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Review

Stress granule and P-body clearance: Seeking coherence in acts of disappearance

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ABSTRACT

Stress granules and P-bodies are conserved cytoplasmic biomolecular condensates whose assembly and composition are well documented, but whose clearance mechanisms remain controversial or poorly described. Such understanding could provide new insight into how cells regulate biomolecular condensate formation and function, and identify therapeutic strategies in disease states where aberrant persistence of stress granules in particular is implicated. Here, I review and compare the contributions of chaperones, the cytoskeleton, post-translational modifications, RNA helicases, granulophagy and the proteasome to stress granule and P-body clearance. Additionally, I highlight the potentially vital role of RNA regulation, cellular energy, and changes in the interaction networks of stress granules and P-bodies as means of eliciting clearance. Finally, I discuss evidence for interplay of distinct clearance mechanisms, suggest future experimental directions, and suggest a simple working model of stress granule clearance.

1. Introduction

Stress granules (SGs) and P-bodies (PBs) are paradigm biomolecular condensates, also referred to as membraneless organelles [1]. They consist largely of non-translating mRNA-protein complexes (mRNPs), and their assembly, composition, dynamics, and function has been the focus of considerable research effort and speculation for almost 25 years [2–6]. In contrast, how SGs and particularly PBs undergo clearance in cells, either via disassembly or degradative means is less well understood, though many pathways and factors have been implicated. SG and PB clearance mechanisms help determine SG and PB abundance, understanding of which may reveal new functional insights, and be pertinent to understanding the dynamics and function of other biomolecular condensates. Additionally, SG clearance defects are linked to the pathogenesis of Amyotrophic Lateral Sclerosis (ALS) and other neurodegenerative diseases [7–9]. Thus, understanding SG and PB clearance may identify future therapeutic targets.

In this review, I will discuss reported and putative mechanisms of SG and PB clearance, assess the importance and integration of distinct clearance pathways, identify gaps in knowledge, and suggest future experimental priorities. A quantitative summary of reported SG and PB clearance effects is presented in Table S1 for additional reference. Finally, a simple working model of SG clearance will be presented.

2. SG and PB clearance - assembly in reverse, or a distinct process?

Several reversible mechanisms exist that oppositely impact assembly and clearance of both SGs and PBs. One example is the entry and exit of mRNAs to and from polysomes. SG and PB assembly is stimulated by translation repression, whereas their clearance correlates with translational recovery [10,11], though sometimes translation recovers with SGs still partly evident [12,13]. Cycloheximide, and other elongation inhibitors, prevent assembly of SGs and PBs by trapping mRNPs in polysomes, but also hasten clearance of already formed SGs [14,15] and non-stress induced PBs [16,17] by presumably the same mechanism. Stress-induced mammalian PBs may clear significantly more slowly than SGs in the presence of cycloheximide [14], unlike in yeast [18], though extended cycloheximide treatment still results in full mammalian PB clearance [19]. Finally, microscopy-based studies [20–25] have

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visualized mRNP entry and exit to/from SGs and PBs during and following stress. Collectively, these data mostly support a model of constant mRNA exchange between polysomes, SGs and PBs.

However, SG (and perhaps PB) clearance is not just a reversal of assembly events. First, whereas SG assembly in human cells is typically described as coalescence of multiple small, spherical foci into fewer, larger, and more irregular condensates, SG clearance is often morphologically distinct in that SGs either dissolve or undergo fracturing with rough-edged SGs sometimes harboring filament-like structures protruding from their surface [15,26–28]. Second, clearance can occur significantly more slowly [11,29] or rapidly [30] than assembly following distinct stresses [5], though this may also depend on where one draws the line on "fully assembled". Third, SGs exhibit an evolving proteomic composition during stress progression [31], with uniquely enriched factors during stress recovery [32]. Finally, some factors impact only clearance, but not assembly (e.g., Hsp70 [13,33], MCM, RVB [34], DYRK3 [35], p62 [36]) or vice versa (e.g., Chaperonin-containing T complex [34]). Different mechanisms of clearance are also utilized depending on stress and cellular context (e.g., [31,37]; discussed later). Thus, SG and PB clearance likely involves a mix of reversing assembly-driving processes, and unique clearance mechanisms whose use depends on cellular and stress context (Fig. 1).

3. Chaperone based mechanisms of SG and PB clearance

Several chaperones localize in and impact SG assembly and clearance [38], including heat shock proteins (Hsps), which generally bind hydrophobic regions of misfolded proteins, and are categorized based on molecular weight, ATPase activity, and function. Hsps and their regulators/co-chaperones can limit accumulation of misfolded proteins in SGs, which can facilitate conversion of SGs to aberrant non-dynamic states, particular under heat shock stress (HS) [18,31,33,38]. Roles for key chaperones in SG (and PB) regulation are discussed below.

3.1. Hsp70s

Hsp70s hydrolyze ATP to bind cyclically to substrates, promote protein disaggregation and refolding, and play integral roles in regulating nascent protein folding during translation [39]. In human cells, Hsp70, and an Hsp70 nucleotide exchange factor BAG3, promote clearance of HS or proteasome inhibition (MG132)-induced SGs, in correlation with translational recovery [10,31,33,40]. Overexpression or prior stress-mediated accumulation of Hsp70 also prevents SG assembly via MG132 or arsenite stress [10]. HS-induced SGs co-localize with misfolded proteins and Hsp70 [31,41], whereas under MG132 or arsenite stress, Hsp70 does not localize in SGs [10,33]. However, in all three stresses, Hsp70 inhibition exacerbates accumulation of ubiquitin signal and misfolded proteins in SGs, including ALS-associated aggregation prone proteins, and misfolded nascent translation products ("Defective ribosomal products"; DRiPs). SGs harboring such misfolded proteins clear more slowly than those that do not [31,33].

Similarly, in yeast and *Drosophila*, Hsp70 and Hsp110 - an Hsp70 subclass that stimulates Hsp70 nucleotide exchange, and has its own ATPase chaperone activity - facilitate SG clearance following HS and sodium azide stress. This again correlates with translational recovery [11,13,33]. Unlike glucose deprivation SGs [18], HS and sodium azide-induced SGs co-localize with Hsp70 and misfolded protein aggregates, again suggesting stress-specific links between SGs and misfolded proteins. Proteins in HS-induced SGs exchange more rapidly with the cytoplasm than aggregate-prone reporter proteins, and thus unsurprisingly, SGs also clear faster than protein aggregates [11,18]. This mirrors in vitro work demonstrating Pab1 (SG marker) biomolecular condensates are more rapidly dispersed by Hsp70 and other chaperones (particularly Hsp104 and 110) than aggregate-prone protein reporters [42]. Inhibiting Hsp70 function genetically or chemically does not induce SG assembly in yeast [13] or human cells [33], or affect assembly under various stresses examined, suggesting a role for Hsp70 primarily in clearance.

Collectively, these findings suggest Hsp70 aids SG clearance by preventing accumulation of misfolded, ubiquitinated proteins in SGs, the importance of which likely depends on the protein misfolding burden caused by a given stress. No SG-localized substrates are known to specifically recruit Hsp70, though many interactions with misfolded proteins, aided by Hsp40s and small Hsps (see below), seem likely. Additionally, Hsp70s and Hsp110 can robustly bind U-rich RNA, and even impact mRNA stability [43,44], suggesting an unexplored recruitment and effector mechanism in clearance.

3.2. Hsp40s

Hsp40 proteins work with Hsp70s, increasing Hsp70 ATPase activity via their J-domains, and conferring Hsp70 substrate specificity via C-terminal client domains [45]. In human cells, several Hsp40s localize in SGs [34,46,47], though no specific substrates are known. However, Hdj1 and Hdj2 rely on a G/F-rich intrinsically disordered region (IDR) sequence present in most Hsp40s to both phase separate and localize in arsenite-induced SGs, suggesting possibly promiscuous interactions. Phase separation or substrate binding via their C-terminal domain may relieve an autoinhibitory interaction within Hsp40s that sequesters the J-domain, thus recruiting and stimulating Hsp70 activity [46,48]. In yeast, the homologs of Hdj1 and 2, Sis1 and Ydj1, localize in sodium azide-induced SGs and regulate specific SG clearance mechanisms. Ydj1 promotes SG disassembly and translation recovery, whereas Sis1 targets SGs to vacuolar compartments, presumably via "granulophagy" (see later) [13].

3.3. Hsp104

In yeast, this AAA+ ATPase "disaggregase" chaperone, absent in metazoa, forms a hexameric ring-like structure through which misfolded substrates are threaded and unfolded [49]. Hsp104 also localizes [18] in and facilitates clearance of HS-induced SGs, and the resumption of translation post-HS, based on genetic and inhibitor studies [11,18]. Intriguingly, Hsp104 (and Hsp70) are recruited to SGs following HS in part by a glycolysis metabolite mediating allosteric modulation, and solubilization, of the SG-localizing amyloid form of pyruvate kinase (Cdc19) [50]. Pyruvate kinase is key for ATP production, and though specific Hsp104 and Hsp70 recruitment mechanisms in this context are unclear, solubilization of pyruvate kinase in SGs, aided by Hsp104 activity, promotes efficient SG clearance. Thus, SG clearance and ATP production are coupled processes [50], which may act in a positive feedback loop to further aid SG clearance via other ATP-dependent mechanisms (see below and Box 1).

3.4. Small Hsps/"Holdases"

Small Hsps, which lack ATPase activity, generally maintain substrates in a conformation for processing by other chaperones [51]. Hspb8 aids clearance of MG132-induced SGs by recruiting Bag3 and Hsp70 to SGs harboring misfolded proteins, particularly DRiPs [33]. Hspb1 (Hsp27) also progressively localizes in HS and Diethyl maleate-induced SGs, but not arsenite, UV or MG132-induced SGs [31,33,41,52]. However, Hspb1 depletion still mildly slows SG clearance during proteasome inhibition [33]. Like Hsp70, SG recruiting mechanisms for small Hsps are unclear, but likely involve multiple promiscuous interactions with misfolded protein substrates, though Hspb1 also can bind RNA [41].

