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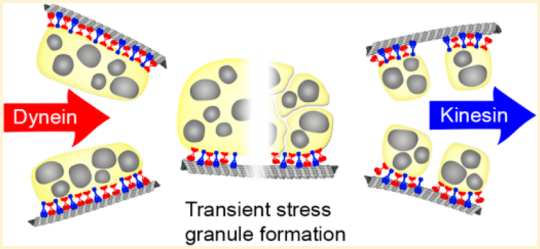
Life and Work of Stress Granules and Processing Bodies: New Insights into Their Formation and Function

Marcelo Perez-Pepe, Ana J. Fernández-Alvarez, and Graciela L. Boccaccio*

Instituto Leloir and Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA)−CONICET, Buenos Aires, Argentina

ABSTRACT: The dynamic formation of stress granules (SGs), processing bodies (PBs), and related RNA organelles regulates diverse cellular processes, including the coordination of functionally connected messengers, the translational regulation at the synapse, and the control of viruses and retrotransposons. Recent studies have shown that pyruvate kinase and other enzymes localize in SGs and PBs, where they become protected from stress insults. These observations may have implications for enzyme regulation and metabolic control exerted by RNA-based organelles. The formation of these cellular bodies is governed by liquid−liquid phase separation (LLPS) processes, and it needs to be strictly controlled to prevent pathogenic aggregation. The intracellular concentration of key metabolites, such as ATP and sterol derivatives, may influence protein solubility, thus affecting the dynamics of liquid organelles. LLPS in vitro depends on the thermal diffusion of macromolecules, which is limited inside cells, where the condensation and dissolution of membrane-less organelles are helped by energy-driven processes. The active transport by the retrograde motor dynein helps SG assembly, whereas the anterograde motor kinesin mediates SG dissolution; a tug of war between these two molecular motors allows transient SG formation. There is evidence that the efficiency of dynein-mediated transport increases with the number of motor molecules associated with the cargo. The dynein-dependent transport may be influenced by cargo size as larger cargos can load a larger number of motors. We propose a model based on this emergent property of dynein motors, which would be collectively stronger during SG condensation and weaker during SG breakdown, thus allowing kinesin-mediated dispersion.



"Cientos de irupés blancos navegaban veloces por el Bermejo. Como enormes balsas las flores se entrechocaban y desaparecían a veces en los remolinos ..." "Hundreds of white flowers sailing swiftly down the Bermejo river. Like huge rafts, the plants clashed one another and sometimes disappeared into the swirls ... Luis Politi, "El Berna", in *Formosa, puros cuentos* (2010).

Since their discovery more than a decade ago, a growing number of membrane-less organelles involved in diverse cellular processes have been described in higher and lower eukaryotes.[1−6] Several nuclear and cytosolic RNA bodies were implicated in alternative splicing, coordination of the expression of functionally related mRNAs, control of the translatable transcriptome at the synapse, and modulation of signaling pathways, among other important functions. In this work, we focus on PBs, SGs, and related structures, which we collectively termed "mRNA silencing foci", as they contain repressed mRNAs.[4] PBs are almost always present in the cytosol, with highly variable numbers and sizes. In contrast, SGs are transiently induced upon acute stress as a consequence of the massive accumulation of repressed messenger ribonucleoparticles (mRNPs) due to the global translational silencing typically elicited by the stress response.[7−11]

Both PBs and SGs are dynamic and exchange molecules with the cytosol. They show a highly variable composition that depends on the cell type and the physiological context. PBs and SGs are frequently in contact and can merge into a single organelle under prolonged or severe stress.[4,12−15] PBs and SGs coexist with discrete protein aggregates that are specifically induced to control the fate of damaged proteins, including JUNQ (juxta nuclear quality control) and IPOD (insoluble protein deposit). The interaction between all these molecular condensates is relevant to neurodegeneration and, as such, intensely discussed.[16−20]

The formation of membrane-less organelles largely depends on multivalent weak interactions among their molecular components. The relevance of protein−protein interactions and protein−RNA interactions in the formation of PBs and SGs is well-known. In addition, RNA−RNA interactions were recently suggested to contribute to SG aggregation.[21] PB and SG proteins are frequently rich in intrinsically disordered regions (IDRs) and low-complexity regions (LCRs). IDRs lack stable secondary structure, and LCRs have a poorly diverse amino acid composition, often with short repeats, and may behave as IDRs. A number of IDRs were shown to bind RNA,[22] and in addition, IDRs mediate protein−protein homotypic interactions, thereby directing a liquid−liquid phase separation (LLPS) process.[7,8,16,20,23−26] Liquid demixing is regulated by multiple post-translational modifications, for example, hyperphosphorylation of LCRs, which interferes with intermolecular

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contacts in vitro and in vivo.[27,28] In contrast, poly-ADP-ribosylation, a protein modification that mimics the RNA backbone, helps SG assembly.[29] More recently, the mutually exclusive methylation and citrullination of RGG motifs was shown to affect protein aggregation.[30]

