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Landscape of post-transcriptional gene regulation during hepatitis C virus infection

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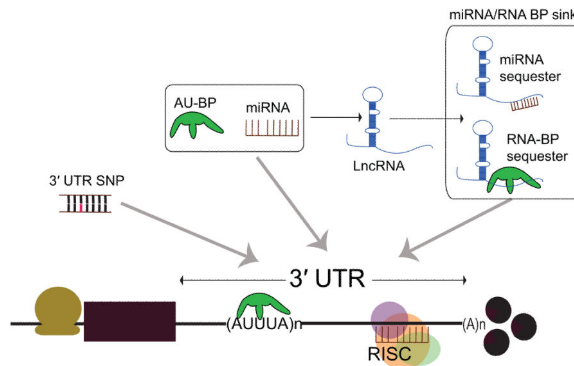
Abstract

Post-transcriptional regulation of gene expression plays a pivotal role in various gene regulatory networks including, but not limited to metabolism, embryogenesis and immune responses. Different mechanisms of post-transcriptional regulation, which can act individually, synergistically, or even in an antagonistic manner have been described. Hepatitis C virus (HCV) is notorious for subverting host immune responses and indeed exploits several components of the host's post-transcriptional regulatory machinery for its own benefit. At the same time, HCV replication is post-transcriptionally targeted by host cell components to blunt viral propagation. This review discusses the interplay of post-transcriptional mechanisms that affect host immune responses in the setting of HCV infection and highlights the sophisticated mechanisms both host and virus have evolved in the race for superiority.

Graphical abstract



Post-transcriptional elements involved in gene regulation during HCV infection. The 3′-untranslated region (UTR) of mRNAs is prone to various post-transcriptional modifications. microRNAs (miRNAs) recruit the RNA-induced silencing complex (RISC) to the target mRNA miRISC which leads to mRNA degradation by ribonucleases or suppression of translation caused

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by steric effects. AU-rich elements (AREs) in the 3′ UTR are targeted by AU-binding proteins (AU-BPs), which results in 3′ to 5′ shortening of the poly(A) tail, a process called ARE-mediated decay (AMD), or to enhanced stability of the transcript, respectively. Single nucleotide polymorphisms (SNP) in the 3′ UTR alter the binding ability of miRNAs and AU-BPs to the mRNA, hence affecting transcript stability. Long non-coding RNAs (lncRNAs) act as sinks for miRNAs and RNA-binding proteins (RBPs) such as AU-BPs, thereby suppressing their function. At the same time, lncRNAs can also target mRNA transcripts directly interact directly and modify translation or splicing.

Introduction

Tight control of gene expression is critical for the organism to maintain metabolic functions, control cell cycle, proliferate and defend against infection. In the last decade the role of post-transcriptional gene regulation during pathogenic infections and host immune responses has become increasingly appreciated. Here we will discuss the roles of post-transcriptional regulation during infection with the *Flavivirus* family member hepatitis C virus (HCV).

Distinct cellular components mediate post-transcriptional control of genes by unique mechanisms. microRNAs (miRNAs) are endogenously encoded, small (20-25 nt), single-stranded RNA molecules that recruit the miRNA-induced silencing complex (miRISC) to target mRNAs [1,2] and repress gene expression. Specific recognition of a target mRNA is mediated by miRNA recognition elements (MREs) in the 3′-untranslated region (UTR) of the mRNA. Binding of the miRISC leads to gene silencing *via* mRNA degradation by ribonucleases or suppression of translation generally by steric effects. Similarly, viruses encode miRNAs, also termed “viral miRNAs,” that blunt expression and/or action of host anti-viral acting genes [3,4].

