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Review Article

Is bRaQCing bad? New roles for ribosome associated quality control factors in stress granule regulation

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Maintenance of proteostasis is of utmost importance to cellular viability and relies on the coordination of many post-transcriptional processes to respond to stressful stimuli. Stress granules (SGs) are RNA–protein condensates that form after translation initiation is inhibited, such as during the integrated stress response (ISR), and may facilitate cellular adaptation to stress. The ribosome-associated quality control (RQC) pathway is a critical translation monitoring system that recognizes aberrant mRNAs encoding potentially toxic nascent peptides to target them for degradation. Both SG regulation and the RQC pathway are directly associated with translation regulation, thus it is of no surprise recent developments have demonstrated a connection between them. VCP's function in the stress activated RQC pathway, ribosome collisions activating the ISR, and the regulation of the 40S ribosomal subunit by canonical SG proteins during the RQC all connect SGs to the RQC pathway. Because mutations in genes that are involved in both SG and RQC regulation are associated with degenerative and neurological diseases, understanding the coordination and interregulation of SGs and RQC may shed light on disease mechanisms. This minireview will highlight recent advances in understanding how SGs and the RQC pathway interact in health and disease contexts.

Introduction

Stress granules (SGs) are membraneless RNA–protein condensates that form in response to many environmental or cell-intrinsic challenges after translation initiation is arrested. The integrated stress response (ISR) is activated during a variety of stress conditions such as ER stress, viral infection, heat, cold, or toxic metalloids. During the ISR, any of four eIF2α (eukaryotic initiation factor 2α) kinases are activated and trigger eIF2α phosphorylation to inhibit ternary complex production. This in turn suppresses translation initiation, resulting in polysome disassembly and the release of mRNAs that are sequestered to SGs by specific RNA binding proteins (RBPs) [1,2]. This process results in SGs composed of non-translating mRNAs, non-coding mRNAs, translation initiation factors, 40S ribosomal subunits, RBPs including G3BP1 (Ras GTPase-activating protein-binding protein 1), TIA1 (TIA1 cytotoxic granule associated RNA binding protein) and UBAP2L (ubiquitin associated protein 2 like), and other proteins with intrinsically disordered regions [3–5]. SGs are disassembled or cleared rapidly once the stress is removed through context-dependent mechanisms such as autophagy [6] or ubiquitin-dependent G3BP1 degradation [7]. Although the exact biological function of SGs is a question of active investigation in the field, advances in uncovering the determinants of their dynamics and composition has given insight into the role of SGs in translation regulation and suggested possible roles in the pathology of several diseases [8].

The ribosome-associated quality control (RQC) pathway facilitates the degradation of nascent peptides that result from translation of aberrant mRNAs, and is a critical mechanism to monitor translation elongation. mRNA features that induce the RQC pathway include premature termination codons

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[9], internal poly(A) tracts [10], absence of stop codons [11,12], and long 3′ untranslated regions [13]. Translation of these aberrant mRNAs can lead to toxic protein aggregates, nonfunctional, and/or dominant-negative proteins that are detrimental to cell viability. Thus mRNAs containing these features are subject to mRNA surveillance machinery including the nonsense-mediated decay, no-go decay, and non-stop decay pathways. Furthermore, during translation elongation, these defective mRNAs cause ribosomes to stall and collide, triggering 40S and 60S ribosomal subunits to split and subsequently activate the RQC pathway [14]. The nascent peptides are then tagged for degradation in a ubiquitin-mediated manner and the ribosomal subunits can be recycled for another round of translation. Mutations in the genes that encode RQC factors are implicated in degenerative diseases of the muscular, skeletal, and nervous systems [15–17] emphasizing the importance of the RQC in human health.

