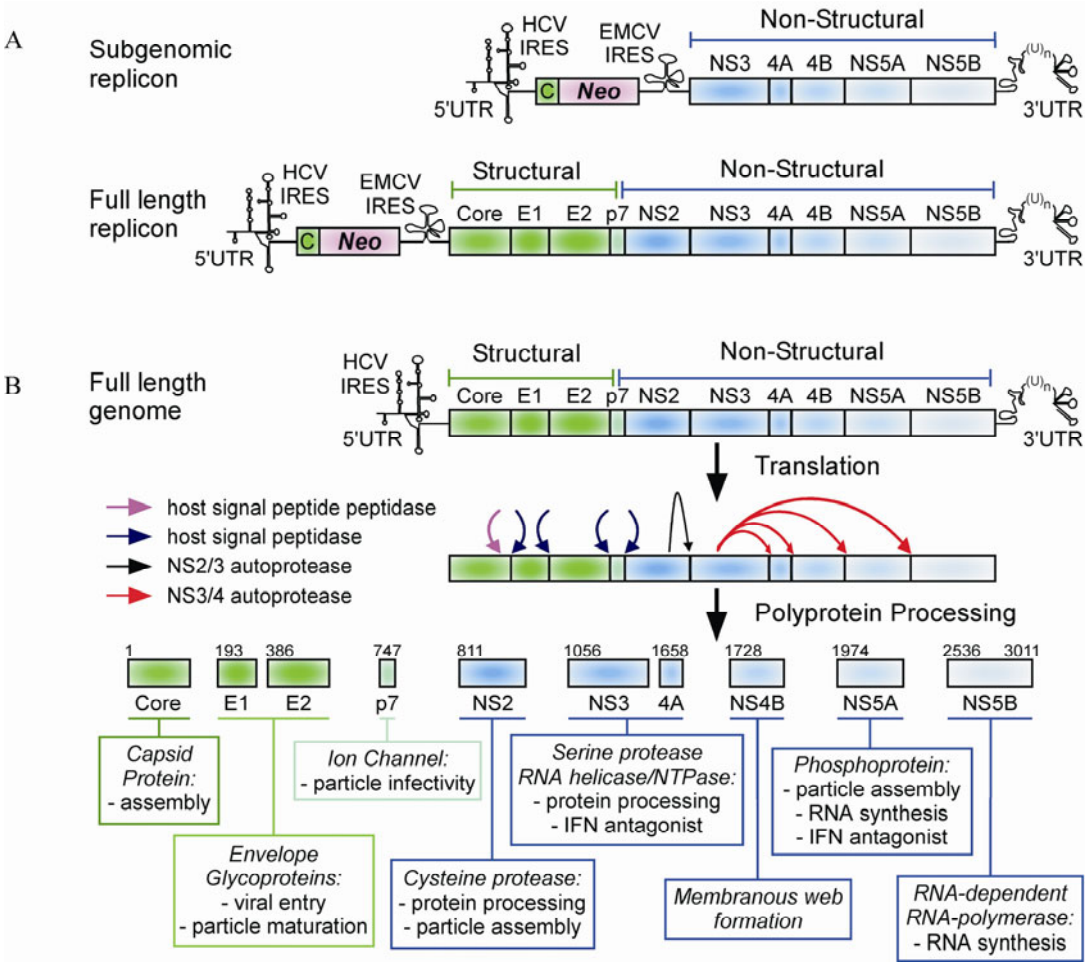

Fig. 2. HCV genomic structure and polyprotein processing. A: Diagram of subgenomic and full length bicistronic HCV replicons. B: Diagram of the HCV RNA genome. The ~9.6kb RNA encodes a single open reading frame for translation of an ~3010 amino acid polyprotein. Both viral and host proteases cleave this polyprotein into at least 10 viral protein products (Amino acid numbers are shown above each protein for HCV H77 strain; genotype 1a; GenBank accession number AF011752). Dark blue arrows denote cleavage sites by the endoplasmic reticulum signal peptidase, purple arrow indicates C-terminal processing of the core protein by signal peptide peptidase and black and red arrows indicate cleavages by the HCV NS2/3 and NS3/4a proteases, respectively. The structural proteins (blue) are located at the 5' end of the ORF, followed by p7 (aqua) and the non-structural (NS) proteins (green). Descriptive titles and known activities for each viral protein are listed.

reported robust HCV infection and production of infectious progeny HCV (termed HCVcc) in Huh7 human hepatoma cell cultures using the HCV genotype 2a JFH-1 consensus genome cloned from a Japanese Fulminant Hepatitis patient or derivative thereof[85,113,167,191]. These systems yield viral titers between 10^4-10^6 infectious units/mL allowing infection to spread throughout a culture within days after inoculation at low multiplicities of infection (MOI) and the serial passage of virus without loss in infectivity. Importantly, HCVcc is infectious in chimpanzees and primary human hepatocytes transplanted into SCID-uPA mice, and virus recovered from these animals is infectious *in vitro*[65,114].

