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*Review*

# A Balancing Act: The Viral-Host Battle over RBPs

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**Abstract:** A defining feature of a productive infection is the co-opting of host cell resources for viral replication. Despite their repertoire of molecular functions, viruses subvert host defenses to take control of cellular factors such as RNA binding proteins (RBPs). RBPs are involved in virtually all steps of mRNA life forming ribonucleoprotein complexes (mRNPs) in a highly ordered and regulated process to control RNA fate and stability in the cell. Thus, the hallmark of this viral takeover is to reshape RNA fate in the cell to modulate host gene expression and evade immune responses. Here we provide an extensive review of work in this area, particularly how the host-viral interplay influences RBP functions to modulate the host cell. Overall, in this review, we highlight the myriad of ways RBPs can regulate RNA stability in either a pro-viral or antiviral manner by gathering novel insights gained from research studies in this field.

**Keywords:** RNA binding proteins; protein-RNA interactions; RNA decay; RNA granules; viruses

## 1. Introduction

Viruses rely on intricate hijacking mechanisms to seize control of the host gene expression machinery for their own replication. Given their important regulatory roles in processes such as splicing, stability, localization, degradation, export, and translation, RNA binding proteins (RBPs) are at the center of this battle to control the gene expression resources [1–3]. Ribonucleoprotein complexes (RNPs) are assembled on mRNA, shaping the fate of transcripts and are recruited via structural elements, sequence motifs, or sequence-independent secondary and tertiary structures [1,4]. Thus, the mRNA-protein interface can vary widely from forming stable RNP complexes to transient interactions. In infected cells, this is further complicated by the presence of viral proteins that may disrupt any number of these post-transcriptional processes. For instance, localization of RBPs to different subcellular compartments has been shown to be highly hijacked by viruses in order to control host gene expression and reprogram the cell machinery towards viral transcripts [5–7]. Likewise, inhibition of 3' end processing by viruses was shown to lead to production of read-through transcripts and failed transcription termination which can affect the repertoire of available mRNAs in the cell and therefore the extent of the host anti-viral defense arsenal [8,9]. Other mechanisms have also been shown to contribute to the viral-host interplay, such as manipulation of nuclear export to allow retention of host mRNAs in the nucleus leading to their targeted degradation [10,11] and therefore freeing up the translational machinery for viral mRNA processing [11,12]. Furthermore, the highly intricate functionality of membrane-less organelles containing RNAs and RBPs are formed as compartmentalized densities for host post-transcriptional regulation or as viral replication factories [13–15]. While an extensive amount of work in the field has shown the importance of RBPs in the regulation of gene expression, and as a particular crux of targeting during viral infection[5,6,16,17], one important gap remains to be explored: how can the same RNP complexes be pro-viral in some context while anti-viral in others? Studies portraying the impact of RNA-protein interactions during viral infection are an exciting avenue of research and will likely yield important information on transcript regulation both in pathogenic and non-pathogenic contexts. In this review we will explore the complex interplay between RNA and proteins and their role(s) in RNA stability during viral infections, shedding light on new avenues to explore RNA fate and RBP modulation during viral infections in the future.

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## 2. RNA Elements and Viral Infection

To rapidly gain access to their host translational resources, certain viruses induce broad RNA degradation events orchestrated directly by their own viral endonucleases. These enzymes cleave processed transcripts, creating unprotected 5′ and 3′ ends that become targets for host exonucleases. Interestingly, recent work has begun to reveal RNA elements that are incorporated into select mRNAs that can drastically impact RNA stability in the face of these viral-induced decay events. Some of these RNA elements serve as target sites that recruit viral nucleases, while others can provide protective measures against viral-induced decay. For example, during Kaposi Sarcoma Associated Herpesvirus (KSHV) lytic replication, the virally encoded endonuclease SOX causes widespread decay of the mRNA transcriptome. However, a fraction of the transcriptome is spared from SOX-mediated degradation which likely ensures cell viability and proper progression viral replication. Thus, it appears that SOX targets are carefully selected and in fact, the Gaglia group has recently mapped SOX targeting motif that is preferentially used to during KSHV infection [18]. This targeting motif does not rely solely on sequence but rather on several key conserved residues alongside a potentially conserved structural motif [18,19]. Similarly, during Influenza A infection, the frameshift product PA-X functions as an endonuclease and contributes to the global viral-induced host shutoff. PA-X activity results in this decay event to free host resources for viral replication and negatively alter host defenses, but how does PA-X specifically locate the right transcripts to degrade? Through 5′ rapid amplification of complementary DNA ends coupled to high-throughput sequencing and predictions of RNA structures, Gaucherand et al. determined that PA-X targets GCUG tetramers in hairpin loops within transcripts for cleavage [20]. This specific element is widely abundant in the host genome but not in the viral genome, thus serving as a highly specific target to specifically differentiate host mRNAs [21]. It therefore emerges that using viral nucleases with a wide range of host transcripts is an effective way for viruses to remodel their host cell environment and that the careful picking of the nucleases targets is a key step in this process. The question then arises of whether the host fights back against this takeover and whether certain RNA elements could instead potentially restrict viral nuclease targeting? This is in fact what has been observed in the context of KSHV infection: the transcript for Interleukin-6 (IL-6) encodes an RNA element within its 3′ UTR shown to provide protection from SOX [22,23]. This RNA element of about 200nt was termed the Sox Resistant Element (SRE) [23]. This SRE appears to fold into a stemloop structure that likely served as a scaffold for recruiting RBPs and modulate the susceptibility to the viral endonuclease [23]. Several RBPs were identified as specifically binding this RNA element and together form a protective RNP. Intriguingly, this protective element appears to dominate over SOX targeting element, as a transcript that contains both the targeting and the protective elements will remain unaffected by SOX. This highlights that the fate of an mRNA and its stability in the face of viral infection is the result of a complex balance of its RBP landscape. This RNP environment on individual transcripts may serve as specific marker in the context of viral-induced decay and provide clues to understand how viruses can distinguish between host and viral genes.