3.5. Cdc48/VCP

Like Hsp104, Cdc48(yeast)/VCP(human) is another hexameric AAA+ ATPase chaperone, though it is conserved throughout eukaryotes

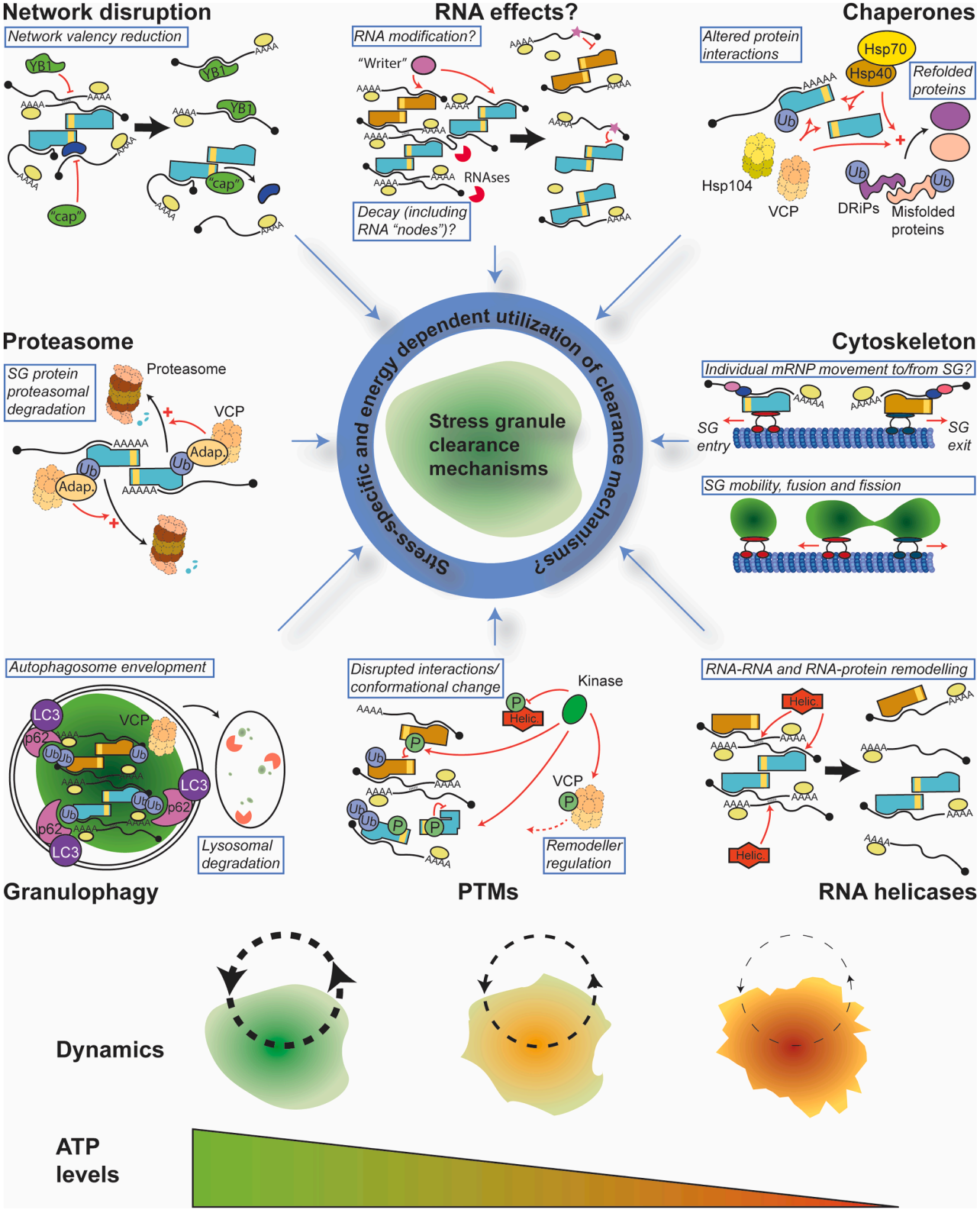

Fig. 1. Reported and putative means of SG clearance. SG clearance is not fully understood, but multiple mechanisms have been reported including chaperone activity, cytoskeletal transport, RNA helicases, post-translational modifications (PTMs), granulophagy, proteasomal activity, and SG network disruption. Direct effects on SG-resident mRNA molecules have not been reported but are feasible given the multivalent, SG-scaffolding nature of mRNA and reported impacts of mRNA modification. A selection of examples of each clearance mechanism are depicted and identified in blue boxes; those with question marks are putative and/or not unambiguously demonstrated. Teal and orange objects with a yellow band indicate RNA-binding proteins with self-interacting domains (analogous to SG scaffolding proteins like G3BP1/2). "Helic.", "Adap." and "cap" refer to RNA helicases, VCP adaptor proteins, and network valency reducing, or "capping" proteins [197], respectively. Black arrows indicate a transition from one state to another, while red arrows/inhibitory lines indicate direct action of a protein or enzyme on a target with functional consequences. Dynamics, meaning the rate of mRNP entry and exit to/from SGs, and their fluidity, likely depends on ATP levels, which is required by almost all of the SG clearance mechanisms proposed. See main text for more details.

Box 1
- THE IMPORTANCE OF ENERGY IN SG CLEARANCE.

ATP levels are a key regulator of both SG assembly and clearance. In human cells and yeast, strongly reducing ATP levels by 50–80% by inhibiting glycolysis (e.g., Glucose depletion, 2-DG, CP91149), or oxidative phosphorylation (e.g., FCCP, CCCP, oligomycin) induces SGs without initially increasing eIF2α phosphorylation [29,97]. In contrast, a combinatorial block to glycolysis and mitochondrial function in human cells, which reduces ATP levels even more, blocks arsenite-induced SG induction and induces cell death [34,97], suggesting SGs are induced by low ATP levels, but still require a minimal level of ATP to form. ATP depletion can also drive SG assembly in *G3BP1/2ΔΔ* (key SG assembly mutant) cells, and greater RNA partitioning in SGs than eIF4A inhibition alone [94]. Following arsenite-induced SG assembly, ATP depletion blocks SG movement, fusion and reduces SG dynamics [34]. Finally, reducing ATP depletion below 50% of normal in human cells causes a near-complete block of arsenite and HS-induced SG clearance over 1.5hrs [37], indicating the importance of ATP-based clearance processes.

Various ATP-dependent mechanisms (e.g., helicases, chaperones) may counteract the inherent tendency of non-translating mRNA and associated RNA-binding proteins to phase separate and form condensates. *In vitro* studies suggest that ATP itself may also play a role as a biological hydrotrope (i.e., solubilizer of hydrophobic proteins) to antagonize condensate formation [132]. Subsequent in vitro work with reconstituted SGs, induced by specific RNA molecules and using yeast lysates, suggests both roles of ATP may limit SG assembly, though hydrolysis of ATP (and GTP) is required to facilitate clearance of already formed SGs, suggesting involvement of ATP and GTP-dependent machineries [133]. What GTP-dependent machineries may be involved is unclear, but the translational apparatus seems a natural candidate.

Most SG clearance mechanisms discussed in this review (Fig. 1) require cellular energy to function. The relative energy requirements of each pathway in SG clearance are unclear but could have a decisive impact on how SGs clear under stress conditions that significantly deplete cellular energy reserves.

and acts more specifically on ubiquitinated substrates [53,54]. Diverse roles including protein refolding, endolysosomal trafficking, and protein degradation (via both proteasomal and autophagic means) depend largely on Cdc48/VCP cofactors that confer substrate specificity.

In yeast, Cdc48 inactivation causes SG accumulation during entry into quiescence, and impairs vacuolar targeting of SG material, implying a role in granulophagy [55]. In human cells, VCP (Valosin-containing protein) localizes in SGs following multiple stresses, and inhibition, either by knockdown or several different inhibitors, slows SG clearance following HS [37] and arsenite stress, though not other stresses [56]. Expression of neurodegenerative disease associated VCP mutants induces constitutive SG assembly [55], and slow SG clearance following HS [37] and arsenite stress in various models [57,58]. VCP localization in HS-induced SGs depends on the VCP adaptor FAF2, and ubiquitination of the key mammalian SG assembly protein G3BP1 [37], whereas another VCP adaptor, ZFAND1, aids VCP recruitment and clearance of SGs following arsenite stress [58]. ZFAND1 depleted cells also accumulate DRiPs in SGs, suggesting VCP may complement Hsp70 functions in SG clearance. As discussed later, VCP is linked to SG clearance via autophagy dependent, independent, and proteasomal-dependent means (Fig. 1), implying a multi-faceted role in regulation of SG dynamics.

3.6. PBs and chaperones

Only a few cases of Hsps impacting PB dynamics are known, most of which differ from SG effects. In yeast, similar to SGs, Hsp104 deletion slows PB clearance, leading to formation of aggregate-like structures harboring SG, PB and misfolded proteins [18]. Unlike SGs, following sodium azide stress, PB clearance is unaffected by Hsp70 inhibition [13]. Ydj1 aids localization of Dhh1 and Lsm1 (core PB proteins) to foci under acute or chronic glucose deprivation, though Edc3 (another core PB protein) is unaffected [59]. Finally, in human cells, inhibition of Hsp90, a homodimeric ATPase chaperone considered more selective than Hsp70 in stabilizing unstable substrates and regulating misfolded proteins [60], aids PB assembly by an unclear mechanism [61,62]. In contrast, Hsp90 inhibition slows SG clearance, primarily due to destabilization of the kinase substrate DYRK3 (see later) [63].

4. Cytoskeletal based mechanisms of SG and PB clearance

Though understudied of late, the cytoskeleton and associated motor proteins impact SG and PB clearance, assembly, mobility, and cellular localization [26,64,65].

4.1. Microtubule-based mechanisms

MT depolymerization reduces the size and mobility of SGs during assembly in many human cell models [12,27,66–70]. However, the effects of MT stabilization on SG assembly are controversial [66,69]. While not required to sustain SG assembly [27], MTs promote SG mobility and coalescence into larger foci [65], and perhaps SG-PB interactions [71]. Conversely, PB assembly in yeast and human cells is stimulated by MT depolymerization, though PBs also become largely immobile and exhibit compositional differences [72–74]. SG clearance following arsenite stress, facilitated by cycloheximide treatment, is strongly impaired by MT depolymerization [27]. The role of MTs in PB clearance is unknown.