With this tool to dissect the complete viral life cycle now available, high throughput screening (HTS) assays utilizing the infectious HCV cell culture system are rapidly being developed as a means to identify

new HCV-specific therapeutics targeting all aspects of infection. In 2008, Zhang *et al* published an HCVcc HTS assay, utilizing an HCVcc construct encoding a luciferase reporter gene[189]. Although HTS assays based on exogenous reporters are common, inherent issues such as specificity (i.e. effect of compound on reporter expression/function) and effects of foreign sequences on viral infection (e.g. decreases in HCVcc infection efficiency) can be problematic. To this end, several groups have recently developed HCVcc-based HTS systems, which do not depend on insertion of a foreign reporter into the viral constructs[77,128,135,186]. For example, Yu *et al*, (2009) recently developed a simple mix-and-measure cell-based HCV infection HTS assay based on an HCV NS3 protease FRET assay[186]. Unlike typical viral HTS assays that infect at a high MOI and are thus focused on a single cycle of virus replication, the assay described by Yu *et al*. (2009) incorporates a low MOI approach allowing inhibitors that target any aspect of the HCV lifecycle (e.g. entry, replication, assembly, egress and spread) to be detected over the course of several rounds of viral replication and spread[186]. Following this low MOI approach, Gastaminza *et al*, recently developed an HCVcc colorimetric assay based on immuno-staining with an anti-E2 antibody to identify 33 inhibitors of HCV at multiple lifecycle stages[51]; however, this approach is not as readily amenable to HTS as it requires numerous antibody incubations and washing steps. To circumvent the need for secondary toxicity screening, Chockalingam *et a*l, (2010) very recently reported the development of an HCVcc cell protection assay, which measures cell viability as a readout of anti-HCV compound activity. As such, viability indicates not only effective blocking of HCV infection, but also the lack of drug-induced cytotoxicity[35]. Using this approach, the authors successfully screened a 1280 compound library and identified 55 compounds targeting HCV at the level of entry, replication and virus production[35].

THE HCV LIFECYCLE AND POTENTIAL ANTIVIRAL DRUG TARGETS

HCV replicons, HCVpp, and most recently infectious HCVcc systems have advanced our understanding of the viral lifecycle highlighting numerous potential antiviral drug targets (Fig. 3), and each step of the HCV life cycle continues to be rigorously studied to identify and test additional HCV-specific therapeutics. While the term "Specifically Targeted Antiviral Therapy for Hepatitis C" (STAT-C) was originally coined to describe inhibitors that directly target HCV proteins[159], inhibitors directed against host proteins involved in HCV infection are also proving to be valuable drug targets and an unprecedented number of putative inhibitors specifically targeting each step of the viral lifecycle have already been identified and are currently being evaluated clinically (Table 2). The two types of STAT-C molecules that have progressed furthest in the pipeline are NS3/4a protease inhibitors [Telapravir (TVR; VX-950; Vertex) [64,122] and Boceprevir (BVR; SCH503034; Schering) [99,153]] and NS5B polymerase inhibitors (reviewed in [162]). The use of these compounds in combination with current HCV SOC have yielded promising results, and their clinical availability in the immediate future will certainly mark the beginning of an exciting period that is anticipated to revolutionize HCV therapy. Further improvements in treatment efficacy in the future will

