Molecular Biology of Hepatitis C Infection

Kenneth E. Drazan

Hepatitis C infection (HCV) is an emerging epidemic. Liver specialists are managing this disease with limited scientific information about the underlying pathogenesis and treatment. The current review offers a molecular dissection of infection, a snapshot of the HCV life cycle, and emerging strategies for antiviral therapy. ***(Liver Transpl 2000;6:396-406.)***

Persistent infection with hepatitis C virus (HCV) is the predominant cause of chronic liver disease in the United States, and the World Health Organization recently estimated that 3% of the world's population has been infected with this pathogen.[1] This statistic suggests those 170 million chronic carriers of HCV are at risk for developing cirrhosis and/or liver cancer. Together with alcoholic liver disease, hepatitis C is the most common cause of cirrhosis and the major indication for liver transplantation in the United States. After liver transplantation, recurrence of HCV infection is common, but in many cases, the hepatitis is mild and the long-term survival rate averages 65% after 5 years.[2] At the current rate of infection, the pool of patients with chronic hepatitis C would increase by 4% in the next decade. At the current attack rate of infection, the number of deaths caused by HCV in the United States is estimated at 8,800/yr, but by the year 2008, this may reach 35,000.[3]

Despite increasing experience in the medical and surgical management of HCV-related disease, this pathogen, its biological characteristics, and the tempo of recurrence using a varying immunosuppressive cocktail are not completely understood. These notions compel liver transplantation physicians to rapidly familiarize themselves with HCV. Toward this end, this review integrates current knowledge of the molecular mechanisms of HCV pathogenesis.

Toward the end of the 1980s, a significant number of cases of parenterally transmitted viral hepatitis could not be ascribed to any of the hepatic viruses known at that time (hepatitis A, hepatitis B, and hepatitis delta viruses). The discovery and characterization of a novel RNA virus with characteristics typical of the family *Flaviviridae*[4] provided the tools to study the epidemiological importance of this agent in these cases of Non A Non B hepatitis (NANBH). The virus, known as HCV, was shown to be the cause of most cases of NANBH. More than 50% of the patients become chronic carriers.[5] The persistent infection commonly results in chronic active hepatitis, which may lead to liver cirrhosis and hepatocellular carcinoma.[6,7] Random screening of blood donor populations has indicated as many as 500 million chronic carriers of the virus worldwide, highlighting HCV as a major human pathogen.[8]

Biological Process of Infection

To discuss the relevance of antiviral therapy and its future, it is necessary to first review current knowledge of the virus. Unfortunately, approaches to the development of in vitro replication systems for propagation of this virus have been particularly difficult, and reliable systems for efficient long-term virus replication are irreproducible. Nevertheless, reports of virus replication have been made using systems from hepatic tissue[9] and peripheral-blood mononuclear cells.[10] In vitro infection systems have been reported from human T- and B-cell lines,[11,12] human fetal liver cells,[13] and chimpanzee hepatocytes.[14]

One of the most promising in vitro infection systems reported to date uses human hepatoma cells in which a subgenomic portion of the virus persisted for up to a year postinfection.[104] Additional evidence was reported by Sun and Feitelson[15] that HepG2 cells are tropic and supportive of a consensus HCV RNA genome. Data reported by Mizutani et al[16] and Sugiyama et al[17] using the human T-lymphotrophic virus type 1–infected T-cell (HTLV-1) line, MT-2, also supported viral expression. In these studies, there was apparent selection from the viral quasispecies of a single genomic sequence, presumably representing a virus naturally adapted for growth in this cell line. Whether the viruses in these studies, which are apparently adapted for tissue culture growth, can replicate in other cell lines remains to be established.

From the Liver Transplant Program, Stanford University, Palo Alto, CA.

Address reprint requests to Kenneth E. Drazan, MD, 750 Welch Rd, Suite 319, Palo Alto, CA 94304. Telephone: 650-498-5688; FAX: 650-498-5690; E-mail: kdrazan@leland.stanford.edu

1527-6465/00/0604-0001$3.00/0

doi:10.1053/jlts.2000.6449

Animal models of HCV infection have been as challenging to devise as the dissection of the virus itself. The chimpanzee was recognized early as a useful study subject in the pathogenesis of HCV. Early in the field of HCV research, the chimpanzee model proved critical to the determination of the physicochemical analysis of the virus. For instance, treatment of infectious human sera with lipid solvents led to viral inactivation, thus identifying its harbored lipid envelope.[18] The model was also used for evaluating a number of procedures for inactivating the virus in biological products, such as formalin, chloroform, heat, and ultraviolet radiation.

The greatest benefit from the chimpanzee model was making genetic characterization of the virus possible. In this model, the nonhuman primate was used as an in vivo propagation system to achieve sufficient quantities of virus to perform sequencing and mutational analysis.

Despite its cost and obvious limitations, information from nonhuman primates has led to a plethora of critical molecular observations. A brief list of these achievements follows: (1) genetic drift occurs in the hypervariable region (HVR) of the envelope proteins, (2) antibodies directed against these epitopes may be protective in naive subjects, (3) regions of the viral genome critical to in vitro infection are also essential in live subjects, and (4) the nature and tempo of the early cellular immune response dictates viral eradication versus chronic infection. A recent presentation by Barry et al[19] from Stanford University on the use of complementary DNA microarray (gene chip) technology showed a pattern of genetic responses within the livers of chimpanzees inoculated with HCV. In this provocative report, the evaluation of hepatic messenger RNA after HCV infection (24 hours) showed a classic inflammatory response, angiogenesis, and attenuation of extracellular matrix degradation. Later time points (3 days) confirmed a T-lymphocyte presence but an attenuated CD4 response.

Two other novel systems are worth mentioning, although their contributions remain to be seen. Xie et al reported on the successful infectivity of primary hepatocytes obtained from the tree shrew, *Tupaia belangeri*, a small primate, using HCV-positive serum. The study only examined short-term parameters, but the prospect of a small-animal model for such studies is hopeful.[20] A unique murine system, referred to as the Trimera Mouse System, has been devised at the Weizmann Institute of Science for the in vivo analysis of human viruses.[21] In this experimental model, normal mice are prepared for implantation of normal or virally infected liver by lethal irradiation, followed by severe combined immunodeficiency mouse bone marrow reconstitution. The durability of this system to show HCV in the serum extended to greater than 30 days.[105] The system is currently being used for the evaluation of antiviral compounds and neutralizing antibody preparations.

Examination of the viral particle itself has shed light on a potential mechanism for viral entry into human tissue. The size of the viral particle (virion) was previously estimated at 80 nm by ultracentrifugation,[22] whereas filtration studies suggested 30 $\pm$ 60 nm.[23] Several studies have shown that the buoyant density of HCV in infectious serum is less than that in noninfectious serum,[24] and the density (1 $\pm$ 06 g/mL) suggested an association with the low-density lipoprotein (LDL) fraction,[25] which was subsequently verified by Prince et al.[26] Typically, HCV-infected serum also contains a higher density fraction in which the virus isadherent to immunoglobulins.[24] As expected, virus in the lipoprotein fraction appears to be relatively more infectious than the antibody-coated fraction. Using very low-density lipoprotein (VLDL)–associated virus, highly purified particles have been observed that appear to have prominent spikes on their surface,[27] probably representing glycoprotein portions of the viral lipid coat. The observation of an association between HCV and lipoproteins may be critical for the virus replication cycle because LDL and VLDL are taken up by hepatocytes through the LDL receptor (LDLR, discussed next).