Additional factors may influence LLPS inside cells, whereas the presence of organelles and macromolecular complexes including several types of cytoskeleton filaments arranged in linear and three-dimensional networks affects movement, solubility, and crowding. For example, LLPS depends on thermal diffusion in vitro, which is rather limited inside cells. Several ATP-dependent mechanisms support intracellular movement of molecules and particles, thus allowing changes in the local concentration of molecules that eventually separate in an immiscible phase.[18−20,23,25,31] In accordance with this, the experimental evidence adduced to date supports the idea that SG formation is governed by phase separation processes and additional forces, including the action of molecular motors, as discussed below. Another related example is the assembly of the nucleolus, which is influenced by both thermodynamically driven processes and active mechanisms that are mostly absent in cell-free systems.[32] In other cellular systems, the actin network prevents liquid nuclear bodies from sedimentation and fusion.[33,34]

Although the conceptual framework that cellular membraneless organelles form via LLPS processes is somehow incomplete, valuable information is being gathered in vitro with the use of chemically defined systems, for example, about the role of normal or pathological LCRs and IDRs.[21,28,35−37] In addition, experiments in vitro have shown that the presence of chemicals that increase the degree of molecular crowding induces the formation of RNA aggregates, which is further facilitated by the addition of SG proteins.[38] More recently, Parker and co-workers showed that purified yeast RNA forms assemblies in vitro when exposed to stress-mimicking conditions, and these RNA assemblies show about the same transcript selectivity as that of SGs induced in yeast cells.[21] The combination of both in vitro and in vivo approaches will help the understanding of this growing family of cellular organelles whose functional relevance we have just begun to explore.

In this work, we focus on the cellular dynamics of SG formation and dissolution highlighting the conserved role of the molecular motors, which are the main source of movement in the cytosol. In addition, we discuss unexpected findings regarding the composition and function of SGs, PBs, and related RNA organelles.

MOLECULAR MOTORS CONTROL SG AND PB DYNAMICS

Super-resolution microscopy of fixed or living mammalian cells revealed that a typical stress granule includes several cores of silenced mRNPs immersed in a less dense shell with liquid phase properties.[39,40] SG cores can be isolated biochemically, and their integrity depends on both hydrophobic and electrostatic interactions.[39,41] A current model proposes that SGs assemble by a stepped pathway. The first step would occur at the nanoscale level, with the formation of accretions that escape microscope detection but can be monitored biochemically. For example, Wallace et al. isolated aggregates of polyadenosine-binding protein 1 (Pab1) from yeast cells exposed to heat shock, while microscopically visible foci were absent.[42]

Microscale SG cores are formed after the primary nucleation, followed by the condensation of a liquid shell that helps fusion and growth, leading to mature SGs.[26] This model implies the movement of macromolecules and relatively large particles, which do not diffuse freely in the cytosol but are actively transported by several mechanisms involving the cytoskeleton and molecular motors.[31,43,44] Briefly, the movement of different organelles along microtubules and microfilaments by the action of kinesin, dynein, and myosin with ATP consumption is well-documented. Motor-driven transports constantly agitate the cytoplasm. Additional ATP-dependent processes, including bending, oscillation, and polymerization and depolymerization of the cytoskeleton filaments, further contribute to cytoplasmic mixing. These mechanisms are collectively termed active diffusion, which unlike thermal diffusion, depends on ATP.[31,45] Whereas the contribution of active diffusion to liquid organelle dynamics remains to be confirmed, the direct role of molecular motors is well-documented. Specifically, the retrograde microtubule-dependent motor dynein is required for SG assembly[46,47] (reviewed in refs 4, 7, 8, and 48). Not surprisingly, dynein also helps the aggregation of abnormal proteins, and this may additionally link SG dynamics to the accumulation of protein deposits relevant to neurodegeneration.[49,50]

In accordance with the requirement for dynein, SG growth in mammalian cells is impaired when microtubules are pharmacologically disrupted.[46,51−55] The exposure of cells to oxidative insults or hyperosmotic stress in the presence of microtubule inhibitors induces small and numerous granules that fail to fuse into mature SGs.[26,38,46] Thus, the condensation of the small nuclei of silenced mRNPs that occurs at early times appears to be independent of microtubules. This step seems to depend on the actin network, as pharmacological disruption of microfilaments leads to the formation of smaller but more numerous granules compared to those formed upon microtubule disruption.[46] Microfilaments are a scaffold for the translational apparatus,[56] and their participation in the first stages of SG formation suggests that the mRNAs may remain in contact with the cytoskeleton after the stress-induced silencing. Repressed mRNPs would be transported by the action of myosins, and the participation of active diffusion supported by microfilaments seems possible, as well.

