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Stress Granules and Virus Replication

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Summary

Viruses are dependent on the cellular translation machinery for protein synthesis. Part of the innate immune response to infection is activation of the stress kinase PKR which phosphorylates the alpha subunit of the initiation factor eIF2. This results in inhibition of translation and is intended to block virus replication. A downstream effect of translational shutoff involves the formation of cytoplasmic granules, termed stress granules (SGs), that contain mRNAs, initiation factors, ribosomal subunits, and other mRNA regulatory proteins. SGs hold mRNAs in a translationally inactive state until cells recover from stress. Recent studies have begun to elucidate the impact of SGs on virus replication. Not surprisingly, viruses from diverse families have been found to modulate SG formation in infected cells by associating with important SG effector proteins. This review describes the current knowledge on SGs and their interaction with and impact on virus replication.

Keywords

Viruses; stress granules; eIF2 α phosphorylation; translation; replication; innate immunity; cellular stress

The most highly regulated stage of protein synthesis occurs at the initiation step. The translation initiation factor eIF2 plays a crucial role in this process. In non-stressed cells, eIF2 complexed with GDP associates with eIF2B, a nucleotide exchange factor, which exchanges GDP for GTP. This exchange allows the eIF2:GTP complex to associate with initiator methionine tRNA to form ternary complex (eIF2:GTP:tRNA^{Met}). Ternary complex brings the initiating methionine to the 43S ribosome pre-initiation complex. The 43S complex contains the 40S ribosome and a variety of additional initiation factors that play important roles in translation. The 43S complex then forms the 48S ribosomal complex by binding mRNA, which has itself been activated for translation via association with additional initiation factors. The 60S ribosomal subunit is then recruited, at which point GTP is hydrolyzed to GDP and eIF2:GDP is released from the ribosomal complex to again associate with eIF2B and be recharged with GTP to start the process anew [reviewed in [1, 2]]. Following cellular stress, the alpha subunit of eIF2 is phosphorylated by one of four stress activated kinases; GCN, which is activated during nutrient starvation, PERK, which is activated following ER stress, HRI, which is activated following oxidative stress and heme deficiency, and PKR, which is activated following virus infection [reviewed in [3]].

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Phosphorylation of eIF2 α increases the affinity of eIF2B binding to eIF2:GDP, which results in eIF2B acting as an inhibitor of eIF2:GDP and preventing its association with the initiating methionine, effectively shutting down most translation initiation [4].

Stress granules: another level of translation regulation

In recent years, an additional level of mRNA translational regulation that occurs following cellular stress has been identified. Following eIF2 α phosphorylation, ternary complex becomes unavailable thereby preventing translation initiation. Elongating ribosomes run off their transcripts leading to polysome disassembly. Stalled 48S ribosomal complexes accumulate and trigger the formation of discrete foci in the cytoplasm termed stress granules (SGs). SGs contain aggregates of the stalled, translationally silent mRNAs, translation initiation factors, small ribosomal subunits, and a wide variety of other mRNA binding proteins.

