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CELL SCIENCE AT A GLANCE

Mammalian stress granules and P bodies at a glance

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ABSTRACT

Stress granules (SGs) and processing bodies (PBs) are membraneless ribonucleoprotein-based cellular compartments that assemble in response to stress. SGs and PBs form through liquid–liquid phase separation that is driven by high local concentrations of key proteins and RNAs, both of which dynamically shuttle between the granules and the cytoplasm. SGs uniquely contain certain translation initiation factors and PBs are uniquely enriched with factors related to mRNA degradation and decay, although recent analyses reveal much broader protein commonality between these granules. Despite detailed knowledge of their composition and dynamics, the function of SGs and PBs remains poorly understood. Both, however, contain mRNAs, implicating their assembly in the regulation of RNA metabolism. SGs may also serve as hubs that rewire signaling events during stress. By contrast, PBs may constitute RNA storage centers, independent of mRNA decay. The aberrant assembly or disassembly of these granules has pathological implications in cancer, viral infection and neurodegeneration. Here, we review the current concepts regarding the formation, composition, dynamics, function and involvement in disease of SGs and PBs.

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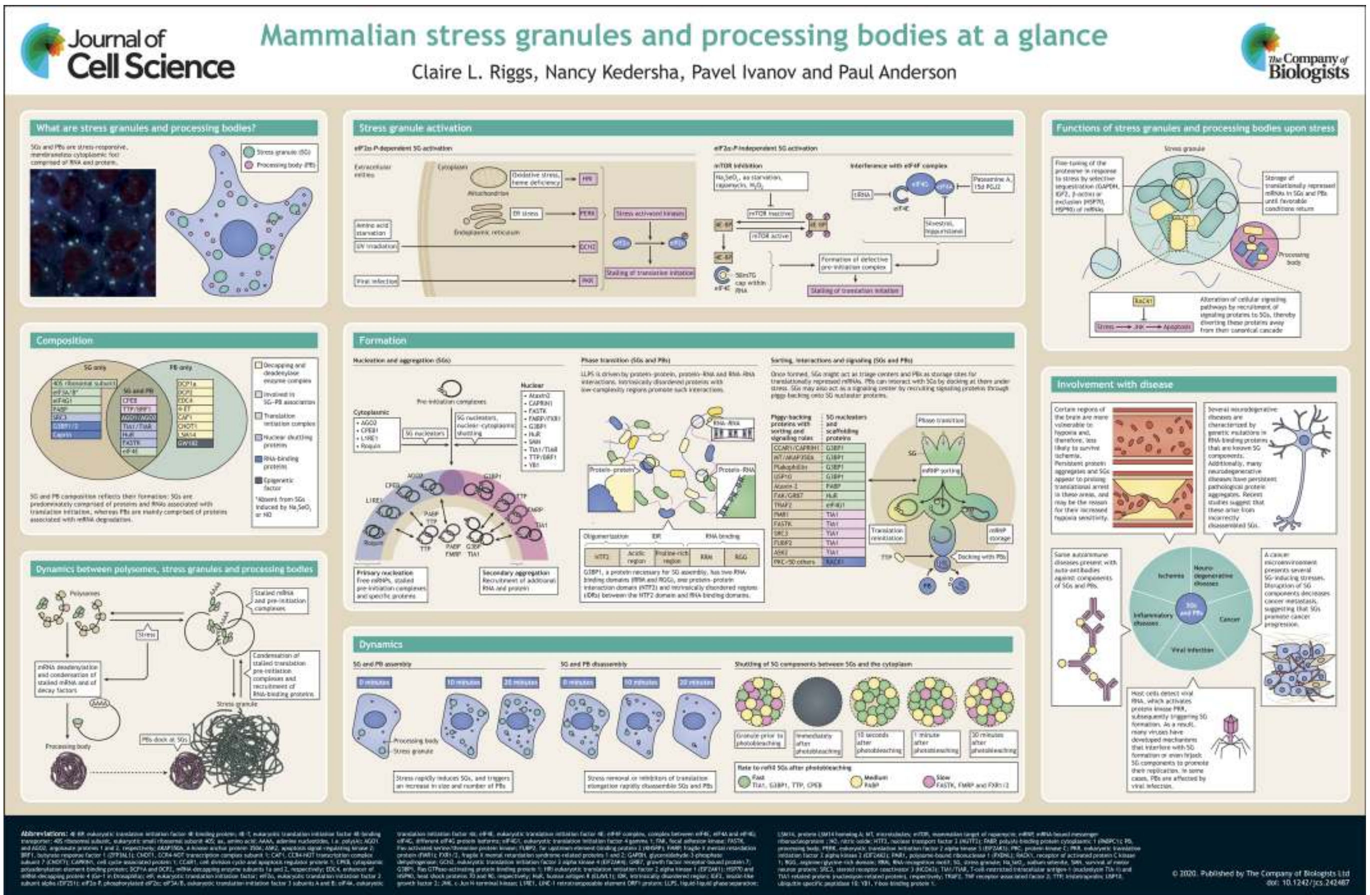
Introduction

