Hepatitis C Virus Suppresses the IRE1-XBP1 Pathway of the Unfolded Protein Response

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Keith D. Tardif†, Kazutoshi Mori§, Randal J. Kaufman†, and Aleem Siddiqui‡*

From the †Department of Microbiology and Program in Molecular Biology, University of Colorado Health Sciences Center, Denver, Colorado 80262, the ‡Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan, and the §Howard Hughes Medical Institute and Department of Biological Chemistry, University of Michigan Medical Center, Ann Arbor, Michigan 48109

Hepatitis C virus (HCV) gene expression disrupts normal endoplasmic reticulum (ER) functions and induces ER stress. ER stress results from the accumulation of unfolded or misfolded proteins in the ER; cells can alleviate this stress by degrading or refolding these proteins. The IRE1-XBP1 pathway directs both protein refolding and degradation in response to ER stress. Like IRE1-XBP1, other branches of the ER stress response mediate protein refolding. However, IRE1-XBP1 can also specifically activate protein degradation. We show here that XBP1 expression is elevated in cells carrying HCV subgenomic replicons, but XBP1 trans-activating activity is repressed. This prevents the IRE1-XBP1 transcriptional induction of EDEM (ER degradation-enhancing α-mannosidase-like protein). The mRNA expression of EDEM is required for the degradation of misfolded proteins. Consequently, misfolded proteins are stable in cells expressing HCV replicons. HCV may suppress the IRE1-XBP1 pathway to stimulate the synthesis of its viral proteins. IRE1α-null MEFs, a cell line with a defective IRE1-XBP1 pathway, show elevated levels of HCV IRES-mediated translation. Therefore, HCV may suppress the IRE1-XBP1 pathway to not only promote HCV expression but also to contribute to the persistence of the virus in infected hepatocytes.

The hepatitis C virus (HCV) poses a significant health problem worldwide as the virus is the causal agent in most cases of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (1). HCV is a positive strand RNA virus of ~9600 nucleotides in length encoding a polyprotein precursor of ~3000 amino acids (2, 3). The HCV polyprotein precursor is co- and post-translationally cleaved to produce at least 10 polypeptides including the structural proteins (core, E1, and E2) and nonstructural proteins (NS2 to NS5B). HCV nonstructural proteins direct viral replication from a ribonucleoprotein (RNP) replication complex that is associated with the endoplasmic reticulum (ER) (4, 5). HCV replication disrupts normal ER functions inducing ER stress (6, 7).

ER stress interrupts protein folding causing the accumulation of unfolded or misfolded proteins in the ER. These misfolded proteins must be refolded or degraded to alleviate the stress placed on the ER. Cells accomplish this by activating the unfolded protein response (UPR) (see Ref. 8 for a recent review). The UPR is linked to at least three different mechanisms used by cells to deal with unfolded proteins: transcriptional induction, protein degradation, and translational attenuation.

Transcriptional induction of ER chaperones and other selective genes of the protein folding machinery improve the ability of the ER to cope with unfolded proteins. ATF6 initiates the UPR by activating the transcription of ER chaperones. ATF6(P) is an ER membrane-bound transcription factor that is activated post-translationally by the presence of unfolded proteins in the ER (9). ER stress causes the proteolytic cleavage of the transcriptionally active N-terminal domain of ATF6, ATF6(N), from the cytoplasmic face of the ER membrane. ATF6(N) stimulates the transcription of ER chaperone genes in the nucleus by directly binding to ER stress response elements (ERSE). These ERSE sequences are necessary for the expression of mRNA from many UPR-regulated genes (9, 10). ERSE-containing genes are activated by ATF6 in cells carrying HCV replicons (7).