Several MT motor and MT-binding proteins localize in SGs and PBs [12,34,47,75–77], but consensus on their role is lacking. Inhibition of dynein motor proteins, which drive retrograde movement on MTs, impairs SG and PB assembly following arsenite and thapsigargin stress [12,67,76], whereas non-stressed PBs are unaffected by MT motor perturbations [12]. Dynein inhibition also weakly inhibits SG clearance in P19 cells [76], though no qualitative effects on SG dynamics were observed in other studies [68,69]. Kinesin, which drives anterograde movement, facilitates SG clearance in NIH3T cells [12]. Finally, the p50 isoform of Nesprin-1 is primarily a PB localizing, MT-binding protein whose expression promotes PB assembly, mobility, SG-PB interactions and SG clearance following H_2O_2 but not arsenite stress [77].

4.2. Actin-based mechanisms

SGs do not localize with, nor are obviously affected by actin-disrupting drugs [67,69], whereas PBs do show some evidence of actin/myosin-based regulation. Specifically, many myosin motor proteins localize in PBs [78,79], and immobile PBs associate with actin bundles in U2-OS cells; mobile PBs, in contrast, associate with MTs [71] (except in plants [80]). In yeast, conditional inactivation of the Myosin protein Myo2 slows PB clearance induced by chronic nutrient deprivation [81]. In HeLa cells, the ortholog Myo5a localizes in PBs, and knockdown reduces PB numbers, whilst a dominant negative Myo5a impairs PB mobility [79]. Though not clarified, this could solely reflect

an actin-based process, or involve microtubule-based function, by virtue of known Myo5-kinesin-MT interactions [82].

Although actin filaments do not bind SGs, lamellar actin retrograde flow during stress in U2-OS cells may complement directed transport to "push" small, nascent SGs towards perinuclear regions. Here, subsequent non-specific capillary-based interactions of SGs with MTs are proposed to facilitate granule fusion independent of motor functions, reduce their mobility and lead to deformation of spherical SGs as they conform around the MT network [83,84].

5. RNA helicase based mechanisms of SG and PB clearance

RNA helicases are strong candidates for regulating SG and PB clearance, given that they can prevent, remodel, or disrupt RNA-RNA and mRNP interactions [85]. Many members of both the non-processive DEAD and processive DExH class of RNA helicases localize in SG and PBs [32,34,47,75,86,87]. Several RNA helicases also harbor IDRs, which often facilitate condensate formation, particularly in the presence of ATP and RNA [88]. Typically, RNA helicases bind ATP and RNA co-operatively, and exhibit low affinity for RNA after ATP-hydrolysis. Thus, regulation of RNA helicase ATPase activity is key to RNP remodeling, and successive rounds of RNA binding. ATPase activity can be activated by helicase-interacting proteins harboring MIF4G domains, such as Not1 (Dhh1) [89] and eIF4G (eIF4A [90]and Ded1 [88]), which in vitro can drive dissolution of helicase/RNA condensates. ATPase mutant versions of Dhh1 [89], Ded1 [91] and DDX3X (human Ded1 homolog) [92] induce formation of PBs (Dhh1) and SGs (Ded1/DDX3), consistent with possible ATPase-reliant clearance functions.

5.1. eIF4A

eIF4A is a SG-localizing helicase best known for its role in unwinding of 5′UTR structure during translation initiation. Inhibition of eIF4A is also a strong eIF2α-phosphorylation independent inducer of SG assembly [93–96]. While eIF4A's translation-enhancing role likely antagonizes SG assembly and may facilitate clearance, a distinct role for eIF4A in limiting RNA recruitment to SGs is suggested by arsenite stress and eIF4A inhibition exhibiting additive effects on SG assembly, without additive impacts on translation repression [94]. ATP-dependent eIF4A binding to RNA also inhibits SG assembly and SG-PB interactions [94]. In contrast, eIF4A inhibition correlates with specific RNAs and G3BP1 concentrating more strongly in arsenite-induced SGs during both assembly and clearance post-stress [94,97]. These findings are consistent with eIF4A limiting SG assembly, and/or aiding clearance, though the latter point remains to be directly examined.

eIF4A is a curiously abundant protein in cells (Top 1%; ~10–100 fold excess of other eIF4F factors) [98,99], with a 3–5 fold excess over other SG-localized helicases, and 5–50 copies per mRNA in human cell models [94]. Thus, it appears well suited to an "RNA chaperone" role [94]. While eIF4A binds and is activated by eIF4G and eIF4B [100], indicating a targeting to mRNAs, the scope of eIF4A specificity and regulation remains unclear, as eIF4A lacks substantial helicase-domain flanking sequence that typically dictate interactions, localization, and regulation of other RNA helicases [101]. Thus, eIF4A may also act somewhat non-specifically as an "RNA disaggregase" [102].

5.2. Ded1/DDX3

Ded1 (yeast)/DDX3 (human) is another SG-localizing DEAD-box helicase best known for unwinding 5′UTR structure during translation initiation. Several studies (with one exception [103]) suggest that Ded1/DDX3 significantly impacts both SG assembly and clearance, albeit via distinct activities. Regarding assembly, overexpression of WT Ded1 [91,104] and DDX3 [92] drives SG assembly in the absence of stress, correlating with reductions in translation rate at the single cell level. This depends not on Ded1/DDX3′s helicase or ATPase activity, but rather interactions with eIF4F factors, resulting in formation of translationally stalled mRNPs [91,105,106]. However, Ded1/DDX3 ATPase mutants, or DDX3 depletion, also induce SGs in the absence of stress [91,105], possibly reflecting a combination of impaired translation and reduced SG clearance (see below). Ded1/DDX3′s IDR domain is also required to form condensates, localize in, and induce SGs in the absence of stress when overexpressed [92,104,105].

Regarding clearance, ATPase-deficient Ded1 exhibits slower clearance of sodium azide-induced SGs in yeast following cycloheximide treatment [88], suggesting DDX3 ATPase/helicase activities facilitate SG clearance. Finally, several DDX3 helicase domain mutations are associated with medulloblastoma and intellectual disability. These mutations inhibit helicase activity, scanning and translation of structured 5′UTR mRNAs, and induce SG-like foci in various models [92,107,108].

5.3. Dhh1/DDX6

Dhh1 (yeast)/DDX6 (human) is a highly studied helicase which promotes translation repression and mRNA decapping [109,110], and is a key marker and assembly protein for PBs [89,111–113]. Dhh1 mutants defective in RNA or ATP binding impair PB assembly [89,114]. In contrast, Dhh1 ATPase mutants, or mutations disrupting Dhh1 binding with Not1 (Dhh1/DDX6 ATPase activator) [115], exhibit constitutive PBs, increased RNA binding, and strongly impair PB clearance [89,114]. Dhh1 ATPase mutants additionally become trapped in PBs unlike WT Dhh1, which cycles in and out of PBs with a half-life of ~30 s [110]. Curiously, in human iPSC cells, catalytically dead DDX6 expression blocks PBs [116]; why similar DDX6 and Dhh1 mutations should oppositely affect PB assembly is unclear but may reflect differences in model systems and approach. Properties of Dhh1/DDX6 that may aid PB assembly include its high RNA binding affinity (Kd 1–2 nM) [114,117], its significant stoichiometric excess to mRNA (~7-fold [117]; Top 10% protein by abundance [98]) and ability to oligomerize [117]. Thus, besides promoting translation repression, Dhh1 may drive PB assembly by interacting with RNA and forming oligomeric scaffolds, whereas ATP hydrolysis likely facilitates PB clearance by reducing Dhh1 RNA binding.

Altered Dhh1-PB protein interactions may also occur due to ATP and RNA binding and ATPase-driven conformational changes that impact PB clearance. For instance, DDX6 binding to Edc3 or Pat1 (other PB assembly proteins) is disrupted upon CNOT1 binding [118]. An ATPase mutant Xp54 (Xenopus Dhh1) also interacts with distinct PB-associated proteins versus WT [119]. Active disruption by Dhh1/DDX6 of RNA-RNA and RNA-protein interactions within PBs is another possible clearance mechanism, albeit direct detection of Dhh1/DDX6 helicase activities remain somewhat controversial [114,117,120].

DDX6 progressively re-localizes from PBs to SGs during arsenite stress and may facilitate a PB-SG maturation process [121–123]. Indeed, DDX6 KO cells, or cells rescued with a DDX6 ATPase mutant, or depleted of CNOT1, all exhibit unusual PB-SG hybrid granules, suggesting DDX6 promotes both PB clearance and separation of SGs from PBs [123], perhaps by facilitating release or remodeling of PB mRNPs into SG mRNP-like states.

5.4. DHX36/RHAU

This human helicase binds G-quadruplex containing mRNAs (rG4s) which are enriched in SGs [124], and promotes translation and decay of such mRNAs [125]. DHX36 localizes to SGs following many stresses [47,86,87], and it's depletion induces SG assembly in the absence of stress [87], and increases SG assembly following arsenite. This may reflect an accumulation of non-translating rG4 RNAs as SGs "seeds", an observed increase in eIF2α phosphorylation, or both [125]. A role for DHX36 in promoting rG4 mRNA exit from SGs, and thus clearance, is possible but

currently unknown.

5.5. "DNA" helicases in SGs

Three DNA helicase complexes that facilitate DNA unwinding and chromatin remodeling for DNA replication, repair and transcription purposes can also regulate SG dynamics. Minichromosome maintenance helicase (MCM) [126] and RuvB-like helicase (RVB) [127] localize in yeast and human SGs [34], and inhibit SG clearance following sodium azide (yeast) or arsenite stress (human cells). MCM and RVB are not known to exert RNA helicase activity, and with DNA not known to be a SG component, their mode of action remains mysterious. Finally, Bloom's syndrome protein [128] can bind and unwind both DNA quadruplex (dG4) and rG4 sequences, localize in human SGs under many stresses, and inhibit SG formation via a proposed mechanism similar to that of DHX36 [87,129].