The assembly and disassembly of SGs has been shown to depend on a number of cellular proteins [5-7]. T cell intracellular antigen-1 (TIA-1) and closely related homologue TIA-1 related protein (TIAR) play important roles in SG formation. TIA-1/R are members of the RNA recognition motif (RRM) family of RNA binding proteins that bind AU-rich regions in RNA and have been implicated in RNA splicing and transcript-specific mRNA silencing. In addition to their three RRM, TIA-1/R also contain glutamine-rich carboxy-terminal prion-related domains (PRD) which allow for their self-aggregation. TIA-1 knockout cells have a diminished capacity to form SGs and overexpression of the PRD acts as a dominant negative inhibitor of SG-formation, strongly suggesting that these proteins play an integral role in SG formation [8]. Another protein that appears to be involved in SG formation is Ras-GTPase activating protein-binding protein-1 (G3BP). G3BP is a phosphorylation-dependent endoribonuclease that contains an RRM for RNA binding and a nuclear transport factor 2 (NTF2) domain for multimerization. G3BP localization to SGs is dependent on both of these domains. Upon cellular stress, a serine residue at amino-acid position 149 in G3BP is dephosphorylated, inducing G3BP oligomerization and SG formation. Overexpression of full-length G3BP induces the formation of SGs and overexpression of the central region of G3BP, which contains residue 149, interferes with SG formation, strongly suggesting that G3BP is an effector of SG assembly that is regulated following cellular stress by dephosphorylation of residue 149 [9]. The histone deacetylase 6 (HDAC6) protein, which is a cytoplasmic protein that deacetylates tubulin and HSP90, has also been implicated as an effector of SG formation. Interestingly, HDAC6 binds G3BP and localizes to SGs under stress conditions. Knockout or pharmacological inhibition of HDAC6 interferes with SG formation. Association of HDAC6 with G3BP is dependent on dephosphorylation of G3BP at residue 149, and the deacetylase activity of HDAC6 also appears to be required for SG activity [10]. Moreover, the microtubule network on which HDAC6 acts by deacetylation of tubulin, also is required for SG formation [10, 11]. The disassembly of SGs appears to rely on heat shock protein (HSP) 70, as overexpression of the TIA-1 PRD induces HSP70, HSP70 localizes to granules formed by the PRD in transfected cells, and overexpression of HSP70 prevents SG formation [8]. While phosphorylation of eIF2 α is sufficient to induce SGs in uninfected cells, treatment with drugs that inhibit translation initiation independent of

eIF2 α phosphorylation (such as Pateamine A and hippouristinol), or siRNA knockdown of proteins involved in translation initiation also induces SGs [12-14].

SGs are dynamic structures that rapidly form, fuse, and dissolve in response to changing cellular conditions [6]. Although as described above there are general core protein constituents of SGs, these structures can be diverse with regards to their protein composition depending on the type of cellular stress [15]. Regulatory proteins involved in mRNA stability localize to SGs, suggesting RNAs may be selected for stabilization or decay in the SG environment. Live cell studies have found that TIA-1 moves rapidly in and out of SGs suggesting that it is actively moving mRNAs in and out of the structures [16]. Moreover, SGs have been shown to interact with a second important set of RNA silencing granules, processing bodies (PBs), which are sites of mRNA degradation [6]. SGs are therefore thought to play an important role in mRNA quality control, translationally silencing some mRNAs and simultaneously targeting specific mRNAs for degradation in PBs [6, 17].

Viruses, protein translation and the cellular stress response

Translational shutoff is a key downstream component of the innate immune response to infection by many viruses, and the activation of PKR (or other stress activated kinases) and phosphorylation of eIF2 α has been shown to play a critical role in this process [18]. Paradoxically, viruses do not encode their own translational machinery and instead depend on the host cell machinery for viral protein synthesis. As a result, viruses that trigger this arm of the innate immune response have evolved elegant strategies to interfere with or otherwise overcome host cell translational shutoff. These strategies include encoding proteins or RNAs that interfere with PKR activation or prevent eIF2 α phosphorylation [19]. Other viruses overcome cellular translation inhibition by utilizing an internal ribosome entry site (IRES) to initiate virus protein translation in an eIF2 independent manner [20]. Viruses that do not trigger host shutoff have also devised methods of effectively competing with host cell mRNAs for the cellular translational machinery during infection.

The discovery of SGs as a downstream consequence of the cellular stress response suggests these structures may also play some part in virus replication, and perhaps may be an additional component of the cellular innate immune response to infection. In fact, many studies published in recent years suggest that many viruses induce and/or modulate SGs in infected cells. Moreover, other viruses that either do not activate, or that subvert the innate immune response and prevent eIF2 α phosphorylation, have also been found to associate with SG components in infected cells. This review aims to summarize the current knowledge on the impact of SGs on virus replication.