## 3. Host - Viral RBP role during viral infections

Through evolutionary pressure and the imperative to fit through their host translational machinery, viruses have often found ways to mimic features of the host to hijack resources and facilitate their escape from the immune system [24–26]. By mimicking host features, especially on their transcripts, viruses also facilitate the hijacking of the RBP of the host and thus create RNP complexes that are indistinguishable from host RNP. For instance, during Dengue Virus (DENV) infection, the Receptor for activated C kinase 1 (RACK1) - a core component of the 40S ribosomal subunit- was shown to interact with host factors SERBP1 and Vigilin to promote viral replication [27]. The authors denoted RACK1 as a platform to bind and recruit SERBP1 and Vigilin to the 40S ribosomal subunit to then collectively connect viral RNAs to the translation machinery to facilitate DENV infection. Furthermore, Diosa-Toro and colleagues observed the host factor Y box binding protein 1 (YBX1) interaction with the structural protein E and the viral protein capsid C to facilitate viral assembly and egress in DENV [28]. These studies, among others, ascertain the role of cellular

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factors to aid processes that would promote viral replication and infection progression in the host cell [29–31].

However, not all cellular RBP favor viral replication, and mimicking host mRNA can be a double edge sword as some RBP can have potent antiviral effects. The host factor Shiftless (SHFL) for example is an RBP that has been shown to restrict expression of a number of viruses through its interaction with viral RNA, from inhibiting HIV frameshifts to manipulating cytoplasmic RNP granules [17,32,33]. Another example is the RNA binding protein polypyrimidine tract-binding protein 1 (PTBP1) which was shown by Qin et al. to target and degrade the viral nucleocapsid (N) protein by activating the MARCH8-NDP52 autophagosome pathway in Porcine epidemic diarrhea virus (PEDV) [34]. Moreover, in this study PTBP1 was also shown to activate type I interferon (IFN) pathway inhibiting PEDV replication [34].

And inevitably, some RBPs can have both pro or anti-viral roles depending on the context: for example, the RNA binding protein Ras GTPase-activating protein binding protein 1 (G3BP1) is known to mediate interactions with innate immune signaling molecules (RIG-I, cGAS, Caprin1) to facilitate the activation of immune pathways to counteract viral infections [7,35–38]. But G3BP1 can also be targeted to favor viral replication by different viruses [7,39] such as during ZIKV infection where G3BP1 is sequestered to disrupt stress granule formation and promote infection [39]. Collectively, these studies illustrate the profound impact cellular RBPs can have on the outcome of an infection. Interestingly, we can further ponder on the mechanisms by which cellular factors might discriminate between an RNA as self or non-self to exert its effects. Yet, we must take into consideration that the notion of what we try to designate as a "host specific RBP" and "viral specific RBP" can become a little obscure to define. Considering that cellular factors can reveal functionalities that are not restricted to viral RNAs or cellular RNAs but merely part of both. More studies in this area are needed to continue to elucidate the mechanisms by which cellular factors modulate processes in a pro-viral or antiviral way during viral infections.

## 4. RBP modulation of RNA stability in subcellular localizations during viral infections

The physical separation between the processes of transcription and translation enables RNA-Protein complexes (RNPs) to adapt and diversify throughout the life of an mRNA contingent on its subcellular localization. However, during viral infections, viral RNAs (vRNAs) compete with cellular RNAs to take over resources from the cell. This can lead to the disruption of common RBPs function and localization between cellular compartments or their reallocation towards vRNAs. To date, several studies have investigated many of the mechanisms in which RBPs modulate key pathways in response to environmental changes, particularly during viral infection [40–43]. In this section, we will review how RBPs regulate RNA stability between cell compartments during viral infections.