5.6. RNA Helicase function and regulation

Despite similar functionality, eIF4A antagonizes SG assembly, DDX6 promotes PB assembly, and Ded1/DDX3 can promote both assembly and clearance of SG assembly depending on expression levels. These distinct behaviors may reflect a combination of protein interactors, RNA binding affinity, abundance, and regulation of ATPase activity, some or all of which may vary within and outside of SGs and PBs. For example, Dhh1 ATPase activity is significantly lower than Ded1 or eIF4A in vitro, due in part to inhibitory intramolecular interactions [114]. Thus, Dhh1 may dissociate from mRNA less often than eIF4A or Ded1, thus predisposing it to maintaining PB assembly. Ded1 condensate formation limits its helicase activity and may aid sequestration of translationally repressed mRNAs with structured 5′UTRs in SG [130]. Finally, all the aforementioned helicases undergo many post-translational modifications (PTMs; see Biogrid/Uniprot), though very few are characterized. One exception is eIF4A Thr164 phosphorylation by cyclin dependent kinase A, which blocks RNA helicase activity, possibly by perturbing RNA binding [131]. Further characterization of helicase regulatory mechanisms, and examination of helicase properties within and outside of SGs and PBs is an important area of future study.

6. Post translational modifications as regulators of SG and PB clearance

Numerous PTMs on proteins besides helicases impact clearance and assembly of both SGs and PBs. During stress, the speed and reversibility of most PTMs offers obvious benefits to altering SGs and PBs in such a way that may aid cell survival [134]. Below, I focus on phosphorylation and ubiquitination events that impact SG and PB clearance, though other modifications (summarized in Table S1) and discussed elsewhere [135,136] also play important roles.

6.1. Phosphorylation

6.1.1. eIF2α phosphorylation and SG-associated translation

Following many stresses, eIF2α phosphorylation by one of four stress-induced kinases in human cells (GCN4, HRI, PERK, PKR) limits translation initiation as part of the integrated stress response. This reduces Met-tRNA ternary complex levels, and thus drives SG and PB assembly by increasing the non-translating mRNP pool [137]. Recently, other eIF2α stress-inducible kinases have been proposed including MARK2, which phosphorylates eIF2α in response to cytoplasmic protein misfolding sensed by a PKC-Hsp90-dependent mechanism [138], and FAM69C, which responds to both HS and arsenite stress, and drives SG assembly in microglia [139].

SGs were originally proposed to exclusively harbor translationally repressed mRNPs, but single molecule mRNA translation studies have modified this view. While most SG-localized mRNAs are non-translating, mRNA reporters with 5′UTRs of genes that are both translationally stimulated (*ATF4*) and repressed (*RPL32*) during stress can accumulate and translate in SGs [140]. For the *ATF4* reporter, translation frequency of SG-associated transcripts approached 30%, with elongation rates similar to non-SG-localized *ATF4* transcripts. While most mRNA reporter transcripts localized close to SG outer edges, consistent with other studies [22], no significant effect of an mRNA's localization on or deep within a SG impacted the likelihood of translation [140]. PBs showed no interaction with the *ATF4* reporter, and thus may be more translationally silent than SGs, though studies with other mRNAs, or sensitive spatial translation assays, are required for more certainty.

Translation of mRNAs localized in SGs or PBs could rapidly facilitate their extraction from either granule, and aid subsequent SG/PB clearance. Despite contrary initial data [29], phosphorylated eIF2α (and perhaps eIF2B) can accumulate in SGs during stress and stress recovery [34,68,141,142]. Once eIF2α is dephosphorylated, translation on SG-associated mRNAs might proceed rapidly given the high local concentration of eIFs and 40S subunits. Specific RNA helicases are involved in all steps of translation [143,144], particularly initiation (e.g., eIF4A, Ded1/DDX3), which could disrupt mRNA-mRNA and mRNA-protein interactions sustaining SG assembly. Elongating ribosomes also display potent helicase activity [145,146]. However, whether translation itself actively disassembles SGs, or occurs after SG clearance remains unclear. Consistent with the second possibility, single molecule translation studies with an mRNA reporter (*KDM5B*) in U2-OS cells suggest that SGs largely undergo clearance a few minutes prior to detection of distinct translation activity. However, transiently SG-localized mRNAs undergoing low levels of translation may also have simply escaped detection [22].

6.1.2. Phosphatases

If translation itself is a key SG disassembling force, and not a downstream consequence, then eIF2α de-phosphorylation could be key to SG clearance. Notably, PP1 phosphatase acts on phospho-eIF2α [147], and treatment with PP1 inhibitors slows trehalose-stimulated SG clearance following arsenite stress [148]. Additionally, chronic MG132 treatment increases PP1 subunit levels, which limits phospho-eIF2α accumulation during subsequent stress. This in turn inhibits SG assembly, and speeds SG clearance [149]. Such preconditioning is distinct from that involving Hsp70 accumulation (also following MG132 treatment) [10], and thus may occur simultaneously to limit SG assembly. PP1 subunits also localize in arsenite-induced SGs [150]. Generally, though, little is known about whether PP1, and the activity of other SG-localizing phosphatases [34,47,86] impacts SG clearance via eIF2α de-phosphorylation, and/or by targeting other phosphorylation substrates. Given the many phosphorylation events that impact SGs, this is an important area of exploration.

6.1.3. DYRK3

Inhibitors, knockdown and catalytic mutants of dual specificity tyrosine-phosphorylation-regulated kinase 3 (DYRK3) impair SG clearance in various stress and cell line contexts [35]. An IDR in DYRK3's N-terminus drives SG localization under stress. While phosphotargets and DYRK3 interaction partners were identified, some of which localize in SGs [35,151], it remains unclear which DYRK3 phosphorylation events aid SG clearance. DYRK3-facilitated SG clearance involves regulation by the chaperone Hsp90, inhibition of which delays SG clearance in various stress and cell line contexts. Hsp90 binds to and stabilizes DYRK3, and the absence of Hsp90 leads to proteasomal-mediated degradation of DYRK3 [63]. Finally, DYRK3 activity also limits formation of SGs, but not PBs, and some nuclear biomolecular condensates (e.g., splicing speckles) but not others (e.g., Cajal bodies). All of these condensates clear during mitosis when DYRK3 abundance relative to substrates is maximal [151].

6.1.4. CDK

In yeast, Cdc28, the cyclin dependent kinase (CDK) that governs cell cycle regulation, localizes in SGs, and aids SG clearance following release from a joint glucose deprivation and HS stress [152]. CDKs localize in SGs in other systems (including human CDK1, CDK2, CDK4 [34,152], and CDKA1 in plants [153]), and CDK2 and CDK4 inhibitors strongly impair SG clearance following arsenite stress in HeLa cells [35]. Additionally, yeast and human SG clearance rates are slower in G1 phase, when CDK activity is lower, than in S, G2 or early M-phase when CDK activity is higher [152]. Many SG-localized proteins are Cdc28 phosphorylation targets, but no specific target is known that explains Cdc28/CDK-mediated SG clearance effects.

6.1.5. Syk

Expression, localization within SGs and the catalytic activity of the tyrosine kinase Syk facilitates clearance of MG132-induced SGs in MCF7 cells [154]. Syk-dependent phospho-tyrosine modified proteins also accumulate within SGs under these conditions. SG clearance correlates with a decrease in eIF2α phosphorylation levels, and an apparent increase in autophagosome levels, suggesting Syk could aid SG clearance both by restoring translation and aiding granulophagy. Indeed, blocking autophagy suppresses Syk-mediated effects on SG clearance [154].

6.1.6. Focal Adhesion Kinase (FAK)

P19 carcinoma cells show some reliance on FAK kinase activity for HS-induced SG clearance [155], which localizes in SGs along with an mRNA-binding protein Grb7, a FAK substrate. Blocking Grb7 phosphorylation by FAK impairs SG clearance following HS. Based on in vivo and in vitro binding assays, it was proposed that Grb7 direct binding to HuR, another SG-localizing protein, is disrupted by FAK-mediated phosphorylation, thus underpinning FAK's SG clearance effect [155].

6.1.7. UNC-51 like autophagy activating kinase 1/2 (ULK1/2)

ULK1 and 2 regulate macroautophagy initiation, but also localize within HS-induced SGs, and interact with several SG localized proteins, including VCP [156]. Inhibiting ULK1/2 slows SG clearance following transient HS and arsenite stress in various cell lines, whereas ULK1/2 stimulation strongly increases HS-induced SG clearance. These effects are autophagy independent, and instead rely on ULK1/2 stress-induced phosphorylation and activation of VCP ATPase activity, which enhances SG clearance [156].

6.1.8. PB regulatory kinases

Fewer examples of regulation of PB clearance and assembly by kinases are known. In yeast, enhanced PKA kinase activity, using a Ras2 constitutive allele or PKA overexpression, promotes clearance of PBs induced by glucose deprivation, in a manner dependent on phosphorylation of Pat1 (a PB assembly and decapping factor) at S456 and S457 [157]. Elevated PKA activity also limits PB assembly under many other PB-inducing stresses but does not impact SGs [157].

In human cells, JNK kinase has been linked to contrasting effects on both PB assembly and clearance that depend on the substrate and PB-inducing stimulus. Following arsenite stress, but not other stresses, the human PB assembly factor and eIF4E binding protein, 4E-T, is phosphorylated at 6 serine residues by JNK, which also localizes in arsenite-induced PBs. Such phosphorylation facilitates assembly of larger PBs and possibly 4E-T self-association [158]. JNK also binds and phosphorylates Dcp1a at S315, with expression of phosphomimetic or phosphonull S315 alleles of Dcp1a strongly reducing or increasing PB levels respectively [159]. Dcp1a is hyperphosphorylated during mitosis, including at the S315 site, which coincides with PB clearance [160]. Finally, in various cancer cell models, based on inhibitor and phosphomimetic/null mutations, Pim kinase 1 and 3 phosphorylate Edc3 at S161, which prevents its localization in PBs and limits PB assembly, possibly via sequestration of other PB-assembly factors [161].

6.2. Ubiquitination

6.2.1. Ubiquitination regulates SG clearance

Most studies, primarily in human cells, indicate an important role for ubiquitination in SG clearance [56,162]. Ubiquitin (Ub) can localize in SGs to varying degrees dependent on the stress applied [31,37,40,56,58,67,163–166]. Free mono-Ub and non-conjugated Ub chains [163,165], as well as Ub-conjugated SG proteins, featuring various types of linkage-specific forms of Ub, have been reported [56,163]. Since distinct Ub-linkage types often specify different outcomes (e.g., K48-linked chains favor proteasomal degradation, K63-linked chains favor autophagy [167,168]), it is noteworthy that different stresses also lead to varying degrees and specificities of Ub-linkages in SGs. HS generally results in a stronger Ub signal within SGs than arsenite stress. Variable levels of K48 and K63 SG-localized Ub signals have been reported in HS, whereas K63 is generally more abundant in arsenite-induced SGs [31,37,56,163,164].