Recent work revealed three major connections between the regulation of SGs and the RQC pathway. First, the proteasome-associated valosin-containing protein (VCP), which aids in the degradation of the nascent peptide chain during translation quality control [18], has also been implicated in regulating SG assembly [19,20] and disassembly [6,7,21]. Second, ribosome stalling and collisions trigger eIF2α phosphorylation subsequently activating the ISR, the key upstream signaling pathway for SG formation [22–24]. Third, G3BP1, a key player in SG assembly and composition, associates with the 40S ribosomal subunit and aids 40S subunit recycling after RQC activation [25]. Given the detrimental disease phenotypes (e.g. degenerative diseases, cancers, and vascular system diseases) of mutations in RQC and SG genes (e.g. *VCP*, *G3BP1*, and *GCN2*), understanding how these processes are related and the functions of their overlapping protein players will be critical for novel therapeutic approaches. These observations together raise the question of whether ribosome stalling, or 'bRaQCing', can trigger and/or perturb the stress response. This minireview will highlight some of the key findings connecting the RQC pathway to SG regulation.

VCP links translation regulation to stress granule dynamics and composition

VCP/p97 is an evolutionarily conserved AAA+ ATPase critical for cellular homeostasis and survival [26]. In addition to its role in the RQC pathway, VCP is a central proteostasis factor that regulates the dynamics of SG assembly and disassembly. Multiple lines of evidence show VCP is crucial for SG clearance and formation in specific stress contexts including heat and arsenite stresses [6,19,21,27,28]. Recent work has connected VCP's role in SG regulation and RQC in a similar yet distinct stress-activated RQC (saRQC) pathway [20]. The following paragraphs will summarize the evidence supporting VCP's role in both pathways as well as introduce its role as an RQC factor regulating SG composition.

Several studies support the model that VCP (Cdc48 in yeast) promotes the degradation of the ubiquitinated nascent peptide in the RQC pathway. First, electron microscopy and biochemical assays identified Cdc48 to be in complex with RQC factors including Ltn1, Rqc1, Rqc2, and the 60s ribosomal subunit [18]. Genetic depletion of these factors in cells expressing non-stop mRNA reporters resulted in aberrant nascent peptides co-sedimenting with the ribosome [29]. Polysome profiling revealed a build up of proteins generated from a nonstop reporter on both 60S and 80S fractions in yeast with non-functional Cdc48 [30]. Other studies identified an accumulation of aggregated proteins encoded by non-stop reporter mRNAs in VCP-depleted cells using western blot analysis, further supporting the model that VCP facilitates the degradation of these nascent chains. Proteasome inhibition with the small molecule MG132 in cells expressing RQC-targeted mRNA reporters mirrored this phenotype, suggesting that VCP aids nascent protein degradation via the proteasome [31]. Given that other studies identified the ubiquitin ligase Ltn1 is responsible for the addition of ubiquitin to nascent peptides during RQC, a proposed model for VCP is the recognition and elimination of these peptides by unfolding and/or releasing nascent proteins to facilitate proteasome-mediated degradation (Figure 1).

Interestingly, VCP is implicated in SG assembly beyond its role in the RQC pathway. siRNA-mediated VCP depletion results in impaired SG assembly or accretion, as fewer large SGs and more small SGs form upon arsenite stress in this condition [19]. Established VCP cofactors UFD1 and PLAA that are implicated in defective ribosomal product (DRiP) clearance [30] and ribosome degradation in yeast [36], respectively, are also required for proper SG assembly upon arsenite stress. siRNA-mediated depletion of UFD1 or PLAA resulted in similar SG phenotypes as VCP depletion [19]. These results suggest that VCP promotes SG assembly and may play a role in determining SG protein composition by modulating the proteome.