As expected, PB dynamics and movement also depend on molecular motors (reviewed in refs 4 and 48). PBs are more or less constitutive and grow upon stress. Under normal conditions, PBs are not significantly affected by microtubule-dependent motors and depend mostly on myosin motors, which operate on actin tracks. A number of reports consistently demonstrate that members of the myosin V family, previously implicated in mRNA transport,[57−59] are involved in PB formation in both yeast and mammalian cells.[46,58,60,61] More recently, four additional myosins were detected among the numerous proteins present in purified human PBs,[9] potentially helping their dynamics. In addition, PBs are highly motile, and their movement occurs mostly along microtubules in animal cells and on microfilaments in plants,[60,62,63] which is the case for many other cargos in these organisms.

Microtubule disruption does not affect the dynamic exchange between PBs and the cytosol as evaluated by fluorescence recovery after photobleaching (FRAP). Furthermore, dynein or kinesin knockdown has no effect on the size or number of basal PBs.[46,60] However, the growth of PBs upon acute stress depends on dynein, thus suggesting that the movement of

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stress-repressed mRNPs by dynein motors is common for both SGs and PBs.[46] SGs frequently form and grow in contact with PBs, and this observation may be related to the shared requirement for dynein.

Molecular motors need adaptors to interact with their cargos. A universal adaptor for dynein is the dynactin complex, which together with Bicaudal D (BicD) proteins activates motor activity.[64,65] The stimulation of dynein processivity by these and related adaptors provides a mechanism for coordinating cargo binding with motor activation. Previous work demonstrated that fly BicD and the mammalian homologue BicD1, but not BicD2, are implicated in SG assembly.[46] Imaging analysis shows that dynein heavy and intermediate chains (DHC and DIC, respectively) are present in SGs, and more recently, dynactin subunit 1 (DCTN1) was found among 317 proteins present in purified SG cores.[39,46,47] These observations support the direct transport of SG cores by dynein. The recruitment of the motor−adaptor complexes may be mediated by RNA-binding proteins normally associated with mRNAs and/or by additional proteins bound during the stress response. In *Drosophila*, the interaction of RNAs with the dynein−BicD motor complex is mediated by Egalitarian, which binds RNA with low specificity and recruits and activates dynein, thus mediating the transport of mRNA toward the microtubule minus end.[59,66−68] Not surprisingly, Egalitarian participates in SG formation in *Drosophila* S2R+ cells (M. Loschi and G. L. Boccaccio, unpublished observations). The vertebrate Egalitarian functional homologue remains elusive, and whether a related secondary adaptor helps SG assembly in vertebrates is unknown. Furthermore, the retrograde motor complex can be recruited by additional adaptors, and among other putative candidates, disrupted in schizophrenia 1 (DISC1) is present in SGs,[69] thus opening new hypotheses to investigate.

SGs are transient and real time microscopy and biochemical studies indicate that SG disassembly is the reverse process of SG formation. Shell dissipation and core dissolution lead to a uniform distribution of their components.[26] The disaggregation involves chaperones and additional ATPases to recover protein solubility.[70,71] However, the weakening of the intermolecular forces is not enough to allow the dispersion of the SG components into the cytosol, and SG dissolution requires specific molecular motors. Kinesins, which move opposite to dyneins along microtubules, are involved. In mammalian cells, kinesin heavy chain 1 (KHC1), also known as KIF5B, and the adaptor protein termed kinesin light chain 1 (KLC1) mediate SG dispersion.[46] Imaging analysis indicates that KHC1/KIF5B and KLC1 are present in SGs, supporting a direct role. Besides KHC1/KIF5B, additional kinesins, specifically, KIF23, KIF13B, and KIF1B, were detected in SGs.[39,72,73] Given the complementary and redundant function of the numerous kinesins described to date, these motors are expected to contribute. However, the knockdown of mammalian KHC1/KIF5B or *Drosophila* KHC seriously affects SG disassembly, suggesting that these are the most important motors for SG dissolution.[46] Their recruitment is likely mediated by primary and secondary adaptors, including several RNA-binding proteins (RBPs) present in SGs that mediate the interaction with kinesins thus facilitating mRNA transport in other cellular contexts. Among others, Staufen, La, fragile X mental retardation protein (FMRP), and zipcode-binding protein 1 (ZBP1) are likely candidates.[11,74−78]

DYNEIN EMERGENT PROPERTIES IN TRANSIENT SG FORMATION

SG formation and dissolution are separate stages, and a given cell displays either growing SGs or vanishing SGs. However, the actions of dynein and kinesin are not separated in time. During SG formation, kinesin counteracts the nucleating action of dynein, and conversely, SG dispersion is helped by kinesin and opposed by dynein.[46] This "tug of war" is frequent in intracellular transport[59,76−80] and suggests that a change in the balance between retrograde and anterograde transport allows SG formation followed by SG dispersion. How is this balance controlled? Motors are regulated at several levels, including cargo recruitment, microtubule binding, and ATPase activity.[80] Microtubule-dependent transport is also influenced by tubulin post-translational modifications, which decorate the microtubule surface thus affecting the interaction with the molecular motors.[81] All these processes respond to several signaling pathways, including glycogen synthase kinase 3 β (GSK3β), casein kinase (CK), protein kinase C (PKC), the stress-activated c-Jun N-terminal kinase (JNK), and p38, and many of these kinases have a known role in SG formation.[82] Several examples have been reported of dysregulation of these signaling routes in age-related neurodegenerative diseases, where defective intracellular transport was suggested to be an important pathogenic factor. In addition, adult-onset diseases frequently involve the accumulation of intracellular aggregates that share components with SGs.[82−85] We speculate that altered SG dynamics likely contributes to the cell damage linked to defective intracellular transport.

