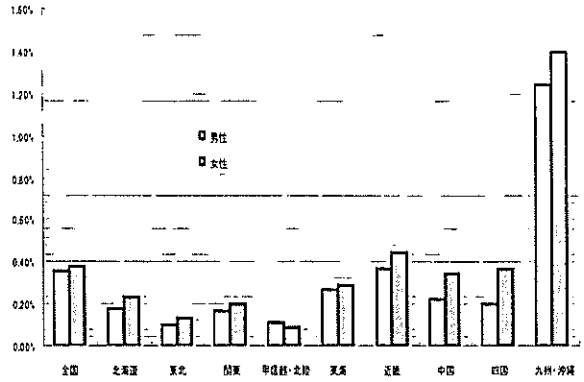
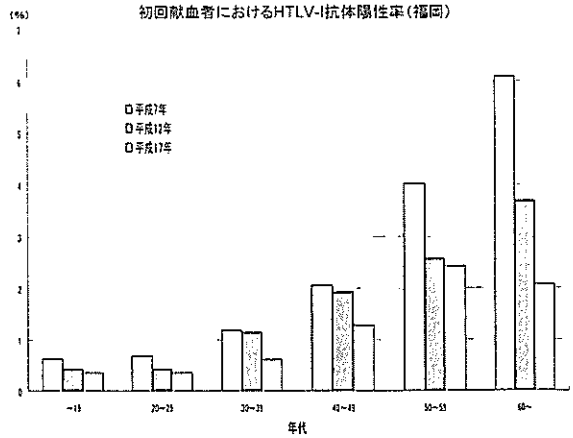
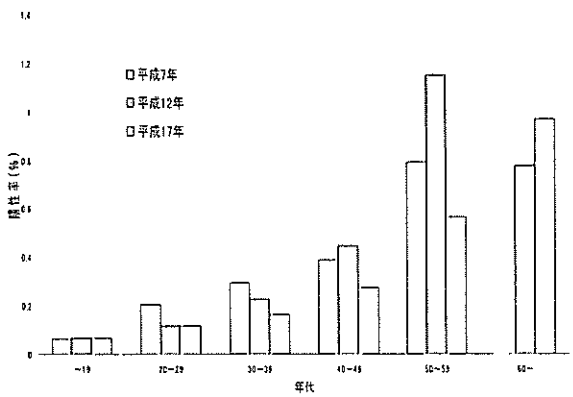
初回献血者におけるHTLV-I抗体陽性率




初回献血者におけるHTLV-I抗体陽性率(福岡)




初回献血者におけるHTLV-I抗体陽性率(関東)



厚生労働科学研究費補助金（第３次対がん総合戦略研究事業）
分担研究報告書

# ２００６年の輸血感染症報告

分担研究者：内田茂治（東京都西赤十字血液センター）
共同研究者：高橋雅彦（東京都赤十字血液センター）

１９９９年より日本赤十字社は全国の献血者血液に対して、ＨＢＶ、ＨＣＶおよびＨＩＶを対象としてミニプール核酸増幅検査（ＮＡＴ）を導入し、これらウイルスの輸血による伝播はＮＡＴ導入以前と比較して大幅に減少した。しかしながら、全国の医療機関からの自発報告例は依然として症例数が多く、輸血と感染との因果関係を明らかにする必要がある。また、２００３年６月からは過去の陽転化例も含めた遡及調査を開始した。

## Ａ．研究目的

輸血によるウイルス伝播の実態を調査することを目的として、２００６年に全国の医療機関から報告のあった自発報告例、ならびに遡及調査を含めた献血者の献血後情報による症例の解析を行った。

## Ｂ．対象と方法

２００６年１月から２００６年１２月までに、全国の医療機関から日本赤十字社血液事業本部に輸血後感染症として報告された自発報告例、遡及調査例、および試験的に行っている輸血実施例の全数調査症例を対象とした。これらの症例における感染と輸血との関連性を調査するため、輸血に使用された血液の保管検体の精査（血清学的検査ならびに個別ＮＡＴ）を行った。

## Ｃ．結果

### ＨＢＶ対象症例とその解析結果

自発報告例と遡及調査ならびに全数調査症例の解析を行い、計５例のＨＢＶ－ＤＮＡ陽性例が確認された（図１）。内訳は自発報告で４例、遡及調査で１例であった。

### ＨＣＶ対象症例とその解析結果

１例のＨＣＶ－ＲＮＡ陽性例が遡及調査により確認された（図１）。この例はＮＡＴスクリーニング検査開始後２例目の輸血感染例である。図２にこの輸血感染例の概略を示す。

## Ｄ．考察

日本赤十字社中央血液センター医薬情報部では、１９９４年から輸血によるウイルス感染疑い例の調査を行っているが、ＨＢＶ感染は１９９８年に２２例（自発報告６例、献血後情報１６）、１９９９年には２０例（自発報告５例、献血後情報１５例）が保管検体精査結果陽性で、輸血による感染の可能性が高い症例と考えられたが、２０００年には５例（自発報告４例、献血後情報１例）、２００１年には７例（自発報告５例、献血後情報２例）、２００２年は８例（自発報告４例、献血後情報３例、追跡調査１例）、２００３年は１２例（自発報告４例、献血後情報８例）、２００４年は２０例（自発報告８例、献血後情報６例、全数調査１例、追跡調査５例）であった。ＨＣＶ感染では１９９８年の７例、１９９９年の５例（いづれも献血後情報）の保管検体精査結果陽性の症例が認められたが、２０００年

以降は２００４年まで１例も確認されていなかった。このように１９９９年のミニプールＮＡＴ導入後、ＨＣＶ、ＨＢＶの輸血による感染は大幅な減少が認められた。

２００６年は２００５年に引き続き１例のＨＣＶ輸血感染例が確認されたが、チンパンジーの感染実験では１０コピーのＨＣＶで感染するといわれており、ＮＡＴ感度以下の低コピー数の血液でも輸血感染の可能性はあり、遡及調査の徹底が重要と考えられた。

図１

献血者検体にウイルスが検出された例

HBV　5
（自発報告 4、 遡及調査 1）

HCV　1
（遡及調査 1）

図２

症例（HCV　遡及調査）

患者　35F　常位胎盤早期剥離　MAP・FFP 6本輸血

輸血後128日　HCVAb　＋

輸血後154日　HCVAb　＋　ALT　12 (9.6×10⁴ copies/mL)

献血者

060601　HCVAb　＋　(6.1×10⁶ copies/mL)

031107　HCVAb　－　(6.8×10³ copies/mL) → 輸血
50PNAT　－

HCV－RNA　＋　genotype 2a(Ⅲ)

解析結果　Core領域前半部(nt.－2～363)の塩基配列はすべて一致

片割れ製剤 MAPは85Mの肺癌患者に輸血→原疾患により死亡

# 研究成果の刊行に関する一覧表

書籍

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者名 | 書籍名 | 出版社名 | 出版地 | 出版年 | ページ |
|---|---|---|---|---|---|---|---|
| Suzuki T, Suzuki R. | Maturation and assembly of hepatitis C virus core protein | Kalitzky M, Borowski P. | Molecular Biology of the Flavivirus | Horizon bioscience | UK | 2006 | 295 -312 |