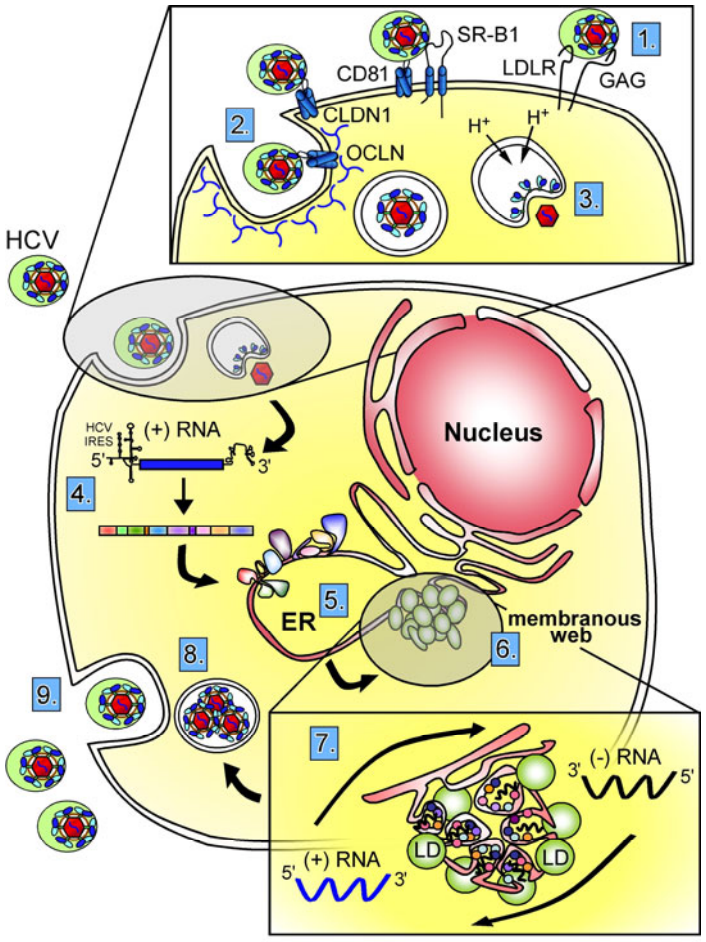

Fig. 3. The HCV Lifecycle. 1. Binding of the virus to cell surface receptors; 2. HCV entry into the cell via clathrin-mediated endocytosis; 3. Endocytic vesicle acidification and release of the viral genome into cytoplasm (i.e. fusion and uncoating); 4. IRES-mediated polyprotein translation; 5. Polyprotein processing; 6. Formation of viral replication complexes (i.e. the membranous web); 7. Viral RNA replication; 8. Packaging and assembly of progeny virions; 9. Virion maturation and release via the cellular secretory system. Although depicted separately, many of these viral functions likely occur concurrently and within close proximity. In particular, we show here lipid droplets (LD), which are thought to be the site of HCV particle assembly, closely associated with the membranous web; however the spatial relationship of these two viral activities remains to be determined. GAG, glycosaminoglycan; LDLR, low density lipoprotein receptor; SR-B1, scavenger receptor class B member I, CLDN1, claudin-1; OCLN, occludin; IRES, internal ribosome entry site; LD, lipid droplets.

likely also be achieved by increasing the repertoire of specifically targeted HCV therapies to include compounds that target additional viral and viral-host interactions. Thus, each step of the viral lifecycle (Fig. 3), each of the 10 viral proteins, and the viral RNA genome itself (Fig. 2B) represent potential drug targets for exploitation.

HCV entry

HCV infection begins with the binding of the viral particle to receptors on the host cell surface. Four cellular receptors have been shown to be necessary, but not sufficient for HCV entry, the tetraspanin protein CD81[10,72,140,174,191], the scavenger receptor class B member I (SR-B1)[10,60,82,154,188], and the tight junction proteins CLDN1[42] and OCLN[15,115,141] (Fig. 3, Step 1). Following a multi-step binding event, the viral particle is taken up into the cell via clathrin-coated mediated endocytosis[20,123] (Fig. 3, Step 2).

Table 2. Specifically Targeted HCV Inhibitors (*March 2010*)