It has been suggested that the retrograde transport may depend on the number of active dynein motors attached to the cargo. In several examples, including the movement of RNPs and membranous organelles, the dynein number influences both the processivity and the average speed.[79,86−89] More recently, Urnavicius et al. showed that the adaptors Bicaudal D-related protein 1 (BicDR1) and Hook3 preferentially recruit two dynein motors thus allowing faster movement, hence adding evidence of an effect of the dynein number on speed.[90] This likely reflects the fact that dynein molecules are prone to detach from microtubules and that they work better in teams, as detachment of a dynein complex is compensated by the action of another. This emergent property affects dynein, and it has been proposed that in contrast, kinesins are efficient motors so that the number of kinesins attached to the cargo has a milder influence on their global performance.[88,89,91] Using different experimental systems, evidence has shown that several active dyneins are required to oppose the dragging force of a single kinesin.[79,86−89] However, a recent in vitro study showed that a single dynein motor fully activated by the binding of dynactin and BicD2 successfully competes with a kinesin motor, thus suggesting that the effect of the dynein number is modulated by the adaptors involved.[92] In addition, given the relatively large size of dynein motors, steric constraints may be expected to affect the interaction with the microtubule of the multiple dyneins bound to a small cargo.[80] All these observations allow the speculation that dynein would move small cargos less efficiently than larger cargos of a similar nature, whereas the transport by kinesin would be less affected by cargo size. This seems to be the case for melanosomes, which are aggregated by dynein and dispersed by kinesin[93] with a differential dependence on organelle size. We speculate that after the primary microtubule-independent accretion of SG

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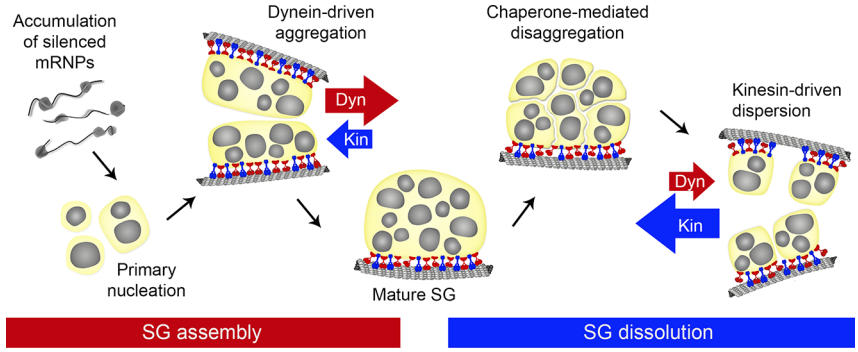

Figure 1. Hypothetical model for SG dynamics based on the emergent properties of dynein motors. Upon acute stress, mRNA translation is immediately repressed, and the resulting silenced mRNPs and abortive translation initiation complexes aggregate at the nanoscale level. This primary nucleation is independent of molecular motors and likely driven by LLPS mechanisms. After this initial condensation, retrograde transport by dynein (red) is facilitated, as the larger number of motors associated with the cargo enhances dynein forces. Particle size does not affect kinesins (blue), which oppose SG formation (see the text). The coordinated transport of numerous SG cores by dynein facilitates their fusion and the recruitment of additional material, giving rise to mature SGs. SGs are always transient, and after a short time, the action of chaperones weakens SG cohesion thus debilitating dynein forces and allowing dispersion by kinesin-dependent transport.

cores, dynein-mediated transport is facilitated by a combination of mechanisms that depend on the cargo size, thus allowing the accumulation of SG cores toward microtubules minus ends and facilitating their fusion and SG growth (Figure 1). Consistently, mature SGs are frequently located in the perinucleus, where the microtubule organizing center is typically located.

Clustering is important in dynein teamwork; for example, specific lipid microdomains in membranous cargo help dynein clustering thus allowing cooperative forces.[89] Dynein clustering is predicted in SGs, and while super-resolution microscopy is pending, confocal imaging shows the presence of SG domains with higher dynein concentrations.[46]

During SG dissolution, the disaggregating action of specific chaperones reduces the absolute number of dynein motors in the cargo, although the ratio of dynein to kinesin remains the same (Figure 1). These conditions would be less favorable for dynein-driven transport, and dispersion by kinesin would take over. This model based on the emergent properties of molecular motors proposes that the tug of war between anterograde and retrograde transports is modulated by the degree of consolidation of the SG. Initial condensation would facilitate retrograde transport by dynein, which allows SG growth. Weakening of the cohesive interactions and incipient breakdown would reduce dynein forces thus facilitating dispersion of SG components by kinesins (Figure 1). Experimental evidence for this working model remains to be presented. For example, the analysis of the direction, speed, and processivity of SG cores and fragments of different sizes during both condensation and dissolution will yield significant information. Real time super-resolution microscopy and FRAP assays in combination with strategies to control SG cohesiveness, for example, the overexpression of chaperones or light-activated IDRs,[36] will help.