IRE1 also activates transcriptional programs in response to ER stress. IRE1 is an ER membrane-anchored endoribonuclease that directs the stress-induced splicing of XBP1 mRNA (11–13). Translation of spliced XBP1 mRNA produces a protein, XBP1(S), that is a potent transcription factor (11). Like ATF6, XBP1(S) can bind to ERSE sequences and induce the transcription of ER chaperone genes (11). XBP1(S) can also activate the transcription of other genes that ATF6 cannot. These genes contain UPR elements (UPRE) that are cis-acting sequences responsive to ER stress and are distinct from the ERSE. Thus, the transcriptional induction of the UPRE is mediated by XBP1(S) through the IRE1-XBP1 pathway (11, 13).

Instead of being refolded, misfolded proteins are also targeted in the ER for degradation by the proteasome. The IRE1-XBP1 pathway stimulates ER-associated protein degradation (ERAD) increasing the capacity of ER-stressed cells to degrade irrevocably misfolded proteins (14). EDEM (ER degradation-enhancing α-mannosidase-like protein) is an important component of the ERAD machinery as the induction of EDEM mRNA expression enhances the degradation of misfolded proteins (15).
The IRE1-XBP1 pathway activates ERAD by stimulating the transcription of EDEM through XBP1(S) and the UPRE (14). Therefore, while the ATF6 pathway is important for protein refolding, the IRE1-XBP1 pathway is required for protein degradation (14).

Translational attenuation also eases the burden that unfolded proteins place on the ER. PERK is an ER membrane-bound protein kinase that reduces the level of protein synthesis by phosphorylating the α subunit of eukaryotic initiation factor 2 in response to ER stress (16). Viruses rely on host-cell translational machinery to upregulate protein synthesis (18–20). Translation is elevated in cells expressing HCV replicons even though these cells are under ER stress (7). In this study, we show that the IRE1-XBP1 pathway is repressed in cells carrying HCV replicons. This deviation in the UPR pathway to promote its survival in hepatocytes.

In this study, we show that the IRE1-XBP1 pathway is repressed in cells carrying HCV replicons. This deviation in the UPR suppresses the degradation of misfolded proteins by ERAD. The overall result of a defect in the IRE1-XBP1 pathway is an increase in HCV IRES (internal ribosome entry site)-mediated translation. HCV may utilize this as a strategy to ensure the translation of its viral mRNA. These intracellular events are ultimately linked to the establishment of chronic hepatitis associated with viral infection.

EXPERIMENTAL PROCEDURES

Cell Culture—The human hepatoma cell lines Huh7, FCA4, and GS4.3 were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml). FCA4 and GS4.3 cells were also cultured in G418 (500 μg/ml) and Geneticin; Invitrogen) and are a generous gift from C. Seeger (Fox Chase Cancer Center). Both the FCA4 and GS4.3 cells are a Huh7 cell line stably expressing a HCV subgenomic replicon (21). The HCV replicons expressed in FCA4 and GS4.3 cells are bicistronic RNA constructs consisting of the HCV-IRES (nucleotides 1 to 377 of the 5’-NCR), the neomycin phosphotransferase (neo) gene, the encephalomyocarditis (EMCV) IRES, which directs the translation of HCV nonstructural proteins NS3 through NS5, and the 3’-NCR (22). Wild-type IRE1α and IRE1α-null mouse embryonic fibroblasts (MEFα) have been described previously (13). Cells were harvested for each experiment at 70% confluence unless otherwise indicated. Cells were maintained in 5% CO2 and 37 °C. Replicon-carrying Huh7 cells were cured of HCV RNA using human leukocyte-derived α-interferon (IFN-α) (100 international units/ml; Sigma-Aldrich) (23). Cells expressing HCV replicons were initially passaged twice in the absence of G418. On the third passage, cells were grown with IFN-α. After 3 days, confluent monolayers of cells were trypsinized, plated, and grown for 24 h before adding IFN-α. Cells were passaged four times in the presence of IFN-α. Before the fourth passage, cells were cultured for 3 days in the absence of IFN-α. Cured cell lines were used for experiments at early passage levels.

