Role of Phosphatidylinositol 4-Phosphate (PI4P) and Its Binding Protein GOLPH3 in Hepatitis C Virus Secretion*

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Hepatitis C virus (HCV) RNA replicates within the ribonucleoprotein complex, assembled on the endoplasmic reticulum (ER)-derived membranous structures closely juxtaposed to the lipid droplets that facilitate the post-replicative events of virion assembly and maturation. It is widely believed that the assembled virions piggy-back onto the very low density lipoprotein particles for secretion. Lipid phosphoinositides are important modulators of intracellular trafficking. Golgi-localized phosphatidylinositol 4-phosphate (PI4P) recruits proteins involved in Golgi trafficking to the Golgi membrane and promotes anterograde transport of secretory proteins. Here, we sought to investigate the role of Golgi-localized PI4P in the HCV secretion process. Depletion of the Golgi-specific PI4P pool by Golgi-targeted PI4P phosphatase hSac1 K2A led to significant reduction in HCV secretion without any effect on replication. We then examined the functional role of a newly identified PI4P binding protein GOLPH3 in the viral secretion process. GOLPH3 is shown to maintain a tensile force on the Golgi, required for vesicle budding via its interaction with an unconventional myosin, MYO18A. Silencing GOLPH3 led to a dramatic reduction in HCV virion secretion, as did the silencing of MYO18A. The reduction in virion secretion was accompanied by a concomitant accumulation of intracellular virions, suggesting a stall in virion egress. HCV-infected cells displayed a fragmented and dispersed Golgi pattern, implicating involvement in virion morphogenesis. These studies establish the role of PI4P and its interacting protein GOLPH3 in HCV secretion and strengthen the significance of the Golgi secretory pathway in this process.

Background: The secretory mechanism of hepatitis C virus (HCV) is currently unknown.

Results: Depletion of the Golgi PI4P levels or PI4P-binding protein GOLPH3 reduces HCV secretion and leads to accumulation of intracellular virions.

Conclusion: PI4P and binding proteins implicate the Golgi as a necessary component of HCV secretion.

Significance: Characterization of the components of the HCV secretion pathway could lead to new therapeutic targets.

Hepatitis C virus (HCV)² infects an estimated 2–3% of the world population (1). HCV is a major cause of various liver diseases such as liver steatosis, fibrosis, and cirrhosis, which can eventually progress into hepatocellular carcinoma (2). HCV replicates within the ribonucleoprotein complexes assembled on the ER-derived membranous structures termed “membranous webs” (3–5). The translation of the positive-strand HCV RNA on the ER is facilitated by an internal ribosome entry site in its 5’ UTR, generating a 3000-amino acid polyprotein which is co- and post-translationally cleaved by viral and cellular proteases to yield structural and non-structural viral proteins (2, 4). HCV infection alters host lipid metabolism to facilitate enrichment of lipids that usually manifest in the accumulation of intracellular lipid droplets or hepatic steatosis (6–8). The lipid droplets serve as platforms for facilitating the post-replicative events of virion assembly and secretion (9). Several studies suggest that HCV co-opts the VLDL secretion pathway for its secretion, although the distinct mechanism involved remains to be fully characterized (10–12). Recent reports have digressed from this general assumption and have implicated the involvement of the endosomal sorting complex required for transport (ESCRT) machinery and endosomal trafficking in HCV secretion analogous to that of HIV virion secretion (13, 14). It should be noted that the Golgi and endocytic pathways intersect and the exit routes from the trans-Golgi network (TGN) also include the early, late, and recycling endosomes (15, 16). A recent study applying siRNA screening highlights the importance of ER-Golgi trafficking in HCV secretion, as well as lipid and protein kinases that function in TGN cargo vesicle budding (17). Rab11A, a small GTP binding protein that modulates trafficking from recycling endosomes and the Golgi and PI4KIIIβ, a PI4-kinase localized to the Golgi were shown to be required for HCV virion secretion (17).