SG induction during virus infection

Many viruses induce the formation of SGs at some point during the replication cycle (Fig. 1). Most of these viruses do so through the activation of PKR and eIF2 α phosphorylation. For example, mammalian orthoreovirus (MRV) activates PKR and eIF2 α phosphorylation in a strain and host cell specific manner [21, 22]. An initial study found that MRV induces SGs and that differences in the extent of SG induction by different strains mirrored that of strain specific differences in eIF2 α phosphorylation [23]. A later study confirmed that MRV

infection induced SGs in a manner dependent on eIF2 α phosphorylation, although in this work SGs were found only at early times in infection, and SG formation was independent of virus strain [24]. Similarly, Sindbis virus (SV) and Semliki Forest virus (SFV) induce the formation of SGs in a manner dependent on PKR and eIF2 α phosphorylation [25, 26], and murine hepatitis coronavirus (MHC), also induces SG formation in infected cells concomitant with increases in eIF2 α phosphorylation [27]. Respiratory syncytial virus (RSV) was also shown to induce the formation of SGs as a result of PKR activation [28], although several other studies have shown that RSV blocks eIF2 α phosphorylation [29] and does not induce SGs [30, 31]. Sendai virus (SeV) also induces SGs, however, the virus has been shown to prevent PKR activation and the mechanism behind SG induction remains unknown [32] [33]. Finally, poliovirus (PV) has also been shown to induce SGs at early times in infection, although, like SeV, it also does not induce eIF2 α phosphorylation [34, 35]. While many viruses remain to be studied, results to date suggest that viruses that are not otherwise capable of preventing translational shutoff by inhibition of PKR activation or eIF2 α phosphorylation are likely to induce SGs at some point during the viral replication cycle. Moreover, as in the case of SeV and PV, infection by some viruses may be capable of inducing SG formation even in the absence of eIF2 α phosphorylation perhaps via inhibition of cellular translation through other virus-directed mechanisms.

SG modulation by viruses

Important initiation factors required for translation of proteins are sequestered in SGs, therefore, when SGs are present it results in inhibition of most mRNA translation [36]. Not surprisingly, many viruses appear to have the capacity to inhibit SG formation or disrupt SGs that have formed in infected cells. For example, although SFV induces SGs, this induction is transient, and as replication proceeds, SGs in the vicinity of viral replication are disassembled [26]. Similarly, while MRV induces SGs in infected cells, these SGs disappear over time in a manner dependent on virus protein translation [24]. At late times, even though eIF2 α remains phosphorylated, SGs are not present in MRV infected cells [37]. Likewise, rotavirus (RV), Cricket paralysis virus (CrPV), and Drosophila C virus (DCV) induce high levels of eIF2 α phosphorylation in infected cells, although SGs do not appear to ever be present [38, 39]. Finally, in PV infected cells, one study provides evidence that SGs similarly dissipate as infection continues [34], although another study suggests that compositionally unique SGs that are void of the G3BP, eIF3, PABP, and TIAR proteins, but contain other cellular proteins such as TIA-1, HSP27, and SAM68 remain present throughout PV infection [35].

In the majority of the above cases, virus infection appears to uncouple cellular stress signals (eIF2 α phosphorylation) from SG assembly. Remarkably, when cells infected with these viruses are treated with other exogenous stressors such as arsenite, which induces SGs via activation of stress kinase HRI in uninfected cells [40, 41], SGs do not form, further suggesting that infection interferes with the cellular stress response and inhibits SG formation (See Fig. 2). A number of other viruses that do not induce SGs are nonetheless also capable of preventing the formation of these structures following cellular stress. West Nile Virus (WNV) [42], Hepatitis C virus (HCV) [43], Theiler's murine encephalitis virus (TMEV) [44], and Human T cell leukemia virus (HTLV) [45] do not induce SGs, but when

cells infected with these viruses are treated with arsenite, SGs do not form. Taken together, these studies suggest that SG formation may be a component of the cellular antiviral response, and that interference with or prevention of SG formation may be necessary for replication of an extremely diverse set of viruses.

Mechanisms of SG modulation

Although interference with SG formation seems to be a common theme among a wide variety of viruses, in many instances it remains unclear how each virus accomplishes this task. The mechanism behind SG modulation has been described for a number of viruses, and in each case, appears to rely on a specific interaction between the virus and important SG effector proteins.

