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Differences between acute and chronic stress granules, and how these differences may impact function in human disease

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Abstract

Stress granules are macromolecular aggregates of mRNA and proteins assembling in response to stresses that promote the repression of protein synthesis. Most of the work characterizing stress granules has been done under acute stress conditions or during viral infection. Comparatively less work has been done to understand stress granule assembly during chronic stress, specifically regarding the composition and function of stress granules in this alternative context. Here, we describe key aspects of stress granule biology under acute stress, and how these stress granule hallmarks differ in the context of chronic stress conditions. We will provide perspective for future work aimed at further uncovering the form and function of both acute and chronic stress granules and discuss aspects of stress granule biology that have the potential to be exploited in human disease.

1. Introduction

RNA granules are a family of macromolecular aggregates that assemble in response to environmental cues. These granules are named based on their constituent parts and/or the process in which they function. The best-studied classes of RNA granules are germ granules, p-bodies, neuronal granules, and stress granules. Germ granules function in germ cells and regulate fertility, while p-bodies are associated with RNA decay and mRNA storage. Neuronal granules have been shown to regulate site-specific translation. Several excellent reviews have been written on these various RNA granule types [11,36,39,66,75]. Here we will focus on stress granules (SGs) because of their relationship with human disease and the gap in knowledge that exists in our understanding of SGs under acute and chronic stress. We will delineate differences between acute and chronic stress granules and highlight potential opportunities for therapies centered on SG disruption.

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2. Acute stress granules

2.1. Components and conditions

Acute stress is generally defined as a stress that is applied transiently (usually for two hours or less) before analysis. Stress granules appear in the cytoplasm of cells under acute stress conditions such as oxidative, metabolic, hypoxic or thermal stress, as well as during cellular infection by many different viruses [2,28,31,58]. The type and duration of the stress can dictate incorporation of constituents within SGs, including or excluding many components of the translation machinery such as translation initiation factors EIF3, EIF4G and even PABP, 40S ribosomes, polyadenylated mRNAs, and SGs also recruit many signal transduction proteins and RNA binding proteins such as the SG nucleating factors G3BP1, G3BP2, TIA1 and TIAR [1]. In other words, SGs contain stalled 48S mRNP complexes which result from translation repression at the initiation step (Fig. 1). However, recent work has illustrated that stress granules can have a composition specific to the particular stress the cells are subjected to [5,56].

2.2. Pathways of induction

Stress granule assembly is a highly regulated, multi-step process[85], typically occurring in response to repression of protein translation. This process can be initiated in multiple ways. The most well-characterized stimuli uniformly induce translation repression by triggering eukaryotic initiation factor 2 α (eIF2α, EIF2S1) phosphorylation and subsequent accumulation of stalled translation initiation complexes [28]. mRNPs within SG rapidly exchange with mRNPs associated with the translation apparatus in the cytoplasm [6,29,32], and some SG components have half-residence times less than 20 s. However, restriction of translation initiation through mechanisms independent of eIF2α, such as inhibition of the RNA helicase EIF4A (with pateamine A, hippuristanol or silvesterol) or the nutrient sensor mTORC1 (with rapamycin or its analogs), has also been shown to promote SG formation in some contexts [10,12,17,76]. Thus, while several mechanisms promote formation of mRNPs that can be packaged into SGs by inhibition of function of the cap-binding complex eIF4f, the most well studied mechanism is through phosphorylation of eIF2α (Fig. 1).

2.3. Key interactions within SG

The critical interactions that have been shown to regulate SG assembly and persistence can be separated into three classes of interactions. The first are stable and specific protein:protein interactions occurring between globular domains that stably recognize interaction partners (Fig. 1, interaction A). Secondly, non-specific and less stable interactions, typically occurring between intrinsically disordered domains of proteins, have also been implicated in phase separation of mRNPs in stress granules (Fig. 1, interaction B). Finally, RNA:RNA interactions that result from exposed mRNA sequences appear upon the uncoating of mRNAs that occurs upon translation arrest have recently been shown to alter the propensity of mRNPs to phase separate (Fig. 1, interaction C). All of these are likely important for proper SG structure and function, but might differ in a stress specific manner.

Most work on specific interactions integral for SG formation (Fig. 1, interaction A) has predominantly focused on SG-nucleating proteins. Several SG-nucleating proteins have been