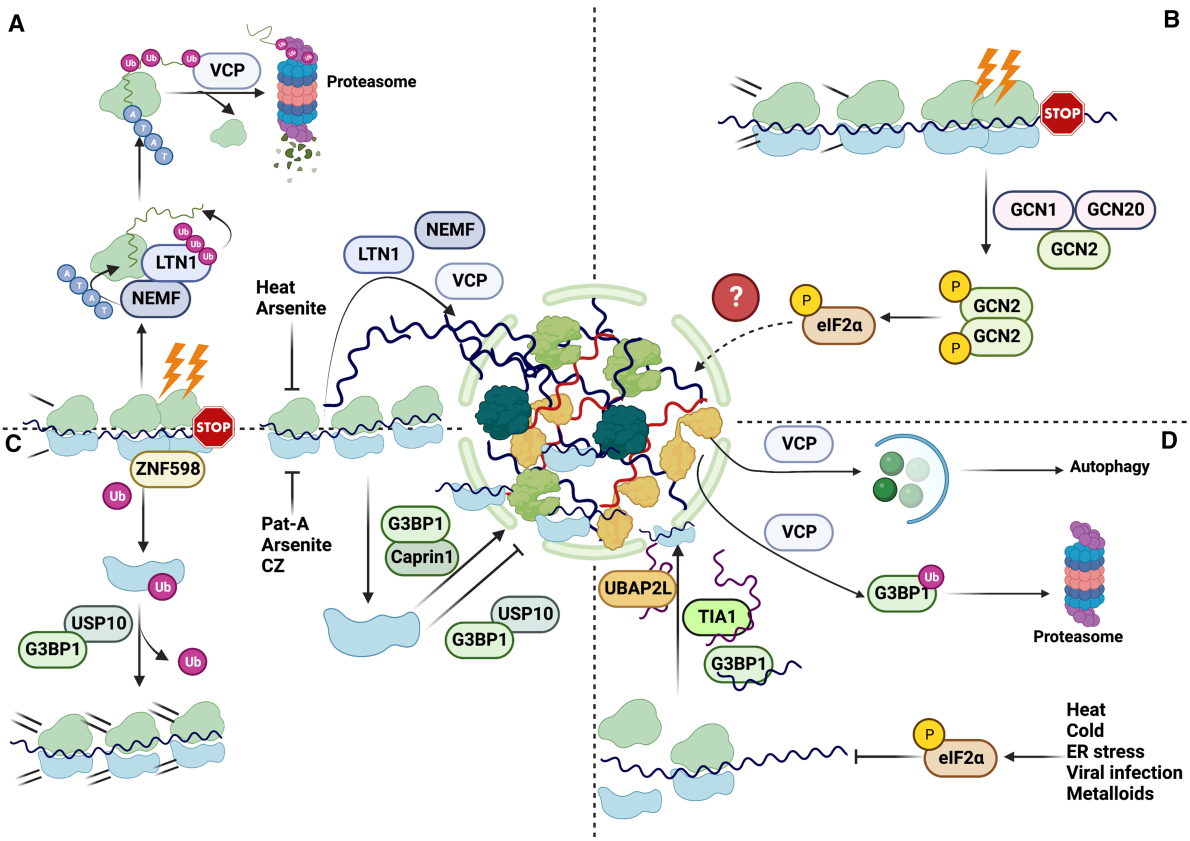

Figure 1. Proposed mechanisms by which stress granules and the RQC pathway intersect.