Viral Lifecycle Step	Viral/Host Target	Agent	Phase
Entry	E1/E2	Civacir	2
	SR-B1	ITX5061	2
Protein Synthesis	NS3/4a	Telepravir	3
		Boceprevir	3
		TM 435	2
		MK 7009	2
		BI 201335	2
		R 7227 (ITMN-191)	2
		SCH 900518	2
		VX 813	1
		VX 985	1
		ABT 450	1
		VX 500	1
		PHX 1766	1
		ACH 1625	1
Genome replication	NS5B	R7128	2
		IDX 184	2
		ANA 598	2
		VCH 759	2
		GS 9190	2
		PSI-7977	2
		BI 207127	1
		A 837093	1
		PF 868554	1
		ABT 333	1
		VCH 916	1
		MK 3281	1
		PSI 7851	1
		VX 222	1
		R05024048	1
		IDX 375	1
		ABT-072	1
	NS5A	BS 790052	2
		AZD2836 (A-831)	1
		AZD7295 (A-689)	1
		PPI-461	pre
	NS4B	Clemizole	1
	miR-122	SPC3649	1
	cyclophilins	Debio025	2
		NIM811	2
		SYC635	1
	HMGCo-A	Statins	2
Assembly & Secretion	Glucosidases	Celgosivir	2
	LDL secretion	Naringenin	pre

Within the acidified endosomal compartment E1/E2-mediated class II fusion occurs between the virion envelope and the endosomal membrane[11,50,61,72,97,103,104,164] resulting in nucleocapsid release into the cell cytoplasm (Fig. 3, Step 3). Although HCV entry is still incompletely defined, the identification of required entry factors and development of neutralizing antibodies against the virus[26,31,105,125] has permitted the in vitro discovery of a number of novel HCV entry inhibitors which may prove to be effective clinically.

For example, the compound Terfenadine has been shown to interfere with the HCV-E2-CD81 receptor interaction[68]. Likewise, serum amyloid A may similarly function by binding to SR-B1 preventing HCV-SR-B1 interactions[32,102]. Post binding inhibition of HCV membrane fusion in vitro has been reported with the broad-spectrum antiviral compound, Arbidol [24,25,137]. More recently, post-binding inhibition was demonstrated both *in vitro* and *in vivo* in the chimeric mouse model using amphipathic DNA polymers, which are hypothesized to block viral entry at the level of virion internalization and/or fusion[121]. Notably, however, several entry inhibitors are already at the clinical trial phase including the human hepatitis C immune globulin Civacir (Nabi Biopharmacueticals)[39] and the SR-B1 inhibitor ITX5061 (iTherX Pharmaceuticals)[120], both of which inhibit HCV entry at the level of virion-receptor interactions.

Viral protein synthesis and processing

The ~9.6 kb viral RNA genome released into the cell cytoplasm encodes a single open reading frame flanked by highly structured 5' and 3' untranslated regions (UTRs). The 5' UTR contains an IRES that is required for translation of a ~3010 amino acid viral polyprotein (Fig. 2B and Fig. 3, Step 4), which is co- and post-translationally cleaved by cellular and viral proteases into mature structural and NS proteins (Fig. 2B and Fig. 3, Step 5). Past efforts to block translation of the HCV polyprotein involved targeting the viral IRES with antisense oligonucleotides, ribozymes, and siRNAs, but the compounds that entered clinical trials were halted due to lack of response and/or toxicity. However, the positive clinical performance of NS3/4a protease inhibitors highlights viral protein production as an effective therapeutic target. Compounds that block the viral NS3/4a protease inhibit polyprotein cleavage and thus HCV infection by prevent the processing needed to generate the individual viral proteins required for viral replication. In addition, inhibition of NS3/4a activity reduces NS3/4a-mediated cleavage of the host IFN simulating proteins Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) and IFN α promoter stimulator (IPS-1, CARDIF, VISA), and thus also restores certain aspects of the host cell innate immune response which is otherwise suppressed by NS3/4a[46,48,108,110]. As such, protease inhibitor development has progressed with two promising protease inhibitors currently in Phase III clinical trials.

Viral replication

Once expressed, the viral proteins NS3 to NS5B, directed at least in part by NS4B[57], assemble on and remodel cytoplasmic ER membranes to form viral RNA replication complexes, which have been termed the membranous web[54] (Fig. 3, Step 6-7). Within this complex, the viral NS5B RNA-dependent RNA polymerase first functions to synthesize complementary negative-strand RNA from the positive RNA genome template. The newly synthesized negative-strand RNA then provides the template for NS5B amplification of ~10-fold excess positive-strand genomic RNA. Currently, six NS5B inhibitors are in Phase II clinical trials (Table 2). Meanwhile, accumulating data continues to reveal that HCV RNA replication is a highly complex process, dependent on both viral and host proteins (reviewed in [23]), all of which could potentially serve as antiviral targets. In fact, the viral hydrophilic phosphoprotein, NS5A is required for HCV RNA replication, most likely via association with host cell proteins[73]. Notably, with