### *4.1. Nuclear and Cytoplasmic Regulation*

RNA fate is highly controlled in the cell and RBPs are at the core of the complex process that regulates targeting between cellular compartments. In particular, in the context of viral infection where resources are re-allocated towards viral needs, the dynamic process of nuclear-cytoplasmic shuttling can be drastically altered. For example, in the context of gamma-herpesvirus infection where host shutoff takes the form of widespread mRNA decay, many RBP find themselves without an RNA target. Hence this massive RNA decay results in a broad scale relocalization of RBPs, many of them finding their way back to the nucleus. This thus creates a measurable "sensing" of this large-scale RNA decay in the cytoplasm, and the message can be relayed to the nucleus through the shuttling of RBPs. It was shown that this creates a feedback mechanism, akin to informing the transcription machinery of the state of RNA decay in the cytoplasm, and resulting in a halt of host transcription. Taken together, studies uncovered an intriguing interconnection between transcription rates in the nucleus and the stability of mRNAs in the cytoplasm [44–47]. Therefore, trafficking of RBPs between cellular compartments during events of stress such as viral-induced RNA decay, could be fundamental to relaying information about cellular mRNA abundance within the cell [44,47,48]. Several studies have detailed the modulation of RBPs during stress and between cellular

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compartments [48–51]. Differences on compartmentalization and functionality are observed primarily dependent on the pathway; compartment specific, shuttling compartments, RNA abundance or stress-regulation [49]. Interestingly, from these studies we can further inquire if host mRNA availability is reduced during targeted viral host mRNA degradation; to what extent protein abundance levels are regulated in subcellular compartments to somehow maintain "homeostasis" when re-localization of RBPs occur to different compartments? And to what extent does the cellular feedback response toward viral targeted RNA influence viral replication?

Another study by Garcia-Moreno and colleagues observed similar dynamics of RBP response reflecting RNA availability during Sindbis Virus (SINV) infection. The authors used a system wide approach known as RNA-interactome capture (RIC) to determine the distribution of RBPs in cells infected with SINV. In the context of high loss of cellular RNAs and concomitant high levels of viral RNAs, they observed a remodeling of the RBP interactome. In particular, host cellular RBPs EIFD3 (cap binding protein) SRPK1(alternative splicing, RNA export and stability), IF16 (enhances RIG-I transcription and activation), IFT5 (antiviral response), TRIM25, TRIM56 (E3 ubiquitin ligase), PPIA (regulation, signaling, apoptosis), HSP90AB1 (RNP remodeling), RTCB, DDX1 and FAM98A (form the tRNA ligase complex) were re-localized to viral factories [29]. Moreover, another affected RBP, GEMIN5, which usually localized to both the nucleus and cytoplasm mediating the assembly of small nuclear RNPs (snRNPs) was re-localized to viral factories and co-localizes with SINV RNA [29].

From these studies and others, we can perceive the battle between host and viruses to take control of cellular gene expression. Notably, RBP function and localization appears to be pivotal during viral infections to co-opt cellular processes. Many questions continue to mount surrounding the mechanisms that regulate the shuttling of RBPs during viral infection, especially during viral-induced RNA decay. With the advent of novel protein labeling methods, we anticipate that we will learn more about the dynamics of these processes in the coming years.

### *4.2. Nuclear Export*

Messenger RNAs (mRNAs) undergo extensive processing before their export from the nucleus to the cytoplasm. Once mature, mRNAs are transported to the cytoplasm through nuclear pore complexes (NPCs) embedded in the nuclear envelope [10,11,52,53]. Unsurprisingly, transport of mRNAs is mediated by mRNP complexes composed of shuttling proteins. Commonly, the transcriptional export (TREX) complex binds mRNAs and recruits other export factors such as the THO complex, UAP56 (also known as HEL), and ALY/REF (also known as THOC4). Subsequently, the ALY/REF complex then interacts with nuclear export factor 1 (NXF1 also known as TAP)-Ntf2 like export factor 1(NXT1 also known as TAP-p15) among other factors to facilitate export of mRNAs from the nucleus to the cytoplasm [11]. NXF1 is known to assist in the export of bulk mRNA, while a subset of transcripts (including unspliced and partially spliced), are exported via the chromosomal region maintenance 1 (CRM1 also known as XPO1) [10]. Interestingly, several studies have shown how viruses target the export machinery to cause a reduction in host gene expression and downregulation of host antiviral responses [11,54–56]. For instance, in Hepatitis B Virus (HBV), the viral core protein HBc contains an NLS sequence and an arginine rich domain (ARD) that allows its physical interaction with NFX1 [57]. This interaction between HBc and NFX1 is suggested to mediate the shuttling of HBc between cellular compartments (Figure 1) and facilitate the export of HBV transcripts [57]. Furthermore, Gong and colleagues in their study identified the interaction of the KSHV encoded ORF10 protein with nuclear export proteins Rae1 and Nup98. This interaction blocked mRNA export leading to nuclear accumulation of transcripts [56]. The accumulation of mRNAs and inhibition of export serves to downregulate expression of host proteins and ensure viral access to the translational machinery [56,58]. Meanwhile, in Murine Leukaemia Virus (MLV) both nuclear export routes (NFX1 and CRM1) are proposed to be exploited by the virus [59]. Mougel *et al.*, show in their study the interaction between MLV full length RNA and NFX1 allowing export of viral RNAs to further load factors for translation initiation. Simultaneously, in their study export by CRM1 marked MLV FL RNAs for viral packaging in the cytoplasm [59]. The use of these two export pathways by MLV highlights the ability of the viral RNA to assemble two different mRNP complexes