雑誌

| 著者氏名 | 論文タイトル名 | 発表誌名 | 刊名 | ページ | 出版年 |
|---|---|---|---|---|---|
| Takizawa,S., Nagasaka,K., Nakagawa,S., Yano,T., Nakagawa,K., Yasugi,T., Takeuchi,T., Kanda,T., Huibregtse,J.M., Akiyama,T., Taketani,Y. | Human scribble, a novel tumor suppressor identified as a target of high-risk HPV E6 for ubiquitin-mediated degradation, interacts with adenomatous polyposis coli | Genes Cells. | 11 | 453-464 | 2006 |
| Mori, S., Ozaki, S., Yasugi, T., Yoshikawa, H., Taketani, Y., Kanda, T. | Inhibitory cis-Element-Mediated Decay of Human Papillomavirus Type 16 L1-Transcript in Undifferentiated Cells. | Mol Cell Biochem. | 288 | 47-57 | 2006 |
| Murata T, Shimotohno K. | Ubiquitination and proteasome-dependent degradation of human eukaryotic translation initiation factor 4E. | J Biol Chem. | 281 | 20788-20800 | 2006 |
| Hamazaki H, Ujino S, Miyano-Kurosaki N, Shimotohno K, Takaku H. | Inhibition of hepatitis C virus RNA replication by short hairpin RNA synthesized by T7 RNA polymerase in hepatitis C virus subgenomic replicons. | Biochem Biophys Res Commun. | 343 | 988-994 | 2006 |
| Goto K, Watashi K, Murata T, Hishiki T, Hijikata M, Shimotohno K. | Evaluation of the anti-hepatitis C virus effects of cyclophilin inhibitors, cyclosporin A, and NIM811. | Biochem Biophys Res Commun. | 343 | 879-884 | 2006 |
| Shimakami T, Honda M, Kusakawa T, Murata T, Shimotohno K, Kaneko S, Murakami S. | Effect of hepatitis C virus (HCV) NS5B-nucleolin interaction on HCV replication with HCV subgenomic replicon. | J Virol. | 80 | 3332-3340 | 2006 |
| Tsumura A, Hayakawa T, Kumaki Y, Takebayashi S, Sakaue M, Matsuoka C, Shimotohno K, Ishikawa F, Li E, Ueda HR, Nakayama J, Okano M. | Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. | Genes Cells. | 11 | 805-814 | 2006 |

| 著者氏名 | 論文タイトル名 | 発表誌名 | 刊名 | ページ | 出版年 |
|---|---|---|---|---|---|
| Ishii N, Watashi K, Hishiki T, Goto K, Inoue D, Hijikata M, Wakita T, Kato N, Shimotohno K. | Diverse effects of cyclosporine on hepatitis C virus strain replication. | J Virol. | 80 | 4510-4520 | 2006 |
| Naka K, Abe K, Takemoto K, Dansako H, Ikeda M, Shimotohno K, Kato N. | Epigenetic silencing of interferon-inducible genes is implicated in interferon resistance of hepatitis C virus replicon-harboring cells. | J Hepatol. | 44 | 869-878 | 2006 |
| Nakai K, Okamoto T, Kimura-Someya T, Ishii K, Lim C-K,Tani H, Matsuo E, Abe T, Mori Y, Suzuki T, Miyamura T, Nunberg JH, Moriishi K, Matsuura Y. | Oligomerization of hepatitis C virus core protein is crucial for interaction with cytoplasmic domain of E1 envelope protein. | J Virol. | 80 | 11265-11273 | 2006 |
| Okamoto T, Nishimura Y, Ichimura T, Suzuki K, Miyamura T, Suzuki T, Moriishi K, Matsuura Y. | Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. | EMBO J. | 25 | 5015-5025 | 2006 |
| Baek K-H, Park H-Y, Kang C-M, Kim S-J, Jeong S-J, Hong E-K, Park J-W, Sung Y-C, Suzuki T, Kim C-M, Lee C-W. | Overexpression of hepatitis C virus NS5A protein induces chromosome instability via mitotic cell cycle dysregulation. | J Mol Biol. | 359 | 22-34 | 2006 |
| Murakami K, Ishii K, Ishihara Y, Yoshizaki S, Tanaka K, Gotoh Y, Aizaki H, Kohara M, Yoshioka H, Mori Y, Manabe N, Shoji I, Sata T, Bartenschlager R, Matsuura Y, Miyamura T, Suzuki T. | Production of infectious hepatitis C virus particles in three-dimensional cultures of the cell line carrying the genome-length dicistronic viral RNA of genotype 1b. | Virology | 351 | 381-392 | 2006 |
| Sato S, Fukasawa M, Yamakawa Y, Natsume T, Suzuki T, Shoji I, Aizaki H, Miyamura T, Nishijima M. | Proteomic Profiling of Lipid Droplet Proteins in Hepatoma Cell Lines Expressing Hepatitis C Virus Core Protein. | J Biochem. | 139 | 921-930 | 2006 |
| Fukasawa M, Tanaka Y, Sato S, Ono Y, Nitahara-Kasahara Y, Suzuki T, Miyamura T, Hanada K, Nishijima M. | Enhancement of de novo fatty acid biosynthesis in hepatic cell line Huh7 expressing hepatitis C virus core protein. | Biol Pharm Bull. | 29 | 1958-1961 | 2006 |
| Masaki T, Matsuura T, Ohkawa K, Miyamura T, Okazaki I, Watanabe T, Suzuki T. | All-trans retinoic acid down-regulates human albumin gene expression through the induction of C/EBPbeta-LIP. | Biochem J. | 397 | 345-353 | 2006 |

| 著者氏名 | 論文タイトル名 | 発表誌名 | 刊名 | ページ | 出版年 |
|---|---|---|---|---|---|
| Shimoike T, Koyama C, Murakami K, Suzuki R, Matsuura Y, Miyamura T, Suzuki T. | Down-regulation of the internal ribosome entry site (IRES)-mediated translation of the hepatitis C virus: critical role of binding of the stem-loop IIId domain of IRES and the viral core protein. | Virology. | 345 | 434-445 | 2006 |
| Saito M, Matsuura T, Masaki T, Maehashi H, Shimuzu K, Hataba Y, Iwahori T, Suzuki T, Braet F. | Reconstruction of liver organoid using a bioreactor. | World J Gastroenterol. | 12 | 1881-1888 | 2006 |
| Inoue M, Kanto T, Miyatake H, Itose I, Miyazaki M, Yakushijin T, Sakakibara M, Kuzushita N, Hiramatsu N, Takehara T, Kasahara A, Hayashi N. | Enhanced ability of peripheral invariant natural killer T cells to produce IL-13 in chronic hepatitis C virus infection. | J Hepatol. | 45 | 190-196 | 2006 |
| Kato H., Takeuchi O., Sato S., Yoneyama M., Yamamoto M., Matsui K., Uematsu S., Jung A., Kawai T., Ishii K. J., Yamaguchi O., Otsu K., Tsujimura T., Koh C.-S., Sousa C. R., Matsuura Y., Fujita T., Akira S. | Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. | Nature. | 441 | 101-105 | 2006 |
| Tajimi M, Ugajin T, Ota M, Hiroishi K, Nakamura I, Imawari M. | Immune responses of liver-infiltrating lymphocytes and peripheral blood mononuclear cells to hepatitis C virus core and NS3 antigens. | Hepatol Res. | 35 | 250-255 | 2006 |
| Ito K, Shiraki K, Funatsuki K, Ishiko H, Sugimoto K, Murata K, Nakano T, Imawari M. | Identification of novel hepatitis C virus-specific cytotoxic T lymphocyte epitope in NS3 region. | Hepatol Res. | 36 | 294-300 | 2006 |
| Yuasa K, Naganuma A, Sato K, Ikeda M, Kato N, Takagi H, Mori M. | Zinc is a negative regulator of hepatitis c virus RNA replication | Liver Int. | 26 | 1111-1118 | 2006 |
| Ikeda M, Abe K, Yamada M, Dansako H, Naka K, Kato N. | Different anti-HCV profiles of statins and their potential for combination therapy with interferon | Hepatology. | 44 | 117-125 | 2006 |
| Deng L, Nagano-Fujii M, Tanaka M, Nomura-Takigawa Y, Ikeda M, Kato N, Sada K, Hotta H. | The NS3 protein of hepatitis C virus associates with the tumour suppressor p53 and inhibits its function in an NS3 sequence-dependent manner. | J Gen Virol. | 87 | 1703-1713 | 2006 |

