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Hepatitis C Virus Exploitation of Processing Bodies

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During infection, positive-strand RNA viruses subvert cellular machinery involved in RNA metabolism to translate viral proteins and replicate viral genomes to avoid or disable the host defense mechanisms. Cytoplasmic RNA granules modulate the stabilities of cellular and viral RNAs. Understanding how hepatitis C virus and other flaviviruses interact with the host machinery required for protein synthesis, localization, and degradation of mRNAs is important for elucidating how these processes occur in both virus-infected and uninfected cells.

The life of a newly synthesized mRNA would be aimless in the absence of RNA binding proteins (RBPs) and microRNAs. These interactions and the formation of ribonucleoprotein particles (RNPs) direct mRNAs from the nucleus to the cytoplasm for translation or to RNA granules for storage and/or degradation (1). To the benefit or detriment of a virus, viral RNA is equally exposed to such fates. From the time that a virus binds and enters a host cell, the virus interfaces with and frequently subverts different host mRNA metabolism pathways. Hepatitis C virus (HCV) is a master of such mechanisms, efficiently co-opting the cellular translation machinery, the microRNA pathway, and components associated with mRNA storage and decay.

The single-stranded positive-sense RNA genome of HCV encodes a single open reading frame. Untranslated regions (UTRs) at the 5′ and 3′ ends of the genome are highly structured and contain elements that are essential for translation and replication (2). The 5′ UTR contains an internal ribosome entry site (IRES) that directly recruits the 40S ribosome and a subset of initiation factors for polyprotein synthesis. Additionally, the liver-specific microRNA miR-122 interacts with two binding sites in the 5′ UTR to maintain viral RNA abundance (3). miR-122 was first shown to modestly affect translation and replication (3). However, the most exciting function described for miR-122 was that of shielding the 5′ UTR from degradation by the 5′-to-3′ exonucleases Xrn1 and Xrn2, thereby increasing the stability of the viral RNA (3). More recently, miR-122 was proposed to facilitate the transition between translation and replication (4), a step that is poorly understood in the life of many viruses. Interestingly, recent work has shown that miR-122 abundance or single point mutations within the miR-122 binding sites facilitate HCV replication independently of miR-122 (5, 6). Many intriguing questions regarding the HCV-miR-122 interaction remain. Although we know that miR-122 must occupy both binding sites to maintain viral RNA abundance, do the sites act independently and synergistically? Does miR-122 binding alter viral RNA structure to affect gene expression, and which RNPs are associated with the miR-122 binding sites? In miR-122-independent gene expression, are the same RNP complexes associated with the HCV 5′ UTR, and how are the different phases in the infectious cycle affected? Translation, localization, storage, or destruction of mRNAs is directed by specific RBPs and microRNAs whose abundance and accessibility are affected by intracellular and extracellular signals, thus raising intriguing questions concerning how, where, and when miR-122-associated proteins might influence HCV gene expression.

PROCESSING BODIES (P-BODIES) AND HCV

P-bodies are RNA granules where nontranslating mRNAs are temporarily stored for later use or are targeted for decapping and degradation. Unlike stress granules, which assemble in response to stress, P-bodies are ubiquitous, cytoplasmic RNA-protein complexes (Fig. 1) (1). P-body components include mRNA transcripts, the decapping proteins (Dcp1/Dcp2), the 5′-to-3′ exonuclease Xrn1, and decapping activators such as Lsm1-7 RCK/p54, RAP55, hEDC3, PatL1, and Ge-1 proteins. Also localized in P-bodies are components of the deadenylase complex, non-sense-mediated decay proteins, and RNA interference (RNAi) machinery (Ago2, GW182, and microRNAs) (Fig. 1) (1). Despite miR-122 and Ago2 localizing in a perilous environment, HCV counterintuitively usurps the microRNA pathway for gene expression. For our research, this posed interesting questions regarding how, where, and when miR-122-associated proteins might influence HCV gene expression.