(**A**) Overview of the RQC and saRQC pathways. In the RQC, translation of aberrant mRNAs induces ribosome stalling and collisions that are recognized by ZNF598, triggering ribosome splitting [32]. NEMF then recognizes the 60S ribosomal subunit and catalyzes the addition of carboxy-terminal alanine threonine ('CAT tails') onto the nascent peptide [33]. NEMF also stabilizes LTN1 which adds ubiquitin to the nascent chain [14]. The ubiquitinated nascent chain is extracted from the 60S ribosome by VCP and unfolded for proteasome- mediated degradation [29]. In the saRQC, VCP, NEMF, and LTN1 may aid in the partitioning of specific RNAs to SGs following arsenite or heat stress [20]. (**B**) Ribosome stalling and collisions activate the ISR through GCN2. GCN2 dimerizes, autophosphorylates, and phosphorylates eIF2α to trigger the ISR and potentially cause SG formation in a mechanism that may depend on GCN1 and GCN20. (**C**) Storage and recycling of the 40S ribosomal subunit is mediated by G3BP1. Upon ribosome collisions, ZNF598 ubiquitinates the 40S ribosomal subunit [34] which can in turn be recognized and removed by G3BP1-family-USP10 to recycle the 40S subunit for another round of translation [25]. In stress conditions such as arsenite, clotrimazole (CZ), and pateamine A (Pat A), 40S subunit proteins co-localize with SGs [35]. When G3BP1 associates with the cytoplasmic activated/proliferation-associated protein 1 (Caprin1), SG formation is promoted. In contrast, when G3BP1 associates with USP10, SG formation is inhibited [35]. (**D**) Roles of RQC factors in SG assembly and disassembly. In response to environmental and cell intrinsic stressors, eIF2α can be phosphorylated thus limiting ternary complex production to suppress translation initiation [2]. This in turn leads to the formation of SGs that are composed of various RBPs, 40S subunit proteins, and RNAs. SGs can be cleared by autophagy [19] or ubiquitination and degradation of the SG protein G3BP1 in stress-specific contexts [7].

Three pieces of evidence suggest VCP is critical for SG clearance (Figure 1). First, VCP colocalizes with heat or arsenite SGs in HeLa cells [6,21] and co-purifies with stable SG sub-structures upon arsenite stress in U-2 OS cells [37]. Interestingly, VCP does not co-localize with arsenite SGs at early time points [20] or at lower arsenite concentrations [38], when SGs are smaller and more dynamic in U-2 OS cells [39]. In-line with this interpretation, VCP accumulates over time in optogenetically induced SG-like G3BP1 granules in U-2 OS cells, which become less dynamic over time [40]. These observations suggest that VCP is likely recruited to stable, less dynamic SGs, perhaps directly aiding in their disassembly by remodeling ribonucleoproteins.

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Second, VCP depletion or inhibition delays SG clearance during the recovery from stress [6,7,41]. A fluorescence microscopy-based gene deletion screen in yeast, in addition to chemical inhibition and siRNA knockdown of VCP experiments in human cells, suggested VCP plays a critical role in heat-induced SG clearance [6]. Live and fixed cell fluorescence microscopy studies have demonstrated delayed SG clearance upon VCP knockdown or VCP inhibition following heat [7] or arsenite stress [6,41]. The proteasome inhibitor bortezomib also caused delayed arsenite and heat SG clearance [41]. These observations together support VCP and the proteasome playing a key role in SG clearance following stress.

Third, disease-associated VCP mutants including A232E and R155H variants may impair SG dynamics in specific contexts. Transient expression of VCP-A232E or VCP-R155H was associated with constitutive SGs in HeLa cells [6], but not in murine skeletal muscle C2C12 cells [27]. Interestingly, over-expression of VCP-A232E or VCP-R155H is associated with increased localization of a representative saRQC target mRNA, *AHNAK*, within SGs in U-2 OS cells, suggesting the RNA composition of SGs may be altered in disease contexts [20]. Transient expression of pathogenic VCP mutants was also associated with persistent SGs after removal of arsenite stress in HeLa cells [21] and C2C12 cells [27]. However, while SG clearance was significantly delayed following heat stress in U-2 OS or HeLa cells expressing VCP-R155H or VCP-A232E [7,28], C2C12 cells expressing these mutants displayed no such phenotype [27]. Therefore, VCP dysfunction impairs SG clearance, seemingly in a stress and/or cell type specific manner.

