Review

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Antiviral innate immunity and stress granule responses

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Viral infection triggers the activation of antiviral innate immune responses in mammalian cells. Viral RNA in the cytoplasm activates signaling pathways that result in the production of interferons (IFNs) and IFN-stimulated genes. Some viral infections have been shown to induce cytoplasmic granular aggregates similar to the dynamic ribonucleoprotein aggregates termed stress granules (SGs), suggesting that these viruses may utilize this stress response for their own benefit. By contrast, some viruses actively inhibit SG formation, suggesting an antiviral function for these structures. We review here the relationship between different viral infections and SG formation. We examine the evidence for antiviral functions for SGs and highlight important areas of inquiry towards understanding cellular stress responses to viral infection.

Viral infection and stress granules

Viral invasion and replication are detected by innate immune sensors in cells, triggering downstream signaling pathways that can ultimately result in the activation of systemic immune responses. Several innate immune sensors recognize cytoplasmic viral RNA [1], and lead to the production of IFNs which in turn trigger various antiviral pathways aimed at halting viral replication and spread. These antiviral effects include double-stranded (ds) RNA-dependent protein kinase (PKR)-dependent inhibition of mRNA translation, and 2′,5′-oligoadenylate synthetase (OAS)/RNase L-mediated RNA degradation [2]. Innate immune responses also trigger the activation of adaptive immunity in the form of T and B cell activation and proliferation, and modulate the phenotype and function of these adaptive responses [3,4].

In some cases, viral infection also induces the formation of cytoplasmic granules similar to those induced by cellular stresses such as heat, oxidation, hypoxia, and osmotic pressure, which are referred to as stress granules (SGs). SGs are ribonucleoprotein (RNP) aggregates that contain translationally stalled mRNAs, 40S ribosomes, and various RNA-binding proteins [5–7] (Box 1).

Activation of the RLR signaling pathways by viral RNA

Innate immune responses are triggered upon recognition of pathogen-associated molecular patterns (PAMPs) which in the case of viruses are often nucleic acid-based, either RNA or DNA. Viral nucleic acid can be detected by sensors including Toll-like receptors (TLR)3, 7/8, and 9, retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), and cytoplasmic DNA sensors such as DNA-dependent activator of IFN-regulator factors (DAI), stimulator of IFN genes (STING, also known as MITA/ERIS/MPYS), DEAD box polypeptide 41 (DDX41), and cGMP/cAMP synthase (cGAS) [8–12]. RLRs, RIG-I, melanoma differentiation-associated protein 5 (MDA5), and DHX58 [DEXH (Asp-Glu-X-His) box polypeptide 58, also known as LGP2] are all RNA helicases and contain the signature DExD/H motif that characterized the DExD/H box family. These proteins are crucial for the detection of cytoplasmic RNA [13,14]. RLRs discriminate self from viral transcripts by recognizing specific biochemical signatures such as ds structure and the presence of a 5′-ppp moiety [15–17]; self-transcipts lack these viral signatures.

The signaling pathways downstream of the founding member of the RLR family, RIG-I, are among the best understood. Upon viral RNA recognition by RIG-I, the signal is relayed to the adaptor protein IFN-β promoter stimulator 1 (IPS-1, also known as MAVS, VISA, or Cardif), which predominantly localizes to the mitochondrial outer membrane [18,19]. When viral RNA binds at the helicase and the C-terminal domain (CTD) of RIG-I, its N-terminal caspase recruitment domains (CARDs) are covalently modified with K63-linked polyubiquitin chains by the E3 ligase, tripartite motif-containing protein 25 (TRIM25) [20], and oligomerize [21]. The ubiquitinated and oligomerized CARDs bind to the CARD domain on IPS-1 on mitochondria, peroxisomes, and/or mitochondrion-associated membrane (MAM) regions in the endoplasmic reticulum (ER) [22]. The translocation of RIG-I to these locales is facilitated by the chaperone protein 14-3-3ε [23]. The requirement for K63-linked polyubiquitin chains is complex because several reports have demonstrated the importance of non-covalent interaction between RIG-I CARDs and the unanchored K63–ubiquitin chains [21,24].

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Box 1. An overview of SG biology

Cells respond to various insults including heat, oxidative stress, nutrient starvation, and proteotoxic stress by forming cytoplasmic nucleoprotein aggregates termed SGs [5,68–70]. Multiple RNA-binding proteins (RBPs) localize to SGs, and some have been used as markers for these cytoplasmic bodies (Table I). Although the formation of SGs in live cells can be detected by monitoring fluorescence-tagged SG marker proteins, biochemical isolation of SGs is notoriously challenging because these are not membrane-sequestered compartments.

