

The virus

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Although some aspects of the molecular biology of hepatitis C virus (HCV) are well defined, the same is not true of the basic virology, the mechanism of virus replication and the functions of some of the viral proteins. The same difficulties that delayed the discovery of the virus in the past now hinder work directed towards these goals: in general, these are the low level of viraemia and poor in vitro cell culture systems. Although HCV is reported to replicate in vitro in some cell types, the level of replication is too low to provide useful information. In addition, as the level of replication in vivo is similarly low, few data have been generated from the study of naturally infected liver samples.

It is not possible to detect viral antigens in the serum of HCV infected persons in a manner analogous to the detection of hepatitis B (HBV) virus surface antigen.

Further, the only direct marker of HCV replication and viraemia is the detection of HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR).

Approximately 80% of individuals who are infected with HCV develop persistent infection. The mechanism(s) for this are still unclear and many hypotheses have been advanced. However, it seems likely to be a strategy adopted by the virus because even in immunosuppressed individuals the levels of viraemia are low. In support of this, recent experiments performed in the HCV laboratory of the Sir Albert Sakzewski Virus Research Centre have detected feedback inhibition by a virus protein on HCV RNA replication.

The basic virology

Before the discovery of HCV, the non A, non B agent was considered to be a 30–60 nm particle with a lipid envelope. [1] More recent electron microscopic studies of virions, purified from the serum of HCV infected individuals, have confirmed HCV to be an enveloped virus of approximately 60 nm diameter. [2] Treatment of the virus with detergent increased the density of the particle, consistent with the removal of a lipid containing glycoprotein envelope and release of a 33 nm nucleocapsid. [3]

Physicochemical studies have identified two HCV populations with different densities. The low density population contains infectious virus associated with low density lipoproteins (LDL), while the high density fraction contains virus that is noninfectious because the particles are coated with antibody. [4] These results explained a discrepancy in the infectivity of different serum samples that contained similar levels of virus (by RT-PCR), and are consistent with immune complex diseases that are often associated with persistent HCV infection.

The HCV genome

The HCV genome is a positive sense, single strand RNA molecule of approximately 9500 nucleotides (nt) that contains a single long open reading frame (gene). This encodes a polyprotein of 3008–3037 amino acids, depending on the genotype, that is flanked by untranslated regions (UTR) at the 5' and 3' ends (Figure 1). As a result, HCV was classified as a separate genus in the Flaviviridae. The core and envelope (E1 and E2) proteins are structural proteins that form the virus particle, while the

remainder, the non-structural (NS), proteins are required for virus replication. The proteins are discussed more fully below.

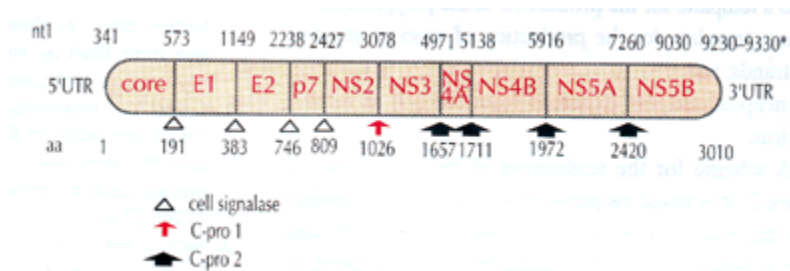


FIGURE 1. THE HCV GENOME AND POLYPROTEIN PROCESSING
 * The length of the 3'UTR varies due to the variable nature of the poly U tract

The nucleotide sequence of certain regions of the genome of different isolates has been shown to differ and, on this basis, six genotypes each containing a number of subtypes have been described. [5] Consequently, this results in the production of proteins that also differ in their degree of identity. However, HCV is typical of many RNA viruses in that the viruses that circulate in the blood of an infected individual usually include a major population of closely related viruses and variants, both derived from a common origin. This population is described as a quasispecies. The variants and the subsequent quasispecies arise as a result of random mutations in the genome during the process of RNA replication. In addition, the genome contains a region, known as hypervariable region 1 (HVR1) at the 5' end of the E2 gene that has been shown to accumulate mutations at a rapid rate; the corresponding region in the E2 protein is thought to contain an epitope for neutralising antibody. The 5' and 3' UTR are the most conserved regions in the genome, although the conservation in the 3' UTR is limited to the 98 nucleotides at the extreme terminus. These regions are thought to be important for the control of RNA transcription/replication and/or protein translation. The 5' UTR contains an internal ribosome entry site (IRES) which permits the translation of proteins from the genome in a cap independent manner. This contrasts with the cap dependent mechanism used by most cellular mRNA molecules. It is unclear why HCV uses such a mechanism for protein expression, but this feature represents a virus specific target that is the subject of intense research in the race to find novel HCV specific antiviral agents.