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and control RNA fate. Strikingly, the nature of an mRNP complex could be changed by the strategies viruses use to exploit cellular pathways such as nuclear export. Ongoing and future studies should continue to elucidate the processes that regulate the fate of viral and cellular mRNAs in these pathways.

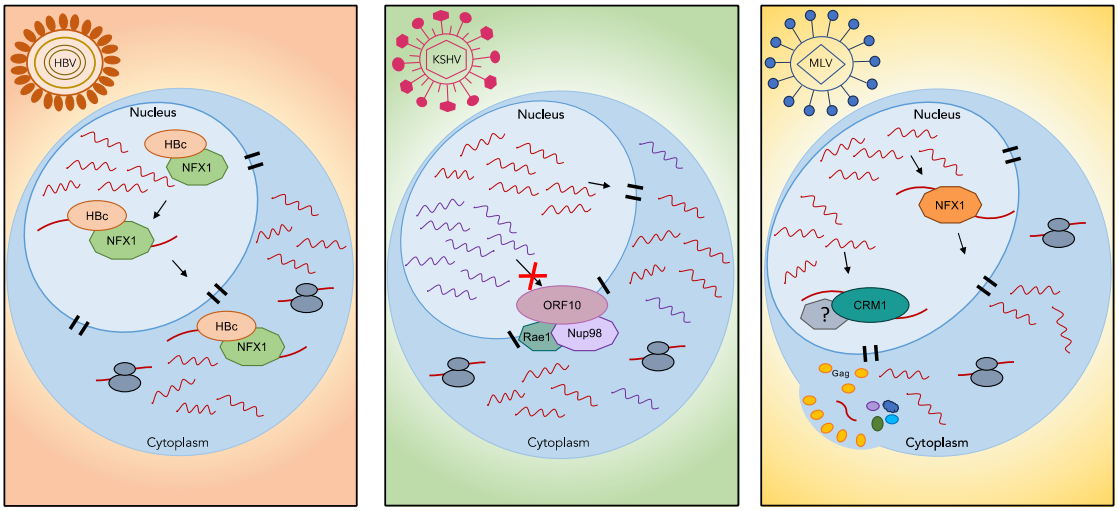


**Fig 1. Viral Manipulation of Nuclear Export Pathways: A.** HBV viral protein HBc binds export proteins NFX1-TAP to facilitate export of viral transcripts (red). **B.** KSHV viral protein OR10 binds export proteins NFX1-NXT1 to block export of cellular transcripts (purple) promoting export of viral transcripts (red). **C.** MLV viral transcripts interact with NFX1 protein to promote their transport from the nucleus into the translation machinery, while other viral transcripts interact with CRM1 which marks them for further viral packaging.

## 5. RNA Granules: A Nexus in the Viral-Host Struggle over RNA

RNA granules are biomolecular condensates of RNA and protein that exist within the cytoplasm. Though these densities are often described to undergo liquid-liquid phase separation (LLPS), the story is more complex. The description of LLPS helps to better illustrate what these foci are: sequestered conglomerates of biomolecules that are separated from the surrounding cytoplasmic matrix without any phospholipid barrier. However, the distinction can be quite gray in such a dynamic system; granules readily exchange materials with their surroundings [60–62]. Granules are not simply defined as viscous liquids but more precisely viscoelastic densities [63–68]. This distinction implies a duality and fluctuation of solid and liquid elements, which helps to better highlight how these subcellular compartments themselves are multifarious along with their functionality. Two classifications of cytoplasmic granules have emerged as important regulatory mechanisms within cells and both heavily rely on complex and dynamic interactions between RBP and RNA: Processing bodies (P-bodies) and Stress Granules (SGs). P-bodies are often comprised of mRNA and RBPs such as DDX6, ET-4, PAT1B, LSM14A, EDC4, DCP1/DCP2, and CPEB1 [69–80]. Along with ribonucleic acids, SGs often contain proteins such as G3BP1/G3BP2, TIA-1, TIAR, Atx2, eIF2, eIF3, eIF4A, eIF4B, eIF4E, eIF4G, and eIF5 [80–88]. Some granules, such as P-bodies, are constitutive, where treatment with a translation elongation inhibitor like cycloheximide will lead to their cytoplasmic loss [69,89,90]. Conversely, Stress Granules must be induced by treatment with a translation terminator such as puromycin [89,91] or via cellular stressors like viral agents [13]. Due to their dynamic nature, diversity in composition, and temporal/environmental sensitivity, RNA granules' functionality varies broadly from mRNA storage, degradation, triage to assisting in cellular processes such as energetic conservation, the stress response, or the inflammatory response. Of note, this multitude of processes can readily be taken advantage of by viruses. Battle wages between virus and host over RNA granules' RBPs to ultimately influence gene expression and arrest pathways, specifically over the control of RNAs.