RNA binding proteins (RBPs) present another class of post-transcriptional regulators of gene expression. RBPs can bind to the 3′ UTR or 5′ UTR of their respective target mRNA to effect translation initiation and transcript stability. RBPs are considered more promiscuous than miRNA, as one binding protein is often able to recognize and bind several unique nucleotide patterns or structures in the UTRs (reviewed in [5]). However, the most prominent of these RBP recognition sites are adenylate uridylate (AU)-rich elements (AREs). The size (40-150 nt) and sequence of AREs can largely vary but a distinct feature is at least one AUUUA pentamer in an AU-rich sequence [6-8]. Depending on the RBP and respective target RNAs this interaction leads to 3′ to 5′ shortening of the poly(A) tail and subsequent mRNA degradation, a process called ARE-mediated decay (AMD), or to enhance stability of the transcript.

Recently discovered long non-coding RNAs (lncRNAs), defined as non-coding RNAs with a size of more than 200 nucleotides, can also have post-transcriptional effects. lncRNAs can directly bind to target mRNA and modify translation or splicing. lncRNAs are also able to act indirectly on mRNA stability *via* modulation of miRNA pathways and the miRISC. A recent review by Fitzgerald & Caffrey provides a comprehensive overview of lncRNA-mediated gene regulation [9].

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The functions of the above regulators can be modulated by genetic variation within the nucleotide sequence of the 3′ UTR of respective target mRNAs. A broad range of single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) are linked to certain diseases. Studies by us and others focusing on the implications of SNPs in the UTRs of mRNAs have documented significant effects on mRNA structure, binding of regulatory elements, and transcript stability [10,11,12**]. Finally, the mRNA structure dictates the expression pattern of genes at various other levels, such as accessibility for ribosomes, translational efficiency, mRNA localization within a cell, and mRNA stability under stress conditions (reviewed in [13]).

Rather than viewing post-transcriptional gene regulation through miRNAs, RBPs and lncRNAs as independent events, we favor a model in which post-transcriptional elements and determinants affect gene expression in a synergistic or antagonistic manner to set the post-transcriptional landscape of an mRNA. In support of this opinion, there is a growing body of evidence for complex crosstalk between post-transcriptional regulatory elements. Competition between miRNAs and RBPs for binding sites on the 3′ UTR of mRNA has been observed by several studies (Savan et al., *unpublished*; [14-16]). Thus it is crucial to consider such complex interactions and all components involved in this post-transcriptional "regulosome" when studying the contribution of a single post-transcriptional regulatory element to the expression pattern of a given gene.

Our understanding of post-transcriptional regulation of host immune responses to HCV infection involves various processes and elements. While host RBPs and miRNAs usually enhance anti-viral responses and block HCV replication, respectively, the virus has evolved sophisticated mechanisms to co-opt some of these components for its own benefit. Additionally, mRNA features such as secondary structure and binding motifs (MREs and/or AREs) for miRISC and RBPs contribute to host and viral gene expression during HCV infection. In this review, we will discuss the concerted actions of post-transcriptional regulatory elements and their implications for the host immune response to HCV infection.

microRNAs

microRNAs (miRNAs) are small, 20-25 nucleotide, non-coding RNAs that act as guides to recruit the miRISC to the 3′ UTR of mRNAs. Once bound to the 3′ UTR, miRISC can interfere with translation or destabilize the mRNA transcript, ultimately leading to reduced gene expression. miRNAs play an important role in many human diseases including cancer, autoimmunity and microbial infection. In the context of HCV infection, miRNAs are appreciated as playing pivotal roles in regulating host immunity and viral pathogenesis. The discovery that miR-122 is required for efficient HCV replication by directly binding the viral RNA set a new precedent for miRNAs as viral RNA stabilizers. The use of deep sequencing and microRNA arrays have since identified numerous miRNAs modulated during HCV infection, suggesting a functional role for these miRNAs in the immune response to HCV [17,18]. Yet despite this advancement in miRNA identification, the role(s) and relative effects of the majority of these miRNAs during HCV infection have yet to be fully elucidated.

