

# Inhibition of the replicative cycle of hepatitis C virus

Richard Bethell, George Kukolj and Peter W. White

It is estimated that 170 million people globally are infected with the hepatitis C virus (HCV). Chronic HCV infection can result in the development of liver cirrhosis and hepatocellular carcinoma, and therefore represents a substantial public health problem. Current treatment for patients infected with HCV is the combination of pegylated interferon-γ and ribavirin, a treatment that can achieve a sustained virological response, that is, a longterm clearance of detectable virus from the plasma. However, both drugs

have poor safety profiles, resulting in their contraindication in many patients, and have limited effectiveness, especially against HCV genotype 1. As a result, there has been considerable interest over the past 15 years in identifying specific inhibitors of HCV replication that could be used either as an adjunct to current therapy or in place of it. This poster summarizes the replicative cycle of HCV and the main targets for specific antiviral agents that are currently being developed.



All proteins encoded in the small HCV genome are essential for

viral propagation. Small molecules that directly or indirectly

inhibit NS4A and NS5A function are in development, although

been demonstrated yet. Small molecules that bind to the

no direct interactions of the molecules with these proteins have

internal ribosome entry site (IRES), p7 and NS4B have also been

reported, but none has yet reached development; development

host-encoded targets have been identified, of which cyclophilin A

of antisense RNAs specific for the IRES has not yet led to a

proof of principle in clinical studies. In addition, a number of

is the most advanced. Non-immunosuppressive analogues of

cyclosporin A that form complexes with cyclophilin A and

do not inhibit calcineurin have clinical antiviral activity

Other possible drug targets

against HCV<sup>10</sup>.

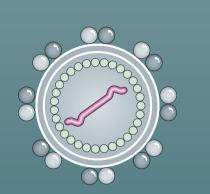
## Viral entry targets

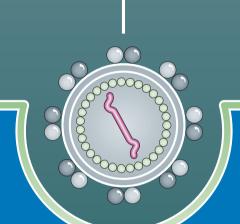
Cell surface contact. The heterodimeric E1 and E2 proteins are expressed on a virus envelope that is associated with low and very low density lipoprotein (vLDL)1. Initial host cell contact is mediated by interaction with the low density lipoprotein receptor and/or glycosaminoglycans. E2 also binds to DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) and L-SIGN (liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin), which is expressed on hepatic endothelial cells and may augment infection<sup>1</sup>. The asialoglycoprotein receptor (ASGP-R) may also interact with HCV structural proteins.

High-affinity receptors and entry. The virus subsequently binds to high-affinity receptors, including scavenger receptor class B type I (SR-BI) and the tetraspanin protein CD81, through the ecto-domain of E2. It then binds to claudin 1 (CLDN1) and occludin<sup>1,2</sup>, both of which are expressed at tight junctions of polarized hepatocytes. Internalization of the virus proceeds through clathrin-dependent endocytosis, and viral membrane fusion takes place in the acidified endosome. This releases the viral core into the host cytosol, where uncoating and disassembly of the virus capsid releases the RNA genome.

## **HCV** morphogenesis targets

HCV assembly starts with recruitment of the core protein and NS5A to the surface of lipid droplets, followed by delivery of HCV RNA from the HCV replication complex to the nascent vira particle. The subsequent intracellular assembly of HCV particles resembles vLDL formation: it takes place at the endoplasmic reticulum and requires apolipoprotein B and the microsomal triglyceride transfer protein (MTP), and leads to the formation of particles with a range of buoyant densities<sup>6,7</sup>. Those particles with a low buoyant density are secreted through the secretory system and circulate in association with triglycerides and lipoproteins B and E, whereas those with higher buoyant densities appear to be targeted for intracellular degradation7. Other HCV proteins, such as p7, NS2 and NS3, participate in virus assembly, and could form additional targets for therapeutic intervention<sup>8,9</sup>.





Target Function Challenges **Attractive** features

Core packaging acids)

> Ion channel or p7 recently

> > Crystal structure Development of of C-terminal cysteine protease domain solved; cysteine

molecule inhibition of activity not shown successfully One class No structural information known, except that the

autoproteolysis

challenging; small

central portion

demonstrated

binds to NS3. Direct

binding of ligands

C-terminal region

assays is

protease structure; of clinically membrane anchoring of replication

Cysteine

Part of NS3

autoprotease

(217

amino

acids)

(54

inhibitors selects for resistance mutations in NS4A, suggesting has not been that this may be target

Possibly, formation ATPases and Hydrophobic and GTPases have previously been targeted

N-terminal half

clinical validation

achieved for at

least one

poorly structured protein a challenge for biochemical successfully; analysis NS4B inhibitors reported recently