Identifying the HCV Receptor

Insight into the mechanism by which HCV gains entry into host cells is critical to understanding primary infection and reinfection posttransplantation. Current research has focused on 2 molecular ligands as loci for viral attachment and entry into human tissue (Fig. 1). As previously mentioned, an early observation in the study of HCV is its association with the lipoprotein fraction of human sera. The observation that HCV is associated with LDL,[26] VLDL,[26,28] and immunoglobulins[24,25,29] has led to the assumption that these complexes favor binding of the virus to target cells. LDL is internalized by LDLR-mediated endocytosis.[30] The LDLR is the main representative of a family of cell-surface receptors (LDLR gene family) that share characteristic sequences; for example, a ligand-binding domain.[31] Members of the LDLR gene family function as receptors for minor-group common cold virus[32] and subgroup A Rous sarcoma virus.[33] This suggests the

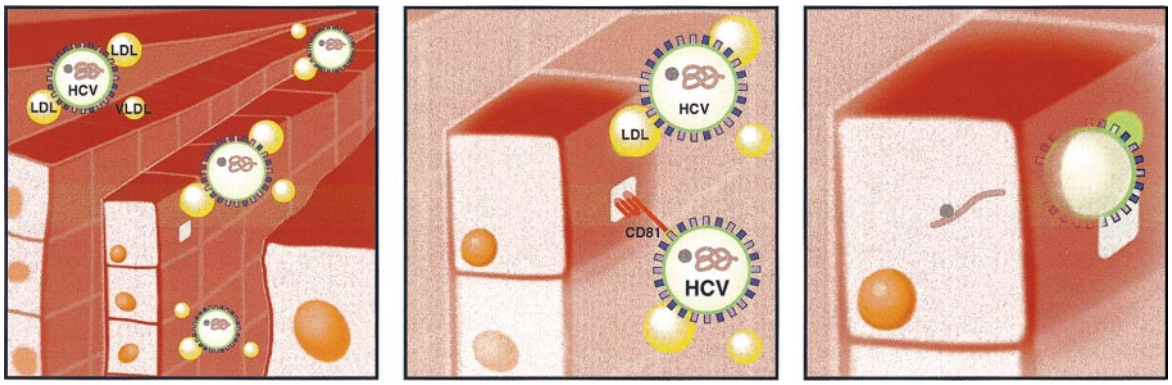

Figure 1.

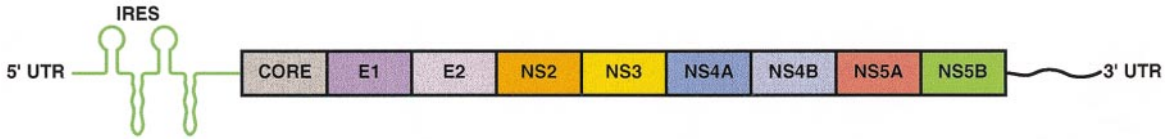

Figure 2.

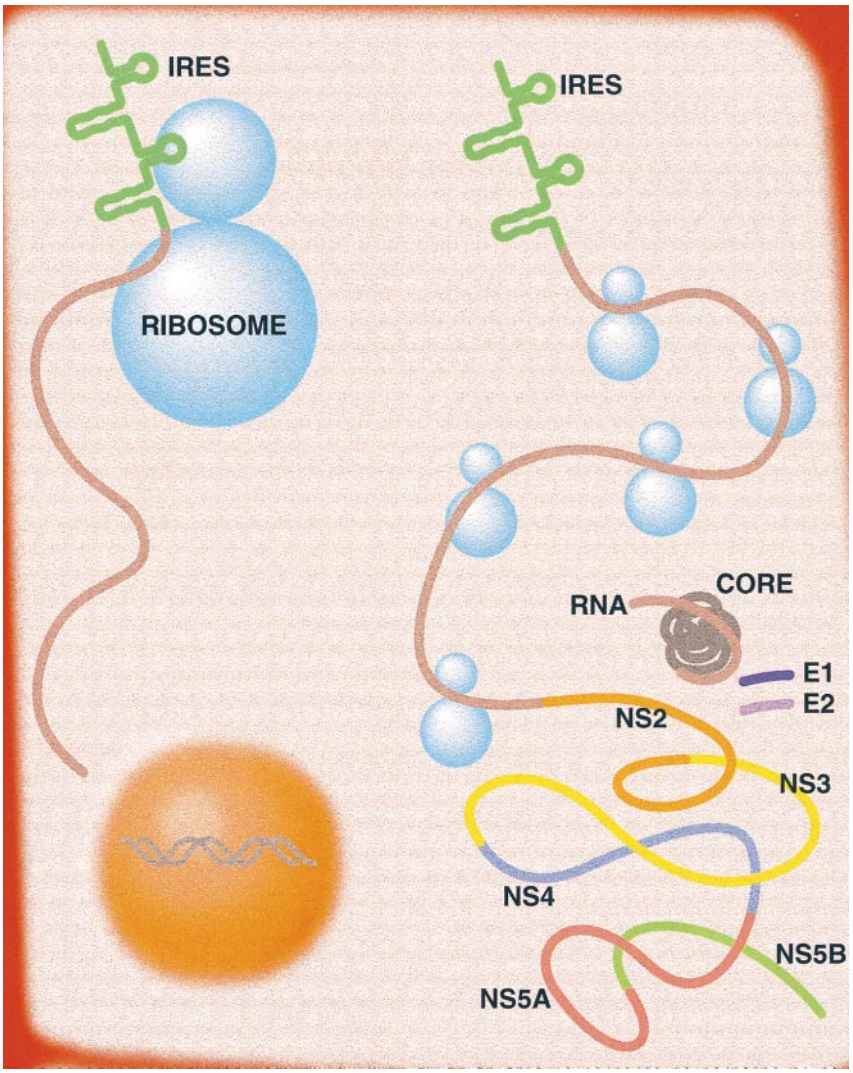

Figure 3.

possibility that the LDLR (or members of the gene family) might also function as a receptor for HCV. Although these studies provide firm evidence for HCV virion binding to the LDLR, internalization through this ligand was not shown.

More convincing evidence was presented in a recent study in which cells and mice null for LDLR that were resistant to viral entry were rendered susceptible to infection by genetic engineering with the human LDLR.[34] At present, it must be considered that other receptors for HCV belonging to the LDLR gene family or others exist that may also mediate the binding and uptake of HCV.