the expansion of siRNA and proteomics technologies the list of host cell proteins involved in HCV replication is rapidly expanding[16,17,33,109,161,163]. As such, compounds which block viral replication by inhibiting other HCV NS proteins or targeting host cell proteins shown to be essential for viral replication are also in development. Those currently in clinical trial (Table 2) include NS5A inhibitors (Bristol Myers Squibb)[106], the NS4B inhibitor Clemizole (Eiger BioPharmaceuticals)[41], HMG Co A reductase inhibitors[74,168,181], cyclophilin inhibitors [e.g. DeBIO-025 (DebioPharm), SCY-635 (ScyNexis) and NIM 811 (Novartis)][47,134], and the liver-specific microRNA miR-122 inhibitor LNA-antimiR/SPC3649 (Santaris Pharma)[81,101], but it is anticipated many more will follow.

Viral assembly and release

The details of HCV virion assembly, maturation and egress are the least understood, as they have only recently become amenable to systematic study with the development of the HCVcc infection system. Nonetheless, based on other flavivirus systems[170], encapsidated HCV virions are believed to become enveloped with E1/E2 bearing cellular membranes upon budding into the ER. Progeny virions are believed to then exit the cell via the secretory pathway, during which glycosylation of viral glycoproteins and association with cellular lipoproteins (e.g. very-low density lipoproteins (vLDL)) occurs. (Fig. 2, Steps 8-9). As the viral proteins and viral-host interactions that mediate capsid assembly, envelopment, and virion maturation are elucidated a plethora of promising drug targets are being identified. In terms of viral targets, a screen to identify compounds that inhibit HCV core dimerization was recently reported[95] and other viral proteins, such as p7[58] and NS2[79,80], are also emerged as essential factors in viral assembly and release. In terms of cellular targets, host factors involved in lipoprotein secretion are prime candidates[144] and it has been shown that infectious HCVcc particle egress is blocked by the vLDL secretion inhibitor naringenin found in grapefruit[131]. One type of HCV virion maturation inhibitor in clinical trials are compounds targeting the cellular α-glucosidase I and II enzymes involved in glycoprotein processing. Because viruses tend to be more sensitive to decreases in the activity of these enzymes than the host cell, α-glucosidase inhibition can be employed to induce misfolding of the HCV envelope glycoproteins preventing virion maturation and release. For example, iminosugars have been shown to inhibit α-glucosidases, resulting in anti- HCV activity in vitro[158]. Celgosivir (Mignex), a prodrug of castanospermine, has also been shown to inhibit α-glucosidase I[40] and has reached Phase II clinical trials.

THE FUTURE OF HCV ANTIVIRAL RESEARCH

Although the development of useful experimental model systems has ushered in a new productive era of HCV molecular virology research and drug discovery, there are still challenges to overcome.

Cell culture infection with other HCV genotypes

Robust HCV infection in cell culture has only been achieved with genotype 2a derivative clones. While attempts to propagate the infectious genotype 1a H77 clone[184] and an infectious genotype 1b virus in cell culture[139] have resulted in detectable HCV levels, de novo virus production was low, limiting the utility of the systems. We do not fully understand the restriction that prevent efficient propagation other HCV

genotypes in vitro, but studies have mapped the 3' end of the JFH-1 clone as sufficient to confer replication permissiveness to other HCV clones, therefore chimeric HCV genomes containing these necessary 3' region of the JFH-1 clone recombined with the corresponding 5' regions from genotypes 1-6 have been developed[55,56,78,138,156].