### *5.1. Viral factors exercise control over P-body RBPs to promote viral replication*

Processing bodies are thought to be sites of RNA metabolism within the cytoplasm of cells. P-bodies have been implicated in mRNA decay, translation repression, nonsense mediated decay

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(NMD), as well as mRNA silencing [76,92–97]. They have commonly been identified as sites of mRNA decay due several constituents' ties to transcript degradation, namely DDX6, DCP1, DCP2, Xrn1, EDC3, EDC4, among others [69–80]. However, they can also serve as compartments to temporarily sequester some transcripts from the translation pool to control pathway activation/deactivation or for energetic conservation. P-bodies are thus prime targets for viruses that need to co-opt these pathways for their own replication. It has previously been shown that viruses such as KSHV, HCV, and WNV all utilize P-body constituents such as DDX6, Lsm-1, DDX3, Ago2, Xrn1 and others to promote viral RNA and protein stability [5,98–105]. Recently, investigation of Enterovirus 71 shows a similar strategy. This single stranded, positive sense RNA virus encodes for two proteases (2A and 3C) that help to cleave the viral polyprotein into 11 smaller proteins; however, some have speculated that these enzymes may be multifunctional especially during infection. Early characterization demonstrated that EV71 induces a loss of P-bodies within human cells; over the course of infection P-body microscopy and quantification revealed fewer foci that were generally smaller in size [106]. Using sodium arsenite (NaAs), an oxidative stress agent known to induce P-body formation, the researchers observed a significantly reduced amount of these granules within viral infected cells. Thus, Fan et al. were able to determine that EV71 blocks the formation of *de novo* P-bodies, where not even an extreme oxidative stressor can lead to their restoration. The group investigated whether the viral proteases played a role in this cytoplasmic granule loss. They ultimately unveiled that protease 2C leads to P-body loss and non-functional 2C has no effect on the granules [106]. Simultaneously, Fan et al. determined that certain scaffold proteins of P-bodies affected viral mRNA transcript levels. The absence of DDX6 and 4E-T lead to decreased viral transcript levels and viral mRNAs were elevated. Thus, they hypothesized that virally induced P-body loss reallocated and repurposed key P-body components for RNA stability and expression. During infection, a DDX6-VP1 (viral capsid protein) interaction was discovered and found to be facilitated by viral RNA; however, this interaction was lost in the absence of protease 2C [106]. The group's final model highlighted that EV71's protease 2C facilitates host RBP interactions with viral mRNA to promote viral gene expression, which ultimately leads to the blockage in formation of P-bodies. It emerges that viruses are masters at seizing control of P-bodies through their interactions with host RBPs and use them to promote virion production. Indirectly, this also likely suppresses host RNA expression and regulation which further remodels the host environment to be more favorable for viruses.

### *5.2. Hosts Manipulate P-body RBPs to Alter RNA Availability/Degradation to Combat Viral Infection*

As previously mentioned, P-bodies are constitutively present RNA granules thought to exist for the purposes of RNA stasis along with some degradation capabilities. It has been widely reported that P-bodies often house transcripts with AU-rich elements (AREs) in their 3' UTR for the purposes of degradation or translational repression [107–112] . Many ARE-bearing transcripts encode for chemokines and cytokines [113,114]. Several groups have demonstrated that following P-body loss, an increase in ARE-mRNAs follows [107–112]. These results suggest that decreases in P-body counts may in fact play an antiviral role with the increased expression of these effectors. To what extent does host and virus individually contribute to the loss of P-bodies during infection remains unclear. Are P-bodies altered to stimulate the host inflammatory response to mount an anti-viral response or to enhance environmental favorability for viral agents? A host RBP that we mentioned previously in this review - Shiftless (SHFL)- is a broad acting and potent antiviral factor [17,32,115–120] with known roles in regulating cytoplasmic granules. In the context of KSHV, SHFL appears to stringently restricts P-body foci [17]. The exact subcellular mechanism through which SHFL accomplishes this P-body loss remains unclear. SHFL localizes to P-bodies [121] and its overexpression causes their downregulation [17]. SHFL-mediated downregulation of P-bodies could subsequently alter the expression levels of certain ARE-mRNAs that are often included in these granules which likely contribute to SHFL overall ability to restrict viral agents. Outside of the context of infection, cellular loss of P-bodies typically occurs through two methods: 1. depletion of core protein components, or 2. expression/phosphorylation of the 68-amino acid microprotein: non-annotated P-body dissociating polypeptide (or "NBDY"). Loss of certain proteins such as LSm14a [71,75], DDX6 [73,75], or EDC4