| 著者氏名 | 論文タイトル名 | 発表誌名 | 刊名 | ページ | 出版年 |
|---|---|---|---|---|---|
| Naka K, Dansako H, Kobayashi N, Ikeda M, Kato N. | Hepatitis C virus NS5B delays cell cycle progression by inducing interferon- via Toll-like receptor 3 signaling pathway without replicating viral genomes. | Virology. | 346 | 348-362 | 2006 |
| 内田 茂治 | 献血者におけるＨＢＶ陽性率の動向とＢ型肝炎感染初期例のＨＢＶ遺伝子型頻度 | 病原微生物検出情報 | 27 | 223-224 | 2006 |
| Kondo, K., Ishii, Y., Ochi, H., Matsumoto, T., Yoshikawa, H., Kanda,T. | Neutralization of HPV16, 18, 31, and 58 Pseudovirions with Antisera Induced by Immunizing Rabbits with Synthetic Peptides Representing Segments of the HPV16 Minor Capsid Protein L2 | Virology. | 358 | 266-272 | 2007 |
| Kawana K, Quayle AJ, Ficarra M, Ibana JA, Shen L, Kawana Y, Greene S, Yang H, Yavagal S, Marrero L, Zhang YX, Pyles RB, Blumberg RS, Schust DJ | CD1d degradation in Chlamydia trachomatis-infected epithelial cells is a result of both cellular and chlamydial proteasomal activity. | J Biol Chem. | 282 | 7368-7375 | 2007 |
| Aly HH, Watashi K, Hijikata M, Kaneko H, Takada Y, Egawa H, Uemoto S, Shimotohno K. | Serum-derived hepatitis C virus infectivity in interferon regulatory factor-7-suppressed human primary hepatocytes. | J Hepatol. | 46 | 26-36 | 2007 |
| Zhang J, Yamada O, Yoshida H, Sakamoto T, Araki H, Shimotohno K. | Helper virus-independent trans-replication of hepatitis C virus-derived minigenome. | Biochem Biophys Res Commun. | 352 | 170-176 | 2007 |
| Shirakura M, Murakami K, Ichimura T, Suzuki R, Shimoji T, Fukuda K, Abe K, Sato S, Fukasawa M, Yamakawa Y, Nishijima M, Moriishi K, Matsuura Y, Wakita T, Suzuki T, Howley PM, Miyamura T, Shoji I. | E6AP ubiquitin ligase mediates ubiquitylation and degradation of hepatitis C virus core protein. | J Virol. | 81 | 1174-1185 | 2007 |
| Moriishi K., Mochizuki R., Moriya K., Miyamoto H., Mori Y., Abe T., Murata S., Tanaka K., Miyamura T., Suzuki T., Koike K., Matsuura Y. | Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. | Proc Natl Acad Sci USA. | 104 | 1661-1666 | 2007 |
| Miyamoto H, Moriishi K, Moriya K, Murata S, Tanaka K, Suzuki T, Miyamura T, Koike K, Matsuura Y. | Involvement of PA28gamma-dependent pathway in insulin resistance induced by hepatitis C virus core protein. | J Virol. | 81 | 1727-1735 | 2007 |

| 著者氏名 | 論文タイトル名 | 発表誌名 | 刊名 | ページ | 出版年 |
|---|---|---|---|---|---|
| Jinushi M, Takehara T, Tatsumi T, Yamaguchi S, Sakamori R, Hiramatsu N, Kanto T, Ohkawa K, Hayashi N. | Natural killer cell and hepatic cell interaction via NKG2A leads to dendritic cell-mediated induction of CD4+ CD25+ T cells with PD-1-dependent regulatory activities. | Immunology. | 120 | 73-82 | 2007 |
| Abe K, Nozaki A, Tamura K, Ikeda M, Naka K, Dansako H, Hoshino HO, Tanaka K, Kato N. | Tandem repeats of lactoferrin-derived anti-hepatitis C virus peptide enhance antiviral activity in cultured human hepatocytes. | Microbiol Immunol. | 51 | 117-125 | 2007 |

# Maturation and Assembly of Hepatitis C Virus Core Protein

10

Tetsuro Suzuki and Ryosuke Suzuki

Abstract

Hepatitis C virus (HCV) core protein, which is derived from the N-terminus of the viral polyprotein, forms the viral nucleocapsid. The amino acid sequence of this protein is well conserved among different HCV strains, compared to other HCV proteins. The N-terminal domain of the core protein is highly basic, while its C-terminus is hydrophobic. The core protein is primarily detected in the cytoplasm in associating with endoplasmic reticulum (ER), lipid droplets, and mitochondria. However, it has also been detected in the nucleus. In fact, a nuclear localization signal has been identified in the N-terminal region of the protein. Its C-terminal hydrophobic region is thought to act as a membrane anchor for the core protein and as a signal sequence for E1 protein.

Nucleocapsid formation presumably involves interactions between the core protein and viral RNA and envelope protein(s), as well as self-interaction of the core protein. Interactions between the HCV core protein and specific regions of viral genomic RNA have been reported. In addition, the core protein has been observed to form homo-multimeric, as well as hetero-dimeric, complexes with E1 protein. These interactions might be important for nucleocapsid formation, and may activate the biological activity of various HCV structural proteins.

## Introduction

Hepatitis C virus (HCV) is the major etiologic agent of posttransfusion- and sporadic non-A, non-B hepatitis (Kuo *et al.*, 1989) and presently infects approximately 170 million people worldwide (Grakoui *et al.*, 2001; Lauer *et al.*, 2001). Persistent infection with HCV is associated with the development of chronic hepatitis, hepatic steatosis, cirrhosis and hepatocellular carcinoma (HCC) (Saito *et al.*, 1990; Alter, 1995; Di Bisceglie, 1997; Lauer *et al.*, 2001; Poynard *et al.*, 2003; Pawlotsky, 2004). Although HCV research has long been hampered by the lack of a cell culture system by which to propagate the virus, molecular cloning and expression of HCV gene products in cell cultures has yielded a lot of information. HCV is classified as belonging to the *Hepacivirus* genus of the Flaviviridae family (Houghton *et al.*, 1991; Robertson *et al.*, 1998). Its genome consists of a single-stranded positive-sense RNA of approximately 9.6 kb, which contains an open reading frame coding for a polyprotein precursor of approximately 3000 residues (Choo *et al.*, 1989). This precursor is co- and post-translationally processed into structural and nonstructural proteins

by cellular and viral proteases (Houghton *et al.*, 1991; Hijikata *et al.*, 1993; Grakoui *et al.*, 1993). The structural proteins are located in the N-terminal one-fourth of the polyprotein and are cleaved by cellular membrane proteinases.