described including Ras-GAP SH3 binding proteins 1 and 2 (G3BP1 and G3BP2), TIA1 and TIA1 cytotoxic granule associated protein like 1 (TIA1 and TIAR), FMRP (FMR1), FUS (TLS) and CAPRIN1 (RNG105). We do not consider all of these true nucleating proteins because some are only recruited to SGs under disease states (FUS; [26,65]) while others appear to function through other nucleating proteins (CAPRIN1 discussed below; [31]), or do not appear to alter SG assembly in their absence (FMR1; [20]). TIA1 and TIAR are *bona fide* nucleating proteins, but they lack discrete globular domains, and we thus focus our discussion on G3BP1. G3BP1 is the best studied nucleating protein, and can induce is SG assembly upon overexpression, and can induce eIF2α phosphorylation through PKR activity [31,61,62]. In some systems, inactivating G3BP1 can completely abrogate SG formation [73,81], while other systems require inactivation of both G3BP1 and its homolog G3BP2 [31,44,63]. Both G3BP1 and G3BP2 are RNA-binding proteins that have globular protein:protein interaction domains in the N-termini and protein:RNA interaction domains in the C-termini, in each case flanking a central intrinsically disordered region [30,59]. The domain layout of G3BP1 suggests a model where G3BP1 and G3BP2 can hold SG together by interacting with SG proteins on one end and RNA on the other. These features ultimately led to interest in the identification of additional proteins with which these two SG-nucleating proteins might be interacting. Subsequent work demonstrated that in addition to homo- and hetero-dimerization, G3BP1 and 2 interact with USP10 and Caprin1 [31,62,72,74]. USP10′s interaction with G3BP1 involves an FGDF motif within USP10 that binds a pocket within the N-terminal NTF2-like domain of G3BP1, and this binding inhibits SG condensation in response to some stresses [31,55]. Caprin1 competes with USP10 for binding to G3BP1, thus favoring SG condensation [31]. However, it is important to note that the interactions between G3BP1 and USP10 or Caprin1 do not fully dictate SG disassembly or assembly, as two different laboratories recently demonstrated that the unstressed G3BP1 interactome significantly overlaps the interactome of G3BP1 during stress [41,90]. Less than half of the G3BP1-interacting proteins interacted with G3BP1 specifically in stress conditions, and both USP10 and Caprin1 were shown to interact with G3BP1 in either context. Further work is required to determine the changes in the nature of these interactions that promote normal function within mRNPs in translation as compared to functions promoting SG condensation.

The second class of interactions involved in SG formation are weak and nonspecific interactions that do not require folded globular domains (Fig. 1, interaction B). Within SGs, these nonspecific interactions are mediated by intrinsically disordered and low complexity sequences intrinsic to many RNA binding proteins [30,38,57]. Despite the contribution of ordered protein-protein interactions, stress granules are phase-separated mRNPs that are largely dependent upon the aggregation of low complexity and intrinsically disordered domains within proteins (Fig. 1). SGs thus contain many RNA binding proteins containing such regions [26,38]. Indeed, recent work indicates that post-translational modification of the intrinsically disordered regions of G3BP1 and FUS by phosphorylation and arginine methylation [63,80] can alter SG formation.

Extending this concept, multiple post-translational modifications (PTMs) including deacetylation, arginine demethylation, poly(ADP) ribosylation, O-GlcNac modification, and phosphorylation have been shown to alter SG dynamics. Several of these modifications occur on specific SG nucleating proteins, and therefore might be expected to influence

SG assembly. For example, G3BP1 is ADP-ribosylated [37], de-phosphorylated [63,78], and de-methylated to promote SG assembly[81]. TIA1 is also ADP-ribosylated under conditions in which SG form[37], and TIA1 oxidation has been shown to inhibit SG assembly [3]. FUS, which assembles into SG in the context of neurological disease, can be phosphorylated by DNA protein kinase thus rendering it less aggregation prone [47]. Finally, O-GlcNac modification of ribosomal proteins appears to regulate SG condensation [51]. On the other hand, while acetylation and ubiquitination have been shown to regulate SG dynamics [35,43], whether these effects are a consequence of modification of SG nucleating proteins or are indirect effects related to cellular stress have not been completely worked out. Importantly, the PTMs on specific proteins that regulate SG dynamics (i.e. G3BP1 and FUS as well as TIA1 to a lesser extent) concentrate in intrinsically disordered regions of the proteins suggesting they may act as a switch to trigger phase separation within a SG. How these PTMs and others in RNA binding proteins not yet discovered affect SG assembly and function remains underexplored.

Finally, consistent with a role for RNA in regulating SG formation, RNA can both self-assemble into RNA aggregates that resemble the SG transcriptome [83] and affect the ability of intrinsically disordered protein fragments to condense into phase separated mRNPs [38]. Thus RNA:RNA interactions are likely an important component of SG structure (Fig. 1, interaction C). G quartets, a well-studied RNA structure, form as a consequence of Hoogsteen base pairing between nearby guanines within RNA. Stacking of G-quartets then forms a right-handed helical structure known as a G-quadruplex [14]. These structures might contribute to SG condensation as guanosine has been shown in certain conditions to aggregate to form gel-like condensates similar to that formed by high concentrations of SG proteins [21,26]. Indeed, many SG-resident RNA binding proteins, such as FUS, YB1, FMRP and hnRNPA1, interact with G quadruplexes [8,40,87,91]. Recently, the interaction between YB1 and G-quadruplex structures within the 5′ cleavage product of a subset of mature tRNAs was speculated to be critical for SG formation, but researchers were unable to test this directly [40]. The involvement of G-quadruplexes in this process is ultimately dependent on conclusively showing that G-quadruplexes assemble *in vivo*. Because SGs assemble under conditions of global translation repression, ribosomes runoff and expose RNA sequences that may contain guanosine stretches involved in G-quadruplex formation, which could assemble into G-quadruplex structures. Assuming the appropriate cations are present within SGs to mediate G-quartet assembly, this process would be further promoted by high concentrations of RNA within SG resulting in an interaction of concentrated G-quadruplex structures that could in theory contribute to maintaining SG structure. Furthermore, hyperfolded, naked RNAs resulting from ribosome runoff could interact through RNA structural elements such as the G-quadruplex. One can surmise that RNA reorganization would also induce allosteric changes in RNA binding proteins containing intrinsically disordered domains to further promote assembly of SGs. Fig. 1 depicts the normally circular state of mRNAs within cells. When cells encounter a stress that abolishes translation initiation, ribosomes dissociate from RNAs causing the RNA to collapse into an aggregate, likely partly mediated by G-quadruplex structures, along with some translation initiation factors and RNA binding proteins. SG disassembly could then potentially occur by dissociation of intrinsically disordered protein interactions, routing of some components