A second suspect responsible for HCV tropism is CD81.[35] The HCV envelope protein, E2, binds human CD81,[36] a ubiquitously expressed transmembrane molecule belonging to the family of tetraspanins. CD81 expression on B lymphocytes has been shown to be critical for HCV envelope protein E2 binding.[37] Does this prove that CD81 is the cellular receptor for HCV? Certainly not, but the findings of CD81 localizing with HCV has interesting implications for the coexistent syndromes manifest in patients with chronic active hepatitis C. HCV infection is associated with B-cell lymphoproliferative disorders, such as mixed-type II cryoglobulinemia[38] and non-Hodgkin's lymphoma.[39] On B cells, it has been observed that CD81 is a member of a signaling complex which regulates the threshold for B-cell activation and proliferation. Could a population of individuals exist with CD81 polypmorphisms such that they may be resistant to HCV tropism? Unfortunately, no clinical studies to date have identified such individuals. Although murine models of CD81 knockouts have been developed without lethality,[40] testing HCV infection in this experimental model is untenable because of lack of species tropism.

←

Figure 1. HCV particles are associated with LDL and VLDL molecules in the circulation of infected humans. In addition, the LDL receptor has been identified as capable of transporting HCV particles across the cell membrane. CD81 is a ubiquitously expressed tetraspan molecule that also associates with HCV. The viral envelope protein, E2, binds 1 of the extracellular domains of CD81; however, the consequence of this discrete interaction is less well understood. After viral binding to its cellular receptor, HCV sheds its lipid envelope to deliver its genetic load (RNA) and structural proteins into the cytosol.

Figure 2. The organization of the hepatitis C genome is similar to other RNA viruses, such as polio and pestivirus. The genome is 9.5 kb in length and has a single internal coding and 2 flanking noncoding regions. The coding region contains the genetic information for 9 viral proteins, 3 structural (core, envelope 1, envelope 2), and 6 nonstructural (NS2 to 5). The nonstructural proteins are members of the replicase complex. Both structural and nonstructural proteins have been implicated in modulating the host immune response and carcinogenesis. The flanking noncoding regions (5′UTR and 3′UTR) are unique RNA molecules that usurp and orchestrate the host cell machinery to convert the viral RNA genome into viral proteins.

Figure 3. The HCV genome, delivered to the cytoplasm after viral infection, harnesses the cell's ribosomes to translate viral RNA into viral proteins. This critical step is initiated by the 5′UTR by virtue of its unique and highly conserved secondary structure, termed the internal ribosome entry site (IRES). This strategy is rarely used by human genes except under stress. Complete translation of the viral genome, proceeding from 5′ to 3′, results in the production of a single viral polyprotein that must be reduced to subunits to effect viral replication.

Molecular Analysis of the HCV Genome

The genomic organization of HCV is shown schematically in Figure 2. Genomic organization of HCV RNA indicated the virus is closely related to the pestivirus genera within the family, *Flaviviridae*, but has now been classified in its own subgenera, *Hepacicvirus.* The viral genome is a single-stranded RNA molecule approximately 9.5 kb in length that is positive sense and possesses a unique open-reading frame, coding for a single polyprotein. The viral genome is flanked by untranslated regions (UTRs) at its 5′ and 3′ ends. The length of the polyprotein-encoding region varies according to the isolate and genotype of the virus from 3,008 to 3,037 amino acids.[41] Apart from differences in length, HCV genotypes (subfamilies) show diversities of approximately 30% in the nucleotide sequences of their whole, showing at least 6 genotypes and more than 30 subtypes throughout the world.[42] The importance of the genomic heterogeneity is that some genotypes appear to be associated with more severe pathological states and are more refractory to treatment.[42] Genotyping is becoming a common clinical assay in the management of newly diagnosed HCV, but not during clinical flare or after liver transplantation.

5′UTR

An obvious characteristic of HCV is the presence of a long UTR at the 5′ end of the genome, and detailed molecular analysis indicates polyprotein synthesis is initiated at the internal end of this region.[43] This region of the genome was predicted by its primary sequence to form extensive secondary structures,[44]

which were biochemically confirmed by Brown et al.[45] Furthermore, a survey of the sequences within the 5′ UTR from 39 different isolates and genotypes of HCV showed remarkable sequence conservation.[46] This region is the most conserved of the genome, a characteristic allowing it to be used as a diagnostic locus (genotype) and marker (HCV RNA) by polymerase chain reaction.[47] Within this region, the majority of genotypes of HCV possess a translation initiation locus closely resembling that of other RNA viruses, such as polio and influenza.[48] This genetic organization is termed an internal ribosomal entry site (IRES), which differentiates the mechanism for viral gene expression (cap independent) from nearly 98% of human gene products (cap dependent).[49] The function of the HCV IRES therefore seems to be to provide a structure able to specifically engage the ribosome for translation initiation (protein production; Fig. 3). As in other IRES examples (poliovirus, influenza virus, hepatitis A), this interaction probably involves complexes with cellular proteins.[50]

Virus-Encoded Proteins

The nascent viral polyprotein is processed (matured and cleaved) by a combination of host and viral proteinases into mature structural proteins and nonstructural (NS) viral enzymes[51] (Fig. 3). Proteolytic processing of the HCV polyprotein results in at least 10 distinct viral proteins: core (capsid), E1 (envelope), E2, NS2, NS3, NS4a, NS4b, NS5a, and NS5b. The structural proteins are located in the amino terminal (proximal) one third, and the replicative enzymes are located within the carboxy terminal (distal) two thirds of the polyprotein. The initial cleavage of the polyprotein is likely mediated by a host (human) signal proteinase.[52] The viral proteins themselves mediate further division of the polyprotein.[53]

Core

The protein located at the amino terminus of the polyprotein is highly basic in nature and considered likely to be the viral core (capsid) protein. It is released from the viral polyprotein by nascent proteolytic cleavage at amino acid 191 by cellular, not viral, proteases.[52] The subcellular trafficking of the core protein has been an area of interest. In particular, the nucleolar localization of core may be caused by its ability to bind to ribosomes assembled in the nucleus.[54]

The biological functions of the core protein found in the nucleus, if this also occurs in natural virus replication, are still unclear. Several studies have reported the suppression by core of transcription of several host immunoregulatory genes,[55] as well as interference in expression of coinfecting genomes of hepatitis B[56] and human immunodeficiency viruses.[57] Perhaps the most interesting recent observations have been that core can specifically suppress apoptotic cell death in artificial systems[58] and also specifically interact with the cytoplasmic tail of the lymphotoxin-β receptor, a member of the tumor necrosis factor family.[59] Because lymphotoxin-β receptor is known to be involved in apoptotic signaling, this strongly suggests that core may have an immunomodulatory function and a critical role in the establishment of persistence and disease pathogenesis.

Finally, a recent report[60] shows an association between the core protein and the surface of lipid droplets within the cytoplasm. Analysis of the triglyceride populations within the cell indicates that core protein expression stimulates a change in cellular metabolism of triglycerides. Because a characteristic of HCV infection is liver steatosis, it is plausible that this is a result of the direct effect of the core protein on lipid metabolism.