We first examined the fate of P-bodies by immunofluorescence assays over the course of a 3-day HCV infection (7). We observed that the number of P-bodies dramatically decreased in HCV-infected cells. Furthermore, this effect was seen in both quickly and slowly replicating HCV strains (JFH-1 and H77), suggesting that it was not RNA abundance that caused it. Depletion of specific P-body components by RNAi is known to disperse these RNA granules, and yet, when we examined the abundance and size of different P-body components during HCV infection, we observed no difference between uninfected and infected cells (7). This phenomenon was unlike that observed during poliovirus infection, where the abundance of Xrn1, Dcp1a, and Pan3 decreased by 5 h postinfection, likely as a result of virus-induced proteolytic cleavage (8). Rather, confocal microscopy showed that HCV likely dispersed P-bodies by relocalizing a number of P-body components (Lsm1, RCK/p54, DDX3, Dcp2, Xrn1, Ago2, and miR-122) to lipid droplets, the proposed sites of HCV assembly (7, 9, 10). Biochemical isolation and immunoblotting of lipid droplets con-

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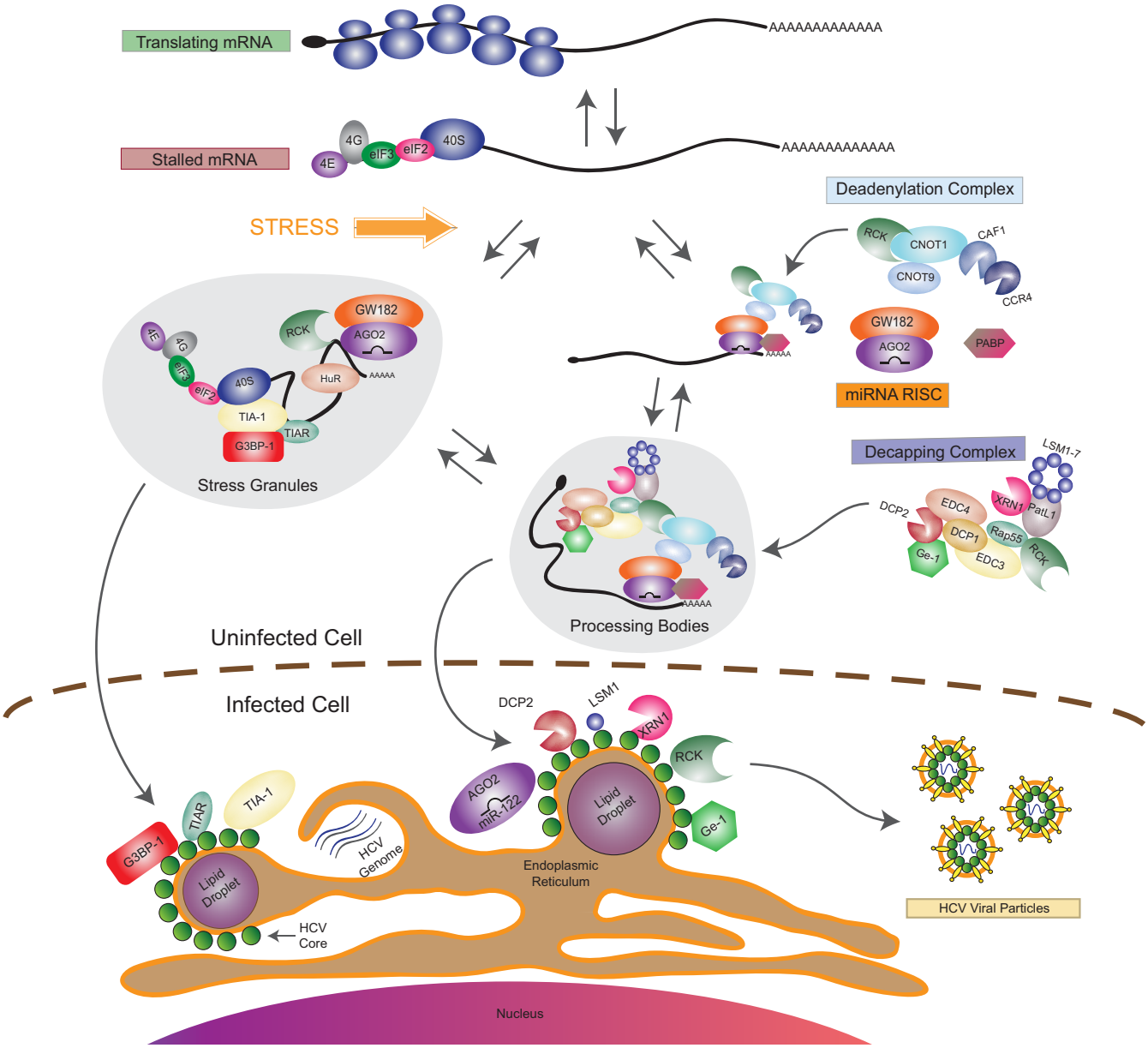

