Cyclophilin A Is an Essential Cofactor for Hepatitis C Virus Infection and the Principal Mediator of Cyclosporine Resistance In Vitro

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Cyclosporine (CsA) and its derivatives potently suppress hepatitis C virus (HCV) replication. Recently, CsA-resistant HCV replicons have been identified in vitro. We examined the dependence of the wild-type and CsA-resistant replicons on various cyclophilins for replication. A strong correlation between CsA resistance and reduced dependency on cyclophilin A (CyPA) for replication was identified. Silencing of CyPB or CyPC expression had no significant effect on replication, whereas various forms of small interfering RNA (siRNA) directed at CyPA inhibited HCV replication of wild-type but not CsA-resistant replicons. The efficiency of a particular siRNA in suppressing CyPA expression was correlated with its potency in inhibiting HCV replication, and expression of an siRNA-resistant CyPA cDNA rescued replication. In addition, an anti-CyPA antibody blocked replication of the wild-type but not the resistant replicon in an in vitro replication assay. Depletion of CyPA alone in the CsA-resistant replicon cells eliminated CsA resistance, indicating that CyPA is the chief mediator of the observed CsA resistance. The dependency on CyPA for replication was observed for both genotype (GT) 1a and 1b replicons as well as a GT 2a infectious virus. An interaction between CyPA and HCV RNA as well as the viral polymerase that is sensitive to CsA treatment in wild-type but not in resistant replicons was detected. These findings reveal the molecular mechanism of CsA resistance and identify CyPA as a critical cellular cofactor for HCV replication and infection.

Hepatitis C virus (HCV), a member of the Flaviviridae family that includes other major human pathogens such as dengue and West Nile viruses, contains a positive-strand RNA genome of 9.6 kb encoding a single polyprotein, which is processed through proteolysis to become at least 10 viral proteins (18). Like other positive-strand RNA viruses, HCV replicates its genomic RNA in association with intracellular membranes (37). The nonstructural proteins, especially NS3, NS5A, and NS5B, directly participate in the replication process and determine replication efficiency from cognate 5’ and 3’ nontranslated regions (3). In addition, HCV replication is regulated by cellular proteins that either directly interact with viral proteins or modulate critical metabolic pathways essential for the virus (7, 11, 31, 40, 43).

Cyclophilins (CyPs) are a family of cellular enzymes possessing the peptidyl-prolyl isomerase activity. The prototypical member of the CyP family is CyPA, the main intracellular ligand of cyclosporine (CsA) (12). The CsA-CyPA complex binds to and inhibits calcineurin, a cellular phosphatase and a key mediator of T-cell activation (19). The role of human CyPs as cellular cofactors in HCV replication was first suggested by studies that showed that CsA is effective in suppressing HCV replication (27, 45). Subsequently, a correlation between the CyP-binding and anti-HCV activity was observed for derivatives of CsA (22, 46). Despite both protein binding and resistance mapping studies suggesting that NS5B is a viral target for CsA (8, 36, 46), the identities and relative contributions of the various CyPs implicated in this interaction remain controversial (28, 36, 46). Furthermore, although CsA and its derivatives efficiently inhibit the infection of JFH-1/HCVcc in vitro (32), the CyP involved has not been identified since CyPB, which has been reported to play a role in the replication of a genotype (GT) 1b replicon, is clearly dispensable for the replication of a JFH-1 replicon (14). Finally, the relationship between the dependence on CyPs and the observed CsA resistance has not been investigated. We report here that CyPA, and not CyPB or CyPC, is an essential cofactor for the replication of various HCV isolates and genotypes. Among these is JFH-1, the GT 2a isolate with the highest efficiency in producing infectious particles in cell culture (6, 17, 42, 47, 49). Our data further indicate that CyPA is the principal mediator of CsA resistance in vitro. Not only is the resistance to CsA correlated with resistance to CyPA suppression, but removal of CyPA from resistant replicon cells also eliminates resistance. Finally, CsA-resistant interaction between NS5B and CyPA contributes to the decreased drug sensitivity of the selected HCV replicons.