WNV was one of the first viruses found to have an association with SGs. Initially, it was found that the 3' stem loop of the virus associates with the RNA recognition motifs (RRM) of SG effector proteins TIAR and TIA-1 [46], and that TIAR/1 also associates with the non-structural protein NS3 from both WNV and Dengue virus (DV). TIAR and TIA-1 were found to colocalize with viral dsRNA and NS3 in perinuclear replication complexes when RNA synthesis was at its maximum levels. Resistance of cells to arsenite-induced SG complexes occurred coincident to TIAR/TIA-1 localization to replication complexes, suggesting that the usurpation of these proteins by the virus plays a role in interference with SG formation [42]. Similar to WNV, SeV appears to specifically associate with the TIAR protein. This interaction occurs through an AU-rich region within virus trailer RNA transcripts that are produced during abortive genome synthesis. The interaction is thought to prevent the formation of SGs in most infected cells, and also to play a role in modulating virus induced apoptosis [32]. RV infection also leads to the relocalization of TIA-1 in infected cells although it remains to be determined if this relocalization is necessary or sufficient for inhibition of SG formation [38].

PV appears to disrupt G3BP-containing SGs via specific cleavage of G3BP by the viral 3c protease. Expression of a cleavage resistant G3BP in infected cells restored formation of G3BP containing SGs, strongly suggesting this cleavage was the mechanism behind SG disruption [34]. HCV, which replicates in complexes that surround cellular lipid droplets, also has been shown to associate with G3BP via interaction with the virus RdRp Nsp5 and the 5' terminus of the minus strand RNA [47]. G3BP and the SG-associated protein PABP are localized to HCV replication complexes at later times during infection [43], although it has not been strictly proven that this recruitment alone prevents SG formation.

Interestingly, the HTLV Tax protein, which plays an important role in cell transformation in human T-cell leukemia, localizes to the cytoplasm following cellular stress and this localization correlates to disruption of SGs. When the protein is localized to the nucleus, SGs spontaneously form. Tax induced SG disruption involves Tax association with the SG effector protein HDAC6. This suggests that Tax binds and sequesters HDAC6, thus preventing SG formation [45].

While most of the evidence so far suggests that viruses interfere with SG formation via a direct interaction with proteins known to be required for SG formation, other cellular

networks or pathways involved in SG formation may also be targeted by viruses. Examples of this are the cellular microtubule network [10, 11], the proteasome degradation pathway [48], the heat shock response [49], and the unfolded protein response [7]. There are numerous examples of viruses that modulate one or more of these important cellular pathways or networks, which may have additional consequences on SG formation.

The role of SGs in the virus replication cycle

The existing evidence on the role SGs play in virus replication supports two divergent ideas, one in which the presence of SGs or interaction of the virus with SG effector proteins appears to be beneficial to the virus, and the other in which the presence of SGs is detrimental to virus infection. There is strong evidence to suggest that SG effector proteins TIAR/1 have a crucial positive function in WNV infection. Mutation of the WNV 3' minus strand stem loop at specific AU-rich regions such that it can no longer bind TIAR and TIA-1 results in loss of virus production with little impact on virus translation. TIAR/1 binding mutants exhibit a low level of initial symmetric RNA amplification that quickly results in the rise of reversion mutants in vivo suggesting TIAR/1 binding is important in the asymmetric amplification of positive strand genomic RNAs from minus-strand intermediate RNA at later times in infection [50]. In support of a positive role of TIAR in infection, WNV grows poorly in TIAR knockout mouse embryo fibroblast (MEF) cells when compared to WT MEFs, again suggesting the association of this SG effector protein is crucial to WNV replication [46]. It remains unclear at this time what specific function the SG effector proteins play in replication, although the association of these cellular proteins with both the RNA and virus RdRp has led to the suggestion that proteins with this binding specificity may be acting as transcription factors, recruiting the RdRp to the RNA minus strand [51]. HCV, another virus that does not itself induce SGs, presents with a similar scenario. In this case the association of G3BP with the 5' terminus of the HCV minus strand RNA as well as the virus RdRp, Nsp5, appears to play a crucial role in virus replication as siRNA knockdown of G3BP results in a substantial decrease in genome amplification [47, 52]. Whether the prevention of SG formation, or SGs themselves, also play a role in WNV or HCV infection remains to be determined.