Most RNAs are highly mobile, and frequently, they are asymmetrically distributed inside cells.[75,78,94,95] SGs contain mRNAs with a diverse range of features.[21,96] The delivery of coding and noncoding RNAs to SGs implies that their localization signals or mechanisms are not functional upon stress or that they are somehow overridden by SG assembly forces. The model described above predicts that RNAs that are not sorted by dynein under normal conditions may be directed to SGs in association with other transcripts that recruit retrograde motors. A similar RNA trans-regulation was described in fly embryos, where different mRNA species can influence others when loaded in the same granule.[97,98] It is relevant that it was recently suggested that RNA−RNA interactions are important in SG formation.[21] All this supports the notion that RNAs may be directed to SGs pulled by forces commanded by other RNAs loaded in the same particle.

CELLULAR METABOLITES AFFECT PROTEIN SOLUBILITY AND REGULATE LIQUID ORGANELLES

SG formation is influenced by protein solubility, which is directly affected by the presence of cellular solubilizers, including small amphiphilic metabolites. A remarkable recent finding is that ATP helps the solubilization of hydrophobic proteins.[99] The protein concentration inside cells may be much higher than that of stable solutions in vitro (>100 mg/mL). The ATP physiological concentration (between 2 and 5 mM) is enough to keep proteins soluble inside cells. Moreover, cellular ATP levels can dissolve aggregates of fused in sarcoma/translocated in liposarcoma (FUS/TLS), an SG component directly relevant to neurodegeneration.[99] ADP is less suited and works at much higher concentrations. These striking observations allow the speculation that the reduction of ATP levels associated with aging[100,101] may be causative of protein aggregation and neurodegeneration.[99]

These novel findings lead to the question of whether SG condensation is facilitated by a stress-induced ATP drop. This possibility is opposed by the evidence that ATP seems to be required during SG condensation. SG formation, their fusion, and the dynamic exchange with the cytosol are abrogated when ATP is depleted by the pharmacological inhibition of glycolysis and mitochondrial respiration. This likely reflects the participation of molecular motors and ATP-driven diffusion. Furthermore, specific ATPases with protein/nucleic acid chaperone activity are required during SG assembly.[39] However, once SGs are formed, control of ATP levels in their surroundings may help keep SG components in the insoluble phase. For example, pharmacological ATP depletion increases the size of the immobile pool of Ras GTPase-activating protein-binding protein (G3BP), a key SG component.[39] In addition, two complexes with ATPase activity, minichromosome maintenance protein complex (MCM) and RuvB-like protein (RVB), are present in SGs, and their knockdown enhances SG dissolution in both yeast and

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mammalian cells.[39] Whether ATP hydrolysis by MCM and RVB locally reduces protein solubility thus helping SG integrity is unknown.

Protein solubility is also influenced by additional cellular metabolites that act as detergents. For example, yeast inclusion bodies that contain damaged proteins are solubilized by sterol derivatives that act as solvent reducing hydrophobic interactions.[102] Specifically, a model unfolded protein forms accumulations that contain a number of SG components, including the RNA binder Mrn1 and several protein chaperones.[39,72,73] These inclusion bodies are cleared by steryl esters emanating from lipid droplets, which store several nonpolar lipids.[102] In mammalian cells, related sterol derivatives reduce folding stress and lipid droplets confer protection against both the endoplasmic reticulum and oxidative stress through several mechanisms.[103,104] Both electrostatic and hydrophobic interactions are important for SG condensation,[39,41] and we speculate that lipid droplets may contact SGs to deliver specific lipid solubilizers that would help SG dissolution. Both the interaction of lipid droplets with SGs and the effect of lipids on SG dynamics remain to be investigated.

SGs AND PBs IN mRNA METABOLISM

Initially believed as organelles for RNA decay, PBs are currently viewed as mRNA storage compartments. Pioneering work by Parker and collaborators demonstrated that yeast PBs store mRNAs transiently repressed that can return to polysomes in response to cellular cues.[105] Afterward, work in mammalian neurons showed that dendritic bodies containing decapping enzyme 1a (DCP1a), a conserved PB component, are also linked to mRNA storage.[106] Subsequently, 5′−3′ exoribonuclease 1 (XRN1), another PB component, was shown to form clusters associated with synapses that dissolve upon stimulation, apparently releasing mRNAs to allow their translation.[107] The orthologue yeast exoribonuclease 1 is reversibly concentrated in specific organelles termed eisosomes, which associate with the cell membrane. Yeast XRN1 is inactive at eisosomes, and the presence of RNA in these organelles is unknown.[108,109] More recently, a number of studies using complementary approaches that combine imaging and biochemical analysis revealed additional compelling evidence that PBs are not involved in mRNA decay but rather in mRNA protection. Single-molecule imaging in yeast and mammalian cells showed that mRNA decay occurs homogeneously through the cytoplasm.[110,111] Moreover, independent work documented the PB transcriptome and suggested a role in translational repression and protection from 5′ decay.[9] Approximately one-third of the cell transcriptome is present in PBs, and it is relevant that mRNAs encoding proteins with related functions are either concentrated in PBs or significantly excluded, as a group. Speculatively, the expression of sets of functionally connected mRNAs, termed regulons, is coordinated by the assembly and disassembly of PBs, which would respond to yet unknown signaling pathways.[9]