Envelope Proteins E1 and E2

The major viral structural envelope proteins are the glycoproteins, E1 and E2, which are released from the viral polyprotein by the action of host-cell signal peptidases. Apart from the interest in understanding the biological processes of assembly and morphogenesis, both E1 and E2 have been extensively studied in terms of antigenic variation and mechanisms of persistence and are obviously major components of prototype vaccine studies for HCV. E2 presumably represents the most variable region of the HCV genome.[61] This variation is assumed to be caused by random mutation and selection of mutants capable of escaping from neutralizing antibodies produced in the host. Furthermore, antibodies against E2 correlate with protection from HCV challenge in chimpanzees. Direct assays are now available for neutralizing antibodies in serum[62] or in vitro culture.[29]

Within the E2 sequence are regions of extreme hypervariability (HVR) that have been the focus of more detailed study; one specific domain is known as HVR-1.[63] This region has been suggested to be particularly important in HCV neutralization because of its extreme variability and because this variability was not observed in a patient with agammaglobulinemia, even over 2 to 5 years.[64] Antibodies to HVR-1 in patient sera have been shown to neutralize binding of E2,[65] whereas peptide antibodies raised to this region are able to block infection in tissue culture[66] and have

shown efficacy in chimpanzee challenge experiments.[67] Despite this observed protective capacity of antibody, the extreme heterogeneity in the sequence of this epitope in diverse virus strains means that its use in a prophylactic vaccine strategy remains doubtful.

The most striking recent observation for E2 is its putative role in obstructing the innate immune response to HCV replication.[68] The HCV envelope protein, E2, contains a sequence identical with phosphorylation sites of the interferon (IFN)-inducible protein kinase (PKR), and the translation initiation factor, eIF2, a target of PKR. In this study, cells engineered to express E2 did not manifest normal kinase activity of PKR, resulting in unregulated protein synthesis and cell growth. This interaction of E2 and PKR may be one mechanism by which HCV circumvents the antiviral effect of interferon.

NS2

The NS2 protein is a transmembrane protein with its carboxy terminus translocated into the lumen of the endoplasmic reticulum (ER) while its amino terminus lies in the cytosol.[69] Although immunoprecipitation studies[51] showed that NS2 is closely associated with the structural proteins, the biological function of the majority of the NS2 protein is still unclear.

NS3

The region coding for the NS3 protein has been the most extensively studied of the entire genome. The NS3 protein has been shown in numerous studies to be approximately 70 kd and to possess several diverse biochemical functions.[70] Initial genomic analysis of this region of the molecule showed that it could be characterized as a serine protease. Studies subsequently showed that the NS3 protease was entirely responsible for proteolytic processing of the entire downstream region of the viral polyprotein.[71] The overall proteolytic pathway for the whole viral genome is shown in Figure 3, implying that the NS3 protease mediates proteolysis at the NS3/NS4a, NS4a/NS4b, NS4b/NS5a, and NS5a/NS5b junctions to release the mature NS3, NS4a, NS4b, NS5a, and NS5b proteins. However, there are subtle biochemical differences in the exact mechanism by which NS3 mediates these cleavages. Further analysis of the NS3 protein also showed motifs characteristic of RNA helicase enzymatic function (unwinding of RNA for replication and protein translation; Fig. 4).[72] This molecule is therefore involved in critical events of viral replication, thus making it an attractive target for antiviral therapy.

NS4

The NS4 region of the polyprotein comprises 2 proteins; namely, NS4a and NS4b. Both these proteins are released from the viral polyprotein by the NS3 serine protease by *cis* cleavage at the NS3/NS4a and *trans* cleavage at the NS4a/NS4b and NS4b/NS5a junction.[73] NS4a is a small protein, approximately 8 kd, and appears to have diverse functions, such as anchorage of replication complexes and a cofactor for the NS3 protease (discussed previously).[74] Currently, there is no ascribed function for the NS4b protein, but it likely has an integral role within HCV replication complexes.

NS5a and NS5b

The NS5 region of the polyprotein is composed of 2 major proteins, NS5a and NS5b, which are released as mature products by the action of the NS3 protease in conjunction with NS4a. The functional role of NS5a is currently unclear. Apart from the probable role of NS5a in the HCV replication cycle, recent evidence suggested that it may be a critical factor in determining the susceptibility of the virus to treatment with IFN. It was initially reported that interferon sensitivity correlated with mutations within a discrete region of NS5a. Subsequently, this domain was named the IFN sensitivity–determining region.[75,76] Further evidence indicated that this likely occurred through a direct interaction of NS5a with the IFN-induced protein kinase, PKR, a cellular mediator of IFN-induced antiviral resistance.[77,78] Because PKR is a critical factor in the host response to IFN,[79] its inactivation by NS5a may be a major mechanism by which HCV evades the host immune response.[80] Furthermore, restrained PKR activity in the face of carcinogenic stress on cells infected with HCV (e.g., hepatocytes and B lymphocytes) may permit transformation into the malignant state.[81,82]

The sequence of the NS5b protein is highly conserved, not only between different strains of HCV, but also in other RNA viruses. In particular, the amino acid motif, G-D-D, is totally conserved in HCV, aviviruses, poliovirus, and tobacco mosaic virus.[83] This motif is a characteristic of all known RNA-dependent RNA polymerases (RdRp), and therefore the function of NS5b in HCV has been speculated to be the viral polymerase. The role of the viral polymerase in HCV replication is to convert the single-strand genome into a faithful intermediate negative strand for subsequent and repetitive production of positive strands for virion production (Fig. 4).[74] This step in the viral replication cycle is responsible for the creation of quasispecies (unfaithfully reproduced genomes).[84] This inefficient