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miRNA processing and viRNAs

miRNAs are predominantly transcribed by RNA polymerase II as primary(pri)-miRNAs. Pri-miRNAs are processed and cleaved in the nucleus by Drosha and DGCR8 then exported to the cytoplasm where the precursor (pre)-miRNA product is cleaved by the endonuclease Dicer into its mature miRNA form. The mature miRNA is then loaded into the RNA binding protein argonaute 2 (Ago2), where it can direct Ago2 and other associated miRISC proteins to specific semi-complementary sequences in the 3′ UTR of mRNAs. By hijacking host miRNA processing machinery, several families of dsDNA viruses and one member of the retrovirus family, bovine leukemia virus (BLV), have been shown to produce mature miRNAs from virus-encoded miRNA precursors (reviewed in [19]). Several of these virus-encoded miRNA are known to bind host and viral mRNAs while the roles of many remain unknown. To date, no ssRNA virus, except BLV, has been shown to encode miRNA. This is likely because processing of miRNA from a ssRNA virus would require cleavage and destruction of the viral genome, and have a negative effect on viral fitness [20,21]. Interestingly, BLV is able to circumvent this limitation by processing miRNAs from subgenomic RNA polymerase III-derived transcripts, avoiding viral genome cleavage by Drosha [22]. While it is possible a similar mechanism may be used by other retroviruses, no precedent exists for processing of viral miRNA from RNA virus lacking a DNA intermediate.

Host miRNAs promote HCV survival

Host-derived miRNAs are known to play important roles during HCV infection including modulating the anti-viral immune response and supporting viral replication. The most well studied miRNA during HCV infection is miR-122, which is highly expressed in hepatocytes where its physiological role is regulating cholesterol metabolism. However miR-122 is more highly recognized for its unusual supportive function in HCV replication [23]. Unlike most miRNAs, whose binding facilitates mRNA decay, binding of miR-122 to the 5′ UTR of the HCV RNA protects the viral RNA from degradation by the host 5′-3′ exonuclease Xrn-1 [24*,25*]. Interestingly, loss of Xrn-1 in the absence of miR-122 cannot rescue HCV RNA accumulation, suggesting another role for miR-122 in supporting HCV RNA accumulation. Indeed, one study reports that miR-122 binding to the 5′ UTR of HCV RNA promotes translation by enhancing association of ribosomes to the viral RNA [26]. Thus it may be that miR-122 both protects the HCV RNA from degradation while also stimulating translation of the viral polyprotein. While additional work is necessary to fully elucidate the functions of miR-122 in HCV replication, the identification of miR-122 has already led to the development and testing of an anti-miR-122 based therapy which is currently in phase 2 clinical trials [27]. Thus identification of miR-122 not only described a new mechanism in virus-miRNA biology but also led to the first anti-miRNA-based therapy. For a more in-depth review of miR-122 and HCV pathogenesis see an earlier review published in this journal [28].

While miR-122 has received much attention in the HCV field, reports of other miRNAs with pro-viral effects are beginning to emerge. The majority of these miRNAs target anti-viral effectors to dampen the host innate immune response. For example, miR-21 enhances HCV replication by targeting members of the Toll-like receptor (TLR) signaling pathway, MyD88

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and IRAK1, leading to decreased type I IFN production [29]. miR-491 also enhances viral replication by inhibiting the PI3K/Akt pathway through a mechanism independent of cell replication and activity at the HCV internal ribosome entry site (IRES) [30]. Yet it is still unclear if HCV infection up- or down-regulates expression of this miRNA, or if expression changes throughout the course of infection [18,30]. Indeed, the exact mechanisms by which many HCV-associated miRNAs support viral pathogenesis remain undescribed. Repression of *DLC-1* by miR-141 correlates with increased HCV replication. However the underlying connection between miR-141, *DLC-1* and HCV replication remains elusive [31]. Additionally, miR-192, miR-215, miR-320c, and miR-483-5p enhance HCV pathogenesis through unknown pathways [30,32]. Thus much remains to be explored even among miRNAs previously identified as playing a role in HCV pathogenesis.