The core protein, which is derived from the N-terminus of the polyprotein, most likely forms the viral nucleocapsid given similarities between its position and that of sequences encoding viral nucleocapsids in the genomes of other flaviviruses. The amino acid sequence of the core protein is highly conserved among different HCV strains, compared with other HCV proteins. This protein has been extensively used in serologic assays since anti-core antibodies are highly prevalent among HCV-infected individuals. The N-terminal domain of the core protein is highly basic, while its C-terminus is hydrophobic (Figure 10.1). Although several core proteins of varying molecular weights have been reported (Harada *et al.*, 1991; Liu *et al.*, 1997; Lo *et al.*, 1994; Lo *et al.*, 1995; Suzuki *et al.*, 2001; Yasui *et al.*, 1998), two processing events result in the predominant production of a 21 kDa core protein. The core protein is primarily detected in the cytoplasm, by associating with the endoplasmic reticulum (ER), lipid droplets, and mitochondria (Harada *et al.*, 1991; Selby *et al.*, 1993; Lo *et al.*, 1995; Suzuki *et al.*, 1995; Suzuki *et al.*, 1996; Moradpour *et al.*, 1996; Barba *et al.*, 1997; Yasui *et al.*, 1998; Moriya *et al.*, 1998; Sabile *et al.*, 1999; Hope *et al.*, 2000; McLauchlan *et al.*, 2002; Okuda *et al.*, 2002; Schwer *et al.*, 2004; Suzuki *et al.*, 2005). In some studies, a fraction of the core protein has also been found in the nucleus (Chang *et al.*, 1994; Suzuki *et al.*, 1995; Lo *et al.*, 1995; Liu *et*

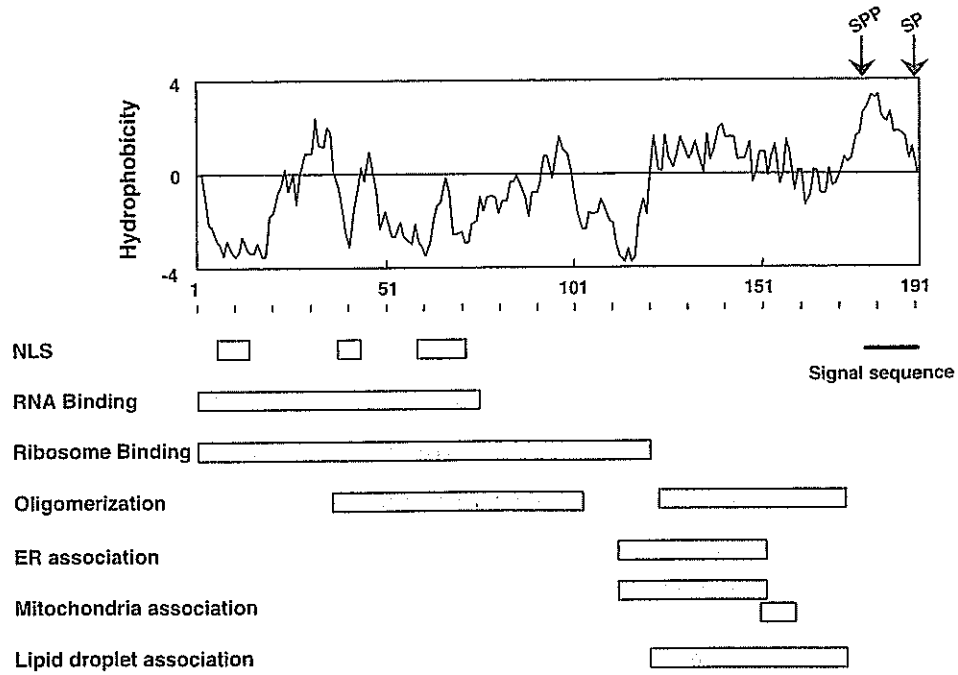



**Figure 10.1** Hydrophobicity profile and regions within HCV core protein involved in subcellular distribution and nucleocapsid assembly. NLS, nuclear localization signal; SPP, signal peptide peptidase; SP, signal peptidase.

*al.*, 1997; Moriya *et al.*, 1998; Yasui *et al.*, 1998, Falcon *et al.*, 2003).

Although the functions of the core protein within various subcellular compartments are unclear to date, the core protein is likely multifunctional and essential for viral replication, maturation, and pathogenesis. It is involved not only in formation of the HCV virion but also has a number of regulatory functions, such as influencing signaling pathways, cellular and viral gene expression, cell transformation, apoptosis, and immune presentation.

## Processing by membrane-associated proteases

A precursor polyprotein, which is translated from an open reading frame of approximately 9 kb, is processed by both host and viral proteases. The HCV structural proteins include the nucleocapsid or core protein and two envelope glycoproteins, E1 and E2. Secondary structure analysis of the core protein has revealed that all major alpha helices are located in the C-terminal half of the protein. A predicted alpha helix encoded by amino acids (aa) 174–191 is extremely hydrophobic and resembles other signal peptide sequences. Membrane and secretory proteins are generally targeted to the appropriate intracellular membrane by their signal peptides. In eukaryotes, signal peptides are 15–50 amino acids long and a typical signal peptide is composed of three distinct regions: a polar N-terminal region that may have a net positive charge, a central hydrophobic sequence that consists of 6–15 hydrophobic residues, and a polar carboxyl-terminal region that contains the cleavage site for the signal peptidase (von Heijne, 1983; von Heijne, 1985; Martoglio *et al.*, 1998).

The N-terminus of E1 protein has been mapped to a signal-peptidase-like cleavage site at aa 192 of the precursor polyprotein (Hijikata *et al.*, 1991). In addition, cleavage occurs on microsomal membranes and depends on a signal recognition particle. Furthermore, inhibition of cleavage by mutations at the possible recognition sites by the signal peptidase has been shown (Hijikata *et al.*, 1991; Santolini *et al.*, 1994).

Cleavage of the core/E1 junction between aa 191 and 192 by the signal peptidase results in anchoring of the core protein within the ER membrane by the C-terminal signal peptide. Further processing within or at the N-terminus of the signal sequence mediated by signal peptide peptidase (SPP) is thought to be involved in maturation of the core protein (Hussy *et al.*, 1996; McLauchlan *et al.*, 2002; Lemberg *et al.*, 2002; Okamoto *et at.*, 2004).

SPP has recently been identified (Welhofen *et al.*, 2002) and exhibits protease activity within cellular membranes, resulting in cleavage of peptide bonds in the plane of lipid bilayers. The peptidase is an aspartic protease and catalyses intramembrane proteolysis of signal sequences and possibly membrane proteins, within the ER (Lemberg *et al.*, 2004). SPP has been reported to cleave human lymphocytic antigen (HLA) molecules, thereby promoting the release of HLA-E epitope-containing peptides from the ER membrane into the cytosol, resulting in recognition by the immune system (Lemberg *et al.*, 2001). The signal sequence at the C-terminus of the core protein is also a substrate for SPP (McLauchlan *et al.*, 2002). It has been shown that (1) intramembrane cleavage by SPP is abolished when helix-breaking and -bending residues in the C-terminal signal sequence are replaced by basic residues, (2) the signal sequence itself and three hydrophobic amino

acids Leu-139, Val-140, and Leu-144 of the core protein are required for SPP cleavage, and (3) none of these residues are essential for cleavage at the core-E1 junction by the signal peptidase, or for translocation of E1 into the ER (Okamoto *et al.*, 2004). Experiments using cDNA clones of HCV isolated from patients with different clinical phenotypes suggest that certain amino acid residues within the C-terminus of the core protein influence processing by SPP (Kato *et al.*, 2003). More work is required to identify the SPP cleavage site within the core protein, although Leu-179 (Hussy *et al.*, 1996), Leu-182 (Hussy *et al.*, 1996), and Ser-173 (Santolini *et al.*, 1994), have all been cited as potential P1 sites of cleavage.

