Review

Regulation of stress granules in virus systems

James P. White* and Richard E. Lloyd

Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA

Virus infection initiates a number of cellular stress responses that modulate gene regulation and compartmentalization of RNA. Viruses must control host gene expression and the localization of viral RNAs to be successful parasites. RNA granules such as stress granules and processing bodies (PBs) contain translationally silenced messenger ribonucleoproteins (mRNPs) and serve as extensions of translation regulation in cells, storing transiently repressed mRNAs. New reports show a growing number of virus families modulate RNA granule function to maximize replication efficiency. This review summarizes recent advances in understanding the relationship between viruses and mRNA stress granules in animal cells and will discuss important questions that remain in this emerging field.

Stress granule formation and composition

Eukaryotic cells can contain multiple types of cytoplasmic mRNA-containing bodies, including processing bodies (PBs, also known as GW bodies) [1], exosome bodies [2,3], neuronal bodies [4,5] and stress granules (SGs) [6,7]. PBs and exosome granules are foci that are constitutively present in cells and contain components involved in mRNA decay [3,8]. Neuronal granules are also constitutively present in neurons but are instead associated with the concentration and transport of translationally silenced messenger ribonucleoproteins (mRNPs) moving along the axons to dendrites [5]. SGs are not constitutively present in cells, but similar to neuronal granules, SGs are concentrations of stable, translationally silent mRNA [9] that are thought to be sites of mRNA storage and triage [10]. SGs and PBs are found in the widest number of cells types. Although PBs are known to be modulated by some viruses, this review will focus on the many more publications describing viral modulation of stress granules.

Based on immunofluorescent microscopic analysis of SG constituents, SGs are defined as macromolecular aggregates of stalled 48S initiation complexes that form in response to stress conditions [11]. The best described pathway of SG formation initiates with phosphorylation of eukaryotic translation initiation factor (eIF) 2α (eIF2α) by the eIF2 kinases PKR, PERK, GCN2 or HRI [12–14], although alternative pathways exist such as inhibition of eIF4A RNA helicase [15–17] or viral infection [15]. PKR, a component of the interferon response, is commonly activated by RNA viruses producing double-stranded RNA as replication intermediates and PERK is activated by endoplasmic reticulum (ER) stress associated with a smaller group of viruses, many that express membrane glycoproteins (e.g. herpes viruses and others). HRI, activated by heme deprivation and oxidative stress, and GCN2, which is activated by nutrient starvation, are not commonly linked to virus infection, although GCN2 binding to Sindbis virus RNA induces its activation [18]. SG are foci of concentrated 48S translation preinitiation complexes, thus SGs are defined by the presence of translation initiation machinery including 40S ribosome subunits, eIF2, eIF3, eIF4A, eIF4B, eIF4E, eIF4G and eIF5 [13,15,19,20]. SGs are also defined by certain key marker RNA binding proteins (RBPs) such as T-cell restricted intracellular antigen 1 (TIA-1), TIA-1-related protein (TIAR) and RasGAP SH3-domain binding protein 1 (G3BP1) [14,21], however, SGs contain many other RBPs (Figure 1). Because SGs contain stable inert mRNA, they represent an intermediate step in the equilibrium between active translation that occurs on free polysomes and mRNA decay, which takes place in PBs. As such, they dynamically release contents for active translation [22–25] as well as interact with PBs in a process that is thought to result in the exchange of mRNA 'cargos' [23]. The movement of RBPs between compartments is rapid, with a full replacement of some SG contents occurring in well under a minute [22,23]. Other evidence suggests that association of mRNA with the ER renders the mRNA resistant to inclusion in SGs [26]. Frequent interaction of SGs with PBs is observed in cells that are actively forming SGs and live cell imaging shows that this process is dynamic and transient [23]. Little is known about the mechanism or purpose of this interaction other than the proposed mRNP cargo exchanges (Figure 1), but the overexpression of tristetraprolin (TTP) and related protein BRF1 is known to promote and stabilize the association of SGs and PBs [23].

The molecular mechanism(s) [27] by which SGs form is undefined, but appears complex and involves several steps that include the self-oligomerization of certain constituent RNA-binding proteins, post-translational modifications of proteins and mRNP transport on microtubules (Table 1, Figure 1). Theoretically, viral inhibition of any of these important steps may block or modulate SG formation in cells. Self-oligomerization of TIA-1 or TIAR and G3BP may play a crucial early role in the SG aggregation process and overexpression of these proteins induces spontaneous SG formation [21,28]. Expression of the C-terminal

Corresponding author: Lloyd, R.E. (rlloyd@bcm.edu)

Keywords: stress granule; antiviral response; translation silencing; G3BP; TIA-1; eIF4G.PKR..

* *Current address:* Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA.