FIG 1 Cytoplasmic RNA granules in uninfected and HCV-infected cells. Different dynamic systems modulate the life of a cellular mRNA. Activation of the translation initiation complex determines whether the mRNA is translated or whether, following stress and phosphorylation of α subunit of eukaryotic initiation factor 2 (eIF2α) and association with different RNA-binding proteins (RBPs), the mRNA is stored in stress granules. Processing bodies (P-bodies) are cytoplasmic RNA-protein structures involved in mRNA storage and decay, microRNA-induced gene silencing (miRISC), and mRNA surveillance through the interaction with stress granules. The balance of these pathways is, however, perturbed when the cell is infected with HCV, which subverts and causes the relocalization of P-body and stress granule components. During HCV infection, different components of stress granules and P-bodies relocalize to lipid droplets, the site of virion assembly.

firmed the localization of RCK/p54 and DDX3 on lipid droplets (9). Although RCK/p54, PatL1, and Dcp1 interact, only some P-body components were relocalized, while others such as Dcp1 and GW182 remained in P-body foci (11). While the composition of P-bodies may have been altered, P-body formation was not required for HCV infection. The disparate dissociations of proteins known to otherwise form functional complexes, such as those in the decapping complex (RCK/p54, PatL1, Dcp1, and Dcp2) or in the microRNA-induced silencing complex (miRISC; RCK/p54, Ago2, and GW182) raise interesting questions of whether proteins at lipid droplets remain complexed together and functional and how they might affect HCV assembly. As most HCV infection studies are done in the hepatoma cell line Huh7, it was significant that, in human liver biopsy specimens from HCV-infected patients, HCV also dispersed P-bodies containing RCK/p54 and Dcp1 and that these RNA granules reformed in those patients who had been given antiviral treatments and had cleared HCV (12). Moreover, RCK/p54 did not colocalize at lipid droplets in hepatocytes from HCV-infected patients. Taken together, the results of these studies show that, rather than indiscriminately ablating

RNA granules, HCV likely subverts specific P-body components to promote gene expression.

P-BODY COMPONENTS AFFECT HCV GENE EXPRESSION

RNAi depletion of Ago2, Xrn1, Lsm1, RCK/p54, Ge-1, and PatL1 altered HCV protein and RNA abundance and virus titers (7, 13, 14), suggesting that HCV subverts specific P-body proteins for HCV gene expression. While the roles of Ago2 and Xrn1 during HCV infection have been extensively studied, less is known about Lsm1, RCK/p54, Ge-1, and PatL1.

As a central component of the microRNA-induced silencing complex, it was not surprising that RNAi depletion of Ago2 decreased HCV gene expression (7). Cross-linking and immunoprecipitation studies demonstrated that Ago2 interacted with HCV RNA not only at the known miR-122 binding sites but also in the IRES, E1, E2, NS5A, and NS5B regions (15). Ago2 and miR-122 colocalization at lipid droplets (10), and the additional Ago2-HCV RNA interactions (15), pose interesting questions concerning whether Ago2 might act beyond the microRNA-directed function during HCV infection. Furthermore, in light of the recent study describing adaptive mutations in HCV impacting miR-122-independent gene expression, would Ago2 still be required for HCV gene expression?