A role for ubiquitination in SG clearance is further suggested by inhibition of the E1 enzyme *UBA1*, which blocks all ubiquitination events in cells. Specifically, three studies using HeLa, HEK293T and iPSC-derived neuronal cell models exhibited significantly impaired SG clearance following HS [56,164] or arsenite stress [56], though another study using similar methods reported no significant effects [165]. Differences here could partly reflect the timing of E1 inhibition and the duration of effective inhibitor action [56]. Finally, chemical inhibition of all deubiquitination events in cells also impairs clearance of arsenite-induced SGs, with a lesser effect on HS SGs [56], suggesting SG ubiquitination alone is not always pro-clearance, and instead plays a complex and stress-specific role.

6.2.2. SG ubiquitination substrates

A key step in deciphering how ubiquitination impacts SG clearance is identifying ubiquitinated SG substrates, and the E3 ligases and deubiquitinase enzymes that regulate such modifications. An unbiased screen of ubiquitination changes in HEK293T cells following different stresses revealed significant ubiquitination of many mRNP proteins and known SG components, particularly following HS stress (less so arsenite). One SG-localized protein that accumulates Ub modifications is the well described SG assembly protein G3BP1 (see later, and Box 2). Specifically, ubiquitination sites in the NTF2L dimerization domain, and the RNA-binding RRM1 domain of G3BP1, were identified through K-R mutagenesis as being ubiquitination sites that aid SG clearance following HS stress [37]. NTF2L ubiquitination also aided G3BP1 interaction with and recruitment of VCP to SGs, suggesting that VCP may remove G3BP1 from SGs. Alternatively, ubiquitination of G3BP1′s NTF2L and RRM domains could simply impair interactions (e.g., dimerization, Caprin1 binding, RNA binding) that sustain SG assembly.

Super-resolution microscopy indicates that K63 and K48 ubiquitination on HS-induced SGs primarily localize on the surface of SGs in the "shell" region, or in cavities directly adjacent to G3BP1 signal. VCP and the proteasome co-localize strongly with SG-associated K48 Ub signal, suggesting a possible role in proteasomal degradation of specific SG substrates [56] (see later). Indeed, inhibition of VCP or the proteasome increases G3BP1 ubiquitination during HS stress recovery [37].

6.2.3. E3 Ub ligases

Several E3 Ub ligases localize in SGs based on IP-MS [150] and proximity ligation data [32,47,86], though none have been clearly shown to promote SG clearance. One candidate is TRIM21, which localizes in arsenite-induced SGs, and at least inhibits SG assembly [169]. TRIM21 ligates K63-linked Ub chains to G3BP1 during arsenite stress, without inducing obvious changes in G3BP1 abundance, suggesting a possible antagonism to SG-sustaining G3BP1 interactions. Consistent with this, K63-ubiquitinated G3BP1 undergoes LLPS less readily than non-ubiquitinated G3BP1 in vitro. However, TRIM21 and G3BP1 also show increased physical interaction with p62 and NDP52 selective

Box 2
G3BP1/2 AND SGs: MORE THAN JUST A SCAFFOLD PROTEIN?

G3BP1/2 RNA binding, protein binding and dimerization activities facilitate SG assembly [217]. However, other reported G3BP1 functions could impact SG clearance, but have not yet been investigated in this context. G3BP1 seemingly harbors an as yet unmapped endonuclease activity that degrades the *c-myc* 3′UTR in vitro [218,219]. G3BP1 also binds and promotes degradation of circular RNAs and mRNAs with highly structured 3′UTRs, in combination with the RNA helicase and nonsense-mediated decay factor, Upf1 [220]. G3BP1 RNA binding domains and S149 phosphorylation are required for these activities. Naturally, an endonuclease activity could clear SGs by targeting SG transcripts for decay. Conversely, G3BP1/2 mRNA binding is also linked to increased mRNA stability [221,222]. Whether S149 phosphostatus, or another means of regulation govern these opposite outcomes remains unclear.

G3BP1 interaction with USP10 reportedly stabilizes levels of both proteins [172,178], but also may inhibit USP10 deubiquitinase activity [223,224]. Thus, beyond USP10 limiting effects on SG network valency [172,197,198], G3BP1-USP10 interaction, or USP10 inhibition by other means, could preserve the ubiquitinated status of several USP10 SG substrates, thus impacting VCP, proteasomal or granulophagy clearance mechanisms. Conversely, interaction of the yeast homologs of G3BP and USP10, namely Bre5 and Ubp3, stimulates Ubp3 deubiquitinase activity [225]. Absence of Ubp3 deubiquitinase activity, or deletion of either Bre5 or Ubp3, blocks SG assembly in yeast under chronic nutrient deprivation [226]. Why USP10 and Ubp3 regulation by G3BP and Bre5 appear opposite, whether USP10 deubiquitinase activity and levels are coordinated by G3BP1/2 or otherwise [227], and the importance of USP10 deubiquitinase to SG clearance remains unclear.

A DNA and RNA helicase activity for G3BP1 has been reported in vitro [228] which could impact SG clearance if functional in vivo. Specifically, a helicase isolated from HeLa cell nuclear fractionation that required ATP and Mg^{2+}, bound ATP, and which could unwind DNA, RNA or RNA/DNA duplexes was identified as G3BP1. No subsequent study has validated this property in vivo, nor identified a putative helicase domain.

Finally, as partly detailed elsewhere [217], G3BP1 has been implicated in RasGAP signaling (though this appears discredited [229,230]), mTOR signaling [231], ribosome quality control [178], regulation of mRNA translation [232] and stability [220–222], all of which could directly or indirectly impact SG dynamics. While roles in SG assembly seem generally similar for G3BP1 and its paralog G3BP2, the latter does bind distinct RNAs, and exhibits tissue-specific expression. However, a clear G3BP2-specific function remains generally elusive [217,233].

autophagy receptors under arsenite stress. TRIM21 also interacts with core autophagic components (e.g., LC3B, ULK1, BECN1) under these same conditions. While suggestive, whether autophagic function is required for TRIM21-mediated effects on SG assembly or clearance remains unclear [169].

6.2.4. Deubiquitinases

Several deubiquitinase enzymes also localize in SGs. USP5 and USP13 localize to HS-induced SGs, but not SGs induced by other stresses [163]. Super resolution microscopy indicates localization to SG shells that only partially overlaps with core SG maker proteins (PABP1, G3BP1). Knockdown of either USP5 or USP13 increases Ub accumulation within HS-induced SGs, accelerates their assembly, and slows clearance in a manner dependent on USP5/13 catalytic activity [163]. USP5 preferentially cleaves free Ub-chains, whereas USP13 acts on protein-conjugated Ub-chains, suggesting that turnover of both types of Ub chain facilitate SG clearance, possibly by disrupting SG protein interactions that rely on Ub-binding.

Ubp3 (*S. cerevisiae* and *S. pombe*)/USP10 (human) is another deubiquitinase that localizes to SGs under many stress conditions [170–174], though it's impacts on SGs apparently differ between species. Specifically, Ubp3 has no impact on SG formation in *S. pombe* [173], but is required, with its cofactor Bre5 (G3BP1 homolog), for SG assembly in *S. cerevisiae* in a manner reliant on its deubiquitinase activity [171]. In human cells, USP10 has been reported to both stimulate [174], in a deubiquitinase-independent manner, and inhibit [172] SG assembly, with reduced G3BP1 dimerization and/or Caprin1 interactions suggested as a possible SG-inhibitory mechanism. Specific Ubp3/USP10 targets in SGs and impacts on SG clearance remain unclear. However, amongst several known substrates and functions [175], ribosomal protein ubiquitination is regulated by Bre5/G3BP1 and Ubp3/USP10 in yeast and human cells under conditions of translational stalling and starvation, which regulates ribophagy [176–178]. Autophagy itself is also stimulated by USP10-mediated deubiquitination and subsequent maintenance of Beclin levels, and thus activity of the Vps34 PI3K complex [179]. Thus, G3BP1's role as deubiquitinase co-factor may play unappreciated roles in regulating SG clearance (see Box 2).

6.2.5. PBs and Ubiquitination

Little is known about PBs and Ubiquitination. However, Ub knockdown or blocking K63 Ub chain formation prevents cytokine-induced Dcp1a phosphorylation by JNK and subsequent PB assembly [180]. The E3 Ub ligase TRAF6 was identified as a Dcp1a binder whose expression maintains levels of other PB proteins (EDC3, XRN1 and DCP2); indeed, TRAF6 KO cells lack PBs entirely. Dcp1a is heavily ubiquitinated by K63 and K29 modifications. However, despite binding Dcp1a, TRAF6 KD does not impact Dcp1a ubiquitination [180], suggesting another E3 ligase is involved, possibly regulated by TRAF6.

7. Clearance of SGs and PBs by granulophagy

Autophagy, or more precisely "macroautophagy", involves sequestration of often large, insoluble substrates (e.g., organelles, protein aggregates) in autophagosomes, which traffic to and fuse with acidic degradative organelles (lysosomes in metazoa, vacuoles in yeast/plants). Here, autophagosome contents are degraded and recycled. Autophagy can act selectively or non-selectively, with the former generally defined and regulated by specific autophagic "receptor" proteins binding to specific "cargos" (i.e., substrate proteins). Autophagic receptor proteins then recruit Atg8, a key autophagosome assembly protein, and additional core autophagy machinery. Several selective autophagic pathways are known that clear damaged or deleterious substrates [181].

"Granulophagy" refers to the selective autophagic clearance of SGs and PBs in eukaryotic cells [55], though SGs are more studied. Granulophagy's role in SG clearance seems to vary depending on stress and cellular context [36,37,40,55,57,148,154,182,183]. In yeast, constitutive SGs accumulate at low levels following autophagy inhibition, with detection of SG protein degradation products in vacuoles under chronic but not transient stress ([55] and our unpublished data). In human cells, SGs also accumulate at low levels in the absence of stress following autophagy blocks [55]. Bafilomycin treatment (lysosomal inhibitor) significantly increases SG accumulation following coxsackievirus A16 (CA16) infection [183], and slows SG clearance following prolonged (90 min) periods of HS stress [37]. In some studies, impairing autophagy significantly slows SG clearance following proteasomal inhibition [154]

or arsenite stress [57,184], but not others [33,156]. Such differences could reflect distinct cell models, inhibitor usage (drug and/or dose), or distinct SG quantification approaches [33,57,154,156].