We have recently shown that HCV infection induces expression of miR-208b and miR-499a-5p, which are able to target *IFNL2* and *IFNL3* genes [12**]. Interestingly a SNP in the 3′ UTR of *IFNL3*, previously identified as one of the strongest predictors of natural and therapy-induced HCV clearance, destroys the binding site for these two miRNAs, allowing the *IFNL3* HCV clearance variant to escape miR-208b and miR-499a-5p regulation. By using inhibitors against miR-208b and miR-499a-5p we were able to rescue *IFNL3* expression during HCV infection and reduce viral load. As mentioned, the *IFNL3* 3′ UTR SNP is also one of the strongest predictors of IFN-α therapy induced clearance of HCV. One explanation for this association arose from recent work in our lab, which discovered that treatment of HCV-infected hepatocytes with type I IFNs amplifies expression of miR-208b and miR-499a-5p. Expanding on this observation, we hypothesize IFN-α amplifies expression of these miRNAs in HCV infected livers. For patients with the viral persistence *IFNL3* genotype, this would further repress expression of IFN-λ3. Conversely, patients with the viral clearance *IFNL3* genotype can escape regulation by these miRNA and would be unaffected by increased miR-208b and miR-499a-5p expression. Thus we purpose miR-208b and miR-499a-5p regulation of *IFNL3* as a mechanism to explain why *IFNL3* genotype associates with natural and IFN-α induced clearance of HCV. Collectively these reports suggest an important role for miRNA in HCV replication and immune evasion.

Anti-viral effects of host miRNAs against HCV infection

Several microRNAs have been reported to bind and degrade the HCV genome including miR-196b, miR-448, miR-199a-3p, and let-7b [33-35]. Many of these miRNAs are induced by interferon stimulation, suggesting miRNA-induced silencing of the HCV genome as an IFN-inducible anti-viral mechanism [35]. Other IFN-inducible miRNAs reduce HCV replication through indirect or yet unidentified mechanisms [17,18,36]. For example, the IFN-inducible miR-30(a-d) family is predicted to bind and degrade the suppressor of cytokine signaling (SOCS) 1 and SOCS3, which negatively regulate Jak/STAT signaling. Decreased SOCS1 and SOCS3 expression in HCV-infected hepatocytes would increase Jak/STAT signaling and the expression of anti-viral IFN-stimulated genes. While miR-30 targeting of SOCS1/3 has yet to be shown in an HCV setting, studies using a gene reporter assay have shown at least miR-30d can bind and degrade SOCS1 in HEK293T cells [37].

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Thus miRNAs reduce HCV pathogenesis by direct degradation of the HCV genome, amplifying anti-viral signaling, and many still unidentified mechanisms.

While it is clear miRNAs play an important role in the immune response against HCV and viral pathogenesis, much remains to be explored in the realm of miRNA-HCV biology. Recent works have highlighted the possibility that changes in Ago2 location or miRNA binding affinity can affect miRNA activity [38-40]. Singaravelu et al. suggest in a previous review in this journal that investigation of such modifications should be explored for their possible role in HCV pathogenesis [41]. Ago2 regulation may have a particularly pronounced effect on HCV as opposed to other viruses, since it is necessary for miR-122-supported HCV replication [41,42]. Along the same lines, post-translational modifications of Drosha and DGCR8 have been reported to control localization and activity of these proteins suggesting regulation of miRNA processing could also be altered during HCV pathogenesis (reviewed in [43]).

The possibility that HCV regulates miRNA transcription factors also remains less widely explored. It has been reported the HCV core protein represses transcription of the p53 transcription factor. p53 is responsible for many cellular processes including transcription of the miR-34 family of miRNAs, which are interestingly up-regulated during HCV infection [44,45]. While to our knowledge no study has investigated the role of HCV regulation of p53 on miRNA biogenesis during HCV infection, this example demonstrates that HCV infection may modulate miRNA expression at even the transcriptional level.