to autophagosomes, enhanced expression or activation of RNA chaperones, or alternatively, degradation of the SG constituents themselves. Future studies are needed to define the importance of G-quadruplexes and other RNA structures in the formation and disassembly of SG.

2.4. Pro-survival functions

The formation of SGs appears to regulate several canonical signaling pathways (NF-κB, mTORC1, PKR, RIGI) within the stress response [30,27,53,59,76]. A decade ago, Takekawa and colleagues showed that acute cellular stresses induce pro-survival SG formation [2]. This seminal work demonstrated that SGs repress pro-apoptotic MAPK signaling through recruitment of RACK1 to SG. Expressing a mutant RACK1 protein, which is not recruited to SG, causes induction of apoptosis in response to some stressors in Cos7 cells. Subsequent studies have generally supported this function. Wei and coworkers showed that recruitment of ROCK1 to SGs inhibits apoptosis and pharmacological inhibition of SG assembly with cycloheximide or EHNA promoted cell death [79], thus affirming that acute SG function in a pro-survival capacity. While this finding is notable, it is unclear whether inhibition of SG themselves, inhibition of protein synthesis, or some other signaling pathway was affected that promoted cell death under their conditions. Finally, recent work indicates that TIA1 oxidation under conditions that promote SG assembly causes impaired SG formation and results in cell death [3]. Conversely, expression of a TIA1 mutant that cannot be oxidized rescues SG formation and impairs apoptosis. Collectively these studies strongly support a pro-survival function for SG formed in response to acute stress (Fig. 2).

Two mechanisms that might explain the pro-survival function of stress granules are the regulation of translation of a specific set of mRNAs or activation of pro-survival cellular signaling pathways independent of the former mechanism. These are not necessarily exclusive possibilities. Evidence for the former mechanism includes the observation that during acute stress, mRNAs for many proto-oncogenes that promote proliferation and survival accumulate in SGs and appear to be sorted based on RNA length and the presence of binding sites for SG resident RNA binding proteins [33,48]. Accumulation of these mRNAs in SGs suggests that they might be specifically regulated by SGs, although ribosome profiling analysis suggests these targets are translated at similar levels as mRNAs not enriched in SGs [48]. This mechanism and its role in promoting the cell survival function of SGs is not well understood; however, it is clear from multiple studies SG do not globally affect mRNA translation. Depletion of SG does not desensitize cells to stress-dependent translation repression.

The latter mechanism, regulation of cellular signaling pathways, is supported by the above-referenced study by Takekawa and colleagues[2]. This work documents repression of MAPK signaling contingent upon RACK1 association with SG. However, a function for acute SG in regulating cellular signaling has also been well-documented in the context of viral infection. SGs induced during viral infection recruit many innate immune proteins, and several studies have revealed that SGs serve as a platform for activation of Protein Kinase R (PKR) and the RIGI dsRNA helicase [53,62,89]. Some picornaviruses including polio-virus, coxsackie viruses, and EMCV cleave the SG nucleating protein G3BP1 to disassemble SG [18,49,86].

This cleavage enhances viral replication and quickly leads to cell death. Ectopic expression of a noncleavable mutant of G3BP1 in this context reduces viral replication and prolonged cell survival [49,86]. This pro-survival function of SGs has also been observed in other contexts, such as some types of cancer. *In vitro*, some sarcoma cells depend on SGs for improved survival [73], and colorectal cancer cells can upregulate SGs as a mechanism to survive chemotherapeutic agents [22]. Together, these studies highlight the need to better understand the specific signaling changes originating from SGs during stress that regulate cell fate. They similarly suggest that other yet-unknown factors may coordinate with SGs in determining cell fate in circumstances where SG regulation may be necessary but not sufficient for eliciting cell death.

3. Chronic stress granules

3.1. Components and conditions

Chronic stress is characterized by prolonged exposure to a given stress condition. Given that the majority of acute SG studies have historically been performed *in vitro* with cells grown on plastic matrices, chronic stress conditions are broadly defined as stresses lasting from four to six hours (or longer) in duration. Studies of chronic stress may intuitively be more relevant to human diseases, given that stresses attributable to pathological circumstances may last days to months dependent on the disease and organ affected. Based on the variation of SG residents under acute stress conditions [5], and data suggesting that chronic SG are fundamentally different from acute SG, there is a current need to better understand the role of SGs during chronic stress conditions and their relevance to disease.