Unlike many viruses, which either do not demonstrably induce SGs or which induce SGs at early times in infection then disrupt them as infection proceeds, one study suggests that SGs are present throughout RSV infection and may play a positive role in RSV replication [28]. These authors found that when RSV is grown in cells that contain a reduced amount of G3BP and are defective in SG formation, RSV replication was substantially diminished. Moreover, RSV protein levels were found to be higher in cells that contained SGs compared to those that did not. These findings suggest that SGs, or the stress response that induces SGs, may be beneficial to RSV infection. However, it remains possible that G3BP itself plays a role in RSV infection independent of SGs, and should again be noted that other studies have found that RSV infected cells do not contain SGs [30, 31].

There is evidence that the cellular stress that results in SG formation positively impacts MRV replication. MRV infection has been found to alter the expression of genes that play a critical role in recovery from stress which includes downregulating an inhibitor of PKR and

PERK (inhibitor of protein kinase R, P58^{IPK}) and upregulating a crucial transcriptional activator of other stress response genes (activating transcription family 4, ATF4) [23]. Moreover, MRV was found to replicate better in 1) wildtype MEFs relative to ATF4 knock-out MEFs [23], 2) P58^{IPK} knock-out MEFs relative to wildtype MEFs [53], and 3) knock-in MEFs expressing a wildtype eIF2 α relative to MEFs expressing a mutant eIF2 α that cannot be phosphorylated [23]. These data suggest that MRV replication may actually benefit from the stress response. On the other hand, SGs induced at early times in MRV infection were recently found to dissipate as infection proceeds and virus-induced SG disruption was found to correlate with the escape of viral but not cellular mRNAs from translational shutoff [37]. These findings suggest that the formation of SGs may be a detrimental consequence of the stress response pathway that the virus must interfere with in order to replicate. In this work, SG disruption occurred in the presence of phosphorylated eIF2 α , which supports the hypothesis that portions of the stress response leading to eIF2 α phosphorylation are beneficial to MRV replication, but that SGs interfere with virus translation and must be dispersed for replication.

Another virus in which disruption of certain SGs may be crucial for virus replication is PV. Cells transfected with a plasmid expressing a mutant form of G3BP that cannot be cleaved by the virus 3c protease are deficient in supporting PV replication when compared to cells expressing wildtype G3BP. This suggests cleavage of G3BP and/or SG disruption by PV 3c protease during infection is necessary for virus replication [34]. It remains unclear at what step in the virus replication cycle G3BP cleavage and/or the associated SG disruption plays a role. It should also again be noted that in a separate study, it was found that granules that are compositionally different from typical SGs, in that they contain TIA-1 but do not contain G3BP, eIF3, PABP, or TIAR, form and are stable in PV infected cells [35]. The function of these unique SGs in PV infection has not yet been determined.

Utilizing an E3L protein mutant of Vaccinia virus (VV) that cannot interfere with PKR activation, a recent study may have shed additional light on the consequences of SG formation in infected cells. Infection with the E3L mutant resulted in the formation of SG-like granules in cells. These granules resembled SGs in protein content and requirement for PKR/eIF2 α phosphorylation for formation, but were more stable than typical SGs and localized around regions of virus replication. The mutant viruses were replication deficient, suggesting the granules were anti-viral in nature. The deficiency in virus replication was partially restored in TIA-1 knockout MEFs, even though eIF2 α phosphorylation was maintained and TIA-1 deficient granules still formed in cells. These findings suggested that when granule formation is not inhibited by E3L suppression of PKR activation, TIA-1 may bind VV RNA specifically and suppress virus translation through sequestration in SG-like antiviral granules [54]. In support of the hypothesis that SGs are acting in an inhibitory manner during infection, perhaps through direct association and sequestration of viral RNAs by TIA-1 or other SG associated proteins, VV, HSV, VSV, and SV have all been shown to replicate to higher levels in TIA-1 knockout MEFs relative to wildtype MEFs, [46].

The consequences of SG modulation during SFV, SV, RV, CrPV, and MHC infection have not been clarified. However, these viruses induce host translational shutoff via activation of the innate immune response and eIF2 α phosphorylation. It seems likely that sequestration of

important translation initiation factors or viral mRNAs within SGs would pose a barrier to translation of viral proteins and that disruption of these structures would be necessary for this aspect of virus replication. In the case of SFV, SG disruption has been shown to correlate with the shift from host cell to viral protein translation [26], suggesting that SGs may interfere with translation of virus proteins. Likewise, SGs may interfere with SV translation as siRNA knockdown of G3BP-1 and the related but non-SG associated G3BP-2 proteins appear to result in an increase in virus polyprotein synthesis [55]. Finally, MHC replication was enhanced in cells that express a mutant eIF2 α that cannot be phosphorylated, suggesting this upstream trigger to SG formation is detrimental to virus infection [27].