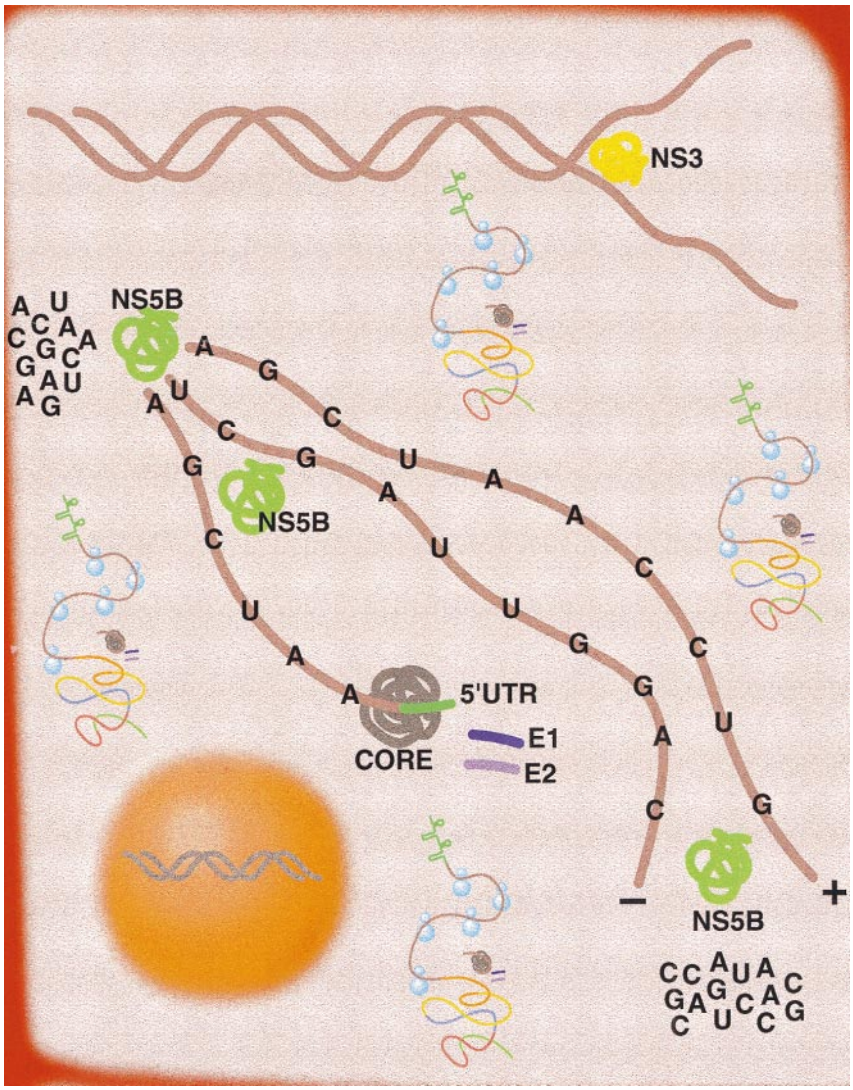

Figure 4. Viral replication occurs after translation of the HCV genome from viral RNA to viral protein. Final liberation of the viral nonstructural protein, NS5b, begins the replication process. NS5b is the viral RNA-dependent RNA polymerase, an enzyme not provided by human cells, that processes the original infecting viral RNA into exact copies through a negative strand intermediate. While these strands of viral RNA are produced, the NS3 protein unravels them to allow their incorporation into developing viral particles containing core and envelope (E1 and E2) proteins.

editing process, also present in HIV infection,[85] may represent a "trojan horse" viral evasion strategy of creating decoy virions or genomes for host cell defense.

3′UTR

A significant recent finding in the HCV area was that the considered 3′ terminal region of the genome was incorrect. A number of studies suggested that the 3′ terminus of the genome terminated in a poly (U) tract[106] or, in another report,[44] as a poly (A) tract. However, more detailed analysis of the 5′ end of the negative antigenomic RNA strand in infected liver cells showed the presence of a novel 98-nucleotide sequence downstream of the presumed genomic terminus.[86,87] Detailed sequence analysis of the complete new 3′UTR shows that it can be considered a tripartite structure comprising the conventional 3′ end, a poly (U) tract, and the new highly conserved sequence known as the 3′X tail.[88] Interestingly, the poly (U) region appears to be extremely heterogeneous between different virus isolates and even within the same infected liver. In contrast, the new sequence is highly conserved even between the 2 most genetically divergent HCV genotypes, 1B and 2A.[88] The 3′UTR, like its 5′ counterpart, is highly associated with cellular host proteins, likely part of the virion assembly and replicase complex.

Emerging Strategies for Therapy

Current approaches to the treatment of NANBH are limited to the use of IFN-α, either alone[89] or in combination with other agents, such as ribavirin.[90] The efficacy of these therapies is extremely poor, and the

posttherapy relapse rate is very high, such that sustained biochemical response is observed in less than 20% of the patients.[91] Consequently, there is an urgent need for the development of novel therapeutics. The antiviral effect of IFN results from both inhibition of viral replication[92,93] and modulation of the immune response to viral epitopes. As recently suggested by mathematical models, the major initial effect of IFN-α is to block virion production or release,[94] but the subsequent second-phase decline is believed to reflect the death rate of productively infected cells, in which HCV-specific cytotoxic T-cell activity could be a major contributor. The mechanism of synergism provided by ribavirin is also unknown, but its benefit has been clearly achieved.[95] The 5′-triphosphate metabolite of ribavirin is believed to act as an inhibitor of the viral RdRp. However, two other non–mutually exclusive mechanisms of antiviral actions of ribavirin have been proposed. Inhibition of the inosine-monophosphate-dehydrogenase enzyme after phosphorylation of ribavirin to ribavirin-5′-triphosphate may deplete intracellular pools of guanosine triphosphate (similar to the activity of mycophenolic acid). The second mechanism proposed is that ribavirin inhibits the guanylyltransferase by ribavirin-5′-triphosphate, which could interfere with the translation of cellular and viral RNA.

The approach that likely will lead to novel therapies is that directed toward unique aspects of the viral life cycle.[96] If one views the spectrum of HCV infection as a continuum from virion binding to cellular receptor on through viral replication and virion export, numerous theoretical opportunities exist that are currently being explored for development. After viral particle entry, HCV RNA targets the host cell ribosome to begin the process of viral translation. The unique mechanism by which HCV performs translation through its IRES may be a highly efficacious target for sterilization of the virus. Targeted destruction of this HCV domain may be achieved by antisense molecules[97] or ribozymes (cleaving RNA molecules).[98]

After the initiation of translation of viral sequences, critical viral proteins emerge that digest the nascent polyprotein into its subunits.[71] This proteolytic function is the target of current and future protease inhibitors. Unquestionably, the emergence of protease inhibitors to the cocktail therapy of HIV has changed the horizon for patients with acquired immunodeficiency syndrome. Recent progress in the proper modeling of the HCV protease, NS3, will also lead to a rationale drug design of prospective compounds.[99] Critical to replication of the viral RNA is the need for one of the HCV proteins, NS3, to unwind (helicase activity) the comingled strands for polymerase activity and translation.[100,101] Unfortunately, helicase inhibitors have not reached the clinic because of toxic cross-reactivity with constitutively active cellular proteins. Another target for antiviral research is the NS5b protein, which functions as the viral RNA polymerase. This editing enzyme may potentially be rendered inactive by drugs that sterically alter its structure through avid binding, compete for its viral RNA target, or poison its substrate (nucleoside analogues).

During viral assembly, some viral proteins require maturation by addition of carbohydrate or lipid molecules. These sites may prove critical for the tropism of the mature virus or its capacity to produce infection. Such maturation events are termed prenylation glycosylation and have been identified as a potential target for antiviral research.[102]

Conclusion

The identification of HCV as the etiologic agent of NANBH is certainly the most significant recent development in any area of viral disease. The clinical importance of the disease and the need to rapidly identify new therapeutic approaches has resulted in intensive study of the molecular properties of the virus. Although there are still large crevasses in our understanding of some aspects of HCV, an aggressive pursuit to bridge these gaps is underway. To develop the proper armamentarium to control HCV, a durable system of long-term viral replication must be devised, accepted, and reproduced. Such an artificial or biologically relevant system must obey important aspects of the host-viral cross-talk yet to be recorded in our patients. The rapidity with which this translational research will reach the clinic and blossom into useful therapy depends on the essential relationship that hepatologists and liver surgeons maintain and develop with those working at the bench.