Various studies of HCV cDNA expression *in vitro* and in cultured cells have generally demonstrated two forms of core protein with 21- and 23-kDa (p21 and p23). The p21 form of the core protein predominates in cultured cells, and also in viral particles isolated from the sera of hepatitis C patients (Yasui *et al.*, 1998). Thus, this form of the core protein, which presumably results from two consecutive membrane-dependent cleavages as described above, is thought to be the mature form of the core protein and to constitute the viral capsid. The other form of the core protein, p23, is a 191-residue product, which contains a signal sequence for directing E1 protein to the ER. In addition, production of a 16 kDa form of the core protein (p16) has been reported by HCV genotype 1a (Lo *et al.*, 1994; Lo *et al.*, 1995). However, later studies have identified this protein as F protein, which is encoded by an alternative reading frame from the core region and is expressed by translational ribosomal frameshift (Xu *et al.*, 2001; Walewski *et al.*, 2001; Varakliotl *et al.*, 2002; Vassllaki *et al.*, 2003).

## Mechanisms of subcellular localization

Analysis of the subcellular localization of HCV within the hepatic tissue of HCV-infected individuals has proven difficult due to low levels of viral replication. Nevertheless, immunostaining of liver biopsy specimens has revealed a predominance of core protein within the cytoplasm of infected hepatocytes, and often shows a punctate granular distribution within cells (Yap *et al.*, 1994; Gonzalez-Peralta *et al.*, 1994; Gowans, 2000; Sansonno *et al.*, 2004). As shown in Figure 10.2, in mammalian cells, when core protein alone or the entire viral polyprotein has been expressed, the core protein has primarily been observed within the cytoplasm. Data from a number of studies have identified its co-localization at the ER (Harada *et al.*, 1991; Selby *et al.*, 1993; Suzuki *et al.*, 1995; Lo *et al.*, 1995; Moradpour *et al.*, 1996; Moriya *et al.*, 1997; Yasui *et al.*, 1998), lipid droplets (Barba *et al.*, 1997; Sabile *et al.*, 1999; Hope *et al.*, 2000; McLauchlan *et al.*, 2002), and mitochondria (Moriya *et al.*, 1998; Okuda *et al.*, 2002; Schwer *et al.*, 2004; Suzuki *et al.*, 2005). In addition, a fraction of the core protein has been detected in the nucleus (Chang *et al.*, 1994; Suzuki *et al.*, 1995; Lo *et al.*, 1995; Liu *et al.*, 1997; Moriya *et al.*, 1998; Yasui *et al.*, 1998; Yamanaka *et al.*, 2002).

It has been proposed that, after SPP cleavage, a large part of the core protein remains within the cytoplasmic leaflets of the ER membrane due to preservation of the original transmembrane domain (McLauchlan *et al.*, 2002). The cytoplasmic leaflets become distended with accumulated lipid between two membrane leaflets. The core protein diffuses freely and is transferred along with part of the ER membrane to the surface of a nascent lipid droplet before the droplet buds off the ER.

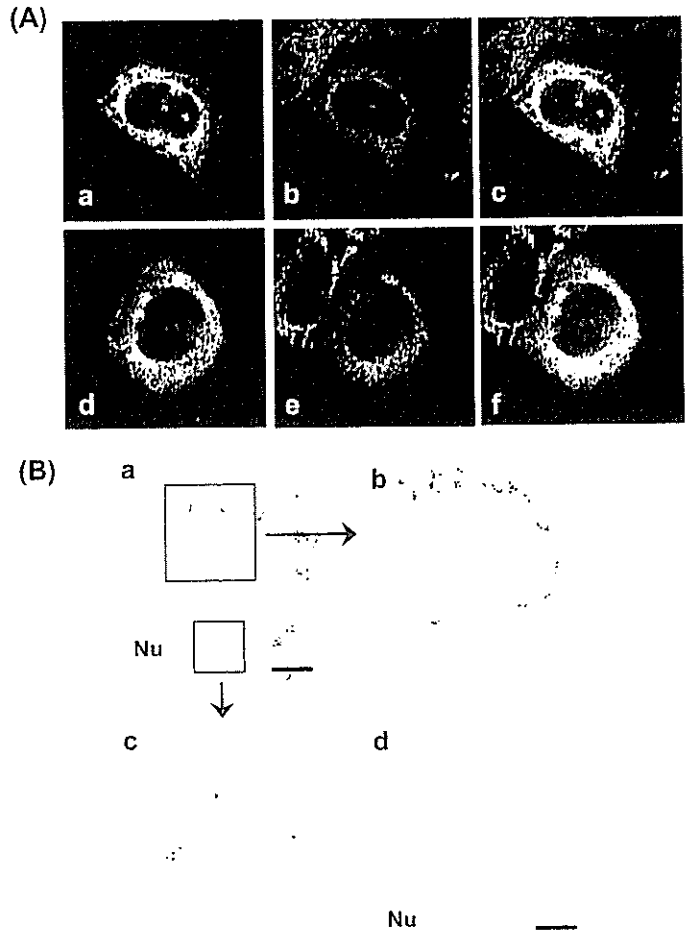



**Figure 10.2** (A) Confocal microscopic images of HepG2 cells transfected with the HCV core (aa 1–191) cDNA. Cells were double stained with anti-core and anti-calregulin, ER marker, or Mitotracker, mitochondrial marker. a, d: core protein, b: calregulin, e: Mitotracker, c, f: overlay. (B) Immunoelectron microscopy. Cells expressing the core protein (a, b, c) or nonexpressing cells (d) were fixed and immunogold labeled with anti-core. Gold particles were found at cytoplasmic membranes (a, b) and in the nucleus (a, c). Bars, 0.5 μm. This figure is reproduced in color in the color section at the end of the book.

This model has recently been modified to indicate that other membranes within the ER network, such as mitochondrion-associated membranes, might be targeted by the mature core protein (Schwer *et al.*, 2004).

Hydrophobic profiling (Figure 10.1) and amino acid sequencing has identified three domains within the core protein (Hope *et al.*, 2000). An N-terminal half domain of 118 residues contains clusters of Lys and Arg residues, which are required for nuclear localization of the core protein and for binding to viral RNA as described below. The 56-residue domain spanning aa 119–174 has a few basic residues and is more hydrophobic compared than the N-terminal domain. The C-terminal 17-residue domain (aa 175–191) is highly hydrophobic and is predicted to have an

alpha-helix structure. This feature is consistent with its role as a signal peptide for E1 protein. Interestingly, the HCV core protein (p21) is much larger than flavi- and pesti-virus capsid proteins. Kunjin virus and classical swine fever virus, for example, are approximately 100 residues in length (Speight *et al.*, 1989; Stark *et al.*, 1993; Rumenapf *et al.*, 1993). Although significant amino acid identity has not been observed between the core protein of HCV and the capsid proteins of other flaviviruses, their N-terminal regions are all rich in basic residues and their C-terminal hydrophobic domains act as signal sequences for the translocation of prM or E1 proteins. A 56-residue domain-like region has also been observed within the core sequence of GB virus-B, which is closely related to HCV, but not among other flavi- and pestiviruses (Hope *et al.*, 2002).