The composition of SGs that assemble under various chronic stress conditions has not been established in the level of detail that has been accomplished for acute stress conditions. However, some recent examples have begun to provide information on the former topic. While many cell culture models of virus infection are acute stresses in which cells are rapidly overcome by the virus and rarely survive, some chronic models also exist. One study utilized a Chikungunya virus replicon to impose chronic stress in the context of a persistent infection. These conditions induced formation of stable NSP3:G3BP1 aggregates lasting days [64]. While these authors did not expect that these RNA granules contained many canonical SG components, this possibility has not yet been rigorously investigated, nor has whether SG formation coincides with global translational repression in this context. During picornavirus infection, normal SGs are disassembled early in infection via cleavage of the SG nucleating protein G3BP1 [18,49,86]. However, EIF4G cleavage by the picornavirus 2A protease during infection may promote formation of persistent, atypical SGs that lack canonical SG components but contain cellular mRNAs. This has led to speculation these persistent SGs would harbor repressed cellular mRNAs, while disassembly of normal SG through G3BP1 cleavage would release viral genomic RNA to promote its translation [88].

Another example where chronic SGs assemble in response to chronic stress is in neurodegenerative disease. Some neurodegenerative diseases are associated with mutations in HNRNPA family members. In this context, these mutant proteins promote the maturation of SGs into irreversible fibrils that enhance the pathology of the disease [23,46]. Previous work indicates that autophagy is partly responsible for SG clearance [7], suggesting that

beneficial effects of autophagy-inducing drugs might be partly owed to enhanced clearance of SG clearance during treatment. Indeed, drugs that promote autophagy have been effective in treatment of neurological disorders [45], and individuals with deleterious mutations in proteins of the autophagic pathway have increased incidence of neurodegenerative disease. These results from the autophagy field suggest that pro-death SG may be induced in the diseased state and might contribute to neuron cell death. Even beyond viral pathogens and deleterious genetic variants, chronic nutrient starvation has been shown to promote SG formation. Similar to acute SGs, chronic SGs arising from a dearth of nutrients contain translation initiation factors, RNA binding proteins, and poly(A) mRNAs. However, these chronic SGs lack 18S rRNA as well as two 40S-associated proteins, RPS6 and RACK1, that are commonly found in SGs under acute stress conditions. SGs forming in response to chronic nutrient starvation do not exchange with cytoplasmic mRNP pools, suggesting their cellular role might vary from the classical paradigm of acute SG function. However, unlike irreversible fibrils in neurodegeneration, the static SGs that form under chronic nutrient starvation are still capable of being cleared once nutrients are restored [60]. This indicates that while nutrient starvation SGs are not dynamic, they do not mature to the state of the irreversible fibrils observed in neurological disease, and therefore may represent a middle ground between dynamic and reversible acute SGs and static and irreversible chronic SGs. This is an emerging area of SG research that will require additional studies to determine the key differences in chronic SG, their relationship with acute SGs, and the contribution of low complexity sequences in each.

3.2. Pathways of induction

While many acute stresses promote SG condensation via eIF2α phosphorylation, chronic stresses have been shown to overcome eIF2α phosphorylation to ultimately reduce SG formation [68]. Alternatively, chronic stress in some contexts can promote increases in eIF2α phosphorylation, such as during the epithelial to mesenchymal transition [15,34] and in neurodegenerative diseases such as frontotemporal dementia and ALS [71]. These conditions are expected to promote SG assembly, although it remains to be formally tested what effect these SGs may have on cell fate.

We recently showed that chronic nutrient starvation conditions in which cells are deprived of glucose, glutamine, and serum induces eIF2α phosphorylation at serine 51. When MEFs homozygous for the S51A mutant of eIF2α were exposed to these conditions, SG did not form and these MEFs were capable of surviving significantly longer than their wild-type counterparts, in which SG readily formed. These data indicate that similar to its role in the context of acute stresses, eIF2α phosphorylation is important for induction of SG formation under chronic nutrient stress conditions (Fig. 1). This likely occurs via activation of PKR-like endoplasmic reticulum kinase (PERK). It is unclear whether impairment of the cap-binding complex important for protein synthesis initiation is also important for SG formation under these conditions; however, it is notable that mTORC1 is rapidly shut down under these conditions despite the presence of amino acids within the culture medium.

While both acute and chronic stress granules require eIF2α phosphorylation, at least under most conditions described to date, there are significant differences in the systems used to

study SG induction. Under acute conditions, the dose of the stress is typically much higher than would be expected to be encountered under physiological conditions. Furthermore, the time frame before analysis is shorter thereby preventing analysis of any adaptive changes the cell may use to counter the effects of the stress. Therefore, while much work has been done to investigate cell survival in response to acute stress conditions, this work may describe only initial sensitivity of a given cell type to a given stress. Thus, future work should delineate how acute and chronic stress yield different responses despite similar translation repression. The differing composition and function of acute and chronic SG is one mechanism that might help to explain these different cellular responses.