The formidability of such a foe as HCV was astutely assessed by Ali Ben Abi Taleb, son-in-law of Mahomet and the fourth caliph, when he stated, ". . . in a single enemy thou hast more than enough."[103]

References

1. Lavanchy D. Hepatitis C: Public health strategies. J Hepatol 1999;31(suppl 1):146-151.
2. Berenguer M, Wright T. Hepatitis C and liver transplantation. Gut 1999;45:159-163.
3. Davis G. Anticipated mortality of hepatitis C and impact on treatment [abstract]. Hepatology 1998;28:390A.

4. Choo QL, Kuo G, Weiner AJ, Bradley LRDW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A non-B viral hepatitis genome. Science 1989;244:359-362.
5. Kuo G, Choo Q-L, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989;244:362-364.
6. Saito I, Miyamura T, Ohbayashi A, Harada H, Katayama T, Kikuchi S, et al. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proc Natl Acad Sci U S A 1990;87:6547-6549.
7. El-Serag H, Mason A. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745.
8. Dhillon AP, Dusheiko GM. Pathology of hepatitis C virus infection. Histopathology 1995;26:297-309.
9. Ito T, Mukaigawa J, Zuo J, Hirabayashi Y, Mitamura K, Yasui K. Cultivation of hepatitis C in primary hepatocyte culture from patients with chronic hepatitis C results in release of high titre infectious virus. J Gen Virol 1996;77:1043-1055.
10. Zignego AL, Macchia D, Monti M, Thiers V, Muzzetti M, Foschi M, et al. Infection of peripheral mononuclear blood cells by hepatitis C virus. J Hepatol 1992;15:382-386.
11. Bertolini L, Iacovacci S, Ponzetto A, Gorini G, Battaglia M, Carloni G. The human bone marrow-derived B cell line CE, susceptible to hepatitis C virus infection. Res Virol 1993;144: 281-285.
12. Shimizu YK, Iwamoto A, Hijikata M, Purcell RH, Yoshikura H. Evidence for in vitro replication of hepatitis C virus genome in a human T-cell line. Proc Natl Acad Sci U S A 1992;89:5477-5481.
13. Iacovacci S, Sargiacomo M, Parolini I, Ponzetto A, Peschle C, Carloni G. Replication and multiplication of hepatitis C virus genome in human foetal liver cells. Res Virol 1993;144:275-279.
14. Lanford RE, Sureau C, Jacob JR, White R, Fuerst TR. Demonstration of in vitro infection of chimpanzee hepatocytes with hepatitis C virus using strand-specific RT-PCR. Virology 1994;202:606-614.
15. Sun BGM, Feitelson MA. Evidence for consistent HCV replication in HepG2 cells. In: Liang TJ, Alter HJ (ed). Sixth International Symposium on Hepatitis C and Related Viruses: Molecular Virology and Pathogenesis, Bethesda, MD, March 16-19, 1999.
16. Mizutani T, Kato N, Saito S, Ikeda M, Sugiyama K, Shimotohno K. Characterization of hepatitis C virus replication in cloned cells obtained from a human T-cell leukaemia virus type I infected cell line, MT-2. J Virol 1996;70:7219-7223.
17. Sugiyama K, Kato N, Mizutani T, Ikeda M, Tanaka T, Shimotohmo K. Genetic analysis of the hepatitis C virus (HCV) genome from HCV-infected human T-cells. J Gen Virol 1997;78:329-336.
18. Bradley D, Maynard J, Popper H, Cook EH, Ebert JW, McCaustland KA, et al. Posttransfusion non-A, non-B hepatitis, physicochemical properties of two distinct agents. J Infect Dis 1983;148:254-265.
19. Barry CWS, Mocarski E, Brown P. Analysis of host viral interactions in CMV infections using cDNA microarray. 25th Annual Meeting of the American Society of Transplant Surgeons, Chicago, IL, May 12-17, 1999.
20. Xie ZC, Riezu-Boj JI, Lasarte JJ, Guillen J, Su JH, Civeira MP, Prieto J. Transmission of hepatitis C virus infection to tree shrews. Virology 1998;244:513-520.
21. Eren R, Lubin I, Terkieltaub D, Ben-Moshe O, Zauberman A, Uhlmann R, et al. Human monoclonal antibodies specific to hepatitis B virus generated in a human/mouse radiation chimera: The Trimera system. Immunology 1998;2:154-161.
22. Bradley DW, McCaustland KA, Krawczynski K, Spelbring J, Humphrey C, Cook EH. Hepatitis C virus: Buoyant density of the factor VIII-derived isolate in sucrose. J Med Virol 1991;34: 206-210.
23. He LF, Alling D, Popkin T, Shapiro M, Alter HJ, Purcell RH. Determining the size of non-A, non-B hepatitis virus by filtration. J Infect Dis 1987;156:636-640.
24. Hijikata M, Shimizu YK, Kato H, Iwamoto A, Shih JW, Alter HJ, et al. Equilibrium centrifugation studies of hepatitis C virus: Evidence for circulating immune complexes. J Virol 1993;67:1953-1958.
25. Thomssen R, Bonk S, Propfe C, Heermann K-H, Kochel HG, Uy A. Association of hepatitis C virus in human sera with b-lipoprotein. Med Microbiol Immunol 1992;181:293-300.
26. Prince AM, Huima-Byron T, Parker TS, Levine DM. Visualization of hepatitis C virions and putative defective interfering particles isolated from low-density lipoproteins. J Viral Hepat 1996;3:11-17.
27. Kaito M, Watanabe S, Tsukiyama-Kohara K, Yamaguchi K, Kobayashi Y, Konishi M, et al. Hepatitis C virus particle detected by immunoelectron microscopic study. J Gen Virol 1994;75:1755-1760.
28. Agnello V. The etiology of mixed cryoglobulinemia associated with hepatitis C virus infection. Scand J Immunol 1995;42:179-184.
29. Shimizu YP, Purcell RH, Yoshikura H. Correlation between the infectivity of hepatitis C virus in vivo and its infectivity in vitro. Proc Natl Acad Sci U S A 1993;90:6037-6041.
30. Brown M, Goldstein J. A receptor-mediated pathway for cholesterol homeostasis. Science 1986;232:34-47.
31. Brown M, Herz J, Goldstein J. Calcium cages, acid baths and recycling receptors. Nature 1997;388:629-630.
32. Hofer FG, Gruenberger M, Kowalski H, Machat H, Huettinger M, Kuechler E, Blaas D. Members of the low-density lipoprotein receptor family mediate cell entry of a minor group cold virus. Proc Natl Acad Sci U S A 1994;91:1839-1842.
33. Bates P, Young J, Varmus H. A receptor for subgroup A sarcoma virus is related to the low-density lipoprotein receptor. Cell 1993;74:1043-1051.
34. Seipp SM, Mueller HM, Pfaff E, Stremmel W, Theilmann L, Goeser T. Establishment of persistent hepatitis C virus infection and replication in vitro. J Gen Virol 1997;78:2467-2477.
35. Levy ST, Todd SC, Maecker HT. CD81 (TAPA-1): A molecule involved in signal transduction and cell adhesion in the immune system. Ann Rev Immunol 1998;16:89-101.
36. Pileri PU, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, et al. Binding of hepatitis C virus to CD81. Science 1998;282:938-941.
37. Flint M, Maidens C, Loomis-Price LD, Shotten C, Levy S, Barclay WS, et al. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. J Virol 1999;72:6235-6244.
38. Agnello V. The etiology and pathophysiology of mixed cryoglobulinemia secondary to hepatitis C virus infection. Springer Semin Immunopathol 1997;19:111-129.
39. Dammacco FG, Gatti P, Sansonno D. Hepatitis C virus infection, mixed cryoglobulinemia, and non-Hodgkin's lym-