MATERIALS AND METHODS

Cells, compounds, and antibodies. GS5 and RS2 cells have been described previously (36). Huh-7.5 cells and the H77 replicon construct were provided by Charles Rice and Apath LLC. CsA was purchased from Alexis Corporation (San Diego, CA). We used the following antibodies: anti-CyPA (Biomol, Plymouth Meeting, PA), anti-CyPB (Affinity BioReagents, Golden, CO), anti-Ku80 and antiactin (Sigma-Aldrich), anti-NS3 (G. George Luo, University of Kentucky), and anticore (Affinity BioReagents, Golden, CO), and antino-ns5 (G. George Luo, University of Kentucky), and antico-ns5 (G. George Luo, University of Kentucky), and anticore (Affinity BioReagents, Golden, CO).

RNA interference. A human immunodeficiency virus (HIV)-based lentiviral vector was used to express all the short hairpin RNAs (shRNAs). The sh-Luc and sh-B710 RNAs have been described previously (36). Target sequences for the
other shRNAs are as follows: A-161, 5′-AAG GGT TCC TGC TTT CAC AGA-3′; A-285, 5′-AAG CAT AGC GGT CCT GCC ATC-3′; A-285′, 5′-AAG CAT ACA GGT CCT GCC ATC-3′; A-459, 5′-AAT GCC AGG AAC AGG AAG AAC-3′; C-454, 5′-AAG ACT GAA GGT GTG CTG GTA-3′; NTC, 5′-AAG GAG GAG TGT ACA TCA CCA CTG-3′. Lentiviral vector production and transduction were performed as described previously (44). Stable cells expressing shRNAs were obtained by selection with 1 μg/ml of puromycin (MP Biomedicals, Solon, OH) for 3 weeks.

In vitro transcription, electroporation, colony formation assays, and quantitative RT-PCR. In vitro transcription, electroporation, colony formation assays, and quantitative reverse transcription-PCR (RT-PCR) have been described previously (36). The primers for detecting CyPA, CyPB, and CyPC have the following sequences: A-Forward, 5′-CGG GTC GTC GCA TCT TGT-3′; and A-Reverse, 5′-GCA GAT GAA AAA CTG GGA ACCA-3′; B-Forward, 5′-GGG CAA CGC AGG ACA A3′-; and B-Reverse, 5′-TGC ACG CAG GCT GTG TTG ACT GT-3′; C-Forward, 5′-GGT GAA GCA TGA TGG CAG TGG-3′; and C-Reverse, 5′-GAA CTG GAC AGC ATT GTC GAT-3′.

Co-IP/RT-PCR. Replicon cells (5 × 10^6) were seeded into a T-75 flask (treated with 4 μg/ml CsA where indicated) 1 day before the immunoprecipitation (IP) experiment. Twenty-four hours later, cells were lysed in 1 ml of IP buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5% NP-40, 1 mM PMSF, 1 mM EDTA, 1 mM DTT, 1 mM EDTA, 100 μM HEPES [pH 7.4], 50 mM NH4Cl, 7 mM KCl, 1 mM spermidine) was added to each plate, and the cell lysates were collected with a CsA-resistant replicon single-cell clone, RS1-2 (data not shown). The same panel of shRNAs was also used in a colony formation assay designed to test their effects on HCV replication. We expressed shRNAs directed at each of these CypVs along with a control shRNA directed at firefly luciferase in either the G5S or RS2 cells. All three CyP-directed shRNAs efficiently knocked down the expression of their respective targets (Fig. 1B and C). The shRNA directed at CyPB (si-B710) or CyPC (sh-C454) had no effect on the NS5A level in either GS5 or RS2 cells, despite similar knock-down levels of CyPA in the two cell lines. The shRNAs directed at CyPB (si-B710) or CyPC (sh-C454) had no effect on the NS5A level in either G5S or RS2 cells. Fluorescence-activated cell sorting (FACS) results with green fluorescent protein (GFP) expression as a readout confirmed these results (data not shown). The same panel of shRNAs was also used in a colony formation assay designed to test their effects on HCV replication. We transduced the replicon cells with shRNA-expressing vectors that carry the puromycin N-acetyltransferase (pac) gene and then subjected the cells to a double selection with both puromycin and G418, which selects for cells that maintain replication. When G5S cells were transduced with sh-A161, a significantly lower number of double-resistant colonies were observed, reflecting the inhibitory effect of sh-A161 on G5S replication. This lower colony formation efficiency was not observed in RS2 cells (Fig. 1D). On the other hand, shRNAs directed at CyPB and CyPC had no significant effect on the number of colonies. Similar results were obtained with a CyPA-resistant replicon single-cell clone, RS1-2 (data not shown).