Stress granules (SGs) and processing bodies (PBs) are microscopically visible cytoplasmic membraneless organelles, consisting of RNA and proteins. SGs are exclusively stress induced, whereas PBs are constitutive in some cell lines but increase in size and number in response to stress (Kedersha and Anderson, 2007; Kedersha et al., 2005; Ohn et al., 2008). SGs have been linked to the pathogenesis of cancer (Choi et al., 2019;



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Somasekharan et al., 2015), viral infections (Lindquist et al., 2010; Panas et al., 2012) and neurodegenerative diseases (Ash et al., 2014; Liu-Yesucevitz et al., 2010; Taylor et al., 2016; Vanderweyde et al., 2012), thus motivating interest in understanding their functions. Although there is less evidence linking PBs to disease, their proposed role in the storage of translationally repressed mRNAs could have pathological implications. Other types of RNA granules include nuclear Cajal bodies, paraspeckles and the nucleolus, as well as cytoplasmic germ cell granules, neuronal transport-dependent and activity-dependent granules and mitochondrial granules (Buchan, 2014; Pichon et al., 2016; Shin and Brangwynne, 2017; Wolozin and Ivanov, 2019).

Stress-induced cytoplasmic RNA–protein granules were first described in mammalian cells (Kedersha et al. 1999), whereas PBs in mammalian cells were first described as cytoplasmic foci enriched in 5′-3′ exoribonuclease 1 (mXRN1p) (Bashkirov et al., 1997). Orthologs of both may be found throughout eukaryota (Guzikowski et al., 2018). Although SGs and PBs are distinct entities, as evidenced by their composition and formation, they spatially interact under certain stresses (Kedersha et al., 2005; Souquere et al., 2009; Stoecklin and Kedersha, 2013; Wilczynska et al., 2004) and some proteins are shared between compartments (Stoecklin and Kedersha, 2013; see poster). In this Cell Science at a Glance article and accompanying poster, we provide an overview of the formation, composition, dynamics, function and disease involvement of mammalian SGs and PBs, highlighting their similarities and differences.

Formation

SG formation is stress induced, whereas PBs may exist constitutively but increase in size and number with stress (Kedersha et al., 2005). Both biotic and abiotic stressors induce translation inhibition, and subsequent SG and PB formation; however, not all stressors trigger both SGs and PBs (see Table 1). Sodium arsenite ($NaAsO_2$), for example, triggers both SG and PB formation (Kedersha et al., 2005; Ohn et al., 2008), and is commonly used in laboratory settings. SGs and PBs form within minutes to hours of exposure, depending on the particular stressor.

SGs and PBs condense into being through liquid–liquid phase separation (LLPS), a fundamental process that creates liquid-like compartments of RNA and proteins, and is crucial to the integrity of SGs, PBs and many similar RNA granules, including the nucleolus and Cajal bodies (Feric et al., 2016; Shin and Brangwynne, 2017; Uversky, 2017). Phase separation is driven by high local concentrations of RNAs and proteins, and by interactions in ways that favor RNA and protein condensation and separation from the cellular milieu – much like the energetically favorable separation of oil and water. Many SG- and PB-nucleating proteins promote phase separation through protein–protein and protein–RNA interactions. SG and PB proteins are enriched in intrinsically disordered (ID) and low complexity (LC) prion-like repetitive regions (ID/LC regions) that drive LLPS *in vitro* (Kato et al., 2012; Lin et al., 2015; Uversky, 2017).