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have all been shown to lead to losses in P-bodies [70]. Otherwise, the expression and phosphorylation of the polypeptide NBDY has been shown to cause the loss of P-bodies, by potentially disrupting the electrostatic networks of these granules [122,123]. These cellular methods of P-body loss likely led to a translational increase in certain mRNAs. However, it has also been posited that this P-body regulation may also release many of these decapping and endonucleolytic enzymes leading to a translation suppression [124]. Therefore, it is possible that during viral infection, the host attempts to control the cytoplasmic gene expression environment by disassembling P-bodies. Viral agents often seek control over P-body RBPs as a tool to influence RNA expression within the cell. All the while, the host attempts to antagonize viral replication through a similar method: utilizing P-body associated RBPs to alter RNA expression for anti-viral pathways.

### *5.3. Viruses Influence Stress Granule RBPs to Suppress Host Immune Response Transcripts and Positively Regulate Viral RNA Fate*

Stress granules (SGs) are thought to be cytoplasmic sites of translational regulation, where specific functionality can range from suppression of cell death pathways [125,126] to serving as a platform for an IFN response activation [127] to viral transcript sequestration foci [127–129]. It has been well characterized that SGs form upon viral infection, and these cytoplasmic granules are often targeted for downregulation by viruses [130–134]. Japanese Encephalitis Virus (JEV) has been shown to alter the localization of SG marker G3BP1 [135]; Dengue virus (DENV), West Nile virus (WNV), Murine Respirovirus (SeV), and the Zika virus (ZIKV) have all been shown to utilize viral biomolecules to sequester SG critical proteins to block granule formation [6,39,136]. Recently, the betacoronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), has been shown to have a similar effect on SG abundance and the re-purposing of the foci's components. SARS-CoV2 encodes for 29 proteins, one of which is the nucleocapsid protein (NP) [137]. NP is responsible for packaging the viral RNA genome and helping virion egress from host cells [138]; it has also been found to interact with the structurally critical SG proteins G3BP1/G3BP2 [139]. Liu et al. observed that this NP-G3BP interaction limits the degree to which SGs can form in response to the viral infection, which in turn limits the host's ability to stall translation and slow viral replication. The group went on the observe that SARS-CoV2's NP sequestration of G3BPs not only resulted in the loss of SGs but also in the downregulation of IFN- β transcript levels and the RIG-I pathway (a critical piece of the innate immune response) as a whole [139]. In an uninhibited immune response system that combats viral infection, G3BP1 typically influences the RIG-I pathway, where IRF3 becomes phosphorylated ultimately causing an increase in IFN-β transcription [38]. The researchers were able to demonstrate how this virus exercises control over SG RBPs, such as G3BP1, to ultimately increase the propensity for viral mRNAs and translation. Through G3BP1 knockdown and knockout experiments, Liu et al. demonstrated that viral mRNAs were significantly elevated, identifying that NP-G3BP1 interactions lead to heightened viral replication states [38]. A genomic intermediate of the SARS-CoV2 genome appears to exist as dsDNA, which has an observable affinity for G3BP1; mechanically, this was hypothesized to be how the virus isolates this RBP from the host's antiviral system [38]. Additionally, this SG component has been shown to be necessary for both murine norovirus (MNV) replication as well as Norwalk norovirus replication (a replicon used to model human norovirus, HuNoV) [140]. Following the knockout of G3BP1 within cells, both MNV and HuNoV were observed to have significantly low transcript levels and were no longer tied to cytotoxic outcomes; cells were seen to be virally resistant [140]. Interestingly, Hosmillo et al. were able to uncover that G3BP1 RNA binding domains directly impacted viral replication; the loss of these domains resulted in less viral yield. In addition, Hosmillo and colleagues discovered that G3BP1 interacts with the viral protein VPg and helps facilitate the loading of ribosomes and polysomes onto norovirus RNA. Thus, certain viruses take advantage of SG RBPs, such as G3BP1, to promote the stability and translation of viral RNAs. SGs present another fascinating focal point for where host and virus compete over RBPs to gain an advantage over the other.