As the quality of small RNA deep sequencing and microRNA arrays continues to improve and these technologies become more widespread, we will likely identify new miRNA networks important in HCV immunity. The challenge then becomes identifying and functionally validating predicted miRNA targets. Traditional approaches include cloning the 3′ UTR of a gene downstream of a luciferase reporter gene and expressing these plasmids in the presence of synthetic miRNA mimics. While this method offers a simple measure of miRNA binding and degradation, overexpression of miRNA or the target mRNA does not recapitulate endogenous levels. Secondly, excising the coding region upstream of the 3′ UTR may alter the 3′ UTR secondary structure leading to gain or loss of an accessible miRNA binding site. One alternative, which circumvents many of these limitations, is the use of an Ago2-miRNA/RNA pulldown. In such a strategy endogenous Ago2 is immune-precipitated from whole cell lysates using antibody-conjugated beads, and then RNA is extracted from the complex and processed for RT-PCR. While limitations still exist in such a system, including the low abundance of some Ago2/miRNA/RNA complexes as well as possible differences in RNA secondary structure *in vitro* versus *in vivo* [46*,47**], the detection of endogenous Ago2/miRNA/RNA complexes is a strong indicator of miRNA regulation.

RNA-binding proteins

Several comprehensive studies identified novel RBPs targeting the HCV 3′ UTR by using RNA capture and subsequent mass spectrometry [48-50]. In the following section we will discuss some RBPs relevant for host immune responses during HCV infection.

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Pro-viral RBPs

Pro-viral RBP-mediated effects can be conferred through interaction of the RBP with the 5′ UTR of the viral RNA where the RBP acts as a cofactor to increase or stabilize translation. The human La antigen has been shown to bind various RNA structures including the 5′ UTRs of RNA transcripts from several different virus families [51-53]. Aleem Siddiqui's group demonstrated a stimulatory role of human La antigen for HCV translation by binding to the IRES element within the HCV 5′ UTR [54,55]. Another pro-viral RBP, the human embryonic-lethal abnormal vision (ELAV)-like protein, Hu antigen R (HuR), is ubiquitously expressed. siRNA knockdown studies revealed that HuR enhances HCV replication [56]. Rivas-Aravena et al. later showed that HuR exerts translational control of HCV and HIV-1 IRES elements [57]. Strikingly, while HuR mediates pro-translational effects on HCV it acts as a negative regulator during HIV-1 infection [57,58]. Similar to HuR, the proteasome alpha subunit PSMA7 is also suggested to enhance HCV translation [59]. Lastly, Vogt and others identified the host lipid droplet-binding protein tail-interacting protein 47 (TIP47) as a critical co-factor facilitating HCV RNA replication [60]. TIP47 interacts with HCV NS3, NS5A, NS5B proteins and viral RNA, and localizes to lower density lipid droplet-rich membrane fractions of HCV RNA-replicating cells. They further showed that shRNA-mediated knockdown of TIP47 in hepatoma cells results in markedly diminished propagation of full-length infectious HCV.

Another mechanism by which RBPs promote HCV replication acts *via* stabilization of viral mRNA, mainly by interaction with the 3′ UTR where RBPs compete with components of the RNA degradation machinery. In addition to its role in enhancement of HCV translation described above, HuR, together with the heterogeneous nuclear ribonucleoprotein C (hnRNP C), has also been reported to interact with the 3′ UTR of sense and antisense HCV genome [61,62]. This observation implies a dual role for some RBPs as both regulators of transcription and transcript stability.

Another important RBP during HCV infection is Staufen 1, a ubiquitously expressed protein shown to interact with cellular mRNAs to regulate trafficking, translation and degradation [63]. Two studies by Kim and colleagues demonstrated that transcript destabilization by Staufen1 is mediated by recruitment of up-frameshift suppressor protein 1 (Upf1) to AREs in the 3′ UTR of several host target mRNAs [64,65]. Interestingly, a recent publication by Blackham & McGarvey unraveled a functional role of Staufen1 in HCV replication [66]. siRNA silencing of Staufen1 led to markedly decreased levels of intracellular HCV RNA, core protein, NS3 protein as well as blunted secretion of virions. Co-localization of Staufen1 with NS3 protein and dsRNA intermediates suggests that Staufen1 has an active role in HCV replication complexes, perhaps by enabling efficient replication, translation or trafficking of the viral RNA genome.