Table 1. Basic attributes of stress granules and processing bodies

		SG	PB
Characteristics	No stress	Not present	Present in some cell types – i.e. HeLa and COS-7 – but not U2OS cells (Kedersha and Anderson, 2007)
	General stress	Formation induced (Kedersha and Anderson, 2007)	Formation induced – and increase in number and size (Kedersha and Anderson, 2007; Ohn et al., 2008)
Physical characteristics	Size	100–2000 nM (Gilks et al., 2004)	400–500 nM (Ayache et al., 2015; Hubstenberger et al., 2017)
	Shape	Irregular with substructure, moderate electron density (Souquere et al., 2009)	Round, with high electron density (Souquere et al., 2009)
Assembly and disassembly	Time of assembly	$NaAsO_2$ induced: within ∼30 min (Kedersha and Anderson, 2002) UV induced: within ∼18 h (Moutaoufik et al., 2014)	$NaAsO_2$ (500 µm) induced: within 15–60 min, i.e. increase in number and size (Kedersha et al., 2008)
	Time of disassembly	Emetine* or cycloheximide* induced: 1–2 h (Kedersha and Anderson, 2007; Ivanov et al. 2019); UV-induced: SGs remain ≤48 h after stress removal (Moutaoufik et al., 2014)	Cycloheximide* induced: ≥5 min to ∼2 h, i.e. gradual disintegration, the majority being dissolved within this time (Andrei et al., 2005; Cougot et al., 2004; Ferraiuolo et al., 2005, Ivanov et al. 2019)
Nature of stress	Viral infection	Formation, subsequently blocked by some viruses (McInerney et al., 2005; Panas et al., 2012)	No effect – but viruses cause disassembly of PBs (Lloyd, 2013; Liu et al., 2019)
	Bacterial infection	Formation due to aa starvation (Tattoli et al., 2012)	Some bacteria, i.e. *salmonella* and *shigella*, cause disassembly of PBs (Eulalio et al., 2011)
	Osmotic stress	Formation (Bounedjah et al., 2012; Kedersha et al., 2016)	Formation (N.K. unpublished observations)
	UV radiation	Formation (Kwon et al., 2007; Moutaoufik et al., 2014)	No effect (N.K. unpublished observations)
	Hypoxia	Formation (Moeller et al., 2004)	Not known
	Oxidative stress, e.g. NO, H_2O_2, $NaAsO_2$	Formation (Aulas et al., 2018; Emara et al., 2012; Kedersha et al., 2005)	Formation – increase in number and association with SGs (Dang et al., 2006; Kedersha et al., 2005)
	Mitochondrial stress (1 h clotrimazole)	Formation (Kedersha et al., 2005)	No effect – neither increase in number nor association with SGs (Kedersha et al., 2005)
	Heat shock	Formation (Kedersha et al., 2005, 1999)	Formation – but neither increase in size nor association with SGs (Kedersha et al., 2005)

*Cycloheximide and emetine are translation elongation inhibitors.
aa, amino acid; H_2O_2, hydrogen peroxide; $NaAsO_2$, sodium arsenite; NO, nitric oxide.

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Table 2. Overview of translation inhibitory pathways that lead to SG formation via LLPS*

Activating stress	Pathway	Mechanism of translation inhibition
eIF2α-*P*-independent		
Na_2SeO_3, aa starvation, rapamycin, H_2O_2	Inhibition of mTOR, leading to hypophosphorylation of 4E-BP (Sonenberg and Hinnebusch, 2009)	Interference with canonical cap-dependent translation
tiRNAs, pateamine A, silvestrol, hippuristanol, rocaglamide A**	Interference with eIF4F complex assembly or functions (Dang et al., 2006; Emara et al., 2010; Ivanov et al., 2011; Kedersha et al., 2016; Kim et al., 2007)	
Osmotic stress, e.g. NaCl, sorbitol (Bounedjah et al 2012; Kedersha et al., 2016)	Molecular crowding due to shrinkage of the cytoplasm increases local concentration of SG-nucleating proteins	
eIF2α-*P*-dependent (eIF2α kinase activation)		
dsRNA exposed during viral infection	PKR (Srivastava et al., 1998)	Inhibition of the GDP-GTP exchange by eIF2B necessary for recycling of ternary complexes that initiate translation (Gray and Wickens, 1998)
ER stress	PERK (Harding et al., 2000)	
aa deprivation	GCN2 (Wek et al., 1995)	
Oxidative stress (e.g. $NaAsO_2$)	HRI (McEwen et al., 2005)	

*Including ribosome run-off and the accumulation of stalled pre-initiation complexes that serve as substrates for LLPS (Kedersha et al., 2002)
**Hippuristanol, pateamine A, rocaglamide A and silvestrol are eIF4A inhibitors.
aa, amino acid; dsRNA, double-stranded RNA; 4E-BP, eIF4E-binding protein; ER, endoplasmic reticulum; GCN2, eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4); HRI, heme-regulated eukaryotic initiation factor eIF-2-alpha kinase (EIF2AK1); LLPS, liquid–liquid phase separation; Na_2SeO_3, sodium selenite; $NaAsO_2$, sodium arsenite; PERK, eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3); PKR, eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2); tiRNAs, tRNA-derived stress-induced RNAs.