VLDL biogenesis is initiated in the ER through the co-translational lipidation of apolipoprotein B-100 (apoB) (18). These

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pre-VLDL particles are subjected to additional lipidation to generate a mature VLDL particle. Although the mechanism and location of this second lipidation event is not clear, it is understood that the VLDL particles are transported from the ER to the Golgi in specialized vesicles (VLDL transport vesicles) and are eventually secreted via the Golgi secretory pathway (19). It has been shown that perturbation of VLDL biogenesis inhibits HCV virion secretion (3, 11, 12). Apolipoprotein E is shown to be a component of lipovirrioparticles and required for HCV secretion (20). Furthermore, the inhibition of ARF-ribosylation factor 1 (ARF1) by brefeldin A and expression of dominant negative mutants of ARF1 abolished HCV secretion (21). ARF1, a small GTPase protein is a modulator of trafficking to and from the Golgi, is also required for the assembly and secretion of VLDL particles (22–24). Together, these studies underscore the importance of VLDL egress through the Golgi secretory pathway for HCV virion secretion.

Phosphoinositides, phosphorylated derivatives of phosphatidylinositol (PI) are important mediators of membrane trafficking and intracellular signaling (25, 26). Recent studies involving genome-wide siRNA screens have identified PI4-kinases (PI4K) as important host factors required for HCV viral proliferation (27, 28). Four known PI4Ks produce PI4P from D-4 phosphatidylinositol. Two of these kinases, PI4KIIα and PI4KIIβ, localize to the Golgi complex; however, PI4KIIβ plays a more dominant role in maintaining important functional and structural attributes of the Golgi complex (29). It was recently shown that HCV induces enrichment of PI4P pools near the ER by PI4KIIα to trigger host membrane rearrangement required for HCV replication (30, 31).

PI4P plays a pivotal role in the Golgi trafficking by recruiting coat proteins and accessory factors required for the vesicular transport process (29, 32). The PI4P binding proteins include the following: OSBP, CERT, and FAPP2, which contain pleckstrin-homology domains that specifically bind to PI4Ps, and mediate the Golgi localization of these proteins (33). We have previously shown that OSBP plays a key role in HCV secretion and that the HCV non-structural protein 5A (NS5A) interacts with OSBP to facilitate this process (34). We have also shown that protein kinase D (PKD), a central modulator of Golgi trafficking, negatively regulates HCV secretion through phosphorylation-mediated inhibition of OSBP and CERT functions and that HCV inhibits PKD activation (35). Intriguingly, PKD is known to activate PI4KIIIβ at the TGN (36). Our findings suggest that HCV tightly regulates the conventional Golgi-secretory pathway and musters the available resources to facilitate selective secretion of the HCV virion. Indeed, we observed that the overall secretion of secretory proteins was hindered in HCV-infected cells (35). A recent addition to the list of PI4P binding proteins is GOLPH3, a Golgi protein that requires PI4P for its Golgi localization through direct binding via a novel domain unrelated to pleckstrin-homology domains (37). GOLPH3 maintains a tensile force on Golgi required for vesicle budding via its interaction with an unconventional myosin MYO18A that binds to F-actin, connecting the Golgi to the cellular actin network (37).

We further investigated the role of TGN-specific PI4P pools, GOLPH3, and its binding partner MYO18A in HCV secretion. Depleting these specific pools of PI4P by either the ectopic expression of a Golgi-targeted PI4P-specific phosphatase, hSac1 K2A, or knockdown of PI4KIIIβ, the kinase primarily responsible for PI4P production at the TGN, resulted in a significant decrease of HCV secretion. This decrease in virus secretion is accompanied by a concomitant increase in the intracellular infectious virus titer. We also observed a similar dramatic decrease of HCV secretion upon siRNA-mediated silencing of GOLPH3 and its binding partner, MYO18A, the proteins that facilitate budding from the TGN via their interactions with the actin network. Taken together, our data establishes the significance of TGN-localized pool of PI4P and its interacting protein GOLPH3 in HCV secretion. Our results also strengthen the notion that HCV utilizes the Golgi secretory pathway for its secretion and in this process may co-opt the VLDL secretory pathway.