To further validate a role of CyPA protein in HCV replication, we performed in vitro replication assays with cell extracts of replicon cells and then tested the ability of an antibody against CyPA to interfere with replication. Anti-CyPA effectively blocked replication when the assay was performed with G5S lysate but failed to inhibit replication if RS2 lysate was used (Fig. 1E). These data corroborate the RNA interference results and suggest that CyPA protein is important for a step in
the HCV replication process that can be measured by the in vitro replication assay.

**HCV replicon expression is correlated with CyPA expression level.** Several other siRNAs directed at CyPA have been evaluated in vitro for anti-HCV effects and have yielded conflicting results. Two shRNAs directed at CyPA were reported by Nakagawa et al. to have both knocked down CyPA and inhibited replication of a GT 1b replicon (28). In contrast, Watashi et al. reported a CyPA siRNA that efficiently suppressed CyPA expression but failed to inhibit HCV replication (46). In an attempt to reconcile these results, we constructed shRNAs directed at the same CyPA mRNA sites as reported by these groups (Fig. 2A) and compared their effects on HCV replication to those of sh-A161. sh-285’s target was the same 21-nucleotide sequence as Watashi’s siRNA (“si-CyPA”) and sh-459’s was a 21-nucleotide sequence that was encompassed by Nakagawa’s shRNA #441, which had a target sequence of 29 nucleotides. We also constructed sh-A285, which is identical to sh-285 except for a mismatch of one nucleotide between the siRNA and the target sequence. Consequently, sh-285′ was expected to lack the ability to knock down CyPA. Finally, we constructed a negative-control shRNA (sh-NTC) that does not recognize any human mRNA in GenBank. When this panel of shRNAs was introduced into GS5 cells, three of them, sh-A161, sh-A285, and sh-A459, knocked down CyPA expression as expected (Fig. 2B). All three cell lines with CyPA knock-downs also had reduced NS5A levels. The shRNAs that did not inhibit CyPA expression also did not affect the replicon. Our original shRNA (sh-A161) was the most effective in both silencing CyPA expression and inhibiting NS5A synthesis (Fig. 2B). Because the different effects of sh-A285 and Watashi’s si-CyPA could potentially be explained by the difference between shRNA and synthesized siRNA duplex, we tested a synthesized form of sh-A161 and sh-A285 in transient-transfection experiments. Both siRNAs (si-A161 and si-A285) inhibited CyPA and NS5A expression (Fig. 2C). In this form, si-A161 is no more potent than si-A285 in suppressing CyPA expression; accordingly, the abilities of these two siRNAs to inhibit HCV replicon were also comparable. The overall reduction of both CyPA and NS5A levels was less dramatic with these preformed siRNA duplexes, likely due to the lower efficiency of transfecting siRNA into cells in comparison to trans-
ducing the shRNAs using lentiviral vectors. To eliminate definitively the possibility that the target of all these siRNAs was a chimeric mRNA containing the entire CyPA mRNA as part of its sequence (38), we performed cDNA rescue experiments. A cDNA of CyPA that contained silent mutations in the recognition site of sh-A161 was constructed and cloned into a mammalian expression vector. A Myc tag was placed at the C terminus of the protein to allow specific detection. When this cDNA (Myc-A#) was introduced into replicon cells together with sh-A161, both NS5A expression (Fig. 2D) and HCV RNA replication (Fig. 2E) were partially rescued, indicating that sh-A161 indeed exerts its inhibitory effect by repressing CyPA expression.

CyPA mediates CsA resistance of RS2 cells. In contrast to the GS5 replicon, RS2 replicated efficiently in the presence of substantial CyPA knock-down (Fig. 1D). This result could be explained by either CyPA-independent replication or a replication strategy that requires much less CyPA protein because shRNAs normally cannot eliminate the gene product completely. To distinguish between these two possibilities, we treated the RS2/sh-A161 replicon cells, recovered after double selection of puromycin and G418 following shRNA transduction, with CsA. If RS2 replicates in a CyP-independent manner in these cells, one would expect no effect of the CsA on the RS2/sh-A161 cells. On the other hand, if the RS2 replicon was resistant to CsA and sh-A161 because it needed less CyPA to replicate (thus requiring a higher concentration of CsA to inhibit its replication), then one would expect a reduced resistance to CsA as the pool of CyPA is smaller in this case. Indeed, the latter appeared to be the case, as the RS2/sh-A161 replicon was six to eight times more sensitive to CsA than was the RS2/sh-Luc replicon (Fig. 3A), essentially reverting to the sensitivity level of the wild-type replicon GS5. Western blotting confirmed the substantial suppression of CyPA in the RS/sh-
A161 cells (Fig. 3B). To control for the possibility that the difference in sensitivity to CsA was caused by some unidentified differences in the antibiotic-selected cells, we applied the double treatment (sh-A161 and CsA) to RS2 cells without selection. When the RS2 cells were treated with 0.5 µg/ml CsA or transduced with sh-A161, no significant suppression was seen in either treatment, but when RS2 cells were first transduced with sh-A161 and then treated with the same concentration of CsA, a dramatic inhibition was achieved (Fig. 3C). Taken together, these data indicate that CyPA is the principal mediator of the CsA resistance observed in RS2 cells, the replication of which requires a much reduced level of CyPA.