Physiological relevant hepatocyte cell cultures

In addition to expanding the repertoire of available infectious HCV clones, improvements in hepatocyte cell culture is required as well. To date, robust HCV infection has only been published in one continuous human hepatoma cell line, Huh7[96,113,132,150]. Studying HCV infection in Huh7 cells has certainly expanded our ability investigate the viral life cycle; however, Huh7 cells are transformed and only marginally mimic the state of hepatocytes in vivo[126,160], which limits our ability to elucidate how HCV interacts with and induces alterations in hepatocytes in vivo to produce clinically observed HCV-associated liver disease. To address this issue, novel two-dimensional[151] and three-dimensional[152] culture systems have been developed to coax Huh7 cells to up-regulate hepatocyte-specific transcripts, become Phase I and Phase II drug metabolism competent[36], and exhibit more specific localization of tight junction, cell adhesion, and polarity markers[152]. While these systems may prove useful in understanding how HCV interacts with polarized cells or disrupts specific aspects of hepatocyte physiology, Huh7 cells by nature remain transformed. As such, primary liver cell cultures remain the most physiologically relevant in vitro model for the study of HCV, and numerous laboratories are trying to develop ways to adapt primary human hepatocytes for HCV research[6,27,192]. One recent publication of note describes a human hepatocyte cell culture system permissible to infection with patient serum of HCV genotypes 1, 2, 3 and 4 mimicking the kinetics of HCV infection in humans and producing infectious virions that can infect naïve human hepatocytes[27]. More recently, HCV infection was established in micropatterned co-cultures (MPCCs) of primary human hepatocytes with supportive stroma[142], which is relevant to antiviral screening because MPCCs can be readily scaled down to a multi-well format for HTS when coupled with appropriate fluorescence- and/or luminescence-based reporter systems. Together, these systems represent important advances in the use of primary human hepatocytes in HCV research and should complement the existing Huh7-based HCV systems by facilitating the understanding of the effects HCV has on it natural host cell, but it remains to be determined if widespread use of these systems will be possible.

HCV small animal model development

The most significant obstacle impeding the preclinical testing of new HCV therapeutics and development of vaccines is the lack of HCV small animal models (reviewed in [143]). Efforts to develop small animal infection models have included trying to transmit HCV to tree shrews [175,190], marmosets/ tamarins[44,83,169], and other primates[1], but only the chimpanzee model of HCV infection, has proven efficacious[93,178]. The species barriers that prevent HCV infection in non-human hosts are not completely defined, but include blocks in viral entry and non-permissiveness for HCV RNA replication. To avoid issues of infectivity and simply study the effects of HCV proteins *in vivo*, several non-replicating

transgenic mouse lineages that express one or more of the HCV proteins have been created[69,86,92,107,127,136]. Although there is a great deal of variation in the HCV protein expression levels observed in these models and in the resulting pathology, these mice have demonstrated that expression of specific HCV proteins at least under some circumstances can induce disturbances in lipid metabolism and possibly contribute to the development of HCC, but it remains unclear how the effects observed in these expression systems relate to clinically observed HCV pathology.

To eliminate issues of transgene expression levels and create a more authentic small animal model of HCV infection, hepatic xenorepopulation approaches have been used to study HCV infection *in vivo* [19,49,59,76,90,91,124,147,157] The basis of these models involves transplanting primary human hepatocytes into immunodeficient mice that loose their endogenous hepatocytes due to either a lethal hepatocyte transgene [49,62,76,147,157] or a hepatotoxic defect in an essential liver enzyme [7,18] allowing the transplanted HCV-permissive human hepatocytes to repopulate the mouse liver. While this approach has been invaluable for HCV antiviral drug development as a means of confirming in vivo efficacy in a true infection model, creating and working with these chimeric mice is technically challenging (reviewed in [89]) making the model impractical for widespread use.

To try and develop an HCV-permissive infectious mouse model, we and others have shown that HCV JFH-1 subgenomic replicons and full length genomes can replicate in mouse cells[34,165] indicating that a species block at the level of HCV RNA replication can be overcome, but that additional blocks in post-replication steps involved in progeny virus production may exist[165]. In terms of HCV entry, Ploss *et al*., recently determined the species-specific determinants for HCVpp entry into mouse cells to be human CD81 and human occludin, as expression of these two human receptors in murine fibroblast cell lines resulted in permissiveness for HCVpp[141]. Notably however, infectious HCVcc entry could not be demonstrated suggesting additional entry factors are required for HCVcc entry into mouse cells. Hence, although we have made advancements towards the development of an HCV-permissive mouse model, this goal has yet to be achieved.

CONCLUSIONS

The lack of targeted HCV-specific treatments to date has hindered the success of HCV treatment strategies. However, the HCV experimental models and HTS assays described in this review have already led to the discovery of numerous potential HCV therapeutics, and ongoing efforts to improve and expand upon these experimental models will continue to benefit the HCV antiviral drug development effort. As such, new HCV therapeutics are on the horizon and promise a future in which effective HCV treatment may be achieved with a “cocktail” approach comprised of compounds that specifically target the virus and critical viral-host interactions.

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