Taken together, it appears that many viruses either utilize SG effector proteins for their own benefit resulting in SG disruption in cells, or interfere with SG formation in order to overcome translational shutoff or other stress responses induced by cells. In this respect, SG disruption may be thought of as an additional weapon in the arsenal used by viruses to overcome the cellular innate immune response.

Conclusions

Viruses from diverse families with very different replication strategies induce or modulate SGs. This suggests that either SGs, or the components of SGs, may play common roles in virus life cycles. At this time, with few exceptions, it appears that while SG effector proteins may play positive roles in the replication of some viruses, SGs themselves play primarily an inhibitory role.

Because inhibition of translation is a known trigger for SG formation, and crucial translation initiation factors and mRNAs are sequestered in SGs, the step in the virus life cycle that is most likely impacted negatively by the presence of SGs is translation of virus proteins. With very few exceptions, even viruses that utilize IRESs or other RNA structures to recruit ribosomes to viral mRNAs for translation initiation require one or more SG sequestered proteins to initiate synthesis of virus proteins. Overwhelmingly, viruses that have been studied appear to disrupt or interfere with SG formation, an action that would be expected to result in freeing initiation factors and ribosomal subunits from the structures into the cytoplasm where they can then be utilized by the virus mRNA for translation initiation. Virus-induced SG disruption may also play other roles in virus replication. For example, virus RNAs may be specifically sequestered in SGs, as is suggested in the case of VV [54], and disruption of SGs may be necessary to release virus mRNAs for translation. In addition, SG formation modulates apoptosis in cells by sequestering apoptosis promoting proteins such as RACK1, ROCK1 and RHOA away from their downstream targets [56, 57], and viruses may manipulate SGs to exert control over the timing of apoptosis to the benefit of virus replication. This appears to be the case with SeV RNA where the ability to modulate SGs by binding and decreasing the availability of TIAR correlates with inhibition of virus-induced apoptosis [32]. Regardless of the outcome of virus induced SG modulation, because SG formation is triggered by cellular signals associated with the innate immune response to virus infection and SGs appear to act in an inhibitory role against most viruses, it is tempting to suggest that these granules are a bona fide downstream component of the cellular innate immune response.

Future Perspectives

Clearly much remains to be discovered about the interplay between virus infection and SGs. It will be important to gain a better understanding of the function(s) of SGs in cells in order to fully understand the role they play in virus infection. Because around one hundred cellular RNA binding proteins possessing multiple functions in mRNA regulation localize in SGs [58, 59], there are likely to be many interesting stories that remain to be discovered in this field. Adding to this complexity, a recent study identified 101 genes that were required for SG formation using an RNAi-based screen [60]. This suggests there are at least that many genes that might be modified by viruses to modulate SG formation during infection. Although many of the identified genes are known to be involved in RNA metabolism, over half of them are not, suggesting that there may be some very unique strategies used by viruses to modulate SGs. This new data may also lead to new strategies to alter cells such that they no longer form SGs, or are able to form very stable SGs, so that the consequences of these conditions on viral infection can be examined. Based on work already completed on TIAR/1, G3BP, and HDAC6, a number of reagents already exist that may assist in understanding the role of SGs in viral infection. These include TIA-1 [8] and HDAC6 [10] knockout MEFs that are deficient in forming SGs, and various mutant plasmid constructs of TIAR/1, G3BP, and HDAC6 that when overexpressed in cells inhibit or induce SGs [8-10]. Examination of virus replication in these knock-out cells, or cells expressing these SG-inhibitory proteins, may shed light on the impact of SGs on virus replication. In addition, future work aiming to identify virus mechanisms involved in SG modulation may not only illuminate the role of these structures in virus infection but also provide a more thorough understanding of the function of SGs in cells. Continued identification of specific virus proteins or virus induced changes in cellular pathways required for SG modulation are warranted to identify the mechanisms used by viruses to uncouple cellular stress signaling and SG formation in infected cells. Upon identification of these mechanisms, experimental approaches such as the creation of virus or cellular protein mutants that interfere with virus-induced SG modulation will further illuminate the importance of these interesting cytoplasmic structures in the replication cycles of viruses.