The viral proteins

The synthesis of a polyprotein which is co and post translationally processed into the mature individual polypeptides is common among viruses, and members of the Flaviviridae (prototype, yellow fever virus) and the Picornaviridae (prototype, poliovirus) provide established models. In vitro replication systems for HCV have not generated new information and our knowledge of HCV polyprotein processing is derived from artificial systems. The structural (S) proteins are cleaved into the mature polypeptides by a cell signalase which is probably located in the lumen of the endoplasmic reticulum, while the NS proteins are cleaved by one of two virus specific proteinases (Figure 1). Specific functions have been assigned to each of the proteins except p7, NS4A and NS5A.

The production of viral proteins by genetic engineering underpins the assays used to diagnose infection with HCV. The individual proteins are coated on a solid phase, typically an ELISA plate, and used as the antigen target in tests to detect anti HCV in individual serum samples ([Appendix 2](#)). The viral proteins can also be detected in liver biopsy and autopsy samples. However, the levels of viral protein expression makes this a difficult task, and these procedures are not performed on a routine basis in the manner used to detect HBV surface and core antigens.

The NS3 and NS5 proteins have helicase/protease and RNA polymerase activities, respectively. [6] These activities are vital for virus replication and represent logical targets for antiviral agents. The protease activity of NS3 is likely to be a particularly suitable target in view of the phenomenal success of specific protease inhibitors which have revolutionised treatment for infection with HIV. NS5A contains a region, the interferon sensitivity determining region (ISDR), thought to influence the response of the virus to treatment with interferon. However, the importance of the ISDR has not been confirmed in other studies, and it is thought that interferon resistance may be multifactorial. [7] Nonetheless, NS5A interacts with and inhibits the double stranded RNA induced protein kinase (PKR), which is part of the immunological cascade leading to the production of interferon. [8]

Virus replication

In the absence of a reproducible cell culture system for HCV, very little is known about the mechanism of virus replication. It has been suggested that the virus may bind to the hepatocyte as a consequence of the LDL component of the envelope binding to the cellular LDL receptor. More recently, the virus has been shown to bind to the CD81 molecule, and it has been proposed that this molecule represents the cellular receptor for the virus. [9] The other events of replication are equally ill defined, but by analogy with other members of the Flaviviridae, particularly the pestiviruses, it is possible to construct a model. After entry and uncoating, the virus RNA acts as mRNA for the synthesis of the virus polyprotein; this is co and post translationally processed to produce the mature viral proteins. The input RNA is then used as a template for the synthesis of nascent RNA in a replicative complex which is thought to contain NS5B and other NS proteins, initially by the synthesis of a negative strand which then base pairs with the input plus strand to form a double strand replicative form (RF), and then by the production of nascent plus strands from the RF. It is thought that the helicase function of NS3B is required to unwind the double stranded RF and/or to remove secondary structure from the plus strand to permit synthesis of the nascent strands. The nascent plus strands can then be used in one of three ways:

- as a template for the production of the polyprotein;
- as a template for the production of nascent negative strands; and
- encapsidated and exported from the cell as mature virus.

A scheme for the replication of HCV is shown in Figure 2. It is based on previous data derived by studies with the flavivirus, Kunjin,¹⁰ and the pestivirus, bovine viral diarrhoea virus. [11] Consequently, the detection of negative strand HCV RNA is thought to be a marker of virus replication, although the detection of negative strand RNA by strand specific RT-PCR is fraught with difficulties. [12] It is thought that the replication complex is closely associated with internal cellular membranes in a similar manner to that proposed for flaviviruses. [10]