Mutational analyses and immunostaining have shown that a considerable length of this domain is indispensable for the associations between the core protein and ER membrane and lipid droplets to occur (Hope *et al.*, 2000; Okamoto *et al.*, 2004; Suzuki *et al.*, 2005). Sequences in the 56-residue domain required for lipid droplet association also facilitate maturation of the core protein (Hope *et al.*, 2000). Results from our laboratory have demonstrated that a region spanning aa 112–152 of the core protein plays a key role in ER retention of the mature core protein (Suzuki *et al.*, 2005). From examination of the secondary structure of the core protein, a long helical segment (aa 116–134) and two short α-helices (aa 146–152, aa 155–159) are predicted. It has been suggested that an amphipathic alpha-helix spanning aa 116–134 may be required for association of the core protein with the ER membrane. A helical wheel plot of this region shows an amphipathic structure with hydrophobic residues on one side and polar residues on the other side of the α-helix, which are often observed in membrane-associated proteins. This helical wheel conformation might be important in directing the core protein to the ER membrane.

Localization of a fraction of the core protein in the mitochondria of cultured cells has been reported (Okuda *et al.*, 2002; Schwer *et al.*, 2004; Suzuki *et al.*, 2005). In addition, mitochondrial localization has been observed in the transgenic mice, following disruption of the bilayer structure of the mitochondrial membrane by expression of the core protein (Moriya *et al.*, 1998; Moriya *et al.*, 2001). Subcellular fractionation and protease protection assays, as well as immunoelectron microscopy, have recently demonstrated localization of the core protein to the mitochondrial outer membrane (Schwer *et al.*, 2004; Suzuki *et al.*, 2005).

Translocation of nuclear-encoded mitochondrial proteins usually depends on N-terminal sequences, known as mitochondrial targeting sequences (Neupert, 1997). However, a significant proportion of mitochondrial proteins lack these N-terminal mitochondrial-targeting sequences. Specifically, a number of outer membrane proteins do not have cleavable sequences at their N-terminus, rather, they are targeted to mitochondria by means of internal or C-terminal signals (Mihara, 2000). There is some controversy regarding the identity of which sequence is responsible for targeting the core protein to the mitochondria, based on results from two groups of researchers. Through fusion experiments with green fluorescent protein, a short stretch extending from aa 149–158 within the C-terminal hydrophobic region of the core protein, has been observed to play a role in mitochondrial targeting (Schwer *et al.*, 2004). However, another study based

on a similar approach has identified a 41-residue region extending from aa 112–152 as the sequence responsible for association between the core protein and mitochondria (Suzuki *et al.*, 2005). This 41-residue region is same as that required for association of the core protein with the ER membrane, as mentioned above. This might suggest that the mature core protein moves freely within the ER network, which might include mitochondria enveloped by cytoplasmic extensions of the ER membrane, after which the core protein might translocate to the mitochondrial surface.

Although eukaryotic proteins which target the ER and mitochondria generally have different signal sequences and follow distinct transport routes, recent evidence suggests that chimeric signals for bimodal targeting might exist, as suggested by the behavior of the core protein. For example, cytochrome P4502E1 (CYP2E1), which has been implicated in cellular pathology and toxicity related to oxidative stress, is able to localize to both the ER and mitochondria. The last 29 residues of the CYP2E1 N-terminal encode a putative transmembrane anchor domain responsible for targeting the ER, while a sequence extending from aa 21–31 might represent a mitochondrial targeting signal activated by cAMP-dependent phosphorylation at Ser-129 of the protein (Robin *et al.*, 2002). Activation of the mitochondrial signal has been associated with increased efficiency of association between the target protein and cytoplasmic chaperones and/or mitochondrial translocases. HCV core protein can be phosphorylated, probably by cellular protein kinase A and C, as described below (Lu *et al.*, 2002). It has been suggested that phosphorylation of the core protein might be required for some of its biological activity. Thus, it would be useful to investigate whether post-translational modification, such as protein phosphorylation, might mediate ER/mitochondria localization of the core protein.

The known association between HCV core protein and the mitochondrial membrane suggests that the core protein has the ability to modulate mitochondrial function, presumably, at least in part, by altering permeability of the mitochondrial membrane. The core protein induces the production of cellular reactive oxygen species (ROS) in the livers of transgenic mice and in cell lines expressing the protein (Moriya *et al.*, 2001). ROS, predominantly generated in the mitochondria, induce genetic mutations and act as secondary messengers to regulate a variety of cellular functions, including gene expression and proliferation (Adler *et al.*, 1999). Although the exact molecular mechanism by which the core protein induces ROS production has not been determined, HCV core protein is known to impair the mitochondrial electron transfer system (Moriya *et al.*, 2001). The core protein may also modulate apoptosis, since mitochondria play a major role in regulating programmed cell death. Expression of HCV proteins, including the core protein, suppresses the release of cytochrome c from mitochondria to the cytoplasm in HCV-transgenic mice, thus inhibiting Fas-mediated apoptosis (Machida *et al.*, 2001).

Finally, in addition to its association with the cytoplasmic membrane, nuclear localization of the core protein has been observed in cultured cells over-expressing the protein, as well as in liver tissue isolated from patients with hepatitis C (Falcon *et al.*, 2003) and in transgenic mice (Moriya *et al.*, 1998). Although three-dimensional structural data regarding the core protein is lacking, studies using a series of monoclonal antibodies have shown that cytoplasmic and nuclear forms of the core protein

are recognized by distinct antibodies, suggesting that cytoplasmic and nuclear forms of the core protein have different tertiary structures (Yasui *et al.*, 1998).

The N-terminal half of the core protein contains three nuclear localization signal sequences, which are composed of three stretches of sequences rich in basic residues (Suzuki *et al.*, 1995; Suzuki *et al.*, 1996; Figure 10.1). C-terminal truncated versions of the core protein, such as that encoded by aa 1–152, are known to localize exclusively to the nucleus, suggesting that the hydrophobic region of the C-terminal might determine whether the core protein localizes to the nucleus or cytoplasm.

In general, the NLS sequences fall into one of two distinct classes termed monopartite NLSs, containing a single cluster of basic residues, and bipartite NLSs, comprising two basic clusters separated by an unconserved spacer sequence of variable length. The conventional NLSs are recognized by the same receptor protein termed importin or karyopherin (reviewed in Damelin *et al.*, 2002; Weis, 2002). Importin α contains the NLS-binding site, and importin β docks importin-substrate complexes to the cytoplasmic filaments of a nuclear pore complex. Thus, importin α functions as an adaptor between the *bona fide* import receptor and the NLS-carrying protein.

All three NLS motifs of the core protein are able to bind to importin α, and at least two NLS motifs are required for efficient nuclear distribution of the core protein. It appears that mutations of two of the three NLS motifs (double mutant proteins) decrease the ability of the core protein to bind importin α. These observations suggest that NLS motifs within the core protein have a bipartite function and binding between double mutants and importin α leads to little, or no, active translocation of the core protein into the nucleus. Double mutants may also block subsequent interactions with importin $\beta_1$, GTPase Ran, and/or NTF2/p10, all of which are required for translocation through nuclear pore complexes.