EXPERIMENTAL PROCEDURES

Reagents and Plasmids—The HCV plasmid pJFH1 was a gift of Dr. T. Wakita (Tokyo, Japan). The plasmids pJC1 and pJC1 RLuc2a, and mouse monoclonal 9E10/A3 for N55A, were gifts of Dr. C. Rice (Rockefeller University). The plasmid of pEGFP hSac1 K2A was a gift of Dr. P. Mayinger (Oregon Health and Science University, Portland, OR). Anti-apoB was purchased from Chemicon Intl. (Temecula, CA). Antibodies against calnexin, MYO18A, and (goat) TGN46 were from Santa Cruz Biotechnology (Santa Cruz, CA). PI4P antibody was from Echelon Bioscience (Logan, UT). Mouse monoclonal anti-core antibody was acquired from Affinity Bioreagents (Golden, CO). Rabbit anti-GOLPH3 antibody was from Abcam (Cambridge, MA). Rabbit polyclonal anti-TGN46 was from Sigma-Aldrich. Fluorescence-conjugated donkey antibodies against mouse, rabbit, goat, and human were acquired from Invitrogen. Primers and probes for quantitative PCR of PI4KIIIβ were acquired from Integrated DNA Technologies (Coralville, IA). Stealth siRNAs were also acquired from Invitrogen. The sequences are as follows: GOLPH3, 5′-UCUGGAUACUGUAGCUAU-3′; MYO18A, 5′-AUUCUACUCCUCACCAUCUA-3′; PI4KIIIb, 5′-ACUCUGUAAUACUCCAC-3′; and PI4KIIIβ2, 5′-AAAGGCUGACGUCAAAGCAGAG CCAGA-3′.

Cell Culture, HCV Infection, Focus-forming Unit (FFU) Assay—Human hepatoma cell lines Huh7 and Huh7.5.1 were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. The Huh7.5.1 cell line was a kind gift of F. Chisari (Scripps Research Institute, La Jolla, CA). The highly infectious HCV genotype 2a chimeric virus JC1 was used to infect cells (38). JC1 infection was performed at a multiplicity of infection of 0.01. These JC1-infected Huh7.5.1 cells were transfected with either plasmids or siRNAs at 6 days post-infection. Media was changed 16 h post-transfection. Culture supernatants and cell lysates were collected at 72 h post-transfection. Cell pellets were subjected to freeze-thaw to release the intact infectious virus, as described previously (11). Virus titers in culture supernatants and lysates were determined by FFU assay as described previously (39). We used the human monoclonal anti-E2 AR3A antibody (kind gift of Dr. M. Law (Scripps Research Institute) for HCV FFU assays (40).
also used Huh7.5.1 cells infected with JC1 RLuc 2A chimeric reporter virus at a multiplicity of infection of 0.1 and used in experiments 6 days post-infection. JC1 RLuc 2A is derived from JC1 by insertion of Renilla luciferase (Rluc) reporter gene between p7 and NS2 of the monocistronic JC1 RNA genome (41). 72 h post-transfection, the Rluc reporter activity in cell lysates was determined to assess HCV replication. HCV virus secretion was estimated by infecting naïve Huh7.5.1 cells with culture supernatants and determining RLuc activity 48 h post-secretion was estimated by infecting naïve Huh7.5.1 cells with lysates was determined to assess HCV replication. HCV virus (41). 72 h post-transfection, the RLuc reporter activity in cell scale RNA production system-T7 (Promega, Madison, WI). 10 

DNA and RNA Transfection—DNA and RNA transfection was performed using the TransIT LT1 reagent according to the manufacturer’s instructions (Mirus Bio, Madison, WI). siRNAs were transfected using the Lipofectamine RNAi Max reagent according to the manufacturer’s instructions (Invitrogen).

Real-time RT-PCR—Total RNA was purified from cells or 200 μl of culture supernatant by guanidinium thiocyanate-phenol-chlorform extraction method (42). HCV RNA was quantified on an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) as described previously (43).

Western Blotting Analysis—Cellular proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotted as described previously (44).

Immunofluorescence—Infected and/or transfected cells grown on glass coverslips were prepared as described previously (34, 35). The slides were analyzed by confocal laser scanning microscopy (Olympus FV1000).

HCV RNA Transcription and Electroporation—The plasmid encoding HCV (JC1) was linearized by XbaI digestion and subsequently subjected for RNA synthesis using a RiboMAX largescale RNA production system-T7 (Promega, Madison, WI). 10 μg of extracted HCV RNA was used for electroporation using a Gene Pulser Xcell electroporation system as described previously (Bio-Rad) (34, 35).

ssHRP-FLAG Secretion Assay—ssHRP-FLAG secretion assay was carried out as described previously (45). HCV-infected and uninfected Huh 7 cells were co-transfected with ssHRP FLAG (kind gift of Dr. V. Malhotra, Barcelona, Spain) and hSac1K2A plasmids. Secreted ssHRP-FLAG in the culture supernatants was quantified 16 h post-transfection by incubation of the clarified supernatants with HRP substrate, and absorbance was measured in an ELISA plate reader at 450 nm.

Statistical Analysis—Statistical analyses (unpaired t test) were performed using GraphPad Prism software.

