Hepatitis C virus (HCV) core protein, a component of viral nucleocapsid, has been shown to modulate cellular and viral promoter activities. To identify potential cellular targets for HCV core protein, a human liver cDNA library was screened for core-interacting proteins using the yeast two-hybrid system. Among the proteins identified was heterogeneous nuclear ribonucleoprotein K (hnRNP K), which has been demonstrated to be a transcriptional regulator. The interaction of HCV core protein with hnRNP K was confirmed by glutathione S-transferase fusion protein binding assay, protein-protein blotting assay, and coimmunoprecipitation in vitro and in vivo. Additionally, these two proteins were shown to be partially colocalized in the nucleus. The hnRNP K-binding site in HCV core protein was mapped to the region from amino acid residues 25–91, a hydrophilic area near the N terminus. The HCV core protein-binding domain was located within amino acid residues 250 to 392, which contain the three proline-rich domains, of hnRNP K. Furthermore, HCV core protein relieved the suppression effect of hnRNP K on the activity of the human thymidine kinase gene promoter. The specific binding of HCV core protein to hnRNP K suggests that multiple functions of hnRNP K may be disrupted by the core protein during HCV infection and thus explains, in part, the pathogenesis of HCV.

Hepatitis C Virus (HCV) is the major cause of transfusion- and community-acquired non-A, non-B hepatitis (1–3), often leading to cirrhosis of the liver, hepatocellular carcinoma (3–5), and some autoimmune diseases (6–10). HCV is an enveloped, single-stranded, positive-sensed RNA virus. HCV belongs to the Flaviviridae family and is related to flaviviruses and pestiviruses with respect to genomic organization and protein processing patterns (11–14). The genome of HCV is approximately 9.5 kilobases and contains a 9-kilobase open reading frame, which encodes a polyprotein of about 3000 amino acids (11, 14, 15). The polyprotein is cleaved by host and viral proteases into three structural proteins, including the core protein and two envelope proteins, and at least six nonstructural proteins (16). The core protein, which is the major component of the viral nucleocapsid, is cleaved from the very N terminus of the polyprotein by a host signal peptidase in the endoplasmic reticulum (17, 18). It contains 191 amino acids; the N-terminal two-thirds is highly hydrophilic, while the C-terminal end is hydrophobic. It is associated with the cytoplasmic side of the endoplasmic reticulum (18, 19), but the various C terminus-truncated forms are found in the nucleus (19–22). It is phosphorylated (23), and binds to DNA, RNA, and ribosomes (18, 24, 25), suggesting that it may have multiple roles in the life cycle of this virus. Recent studies have shown that HCV core protein can modulate the expression of several viral and cellular genes (23, 25–27), including c-myc, p53, and hepatitis B virus. These properties together suggest that HCV core protein may affect normal cellular functions and thus contribute to the pathogenesis of HCV.

To explore the potential targets of the core protein during HCV infection, we have used the yeast two-hybrid system to screen a human liver cDNA library for cellular proteins that can interact with HCV core protein. We have previously characterized one of the major proteins detected, namely, lymphocyte β-receptor (28), which is a member of the tumor necrosis factor receptor family. In this study, we report the second molecule detected in the yeast two-hybrid screening. This molecule, heterogeneous nuclear ribonucleoprotein K (hnRNP K), was isolated in two independently derived cDNA clones.

hnRNP K has both RNA-binding and DNA-binding properties, with preference for polyPyrimidine sequences (29, 30). It is a component of the hnRNP complex and is predominantly present in the nucleus, but shuttles between the nucleus and the cytoplasm (31, 32). Therefore, hnRNP K is thought to participate in the processing and transport of pre-mRNA (33). hnRNP K has also been demonstrated to be a transcriptional regulator, which stimulates the c-myc promoter in vitro (30, 34, 35). In this paper, we have characterized the interaction between HCV core protein and hnRNP K. We showed that HCV core protein can relieve the suppression effect of hnRNP K on the activities of the human thymidine kinase gene promoter in vivo. These results suggest that HCV core protein can disrupt the multiple functions of hnRNP K and thus contribute to the pathogenesis of HCV.

MATERIALS AND METHODS

Cells and Media—COS-7, a monkey kidney cell line, and K12 (36), a Chinese hamster fibroblast cell line, were used in this study. Cells were
Hepatitis C Virus Core Protein and hnRNPK

grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with nonessential amino acids (Life Technologies, Inc.) and 10% fetal bovine serum.