3.3. Key interactions within SG

The protein:protein and RNA:RNA interactions that serve to hold chronic SG together are likely to be similar to those that have been described for acute SGs. While the interactions that serve to hold chronic SGs together have not yet been well-defined, yet some basic principles have been observed. As in the context of acute stresses, depletion of G3BP1 and G3BP2 completely impairs SG condensation under chronic stresses. This suggests that specific protein:protein interactions associated with these proteins reprise some role in SGs arising from chronic nutrient starvation. However, a role for the G3BP1 and G3BP2 interactions with CAPRIN1 and/or USP10 under chronic nutrient starvation has not yet been investigated. Yet, structural differences between chronic and acute SGs may mediate differential recruitment or exclusion of distinct factors that ultimately engender an altered impact on cell fate. Indeed, RACK1 is absent from chronic nutrient starvation SGs. Therefore, while the interactions between the SG nucleating factors G3BP1 and G3BP2 are likely similar between acute and chronic SG, one can reasonably conclude that these interactions are unlikely to affect cell fate by themselves.

These initial findings in chronic nutrient starvation models parallel results from studies in neurodegenerative diseases suggesting that prolonged aggregation of SG proteins promote assembly of fibrils that eventually recruit pathological aggregates that induce neuronal cell death [4,84]. In fact, expression of RNA binding protein mutants associated with neurodegenerative disease (e.g. FUS, HNRNPA1, C9ORF72, and ATAXIN2) all promote SG aggregation. This is in large part associated with intrinsically disordered domains, and these interactions have been suggested to promote the fibril assembly associated with disease [23,46,47].

Finally, as with acute SGs, it is reasonable to speculate that RNA:RNA interactions are involved in chronic SG formation. Nucleotide repeats within the C9ORF72 RNA, again associated with neurological disease, have been shown to assemble into G-quadruplexes that promote assembly of stress granule-like structures [13]. However, further studies are required to investigate the relative contribution of RNA and protein in phase separation of SGs. While the SG that form in neurological disease differ from those forming under acute stresses, they also differ from those that form under chronic nutrient starvation. Unlike in neurodegenerative diseases, chronic nutrient starvation induces SG that are reversible despite not being dynamic as observed under conditions of acute stress. To explore the possibility of targeting chronic SGs for specific pharmacological interventions, much work remains.

3.4. Pro-death function

While the individual signals and mechanisms remain largely unclear, it is clear that cellular context, stress type, and stress duration may have largely different effects on SG composition and function. SGs are important for cancer cell viability and stress resistance [22,73] when cells are exposed to acute stresses *in vitro*. However, in an *in vivo* tumor setting cells are often exposed to chronic stress, leading to the question of whether chronic nutrient starvation induced pro-survival SG similar to acute stress conditions. One of the most well-accepted strategies for inactivating SG is to deplete the SG nucleating proteins G3BP1 and G3BP2. Using this model in the context of chronic nutrient starvation, we found that SG depletion resulted in improved survival. These results suggest that at least some chronic stresses induce pro-death SG in contrast to acute stress conditions (Fig. 2).

The pro-death function we observed during chronic nutrient starvation is reminiscent of studies of acute stress with either H_2O_2 or selenite. These stresses induce non-canonical SG formation, and selenite-induced SG have been suggested to function in a pro-death capacity [12,17]. However, the SGs described in these previous studies differ in composition and are induced by different pathways of translation repression as compared to chronic nutrient starvation. Given that these studies also predated a robust strategy for SG depletion, it remains unclear whether SGs themselves or regulation of translation of subsets of mRNAs is primarily responsible for the pro-death effects observed within this context.

4. Targeting SG or SG subcomplexes in disease

4.1. Rationale for targeting SGs in disease

Many therapies target single proteins that are overexpressed or activated in a given disease state. However, despite the protein concentration or activity being elevated in diseased cells, the therapeutic index of these approaches may be suboptimal since the protein may also be expressed (at lower levels) in unaffected tissues. Because SGs appear only during stress conditions, they have the potential to be a comparatively tractable target for drug discovery (under the rationale that only stressed cells would be expected to be vulnerable to drugs targeting SG-dependent functions). Specific interactions within SGs that depend on interaction of globular domains which are critical for SG assembly could be targeted using conventional drug design platforms. Nonspecific, weak interactions contributed by intrinsically disordered domains could also potentially be exploited to interfere with SG assembly or function. While this strategy would be more difficult because of the importance of these domains in cellular signaling and other processes, recent work has suggested it would be both possible and beneficial to disrupt nonspecific and weak interactions within SGs in the context of neurological disease [23]. Finally, regulation of subsets of mRNAs by SG resident complexes is another mechanism that could be exploited to influence cell fate of diseased cells. Together, these strategies reveal a vast array of strategies that can be exploited to target SG.