Intriguingly, VCP, LTN1, and NEMF may also regulate the mRNA composition of SGs in a unique saRQC pathway. Live and fixed cell fluorescence microscopy experiments revealed a novel role for VCP, LTN1, and NEMF in regulating the partitioning of certain mRNAs to SGs [20]. Chemical inhibition of VCP resulted in the accumulation of reporter mRNAs associated with nascent protein chains during arsenite stress. Furthermore, VCP inhibition caused a decrease in the localization of specific mRNAs to SGs that could be rescued by co-treatment with puromycin, which ejects mRNAs and nascent proteins from ribosomes [20]. Inhibition of the proteasome or knockdown of LTN1 or NEMF phenocopied VCP inhibition [20]. These observations suggest that VCP and other canonical RQC factors play a role in releasing mRNAs from translation complexes to facilitate their localization to stress granules. Because a poly-lysine tract-containing RQC target transcript did not depend on VCP for partitioning into SGs, and intact translation complexes accumulated upon VCP or proteasome inhibition during arsenite stress, VCP may be part of a unique saRQC pathway that targets different mRNAs than the canonical RQC pathway. Taken together, these observations underscore a critical role for VCP and other RQC factors in the regulation of SG assembly, composition, and disassembly that must be further explored.

Ribosome stalling and collisions trigger the ISR

GCN2 (general control nonderepressible 2) is a serine/threonine kinase that phosphorylates eIF2α upon amino acid starvation and other stresses to activate the ISR. Through microarray analysis coupled with gel shift and northern blot assays, GCN2 was shown to be activated by binding uncharged tRNAs [42–45]. The ribosomal P-stalk was also shown to activate GCN2 both *in vitro* and *in vivo* [46,47], exemplifying a second distinct mechanism of GCN2 activation independent of uncharged tRNAs. Exciting recent advances that we detail below now point to a role for GCN2 in detecting ribosome collisions, which can result from strong ribosome stall sites. The results of these studies together suggest a mechanism by which the ribosome can activate GCN2 and subsequently induce the ISR to inhibit translation initiation, a key signaling pathway that drives SG formation. This section will highlight the recent evidence that the canonical RQC triggers of ribosome stalling and collisions activate the ISR through GCN2.

Various lines of evidence support the notion that ribosome stalling and collisions activate GCN2 and subsequently activate the ISR (Figure 1). First, analysis of a mouse model lacking the nervous system-specific $tRNA^{Arg}_{UCU}$ and the ribosome rescue factor *Gtpbp2* (GTP-binding protein 2) by Ishimura and colleagues identified ribosome stalling at arginine codons as well as ISR hallmarks including P-eIF2α and increased levels of the stress-induced gene ATF4 (activating transcription factor 4) [22]. ISR induction was shown to be triggered by GCN2 activation, as *Gcn2*$^{-/-}$ mice did not display the increased P-eIF2α levels [22]. These data demonstrated that ribosome stalling can trigger the ISR through GCN2.

Second, intermediate levels of ribosome collisions by low-intermediate concentrations of chemical inhibitors of translation elongation activate GCN2 and subsequently the ISR. The elongation inhibitors including anisomycin (ANS), emetine (EME), methyl methanesulfonate (MMS), and cisplatin were shown to activate GCN2 at low-intermediate concentrations predicted to stall some, but not all, ribosomes and thus leads to collisions