Immunoblotting—XBP1(S) and XBP1(U) were detected with anti-XBP1 polyclonal antibody (Santa Cruz Biotechnologies) and anti-XBP1-A polyclonal antibody (11). Rabbit anti-neomycin phosphotransferase polyclonal antibody was obtained from Cortex Biochem, mouse anti-actin monoclonal antibody was obtained from Neomarkers, and rabbit anti-HCV NS5A polyclonal antibody was a generous gift from S. Sharma (Pennsylvania State University). Cell lysates were prepared and analyzed as described previously (7). The membranes were probed and developed using the ECL chemiluminescence kit (Amersham Biosciences). The band intensities from Western blots were measured with Quantity One software from BioRad.

Plasmids—The mammalian expression vectors for human XBP(U), pcDNA-XBP1(unspliced), and human XBP(S), pcDNA-XBP1(spliced) were as described (11). XBP1 transcriptional activity was measured using the 5X UPRE luciferase reporter (a kind gift from R. Prywes, Columbia University), a reporter plasmid containing the luciferase gene under the control of five UPRE sequences. This 5X UPRE reporter is identical to pSX ATF6GL3 (24). The expression vector for the α1-antitrypsin folding incompetent variant NHK, pREP9-NHK, was sup-

Fig. 1. HCV replicon induces the expression of XBP1. A, schematic illustration of the unspliced and spliced forms of human XBP1 mRNA and protein. The protein products from the two open reading frames and the 26-bp intron from XBP1 mRNA are shown. B, cells carrying HCV replicons have elevated levels of XBP1(S) protein. XBP1(S) was identified by Western blot analysis using anti-XBP1 polyclonal antibody, and XBP1(U) was detected using anti-XBP1-A (11). The actin protein band in the same Western blot was detected with the anti-(Pan)actin antibody. Cell lysates were prepared from Huh7, FCA4, and GS4.3 cells cultured with or without 25 μM MG132 for 6 h before harvest. C, semiquantitative RT-PCR analysis of XBP1 spliced and unspliced mRNA. Total RNA was prepared from Huh7, FCA4, and GS4.3 cells cultured in the presence or absence of tunicamycin (10 μg/ml) for 6 h before harvest. RNA was then amplified by RT-PCR. PCR products synthesized from pcDNA-XBP1(unspliced) and pcDNA-XBP1(spliced) were presented in lanes 1 and 2 for size comparison to experimental data. The resulting products were subjected to 5% polyacrylamide gel electrophoresis. RT-PCR products of β-actin mRNA are provided as a standard for normalization.
plified by N. Hosokawa (Kyoto University) (15). Cap-dependent and HCV IRES-dependent translation were monitored with the luciferase reporter plasmid, plRL HCV 1b, as described previously (7). This construct contains a firefly luciferase gene, the HCV IRES, and a Renilla luciferase gene.

**RT-PCR**—Total RNA was extracted from Huh7, FCA4, and GS4.3 cells treated with or without tunicamycin (10 μg/ml, 6 h) using RNA STAT-60 (Tel-Test) and amplified by semiquantitative RT-PCR. Total RNA (10 μg) was amplified with the Access Quick RT-PCR System (Promega) using the primers corresponding to nucleotides 412–431 (5′-CTG AAT TTG TGA GAA CCA GG-3′) and 834–853 (5′-GGG GCT TGG TAT TAT GTA TGT (GG-3′) of the XBP1 gene. The primers 5′-CAC CCA CAC TGT GCC CAT CTA CG-3′ and 5′-CGA TTT CCC GCT CGG CCG TG-3′ were used to amplify β-actin. RT-PCR products produced from β-actin mRNA are used as a standard for normalization to RT-PCR products produced from XBP1 mRNA. Reactions were carried out in the presence of [α-32P]dCTP (2.7 μCi/sample).