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Executive Summary

Stress granules (SGs) are a regulatory component of the cellular stress response to viral infection.

- Infection by many viruses induces the activation of PKR and eIF2 α phosphorylation, which leads to inhibition of translation and induction of cytoplasmic granules termed stress granules (SGs).
- SGs contain stalled 48S translation initiation complexes, including mRNAs, translation initiation factors, and small ribosomal subunits in addition to many other important RNA binding proteins.
- eIF2 α phosphorylation is sufficient to induce SGs in uninfected cells, however, inhibition of translation initiation at many other steps can also lead to SG formation.
- Cellular proteins that play important roles in effecting SG formation include the RNA binding proteins TIA-1, TIAR, and G3BP, as well as the histone deacetylase protein HDAC6.

Viruses from diverse families induce and/or modulate SGs in infected cells.

- Many divergent viruses induce SG formation in cells, either in an eIF2 α phosphorylation dependent or independent manner. Other viruses induce eIF2 α phosphorylation, yet SGs never form in infected cells.
- SGs generally dissipate at later times in infection even though eIF2 α remains phosphorylated. External stressors, such as sodium arsenite, are unable to induce SGs in cells infected with many viruses suggesting viruses uncouple stress signals from SG formation.

Viruses modulate SGs by interaction with SG effector proteins.

- West Nile virus RNA is bound by SG effector TIAR/1 proteins, which play a role in virus replication. This association likely leads to virus interference with SG formation in infected cells.
- Poliovirus 3C protease cleaves SG effector protein G3BP resulting in loss of G3BP-containing SG formation.
- Hepatitis C virus RNA and Nsp5 protein associate with SG effector G3BP, which is recruited to virus replication centers. This association may play a role in SG disruption by this virus.

SGs may be beneficial to rare viruses but seem to generally be detrimental to virus replication.

- Although mammalian orthoreovirus appears to replicate better in stressed cells, SG presence correlates with inhibition of virus protein synthesis, and disruption of SGs correlates with escape of virus mRNA from translational inhibition in the presence of eIF2 α . This suggests SGs are not beneficial.

- Inhibition of disruption of G3BP-containing SGs in poliovirus infected cells leads to decreased levels of virus, suggesting these SGs are inhibitory to replication.
- Downregulation of SG effector protein G3BP leads to increased levels of Sindbis virus polyprotein synthesis, suggesting SGs play an inhibitory role in infection.

The future of stress granules and virus replication

- Gaining a better understanding of the function of SGs in cells will assist in understanding their role in virus replication.
- Over 100 genes are involved in SG formation, and over 100 proteins localize in SGs, therefore there are multiple cellular targets that may be modified by viruses to modulate SGs.
- Continued identification and mutation of virus proteins involved in SG modulation will assist in our understanding of the impact of SGs on virus replication.

Figure 1. Viruses that induce or modulate SGs during infection or following other cellular stress
 Viruses that induce SGs during infection are listed on the left. Viruses that modulate SGs by disrupting their formation during infection or by interfering with their formation following other types of cellular stress such as sodium arsenite treatment are listed on the right. SG components implicated in virus-induced SG modulation are indicated in parenthesis. Asterisks identify viruses that interfere with SG formation induced by virus infection and other types of cellular stress. A carat indicates SG induction or modulation remains controversial.

Figure 2. Virus infection interferes with sodium arsenite induced SG formation

HeLa cells were infected with mammalian orthoreovirus, and at 24 h p.i., cells were treated with sodium arsenite for 1 h. Cells were then fixed and stained with antibodies against virus non-structural protein μ NS (left panel, red) and TIAR (middle panel, green) followed by Alexa 594- and Alexa 488-conjugated secondary antibodies to visualize infected cells and SGs respectively. A merged image is shown on the right (DAPI, blue). Arrowheads indicate infected cells that do not contain SGs. Arrows indicate uninfected cells that contain SGs.