[23,24,48]. Polysome profiling revealed an increase in disomes at intermediate levels of ANS and EME, suggesting the induction of ribosome collisions [23]. Interestingly, amino acid starvation also induced collisions [23]. Analysis of P-eIF2α levels under these conditions in GCN2 depleted or inhibited cells demonstrated that GCN2 is responsible for eIF2α phosphorylation and ISR activation [23,49]. Polysome profiling demonstrated that the GCN2 co-activator, GCN1, associated with disomes [23] and cryo-electron microscopy structures of collided disomes revealed a specific interaction of GCN1 with the ribosomal P-stalk [50]. GCN20, another GCN2 cofactor, was not found to be associated with disomes [23] and the GCN20 binding domain of GCN1 was poorly resolved in structural analysis [50]. These observations may suggest GCN20 transiently associates with GCN1 and GCN2, as depletion of GCN20 was found to increase cell survival upon ANS treatment and protein interactome studies revealed overlapping GCN20 protein interactomes with GCN1 and the ribosome collision sensor ZAKα [23]. These data suggest that when ribosomes stall and collide, GCN2 is activated by GCN1 and GCN20, potentially through a mechanism involving the ribosome P-stalk and its associated proteins (Figure 1). Interestingly, western blot analysis detected activated forms of p38 and c-Jun N-terminal kinase (JNK) at intermediate elongation inhibitor concentrations, suggesting that these collisions can also trigger cell-cycle arrest and apoptosis in addition to elevated P-eIF2α levels [23]. These data together suggested the model that while low levels of ribosome stalling and collisions can trigger the ISR to feedback and inhibit translation initiation when elongation is perturbed, apoptosis could be triggered through JNK activation when severe ribosome stalls and collisions cannot be resolved.

Another proposed model of the intersection of the RQC and the ISR suggests that the RQC and ISR pathways act antagonistically. Specifically, low levels of ribosome stalling and collisions are proposed to activate the RQC, while high ribosome stalling and collision frequency activates the ISR [24]. The data supporting this model are as follows. In Hel2 (*ZNF598*) knockout yeast, ribosome collisions caused by intermediate concentrations of MMS, which inhibits translation elongation by alkylative damage of mRNA [51], resulted in increased P-eIF2α levels [24]. Interestingly, ubiquitinated ribosomal protein levels within the cell increased in both *Gcn2* knockout yeast and yeast expressing a phosphorylation-deficient serine to alanine mutation at residue 52 in Sui2 (the eIF2α homolog in yeast) upon MMS treatment [24,48]. This suggests a mechanism where GCN2 is responsible for inhibiting translation initiation via the ISR to prevent translation of aberrant mRNAs and increased RQC activation in these conditions is a result of continued translation as translation initiation cannot be suppressed via the ISR [24].

Collectively, these studies of GCN2 activation by ribosome collisions suggest that at low levels of ribosome stalling and collisions, the RQC is activated and thus allows bulk translation to resume. Intermediate or high levels of ribosome stalling and collisions then activate the ISR potentially through GCN1/20 and GCN2, thus inhibiting canonical translation initiation and promoting cell survival. Finally, high levels of ribosome collisions correlating with high levels of mRNA damage in the cell may overwhelm the RQC and the ISR, in turn inducing apoptosis [23]. Therefore, these studies demonstrate that the RQC pathway intersects with the ISR, a key upstream pathway for stress granule formation. However, more work needs to be done to understand precisely how the ISR and RQC pathways are coordinated across eukaryotes and determine whether ribosome collisions can trigger SG formation.

G3BP1 regulates storage and recycling of the 40S ribosomal subunit

G3BP1 is a key stress granule protein through its RNA-binding and phase-separating functionalities [52]. G3BP1 is a central node of the RNA–protein network in stress granules, and is essential for SG assembly in numerous stress contexts [35]. Multiple lines of evidence have also implicated G3BP1 in recycling of the 40S ribosome after RQC activation. When ribosome collisions occur, the E3 ubiquitin-protein ligase ZNF598 recruits UBE2D3 (ubiquitin conjugating enzyme E2 D3) [53] to monoubiquitinate two lysine residues on ribosomal protein S10 (RPS10) [34]. ZNF598 also ubiquitinates RPS3 [32,54] and RPS20 [54], yet mutagenesis studies of these proteins with poly(A) reporter mRNAs suggest this regulation may not be directly related to RQC [32,54]. As ubiquitination generally targets proteins for degradation, these events could be interpreted as a destruction signal for a potentially defective 40S ribosome.