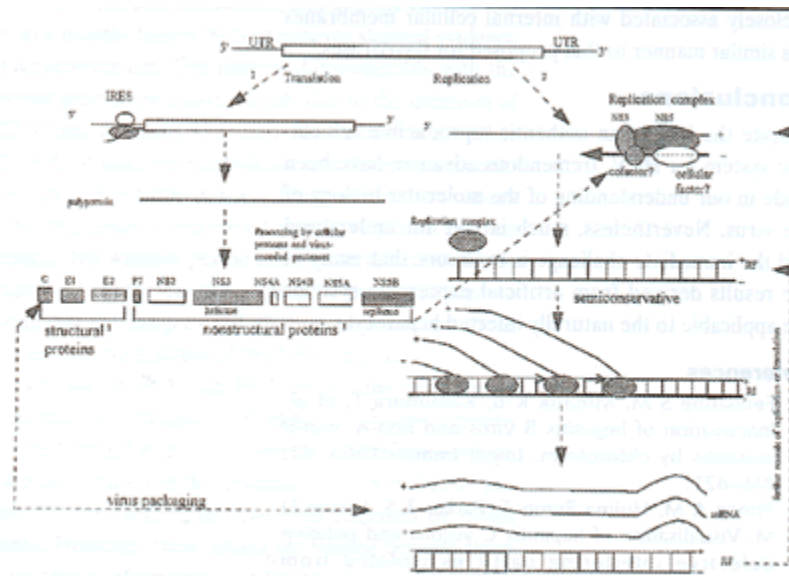


FIGURE 2. THE REPLICATION OF HCV

UTR=untranslated region; IRES=internal ribosome entry site; RF=replicative form; RI= replicative intermediate; ssRNA=single strand RNA; This figure was modified from a figure produced by Dr Yunhao Gong (PhD Thesis, University of Queensland)

Conclusions

Despite the lack of an authentic reproducible cell culture system for HCV, tremendous advances have been made in our understanding of the molecular biology of the virus. Nevertheless, much is still not understood and the immediate challenge is to ensure that many of the results derived from artificial expression systems are applicable to the naturally infected hepatocyte.

References

1. Feinstone S M, Mihalik K B, Kamimura T, et al. Inactivation of hepatitis B virus and non-A, non-B hepatitis by chloroform. *Infect Immun* 1983; 41: 816–821.
2. Prince A M, Huima-Byron T, Parker T S, Levine D M. Visualisation of hepatitis C virions and putative defective interfering particles isolated from low-density lipoproteins. *J Viral Hepatitis* 1996; 3: 11–17.
3. Takahashi K, Kishimoto S, Yoshizawa H, Okamoto H, Yoshikawa A, Mishoro S. p26 protein and 33 nm particle associated with nucleocapsid of hepatitis C virus recovered from the circulation of infected hosts. *Virology* 1992; 191: 431–434.
4. Hijikata M, Shimizu Y K, Kato H et al. Equilibrium centrifugation studies of hepatitis C virus: evidence for circulating immune complexes. *J Virol* 1993; 67: 1953–1958.
5. Simmonds P E, Alberti A, Alter H J, et al. A proposed system for the nomenclature of hepatitis C virus genotypes. *Proc Natl Acad Sci, USA* 1994; 91: 1321–1324.
6. Major M E, Feinstone S M. The molecular biology of hepatitis C. *Hepatology* 1997; 25: 1527–1538.
7. Duverlie G, Khorsi H, Castelain S, et al. Sequence analysis of the NS5A protein of European hepatitis C virus 1b isolates and relation to interferon sensitivity. *J Gen Virol* 1998; 79: 1373–1381.
8. Gale M J, Korth M J, Tang N M, 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.

9. Pileri P, Uematsu Y, Campagnoli S, et al. Binding of Hepatitis C virus to CD81. *Science* 1998; 282: 938–941
10. Chu P W G, Westaway E G. Replicative strategy of Kunjin virus: evidence for recycling role of replicative form RNA as template in semiconservative and asymmetric replication. *Virology* 1985; 140: 68–79.
11. Gong Y, Trowbridge R, Macnaughton TB, et al. Characterisation of RNA synthesis during a one–step growth curve and of the replication mechanism of bovine viral diarrhoea virus. *J Gen Virol* 1996; 77: 2729–2736.
12. McGuinness PH, Bishop GA, McCaughan G, Trowbridge R and Gowans EJ. False detection of negative–strand hepatitis C RNA. *Lancet* 1994; 343: 551–552.