RESULTS

PI4P Depletion from TGN Inhibits Vesicular Trafficking—The bulk of total cellular PI4P is localized at the Golgi compartment and plays a pivotal role in the Golgi secretory functions by facilitating recruitment of proteins that promote vesicular transport (32). The PI4P-binding proteins OSBP and CERT that function in the Golgi trafficking were shown to affect HCV secretion (34, 35, 46, 47). To investigate the functional role of Golgi-localized PI4P in the HCV secretion process, we used a mutant version of the hSac1 protein, hSac1 K2A. hSac1 is a phosphatase that specifically dephosphorylates PI4P to PI (48). Two lysine residues in the KEKID domain, responsible for the protein’s ability to localize to the ER, of the wild-type hSac1 protein were changed to alanine. The hSac1 K2A protein accumulates almost entirely in the Golgi (Fig. 1A, see the hSac1K2A-GFP+TGN46 panel) (48). Expression of hSac1 K2A led to selective reduction of Golgi-localized PI4P pools (Fig. 1A; see TGN46+PI4P panel). The ER-localized pools of PI4P have been shown to induce membrane alterations and promote HCV replication (30, 31). Thus, we sought to investigate the potential role of Golgi-localized PI4P in the HCV secretion process by employing the hSac1 K2A expression strategy without affecting HCV replication. In agreement with previous reports, we observed the robust stimulation of total cellular PI4P pools in HCV-infected cells (Fig. 1B and see Fig. 6C; see the uninfected and HCV-infected cells in PI4P panels) (30). Expression of hSac1 K2A effectively reduced the levels of Golgi-localized PI4P in HCV-infected Huh7 cells, as shown by immunofluorescence imaging of PI4P and the TGN (Fig. 1B; see TGN46+PI4P and hSac1K2A-GFP+PI4P panels). Total PI4P levels in HCV-infected cells are not reduced as effectively as in uninfected; this reflects the induction of non-Golgi localized PI4P by HCV. In one of the hSac1 K2A expressing cells (Fig. 1B; lower cell marked with white arrow), the continued presence of PI4P (red) represents non-Golgi-localized PI4P pools, which are stimulated by HCV infection. This PI4P staining fails to co-localize with TGN46 and most likely represents ER or ER-associated PI4P pools. The PI4P levels specifically at the Golgi, however, remain depleted by hSac1 K2A expression (Fig. 1B; hSac1 K2A+PI4P panel). Because PI4P is a major modulator of Golgi secretory capacity, we investigated whether the Golgi-specific depletion of PI4P by ectopic expression of hSac1 K2A affected the conventional secretory capacity of the cell. As expected, we observed a dramatic reduction in the secretion of ssHRP (secretory form of HRP protein) in cells expressing hSac1 K2A compared with the untransfected control cells (Fig. 2A). ssHRP secretion in the culture supernatants is determined by assaying for HRP activity as described under “Experimental Procedures.” Given the preponderance of evidence suggesting a link between HCV secretion and VLDL, we also sought to establish whether PI4P depletion in the TGN had any effect VLDL secretion. VLDL secretion is evaluated by Western blot analysis of apolipoproteinB-100 (apoB), the major component of VLDL, in the cell culture supernatants. hSac1 K2A expression resulted in a modest decrease in the levels of secreted apoB in the culture supernatants compared with untransfected control cells (Fig. 2, B and D). Intracellular apoB levels were unchanged (Fig. 2, C and E). Collectively, these results demonstrate that the depletion of Golgi-localized PI4P disrupts the secretion capacity of the Golgi apparatus resulting in reduced secretion of cellular secretory components traversing the Golgi secretory pathway.

hSac1 K2A Inhibits HCV Secretion without Affecting Replication—To investigate the effect of Golgi-specific depletion of PI4P on HCV RNA replication and virion secretion, we transiently expressed hSac1 K2A into HCV JC1 RLuc 2A-infected Huh7.5.1 cells (41). Quantification of luciferase reporter activity in the lysates of the transfected cells was used to assess HCV RNA replication. hSac1 K2A expression had no significant
effect on the luciferase activity of the cell lysates, suggesting that Golgi-specific depletion of PI4P pools did not affect HCV replication (Fig. 3A). We then examined the effect of hSac1 K2A-mediated depletion of the Golgi-localized PI4P pool on HCV virion secretion in Huh7.5.1 cells infected with the highly infectious HCV JC1 virus by FFU assay. Expression of hSac1 K2A resulted in a significant decrease in the extracellular infectivity (extracellular infectious virus titer) of the culture supernatants.
This reduction was accompanied by an increase in the intracellular infectivity (intracellular virus titer) (Fig. 3C). Together, these results suggest that depletion of Golgi-specific PI4P pools lead to a stall in secretion of HCV virion, with a resultant accumulation of intracellular virus particles (Fig. 3, B and C).