NS5A Possibly,

regulation of RNA replication and assembly; multiple viral-viral and viral-host protein

of membranous

web structure

and assembly

of replication

NTPase

complex and/or

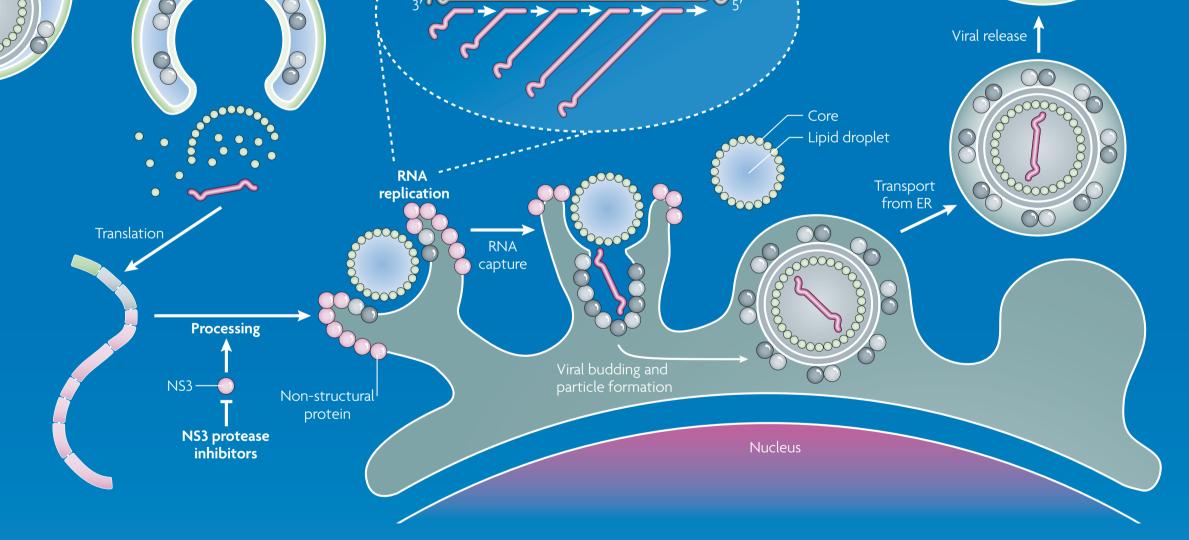
not well structured in vitro; direct crystal structure published. Many binding of ligands to protein has not viral replication inhibitors select been demonstrated for resistance in NS5A and strong

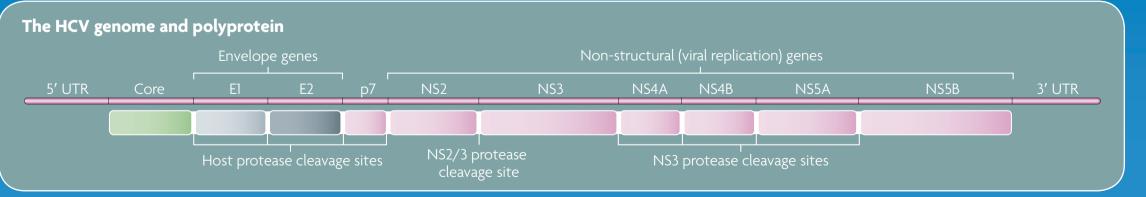
## **RNA** replication targets

NS3 protease helicase. The amino-terminal third of the NS3 protein possesses serine protease activity that is essential for viral replication. This is the target of BILN 2061, the first small molecule inhibitor of HCV to be tested in humans<sup>3</sup>. Two other protease inhibitors, telaprevir and boceprevir, are in Phase III clinical trials and may become the first anti-HCV drugs, whereas several other protease inhibitors are in earlier-stage human trials⁴. The carboxy-terminal two-thirds of NS3 constitute the helicase domain, which is also essential for the replication of viral RNA. The helicase domain might stimulate the activity of NS5B polymerase, resolve RNA secondary structure immediately prior to replication by the polymerase and/ or separate newly synthesized double-stranded RNA into separate positive and negative strands. No helicase inhibitors are currently reported in clinical trials or advanced stages of preclinical development, although this is an active area of research.

NS5B polymerase. The NS5B RNA-dependent RNA polymerase catalyses viral RNA synthesis and genome replication. lt shares a common fold with other nucleic acid polymerases with characteristic thumb, finger and palm domains<sup>5</sup>. A well-conserved active site for nucleotide and metal cofactor binding allows for selective incorporation of nucleoside analogues, a promising class of anti-HCV compounds. Several pockets form binding sites for allosteric inhibitors that interfere with conformational changes required during the initiation of RNA synthesis. Two loops ( $\lambda 1$  and  $\lambda 2$ ) bridge the fingers and the top surface of the thumb domain and

might regulate RNA binding. Benzimidazole and indole-based inhibitors prevent the association of the N-terminal finger loop with the thumb domain by binding to thumb pocket 1, which is situated at the top of the thumb domain. Thiophenecarboxylic acid and dihydropyrone-based inhibitors bind to thumb pocket 2 at the base of the thumb domain, proximal to thumb pocket 1, and induce conformational changes that prevent the initiation of RNA synthesis. Two overlapping pockets, palm site 1 and palm site 2, are located near the enzyme active site. Palm site 1 binds benzothiadiazine and acylpyrrolidine analogues, whereas benzofuran-based derivatives bind to palm site 2.





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For further reading, see http://www.nature.com/ nrmicro/posters/hepatitis-c/

## Supplementary information S1 | Further reading

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