SG formation

Stress triggers translational arrest that stalls the translational machinery and causes the rapid release of mRNA-bound messenger ribonucleoproteins (mRNPs) from polysomes (a cluster of ribosomes on actively translating mRNAs) (Advani and Ivanov, 2019). Translation stalling may occur at different stages in the translation initiation process, depending on the type of stress (see Table 2 and poster) but, in most physiological cases, stalling is triggered by phosphorylation of the eukaryotic translation initiation factor 2 subunit alpha (EIF2S1, hereafter referred to as eIF2α). Hence, SG-inducing stresses are broadly subdivided into those that depend on phosphorylated eIF2α (eIF2α-*P*) and those that do not (i.e. eIF2α-*P*-dependent and eIF2α-*P*-independent stresses) (Panas et al., 2016; see Table 2 and poster) – all of which stall translation initiation but allow elongation, thereby, causing ribosomes engaged in translation to dissociate from mRNA ('ribosome run-off') and polysomes to disassemble. The released mRNA recruits RNA-binding proteins (RBPs) and serves as a substrate for LLPS-mediated SG formation (Kedersha et al., 2005, 1999; Sanders et al., 2020).

SG formation entails the condensation of stalled pre-initiation complexes and associated proteins into discrete immiscible granules (see poster). This process is mediated by specific RBPs that bind to the stalled translation pre-initiation complexes, increasing their local concentration and facilitating LLPS. Overexpression of some RBPs nucleates SGs in the absence of stress (Kedersha et al., 2005; Tourrière et al., 2003), demonstrating that LLPS is driven by high local concentration of nucleating proteins. SG-nucleating RBPs include, among others, Ras GTPase-activating protein-binding protein 1 (G3BP1) (Tourrière et al., 2003), T-cell-restricted intracellular antigen-1 and TIA1-related protein (TIA1 and TIAL1, respectively; hereafter referred to as TIA1/TIAR) (Gilks et al., 2004), tristetraprolin (TTP) (Stoecklin et al., 2004), fragile X mental retardation protein (FMRP; officially known as FMR1) (Mazroui et al., 2002) and cell cycle-associated protein 1 (CAPRIN1) (Solomon et al., 2007). Proteins enriched in ID/LC regions may facilitate SG condensation by promoting protein–protein interactions (Molliex et al., 2015). 'Piggybacking' proteins, which are not involved in the initial SG nucleation and often are not RBPs, are subsequently recruited (Anderson and Kedersha, 2007); for example by binding to nucleators TIA1 (Yang et al., 2006; Yu et al., 2007) and G3BP1 (Hofmann et al., 2006). Additionally, RNA–RNA interactions also contribute to SG assembly (Van Treeck et al., 2018). Post-translational modifications (PTMs) of SG-associated proteins include sumoylation (Jongjitwimol et al., 2016), NEDDylation (Jayabalan et al., 2016), methylation (Huang et al., 2019; Tsai et al., 2016), polyADP-ribosylation (PARylation) (Duan et al., 2019) and *O*-linked *N*-acetylglucosamine modification (Ohn et al., 2008), all of which can influence the formation of SGs.

PB formation

Although mechanistic details of SG formation are emerging, less is known about PB formation. PB proteins are also enriched in ID/LC regions that promote LLPS (Jonas and Izaurralde, 2013; Lin et al., 2015; Schütz et al., 2017; Uversky, 2017). The influx of stalled pre-initiation complexes during stress might directly contribute to the increased size and number of PBs; however, what gives rise to high local concentrations of proteins that form constitutive PBs in some cell lines is unknown. Knockdown of many proteins decreases constitutive and stress-induced PBs, but only probable ATP-dependent RNA helicase DDX6 (DDX6), protein LSM14 homolog A (LSM14A) or eukaryotic translation initiation factor 4E transporter (EIF4ENIF1; also known as 4E-T) appear essential for PB assembly induced by $NaAsO_2$ (Ayache et al., 2015; Ohn et al., 2008). In the case of DDX6, its helicase activity appears crucial (Minshall et al., 2009). Furthermore, as with SGs, PTMs also influence the formation of PBs (Cargnello et al., 2012; Rzeczkowski et al., 2011; Matsumoto et al., 2012).

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SG and PB composition

Methods including immunofluorescence, fluorescence *in situ* hybridization (FISH), fluorescence-activated cell sorting (FACS), fluorescence-activated particle sorting (FAPS) and biochemical/ immunological isolation of putative cores have been used to study the composition of RNA granules. Although the composition of SGs and PBs is distinct, wherein SGs uniquely contain certain translation initiation factors and PBs are enriched in factors associated with RNA stabilization or mRNA silencing (Hubstenberger et al., 2017), recent omics analyses illuminate commonalities between SGs and PBs.