RNAi presents a major mechanism of host anti-viral response. During the last decade many studies focused on the role of host encoded siRNAs during HCV infection as well as on the potential use of synthetic siRNAs for HCV therapy (reviewed in [67]). As a consequence, several reports identified viral RBPs that have pro-viral functions. The HCV envelope protein 2 (E2) has been shown to interact with the RISC family member Ago2 hence

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suppressing RNAi [68]. Furthermore, there are two studies proving downregulation of RNAi *via* direct interaction of HCV core protein with Dicer, which processes host siRNA in addition to miRNA [69,70]. In addition to its original function in siRNA processing, Dicer has been identified to directly interfere with HCV replication by targeting and digesting the HCV IRES element and replicative intermediates. The HCV core protein in turn was shown to counteract the anti-viral activity of Dicer by direct binding to it [70]. This HCV core-associated inhibition of RNAi is specific for endogenously encoded siRNAs and their processing by Dicer as it does not affect RNAi mediated by exogenous siRNAs [69].

Acute HCV infection triggers a strong innate immune response within the first days following infection, including induction of type I and III IFN. HCV in turn evolved sophisticated immune evasion mechanisms to subvert the host IFN responses (reviewed in [71]). Many cytokines, including but not limited to *IL-2*, *IL-6*, *TNFA*, *IFNA* and *IFNG* contain AREs in their 3′ UTRs (reviewed in [72]). So far little is known about the role of RBPs in type I and III IFN responses to HCV infection. Studies by Tom Maniatis' group and by Pasté et al. confirmed presence of an ARE in the *IFNB* 3′ UTR and destabilization of *IFNB* transcript through this ARE [73-75]. Just recently, HuR has been shown to be the predominant RBP targeting *IFNB* through its ARE [76].

Anti-viral RBPs

There are only a few reports available that suggest anti-viral functions of RBPs in the context of HCV infection and post-transcriptional regulation of immune responses. Just recently, a study by Takashi Fujita's group uncovered a positive regulatory effect of the PUF family proteins Pumilio 1 and 2 (PUM1 & PUM2) on IFN-β expression in a Newcastle disease virus infection model [77*]. PUM1 and PUM2 specifically interact with laboratory of genetics and physiology 2 (LGP2), but not with retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (MDA5), to enhance LGP2 binding to dsRNA by a yet unknown mechanism. Formation of a ternary PUM1/2-LGP2-dsRNA complex was not observed. Most likely, PUM1 and PUM2 act as recruiting factors that enhance binding affinity of LGP2 to dsRNA and thereby facilitate RIG-I-like receptor (RLR) signaling through interaction of LGP2 with RIG-I and MDA5.

Xin et al. showed that poly(rC) binding protein (PCBP2) enhances anti-viral activity of IFN-α against HCV *via* interaction with the 3′ UTR of *STAT1* and *STAT2* mRNA [78]. Similarly, recent work by Bidet and co-workers uncovered novel RBPs that exert anti-viral activity during dengue virus serotype 2 (DENV-2) infection, a family member of the flaviviruses [79*]. Three ubiquitous host RBPs (Ras GTPase-activating protein-binding proteins 1 and 2 [G3BP1, G3BP2] and cell cycle-associated protein 1 [CAPRIN1]) act as co-factors for efficient IFN-β mediated anti-viral immune response by specific activation of anti-viral ISG mRNA translation. Deficiency of those RBPs leads to blunted anti-viral activity upon IFN-β pre-treatment and results increased DENV-2 propagation. Non-coding DENV-2 subgenomic flaviviral RNA (sfRNA) in turn subverts the anti-viral function of G3BP1, G3BP2 and CAPRIN1 by direct binding.