Crystallographic studies of the structural basis of the NLS recognition by importin α have shown that the two basic residue clusters of bipartite NLSs occupy separate binding sites on importin α. In contrast, monopartite NLSs can bind to the same sites but primarily use the binding site for the C-terminal basic cluster of the bipartite NLSs, which is known as the major NLS binding site on importin α (Conti *et al.*, 1998; Fontes *et al.*, 2000). An importin α variant containing a mutation in its major binding site has shown a decreased ability to bind both monopartite and bipartite NLSs. Another importin α variant with a mutation in its minor binding site, has shown a decreased ability to bind bipartite NLS-containing proteins, making importin α nonfunctional *in vivo* (Leung *et al.*, 2003). Thus, we favor a model in which the core protein bipartite NLS, composed of any two of the three basic clusters, occupies both major and minor binding sites on importin α, resulting in efficient nuclear translocation. Importin α may be equally accessible to all clusters given their close proximity to one another, as well as the distinct conformational flexibility of the approximately 70-residue N-terminal region of the core protein.

A proteasome activator, PA28γ, has been implicated in nuclear localization of the core protein. Yeast two-hybrid screening has identified PA28γ as the core-binding protein. PA28γ specifically interacts with the core protein in cultured cells, as well as in the livers of transgenic mice and hepatitis C patients. Interaction of the core protein with PA28γ plays an important role

in retention of the core protein, especially the C-terminal truncated form, in the nucleus (Moriishi *et al.*, 2003). A yeast model system has shown a requirement for small GTPase Ran/Gsp1p activity mediated by Kap123p, for nuclear transport of the core protein, however, neither importin α nor importin β are (Isoyama *et al.*, 2002).

Thus, multiple functional domains within the core protein appear to play a role in its subcellular localization, which might ultimately depend on the balance achieved between competing signals.

## Possible post-translational modification

The core protein can be phosphorylated in insect cells (Lanford *et al.*, 1993), reticulocyte lysates (Shih *et al.*, 1995), and mammalian cells (Lu *et al.*, 2002). Possible sites of phosphorylation include Ser-53, -93, -96, and -116 of the core protein (Shih *et al.*, 1995), and the basal phosphorylated residues have been identified as Ser-53 and -116 (Lu *et al.*, 2002). Cellular protein kinase A (PKA) and C (PKC) are presumably responsible for their phosphorylation since activation of PKA and PKC enhances phosphorylation, while inhibition of PKA and PKC negates this effect (Shih *et al.*, 1995; Lu *et al.*, 2002). Results from mutational analyses suggest that phosphorylation at Ser-116 may regulate nuclear localization of the core protein (Lu *et al.*, 2002). Phosphorylation of the core protein might also be required for its biological activity, including inhibition of replication and gene expression of hepatitis B virus (Shih *et al.*, 1995).

The core protein is a substrate of tissue transglutaminase (Lu *et al.*, 2001), which catalyzes calcium-dependent acyl transfer reactions between the γ-carboxamide groups of Gln residues and the ε-amino groups of Lys residues within peptides, resulting in the formation of a gamma-glutamyl-ε-lysine isopeptide bond (Lorand *et al.*, 1984; Greenberg *et al.*, 1991). Presumably, a primary function of the core protein is formation of the viral nucleocapsid. Indeed, the core protein has been observed to form dimers and multimers (Baumert *et al.*, 1998; Nolandt *et al.*, 1997; Kunkel *et al.*, 2001; Matsumoto *et al.*, 1996). A small fraction of the core dimer is highly stable and resistant to denaturation and reduction by SDS and β-mercaptoethanol (Lu *et al.*, 2001). A potential role for tissue transglutaminase in core protein dimer formation has been proposed, based on the results of induction and inhibition experiments. Also, post-translational modification of the core protein by tissue transglutaminase has been observed to generate multimers *in vitro* (Lu *et al.*, 2001).

The ubiquitin-proteasome pathway is the major route by which selective protein degradation occurs in eukaryotic cells and is now emerging as an essential mechanism of cellular regulation (reviewed in Hershko *et al.*, 1998; Finley *et al.*, 2004). This pathway is also involved in the post-translational regulation of the core protein. The core protein is unstable in cells when expressed as the C-terminal truncated forms such as aa 1–173 (21 kDa) and 1–152 (17 kDa) (Suzuki *et al.*, 2001; Moriishi *et al.*, 2003). Specific inhibitors of the 20S proteasome stabilize these short-lived forms of the core protein, suggesting that the proteasome machinery is responsible for their degradation. By contrast, the full-length form of the core protein (aa 1–191) is long-lived and its life is only minimally prolonged by treatment with proteasome inhibitors. Although both C-terminal truncated and full-length forms of the core protein can be ubiquitylated, only conjugation of C-terminal truncated forms to form multiubiquitin chains has been observed. The

predominant stable form of the core protein links to a single or only a few ubiquitin moieties.

The homopolymeric multiubiquitin chain, which links multiple ubiquitin molecules through isopeptide bonds between Lys-48 and Gly-76, is a signal for targeting various substrates to the proteasome. Studies of structurally defined multiubiquitylated substrates have revealed that a chain of four or longer is required for efficient proteasomal targeting (Thrower *et al.*, 2000). The same scenario has been suggested for destabilization of the core protein. Differences in the stability of various forms of the core protein depend on how the ubiquitin chain is assembled, such as the number of ubiquitin molecules conjugated to the core protein. Diversity in ubiquitin-chain assembly might result from variations in the conformation of the core protein. The conformation of C-terminal truncated versions of the core protein might favor multiubiquitylation. Although more stable forms of the core protein are recognized by the ubiquitin-conjugating system, ubiquitylation ceases after only one or a few cycles. This may be due to steric constraints. It has been hypothesized that the conformation of stable forms of the core protein might interfere with elongation of the ubiquitin chain.

Recently, a role for the core-binding protein PA28γ in degradation of the core protein has been suggested (Moriishi *et al.*, 2003). Overexpression of PA28γ promotes proteolysis of the core protein. PA28γ predominates in the nucleus and forms a homopolymer, which associates with the 20S proteasome (Tanahashi *et al.*, 1997), thereby enhancing proteasomal activity (Realini *et al.*, 1997). Both nuclear retention and core protein stability are regulated via a PA28γ-dependent pathway.

The biological significance of ubiquitylation of the core protein is not well understood. In eukaryotic cells, targeted protein degradation is increasingly understood to be an important mechanism by which cells regulate levels of specific proteins, and thereby regulate their function. Presumably, the core protein plays a key role in viral replication and pathogenesis since it forms the viral particle and regulates a number of host cell functions. Degradation of the core protein by the ubiquitin-proteasome pathway might result in downregulation of viral assembly and/or inhibition of a number of intracellular processes mediated by the core protein.

## Viral assembly

A crucial function of the core protein is assembly of the viral nucleocapsid. However, the molecular mechanism by which this occurs is still uncertain. In addition to mature, enveloped HCV virions (Kaito *et al.*, 1994; Kanto *et al.*, 1994), nonenveloped nucleocapsid-like particles and viral structures expressing the core protein on their surface are present in the plasma of HCV-infected patients (Takahashi *et al.*, 1992; Ishida *et al.*, 2001; Maillard *et al.*, 2001). Thus, in spite of an internal component of infectious particles, the HCV nucleocapsid may be a feature of the viral morphogenesis and be released from the cells. Nucleocapsid-like particles obtained from patient plasma are spherical particles, 33–40 nm in diameter, with a buoyant density of 1.22–1.25 g/ml in sucrose density gradients (Ishida *et al.*, 2001) or those with 38–43 or 54–62 nm appeared at 1.32–1.34 g/ml in CsCl (Malliad *et al.*, 2001). They have similar morphology and physicochemical properties to HCV nucleocapsids isolated by detergent treatment of putative virions.