**DNA Sequence Analysis**—DNA sequences were determined by the dideoxynucleotide chain termination sequencing method (37) using the appropriate oligonucleotides. DNA sequences were analyzed using the data base of the National Center for Biotechnology Information using the BLAST program.

**Plasmid Construction**—The plasmid pGSTM/HCVcore (1–115), derived from a Taiwan HCV isolate (15), was constructed as described previously (28) and used in the yeast two-hybrid screening. The construct pET-2/HCVcore K, which expresses a C-terminal 173 amino acid fragment of the HCV core protein (39) for 4 h at 4 °C. The protein A-Sepharose-bound rabbit preimmune serum, and then incubated with protein A-Sepharose-bound rabbit polyclonal antibody against HCV core protein (39) for 10 h at 4 °C. The protein A-Sepharose beads (Amersham Pharmacia Biotech) were washed four times with binding buffer, and bound proteins were separated by SDS-PAGE and visualized by autoradiography.

**In Vivo Coimmunoprecipitation**—COS-7 cells were infected with recombinant vaccinia virus vTF7–3 expressing T7 RNA polymerase (44). At 2 h post-infection, cells were cotransfected with HCV core protein and N-Flag-hnRN KP-expressing vectors by the calcium phosphate precipitation method (45). Cells were harvested and lysed in lysis buffer (46) (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM MgCl2, 1 mM EDTA, 5% glycerol, 1 mM 2-mercaptoethanol, 0.5% non-fat milk, and 0.1% Triton X-100) 12 h after transfection. Lysates were incubated for 4 h at 4 °C with protein A-Sepharose-bound anti-Flag (Eastman Kodak Co.) or anti-hemagglutinin monoclonal antibodies (Boehringer Mannheim). Beads were washed four times with binding buffer, and bound proteins were separated by SDS-PAGE and electrotransferred to a nitrocellulose membrane. The membrane was blocked with 5% non-fat milk, washed three times with PBS, and incubated with rabbit polyclonal antibody (1:250) against HCV core protein in PBS for 2 h at room temperature. Subsequently, the membrane was washed again with PBS, incubated with 125I-protein A for 1 h at room temperature, and washed again with PBS. The membrane was dried and subjected to autoradiography.

**Yeast Two-hybrid System**—The procedures for the yeast two-hybrid screening were described previously (28). Briefly, the Saccharomyces cerevisiae strain SPY526 was grown in YPD (containing 1% yeast extract, 2% of peptone, 2% of dextrose) or synthetic minimal medium (containing 0.67% yeast nitrogen base, 2% dextrose, and appropriate auxotrophic supplements). Yeast was cotransformed with both GAL4 and a reporter plasmid, phTK-CAT, which contains the chloramphenicol acetyltransferase (CAT) gene driven by the human thymidine kinase promoter containing 474 base pairs of promoter sequence, respectively. The plasmid pHK-TK-CAT, which contains the chloramphenicol acetyltransferase (CAT) gene driven by the human thymidine kinase promoter containing 474 base pairs of promoter sequence, was constructed as described previously (40).

**Immunofluorescence Staining**—COS-7 cells grown on coverslips were cotransfected with GFP-HCV core protein- and N-Flag-hnRN KP-expressing plasmids, by the (N-1-2,3-dioleoylxylo)propyl-N,N,N-trimethylammonium salts method (Boehringer Mannheim). Thirty-six hours after transfection, cells were washed with PBS, fixed with 2% formaldehyde in PBS for 30 min at room temperature, and stained with the Anti-Flag monoclonal antibody (American Qualex) (1:1000) as primary and secondary antibodies, respectively. The stained cells were mounted with Vectashield mounting medium (Vector Laboratories Inc.) and examined by confocal laser scanning microscopy.

**CAT Assays**—The CAT reporter plasmid pHK-TK-CAT, and a β-galactosidase reporter plasmid pCMVβ-galactosidase, under the control of a CMV promoter, were cotransfected into K12 cells together with hnRNKP K-expressing and the various HCV core protein-expressing vectors using the SuperFeet transfection reagent (Qiagen). Thirty-six hours after transfection, cells were harvested and lysed in reporter lysis buffer (Promega). Lysates were incubated at 65 °C for 10 min prior to CAT assays. CAT activities were measured according to the published method (47) and normalized on the basis of β-galactosidase expression.