SG composition

Translation-associated factors and RNAs define SGs. Immunofluorescence and FISH experiments reveal many components of the translation initiation complex – i.e. 40S ribosomal subunit, eIF3, eIF4G (officially known as eIF4G1) and poly(A)-binding protein cytoplasmic 1 (PABPC1, hereafter referred to as PABP) – in SGs (Kedersha et al., 2002, 2005). Proximity-labeling studies, in which enzymatic tagging of nearby proteins is used to allow their subsequent identification by mass-spectrometry, identified about 150 novel SG-associated proteins and confirmed 238 known SG proteins (Markmiller et al., 2018). These SG proteins fall into different gene ontology categories; however, many are related to RNA processing, including mRNA stability, translational control, regulation of metabolic processes, spliceosome assembly and mRNA splicing (Markmiller et al., 2018; Youn et al., 2018). Additionally, many SG proteins are enriched in disordered regions (Youn et al., 2018). Studies of isolated SG cores – i.e. separated from their dynamic shell by differential centrifugation and immunoprecipitation – have identified proteins with RNA-binding activity, ATPase activity and protein-modification activity (Jain et al., 2016). Although some SG proteins are predominantly nuclear, such as Fas-activated serine/threonine kinase (FASTK) (Li et al., 2004), TIA1/TIAR (Kedersha et al., 1999), human antigen R (HuR, officially known as ELAVL1) (Gallouzi et al., 2008) and steroid receptor co-activator 3 (SRC3, officially known as NCOA3) (Yu et al., 2007), they move into the cytoplasm under stress, thereby increasing their local concentration and facilitating SG formation. Importantly, SG composition varies by cell type, stress and disease state (Markmiller et al., 2018). For example, SGs induced by sodium selenite (Na_2SeO_3) (Fujimura et al., 2012) or nitric oxide (NO) (Aulas et al., 2018) lack EIF3 subunits and correlate with increased cell death, suggesting that SG composition is associated with cell survival.

SGs contain untranslated polyadenylated mRNAs, a finding based on immunofluorescence against endogenous PABP and *in situ* hybridization for polyA mRNA (Kedersha et al., 1999, 2002, 2005; Souquere et al., 2009). *In situ* hybridization studies identified specific mRNAs recruited into or excluded from SGs (Kedersha and Anderson, 2002; Nelles et al., 2016; Stöhr et al., 2006), and recent transcriptomic analyses of SGs confirm these findings (Khong et al., 2017; Matheny et al., 2019). However, these transcriptomic studies relied on isolating putative cores by using differential centrifugation and/or immunoprecipitation of tagged overexpressed G3BP1 and are, thus, perhaps more indicative of the G3BP1-interactome and granule core, than the SG per se. With the exception of mRNAs coding for the heat shock proteins 70 and 90 (HSP70 and HSP90, respectively) (Kedersha and Anderson, 2002; Stöhr et al., 2006) that are excluded from SGs, mRNA targeting to SGs appears to be non-specific (Khong et al., 2017; Stöhr et al., 2006), although SG-associated mRNAs generally contain longer coding and untranslated regions (Khong et al., 2017). Approximately 10% of the total mRNA in the cell are present at SGs during stress, but the percentage of transcripts localizing to SGs for any given RNA varies widely from <1% to >95% (Khong et al., 2017). Therefore, the translation of certain transcripts or groups of transcripts may be dramatically altered by SGs and, thereby, change the proteome and physiology of the cell.

PB composition

PBs are characterized by proteins involved in mRNA decay or mRNA silencing. Canonical mRNA decay begins when the cap is removed from the 5′ end of the mRNA by the protein-decapping complex, which contains several PB-specific proteins, i.e. mRNA-decapping enzyme subunits 1a or 2 (DCP1A or DCP2, respectively), enhancer of mRNA-decapping protein (EDC4, also known as Hedls, Ge-1 in *Drosophila*) and one protein – DDX6 – shared with SGs. PBs, like SGs, contain translationally repressed, stable mRNAs (Hubstenberger et al., 2017). Transcriptomic analysis revealed that PB-enriched mRNAs encode proteins that are about 20 times less abundant in the cytoplasm than proteins encoded by mRNAs that are not enriched in PBs (Hubstenberger et al., 2017). The adenylation state of mRNAs in PBs remains ambiguous. Although the absence of PABP in PBs provides evidence for deadenylated mRNAs in PBs within U2OS cells (Kedersha et al., 2005), a recent analysis of poly(A) lengths of mRNAs enriched in PBs within HEK293 cells did not reveal a correlation between poly(A) length and enrichment in PBs, suggesting that shorter (i.e. deadenylated) mRNA is not a hallmark of PBs (Hubstenberger et al., 2017). RNA type and function influence enrichment in PBs (Pitchiaya et al., 2019; Hubstenberger et al., 2017). PBs appear selectively enriched for mRNAs encoding regulons, i.e. groups of genes that work together, and seem depleted of mRNAs encoding housekeeping proteins, including mRNA decay and translational repression factors, miRNA pathway components, decapping components and nonsense-mediated mRNA decay (NMD) factors (Hubstenberger et al., 2017). Under non-stress conditions, poorly translated mRNAs dominate the transcriptome of constitutive PBs, which may be indicative of a storage function (Matheny et al., 2019). PB-mRNAs, typically, have a low GC content and are enriched in particular codons associated with low translation rates (Courel et al., 2019).