Knockdown of PI4KIIβ Results in Decreased Extracellular Infectivity and Increased Intracellular Infectivity—PI4KIIβ is the kinase predominantly responsible for production of PI4P in the Golgi, without affecting ER or other pools (32). As an alternative approach to reduce Golgi-specific PI4P pools, we used the siRNA strategy to knock down PI4KIIβ. We used three different siRNAs to knockdown PI4KIIβ, of which two were effective. The most effective knockdown was achieved using a pool of siRNA 1 and 2 (Fig. 4A). Knockdown of PI4KIIβ also resulted in depletion of PI4P levels at the TGN and abrogated ssHRP secretion in a manner similar to the effect of hSac1 K2A expression (supplemental Figs. S2 and S4). The transient knockdown of PI4KIIβ in the HCV JC1-infected Huh7.5.1 cells did not effect HCV replication levels (Fig. 4B), and replication levels were not affected when PI4KIIβ was presilenced prior to HCV infection (supplemental Fig. S3). However, the knockdown did lead to drastic reduction in extracellular infectivity (Fig. 4C) with a concomitant increase in intracellular infectivity compared with untransfected control cells (Fig. 4D). The decrease in extracellular infectivity by PI4KIIβ knockdown suggests that HCV virion secretion was hindered (Fig. 4C). In contrast, the retention of completely assembled infectious viral
particles in these cells suggests that the Golgi-associated PI4P pools or the conventional secretory functions of Golgi are not required for efficient assembly of the virus particles. Collectively, these results suggest an essential role of PI4P in the HCV secretion process.

GOLPH3 and MYO18A Affect HCV Secretion—GOLPH3 is a protein that localizes to the TGN via its interaction with PI4P, which functions as a membrane anchor (25, 37). GOLPH3 also interacts with an unconventional myosin, MYO18A. The interactions between PI4P, GOLPH3, and MYO18A are required for characteristic “flattened stack” Golgi morphology as well as vesicle budding from the TGN, as proposed by Dippold et al. (37).

We sought to investigate the role of GOLPH3 in HCV secretion. siRNA against GOLPH3 was used to achieve a significant reduction in GOLPH3 expression levels (Fig. 5A). GOLPH3 knockdown in HCV JCI-infected Huh7.5.1 cells had no significant effect on the intracellular viral RNA levels, suggesting that GOLPH3 knockdown did not effect HCV replication, which occurs on the ER modified membranes (Fig. 5B) (30, 31, 49).

However, the knockdown of GOLPH3 significantly reduced the amount of viral RNA secreted in the culture medium (Fig. 5C), suggesting that GOLPH3 effectively participates or facilitates the HCV secretion process. Next, we investigated the role of MYO18A, the binding partner of GOLPH3 and an integral component of the GOLPH3-MYO18A-F-actin complex required for maintaining Golgi morphology and function (37).

Using siRNA, we achieved a substantial knockdown of MYO18A expression levels (Fig. 5A). Knockdown of either GOLPH3 or MYO18A resulted in a decrease in the extracellular infectivity (virus titers in culture supernatants) (Fig. 5D). This was accompanied by a corresponding increase in the levels of intracellular infectivity (virus titers within the cells) (Fig. 5E). GOLPH3 and MYO18A silencing also resulted in reduced ssHRP secretion (supplemental Fig. S4). These results suggest that in GOLPH3 or MYO18A knockdown, the infectious virions are stalled from secretion and retained within the cell, resulting in higher levels of intracellular infectivity. To determine the effect of HCV infection on the Golgi, and the protein distribution in infected cells, we immunostained the cells for GOLPH3, PI4KIII/H9252, and PI4P in infected cells, respectively. GOLPH3 and PI4KIII/H9252 proteins both co-localize with the TGN, as usual; however, we also noted in increased level of Golgi fragmentation in the infected cells, which is reflected in the localizations of these proteins (Fig. 6, A and B). In addition, the levels of non-Golgi PI4P induction by HCV infection can easily be seen, consistent with previous reports (Fig. 6C) (30).