Deadenylated mRNAs targeted to P-bodies may be stored or degraded. For those mRNAs slated to be degraded, Dcp1/Dcp2 cleaves the m^7G-cap, leaving 5′monophosphorylated RNA, which is then degraded by Xrn1 (1). miR-122 has been proposed to shield the HCV 5′ end from Xrn1 and Xrn2 (3, 16). Indeed, depletion of Xrn1 increased the half-life of H77 RNA when miR-122 was sequestered with an antisense oligonucleotide. Interestingly, following miR-122 sequestration, specific H77 viral RNAs trimmed at the 5′ end were also detected (16). While incongruous with the masking role of miR-122 from Xrn1 degradation, an elegant RNA knot structure in the 3′ UTR of other flaviviruses is known to stall and repress Xrn1 exonuclease activity to modulate the stability of cellular mRNAs (17). This strategy, applied to regions in the HCV 5′ UTR, similarly increased the overall stability of cellular mRNAs, including those mRNAs with short half-lives that encode different oncogenes and angiogenesis factors (18). Last, localization of Xrn1 at lipid droplets during HCV infection is intriguing (7) and suggests that Xrn1 might have a role in regulating the HCV RNA genome during virion assembly.

Lsm1, RCK/p54, Ge-1, and PatL1 are all components of the decapping complex, and it is unclear why a virus such as HCV, which lacks a cap, might subvert these proteins. Lsm1-7, a heptameric ring complex in P-bodies, interacts with deadenylated mRNAs and other RNA decay components to stimulate decapping and 5′-to-3′ degradation and to prevent exonuclease trimming at the 3′ end of the deadenylated mRNAs. Lsm1 was shown to stimulate miR-122-directed translation of HCV, and recombinant Lsm1-7 binds HCV 5′ and 3′ UTRs (14, 19). While the interactions with the 5′ UTR likely impact HCV translation, the 3′ UTR might direct unique molecular events during HCV gene expression.

RCK/p54, a DEAD-box RNA helicase, associates with deadenylation, decapping, and miRISC complexes to modulate translational repression, decapping of cellular mRNAs, and microRNA-mediated gene regulation (20). Of the P-body proteins depleted, RCK/p54 was shown to have the most dramatic effect on HCV gene expression (7). The role of RCK/p54 during HCV infection is, however, still unclear. *Renilla* luciferase replicon studies showed RCK/p54 affecting HCV translation, while another suggested effects on replication (13, 14). Interestingly, decreased HCV gene expression following RCK/p54 depletion was rescued by increasing amounts of miR-122, suggesting that RCK/p54 might act upstream of miR-122 (13). Knowing the intricate role of miR-122, it would not be surprising if RCK/p54 were shown to similarly modulate HCV RNA stability, miR-122-RISC interactions with HCV RNA, or the translation-replication switch. That RCK/p54 interacted with core protein and localized at lipid droplets suggests that RCK/p54 might additionally modulate virion assembly (7, 9, 13). RCK/p54 contains RNA binding and ATPase activities, but specific helicase activity has not been demonstrated (20). Yet RCK/p54 containing a mutation in the DEAD box helicase motif did not rescue HCV gene expression (13). We might speculate that, similar to other DEAD-box helicases, RCK/p54 modulates RNA clamping, RNA structures, displacement of RBPs, aggregation of other protein cofactors, or even metabolite sensing. Such activities would facilitate shuttling HCV genomic RNA to lipid droplets and stripping of replication proteins or even of miR-122 from the HCV genome prior to assembly or during virion assembly to assist the folding of the HCV genome or to mediate HCV RNA-core (or even host) protein interactions.

Several viruses exploit RNA granules during infection (1). Though much is known about the destination of P-body components during HCV infection, their exact role in HCV gene expression is unclear. However, understanding the dynamic interactions between HCV and RNA granules will likely inform the temporal, spatial, and functional dynamics regulating viral and cellular RNAs.

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