### *5.4. Host Agents Orchestrate Stress Granule RBPs to Effectively Quench Viral Replication*

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Stress Granules have been widely characterized as antiviral subcellular compartments; SG RBPs, such as G3BP1 and TIA-1, are non-traditional components of the host immune-response in the context of these granules. Functionally, SGs exist to help sequester transcripts out of the translation pool and otherwise slow protein synthesis. Liu et al. implicitly define this purpose. SARS-CoV2 must manipulate G3BP1 to improve viral replication, meaning hosts use of G3BP1 actively and effectively mutes RNA expression enough to hinder viral replication [38]. Through the knockdown of G3BP1, they observed a significant increase in viral mRNAs, again implicitly demonstrating this host RBP usefulness in gene expression control via granular formation [38]. Alternatively, the host-defense significance of SG RBPs may be seen in the case of the $\alpha$-coronavirus porcine epidemic diarrhea virus (PEDV). PEDV threatens suckling piglets globally. Following PEDV infection, SGs form and eventually are lost towards the latter stages (i.e., around 36 hours) [141]. Sun et al. identified that overexpression of G3BP1 resulted in significantly decreased levels of viral mRNA [141]. They further discovered that PEDV encodes a protease that cleaves G3BP1 in the late stages of infection which causes this loss. They were able to pinpoint the specific cleavage sites that viral caspase-8 targets down to two aspartic acid residues. Interestingly, robust SGs with cleavage resistant G3BP1 stringently limited viral transcripts as well as overall viral titers, indicating that SGs can efficiently limit viral transcription and translation. Studies like these demonstrate the capability and anti-viral functionality of SGs and how the host attempts to use RBPs like G3BP1 to restrict viral agents.

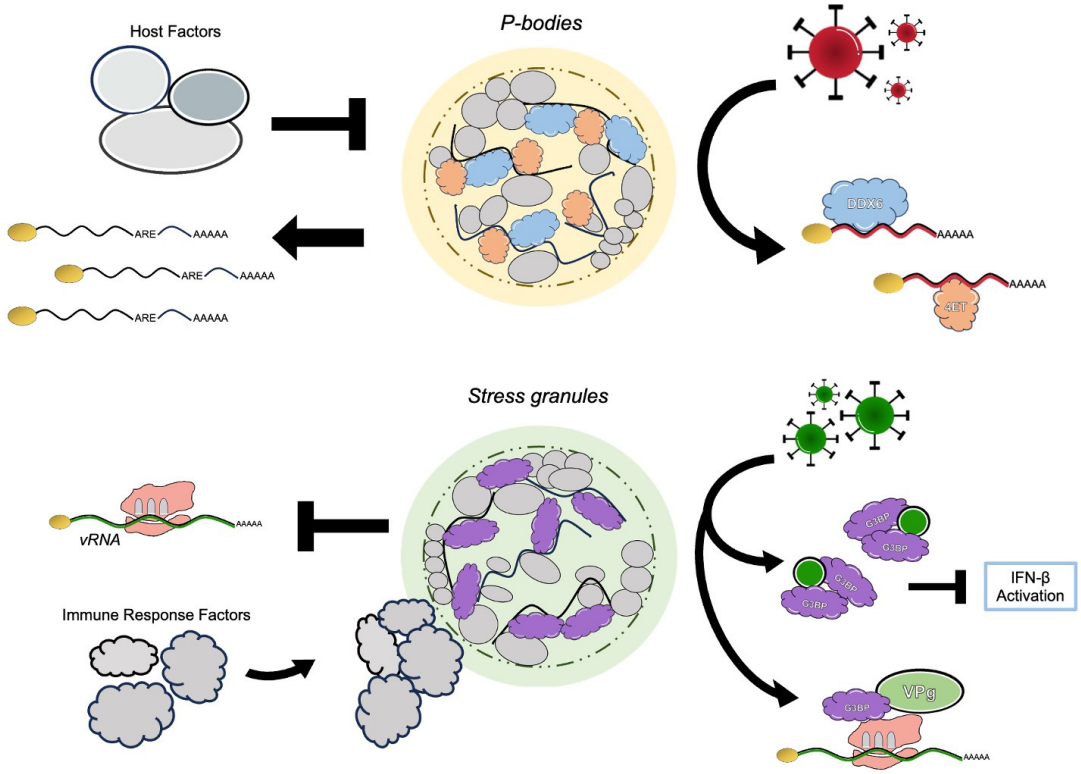


**Figure 2:** RNA Granule Functionality in the context of Viral Infection. **(1).** Host factors, such as SHFL or NBDY, result in P-body loss, which can impact the availability of ARE-mRNA transcript levels. **(2).** Viral agents like KSHV, HCV, WNV, and EV71 recruit P-body RBPs to promote viral RNA stability and translation. **(3).** Stress granule induction helps to slow viral translation, while serving as a scaffold to mount an immune response and combat infection. **(4).** Viruses, such as SARS-CoV2, MNV, and HuNoV, have been observed to re-purpose G3BP1 leading to a blockage for certain anti-viral pathways. G3BP1 may also be repurposed during infection to promote stability for viral translation complexes.