The relevance of SGs to mRNA metabolism is less clear. As an integral part of the cellular defense response, SGs are involved in the activation of the antiviral program and in the control of retrotransposons.[112−114] Whereas a number of transcripts from retrotransposons associate with SGs in a repressed state, work from several laboratories strongly suggests that the presence of SGs is not required for the translational silencing triggered by acute cellular stress. Moreover, it has been suggested that SGs protect mRNA from entering decay.[3,4,46,115−117]

When analyzing either specific mRNAs or polyadenylated RNA, several laboratories came to the conclusion that microscopically visible SGs contain between 3 and 30% of the cellular RNA content (reviewed in refs 24 and 39). However, the amount of mRNA associated with nanoscale SGs is unknown, and whether submicroscopic organelles help mRNA repression or stability remains to be determined. In addition, mRNAs rapidly shuttle between SGs and the cytosol. A specific report indicates an immobile fraction of ≤30% of polyadenylated RNA, which includes mRNAs and noncoding RNAs that may serve as a scaffold.[21,118] However, most mRNAs show a residence time in the range of 1−5 min (reviewed in ref 24). Thus, the influence of SGs on mRNA metabolism might depend on their transit through SGs rather than on their permanence in these foci. Among other possibilities that remain open, specific biochemical interactions and reactions, for example, nucleotide modifications, may be facilitated within SGs.

In contrast to the case of PBs, the SG transcriptome shows no strong selectivity. The common features among mRNAs sorted to SGs are low translatability and large size.[21,96] Besides the well-known lack of polysomes, mRNA sorting to SGs may involve additional mechanisms. For example, unexpected observations by Zid and O'Shea[119] indicate that yeast mRNAs transcribed by stress-activated promoters are excluded from PBs and SGs. This suggests that co-transcriptional events, for example, the binding of specific RBPs or the chemical modification of specific bases, influence the fate of mRNAs in the cytosol. Dynamic RNA methylation is connected to regulation upon stress and may modulate recruitment of mRNA to SGs. For example, the modification *N*(6)-methyladenosine (m6A) is recognized by specific YTH proteins, and members of this family of "readers" are present in both PBs and SGs in mammalian cells.[120−123] However, a recent report shows that RNA binding by G3BP1, a key SG component that helps assembly, is prevented by m6A, thereby providing a mechanism for the exclusion of m6A-modified mRNAs from SGs.[124] Finally, whereas m6A at the 5′ end of heat shock protein mRNAs facilitates their translation upon stress, the stress-induced demethylation of specific m6A sites allows the translation of stress-specific alternative open reading frames in other cases.[121,125] The connection among SGs, RNA methylation, and stress-specific translation is complex, and this novel field warrants further investigation.

While the role of SGs in RNA metabolism is still unclear, recent striking discoveries about SG functions arose from the study of their protein composition. As discussed next, SGs protect specific enzymes from stress-induced degradation, and a similar function is emerging for PBs.

SGs AND PBs AS PROTEIN DEPOTS

A number of recent reports offer a systematic study of the protein composition of PBs and SGs.[9,14,39,42,72,73] Differences between the output of imaging and biochemical analyses indicate that a number of granule proteins are lost during cell fractionation procedures. Thus, rather than being an integral part of SGs or PBs, several proteins associate weakly with these bodies, and moreover, they may be recruited after condensation.

The presence of a given protein in SGs or PBs may affect granule physiology, and conversely, these organelles may

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Table 1. Metabolic Enzymes Associated with SGs and Macromolecular Complexes[a]

