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Ribonucleoprotein bodies are phased in

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Intracellular compartments are necessary for the regulation of many biochemical processes that ensure cell survival, growth and proliferation. Compartmentalisation is commonly achieved in organelles with defined lipid membranes, such as mitochondria, endoplasmic reticulum or the Golgi apparatus. While these organelles are responsible for many localised biochemical processes, recent evidence points to another class of compartments that lack membrane boundaries. The structure and content of these bodies depend on their function and subcellular localisation, but they mainly incorporate proteins and RNA. Examples of these ribonucleoprotein bodies (RNPBs) include eukaryotic mRNA processing bodies (P-bodies) and stress granules (SGs). While most of these structures have been widely studied for their capacity to bind, store and process mRNAs under different conditions, their biological functions and physical properties are poorly understood. Recent intriguing data suggest that liquid–liquid phase separation (LLPS) represents an important mechanism seeding the formation and defining the function of RNPBs. In this review, we discuss how LLPS is transforming our ideas about the biological functions of SGs and P-bodies and their link to diseases.

Adaptation to changing external conditions is a fundamental aspect of survival in biological systems. Across eukaryotes, a range of adaptation systems have evolved to enable such survival. One such adaptive mechanism involves the rapid inhibition of protein synthesis in response to stress, commonly via targeted regulation of translation initiation [1,2]. This inhibition creates a mass of non-translated mRNA that appears to seed the formation of ribonucleoprotein bodies (RNPBs) to control the expression, repression or decay of specific mRNAs [3,4]. There are two major types of cytoplasmic bodies that amass mRNAs under stressful conditions, eukaryotic RNA processing bodies (P-bodies) and stress granules (SGs) [5]. P-bodies are associated with mRNA processing and decay, since they harbour many of the proteins participating in mRNA degradation and have been shown to accumulate mRNA decay intermediates [6,7]. On the other hand, SGs are thought to act as sites of storage for mRNAs and the machinery associated with protein synthesis [8]. However, even in unstressed cells, mRNAs can be present in distinct granules that, in this case, are associated with active translation [9].

One of the first observations of stress-induced granules was in heat-treated tomato cells, where heat shock proteins were observed to localise to granules [10]. Later studies showed that, following stress, two related RNA-binding proteins, TIA1 and TIAR, could change their subcellular distribution and accumulate in phase-dense structures, termed SGs [11]. SGs commonly form as a result of the inhibition of translation initiation via phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2) by eIF2α kinases [12]. eIF2 is a G-protein and its phosphorylation increases its affinity for the guanine nucleotide exchange factor, eIF2B, leading to a failure to exchange GDP to GTP and therefore a decrease in the levels of the eIF2/tRNAMeti/GTP ternary complex [13]. As a result, translation initiation is inhibited and RNA-binding proteins, such as TIA1, TIAR and G3BP1, associate with stalled or partially formed mRNA translation complexes and promote the formation of SGs [12]. SGs can also be formed as a consequence of eIF4A inhibition. eIF4A is an ATP-dependent RNA helicase that can unwind double-stranded RNA. eIF4A interacts with the mRNA selection machinery via the scaffold protein, eIF4G, along with the mRNA cap-binding protein, eIF4E [8]. Thus, application of eIF4A inhibitors, such as hippuristanol [14], pateamine A [15] and episilvestrol [16], or the depletion

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of various translation initiation factors associated with helicase activity [17] can inhibit translation and induce the assembly of SGs. Indeed, many components of the translation initiation machinery, such as eIF2, eIF3, eIF4E, eIF4G, eIF4B and poly(A)-binding protein, have been identified as part of SGs [8]. While, the protein composition of SGs has been well studied, it is still not clear whether SGs harbour bulk mRNA, or if SG proteins, such as TIA1, TIAR and hnRNPA1, dictate a level of specificity in the mRNAs targeted [18]. Perhaps as a result, the biological function of SGs remains unclear with most reports focusing on potential roles in mRNA storage and/or triage to other mRNA bodies and fates. Indeed, there has been much debate about the relationship between SGs and other protein aggregates that form as a consequence of stress [19].