Furthermore, HCV RNA editing by the IFN-α inducible adenosine deaminase acting on RNA (ADAR1) has been reported as an efficient mechanism to eliminate HCV RNA from

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the cell [80]. ADAR1 converts adenosine to inosine in targeted dsRNA, which in turn undergoes rapid decay mediated by a specific RNAse. Besides its deaminase activity ADAR1 also recruits HuR to mRNA in an ARE-dependent manner [81]. It is tempting to speculate that ADAR1 might also be involved in recruitment of other RBPs to HCV RNA.

We have recently unraveled the molecular mechanism by which a functional polymorphism in the 3′ UTR of *IFNL3* mRNA dictates transcript stability and how this is associated with outcome of HCV infection [12]. We observed that the unfavorable T/T genotype (associated with HCV persistence) confers a markedly decreased stability of *IFNL3* mRNA compared to the favorable G/G genotype (associated with HCV clearance) even in the absence of miRNA inhibition. The destabilization of *IFNL3* mRNA depends on three AREs in the *IFNL3* 3′ UTR. However, the RBP responsible for AMD is yet to be defined.

Long non-coding (lnc) RNAs

The role of lncRNAs in post-transcriptional gene regulation represents a largely unexplored realm of RNA regulatory activity. Cytoplasmic lncRNAs may participate in the post-transcriptional layer of gene regulation through a variety of mechanisms which include lncRNA-miRNA, lncRNA-mRNA interactions and modulation of RBP activity (reviewed in [82]). lncRNAs are capable of controlling miRNA-mediated gene repression by competing with target mRNAs for miRNA binding or by masking miRNA binding sites on target mRNAs through sequence complementarity. Similar to the activity of miRNAs, lncRNAs are capable of modulating target mRNA stability through recruitment of RBPs, as well as disrupting specific or global mRNA translation. lncRNAs may also generate miRNAs and indirectly modify post-transcriptional processes by regulating expression of key elements of the miRNA processing machinery.

To date, a number of lncRNAs have been implicated in hepatocyte cellular activity and in the function of immune responses to viral infection. Several examples have demonstrated the potential of lncRNA to act at the post-transcriptional level as competing endogenous RNA (ceRNA) to alter protein production of target genes containing similar miRNA binding sites by acting as a sponge for regulatory small (smRNAs) targeting these genes (reviewed in [83]). In hepatocytes, the lncRNA *HULC* is upregulated by hepatitis B virus (HBV) infection and is highly expressed in hepatocellular carcinoma (HCC) tissue [84,85]. cAMP response element-binding protein (CREB)-directed transcription of *HULC* generates transcripts, which act as a ceRNA sponge for miR-372, relieving its translational repression of the protein kinase A (PKA) catalytic subunit PRKACB and enhancing PKA signaling. In a possibly tumorigenic positive feedback loop, increased PKA signaling enhances CREB activity, driving further *HULC* expression and replacement of transcripts decayed through miR-372 binding [86]. Additionally, *HULC* is subjected to further destabilization by RNA-BPs insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) and CCR4-NOT transcription complex subunit 1 (CNOT1) [87]. While the role of *HULC* during the hepatocyte innate immune response to HCV has not been explored, there is evidence indicating that other lncRNAs may act as positive or negative regulators of the hepatocyte IFN response during infection. Several studies have profiled lncRNA expression signatures in response to virus infection in various tissues and found marked differences, many of