### *5.5. Granule Functionality/Affects are Convoluted; Both Virus and Host Wield SG RBPs for Their Own Benefit*

The above sections detailing the great virus and host struggle over P-body and SG RBPs already outlines how ambiguous the functionality of granules can be. One other fascinating viral case study that depicts this murkiness can be seen in the context of Viral Hemorrhagic Septicemia Virus (VHSV); a member of Rhabdoviridae and threatens dozens of fish species globally. [142]. And appears to

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trigger the formation of Stress Granules [143]. Interestingly, G3BP1 was shown to be redistributed to viral replication complexes and prove to be essential for efficient virion production [143]. Even though viral infection results in SG formation, VHSV still influences G3BP1 to facilitate its own replication. However, SGs also seem to play an anti-viral role within the system. Though IFN output was markedly higher under G3BP1 knockdown conditions, viral mRNA levels were also significantly higher. This suggests that G3BP1 and SGs can limit viral replication to an extent, likely via translational arrest and sequestration [143]. VHSV utilizes SG RBPs such as G3BP1 to enhance replication, yet the host also exercises some control over these RBPs to simultaneously limit viral replication [143]. This case of viral infection and SG dynamics captures the convoluted subcellular environment and depicts how RNA granules exist at the axis where pro-viral and pro-host states teeter. Have viral agents robustly conquered this host defense and adapted to largely benefit from granules? Will host mechanisms adapt or can they be shaped to re-purpose the likes of P-bodies and SGs to effectively combat viral replication?

## 6. Conclusions and Future Perspectives

RNA binding proteins comprise the subcellular foundation for host survival and viral replicative strategies. While this review covers many of the most recent findings of how both sides of this conflict attempt to take advantage of these proteins, new studies continue to unveil different viral case studies and mechanisms of exploit. mRNP complexes present an exciting avenue of study to better understand viral replication and takeover. The first major component of these complexes, mRNA, directly influence gene expression and thus serve as a major target for viruses. Certain elements within transcripts can convey protection from nucleolytic activity or even help distinguish between virus and host. Proper gene expression requires fine-tuned RNA processing, and this multifaceted quality control system helps to regulate the cell homeostasis. However, it can prove to be disastrous when certain viral agents target one juncture of RNA processing and critical information is lost. The other component of these mRNP complexes, RBPs, also directly contribute to gene expression and are critical subcellular components that must be subjected to control for successful viral replication. Often these proteins are thought control stability to transcripts and even impact their localization. Certain host factors, such as NFX1 and CRM1, critically regulate nuclear egress of transcripts, which can largely impact host survival dependent upon whether virus or host control these export pathways. Interestingly, RBPs also can help regulate the dynamics of certain RNA granules, which can significantly impact gene expression. Through P-bodies and stress granules, RBPs influence mRNA sequestration, decay, and release into the translation pool.

Furthermore, from the studies reviewed here we can continue to interrogate how cellular pathways are affected by viral takeover, but also how the host responds to the viral attack. As mRNA abundance and availability can be drastically altered during viral infection. Cellular feedback pathways can potentially serve to relay stress signals during infection. This area is still ripe for further study since it is still unclear which cellular factors are involved and to what extent communication is carried out in the cell between cellular compartments. Further work to elucidate the effects on transcription and translation are also still needed. Since viral infection is an event characterized by unique mechanisms that vary from agent-to-agent; certain homologies and divergence would be expected. Moreover, more studies to discern RNA-Protein interactions when RBP expression levels fluctuate in cells during viral infections are imperative to shed light into the unknown biological significance of RBP localization to more than one cellular compartment, multifunctionality, and response to target availability. Studies in these areas could prove exceptionally fruitful, providing a deeper understanding of these processes which could lead to the discovery of new antiviral targets and the development of therapeutic agents.

Regarding the RBPs associated with RNA granules, many factors and mechanisms are yet to be discovered. Specifically, within the context of viral infection, granule RBPs and their contribution appear to be quite convoluted and dependent from virus to virus. For viral agents, such as EV71, that lead to P-body loss, it remains to be seen mechanistically how the virus utilizes and recruits RBPs like DDX6 to provide stability and promote viral gene expression. Further characterization also ought

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to be conducted on exactly how the host wields P-bodies and their biomolecules specifically to alter its own gene expression to ultimately combat viral gene expression. For stress granule RBPs, how prevalent is this viral hijacking strategy? What other viral agents have evolved mechanisms to disrupt SG formation for purposes other than a lack of transcript sequestration? Additionally, how may host defense counteract this disassembly of the widely regarded anti-viral granule type? The characterization of RNA granule functionality during viral infection has emerged as an exciting frontier for better understanding virus-host interactions at the subcellular level.

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