4.2. Molecular targets to exploit

In order to effectively target SG within disease, it will be important to understand which type and function of SG might need to be targeted in a given context. For example, while it would

be useful to inhibit pro-death chronic SGs to preserve functioning neurons in the context of neurodegenerative disease, inhibition of SGs functioning as pro-survival factors during chemotherapeutic stress would render cancer cells more sensitive to existing interventions.

Both acute and chronic SGs could be concomitantly targeted by the inhibition of protein:protein interactions common to both entities. This would be useful in cases where SG can be locally targeted with nano-particles or similar approaches, or in cases in which only one of the acute or chronic SGs are forming. For example, G3BP1 and G3BP2 are present in both acute and chronic SG and regulate their assembly. G3BP1 is bound by the small molecules resveratrol and the polyphenol epigallocatechin gallate (EGCG) [52,69]. While a role for these small molecules in regulating SG assembly has not been directly shown, Oi et al did demonstrate that resveratrol interacts with the same N-terminal pocket in G3BP1 mediating the interaction of this protein with CAPRIN1 to promote SG assembly [31,52,55]. Therefore, inhibition of SG via G3BP1 (and likely also G3BP2) might be achieved by employing derivatives of these compounds, which have already been proven to be safe (Table 1). Interestingly, this same N-terminal pocket bound by resveratrol has also been shown to interact with the FGDF domains in USP10 and the Alphavirus protein NSP3 [55], which inhibit SG assembly by competing with CAPRIN1 binding. Alphaviruses subvert G3BP1 function by recruiting this protein from SGs and into replication factories via NSP3, promoting viral genome replication [16,54]. Peptides derived from NSP3 or other FGDF-containing proteins have the potential to specifically target cells under stress without affecting adjacent unstressed cells. Because recent research has shed light onto the structural determinants of SG formation, it is now feasible to develop drugs that target key interactions within the SG similar to those described above (Table 1). Of course, this possibility will become more attractive as additional studies are published in this area, and additional protein:protein interactions critical to SG formation that might confer vulnerability to this process may remain to be discovered.

One strategy to specifically perturb pro-survival SGs would be to target the PTMs needed for SG assembly and signaling. For example, HDAC6 and the arginine demethylase JMJD6 have been shown to promote SG formation [35,81]. In contrast, the arginine methyltransferases PRMT1, 5 and 8 have been shown to antagonize SG assembly. Therefore, inhibition of HDAC6 or JMJD6 and/or activation or PRMT 1, 5 or 8 may be a feasible approach to SG inhibition. It is important to note, however, that strategies targeting these molecules may suffer from drawbacks associated with indirect and epigenetic effects, and it is too early to tell whether the same PTMs are important in facilitating assembly of chronic SGs.

In contrast, specific inhibition of pro-death chronic SGs may be achieved via multiple approaches. Shorter and colleagues show that pathological aggregates in neurodegenerative disease can be reversed by transient expression of the nuclear import receptor Karyopherin-β2, which appears to interfere with intermolecular interactions responsible for fibril formation [23]. While the understanding of this mechanism is in its infancy, it is possible that small peptides that phenocopy this effect could be developed to improve survival of neurons harboring toxic fibrils (Table 1). Another strategy to target chronic SGs in neuro-degenerative disease would be to pharmacologically promote autophagy, which is known to

contribute to SG clearance, would eliminate pro-death granules and resulting toxic fibrils, and has received attention for beneficial responses in mouse models of various neurological diseases (reviewed in [45]). Additionally, variants of the yeast disaggregase Hsp104 have been shown to disassemble FUS and TDP-43 aggregates that have been associated with ALS and FTD [70], and the human nuclear transport Karyopherin-β2 has been shown to reverse FUS, TAF15, EWSR1, HNRNPA1, and HNRNPA2 aggregates associated with disease [23]. Modifications of disaggregation by these molecules may prove useful in the clinic in the near future.

In metastatic cells, one would most likely want to promote assembly of pro-death SGs in an attempt to cull migrating cells responsible for disease progression. While pro-survival SGs are thought to form in response to chemotherapeutic agents [22], it might be possible to convert these SGs to pro-death SGs as more studies are done to characterize and mechanistically define chronic pro-death SGs. If treatment exploited the different characteristics of pro-death and pro-survival SGs to inhibit the pro-survival ones, drug resistant cell populations could likely be curbed resulting in treatment with higher efficacy.