Several expression systems have been used to investigate HCV capsid assembly using mammalian, insect, yeast, bacteria, and reticulocyte lysates, as well as puri-

fied recombinant proteins (Baumert *et al.*, 1998; Falcon *et al.*, 1999; Shimizu *et al.*, 1996; Kunkel *et al.*, 2001; Lorenzo *et al.*, 2001; Acosta-Rivero *et al.*, 2001; Kunkel *et al.*, 2002; Acosta-Rivero *et al.*, 2003; Blanchard *et al.*, 2003; Majeau *et al.*, 2004; Klein *et al.*, 2004). The results suggest that immunogenic nucleocapsid-like particles are heterologous in size and range from 30 to 80 nm in diameter. The N-terminal half of the core protein is important for nucleocapsid formation (Kunkel *et al.*, 2001; Majeau *et al.*, 2004; Klein *et al.*, 2004). Assembly of the nucleocapsid does not require presence of the viral envelope or nonstructural proteins. In addition, HCV capsid formation occurs in the presence or absence of ER-derived membrane, which supports cleavage of the signal peptide at the C-terminus (Klein *et al.*, 2004).

Nucleocapsid assembly generally involves oligomerization of the capsid protein and encapsidation of genomic RNA. In fact, study of a recombinant mature core protein has shown it to exist as a large multimer in solution under physiological conditions, within which stable secondary structures have been observed (Kunkel *et al.*, 2004). There is conflicting data regarding the sequence responsible for self-interaction of the core protein. Studies using yeast two-hybrid systems have identified a potential homotypic interaction domain within the N-terminal region of the core protein (aa 1–115 or–122), with particular emphasis on the region encompassing aa 82–102 (Matsumoto *et al.*, 1996; Nolandt *et al.*, 1997). However, more recent studies have identified two C-terminal regions, extending from aa 123–191 and 125–179, as responsible for self-interaction. Furthermore, Pro substitution within these C-terminal regions has been observed to abolish core protein self-interaction (Yan *et al.*, 1998, Kunkel *et al.*, 2004). Circular dichroism spectroscopy has further shown that a Trp-rich region spanning aa 76–113 is largely solvent-exposed and unlikely to play a role in multimerization (Kunkel *et al.*, 2004).

Once a HCV nucleocapsid is formed in the cytoplasm, it acquires an envelope as it buds through intracellular membranes. Interactions between the core protein and E1/E2 envelope proteins are thought to determine viral morphology. Expression of HCV structural proteins using recombinant virus vectors has succeeded in generating virus-like particles with similar ultrastructural properties to HCV virions. Packaging of these HCV-like particles into intracellular vesicles as a result of budding from the ER has been noted (Baumert *et al.*, 1998; Ezelle *et al.*, 2002; Clayton *et al.*, 2002). Mapping studies to determine the nature of interaction between core and E1 proteins have demonstrated the importance of C-terminal regions in this interaction (Lo *et al.*, 1996; Ma *et al.*, 2002). Since corresponding sequences are not well conserved among various HCV isolates, interactions between core and E1 proteins might depend more on hydrophobicity than specific sequences.

The core protein is highly basic, especially its N-terminal half. Therefore, the protein may encapsulate the viral genome within the nucleocapsid and/or associate with RNA in infected cells. Interactions between the core protein and homologous and heterologous RNA have been analyzed. The results of an *in vitro* North-Western analysis suggests that the core protein binds to ribosomes and the 5′ untranslated region (5′ UTR) of viral genomic RNA, regardless of the specific RNA sequences involved (Santolini *et al.*, 1994; Hwang *et al.*, 1995; Fan *et al.*, 1999). By contrast, an *in vivo* system has been used to show preferential interaction between the core protein and positive-sense viral RNA containing the 5′ UTR and

part of the structural protein coding region (Shimoike *et al.*, 1999). A study employing surface plasmon resonance technology has demonstrated selective binding between the core protein and synthetic oligonucleotides corresponding to the 5′ UTR, as well as short homopolymeric oligomers. In addition, it has been shown that the core protein has strong affinity for the stem–loop IIId domain of the 5′ UTR and for (G)-rich sequences (Tanaka *et al.*, 2000). Formation of HCV nucleocapsid-like particles by purified core protein occurs in the presence of full-length or partial 5′ UTR RNA, but not in the absence of nucleic acids, indicating that interaction between the core protein and RNA is essential for HCV assembly (Kunkel *et al.*, 2001). Recently, the core protein has been reported to have nucleic acid chaperone activity (Cristofari *et al.*, 2004). The core protein enhances hybridization of complementary DNA and RNA sequences, and allows the formation of stable structures by strand exchange. The core protein also mediates dimerization of viral positive-stranded 3′ UTR RNA

In addition to the importance of core-RNA interactions for virion formation, these interactions are also thought to regulate 5′ UTR internal ribosome entry site (IRES)-mediated viral translation, which is required for the initiation of cap-independent translation. It has been shown that the core protein down-regulates HCV translation through interactions with viral IRES RNA (Shimoike *et al.*, 1999). Although a conflicting report has suggested that inhibition of HCV translation is due to an RNA–RNA interaction, rather than an interaction between RNA and the core protein (Wang *et al.*, 2000), later studies support the role of a core protein sequence spanning aa 34–44 in inhibition of viral translation through its interaction with IRES (Zhang *et al.*, 2002). Furthermore, the N-terminal 20 residues of the core protein have been shown to selectively inhibit translation mediated by HCV IRES in a cell type-specific manner (Li *et al.*, 2003). This implies that the core protein might contribute to virus persistence by maintaining a low level of HCV replication and expression.

## Future perspectives

Although progress is being made in understanding biosynthesis and biochemical properties of the HCV core protein, a number of questions remain regarding its maturation and morphogenesis into the viral nucleocapsid. The development of subgenomic- and full-length replicons has greatly contributed to our knowledge regarding the molecular biology of HCV. However, cells expressing HCV replicons do not support virion formation, including the nucleocapsid assembly. There is particular need for the development of an improved cell culture system, by which HCV virions can be efficiently produced and released.

## References

Acosta-Rivero, N., Aguilar, J.C., Musacchio, A., Falcon, V., Vina, A., de la Rosa, M.C., and Morales, J. (2001). Characterization of the HCV core virus-like particles produced in the methylotrophic yeast Pichia pastoris. Biochem. Biophys. Res. Commun. *287*, 122–125.

Acosta-Rivero, N., Falcon, V., Alvarez, C., Musacchio, A., Chinea, G., Cristina de la Rosa, M., Rodriguez, A., Duenas-Carrera, S., Tsutsumi, V., Shibayama, M., *et al.* (2003). Structured HCV nucleocapsids composed of P21 core protein assemble primary in the nucleus of Pichia pastoris yeast. Biochem. Biophys. Res. Commun. *310*, 48–53.

Adler, V., Yin, Z., Tew, K.D., and Ronai, Z. (1999). Role of redox potential and reactive oxygen species in stress signaling. Oncogene *18*, 6104–6111.

Alter, M.J. (1995). Epidemiology of hepatitis C in the West. Semin Liver Dis. *15*, 5–14.