Shared SG and PB components

Proteins shared between SGs and PBs include those that promote SG–PB fusion or tethering, such as TTP and butyrate response factor-1 (BRF1; officially known as ZFP36L1), as well as eIF4E, which is required for translation of capped mRNA (Kedersha et al., 2005). A proximity-labeling study indicates that PBs share 144 SG-associated proteins, many of which contain extensive disordered regions (Youn et al., 2018) associated with LLPS. In addition, when PBs are eliminated, some proteins that are typically found in PBs relocate to SGs (Mollet et al., 2008). Transcriptomes associated with PBs change upon stress, with SG and PB transcriptomes actually becoming more similar under stress (Matheny et al., 2019).

Dynamics

SGs rapidly assemble and disassemble in response to the onset and removal of environmental stress, although the kinetics are stress specific. Whereas constitutive PBs exist in some cells, stress-induced PBs also form rapidly (within 15 min) upon environmental stress (Kedersha et al., 2008) (see Table 1 and poster).

Both SGs and PBs are in a dynamic equilibrium with polysomes (see poster), which can be demonstrated by the pharmacological inhibition of translation. Stabilization of polysomes in response to the

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elongation inhibitors emetine or cycloheximide prevents assembly of SGs and PBs (Andrei et al., 2005; Kedersha et al., 2000). Puromycin, by contrast, promotes premature termination of translation, destabilizes polysomes and enhances SG assembly (Kedersha et al., 2000). Similarly, PBs are disassembled upon treatment with cycloheximide, albeit more slowly than SGs (Andrei et al., 2005; Cougot et al., 2004; Ivanov et al., 2019), suggesting that PB maintenance also requires the presence of stalled pre-initiation complexes.

The contents of SGs and PBs are dynamic, as both proteins and RNAs shuttle in and out. Protein shuttling rates can be experimentally determined by using fluorescence recovery after photobleaching (FRAP), in which a fluorescently tagged SG or PB protein is photobleached and its exchange rate monitored. Exchange rates of individual proteins are in the range of seconds to several minutes (Andrei et al., 2005; Bley et al., 2015; Kedersha et al., 2005; Mollet et al., 2008) (see poster). Less is known about RNA dynamics within SGs and PBs. A few SG-associated transcripts shuttle with a half-life of 1 min (Mollet et al., 2008). Continuous shuttling of protein and RNA, into and out of SGs and PBs, allows their assembly and disassembly to be highly dynamic, which is likely to be important in rapid stress response and avoidance of disease states associated with persistent granules.

Function

Little is known – and much speculated – regarding SG and PB function. Cells and organisms exposed to stress must use their resources judiciously, if they are to adapt to, and survive under, changing conditions. Stress granules may maximize the efficiency of translation reprogramming by dynamically partitioning silenced mRNAs away from cytoplasmic domains at which translation occurs, thus facilitating translation of high-priority mRNAs. House-keeping mRNAs, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin and insulin-like growth factor 2 (IGF2) (Stöhr et al., 2006), are sorted into SGs and, thereby, silenced during stress. In contrast, mRNAs coding for proteins necessary to respond to stress, such as HSP70 (Kedersha and Anderson, 2002) during heat shock, are simultaneously excluded from SGs and transcriptionally activated, thereby promoting their expression. This facilitates rapid and reversible changes in the cellular proteome and, consequently, the physiology in the face of environmental stress.

Independent of translation reprogramming, SGs may act as signaling hubs since they recruit many signaling proteins unrelated to RNA metabolism. Diverting such proteins away from their canonical signaling roles may rewire the metabolism, growth and survival of the cell (Kedersha et al., 2013). For example, receptor of activated protein C kinase 1 (RACK1) integrates cell adhesion, polarity and motility. RACK1 sequestration in eIF2α-*P*-dependent SGs inhibits stress-induced activation of the c-Jun N-terminal kinase (JNK) pathway that triggers apoptotic death, thereby, preventing apoptosis (Arimoto et al., 2008).