7.1. Granulophagy receptor candidates

Various autophagic receptors may aid SG and PB clearance in a manner dependent on the composition and dynamic state of SGs. p62 and NDP52 both localize with SGs under arsenite stress in a manner dependent on their Ub-binding domains, and physically interact with G3BP1 [169]. p62 also increasingly localizes over time in SGs chronically induced via an optogenetic-based G3BP1-driven assembly mechanism in U2-OS and iPSC-derived neurons; such co-localization correlates with SGs becoming less dynamic [185]. Knockdown of p62 and NDP52 impairs SG clearance following arsenite stress [169,182]. p62 knockdown also increases SG levels following CA16 viral infection, with p62 Ub-binding domain mutants exhibiting a similar effect [183].

NDP52 may be a preferential PB granulophagy receptor as PB levels under non-stress conditions are significantly increased following NDP52 knockdown. NDP52 also localizes in PBs to a greater extent than p62 [182]. NDP52 binds Pat1 and facilitates autophagy-dependent clearance of PBs following Kaposi's sarcoma associated herpesvirus (KSHV) infection [186,187].

Recently, the chaperonin subunit CCT2 was identified as a non-Ub binding autophagy receptor that preferentially acts on protein aggregates with very low dynamics [188]. CCT2 localizes in SGs [34], and thus may harbor a SG clearance role involving granulophagy [189].

7.2. Granulophagy cargo candidates

Several SG proteins have emerged as putative granulophagy cargos, whose utilization and importance may also be context specific. Histone Deacetylase 6 (*HDAC6*) interacts with p62 during CA16 viral infection in a manner stimulated by *HDAC6*'s Ub binding domain (UBD), which also drives SG poly-Ub enrichment in CA16 infected cells [183]. *HDAC6* UBDΔ cells also exhibit higher SG levels following CA16 infection.

C9orf72 is another reported p62 interactor and SG and PB-localizing protein [36,190], repeat expansion mutations in which are associated with ALS [191]. KD of *C9ORF72* impairs SG clearance following arsenite stress [36] to a similar degree as p62 KD, which could reflect a p62 SG recruitment function, a role for *C9ORF72* in autophagic flux [192–195], or other novel functions.

G3BP1 is another putative granulophagy cargo given its critical SG assembly role, K63-ubiquitination status under HS stress [37], p62-interaction, and ubiquitination by TRIM21 [169]. However, no study has clearly shown that G3BP1-p62 interaction, or G3BP ubiquitination events are required for granulophagy.

7.3. SG/PB physical juxtaposition with autophagic machinery

It is unclear if SGs and PBs are enveloped whole by autophagosomes, or whether partial fragmentation of the granule is required; both processes may also occur. Autophagosomes can be large enough (0.5–1.5 μm) [196] for engulfment of most SG and PBs (0.1–2 μm)[1]. Indeed, LC3 (Atg8) foci co-localization with SGs has been commonly observed [36,182–184], with super resolution microscopy suggesting p62, NDP52 and LC3 appear on the surface of some arsenite induced SGs [36,169]. Furthermore, FUS (Fused in Sarcoma; an ALS-associated SG-localizing protein) and p62 labelled structures presumed to be SGs show evidence by electron microscopy of autophagosome engulfment [36].

8. Proteasomal-based clearance of SGs

The proteasome, which localizes in both human [58] and yeast SGs [150], is implicated in regulation of SG and PB dynamics. Specifically, in human cells, proteasomal activity aids SG clearance in a stress-dependent manner (e.g., arsenite-induced SGs strongly reliant; HS-induced SGs moderately reliant; sorbitol-induced SGs not reliant) [56,58]. A key factor in proteasome-based SG clearance is ZFAND1, which drives proteasome localization to SGs and their clearance following arsenite stress [58]. Proteasomal inhibition also induces SGs, likely due to accumulation of misfolded proteins with SG-seeding potential [10,33,38]. Unlike SGs, proteasomal inhibition reduces PB levels by an unknown mechanism [10]. Currently, no specific substrates of proteasomal activity in SGs critical for clearance are known.

9. Clearance via alterations in the network strength of SGs and PBs

SGs and PBs are sustained by a network of protein-protein, protein-RNA and RNA-RNA interactions. Given this, certain proteins or RNAs may serve roles as core interaction "nodes" that sustain part or all of the condensate network via a combination of possessing a high valency, high affinity interactions, high local concentration, and favorable entry/exit dynamics. Molecules that "bridge" nodes may connect subnetworks together, helping sustain a larger network, or underpin interactions of distinct condensates such as SGs and PBs. Targeting nodes and bridges for degradation or inhibiting their interactions could therefore efficiently promote SG and PB clearance. Disrupting SG and PB networks with incorporation of molecules that "cap" and thus reduce the network interconnectivity is another possible clearance mechanism. Recent publications focusing on G3BP1 and 2 [197–199] have driven a focus on these concepts and are excellently reviewed here [5]. Below, only known, and putative examples of SG network disruption that may promote clearance are discussed, as knowledge for PBs is lacking, though similar concepts likely apply.

9.1. G3BP1/2 – a regulated central node sustaining SG networks

Characterized SG and PB assembly proteins often exhibit high valency and harbor some or all of the following [5]: dimerization or oligomerization domains, IDRs, and RNA binding domains. Possessing all of these features, G3BP1 and 2 represent the most important known human SG assembly proteins, being essential for SG assembly under many (e.g., arsenite, thapsigargin, eIF4A inhibition [172,200]) but not all tested stresses (e.g., HS, sorbitol) [198]. G3BP1/2 exhibit domain homology and redundancy in rescuing SG assembly in *G3BP1/2ΔΔ* backgrounds when expressed ectopically, with each harboring an NTF2L dimerization domain, an RRM RNA binding domain, and 3 IDRs, the 3rd of which also harbors RNA binding activity. G3BP1 NTF2L and RNA binding domains are essential to SG assembly [197–199].

G3BP1 exists in either a closed or open state, the latter of which promotes SG assembly. In the closed state, IDR1-IDR3 bind via electrostatic interactions that limit RNA binding. In response to long relatively unstructured RNAs, which accumulate during stress-induced polysome collapse, G3BP1 adopts an open state, allowing the IDR3 and RRM domains to bind RNA and promote SG assembly [198,199]. *In vitro* and in vivo data with phosphomutant alleles suggests IDR1 phosphorylation, particularly at S149, favors IDR1-IDR3 interaction, and thus impedes SG assembly by reducing RNA binding and RNA-induced condensate formation [198].

Whether G3BP S149 undergoes stress-induced changes in phosphorylation is controversial [200–202]. One study demonstrated that Casein Kinase 2 localizes in SGs following arsenite stress and phosphorylates G3BP1 at S149 in vitro and in vivo. G3BP1 S149 exhibited lower phosphorylation levels during arsenite stress versus unstressed cells, with phosphorylation rebounding during recovery [202]. This supports a proposed "tunable" switch model of RNA-mediated condensation of G3BP1, and subsequent SG assembly [198,199].

Regardless, PTM of G3BP1 (also including acetylation [203], methylation [204,205] and ubiquitination [37]) offers many means to

facilitate SG clearance by altering G3BP1 RNA binding and RNA-induced condensation, disrupting interactions with other proteins (including itself), degrading, or extracting G3BP1 from SGs. All of these possibilities could reduce the strength of the SG interaction network, thus promoting clearance.

9.2. G3BP1/2 binding partners that disrupt SG networks

Distinct protein interactions with G3BP1/2 may also alter SG network strength and favor assembly or clearance. The NTF2L domain not only aids G3BP1/2 dimerization, but also binds proteins that positively (e.g., Caprin1, UBAP2L) or negatively (e.g., USP10) impact SG formation, potentially by adding additional RNA or protein binding valency to G3BP1/2 complexes, thus potentially connecting (Caprin1, UBAP2L) or blocking (USP10) G3BP1/2 interactions with other SG subnetworks [5,197–199]. Caprin1 and USP10 bind competitively to the NTF2L domain [172,206], without disrupting G3BP1 dimerization [207, 208]. USP10 overexpression blocks SG assembly [172,208], and is hypothesized to act as a capping protein that limits G3BP1/2 interaction valency in the SG network [5,197].

The viral nonstructural protein 3 (nsP3) of Semliki Forest virus binds at the same site as USP10 via a pair of FGDF motifs in its C-terminus, sequestering G3BP1/2 into viral replication centers and aiding viral replication by disrupting SG assembly [209]. USP10 harbors its own single FGDF motif, and over-expression of either USP10 or nsP3 blocks SG assembly following multiple stresses in an FGDF-dependent manner [208], likely by blocking G3BP1 interactions with other SG-promoting proteins (e.g., Caprin1, UBAP2L). Recently, small molecule FGDF-peptide mimics have been developed that prevent in vitro condensation of G3BP1, RNA and Caprin1, block assembly of arsenite and HS-induced SGs, and readily clear already formed SGs induced by various stress and mutant stimuli [210].

9.3. General protein-mediated disruption of SG networks

YB-1 may disrupt SG networks by targeting SG interactions involving RNA. YB-1 is a highly abundant multimer-forming protein that strongly localizes in SGs [211], and for which contrasting effects on SG assembly have been reported [166,212–214]. Regardless, YB-1 preferentially binds non-translating mRNA, can disrupt TIA1-mRNA aggregates in vitro, and at endogenous levels, YB-1 facilitates translation and SG clearance following arsenite stress [211,215]. YB-1 SG clearance effects rely at least partly on it's cold-shock domain that disrupts RNA structure in an ATP-independent manner [215,216]. Thus, like eIF4A, it has been described as an RNA chaperone [211]. SG network strength may also be disrupted by broader compositional changes during recovery. Following arsenite stress, SG recovery enriched proteins generally harbor fewer IDR domains, and exhibit lower phase separation potential than SG proteins localized during assembly [32].