	enzyme	stress granules	yeast quiescent foci	metabolic complex	RNA binding
glycolysis	pyruvate kinase	Y	+	Glyco (Y, M)	Y M F
	phosphoglycerate mutase	Y	+ (i)	Glyco (Y)	Y M F
	aldolase	Y		Glyco (Y, M)	Y M
	triosephosphate isomerase	Y		Glyco (Y)	Y M F
	glyceraldehyde-3-phosphate dehydrogenase			Glyco (Y, M)	Y M
	enolase			Glyco (Y)	Y M
	phosphofructokinase			Glyco (Y)	Y M F
	glucokinase			Glyco (Y)	Y
	glucose-6-phosphate isomerase			Glyco (Y)	Y
	fructose 1,6-bisphosphatase			Glyco (M)	
	phosphoglycerate kinase				Y M
	lactate dehydrogenase				M
aminoacyl-tRNA ligases	Tyr-tRNA ligase	Y M	+ (i)		Y M
	Met-tRNA ligase	Y M	+ (i)	MSC (Y, M)	Y M
	tRNA-aminoacylation cofactor ARC1	Y	+	MSC (Y, M)	Y M
	Glu-tRNA ligase	Y	+ (i)	MSC (Y, M)	Y
	Gln-tRNA ligase	Y	+	MSC (M)	Y
	Ile-tRNA ligase	Y	+ (i)	MSC (M)	Y F
	Lys-tRNA ligase	M	+ (i)	MSC (M)	Y M F
	Arg-tRNA ligase		+ (i)	MSC (M)	Y M F
	Asp-tRNA ligase		+ (i)	MSC (M)	Y M
	Leu-tRNA ligase		+	MSC (M)	Y
	Pro-tRNA ligase		+ (i)	MSC (M)	Y
	Ala-tRNA ligase		+		Y M F
	Val-tRNA ligase		+		Y M F
	His-tRNA ligase		+		Y F

[a]The current SG proteome is based on imaging analyses and biochemical strategies that collectively revealed the presence of 674 mammalian and 254 yeast SG proteins.[14,39,42,72,73] Yeast quiescent foci include both reversible and irreversible (i) protein assemblies specific to quiescent cultures.[133] The composition of specific metabolic complexes, namely, the glycosome (Glyco) or the multisynthetase complex (MSC), is described in refs 129 and 134. Yeast (Y), fly (F), and mammalian (M) RNA-binding proteins were identified in multiple works.[22,128,130−132]

modulate the function or stability of the associated proteins. One remarkable example is yeast pyruvate kinase (yPK), also termed Cdc19. This enzyme transfers one phosphate group from phosphoenolpyruvate to ADP and has a key role in the energetic metabolism. It was recently shown that yPK is recruited to SGs, where it is protected from degradation thus facilitating recovery after stress.[126] Recruitment of yeast PK to SGs involves an LCR that is normally occluded and becomes exposed and dephosphorylated upon stress. In addition, yPK binds RNA in vitro and in vivo,[127,128] and RNA binding facilitates its aggregation upon stress.[126] The identity of the transcript(s) bound by yPK hypothetically helping the recruitment of yPK to SGs remains unknown.

PK is part of a dynamic multienzymatic complex termed the glycosome or glucosome that can be visualized in several cell types and organisms, including trypanosomatids, with variable dimensions.[129] Besides yPK, half of the yeast glycosome enzymes were detected in heat-induced SGs[14] (Table 1), likely suggesting the coordinated protection or regulation of several glycolytic enzymes in SGs (Table 1). Furthermore, almost all the protein components of the glycosome are RNA binders[22,128,130−132] (Table 1), suggesting that the glycosome may include RNA molecules that would serve as a scaffold or that would allow the co-regulation of mRNAs and cognate enzymes.

Besides the glycosome, other multimolecular complexes that contain defined groups of enzymes linked to specific metabolic pathways were reported in several organisms[129] (Table 1). For example, in both yeast and mammals, a number of aminoacyl-tRNA synthetases (ARSs) and cofactors form a complex termed the multisynthetase complex (MSC). Several ARSs are present in SGs, and a number of them bind their own mRNA or mRNAs of other enzymes in the complex.[135−137] The relevance of binding of RNA to MSC formation and to the association of ARSs to SGs is unknown. There has been speculation that recruitment of ARS to SGs may protect the enzymes from stress-associated damage.

In addition, the gene ontology group "nucleoside metabolic process" is 5-fold over-represented in the yeast SG proteome, and the "de novo pyrimidine synthesis" is 11-fold enriched.[14,39,72,73] Remarkably, all these enzymes present in SGs bind RNA.[128] In contrast, the purinosome, a macromolecular complex of microscopic dimensions that recruits the six enzymes of the purine biosynthetic pathway[138−140] (reviewed in 141 and 142), is underrepresented in both the RNA interactome and the SG proteome known to date.[14,22,72,73,128] Altogether, these observations suggest that the RNA binding capacity of metabolic enzymes is connected to their recruitment to SGs, where enzymes would be regulated or protected from damage and degradation.

Similar to SGs, PBs were implicated in protecting specific proteins from decay. Recent work shows the conserved recruitment of the yeast casein kinase 1 (CK1) isoform Hrr25, CK1δ in mammals, to PBs after a variety of stress insults. For example, in yeast pyruvate kinase and SGs, Hrr25 is protected from the proteasome-mediated degradation when recruited to PBs.[143]

All these observations are inspiring, and it is anticipated that additional enzymes that bind RNA might be recruited to SGs