5. Future directions

The significant recent advances in the understanding of SG function have tremendous implications for the importance of these cellular compartments in cellular function and disease pathology; however, these advances also highlight how much there still is to learn. Chronic stress is highly relevant to disease states including ischemia, neurological disease, cancer and even prolonged viral infection. Many signaling molecules relevant to disease states have been shown to concentrate in acute SGs, but only a few have been shown to be regulated by SGs (e.g. PKR, mTORC1, RIG-I and TRAF2), and none have been characterized within the context of chronic stress. Chronic stress involves adaptive mechanisms to promote cellular fate and function that rely on changes in gene expression both at the transcriptional and post-transcriptional levels. Recent studies in U2OS cells show that acute SGs do not regulate global translation [31,48,63], although regulation of subsets of mRNAs within these contexts remain to be extensively characterized. Do chronic SGs regulate translation globally, or that of context-specific functional subsets of mRNAs? The latter possibility has the potential to be crucial to adaptive mechanisms impacting the response to the stress, cell fate decisions, and ultimately to the progression of disease. Acute SGs concentrate many mRNAs for protooncogenes, and enhanced translation of subsets of mRNAs in cancer models has previously been shown to promote cancer progression [9,24], suggesting the SGs arising from chronic stress conditions may function via analogous mechanisms. However, the mechanisms responsible for selecting mRNAs for SG incorporation are not known. Finally, robust studies showing that SG are important in whole animals in the context of disease are sparse. It is imperative moving forward to explore these topics as well as to better understand the regulatory mechanisms and context of chronic SG function in disease to effectively target SGs and promote a desired cellular response. Studies of acute SG will provide insight into the mechanisms for study under chronic stress conditions and have the potential to be used as a roadmap in these newer studies. Completion of these studies are necessary before extensive resources should be committed to development of drugs targeting SG in disease.

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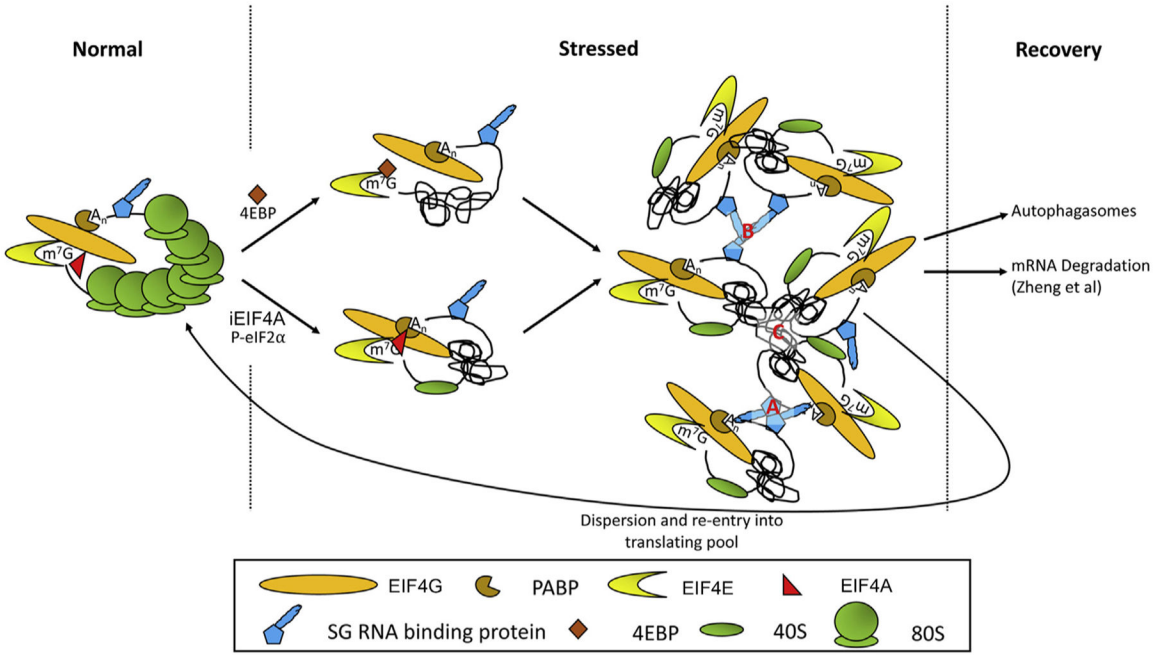

Fig. 1.

General features of acute stress granules. During unstressed conditions, mRNAs generally exist in a complex with ribosomes and translation initiation factors, as well as RNA binding proteins that stabilize the mRNA and promote its translation. During stress conditions, the cap-binding complex is disassembled or 40S ribosomes are stalled within the 5′UTR. Stress promotes disassembly of the cap-binding complex or inhibits 40S scanning via activation of 4EBPs, inhibition of EIF4A activity (iEIF4A), or inactivation of eIF2 by promoting eIF2α phosphorylation. Bound 80S ribosomes present within the open reading frame run off the mRNA under these conditions leaving exposed mRNA that collapses onto itself and is subject to RNA folding. At this point, RNA binding proteins also associate with the mRNA and the resulting complexes assemble into SG dependent on specific (A) and non-specific (B) protein:protein interactions requiring globular and disordered domains, respectively, as well as RNA:RNA interactions (C) resulting from free mRNA.