Quantitative RT-PCR was performed using an ABI PRISM 7700 Sequence Detector (PerkinElmer Life Sciences/Applied Biosystems). Amplifications were conducted in duplicate using the following primers and 6-carboxyfluorescein (6FAM)- and tetrachloro-6-carboxyfluorescein (TAMRA)-labeled probes (PerkinElmer Life Sciences): KIAA0212 Taqman probe, 5′-6FAM-AAG AGG GAG GCC AGG ACC AAG GG-TAMRA-3′; KIAA0212-1823, 5′-CAT CTT CGG GAA TTG CCA TG-3′; KIAA0212-1912, 5′-ATG AGG TTT CGG CCT GTG C-3′; HCV Replicon Taqman probe, 5′-6FAM-CCT TCA TCT TCT TCG GCA CGT CCC G-TAMRA-3′; HCV Replicon RNA-FWD, CTT TCA CAG ACT GCA GGG CCT G; HCV Replicon RNA-REV, GCC TTA ACT GTG GAC GCC TTC; 18 S rRNA Taqman probe, 5′-6FAM-TGC TGG CAC CAG ACT TGG CCT C-TAMRA; 18 S rRNA-FWD, 5′-CGG CTA CATCCA CAA AGG AA-3′; and 18 S rRNA-REV, 5′-CTG GGA ATT ACC GCG GCT-3′. Amplifications were performed in a 50 μl mix containing 8% glycerol, 1× TaqMan buffer A (500 mM KCl, 100 mM Tris-HCl, 0.1 mM EDTA, 600 mM passive reference dye ROX, pH 8.3), 300 μM each of dATP, dGTP, dCTP, and dUTP, 5.5 mM MgCl2, 900 nM forward primer, 900 μM reverse primer, 200 nM probe, 1.25 units of AmpliTaq Gold DNA polymerase (PerkinElmer Life Sciences), 12.5 units of Moloney Murine leukemia virus reverse transcriptase (Invitrogen), 20 units of RNasin ribonuclease inhibitor (Promega), and the template RNA. Reactions were performed under the following conditions: 30 min at 48 °C (reverse transcription reaction); 10 min at 95 °C (heat inactivation of reverse transcriptase and activation of TaqGold polymerase) cycles of 15 s at 95 °C and 1 min at 60 °C (PCR amplification). The amounts of KIAA0212 RNA and HCV replicon RNA were calculated by comparison to serially diluted pcDNA3.1-mycKIAA0212 and pS1/AS-BM4–5 plasmids (21). The amounts of KIAA0212 or HCV replicon RNA were also normalized to 18 S RNA.

**Transfections and Luciferase Assays**—Cells at ~70% confluency were transfected using LipofectAMINE reagent (Invitrogen). Reporter assays were performed as described previously (7). In each assay, a reporter plasmid containing β-galactosidase under the control of the CMV promoter was used to correct for transfection efficiency.

**Pulse-Chase Experiments**—Huh7 and FCA4 cells were cultured in 60-mm dishes and transfected with 4 μg of pREP9-NHK. Twenty-four hours after transfection, cells were incubated for 1 h in methionine- and cysteine-free DMEM supplemented with 2 mM glutamine, antibiotics, and 10% fetal bovine serum. Cells were then labeled with [35S]methionine and [35S]cysteine for 1 h using 200 μCi/ml Trans 35S-label (1175 Ci/mmol; ICN) and chased with complete medium for the indicated times. Cells were lysed in Nonidet P-40 lysis buffer (50 mM Tris-HCl (pH 7.4), 0.5% Nonidet P-40, 5 mM MgCl2) supplemented with protease inhibitors (Complete EDTA-free, Roche Applied Science). Lysates used for immunoprecipitations were normalized to equal trichloroacetic acid-precipitable [35S]-labeled protein. Lysates were incubated with goat anti-α1-antitrypsin antibody (US Biologicals) at 4 °C for 2 h. The immune complexes were absorbed to protein G-Sepharose (Amersham Biosciences), then washed with Nonidet P-40 wash buffer (50 mM Tris-HCl (pH 7.4), 0.5% Nonidet P-40, 5 mM EDTA, 150 mM NaCl), and analyzed by SDS-PAGE.
RESULTS