SGs and PBs contain RNA-induced silencing complex (RISC) components, such as microRNAs and argonaute proteins (Leung and Sharp, 2006; Liu et al., 2005; Sen and Blau, 2005; Gallois-Montbrun et al., 2007), which target and silence specific mRNAs. SGs and PBs also house RNA-editing enzymes with antiviral activity, such as apolipoprotein B mRNA-editing enzyme catalytic subunit 3G (APOBEC3G) (Gallois-Montbrun et al., 2007). Moreover, a retrotransposon-derived RNA, LINE-1 retrotransposable element ORF1 (L1RE1), which encodes an RNA binding protein, is found only in SGs (Goodier et al., 2007). The presence of these epigenetic regulators suggests that SGs and PBs may modulate gene expression through epigenetic-related mechanisms.

PBs are enriched in RNA decay enzymes (Cougot et al., 2004; Sheth and Parker, 2003) but are not required for constitutive mRNA decay, miRNA-induced silencing or NMD (Eulalio et al., 2007; Horvathova et al., 2017; Pitchiaya et al., 2019). Recent studies posit that the partitioning of mRNAs into PBs determines the extent to which mRNAs are translated (Hubstenberger et al., 2017). This model is consistent with data showing that PB-associated mRNAs can re-enter the actively translating polysome fraction in response to changes in the environment of the cell (Bhattacharyya et al., 2006; Brengues et al., 2005; Hubstenberger et al., 2017). The coordinated sequestration of regulons – such as mRNAs encoding subunits of the same complex or regulatory switches, like histone modifiers, in PBs – may allow for the coordinated translation of key sets of proteins during recovery from stress (Hubstenberger et al., 2017). These results support the claim that PB formation is a consequence of decay (Eulalio et al., 2007) rather than a mediator of mRNA decay. Therefore, PBs may store mRNA to prioritize its translation or decay when resources permit. Furthermore, recent work also suggests that PBs are involved in differentiation and self-renewal of stem cells (Di Stefano et al., 2019). By storing specific mRNAs in PBs, cells may be poised for rapid plasticity in response to developmental cues and environmental changes alike.

Involvement with disease

Whereas SGs and PBs may be involved in translation regulation, signaling and epigenetic modifications during stress, their functions appear context dependent. In many cases, SGs are coopted to advance disease progression, or their aberrant formation is associated with disease (see poster); however we still lack data regarding the involvement of PBs in disease.

SGs and disease

SGs are associated with cancers, ischemic events (heart attack and stroke), neurodegenerative diseases, inflammatory diseases and infections. In cancer, the tumor microenvironment exposes cancer cells to diverse and rapidly changing stresses, including hypoxia, ER stress and osmotic stress, in addition to the stress resulting from chemotherapy drugs (Ackerman and Simon, 2014; Anderson et al., 2015). This environment induces SG formation through phosphorylation of eIF2α by stress-induced kinases. SG assembly is linked to cancer-cell survival during chemotherapy, suggesting that SGs promotes the development of chemo-resistant cancer and aids metastasis (Somasekharan et al., 2015). At the same time, some synthetic and natural compounds that induce SG assembly through eIF2α-*P*-independent mechanisms appear to be therapeutic, as they encourage apoptosis and halt proliferation (Bordeleau et al., 2008; Kuznetsov et al., 2009; Shin et al., 2009; Tsumuraya et al., 2011). One such compound is 15-deoxy-Δ(12,14) prostaglandin J2 (15d-PGJ2), which targets the ATP-dependent RNA helicase eukaryotic initiation factor 4A-I (eIF4A1) (Kim et al., 2007), stops cancer proliferation and induces apoptosis during leukemia and colorectal cancer (Shin et al., 2009).

RNA granules are part of the cellular defense against viruses (Lloyd, 2013; McInerney et al., 2005), and may contribute to sensing the virus and triggering an immune response (reviewed in Tsai and Lloyd, 2014). Viral infection activates eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2, hereafter referred to as PKR), which phosphorylates eIF2α (Srivastava et al., 1998), inhibits translation of both viral and cellular mRNAs (Balachandran et al., 2000) and, consistently, triggers SG formation. To evade repression of viral translation, many viruses suppress SGs by using various mechanisms, such as cleavage of key SG proteins

(such as G3BP1) through a viral proteinase (White et al., 2007), inactivation of PKR (Khaperskyy et al., 2011) or inclusion of SG components by redirecting them to viral replication centers (Panas et al., 2012). Viral interference with SGs suggests that SGs are an essential part of the cellular defense against viruses.