 doi:10.1016/j.tim.2012.02.001

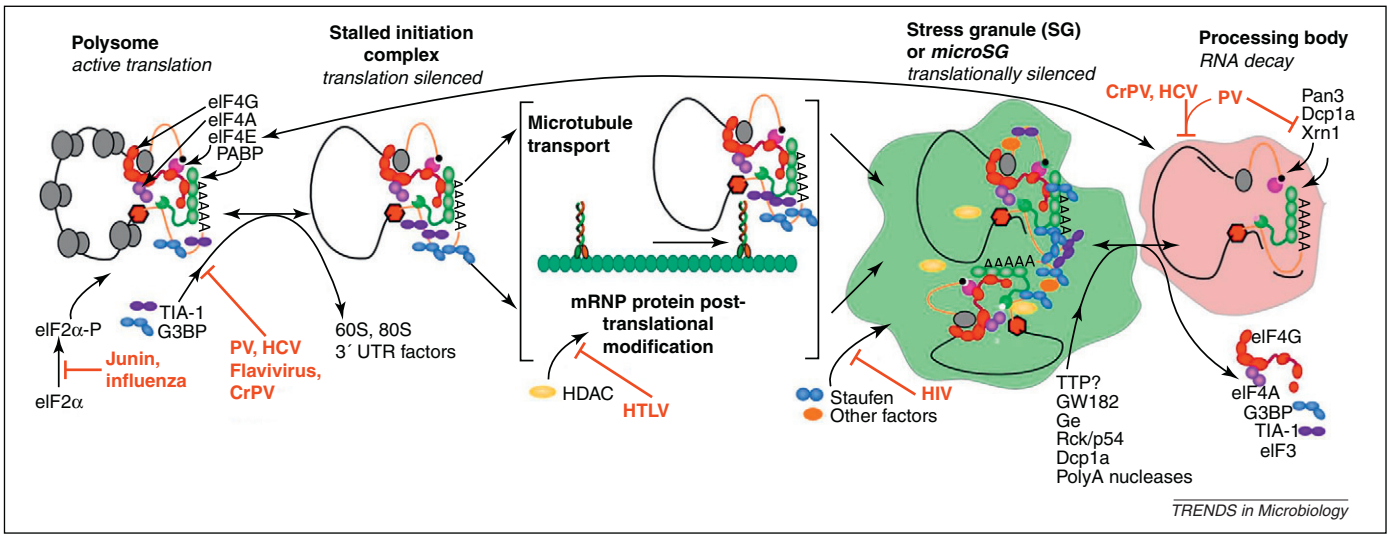

Figure 1. Stress granules (SGs) are intermediate compartments in mRNA metabolism. Inhibition of translation initiation leads to the disassembly of polysomes and the formation of stalled 48S initiation complexes. These messenger ribonucleoprotein (mRNP) complexes are recognized via an unknown mechanism and are remodeled, marking them for inclusion in SGs despite continued association with pro-translation initiation factors. SG components such as RasGAP SH3-domain binding protein 1 (G3BP1), Fragile X mental retardation protein (FMRP) and others are post-translationally modified, and small dispersed aggregates of remodeled mRNP complexes are transported by microtubule-associated motor proteins into larger SGs. The brackets around this central step indicate that it is not currently known which process is initially undertaken. SGs are thought to be sites of storage of stabilized mRNA, although it is known that mRNA can be released for translation or transported to processing bodies (PBs) for active decay by an unknown mechanism. Multiple virus systems (in red) have been found to interfere with the process of SG and PB formation and the points of interaction with the process are indicated. Stress granules also dock with PBs where mRNP modification and cargo exchange takes place. Initiation factors are lost except eukaryotic translation initiation factor (eIF4E) and deadenylase complexes (Pan2/3, Caf1/Ccr4) decapping complexes (Dcp1a/2) and exonucleases (Xrn1) become associated. Some viruses inhibit PB formation as indicated and poliovirus (PV) antagonizes specific PB components [43,45,71].

glutamine-rich prion related domain (PRD) of TIA-1 inhibits the formation of SGs and overexpression of TIA-1 lacking the PRD does not spontaneously induce SGs [28]. Additionally, murine embryonic fibroblasts (MEFs) that are null for TIA-1 or TIAR display deficient SG formation in response to various stressors [28]. G3BP can self-oligomerize in a phosphorylation-dependent manner and overexpression of the central domain of G3BP containing the arginine-rich and PxxP domains inhibits SG formation [21]. As is the case with TIA-1, cells with

Table 1. Stress granule mechanistic processes that viruses can potentially modulate[a]

Process	How it can be modulated	Refs.
Cell Insult		
Inhibit translation ternary complex formation	Phosphorylate eIF2α via activation of PKR, HRI, GCN2 and PERK	[14]
Block eIF4F function (scanning)	Hippuristanol inhibition of eIF4A, viral cleavage of eIF4G	[15]
Assembly/mRNP infiltration of key proteins		
G3BP	RNA-binding protein can self-oligomerize, may sequester mRNA in SG, overexpression of mutants blocks SG formation	[21]
TIA-1, TIAR	RNA-binding protein can self-oligomerize, may sequester mRNA in SG, overexpression of mutants blocks SG formation	[14,22,28]
HDAC6	Deacetylase function and SG infiltration associated with SG assembly	[36]
Movement and post-translational modifications of proteins		
Microtubule transport	Required for assembly but not maintenance	[31–36]
O-GlcNAc on ribosomal proteins	Required for SG assembly, multiple proteins modified	[72]
Acetylation	HDAC6 function associated with SG assembly	[36]
Methylation	Methylation is recruitment tag that controls SG assembly TDRD3 tudor domain interacts with methylated proteins Cold-inducible RNA binding protein (CIRBP) methylation controls nuclear translocation and SG entry FMRP methylation for RNA binding and SG localization	[73] [74] [75] [76]
Phosphorylation/dephosphorylation	G3BP dephosphorylation required for SG assembly Grb7 non-phosphorylatable double mutant stabilizes SGs, focal adhesion kinase mutant stabilizes SGs	[21] [77]
Ubiquitination	SGs contain ubiquitinated proteins, Ub-binding domain of HDAC6 required for localization to SGs	[36]
Interruption of SG disassembly		
OGFOD	Interacts with G3BP, HRI, regulates eIF2α phosphorylation	[78]

[a]Abbreviated list only, particularly in terms of factors that infiltrate SGs.