XBP1 Expression Is Elevated by HCV Replicons—Human XBP1 mRNA has two open reading frames (ORFs), an intron, and a bZIP domain (Fig. 1A). The 26-base intron of XBP1 mRNA is spliced in response to ER stress (11). This produces a frameshift combining ORF1 with ORF2 and permits the translation of a 376-amino acid transcriptional activator, XBP1(S) (Fig. 1A). Western blot analysis using antibodies specific to XBP1(S) demonstrated that the level of XBP1(S) expression is higher in HCV replicon-expressing FCA4 and GS4.3 cells than in Huh7 control cells (Fig. 1B). XBP1(U) is rapidly degraded once the protein is translated from unspliced XBP1 mRNA (11). Consequently, XBP1(U) was detected only in the presence of the proteasome inhibitor MG132 (Fig. 1B). Overall, these results indicate that XBP1(S) expression is induced in response to ER stress produced from HCV replicons.

RT-PCR analysis of RNA isolated from cells expressing HCV replicons was performed using primers bordering the 26-base intron and the overlapping region of ORF1 and ORF2 in XBP1 mRNA. The 416-bp fragment amplified from spliced XBP1 mRNA was detected in cells carrying HCV replicons, FCA4 and GS4.3 cells, but not in Huh7 cells (Fig. 1C, compare lane 4 with lanes 5 and 6). A similar band was found in Huh7 cells treated with tunicamycin (Tm) (Fig. 1C, lane 3), a compound that causes ER stress by inhibiting protein N-glycosylation (25). A 442-bp RT-PCR product corresponding to unspliced XBP1 mRNA was also generated from RNA prepared from Huh7, FCA4, and GS4.3 cells (Fig. 1C). Overall, RT-PCR confirms that XBP1 mRNA is spliced and that the IRE1-XBP1 pathway is activated in cells carrying HCV replicons.

XBP1 Trans-activating Activity Is Inhibited by HCV Replicons—The IRE1-XBP1 pathway and XBP1(S) activate transcription through UPRE sequences. To test the trans-activating activity of XBP1, we used a luciferase reporter plasmid under the transcriptional control of five consecutive UPRE motifs. The UPRE sequence contains the XBP1 binding site (11). FCA4 and GS4.3 cells expressing HCV replicons show reduced 5× UPRE luciferase reporter activity (Fig. 2A). In fact, UPRE-mediated transcription in FCA4 and GS4.3 cells is 4-fold lower than that found in Huh7 cells (Fig. 2A), suggesting that XBP1(S) activity is repressed in cells stably expressing HCV replicons. To determine if this observation is due to clonal differences between the parental Huh7 cells and the Huh7 cells harboring the HCV replicon, we cured GS4.3 cells of the HCV replicon-carrying cells, FCA4 and GS4.3 cells while tunicamycin treatment of Huh7 cells resulted in a 4-fold increase in KIAA0212 mRNA as expected (Fig. 3A). Thus, the suppression of the IRE1-XBP1 pathway inhibits the transcription of KIAA0212 to elevated levels in response to ER stress.

We next investigated whether a defect in the IRE1-XBP1 pathway would also influence ERAD and the degradation of misfolded glycoproteins in the ER. The null Hong Kong (NHK) variant is a mutant form of the α1-antitrypsin protease inhibitor that is misfolded in the ER and degraded through ERAD (26, 27). This makes the stability of the NHK protein an excellent measure of ERAD activity. To analyze NHK protein stability in FCA4 and Huh7 cells, cells were transfected with a NHK expression plasmid, 35S-pulse-labeled for 1 h, and chased for the indicated times. NHK was immunoprecipitated with anti-α1-antitrypsin antibody. The immunoprecipitated samples were subjected to SDS-PAGE.