P-bodies contain many mRNA decay factors and have hence been associated with mRNA processing/degradation functions [7]. Many P-body components are conserved among eukaryotes, from yeast to mammals [20]. In yeast, P-bodies form in response to stress, whereas in mammals, P-bodies can be present in unstressed cells although numbers do increase after stress [21]. P-body components include mRNA decay factors, such as the mRNA decapping enzymes, decapping activators, the 5′–3′-exoribonuclease [7], as well as many other RNA-binding proteins [20]. P-bodies also contain the nonsense-mediated mRNA decay machinery and components necessary for miRNA function [22,23]. In contrast with SGs, P-bodies harbour only a limited number of translation initiation factors and regulators [24–26]. Recent evidence in yeast suggests that pre-existing mRNA-containing bodies associated with active protein synthesis mature into P-bodies after stress, highlighting the likelihood that RNA plays a key role in their formation [9]. Moreover, other mRNA species enter P-bodies at much later time points after stress in a process reliant upon a specific mRNA-binding protein, Bfr1p [27]. Intriguingly, mRNAs can also leave P-bodies to return to the translated pool, indicating that they not only mediate mRNA degradation, but can also store mRNA for subsequent translation [26].

There exists some controversy in the literature regarding the functional relationship between P-bodies and SGs. In yeast, based on epigenetics, it has been proposed that P-bodies precede SGs, so that if a stress endures, P-bodies evolve to become SGs [28]. However, there is also evidence that P-bodies and SGs can form independently [24,29]. In mammalian cells, these two RNPBs are able to interact, and it has been proposed that mRNA is able to transit SGs to P-bodies and vice versa [21]. In addition, SGs can recruit P-bodies upon overexpression of CPEB1, a protein important for cytoplasmic polyadenylation and the regulation of protein synthesis [30]. Therefore, it seems that although P-bodies and SGs can interact and have an impact on each other's formation, the actual nature of this interaction is yet to be fully elucidated. Some of the apparent contradictions regarding the relationship between P-bodies and SGs probably stem from differences in the systems and stresses used to induce them. Overall, the most striking contrast between them appears to be their components, with P-bodies harbouring mRNA decay factors while SGs contain translation factors [6,8,20].

Recently, a concept has emerged that relates to the physical properties of RNPBs and has important implications for their function and relationship to one another [31,32]. Studies in *Caenorhabditis elegans* on both nucleoli and P-granules (also termed germ granules, a class of RNA granule specific to the germline with some similarities to both SGs and P-bodies [33]) indicated that they form via liquid–liquid phase separation (LLPS) and exist as liquid droplets [34,35]. These droplets were found to be spherical, to fuse upon contact and their formation was promoted by low salt concentration [34,35]. However, even though the physical properties of these bodies appeared quite nebulous, their formation was found to be regulated, for instance MEG-1 and MEG-3 regulated the assembly and disassembly of P-granules depending on their phosphorylation status [36]. Furthermore, it was noted that factors involved in the formation of P-granules, such as LAF-1, MEG-1 and MEG-3, harbour structurally disordered domains variably called low complexity domains (LCDs), intrinsically disordered regions (IDRs) or prion-like domains (PLDs) [36,37]. LCDs are proposed to promote LLPS via multivalent weak protein–protein interactions that cause liquid demixing and formation of phase-separated liquid droplets [38–40].

Given that many protein components of SGs and P-bodies contain LCDs (Figure 1), an obvious question pertains to the role of these proteins in regulating the content and genesis of these RNPBs. To address this, recent studies have focused on the ability of isolated LCD-containing SG proteins to promote LLPS [41,42]. The LCDs of proteins, such as Pub1, eIF4GII, hnRNPA1 and FUS, were found to assemble into liquid droplets under low salt conditions. The morphologies of the phase-separated liquid droplets varied depending upon the LCD being studied. Some, such as those formed by the RNA-binding protein FUS, matured over time from liquid droplets to hydrogels in the form of aggregates or fibres [43]. Interestingly, when these LCDs were fused to an RNA-binding protein, such as PTB, phase separation was apparent in the presence of a lower concentration of RNA [42], while experiments on hnRNPA1 showed that the addition of RNA in a manner dependent