**RESULTS**

Two hnRNKP K-encoding cDNA Clones Were Identified to Interact with HCV Core Protein Using the Yeast Two-hybrid Screening—To identify possible cellular proteins interacting with HCV core protein using the yeast two-hybrid system, we employed to screen a human liver cDNA library, using the hydrophilic region of HCV core protein (aa 1–115) as a bait as reported previously (28). Twenty positive clones were obtained from a total of 4.7 × 106 transformants screened. DNA sequence analysis showed that two of the 20 positive clones
matched the cDNA sequence of hnRNP K. Both of these two clones represent nucleotides 231–1282 of hnRNP K cDNA sequence, corresponding to amino acid residues 42–392 of hnRNP K, fused in frame to the GAL4 activation domain (Fig. 1). Re-transformation of these two clones into the yeast host produced blue colonies only in the presence of the pGBT9/HCVcore(1–115) plasmid, but not the empty vector, indicating that this hnRNP K clone specifically interacts with HCV core protein. As shown previously, the full-length HCV core protein (aa 1–191) did not interact with hnRNP K in the yeast two-hybrid screen, probably because the strong hydrophobicity of the C-terminal residues of the core protein prevented its function in the yeast two-hybrid system. But the full-length core protein did have a similar binding properties to those of the truncated (aa 1–115) core protein (see below).

**HCV Core Protein from Different HCV Isolates Binds to hnRNP K in Vitro—**To confirm the binding between HCV core protein and hnRNP K, we first performed in vitro binding studies between hnRNP K and HCV core protein using GST fusion protein binding assay. The in vitro translated, [35S]Met-labeled HCV core protein was incubated with partially purified GST-hnRNP K fusion protein, and bound proteins were detected by SDS-PAGE. As shown in Fig. 2B, the core protein bound to GST-hnRNP K but not to GST. The in vitro translated core protein consisted of two major species (21 and 18 kDa) and several high molecular weight core protein aggregates, as demonstrated previously (39). All of these protein species bound to GST-hnRNP K. Furthermore, C terminus-truncated core proteins, including aa 1–115, 1–153, and 1–173, bound to the GST-hnRNP K to the same extent, indicating that the binding domain is located within the N-terminal 115 amino acids of the core protein (data not shown). This finding is consistent with the result of the yeast two-hybrid screening, which showed that the aa 1–115 of the HCV core protein is sufficient for binding to hnRNP K (see below). The remaining sequences (aa 116–191) did not affect the in vitro binding properties of the core protein, but did affect its hydrophobic properties and subcellular distribution (see below). For all of the full-length and truncated HCV core proteins used, approximately 10–15% of input radioactivity bound to GST-hnRNP K. There was no difference in the extent of binding among the various constructs. To establish that the hnRNP K binding was not unique to the sequence of

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**Fig. 1. Schematic representation of hnRNP K structure and of the clones obtained from the yeast two-hybrid screening.** Boxes represent open reading frame, and bars indicate 5‘- and 3‘-untranslated regions of hnRNP K cDNA. Nucleotide and amino acid numbers are indicated above and below the boxes, respectively.

**Fig. 2. Interaction of HCV core protein with hnRNP K in the GST fusion protein binding assay.** In vitro translated, [35S]Met-labeled HCV core proteins from different isolates (Taiwan (Tw), RH, and Chiron) were mixed with GST and GST-hnRNP K fusion proteins bound to glutathione-Sepharose 4B beads. Bound proteins were then analyzed by SDS-PAGE followed by autoradiography. A, Coomassie Blue staining of GST and GST-hnRNP K fusion proteins used in each experiment. B–D, autoradiography of [35S]Met-labeled HCV core proteins from Taiwan (Tw), Southern California (RH), and Chiron (Chiron) isolate, respectively, bound to GST-hnRNP K proteins. IVT, input in vitro translated, [35S]Met-labeled proteins. M, molecular weight markers in kilodaltons.
this particular HCV clone, we examined the core proteins from two other HCV isolates (Southern California (RH) and the prototype Chiron isolates) (48). Both of these proteins bound to GST-hnRNP K but not to GST (Fig. 2, C and D). These results indicated that the ability to bind to hnRNP K is a general property of the core protein from different HCV isolates.

We next used a far-Western protein-protein blotting assay to confirm the interaction between the two proteins. Partially purified GST and GST-hnRNP K from E. coli were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with [35S]-labeled HCV core protein. A reverse experiment was also performed using E. coli-expressed HCV core protein and [35S]-labeled hnRNP K. A, Coomassie Blue staining of GST and GST-hnRNP K fusion proteins used. B, autoradiography of bound [35S]-labeled HCV core protein. C, Coomassie Blue staining of total bacterial lysates used. D, bound [35S]-labeled hnRNP K.