Collectively, our results suggest that PI4P and PI4P-interacting GOLPH3 and GOLPH3-interacting MYO18A, are required for efficient functioning of Golgi-associated secretion and in secretion of HCV virions from the TGN. The intracellular accumulation of completely assembled infectious virions (intracellular infectivity) in similar conditions also suggests that Golgi-
associated vesicle sorting and secretory function are not required for the virus assembly process.

DISCUSSION

It has been well demonstrated that HCV depends on VLDL biogenesis for its secretion (3, 7, 10–12, 20). The occurrence of VLDL-associated viral particles (lipoviroparticles) in patient sera further strengthen the notion that HCV and VLDL secretion overlap and that HCV co-opts the VLDL secretory pathway for its secretion (50). Although the intricate details of VLDL biogenesis are still unclear, it is well known that the VLDL particles traffic through the Golgi compartments, implicating a similar pathway for HCV virion egress (19, 51).

Nonvesicular lipid transport between the ER and Golgi affects the lipid composition of the Golgi membranes (46). We and others (34, 52) have shown that Golgi-associated OSBP and CERT are required for HCV virion secretion. OSBP is a sterol sensor and transporter and maintains cholesterol levels in the Golgi membrane (53). CERT transfers ceramide from ER to the Golgi where it is used for synthesis of sphingomyelin (54). Both cholesterol and sphingomyelin are enriched in HCV virions, suggesting that the HCV virion envelope becomes enriched in these lipid molecules during egress or that the virion envelope is derived from trans-Golgi membrane (55). We further reported that PKD, a key component of PI4P-regulated pathways at TGN, negatively regulates HCV secretion by triggering phosphorylation of OSBP and CERT, thereby inhibiting their function. In correlation with this hypothesis, we observed that HCV inhibits PKD activity (35). Because PKD activates

![Figure 5. Effect of GOLPH3 knockdown on HCV replication and secretion.](image)
PI4KIIIβ at the TGN, our observations suggest that although HCV reduces the general secretion via TGN, it modulates the local lipid composition of the TGN to specifically enhance HCV virion secretion (35). Interestingly, we also observed that in HCV-infected cells the Golgi apparatus often displays a dispersed and fragmented pattern, which might contribute to the decrease in general secretion capacity of the cell (Fig. 6). However, this fragmentation may also hint at the plausible sequestration of Golgi compartments at the site of virion assembly for efficient secretion of the assembled virions. These observations strongly emphasize the major contribution of the Golgi network in HCV virion secretion. To further strengthen our hypothesis, in this study, we have investigated the role of Golgi-localized PI4P pools in HCV secretion.

PI4P pools at the TGN regulate important functional and structural attributes of the Golgi complex (25, 26, 32, 33). siRNA screening for host factors has revealed that PI4Ks are essential for HCV proliferation (28). Because the ER-localized PI4 kinase PI4KIIIβ has been shown to be important for viral induced membrane rearrangement and HCV replication, we primarily focused on investigating the role of Golgi-localized PI4P pools (30, 31). We took advantage of Golgi-specific hSac1 K2A phosphatase to specifically deplete PI4P from the TGN. The depletion of the Golgi-localized PI4P pools reduced HCV secretion, but had no notable effect on viral replication. Although the extracellular infectivity (secreted virus titers) decreased, we observed a concomitant increase in the intracellular infectivity (intracellular virus titers) upon ectopic expression of hSac1 K2A, suggesting that the viral replication and assembly processes are not affected but that there is stalling in

FIGURE 6. Effect of HCV infection on the Golgi network. HCV-infected and uninfected cells are stained with various Golgi-specific antibodies. Cells were also stained with antibodies specific to PI4P, GOLPH3, and PI4KIIIβ. Nuclei were counterstained with DAPI. Infected cells are marked with white arrows, whereas uninfected cells are denoted with an X.
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The Golgi apparatus in HCV secretion is critically involved in the maturation and egress of viral particles. HCV uses the Golgi apparatus for the secretion of its viral components, and defects in Golgi function can result in the inhibition of viral replication. The Golgi complex plays a pivotal role in the transport of membrane proteins and lipids, which are essential for HCV assembly and secretion. Disruption of Golgi function can lead to the accumulation of viral particles within infected cells, highlighting the importance of the Golgi apparatus in HCV replication.

KEYWORDS:
HCV, Golgi apparatus, secretion, viral replication, cellular trafficking.

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