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and/or PBs. Work by the laboratories of Castello, Baltz, and others allowed the identification of hundreds of previously unknown RNA binders in several organisms, including human, mouse, yeast, and fly.[22,128,130−132] Almost one-third of the human and 40% of the yeast RNA interactomes have no known link to RNA biology, and many of these novel RNA binders are metabolic enzymes collectively termed enigmRBPs (discussed in refs 22 and 128). Several of them include IDRs,[144] and a significant number are present in SGs with unknown implications (Table 1). The proportion of each enigmRBP recruited to SGs and the residence time are unknown. Either sequestration in SGs or facilitation of specific biochemical reactions involving enigmRBP is a potential mechanism. For example, the presence of ARSs in SGs might be connected to the recently reported role of ARSs in protein modification.[136] Briefly, in addition to being key factors for tRNA charging, all human ARSs are able to transfer their cognate amino acid to the ϵ-amine of specific lysines. Remarkably, 126 proteins previously identified as SGs components (19% of the SG proteome, 12% of the total aminoacylated proteins) are modified by this novel mechanism.[39,72,73] Whether protein aminoacylation by ARSs is regulated by SG formation is currently unknown. A tempting speculation is that the concurrent presence of enzymes and cognate substrates in the liquid organelle may facilitate the reaction.

Besides SGs and PBs, additional cellular granules likely containing RNA appear to concentrate enigmRBPs. Early work by Marcotte and co-workers[133] describes that 33 metabolic enzymes reversibly form foci in yeast cells entering quiescence. Recent reports show that all these enzymes bind polyadenylated RNA[22,128,132] (Table 1), thus suggesting that the interaction with mRNAs may help in the formation of foci. Although the presence of RNA in these bodies was not directly assessed, Pab1—a surrogate marker for polyadenylated RNA—is present in the foci formed during yeast quiescence.[133] In addition, six of these metabolic enzymes are recruited to SGs[14,39,72,73] (Table 1), thus opening the possibility that the yeast stationary phase assemblies may be related to SGs. However, the presence of client proteins in cellular bodies needs to be carefully validated.[145] As a striking example, an unwanted cross-reaction of an antibody against p53 with unknown human PB components mistakenly informs the presence of p53 in PBs in human cells (ref 146 and references therein).

The role of RNA liquid organelles as protein depots is a common theme also in the nucleus. A remarkable example is the nucleolus, which regulates p53 transcriptional activity.[147] Further work will illuminate the significance of the association of metabolic enzymes and signaling molecules to SGs, PBs, and other RNA bodies.

CONCLUDING REMARKS

The cytoskeleton network and active transport by molecular motors provide mechanisms for controlling the size, position, fusion, and fission of membrane-less organelles. SGs are among the most dynamic RNA-liquid organelles, and their transient formation depends on the balance between the transports driven by dynein and kinesin. We propose that this tug of war is governed by the emergent properties of the molecular motors. In addition, the contribution of the active diffusion linked to cytoplasmic agitation is likely and remains to be investigated. Further analysis of the condensation of SG cores and their movement during SG assembly and dissolution will help to answer these open questions.

Another important line that warrants future research is the biological significance of these RNA-liquid organelles. Whereas PBs coordinate the expression of regulons, the role of SGs in RNA metabolism is less clear. An extreme paradigm-shifting hypothesis is that SGs have a mild influence on the RNA component, which would serve as a scaffold or regulator of granule dynamics, and that SGs are mostly dedicated to protein regulation. Future studies are required to define where SG functions are positioned between these two contrasting scenarios.

AUTHOR INFORMATION

Corresponding Author

*Instituto Leloir, IIBBA-CONICET and Facultad de Ciencias Exactas y Naturales, University of Buenos Aires, Buenos Aires, Argentina. Telephone: 54 11 5238 7500. Fax: 54 11 5238 7501. E-mail: gboccaccio@leloir.org.ar.

ORCID

Graciela L. Boccaccio: 0000-0003-1349-898X

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ABBREVIATIONS

ARS, aminoacyl-tRNA synthetase; BicD, Bicaudal D; BicDR1, BicD-related protein 1; CK, casein kinase; DCP1a, decapping enzyme 1a; DCTN1, dynactin subunit 1; DHC, dynein heavy chain; DIC, dynein intermediate chain; DISC1, disrupted in schizophrenia 1; FMRP, fragile X mental retardation protein; FRAP, fluorescence recovery after photobleaching; G3BP, Ras GTPase-activating protein-binding protein; GSK3β, glycogen synthase kinase 3 β; Hrr25, casein kinase 1, isoform Hrr25; IDR, intrinsically disordered region; IPOD, insoluble protein deposit; JNK, c-Jun N-terminal; JUNQ, juxta nuclear quality control; KHC, kinesin heavy chain; KLC, kinesin light chain; LCR, low-complexity region; Line-1, long interspersed element-1; LLPS, liquid−liquid phase separation; MCM, minichromosome maintenance protein complex; m6A, *N*(6)-methyladenosine; Pab1, polyadenosine-binding protein 1; PB, processing body; PK, pyruvate kinase; PKC, protein kinase C; RBP, RNA-binding protein; RNP, ribonucleoparticle; RVB, RuvB-like protein; SG, stress granule; XRN1, 5′−3′ exoribonuclease 1; ZBP1, zipcode-binding protein 1.

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