However, some findings suggest that these ubiquitination events are instead a regulatory mechanism for 40S ribosome recycling involving G3BP1 and the deubiquitinase ubiquitin specific peptidase 10 (USP10). USP10 plays a role in a diverse array of cellular processes [55], but recently was identified to be in a

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G3BP1-family-USP10 complex on the 40S ribosomal subunit based on co-immunoprecipitation experiments [25]. Examination of ubiquitination, stoichiometry, and degradation of 40S subunits in cells expressing RQC substrate transcripts (e.g. those with internal poly(A) tracts) and carboxy-terminal alanine threonine (CAT) tail activators (see Figure 1) suggested that G3BP1-family-USP10 complex deubiquitinates the 40S ribosomal subunit to prevent it from lysosomal degradation [25]. Low doses of translation elongation inhibitors that result in RQC activation showed similar results further supporting this model [25]. Interestingly, SGs are enriched with 40S ribosomal subunits [56] and co-immunoprecipitation under various stress conditions revealed a G3BP1-40S subunit interaction [35]. Further characterization of this process demonstrated that G3BP1 interacts with Caprin and USP10 under stress conditions and these proteins act in an antagonistic manner to promote and impair SG formation, respectively [35]. Taken together, these results suggest a balance between 40S ubiquitination-mediated rescue and degradation that may depend on cellular context and rely on G3BP1, as well as connecting these ribosomes to be localized to SGs (Figure 1). Thus, this connection between G3BP1 and the 40S subunit further extends the network of interactions between the RQC pathway and SGs.

Diseases associated with stress granules and the RQC pathway

Genes involved in both the RQC pathway and SGs have been implicated in many diseases including vacuolar tauopathy reminiscent of Alzheimer's disease (AD), frontotemporal dementia (FTD), inclusion body myopathies, Paget's disease of the bone, amyotrophic lateral sclerosis (ALS), various cancers, and pulmonary vascular diseases [17,57–61]. Mutations in RQC genes could result in the accumulation of potential gain-of-function toxic aggregates that cells have difficulty clearing, a common hallmark of degenerative diseases. In support of this notion, VCP mutations (e.g. A232E and R155H) are associated with diseases characterized by protein inclusion bodies including inclusion body myopathies, Paget's disease of the bone, ALS, and FTD [62]. Furthermore, VCP is implicated in the disaggregation of tau fibrils, and a VCP mutation (D395G) is associated with vacuolar tauopathy reminiscent of AD [57,63]. Patients with pathogenic VCP mutations exhibit TDP-43 neuropathology including TDP-43+ inclusion bodies and accumulation of TDP-43 in the cytoplasm [58,64]. Importantly, a transgenic mouse model expressing either VCP-R155H or VCP-A232E exhibits aberrant cytoplasmic TDP-43 localization [65]. Additionally, expression of disease-associated VCP mutations including A232E or R155H in human SH-SY5Y cells [64] or mouse cortical neurons [66], recapitulates these TDP-43 neuropathology phenotypes. Furthermore, exogenous expression of the VCP-R155H homolog VCP-R152H in a *Drosophila* model also causes aberrant TDP-43 localization to the cytoplasm [66]. Interestingly, a genetic screen in *Drosophila* identified Znf598 as a genetic modifier enhancing the progression of *C9ORF72*-associated ALS/FTD [67]. Furthermore, an *Ltn1* allele identified in a forward genetics screen was also shown to cause neurodegenerative hallmarks in mouse models including accumulation of hyperphosphorylated tau protein [15]. *LTN1* mRNA levels are increased in tissues from individuals with Down Syndrome and the Ts65Dn Down Syndrome mouse model [68–70], and LTN1 is differentially expressed in Huntington's disease patient samples [71]. Recessive loss-of-function mutations in *Nemf* result in deficits in motor function and lifespan as well as key hallmarks of progressive neuromuscular and neurological degeneration in mice [16]. Furthermore, pedigree analysis from seven families identified specific *NEMF* alleles that phenocopy mouse models [16]. Pedigree analysis of a family with ataxia and dystonia phenotypes implicated *GTPBP2* alleles in their disease pathology [72], and *Gtpbp2*-null mouse models display increased neuronal death [22]. Thus, mutations in the genes that encode numerous proteins that play key roles in translation quality control pathways including RQC are implicated in neurological and degenerative disease phenotypes.