phoma: An emerging picture. Leuk Lymphoma 1998;31:463-476.

40. Maecker HL, Levy ST. Normal lymphocyte development but delayed humoral immune response in CD81-null mice. J Exp Med 1997;185:1505-1510.
41. Houghton M (ed). Hepatitis C viruses (ed 3). Philadelphia: Lippincott-Raven, 1996.
42. Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: Quasispecies and genotypes. Semin Liver Dis 1995;15:41-63.
43. Han JH, Shyamala V, Richman KH, Brauer MJ, Irvine B, Urdea MS, et al. Characterization of the terminal regions of hepatitis C viral RNA: Identification of conserved sequences in the 5′ untranslated region and poly(A) tails at the 3′ end. Proc Natl Acad Sci U S A 1991;88:1711-1715.
44. Tsukiyama-Kohara K, Iizuka N, Kohara M, Nomoto A. Internal ribosomal entry site within hepatitis C virus RNA. J Virol 1992;66:1476-1483.
45. Brown EA, Zhang H, Ping L-H, Lemon SM. Secondary structure of the 5′ nontranslated regions of the hepatitis C virus and pestivirus genomic RNAs. Nucleic Acids Res 1992; 20:5041-5045.
46. Bukh J, Purcell RH, Miller RH. Sequence analysis of the 5′ non-coding region of hepatitis C virus. Proc Natl Acad Sci U S A 1992;89:4942-4946.
47. Furione MS, Simoncini L, Gatti M, Baldanti F, Grazia Revello M, Gerna G. HCV genotyping by three methods: Analysis of discordant results based on sequencing. J Clin Virol 1999;13: 121-130.
48. Lemon SM, Honda M. Internal ribosome entry sites within the RNA genomes of hepatitis C virus and other flaviviruses. Semin Virol 1997;8:274-289.
49. Sachs AB, Sarnow P, Hentze MW. Starting at the beginning, middle and end: Translation initiation in eukaryotes. Cell 1997;89:831-838.
50. Lai MMC. Cellular factors in the transcription and regulation of viral RNA genomes: A parallel to DNA-dependent RNA transcription. Virology 1998;244:1-12.
51. Grakoui A, McCourt DW, Wychowski C, Feinstone SM, Rice CM. Characterization of the hepatitis C virus-encoded serine proteinase: Determination of proteinase-dependent polyprotein cleavage sites. J Virol 1993;67:2832-2843.
52. Hijikata MK, Kato N, Ootsuyama Y, Nakagawa M, Shimotohno K. Gene mapping of the putative structural region of the hepatitis C virus genome by in vitro processing analysis. J Virol 1991;67:4665-4671.
53. Hijikata MM, Mizushima H, Tanji Y, Komoda Y, Hirowatari Y, Akagi T, Kato N, et al. Proteolytic processing and membrane association of putative nonstructural proteins of hepatitis C virus. Proc Natl Acad Sci U S A 1993;90:10773-10777.
54. Santolini E, Migliaccio G, La Monica N. Biosynthesis and biochemical properties of the hepatitis C virus core protein. J Virol 1994;68:3631-3641.
55. Large M, Kittleson D, Hahn Y. Suppression of host immune response by the core protein of hepatitis C virus: Implications for hepatitis C virus persistence. J Immunol 1999;162:931-938.
56. Shih CM, Lo SJ, Miyamura T, Chen SY, Lee YW. Suppression of hepatitis B virus expression and replication by hepatitis C virus core protein in Huh-7 cells. J Virol 1993;67:5823-5832.
57. Srinivas R, Ray R, Meyer K, Ray R. Hepatitis C virus core protein inhibits human immunodeficiency virus type 1 replication. Virus Res 1996;45:87-92.
58. Ray RB, Steele R, Meyer K, Ray R. Transcriptional repression of P53 promoter by hepatitis C virus core protein. J Biol Chem 1997;272:10983-10986.
59. Matsumoto M, Hsieh T-Y, Zhu N, Vanarsdale T, Hwang SB, Jeng K-S, et al. Hepatitis C virus core protein interacts with the cytoplasmic tail of lymphotoxin-β receptor. J Virol 1997;71: 1301-1309.
60. Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, et al. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci U S A 1997;94:1200-1205.
61. Weiner A, Brauer M, Rosenblatt J, Richman KH, Tung J, Crawford K, et al. Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. Virology 1991;180:842-848.
62. Rosa D, Campagnoli S, Moretto C, Guenzi E, Cousens L, Chin M, et al. A quantitative test to estimate neutralizing antibodies to the hepatitis C virus: Cytofluorometric assessment of envelope glycoprotein 2 binding to target cells. Proc Natl Acad Sci U S A 1996;93:1759-1763.
63. Kumar U, Brown J, Monjardino J, Thomas HC. Sequence variation in the large envelope glycoprotein (E2/NS1) of hepatitis C virus during chronic infection. J Infect Dis 1993;167:726-730.
64. Kumar U, Monjardino J, Thomas HC. Hypervariable region of hepatitis C virus envelope glycoprotein (E2/NS1) in an agammaglobulinemic patient. Gastroenterology 1994;106: 1072-1075.
65. Zibert A, Schreier E, Roggendorf M. Antibodies in human sera specific to hypervariable region 1 of hepatitis C virus block viral attachment. Virology 1995;208:653-661.
66. Shimizu YK, Igarashi H, Kiyohara T, Cabezon T, Farci P, Purcell RHY. A hyperimmune serum against a synthetic peptide corresponding to the hypervariable region 1 of hepatitis C virus can prevent viral infection in cell cultures. Virology 1996;223:409-412.
67. Farci P, Shimoda A, Wong D, Cabezon T, De Gioannis D, Strazzera A, et al. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. Proc Natl Acad Sci U S A 1996;93:15394-15399.
68. Taylor DR, Shi ST, Romano PR, Barber GN, Lai MM. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. Science 1999;285:107-110.
69. Santolini E, Pacini L, Fipaldini C, Migliaccio G, La Monica N. The NS2 protein of hepatitis C virus is a transmembrane polypeptide. J Virol 1995;69:7461-7471.
70. Kwong AK, Kim JL, Rao G, Lipovsek D, Raybuck SA. Hepatitis C virus NS3/4A protease. Antiviral Res 1999;41:67-84.
71. Gallinari PB, Brennan D, Nardi C, Brunetti M, Tomei L, Steinkühler C, De Francesco R. Multiple enzymatic activities associated with recombinant NS3 protein of hepatitis C virus. J Virol 1998;72:6758-6769.
72. Tai CLCW, Chen DS, Hwang LH. The helicase activity associated with hepatitis C virus nonstructural protein 3 (NS3). J Virol 1996;70:8477-8484.
73. Hahm BH, Han DS, Back SH, Song OK, Cho MJ, Kim CJ,