Fig. 3. ER-associated protein degradation is reduced in cells carrying HCV replicons. A, QT-RT-PCR analysis of KIAA0212 mRNA (hEDEM). Total RNA was isolated from Huh7, FCA4, and GS4.3 cells cultured in the presence or absence of tunicamycin (10 μg/ml) for 6 h before harvest. The amounts of KIAA0212 were normalized to 18 S rRNA. B, degradation of the NHK variant of α1-antitrypsin in Huh7 and FCA4 cells. Huh7 and FCA4 cells were transfected with pREP9-NHK, 35S-pulse-labeled for 1 h, and chased for the indicated times. NHK was immunoprecipitated with anti-α1-antitrypsin antibody. The immunoprecipitated samples were subjected to SDS-PAGE.

HCV Replicons Inhibit ER-associated Protein Degradation—The IRE1-XBP1 pathway stimulates ERAD by up-regulating the stress-induced transcription of EDEM (14). Mouse EDEM is homologous to KIAA0212 in humans sharing 92% identity (15). Quantitative RT-PCR analysis of KIAA0212 revealed that mRNA expression of KIAA0212 is not induced in FCA4 and GS4.3 cells while tunicamycin treatment of Huh7 cells resulted in a 4-fold increase in KIAA0212 mRNA as expected (Fig. 3A). Thus, the suppression of the IRE1-XBP1 pathway inhibits the transcription of KIAA0212 to elevated levels in response to ER stress.
MEFs cannot effectively degrade misfolded glycoproteins because these cells are unable to stimulate the transcription of EDEM to higher levels in response to ER stress (14). Without IRE1α, IRE1α-null MEFs can neither splice XBP1 mRNA nor produce any XBP1(S) to activate EDEM transcription (13, 14). This makes this cell line a good model to study the effects of a defective IRE1-XBP1 pathway on HCV expression. HCV translation is mediated through an internal ribosome entry site (IRES) and a cap-independent mechanism (28–30). HCV IRES translation in IRE1α-null MEFs was 2–3-fold higher than in wild-type IRE1α MEFs (Fig. 4, left panel). The increase in translation was not specific to cap-dependent translation. Cap-dependent translation was also 18 times higher in IRE1α-null MEFs (Fig. 4, right panel). The elevation in HCV IRES translation and cap-dependent translation in IRE1α-null MEFs was unchanged when cells were transfected with an XBP1(S) expression vector (Fig. 4), suggesting the increase in translation cannot be reversed with XBP1(S) expression. Instead, this increase in translation may be due to a reduction in PERK-eIF2α phosphorylation since PERK activity could require IRE1α. Nevertheless, these results indicate that HCV pressurizes the IRE1-XBP1 pathway to enhance the translation of its viral proteins marking another strategy used by this virus to manipulate host-cell translation to promote the expression of its viral mRNA.

The effects of a deficiency in the IRE1-XBP1 pathway on HCV translation were further analyzed in the context of HCV replicon-expressing cells. The HCV subgenomic replicon stably expressed in FCA4 cells is a bicistronic construct (Fig. 5). In this RNA form, HCV IRES-directed translation can be measured by neomycin phosphotransferase protein expression and the degree of EMCV IRES translation can be evaluated by the translation of HCV NS5A protein. Based on the observations in Fig. 4, tunicamycin treatment of FCA4 cells should enhance any effects that a defect in the IRE1-XBP1 pathway has on HCV translation, even though these cells are already under HCV induced-ER stress. Treating FCA4 cells with tunicamycin resulted in an increase in neomycin phosphotransferase expression (Fig. 5A). A quantitative measurement of the neomycin phosphotransferase protein reveals a 1.7-fold rise in HCV IRES translation (Fig. 5B). Although HCV IRES translation was higher, HCV NS5A expression from the EMCV IRES appeared to be unaffected (Fig. 5A). This implies that the effects of down-regulating the IRE1-XBP1 pathway on protein synthesis may be more tailored to promoting HCV IRES-directed translation. Treating FCA4 cells with MG132 reduced some of the gains in HCV IRES translation (Fig. 5A, compare lanes 3 and 4). This suggests that the increase in HCV translation may partially depend upon an active proteasome even as ERAD is suppressed in FCA4 cells.