FIG. 3. Association of HCV core protein with hnRNP K detected by far-Western protein-protein blotting assay. GST and GST-hnRNP K fusion proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with [35S]-labeled HCV core protein. A, Coomassie Blue staining of GST and GST-hnRNP K fusion proteins used. B, autoradiography of bound [35S]-labeled HCV core protein. C, Coomassie Blue staining of total bacterial lysates used. D, bound [35S]-labeled hnRNP K.

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FIG. 4. In vitro coimmunoprecipitation of HCV core protein and hnRNP K. In vitro translated, [35S]-labeled HCV core protein and hnRNP K were mixed and precipitated with an antibody against HCV core protein. IVT, input in vitro translated, [35S]-labeled proteins without immunoprecipitation.

HCV Core Protein Interacts with hnRNP K in Vivo—To determine whether HCV core protein is capable of binding to hnRNP K in cells, coimmunoprecipitation was performed on cells expressing both proteins. It has been shown that the full-length core protein (aa 1–191) is localized in the cytoplasm, whereas the various C-terminal truncated core proteins are localized in the nucleus (18–22). Because hnRNP K is mainly localized in the nucleus (29), we reasoned that the nuclear forms of the HCV core protein will be the one that interacts with hnRNP K. Therefore, we used a truncated HCV core protein (aa 1–153) for in vivo binding study. COS-7 cells were infected with recombinant vaccinia virus vTF7–3 and transfected with vectors expressing a truncated HCV core protein (aa 1–153) and/or expressing hnRNP K tagged with a Flag epitope. Lysates were subjected to coimmunoprecipitation using the anti-Flag monoclonal antibody. Immunoprecipitates were separated by SDS-PAGE and transferred to a nitrocellulose membrane for Western blotting assay using an antibody against HCV core protein. The results showed that HCV core protein was precipitated by the anti-Flag antibody but not by an unrelated anti-hemagglutinin antibody, when these two proteins were coexpressed in cells (Fig. 5, lanes 3 and 4). As a

FIG. 4. In vitro coimmunoprecipitation of HCV core protein and hnRNP K. In vitro translated, [35S]-labeled HCV core protein and hnRNP K were mixed and precipitated with an antibody against HCV core protein. IVT, input in vitro translated, [35S]-labeled proteins without immunoprecipitation.
To define the region of HCV core protein critical for specific binding to hnRNP K, a series of deletion mutants of HCV core protein were fused to the GAL4 DNA-binding domain plasmid and cotransformed with the hnRNP K-GAL4 activation domain fusion plasmid in the yeast two-hybrid system (Fig. 7). The results showed that the minimum region, which yielded strong positive interaction between the two proteins, was in amino acid residues 25–91. Although truncated HCV core proteins aa 36–91 and aa 36–115 also showed positive interaction with hnRNP K, the binding was weak, as indicated by the light blue yeast colonies (data not shown). It is interesting to note that aa 36–91 of HCV core protein is the most hydrophilic region of the protein and is also the region that binds the lymphotoxin-β receptor (28).

The HCV Core Protein-binding Domain of hnRNP K Includes the Three Proline-rich Regions—To determine which region of hnRNP K is required for interaction with HCV core protein, a series of truncation mutants of hnRNP K were fused to the GAL4 activation domain plasmid and cotransformed with the plasmid encoding GAL4 DNA-binding domain-HCV core protein (aa 1–115) using the yeast two-hybrid system (Fig. 8). The results showed that the minimum domain, which yielded strong positive interaction, was from amino acid residues 250–392. The hnRNP K mutant aa 276–463 yielded only weak interaction with HCV core protein, as indicated by the light blue yeast colonies (data not shown). Because the original clones (aa 42–392) identified in the yeast two-hybrid screening produced very strong interaction, we conclude that the region from amino acids 250 to 392, which includes the three proline-rich domains, is the HCV core protein-binding domain in hnRNP K. The binding affinity appeared to be affected by neighboring amino acid sequences.