SG formation has been interpreted as a response aimed at preventing the generation of abnormal proteins by transient stalling of translation in times of cellular stress. Stalled transcripts undergo translation upon recovery from stress, or alternatively they are degraded in another granular compartment termed the processing body (P-body, PB) [71,72]. Unlike SGs, PBs are present in the unstressed cell, and contain enzymes for mRNA degradation such as decapping enzymes and 5′–3′ exonucleases (Table I). It is thought that transcripts and proteins can move from SGs to PBs (and vice versa), and these aggregates share some of their components (Table I) [60]; however, the mechanisms underlying the proposed exchange of contents are unclear.

SG proteins, as defined by studies using proteins characteristic to SGs as markers [73], are either diffusely distributed in the cytoplasm or localized in the nucleus in normal conditions; stress triggers their aggregation in the cytoplasm [5,74]. A common event downstream from the aforementioned cellular stresses is phosphorylation of eIF2α at serine 51, which is considered to be an initial trigger for SG formation. Four eIF2α kinases, PKR, GCN2, PKR-like endoplasmic reticulum kinase (PERK), and heme-regulated eIF2α kinase (HRI), can phosphorylate eIF2α in mammals (Table II). The mechanisms that connect eIF2α phosphorylation to SG formation remain to be elucidated.

Some proteins have been shown to be crucial for the formation and or stability of SGs. These include G3BP1, a phosphorylation-dependent endonuclease [30,75], and T cell restricted intracellular antigen-1 (TIA1) and TIA-related protein (TIAR) that are collectively termed TIA1/TIAR [74,76]. Removal of these regulators by genomic deletion or RNAi blocks SG formation by sodium arsenite, and a mutant form of G3BP1 (S149E) acted as a dominant inhibitor of SG formation [75]. However, because of analytical constraints, the molecular machinery underlying the formation of SGs remains unclear.

Table I. Protein components of SGs and P-bodies.

Factor	Full name	Functions	Refs
SG components			
ADAR	Adenosine deaminase, RNA-specific	RNA editing, RNA stability	[77]
Caprin-1	Cell cycle associated protein 1	Cell growth, SG assembly	[44,78]
phospho-eIF2α	Eukaryotic translation initiation factor 2A	Initiation factor, SG assembly	[60,79]
eIF3	Eukaryotic translation initiation factor 3	Multisubunit initiation factor	[5]
eIF4G	Eukaryotic translation initiation factor 4G	Initiation factor	[5]
G3BP1	Ras-GTPase-activating protein SH3-domain-binding protein 1	Endoribonuclease, ras signaling, SG assembly	[75]
HDAC6	Histone deacetylase 6	Translation regulator, SG assembly	[80]
HuR/ELAVL1	Hu antigen R/ELAV-like RNA-binding protein 1	mRNA stability, translation regulator	[5,81]
OGFOD1	2-Oxoglutarate and iron-dependent oxygenase domain containing 1	Translation regulator, SG assembly	[82]
PABP1	PolyA-binding protein 1	mRNA stability, translation regulator	[74]
Pum1	Pumilio RNA-binding family member 1	Translation regulator, cell growth	[83]
Pum2	Pumilio RNA-binding family member 2	Translation regulator, SG assembly	[84]
RHAU/DHX36	RNA helicase associated with AU-rich element/DEAH box polypeptide 36	RNA helicase, SG assembly, antiviral activity	[49,85]
SMN	Survival of motor neuron	RNA metabolism, SG assembly	[86,87]
STAU1	Staufen dsRNA-binding protein 1	RNA transport, SG assembly	[88]
TIA1	T cell restricted intracellular antigen-1	Translation regulator, SG assembly	[74,76]
TIAR	TIA-1-related protein	Translation regulator	[74]
ZBP1	Z-DNA-binding protein 1	DNA sensor, translational regulator	[11,89]
40S	Eukaryotic small ribosomal subunit	Ribosome	[5]
P-body components			
CNOT6/CCR4	CCR4–NOT transcription complex, subunit 6	mRNA deadenylation, PB assembly	[90,91]
DCP1a	Decapping mRNA 1A	mRNA decapping	[60,92]
DCP2a	Decapping mRNA 2A	mRNA decapping	[60,92]
EDC4/GE-1/Hedls	Enhancer of mRNA decapping 4	Decapping coactivator, PB assembly	[93,94]
TNRC6A/GW182	Trinucleotide repeat-containing 6A/GW bodies 182	RNA silencing, PB assembly	[95,96]
Lsm1	Lsm1, U6 small nuclear RNA associated	Decapping coactivator, PB assembly	[90,92,97]
SG and PB components			
APOBEC3G	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G	Antiviral activity	[98,99]
Ago2	Argonaute RISC catalytic component 2	RNA silencing, PB assembly	[100,101]
BRF1	Butyrate response factor 1	ARE-mediated mRNA decay	[60]
CPEB	Cytoplasmic polyadenylation element binding protein	Polyadenylation, translation regulator	[102]
DDX3	DEAD box helicase 3	RNA helicase, antiviral activity	[103,104]