10. RNA-based regulation of SG and PB clearance

RNA is an essential component of the networks underlying SG and PB formation. This reflects a combinatorial effect of RNA-protein and intermolecular RNA-RNA interactions. Supporting this, trapping mRNA in polysomes with cycloheximide blocks assembly and facilitates clearance of both granule types (see earlier), whereas introducing an excess of non-translating mRNA (or ssDNA) induces SGs [166]. Interestingly, expression of RNAse L, a viral-induced ssRNA endonuclease, strongly reduces SGs in vivo, but does not impact PBs [234]. Whether this indicates RNAse L accessibility issues to PB RNAs, or that PBs, once assembled, are not reliant on RNA to sustain them, is unclear. Paradoxically, in vitro, isolated PBs are fully cleared by RNAse A treatment [16], whereas SGs are RNAse resistant [34]. Regardless, known, and putative examples of how RNAs impact SG and PB assembly and thus potentially clearance are discussed below.

10.1. RNA nodes in SG and PB networks?

Whilst the lncRNA NEAT1 provides a clear example of an RNA scaffold driving formation of a biomolecular condensate (paraspeckles) [235,236], there is no known singular RNA that drives SG assembly. This is perhaps unsurprising given the number of RNA molecules that localize in and presumably drive SG assembly redundantly (e.g., approximately 42,000 SG-localized RNAs in each U2-OS cell under arsenite stress [124]). However, certain RNA molecules could be more important than others, as variables such as length, lack of structure, low levels of translation, and binding sites for SG-enriched proteins all predispose RNAs to SG enrichment [124]. Recently, snoRNAs have been proposed to help bridge G3BP1 and UBAP2L interactions and aid SG assembly, based on UBAP2L RNA-IP and sequencing, snoRNA KDs, and a reduced UBAP2L-G3BP1 IP interaction with RNAse present [237]. This contrasts with an absence of detectable snoRNAs in SGs [124], and a robust RNase-insensitive G3BP1-UBAP2L IP interaction described elsewhere [197].

In yeast, under non-stress conditions, the *RPS28B* mRNA is required for PB assembly, and facilities PB assembly under stress [238]. This reflects a role for the long 3′UTR of *RPS28B* which binds Edc3 (enhancer of decapping 3; a yeast PB assembly factor) and Dhh1, possibly acting as a nucleating PB scaffold, and a 3′UTR-mediated establishment of an Edc3-Rsp28b protein interaction that also aids PB assembly by an unknown mechanism [238].

10.2. Impact of mRNA modifications

mRNAs can undergo numerous modifications due to the activity of "writer" enzymes. "Readers" bind these modified bases and may confer altered regulation of modified mRNAs, while "erasers" remove mRNA modifications. Many writer, reader and eraser enzymes localize in SGs and PBs based on compositional and microscopy datasets [34,75, 239–242], though in most cases the significance of this is unknown.

m^6A, which is enriched in 3′UTRs and near stop codons, and occurs on 0.1–1.8% of A bases (dependent on context, study) [243], may impact SG and PB composition and dynamics, though findings remain controversial. First, several labs using FISH and SG RNA-seq approaches sensitive to methylation observe that m^6A-modified RNAs are enriched in SGs in various cell and stress contexts [239,240,242]; analogous findings have been made for m^7G [244] and m^1A[245]-modifeid RNA. In contrast, another study argued mRNAs with multiple m^6A modification sites show no SG enrichment [246]. Second, KO or depletion of the m6A writer (METTL3/14) enzyme does not impact SG assembly [239, 240]. Third, KD of three m^6A reader proteins (YTHDF1, 2 and 3) singly or in combination impairs arsenite-induced SG assembly [242], though another study saw no effect with YTHDF3 KD [239], possibly reflective of use of a G3BP1 over-expression cell line model. How might m6A readers impact SG assembly, but the m6A modification be dispersible? One possibility is that YTHDF1, 2 and 3, perhaps via their IDRs, stimulate interactions aiding SG assembly [246], though this remains untested. In contrast, triple KD of YTHDF1, 2 and 3 induces PBs, possibly related to effects of YTHDF proteins on mRNA decay, though preventing m^6A modification itself again has no PB impacts [247].

IP-MS approaches to identify RNA binding proteins influenced by m^6A modification discovered that in certain sequence contexts, G3BP1/2 RNA binding is repelled by m^6A, whereas other SG proteins (e.g., FMR, FXR1/2) and the PB-localized 5′– 3′ Exonuclease XRN1, preferentially bind m^6A [221,248]. >1000 G3BP1/2 RNA binding sites in 3′UTRs overlap with m^6A sites, thus m^6A-driven dissociation of G3BP1/2 mRNA binding could theoretically weaken SG networks and facilitate SG clearance.

10.3. Impact of RNA structure

Intermolecular RNA-RNA interactions promote SG assembly, and

extensively structured RNAs (e.g., tRNAs) are generally excluded from SGs [124,249]. However, specific intramolecular RNA secondary structures, particularly G-quadruplexes (rG4s), can impact SG mRNA targeting and SG dynamics. Transfection of rG4 RNAs promotes stress-independent SG assembly in a small fraction of U2-OS cells in an RNA length and eIF2α phosphorylation-dependent manner [250]. rG4 RNAs accumulate and are enriched within SGs [251]. rG4 RNA derived from a C9ORF72 repeat expansion is notably static in SGs [250], in contrast to G3BP1/2, which along with other SG proteins (DDX3X, DHX36, FMRP) directly binds rG4 RNAs via their RRM and RGG domains [222]. Finally, pre-incubation of small molecules that bind rG4 structures slow arsenite-induced SG assembly, possibly due to impaired rG4 binding by G3BP1/2 and other SG proteins [251]. Thus, disrupting rG4 structures and interactions could theoretically be a SG clearance promoting mechanism.

10.4. RNA degradation as a clearance mechanism?

Since mRNA is an abundant, high-valency molecule sustaining SG and PB formation, a simple way to clear granules, besides mRNA exit, would be to promote mRNA decay. Given enrichment of mRNA decay factors in PBs, this seems at first glance a highly probable as a PB clearance mechanism, though whether mRNA decay occurs in PBs remains controversial.

Supporting PBs as mRNA decay sites, PB numbers and volume in yeast increase significantly following mutations in mRNA decapping factors (Dcp1/2) or Xrn1 (5′−3′ major cytoplasmic exonuclease) [252]. Similarly, Dcp2 KD in U2-OS cells increases PB numbers and increases PB accumulation of an mRNA reporter [19]. mRNAs stalled in decay due to strong secondary structures hindering Xrn1 progress also accumulate in PBs [252]. However, these results do not preclude decay having initiated outside of the PB. Finally, in vitro data suggests Dcp1/2 mRNA decapping activities are facilitated in condensates in the presence of Edc3 [253].

Other evidence argues against PBs as a site of mRNA decay. First, normal mRNA decay rates for various reporters are typically observed in models where visible PBs are genetically blocked [254,255]. Second, mRNA decay intermediates are not detected in isolated PB transcriptomes in cells with functioning mRNA decay [75]. Third, PB-localized mRNAs can return to translation during stress recovery [21,256,257]. Fourth, some mRNAs undergo co-translational decay in yeast [258]. Finally, single molecule analyses for individual reporters indicates mRNA decay does not occur in PBs [257,259].

It remains possible that a subset of mRNAs degrade in PBs. Whether a sufficient fraction of the PB mRNA network is degraded to facilitate PB clearance, versus simply returned to translation, is unknown. In contrast, preferential targeting of mRNAs with a key PB scaffolding role (e.g., *RPS28B* in yeast [238]) could be an efficient means to stimulate PB clearance.

SGs also harbor both RNA exonucleolytic and endonucleolytic enzymes (e.g., Xrn1, Angiogenin, Eri1, and perhaps G3BP1/2; see Box 2), and the ribonuclease/angiogenin inhibitor RNH1 [32,34,260], though no role for these factors in facilitating SG clearance via mRNA decay, targeted or otherwise, has been described.

10.5. RNA dynamics during SG and PB clearance

The extent, rate and specificity with which mRNAs exit SGs and PBs during clearance, particularly following distinct stresses, remains largely unclear. Based on specific mRNA reporter studies, a significant fraction of SG and PB-localized transcripts can exit during stress recovery [14,20–22,261]. The dynamics of all poly(A) SG-localized mRNA in live cells has also been examined under arsenite stress, using injection of fluorescently-labelled poly(U) oligos. Interestingly, 1/3rd of mRNAs diffuse rapidly (half-life in SGs of 40 s), 1/3rd diffuse slowly (half-life of 275 s) and the remaining 1/3rd do not exchange at all [25]. Unfortunately, this method was not applied to the study of mRNA exit during SG or PB clearance, but it would be informative to do so. Recently, a study utilized RNA-seq of transient arsenite-induced SGs, and polysome-associated RNA following stress recovery to suggest that $>$ 95% of SG-associated mRNA re-enter translation, particularly if subject to m^6A modification [262]. However, this work did not determine whether mRNAs entering translation post stress previously resided in SGs or were simply repressed elsewhere in the cytosol (or derived from nuclear export). Finally, single molecule mRNA reporter imaging combined with FRAP indicates that mRNA exchange dynamics in PBs decreases with stress (chronic nutrient deprivation), with a larger fraction becoming nearly immobile in PBs under stress versus non-stress conditions [19].

Collectively, these data highlight a fundamental gap in understanding with clearance implications; namely, to what extent SG and PB mRNA populations exit and re-enter translation, versus other possible fates including targeted mRNA decay or degradation via granulophagy. It is often assumed that mRNAs exit SGs and PBs en masse, at least following transient stresses. As stated earlier, this usually correlates with translational recovery. However, given that the fraction of bulk cytoplasmic mRNA localization in SG ranges from 10–15% with RNA-seq [124] or 5–50% with poly(A)-FISH studies [263], translation recovery is likely driven mostly by non-SG/PB mRNAs being relieved of their repression. Closer study of this issue is warranted.

11. Independence and interplay of SG and PB clearance mechanisms

Given the many SG and PB clearance mechanisms identified to date, it is useful to discern under what conditions one clearance mechanism is particularly favored over others, or whether several clearance pathways work redundantly or in combination. Evidence supporting all these scenarios currently exists.