In the realm of SG proteins, multiple studies have implicated G3BP1 in the pathogenesis of several types of cancer, cardiovascular diseases, and nervous system disease [61]. G3BP1 knockout is associated with an ataxia-like phenotype in mice [73]. Furthermore, G3BP1 is up-regulated at the mRNA level in prefrontal cortex samples from Huntington's disease patients and is increased at the protein level in cortex and hippocampus samples of the R6/2 Huntington's disease mouse model [74]. SG protein-encoding genes including *G3BP1*, *G3BP2, CAPRIN1*, and *UBAP2L* are genetically implicated in neurodevelopmental diseases and further analysis showed these variants decreased SG formation in cells treated with arsenite [75]. *CAPRIN1* haploinsufficiency has been identified in patients with language impairment, ADHD, and ASD and impaired neuronal organization along with abnormal neuronal firing [76]. Pedigree analysis of two families have associated *CAPRIN1* mutations with aberrant protein aggregation and early onset ataxia [77]. Genetic mutations in *GCN2* are

primarily implicated in pulmonary diseases such as pulmonary veno-occlusive disease [60] and pulmonary capillary hemangiomatosis [59]. However, one study identified that Gcn2 down-regulation may alleviate neuronal plasticity and memory deficits in an AD mouse model [78]. Together, the detrimental effects of mutations in both RQC factors and SG proteins stresses the importance of these pathways in the maintenance of protein homeostasis and cellular health.

Conclusion

The interconnection between the RQC pathway and SGs is an emerging field linking cellular mechanisms of proteostasis. However, many unanswered questions remain. Human genetics supports an important role for VCP and other RQC factors in cellular and organism health, yet, the mechanisms by which VCP mediates nascent protein degradation, SG assembly, and SG disassembly must be further explored. Beyond this, it is unknown whether ribosome collisions trigger SG formation. Understanding whether and how ribosome collisions lead to SG formation may inform our knowledge of the balance between RQC, the ISR, and cell fate. Finally, the mechanisms and outcomes of ubiquitinated 40S subunits in the context of both RQC and ISR activation or SG formation must be further studied. Further elucidation of 40S ribosome regulation will be critical in deciphering mechanisms of ribosome recycling and recovery from stress. Overall, there is significant interplay between the RQC pathway and SG regulation. Further study of these connections holds promise for uncovering novel mechanisms of proteostasis and stress resilience in health and disease.

Perspectives

- Understanding the interconnections between the RQC pathway and SG regulation is critical for improving our knowledge of proteostasis and stress adaptation.
- RQC proteins regulate SG dynamics via activation of a major stress signaling pathway upstream of SG formation, contribute to SG mRNA and protein composition, and drive SG disassembly.
- Further investigation of how RQC factors regulate SGs will likely identify therapeutic targets for neurological and degenerative diseases and give further insight into how the cell maintains proteostasis.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contributions

N.S.H. drafted the majority of the article, prepared the figure, edited and revised the article, and approved the final version of the article. S.L.M. drafted parts of the article, edited and revised the article, and approved the final version.

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Abbreviations

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ANS, anisomycin; CAT, carboxy-terminal arginine threonine; EME, emetine; FTD, frontotemporal dementia; ISR, integrated stress response; JNK, c-Jun N-terminal

kinase; MMS, methyl methanesulfonate; RBPs, RNA binding proteins; RQC, ribosome-associated quality control; SGs, stress granules; USP10, ubiquitin specific peptidase 10; VCP, valosin-containing protein.

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