**CyPA is essential for the replication of multiple HCV isolates.** GS5 and RS2 cells contain GT 1b replicons with the GFP gene inserted into the NS5A region (25, 29). We next examined the effect of CyPA knock-down on replicons without GFP. Both GT 1a and 1b replicons are tested. To this end, we established stable Huh-7.5 cell lines that expressed the various shRNAs and then challenged them, by electroporation, with in vitro-transcribed GT 1a (H77) and 1b (Con1) subgenomic replicon RNAs. The shRNAs effectively silenced the expression of their respective targets in the stable cells as expected (Fig. 4A). No defect in morphology or growth rate was detected for any of the stable cell lines (data not shown), confirming that these CyPs are dispensable for cell survival in vitro (5). Expression of sh-A161 completely inhibited the ability of either replicon RNA to form colonies, whereas sh-Luc had no effect (Fig. 4B). An inhibitory effect (~50%) of sh-B710 on the GT 1a replicon was sometimes observed, but the colony formation efficiency was still much higher than that in sh-A161 cells. No other shRNA showed any consistent inhibitory effect on any of the replicons. Transient-transduction experiments again confirmed the inhibitory effect of sh-A161 on the expression of NS5A of both GT 1a and 1b replicons (Fig. 4C).

The complete cycle of HCV infection can now be studied in cell culture with infectious viruses produced in vitro (6, 17, 42, 47, 49), so we next determined whether CyPA is required for HCV infection in vitro. Stable Huh-7.5 cells harboring control or sh-A161 were infected with HCVcc particles produced with the JFH-1 genome. Infection of Huh-7.5 cells was efficient; viral RNA and antigens became readily detectable in the target cells within a few days after infection. Expression of sh-Luc or sh-B710 had no effect on HCV infection, whereas the sh-A161 cells were highly refractory to infection (Fig. 5A). The protection provided by sh-A161 was observed by several methods: RT-PCR, fluorescence staining for core protein, or Western blot detection of NS3 (Fig. 5B and C). The sh-A161 cells remained fully susceptible to infection by vesicular stomatitis virus, a negative-strand RNA virus that is sensitive to nonspecific antiviral responses (Fig. 5D).

Last, to determine whether sh-A161 could repress an existing infection, we infected Huh-7.5 cells with JFH-1 virus for 10 days and then introduced sh-A161 by transduction. The expression of NS3 was measured 7 days after transduction. As shown in Fig. 5E, delivery of sh-A161 into infected cells suppressed viral replication, parallel to the results obtained with transient transduction of replicon cells.