Several preferential SG clearance mechanisms following a given stress are known. HS-induced SGs in human cells that do not accumulate significant quantities of misfolded proteins are preferentially cleared by Hsp70 during recovery. In contrast, more persistent aberrant SGs enriched in misfolded proteins, which also exhibit reduced dynamics based on FRAP data, increasingly undergo MT-based transport to the aggresome, followed by autophagic (granulophagy) clearance [31,33]. Similarly, transient (30 min) HS stress induces SG clearance that is insensitive to bafilomycin, implying autophagy independence, whereas SGs induced by longer HS (90 min) are bafilomycin sensitive, implying autophagic dependance [37]. Clearance of arsenite and HS-induced SGs are sensitive to VCP and proteasomal inhibition, whereas SGs induced by osmotic stress are insensitive. Clearance of arsenite-induced SGs is also more sensitive to deubiquitinase inhibition than HS-induced SG clearance [56]. Distinct VCP adaptor proteins (ZFAND1 and FAF2) aid clearance of arsenite and HS-induced SGs respectively [37,58]. In ZFAND1 KD cells, or proteasome-inhibited cells, arsenite also leads to the formation of aberrant SGs in which misfolded, nascently translated proteins and autophagic proteins accumulate [58]. In ZFAND1 KD cells, treatment with bafilomycin following arsenite causes a 5-fold accumulation in aberrant SGs 3hrs after arsenite removal. Collectively, these data demonstrate stress-specific SG clearance mechanisms, and suggest that granulophagy may compensate for the inability to clear SGs via Hsp chaperones [31,33] or proteasomal/ZFAND1/VCP-dependent mechanisms [58].

Other studies suggest that multiple SG clearance mechanisms may act together under specific conditions. In yeast, Hsp40 proteins Ydj1 and Sis1, in tandem with Hsp70, simultaneously promote clearance of sodium azide-induced SGs, albeit Ydj1 facilitates SG disassembly and translational recovery, whereas Sis1 aids granulophagy [13]. Similarly, in human cells, both Hsp70 and autophagy are implicated via knockdown studies in weakly aiding SG clearance in proliferating cells subject to daily low-dose arsenite stress. Notably, combinatorial Hsp70 and

autophagy inhibition via KDs does not enhance SG clearance defects seen with single blocks alone, possibly suggesting an epistatic relationship (or incomplete KDs) [264]. More strikingly, senescent cells show upregulation of both Hsp70 and autophagic activity and are strongly inhibited in SG assembly. Knockdown of either Hsp70 or Atg5 (autophagy block) equally rescues SG assembly in senescent cells following arsenite stress, again suggesting epistasis. However, neither autophagy nor Hsp70 inhibition impacts SG clearance in senescent cells [264]. Thus, cellular growth state, and the nature of the stress, impact SG clearance mechanisms.

Given the compositional and structural complexity of SGs, it is likely that under some conditions, >1 clearance mechanism may facilitate complete SG clearance. Specific clearance mechanisms (e.g., VCP activity and the proteasome [58]; MTs and autophagy [31], Syk kinase, VCP and autophagy [154], ULK kinase and VCP [156]) may also function together and thus be epistatic, though with rare exceptions [264], combinatorial inhibition studies of distinct SG clearance mechanisms to assess this are lacking. Alternatively, heterogeneous populations of SGs may exist in most stress contexts, each of which has their specific preferred clearance mechanism. Importantly, no single clearance mechanism has been identified that completely blocks SG clearance under any given stress (Table S1). Thus, in the absence of a known homogenous SG population, careful analysis of the physical state, composition, and dynamics of individual SGs may be necessary to distinguish whether multiple clearance pathways indeed function simultaneously [31,38] or in a compensatory manner. Such questions equally apply to PBs, though better foundational knowledge of PB clearance pathways is required in the first instance.

12. Gaps in knowledge and future experimental directions

Many processes are known that impact SG clearance in specific contexts, with only a handful well described for PBs. This may reflect in part the greater connection in the literature of aberrant SG dynamics and clearance to disease states, though altered PB dynamics have also recently been linked to cancer [161], Parkinson's disease [265] and forms of intellectual disability [266]. Thus, an obvious area for future progress is to better characterize PB clearance. Initial comparisons of whether PB clearance is acted upon by similar mechanism that underly SG clearance may yield general insights for other biomolecular condensates. As discussed throughout the review, several observations suggest that PBs may be surprisingly distinct in their clearance (Table S2), though more work is necessary to understand the significance of these differences, and their underlying mechanisms. Regardless, many key questions remain regarding the mechanisms and context under which specific processes impact clearance of both SGs and PBs (Box 3).

What experimental approaches are preferable to better understand SG and PB clearance? Whilst strong progress has been made in identifying SG clearance mechanisms in particular, most studies have focused on candidate-based analyses rather than utilizing unbiased genome-wide genetic screening approaches to identify key mechanisms. Thus, unbiased genetic and chemical screening methods remain one obvious area to pursue. However, given issues of redundancy, adaptation, or incomplete knockdown, complementing screening methods with biochemical purification studies to assess changes in condensate composition and modification status during and following stress are especially welcome [32,267]. A notable study of this type examined SG composition during and following arsenite stress, over a 2hr recovery time course, using APEX2-based proximity labelling [32]. This approach revealed over 200 "disassembly-engaged proteins" (DEPs) that preferentially enrich in SGs during clearance. These included autophagy proteins, ubiquitination factors, chaperones, RNA helicases, SUMOlyation enzymes, cytoskeletal proteins and interestingly mitochondrial proteins, the significance of which remains unclear. Another recent temporally resolved compositional analysis of HS-induced SGs also revealed that the proteasome, VCP and select translation initiation components only enrich within SGs following $>$ 1hr of HS stress [267], further suggesting temporally and composition-specific clearance mechanisms may operate. Super-resolution and live cell microscopy methods, including single molecule and FRAP studies will remain pivotal in revealing the underlining mechanisms of SG and PB clearance under a given set of experimental conditions. Finally, increased testing of putative SG and PB clearance mechanisms singly and in combination, over a range of stress types, doses and durations would greatly help elucidate key clearance mechanisms, redundancies and epistatic relationships.

Several factors complicate the interpretation and comparison of SG and PB clearance data in prior studies that could be improved in future work. First, clearly distinguishing assembly versus clearance effects is often difficult when using KOs or treatments (e.g., inhibitors) administered prior or coincident with the addition of the inducing stress. Such

Box 3
KEY UNANSWERED QUESTIONS REGARDING SG AND PB CLEARANCE.

1. Do Hsp chaperones bind and act on specific protein (or RNA?) substrates in SGs to facilitate clearance?
2. What factors dictate VCP-mediated clearance of SGs via autophagy, proteasomal or other means?
3. How do cytoskeletal elements and associated motor proteins interface with SG and PB components and regulate clearance?
4. Is eIF4A a non-specific RNA disaggregase that limits assembly, and facilitates clearance of SGs?
5. What are the regulatory mechanisms affecting specificity and activity of SG/PB-resident helicases, and how does this impact SG and PB clearance rates?
6. Does mRNA translation within SGs facilitate their clearance via disruption of RNA-RNA and RNA-protein contacts?
7. Many protein (and RNA?) modifications impact SG and PB dynamics; what are the roles and regulatory mechanisms of erasers of such modifications in SG and PB clearance?
8. What E3 Ub ligases impact SG clearance, and what are their substrates?
9. What are the key receptor and cargo interactions that underpin granulophagy?
10. What are the key substrates of proteasome-mediated SG clearance?
11. Is G3BP1′s role in SG dynamics limited to its multivalent RNA-protein scaffolding function, or do other reported activities contribute?
12. Does RNA decay play any role in SG or PB clearance? If so, do specific RNA molecules exist that preferentially scaffold SGs and PBs, and whose targeted decay would have significant impact?
13. What are the dynamics of bulk mRNA exit (or decay) within SGs and PBs during clearance?
14. What factors (e.g., stress, condensate composition, physical state) dictate the use of specific clearance pathways either singly or in combination?
15. Do cellular energy levels impact the use of specific SG and PB clearance mechanisms?

approaches can significantly alter the levels of SGs or PBs induced between control and experimental cells, thus distorting interpretation of clearance phenotypes. Second, related to this, rates of SG or PB clearance are rarely calculated, and well resolved clearance time courses are often lacking. Finally, reported metrics of SG or PB abundance often do not fully convey potential differences in datasets. For example, % cells with foci does not reveal differences in the average number condensates/cell, their intensity, size, or area relative to cell volume. While experimental practicality often underlies these issues, ideally, quick inactivation strategies for a process of interest (e.g., rapidly acting drugs, conditional inactivation alleles) should be utilized at the point of stress recovery commencing, with detailed time course data being collected. No single metric is perfect for measuring SG and PB levels, and accurate unbiased quantification can be challenging [268]. Nonetheless, use of multiple metrics and automated quantification methods should be pursued whenever reasonably possible.

13. Working model of SG clearance and summary

Although not all SG clearance observations are consistent (Table S1), a general working model (Fig. 1) is that stress-specific variations in SG composition and physical state (e.g., the extent of misfolded or modified proteins/mRNAs) likely elicits stress-specific clearance mechanisms; this would mirror the existence of stress-specific assembly mechanisms [269,270]. SG clearance mechanisms likely vary in importance and redundancy based on stress duration, dose, and cellular context. Granulophagy may become more critical as SGs transition into aberrant, less dynamic states [38]. Maintenance of SG dynamics [34,97], and indeed most reported SG clearance mechanisms also require cellular energy, but the relative energy demands of each pathway, and how this impacts their usage, remain unclear. Finally, RNA itself represents a potentially appealing target for directly effecting SG clearance, possibly through the act of SG-localized translation, modification, structural alteration, or degradation, though these areas remain poorly explored for now.

Understanding of PB clearance remains relatively nascent, but this is an important area for future study which may also impact SGs, given that PBs and SGs physically interact, may exchange components [134] and share fundamental assembly mechanisms and compositional elements (i.e., non-translating mRNPs). Despite this, differences in PB versus SG clearance (Table S2) suggests we may be missing important insight into condensate clearance mechanisms. Thus, to achieve conceptual coherence, in SG and PB clearance, the field needs continued perseverance.

Declaration of Competing Interest

I declare that I have no competing interests with regards to publication of the review “Stress granule and P-body clearance: seeking coherence in acts of disappearance”.

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Competing interests statement

I declare that I have no competing interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.semcdb.2024.01.002.

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