HCV Core Protein Reversed the Suppression Effect of hnRNP K on the Promoter Activity of the Human Thymidine Kinase Gene in Vivo—HCV core protein has been shown to modulate promoter activities of several cellular and viral genes (23, 25–27), and hnRNP K also has been reported to exhibit a significant, but weak, trans-acting enhancement effect on the c-myc promoter mediated by a CT element (30, 34, 35). The human thymidine kinase (TK) promoter also contained a CT motif about 100 bp upstream of its TATA element (49). Recently, hnRNP K was found to have a repressive effect on the promoter activity of the human TK gene. To investigate whether HCV core protein can affect the activity of hnRNP K by modulating transcription, we thus used a TK-promoter-driven CAT reporter system. This reporter plasmid (pH-TK-CAT) and a β-galactosidase-expressing plasmid were cotransfected with the HCV core protein- and hnRNP K-expressing plasmids into K12 cells, a Chinese hamster fibroblast cell line. CAT activities were determined and normalized to β-galactosidase expression. The results showed that hnRNP K suppressed the TK promoter...
activity around 5-fold (Fig. 9, lanes 1 and 2). The suppression effect of hnRNP K was partially reversed by the expression of a truncated HCV core protein (aa 1–153) (Fig. 9, lane 3). Another truncated core protein (aa 1–115), which was expressed in lower amount, reversed the suppression effect of hnRNP K to a slightly lower extent (Fig. 9, lane 4). Interestingly, a further truncation of the core protein (aa 1–75), which did not bind to hnRNP K (Fig. 7), did not affect the suppression activity of hnRNP K on the TK promoter (Fig. 9, lane 5). However, the expression level of the core protein (aa 1–75) was slightly lower; thus, it is not certain whether the failure of this protein to reverse the hnRNP K effect was entirely due to its failure to bind hnRNP K. These results, nevertheless, suggested that the binding of the HCV core protein to hnRNP K reversed the transcription-regulatory functions of hnRNP K. As a control, HCV core protein by itself did not affect the TK promoter activity (Fig. 9, lanes 6 and 7). This result provides confirming evidence that HCV core protein has a biologically significant interaction with hnRNP K.

**DISCUSSION**

The data presented in this report demonstrated that HCV core protein specifically associates with hnRNP K and may affect the function of hnRNP K in cells. This observation adds to the growing body of evidence that HCV core protein is a multifunctional viral protein that may affect normal cellular functions. Besides being a viral nucleocapsid protein, HCV core protein has RNA-binding properties and can oligomerize (39). It binds to the cytoplasmic tails of lymphotoxin-β-receptor (28) and tumor necrosis factor receptor (63) and sensitizes cells to tumor necrosis factor- and Fas-mediated cell death (51, 63). In addition, it also has been demonstrated to associate with apolipoprotein AII at the surface of the lipid storage droplets, linking HCV core protein to cellular lipid metabolism (52).
Indeed, a recent study demonstrated that expression of HCV core protein in transgenic mice results in hepatic steatosis (fatty degeneration), one of the histological features of the liver in chronic hepatitis C (53). The finding presented here adds another aspect to the potential effects of HCV core protein, namely, the possible regulation of cellular gene expression and RNA metabolism.

HCV core protein has been demonstrated to modulate (either stimulate or suppress) the promoter activities of various viral and cellular genes, including suppression of gene expression and replication of hepatitis B virus (23, 25), inhibition of human immunodeficiency virus type 1 long terminal repeat activity and c-fos promoter (26), and activation of Rous sarcoma virus long terminal repeat, human c-myc, and simian virus 40 early promoters (26), although these effects are modest at best (usually 3–4-fold). It is not clear whether the core protein binds directly to these promoters or acts indirectly through interaction with transcription factors. The multitude of promoters affected would argue for an indirect effect through a common transcription factor. In this study, we found that HCV core protein binds to hnRNP K, which, by itself, modestly stimulates the c-myc promoter (30, 34, 35), but suppresses the TK promoter. The finding that HCV core protein partially relieved this suppression activity of hnRNP K on the TK promoter suggests that the core protein has potential effects on gene expression through interaction with cellular factors. It is interesting to note that the HCV core-binding domain is located within the region from amino acid residues 250–392 of hnRNP K, including the three proline-rich domains, which are critical for interaction of hnRNP K with other cellular protein partners (54–56). hnRNP K has been shown to bind to the transcription factor TBP (57), a transcription repressor Zikl (58), a proto-oncoprotein Vav (54, 55), and the protein-tyrosine kinases Src, Fyn, and Lyn (56, 59, 60), suggesting the potential roles of hnRNP K in gene regulation and signal transduction. The binding of HCV core protein to hnRNP K in the proline-rich regions may disrupt the association of hnRNP K with its protein partners and therefore affect the function of hnRNP K. The
CAT activities were determined and normalized on the basis of plasmids. Thirty-six hours after transfection, cells were lysed, and protein and hnRNP K can potentially affect the function of biological roles in normal cellular functions. Nevertheless, our modest; thus we cannot attach too much significance to its effect of HCV core protein on this suppression effect was also growth. However, it should be cautioned that the suppression mechanism by which HCV core protein can regulate cell transition, by binding to hnRNP K suggests an additional possibility.