**DISCUSSION**

HCV replication interrupts typical ER operations inducing ER stress. These cells alleviate this strain placed on the ER by activating the UPR (7). The UPR consists of two transcriptional programs, the ATF6 and IRE1-XBP1 pathways (Fig. 6). Although these branches of the UPR are both induced by the presence of unfolded proteins in the ER, the end result of their activation is quite different. ATF6 stimulates the transcription of ER chaperones and other components of the protein folding machinery (9, 10, 31). Unfolded proteins that are still not folded correctly after the activation of ATF6 can be degraded by ERAD (14). The IRE1-XBP1 pathway is responsible for stimulating ERAD activity in response to ER stress (14). While ATF6 functions properly in cells carrying HCV replicons, the IRE1-XBP1 pathway is suppressed and ERAD is not turned on (Fig. 6).

In this study, we find XBP1(S) trans-activating activity is inhibited as measured by UPRE reporter activity even though XBP1 mRNA is spliced and XBP1(S) expression is elevated in HCV replicon-expressing cells. This prevents the transcriptional induction of EDEM (Fig. 3A). EDEM is a target gene of the IRE1-XBP1 pathway and a part of the ERAD machinery (14, 32). It is not entirely clear why EDEM mRNA expression did not decline with XBP1 activity (compare Figs. 2A and 3A). One explanation for this is that the small amount of XBP1(S) expression in Huh7 cells (Fig. 1B) is not adequate enough to stimulate EDEM mRNA expression while it is capable of simulating a low level of UPRE reporter activity. Although the suppression of XBP1-induced transcription did not reduce EDEM mRNA expression, ERAD did decline with XBP1 activity in cells carrying HCV replicons (compare Fig. 2A and Fig. 3B). This suggests that a deficiency in the IRE1-XBP1 pathway may repress ERAD through other UPR regulated-components associated with ERAD activity.

HCV may use the disruption in the IRE1-XBP1 pathway as
a strategy to promote the translation of its viral RNA. HCV IRES translation is elevated in cells where the IRE1-XBP1 pathway is malfunctioning. HCV translation is higher in both IRE1/H9251-null MEFs and Huh7 cells carrying HCV replicons. HCV has evolved numerous mechanisms to enhance translation of its viral proteins. HCV E2 has been shown to inhibit the double-stranded RNA-dependent eIF2/H9251 kinase, PKR (19). HCV NS5A also inhibits PKR activity by binding to PKR and preventing its dimerization (18). Additional studies will be required to determine the precise mechanism by which HCV IRES-directed translation is elevated in cells with a defective IRE1-XBP1 pathway.

Numerous viruses induce ER stress, including Japanese encephalitis virus (JEV) (33), bovine viral diarrhea virus (BVDV) (34), hepatitis B virus (HBV) (35), adenovirus (36), and influenza (37). The stress caused by each virus provokes a response from infected cells to ease the strain the viruses place on the ER. However, HCV is the only virus shown to either use ER stress or manipulate the ER stress response to promote its survival in cells that support HCV replication. HCV induced-ER stress inhibits major histocompatibility complex (MHC) class I antigen presentation to cytotoxic T lymphocytes (CTLs) (38). This is caused by a disruption of MHC class I glycosylation and folding in the ER (38), a process necessary for the expression of MHC class I on the cell surface (see Ref. 39 for a recent review). Furthermore, HCV E2 alters the normal course of the UPR. Although the HCV E2 protein can induce ER stress (40), E2 binds to and inhibits PERK (20), an eIF2/H9251 kinase responsive to ER stress (16). This promotes the synthesis of viral proteins even in the presence of ER stress.

In summary, we show that XBP1 and ERAD activity are inhibited in cells expressing HCV replicons. This defect in the...
IRE1-XBP1 augments the translation of HCV viral RNA. This is one way HCV uses ER stress and subsequent intracellular responses to promote its survival in hepatocytes.

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