**The association of CyPA with HCV polymerase and RNA in replicon cells is correlated with CsA resistance.** In vitro replication results suggested that CyPA is directly involved in the replication process (Fig. 1E). To determine whether CyPA is associated with HCV genome in replicon cells, we precipitated CyPA from replicon lysates and extracted the coprecipitated RNA, which was then subjected to RT-PCR analysis with primers complementary to the 5' nontranslated region of HCV. HCV RNA was found to be precipitated by an anti-CyPA antibody but not by an IgG control antibody (Fig. 6A). This association was inhibited by CsA treatment when the experiment was performed with the GS5 replicon (Fig. 6B, left). For the RS2 replicon, association with CyPA was resistant to CsA treatment (Fig. 6B, right). We next examined the interaction between CyPA and the HCV polymerase NS5B. GST-CyPA specifically bound NS5B while GST protein alone was not able to bind (Fig. 6C). The interaction between GS5 NS5B and GST-CyPA was reduced by CsA treatment and became abolished at 2.4 µg/ml CsA (Fig. 6D, left). NS5B from the RS2 cells, however, retained binding to GST-CyPA even at this concentration of the drug (Fig. 6D, right). These results suggest that the association of CyPA with the HCV replication machinery is targeted by CsA and the CsA-resistant interaction
between NS5B and CyPA contributes to the CsA-resistant replication of the RS2 replicon. To examine if NS5B could interact with CyPA in vivo in the absence of any other HCV proteins and viral RNA, we performed co-IP experiments with NS5B and Flag-tagged CyPA transiently expressed in 293-T cells. NS5B coprecipitated with CyPA in this setting (Fig. 6E), indicating that the CyPA-NS5B interaction in vivo is not mediated by any other viral protein or RNA. Only a fraction of the total NS5B was precipitated by the anti-Flag beads as expected because the expressed NS5B proteins were expected to interact with both Flag-tagged and untagged, endogenous CyPA.

Different expression levels of CyP isoforms in replicon cells. Consistent with the critical role of CyPA in mediating CsA’s action in regulating a variety of biological activities such as immunosuppression (12), HIV infection (5), and HCV replication (this study), it has been shown that the expression level of CyPA is 10 to 100 times higher than that of other CyPs in various tissues (2). Here we examined the endogenous expression level of the three CyP isoforms in the replicon cells. Using quantitative RT-PCR and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as an internal control, we discovered a significant difference in the expression levels of CyPA, CyPB, and CyPC in the replicon cells. The CyPA mRNA was expressed approximately 10 and 150 times higher than CyPB and CyPC mRNAs, respectively (Fig. 7). Moreover, the expression levels of the CyPs were found to be very similar in GS5 and RS2 cells, ruling out the possibility that RS2 cells are more resistant to CsA because of higher endogenous level of CyPs.
DISCUSSION

CsA-resistant HCV replicons have been isolated and characterized in vitro. Viral mutations, particularly those in the NS5B coding region, are correlated with CsA resistance (8, 36). In the study reported here, we demonstrated that the mechanism of this resistance lies in a reduced dependency on a specific target protein of CsA, CyPA, by the mutant replicons. Replication of wild-type replicons (GS5, Con1/SGR, and H77/SGR) was readily inhibited when CyPA expression was silenced, whereas the CsA-resistant replicons (RS2 and RS1-2) remained replication competent even in those cells with substantial CyPA knock-down. Although the correlated resistance to either CsA treatment or CyPA knock-down immediately suggested that RS2 cells are able to replicate independently of CyPs, in actuality, the RS2/sh-A161 replicon reverted to being CsA sensitive compared to RS2 and RS2/sh-Luc replicons. Moreover, treating the RS2 cells with a combination of 0.5 μg/ml CsA and sh-A161 inhibited replication, although neither treatment alone was effective. These results are best explained by a model in which CsA directly competes with HCV for binding to CyPA and an increase in affinity for CyPA as a result of the mutations in the RS2 replicon. The wild-type replicons are sensitive to CsA because they require a particular level of interaction with CyPA, which is disrupted upon CsA or siRNA treatment. If the mutations in the resistant replicons confer increased affinity for CyPA, these replicons would then require a lower amount of CyPA to achieve the same level of interaction. This trait is manifested as resistance to CyPA depletion and to the otherwise inhibitory concentrations of CsA. This hypothesis is supported by in vitro binding experiments involving NS5B and CyPA. NS5B from RS2 cells bound to GST-CyPA in a CsA-resistant manner while the interaction between the GS5 NS5B protein and CyPA was readily disrupted by the compound (Fig. 6C). It is also consistent with the finding that RS2’s resistance to CsA is relative in that high concentrations of the compound can still inhibit the replication of the RS2 replicon, although high concentrations of CsA may have pleiotropic effects that have not been monitored by our experiments. Finally, in addition to enhancing binding affinity, the mutations may also alter the NS5B conformation in a way that requires less CyPA to properly function.