The effect of HCV core protein on the suppression activity of hnRNP K on the human thymidine kinase gene promoter. K12 cells were cotransfected with the reporter plasmid (pHTK-CAT), a β-galactosidase-expressing plasmid, and the indicated expression plasmids. Thirty-six hours after transfection, cells were lysed, and CAT activities were determined and normalized on the basis of β-β-galactosidase expression. CAT activities were averaged from three independent experiments. The CAT chromatography of one of the experiments is shown in the upper panel. The relative CAT activities are shown in the middle panel. Lower panels, various HCV core proteins expressed in the cells were determined by immunoblotting using a rabbit polyclonal antibody against the core protein. The same blot was probed with an antibody against a cellular protein Sam68 to serve as a loading control.

data presented in this report demonstrating that HCV core protein binds to hnRNP K, and may affect the functions of hnRNP K as a transcription regulator, further support this possibility.

HCV core protein may also have a potential role in the regulation of cell growth, because HCV core protein has a suppression effect on the promoter activity of p53 (27), a tumor suppressor gene, and it can transform primary rat embryo fibroblasts in cooperation with the ras oncogene (61). Our finding that HCV core protein could regulate promoter activity of the human thymidine kinase gene, which is important for G1-S transition, by binding to hnRNP K suggests an additional mechanism by which HCV core protein can regulate cell growth. However, it should be cautioned that the suppression effect of hnRNP K on TK promoter activity is modest, and the effect of HCV core protein on this suppression effect was also modest; thus we cannot attach too much significance to its biological roles in normal cellular functions. Nevertheless, our finding clearly suggests that interaction between HCV core protein and hnRNP K can potentially affect the function of hnRNP K. An additional potential function of hnRNP K is in pre-mRNA processing (33). The possible effects of HCV core protein on this activity have not yet been assessed.

The full-length core protein of HCV is localized to the cytoplasm, particularly in association with the endoplasmic reticulum (18, 19). However, HCV core protein contains a nuclear localization signal near the N terminus (20, 62), and various truncated forms (aa 1–173 or shorter) of the core protein were detected in the nucleus (19–22). Nuclear localization of these proteins is consistent with their potential roles in gene regulation. In this study, we used various truncated proteins for different in vivo binding and localization studies, because we found that the stability and expression levels of the various truncated forms were different when they were fused with different proteins. We used the more stable forms for our experiments. Regardless, the hnRNP K-binding domain is localized within the N-terminal aa 1–115, and in vitro binding studies showed that there was no difference in binding properties among the various truncated core proteins. Thus, these various truncated forms should have comparable properties. Although the functional importance of the truncated core proteins in the HCV life cycle is not yet clear, these truncated proteins were invariably detected in numerous studies on expression of HCV core protein (19, 21, 22, 62). Thus, it is very likely that these truncated proteins are produced during natural HCV infection. Because hnRNP K is located mainly in the nucleus, only the truncated HCV core proteins are expected to have functionally significant interaction with hnRNP K in the cells. Therefore, all of the in vitro interaction studies were performed using various truncated core proteins. The observation that HCV core protein (aa 1–173) only partially colocalized with hnRNP K in the nucleus may explain why HCV core protein only partially relieved the suppression effect of hnRNP K on the human TK promoter activity. It appears that HCV core protein and hnRNP K colocalized in granules in the nucleus. It will be interesting to know what kind of structure these granules represent.

Both HCV core protein and hnRNP K are functionally diverse (23, 25–27, 50). The interaction between HCV core protein and hnRNP K suggests that HCV core protein has multiple effects in virus-infected cells and may thus help to explain the viral pathogenesis of HCV. However, because no in vitro HCV culture model is currently available, the biological significance of these various properties of the core protein in HCV infection will await future studies.

Acknowledgments—We thank Dr. Gideon Dreyfuss and James Jing-Hsiong Ou for providing plasmids to express GST-hnRNP K fusion protein and HCV core proteins (RH and Chiron isolates), respectively. We also thank Dr. King-Song Jeng, Pei-Yun Su, Shih-Yen Lo, and Julie Lau for their advice in this study.