In neurodegenerative diseases, impaired or aberrant SG composition, formation and disassembly are genetically linked to pathology. Genetic mutations in RBPs that are central to SG assembly and composition connect SG biology with neurodegenerative diseases: a mutation in the RNA-binding protein FMRP is the most common cause of the fragile X mental retardation phenotype (Bassell and Warren, 2008), and mutant forms of FMRP impair SG assembly (Didiot et al., 2009). Alzheimer's disease is characterized by pathological protein aggregates that contain core SG-nucleating proteins, such as TIA1/R, TTP and G3BP1 (Ash et al., 2014; Vanderweyde et al., 2012). In patients with amyotrophic lateral sclerosis (ALS), TAR DNA-binding protein-43 (TDP43) is a prominent component of the pathological protein aggregates and colocalizes with SGs in CNS tissue (Liu-Yesucevitz et al., 2010). Moreover, as in the case of fragile X syndrome and Alzheimer's disease, mutations in different SG-associated RBPs are found in ALS patients (reviewed in Taylor et al., 2016).

In addition to them correlating with mutations in SG proteins, all these neurodegenerative diseases are associated with the persistent occurrence of 'pathological SGs' that seem to have an extended life span compared with typical SGs (reviewed in detail by Wolozin and Ivanov, 2019). ALS- and/or frontotemporal dementia-associated mutations identified in the low complexity domain – a region of little diversity regarding its aa composition – of TIA increase its phase separation *in vitro* and *in vivo* and promote large SG-like inclusions enriched with TDP-43 (Mackenzie et al., 2017). Whereas SGs may be an adaptive response to acute stress, abnormal SGs that persist in neurodegenerative diseases might result from (or indicate) pathological states.

One distinction between cancer and neurodegenerative diseases is the ability of cells to undergo division. Cancer cells divide continuously and rapidly, and thus effectively dilute out stress-induced injuries of the microenvironment. In contrast, neurons do not divide, may accumulate malfunctional SG proteins and, thus, lose their ability to mount a stress response (Wolozin and Ivanov, 2019).

SGs cannot simply be characterized as promoting or counteracting disease progression. Disease-specific differences in SG composition and dynamics, and altered biology due to mutations in known SG RBPs indicate that the roles of SGs in disease progression are complex yet significant. Further investigation is warranted to dissect the role of RNA granules in disease.

PBs and disease

Research linking PBs to disease is minimal but some evidence links PBs to viral infection, neurodegenerative diseases and autoimmune diseases. Some viruses, predominantly RNA viruses, affect PB number and stability, and can recruit PB components to viral replication centers (Chahar et al., 2013; Emara and Brinton, 2007; Liu et al., 2019). However, whether these changes affect viral replication rates is unknown. Furthermore, point mutations in DDX6, a key protein required for PB assembly (Ayache et al., 2015), abrogate PB assembly and are linked to intellectual developmental disorder with impaired language and dysmorphic facies (IDDILF) (Balak et al., 2019). Finally, in the context of autoimmune diseases, autoantibodies against different PB components have been recognized and these, potentially, contribute to the autoimmunity phenotype (Bhanji et al., 2007; Johnson et al., 2016).

CONCLUSIONS

SGs and PBs are transient cytoplasmic membraneless compartments of RNA and protein, and represent new frontiers for understanding translational control. When cells are faced with environmental stress, adapting to maintain homeostasis is paramount. Stresses induce global inhibition of translation, which triggers formation of SGs and PBs, both of which may then contribute to the acute response to stress.

Technological advances since the discovery of mammalian SGs and PBs have greatly enhanced our ability to probe their basic biology, yet many outstanding questions remain regarding their function(s) and role(s) in disease. To better understand RNA granule biology, we must consider multiple levels of biological organization – from single molecules to signaling networks and protein interactomes, to cellular and organismal physiology. Understanding what determines whether a specific mRNA or protein is sorted into a SG or PB, how it is recruited there and how this partitioning alters its behavior or function is crucial. Developing methods in order to assess the activity and function of proteins when condensed into SGs and PBs versus proteins outside of these compartments might clarify the specific roles of SGs and PBs. Deciphering how the presence of SGs and PBs changes the biology of the cell might clarify the emergent properties of RNA granules. Ultimately, expansion into whole-organism models will be essential to develop a comprehensive understanding of the role of SGs and PBs in disease.

Over the last two decades, SGs and PBs have emerged in cellular biology as complex, dynamic and context-specific byproducts of cellular stress, likely to be involved in adaptive reprogramming of cellular translation. Their full contribution to cellular homeostasis remains to be seen. Nonetheless, the discovery of SGs and PBs, and their transient, dynamic nature as subcellular compartments highlight the complexity of cellular transcriptomics and proteomics. As such, work on SGs and PBs is relevant to the fundamentals of cellular biology, and has the potential to impact our understanding of cellular biology beyond the stress response.

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