Our data also show that CyPA is an essential cofactor for both the subgenomic replicon and the infectious virus of wild-type HCV. Silencing of CyPA not only protected naïve Huh-7.5 cells from challenges by replicon RNA or JFH/JFH-1 but also suppressed replication in cells with a preexisting replicon or viral infection, indicating a continuing requirement for CyPA in replicon or infected cells. When multiple shRNAs against CyPA were tested, a direct correlation was observed between the ability of a particular shRNA to knock down CyPA and the level of HCV inhibition. The replication efficiency of the GS5 replicon appeared to be exquisitely sensitive to the expression level of CyPA, as even small changes in the latter had a pronounced effect on the former (Fig. 2B). The
requirement for CyPA was also observed with HCV of the GT 1b, 1a, and 2a genomes. This result contrasts with the reported role of CyPB in HCV replication, which appeared to be restricted to GT 1b replicons (14), and perhaps even to a specific subset of GT 1b replicons (36). A putative role of CyPC in the replication of a GT 1b replicon was also suggested (28). In our experiments, silencing of either of these CyPs did not have any consistent effect on HCV replication or infection in the various assays and viral genomes that we used. The structures of these CyPs, especially in complex with CsA, are quite similar (15, 23, 30, 34). In fact, the CsA-binding pocket in CyPB has the same structure as that in CyPA (23, 30). Since CsA competes with NS5B for CyP binding, one might expect all these CyPs to be able to bind NS5B and support HCV replication. However, the CyPA-directed shRNAs and the anti-CyPA antibody, all of which affected only CyPA and not CyPB or CyPC, were sufficient to suppress and in some cases completely abolish replication or infection, suggesting that other CyPs were not able to fulfill CyPA’s essential role in HCV replication. An analogous observation has been made for HIV replication in T cells. Although both CyPA and CyPB bind to HIV Gag in vitro (4, 21), disrupting the CyPA gene alone by homologous recombination in a T-cell line completely abolished any effect of CsA on HIV, indicating that no other CyP could substitute for CyPA in these PPIA−/− cells (5). Subtle differences in structure and/or subcellular localization between CyPA and other members of the CyP family may contribute to this phenomenon. More importantly, the expression levels of these CyPs differ significantly both in the replicon cells (Fig. 7) and in liver tissue (2) in that CyPA is expressed 8 to 10 times more highly than CyPB and CyPC combined. As a result, even if CyPB or CyPC can interact with HCV in biochemical assays, neither comprises a large enough fraction of the total CyP pool in vivo to make significant contributions to HCV replication. In fact, CyPB has been found to interact with the NS5B protein of JFH-1 but its knock-down failed to suppress JFH-1 replication (14). Since JFH-1 is still inhibited by CsA, the authors of that report speculated that another member of the CyP family mediates the effect of the compound. Here we identify CyPA as the cellular factor that mediates the CsA inhibition of JFH-1 in addition to GT 1a and 1b replicons.

Biochemical experiments including in vitro replication and binding assays reveal an interaction between CyPA and the replication complex of HCV. An antibody against CyPA blocked replication directed by lysates from GS5 but not from RS2 replicon; moreover, CyPA was found to associate with HCV RNA in replicon cells in a CsA-sensitive manner. This association is mediated by an interaction between CyPA and NS5B. The CyPA-NS5B interaction appears to be direct and independent of other nonstructural proteins since recombinant NS5B produced from Escherichia coli also binds CyPA (data not shown). A direct interaction between CyPA and HIV capsid (CA) protein has also been demonstrated (21). The CyPA-CA complex not only constitutes the molecular target of CsA-mediated modulation of HIV infection in T cells (21) but also participates in innate immunity pathways that restrict HIV replication (20). CyPA binding either shields HIV capsid from
an endogenous restriction factor or renders the viral core vulnerable to the TRIM-5α-mediated inhibition, depending on the host cell type (39). Similar to the CyPA-NS5B interaction, binding of CyPA to HIV CA or its precursor protein, Gag, is also sensitive to CsA. The well-characterized crystal structure of the CyPA-CA complex (9, 41, 48) may provide relevant insight and further guidance for complex characterization of the CyPA-NS5B interface.

Precisely how HCV requires CyPA to replicate is not yet understood. CsA has been shown to interrupt RNA binding by NS5B synthesized in replicon cells (36, 46). In addition, CsA treatment induces the unfolded protein response, resulting from protein misfolding and accumulation in the endoplasmic reticulum (16, 26, 28, 33). CsA’s effect on protein folding is consistent with the proposed chaperone role of immunophilins (9, 41, 48) and also sensitive to CsA. The well-characterized crystal structure of CyP-CA complex in Huh-7 cells harboring subgenomic replicons. J. Virol. 79:5487–5492.