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which overlap with IFN-inducibility [88-95]. A number of lncRNAs identified in such studies have demonstrated transcriptional roles in immune function which benefit or inhibit viral infection, but the modulation of antiviral cellular activities by virus or IFN-inducible lncRNAs through post-transcriptional means remains generally unexplored. The lncRNA *NRAV* was recently shown to be a potent transcriptional negative regulator of innate antiviral responses and found to be downregulated during infection with RNA and DNA viruses [94**]. Conversely, several studies have identified IFN-inducible host lncRNAs which are upregulated in HCV-infected liver tissue, including lncRNA-*CMPK2* [92**], ISR2 and ISR8 [93*], BISPR and lncISG15 [96*], each of which appear to play transcriptional roles in the regulation of IFN responses. lncRNA-*CMPK2* is an IFN-inducible transcript which negatively regulates the antiviral activity of IFN via decreased ISG expression. Silencing of lncRNA-*CMPK2* in hepatocytes resulted in decreased HCV replication, illustrating the potential of lncRNA-targeted therapeutic interventions to limit viral infection. The advantage of lncRNA-*CMPK2* expression to HCV replication also points to the likelihood that both virus-induced endogenous and virus-encoded lncRNAs function in the evasion of host responses. Precedent for functional cytoplasmic virus-encoded lncRNAs is found in several examples (reviewed in [97,98]). The human cytomegalovirus (CMV) RNA β2.7 was found to directly bind a host mitochondrial protein and limit apoptosis [99]. Among flaviviruses, the 3′ UTR-derived sfRNA has a stable secondary structure, which prevents its degradation by exonucleases and it has been shown to promote WNV replication and pathogenicity by evading the type I IFN response [100,101]. Similarly, DENV-2 sfRNA has been shown to block type I IFN mediated antiviral responses (see above, [79*]). HCV infection results in production of viral small (vs)RNAs in the cytoplasm, and while they could be a form of RNAi [102], the functional roles of HCV-encoded vsRNAs and lncRNAs have yet to be described.

The role of lncRNAs as post-transcriptionally acting regulatory factors has become increasingly appreciated in recent years. However, optimal approaches to identify novel lncRNAs and to investigate their roles in post-transcriptional gene regulation are still being developed. Immunoprecipitation experiments of RBPs or Ago2 will help to elucidate possible sequestration of RBPs and miRISC, respectively, by lncRNAs, thereby revealing their roles as molecular sinks. In addition, due to their relatively large length lncRNAs can form complex secondary RNA structures, which determine their function as post-transcriptional modulators. It is key to establish a correlation between lncRNA structure and respective function in order to obtain a detailed understanding of their mode(s) of post-transcriptional regulation. Given the novelty of lncRNAs, complexity of post-transcriptional processes and the inherent challenges in these studies, much remains to be learned about the post-transcriptional roles of lncRNAs in innate immune responses to HCV infection.

Conclusion and challenges ahead

The interaction of various host and viral-induced post-transcriptional regulatory elements shape the immune response to HCV infection. Intracellular host components target virus replication, hence blunting virus propagation, while at the same time HCV co-opts some of those elements for its own purpose to inhibit efficient mounting of an immune response. Unambiguous identification of novel post-transcriptional regulatory elements affecting this

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complex host-virus interplay and a detailed understanding of their mechanism(s) will be crucial to identify potential drug targets, develop new therapeutic approaches, and design genotype-specific, custom-tailored patient treatment. Recent advances in deep-sequencing based approaches like HITS-CLIP will help to reveal novel RNA-RBP interactions during HCV infection. Additionally, alternative polyadenylation generates isoforms of a single gene transcript, eventually leading to expression of mRNAs with different coding sequences, altered 3′ UTR, and thus distinct composition of post-transcriptionally acting elements. However, how such transcript variants of host anti-viral genes affect the immune response to HCV and whether the virus potentially manipulates 3′ UTR compositions remains elusive. The use of RNA-seq technology will shed more light on putative HCV-induced alternative polyadenylation as well as on the presence of novel miRNAs and their possible target sites within 3′ UTRs. A complex regulatory network of factors involved in this post-transcriptional "regulosome" that act synergistically or antagonistically in a pro- and anti-viral manner has co-evolved. However, the current state of knowledge about the regulatory elements involved herein and their specific mode(s) of action appears to be just the tip of the iceberg.

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Highlights

- Interplay of post-transcriptional elements affects immune response to HCV
- HCV co-opts host post-transcriptional elements for its propagation
- lncRNAs as novel regulators of host immune responses