Shimotohno K, Jang SK. NS3-4A of hepatitis C virus is a chymotrypsin-like protease. J Virol 1995;69:2534-2539.
74. Rice CM. Flaviviridae: The viruses and their replication (ed 3). Philadelphia: Lippincott-Raven, 1996.
75. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred with amino acid and substitutions in the NS5A gene. J Clin Invest 1995;96:224-230.
76. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in the nonstructural protein NS5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77-81.
77. Gale MJ, Korth MJ, Tang NM, Tau SL, Hopkins DA, Dever TE, et al. Evidence that hepatitis C virus resistance to interferon is mediated through repression of the PKR protein kinase by the nonstructural 5A protein. Virology 1997;230:217-227.
78. Gale MJ, Blakeley CM, Kwieciszewski B, Tan S-L, Dossett M, Tang NM, et al. Control of PKR protein kinase by the hepatitis C virus nonstructural 5A protein: Molecular mechanisms of kinase regulation. Mol Cell Biol 1998;18:5208-5218.
79. Williams B. Role of the double-stranded RNA-activated protein kinase (PKR) in cell regulation. Biochem Soc Trans 1997;25:509-513.
80. Gale MJK, Korth MJ, Katze MG. Repression of the PKR protein kinase by the hepatitis C virus NS5A protein: A potential mechanism of interferon resistance. Clin Diagn Virol 1998;10:157-162.
81. Jagus R, Joshi B, Barber GN. PKR, apoptosis and cancer. Int J Biochem Cell Biol 1999;31:128-138.
82. Clemens MJ, Bommer UA. Translational control: The cancer connection. Int J Biochem Cell Biol 1999;31:1-23.
83. Kamer G, Argos P. Primary structural comparison of RNA-dependent polymerases from plant, animal and bacterial viruses. Nucleic Acids Res 1984;12:7269-7282.
84. Oh JW, Ito T, Lai MM. A recombinant hepatitis C virus RNA-dependent RNA polymerase capable of copying the full-length viral RNA. J Virol 1999;73:7694-7702.
85. Berkhout B. HIV-1 evolution under pressure of protease inhibitors: Climbing the stairs of viral fitness. J Biomed Sci 1999;6:298-305.
86. Tanaka T, Kato N, Cho M-J, Sugiyama K, Shimotohno K. Structure of the 3′ terminus of the hepatitis C virus genome. J Virol 1996;70:3307-3312.
87. Kolykhalov AA, Feinstone SM, Rice CM. Identification of a highly conserved sequence element at the 3′ terminus of hepatitis C virus genome RNA. J Virol 1996;70:3363-3371.
88. Yanagi M, St Claire M, Emerson SU, Purcell RH, Bukh J. In vivo analysis of the 3′ untranslated region of the hepatitis C virus after in vitro mutagenesis of an infectious cDNA clone. Proc Natl Acad Sci U S A 1999;96:2291-2295.
89. Davis GL, Balart LA, Schiff EL, Lindsay K, Bodenheimer HC, Perrillo H, et al. Treatment of chronic hepatitis C with recombinant interferon-alfa. A multicenter randomized controlled trial. N Engl J Med 1989;321:1501-1506.
90. Brillanti S, Miglioli M, Barbara L. Combination antiviral therapy with ribavirin and interferon alpha in interferon alpha relapsers and non-responders: Italian experience. J Hepatol 1995;23:13-15.
91. Lindsay KL. Therapy of hepatitis C: Overview. Hepatology 1997;26:715-755.
92. Castelli JW, Wood KA, Youle RJ. The 2-5A system in viral infection and apoptosis. Biomed Pharmacother 1998;52:386-390.
93. Jacobs BL, Langland JO. When two strands are better than one: The mediators and modulators of the cellular responses to double-stranded RNA. Virology 1996;219:339-349.
94. Neumann A, Lam N, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon alfa therapy. Science 1998;282:103-107.
95. Glue P. The clinical pharmacology of ribavirin. Semin Liver Dis 1999;19:17-24.
96. Jones P. Strategies for antiviral drug discovery. Antiviral Chem Chemother 1998;9:283-297.
97. Brown-Driver VE, Eto T, Lesnik E, Anderson KP, Hanecak RC. Inhibition of translation of hepatitis C virus RNA by 2-modified antisense oligonucleotides. Antisense Nucleic Acid Drug Dev 1999;9:145-154.
98. Lieber AH, He CY, Polyak SJ, Gretch DR, Barr D, Kay MA. Elimination of hepatitis C virus RNA in infected human hepatocytes by adenovirus-mediated expression of ribozymes. J Virol 1996;70:8782-8791.
99. Kim JL, Morgenstern KA, Lin C, Fox T, Dwyer MO, Landro JA, et al. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. Cell 1996;87:343-355.
100. Kim DW, Gwack Y, Han JH, Choe J. C-terminal domain of the hepatitis C virus NS3 protein contains an RNA helicase activity. Biochem Biophys Res Commun 1995;215:160-166.
101. Kanai A, Tanabe K, Kohara M. Poly(U) binding activity of hepatitis C virus NS3 protein, a putative RNA helicase. FEBS Lett 1995;376:221-224.
102. Glenn JM, Marsters JC Jr, Greenberg HB. Use of a prenylation inhibitor as a novel antiviral agent. J Virol 1998;72:9303-9306.
103. Bartlett J. Familiar quotations: A collection of passages, phrases, and proverbs traced to their sources in ancient and modern literature (ed 9). Boston: Little, Brown, 1901.
104. Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 1999;285:110-113.
105. Galun E. The Trimera Mouse System: A model for HCV infection and evaluation of antiviral drugs. In: Liang TJ, Alter HJ, eds. Seventh International Symposium on Hepatitis C and Related Viruses: Molecular Virology and Pathogenesis. Atlanta, GA, April 9-13, 2000.
106. Okamato H, Okada S, Sugiyama Y, Kurai K, Iizuka H, Machida A, et al. Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: Comparison with reported isolates for conserved and divergent regions. J Gen Virol 1991;72:2697-2704.