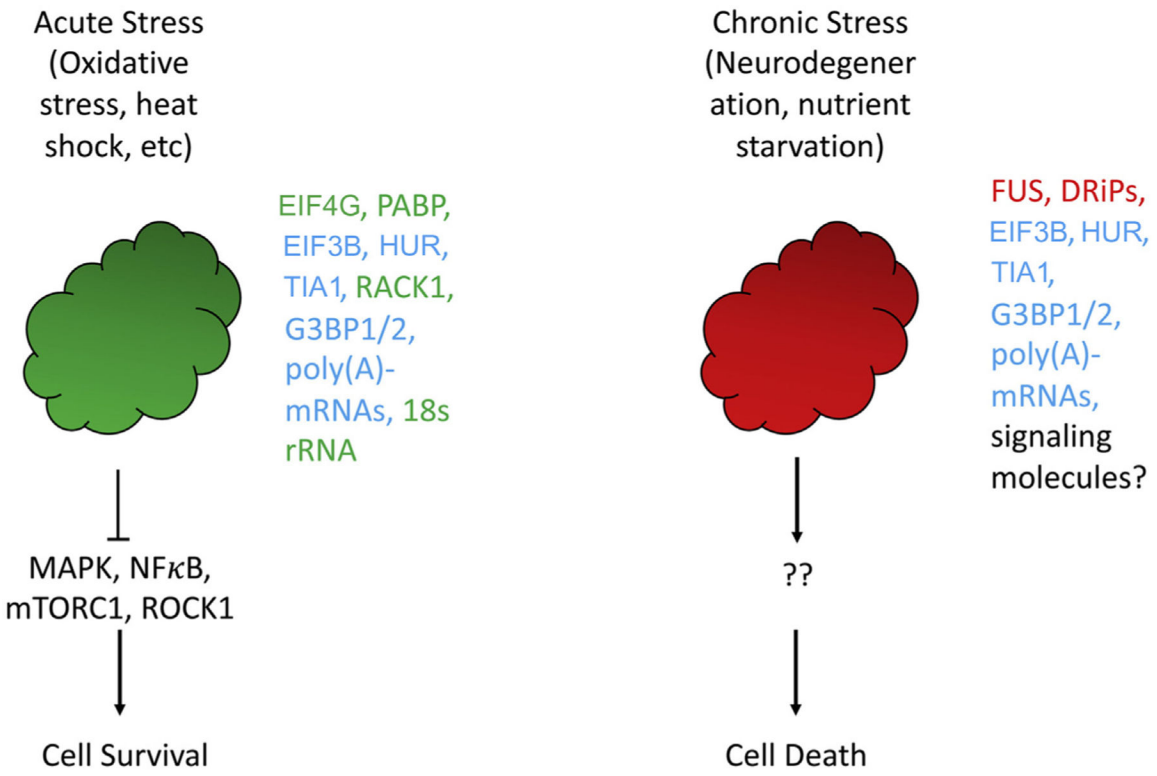

Fig. 2.

Acute and chronic SG differ in composition and effects on cell fate. Acute stress granules (left) contain many signaling components as well as 40S ribosomes, are very dynamic, and have a pro-survival function. In contrast, chronic SG (right) lack 40S ribosomes, are static and have a pro-death function. Components known to exist within each type of SG are listed (green, acute SG-specific; blue, present in both acute and chronic SG; red, chronic SG-specific).

Table 1

Proteins and treatments that can interfere with SG assembly.

SG Protein	Treatment/Effectors	Description	Reference
TDP43/FUS	VEGF	VEGF treatment of cells in CSF from ALS patients	Shantanu et al. [67]. J. Chem. Neuro.
C9ORF72 *	Antisense Therapy	Development of antisense oligonucleotides against *C9ORF72*	Andrew Scott. (2017). Nature. Ionis Pharmaceuticals
FUS	Rapamycin, Torkinib, Paroxetine, promethazine	Autophagy Inducers	Marrone et al. [42]. Stem Cell Reports.
TDP43	Auranofin, chlerythrine, riluzole	Thioredoxin reductase inhibitor, TTX-sensitive sodium channels	Oberstadt et al. [50]. Scientific Reports.
G3BP1 *	Epigallocatechin gallate	Direct G3BP1 binding	Sim et al. [69]. Cane. Prev. Res.
G3BP1 *	Resveratrol	NTF2-like domain of G3BP1	Oi et al. [52]. Oncogene.
G3BP1	NSP3 and USP10 FGDF tetrapeptides	Interacts with the NTF2-like domain of G3BP1	Panas et al. [55], Plos Pathog.
TDP43, FUS, α-synuclein *	Hsp104p variants	Disaggregation of toxic proteins	Jackrel and Shorter [25]. Prion.
FUS, TAF15, EWSR1, hnRNPA1, hnRNPA2	Karyopherinβ2 and Importinα-karyopherinβ1	Disaggregation and relocalization of RNA binding proteins to the nucleus	Guoet al. [23]. Cell.
Defective ribosomal products (DRiPs)	HSPB8-BAG3-HSP70 and HSP70	Disrupts toxic protein aggregation	Ganassi et al. [19]. Mol. Cell, [43]. EMBO J.
G3BP1	PRMT1 and 5	Antagonizes SG condensation	Tsai et al. [74,80]. J. Biol. Chem.
G3BP1	JMJD6	Depletion enhances SG assembly	Tsai et al. [82]. J. Biol. Chem.
Unknown	Staufen1	Binds dsRNA to inhibit SG condensation	Thomas et al. [77]. J. Cell Sci.

* These studies did not specifically look at SGs, but it can be inferred that these might be useful in their inhibition.