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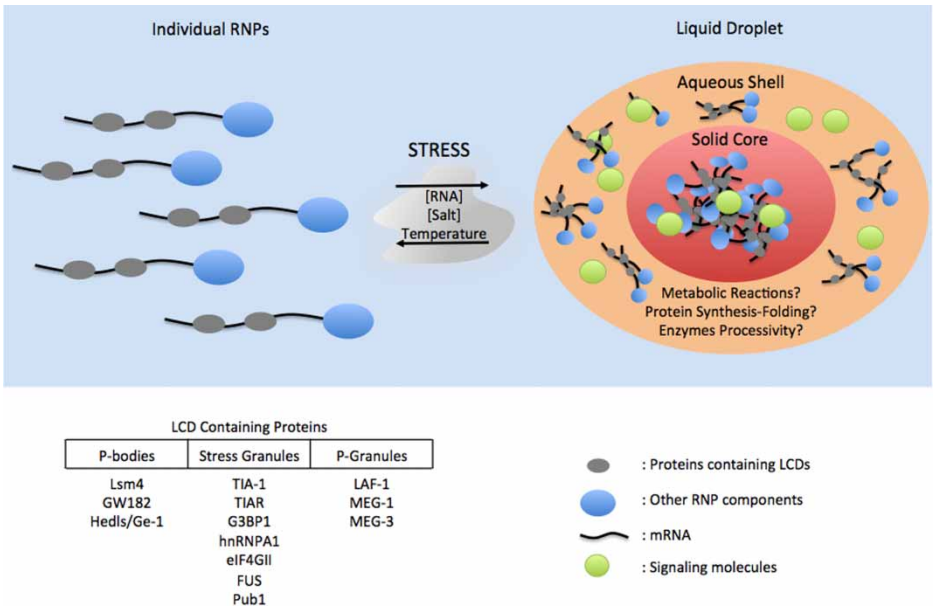

Figure 1. LLPS and its role in RNP body formation.

During translation, and in particular following inhibition of translation, individual RNPs can co-localise into non-membrane bound bodies. These bodies form as a result of liquid–liquid phase transition and have the consistency of liquid droplets. Their formation is affected by many biophysical properties, such as RNA and salt concentration, as well as temperature. The liquid droplets are spherical, mobile and fuse upon contact. Their formation relies on proteins featuring LCDs and RNA-binding domains, and may be influenced by the concentration of specific mRNAs. Some of the droplets, such as SGs, are thought to contain a solid protein dense core with a more liquid outer shell. The liquid environment may facilitate metabolic and enzymatic reactions or promote protein folding.

upon its RNA interaction motifs reduced the effective concentration of hnRNPA1 needed to achieve phase separation [41]. Overall, these studies demonstrate that the concentrations of both RNA and LCD-containing proteins are crucial in the formation of SGs and P-bodies, but what dictates any specificity in content and formation is less clear [9,28,38,44,45]. However, there is evidence emerging that specific mRNAs may be able to drive LLPS and regulate the viscosity of the liquid droplets [46].

As indicated above, liquid droplets can mature into more fibrillar and aggregated cellular compartments that are composed of dynamic amyloid-like fibres [41–43,47]. The role of liquid droplets in promoting the formation of these more solid compartments likely stems from increased molecular crowding due to high local concentrations of specific proteins and RNA (Figure 1). To distinguish between liquid droplets and fibrillar aggregates, a range of studies have made use of 1,6-hexanediol, an aliphatic alcohol that disrupts liquid structures by disturbing weak hydrophobic bonds while leaving aggregates largely unaffected [48]. 1,6-Hexanediol has been used to demonstrate that yeast P-bodies are liquid in nature, whereas yeast SGs form or mature into solid aggregates [49]. In contrast, 1,6-hexanediol treatment of HeLa cells disrupts both SGs and P-bodies suggesting they are predominantly liquid in nature, with P-bodies disappearing faster than SGs [49]. In complementary studies, SGs were suggested to consist of a solid core assembled by both electrostatic interactions and hydrophobic bonds, and an aqueous shell that is maintained by weaker hydrophobic interactions. Thus, the shell is sensitive to 1,6-hexanediol treatment, whereas the SG core is only disrupted in the presence of both high salt and 1,6-hexanediol [18]. The functional significance of these different physical arrangements and the mechanism by which they are established are still largely unanswered questions. Similarly, the full range of parameters that can affect LLPS and the physical properties of RNPBs are also not clear [34,42,50,51] (Figure 1).

Proteins involved in various diseases have been studied for their association with membraneless organelles and their ability to promote LLPS [50]. For example, mutations in the *FUS* gene are linked to amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases by increasing amyloid-like fibre formations via its LCD [52]. However, since FUS is also associated with various other functions, it is likely that these mutations

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could have an impact on cellular activities besides the response to stress [43,52,53]. Another SG component with mutants associated with human disease is hnRNPA1 [54]. In particular, the hnRNPA1 D262V mutation is associated with ALS, frontotemporal disorder and myopathy [41]. While this mutation does not appear to affect hnRNAPA1 localisation to liquid droplets or the formation of such droplets, it accelerates the formation of fibril structures [41,42,55]. Analysis of other known SG components reveals that they include proteins involved in a range of other diseases [8]. Recent proteomic data have identified proteins such as YARS and HSBP1, where mutations can cause Charcot-Marie-Tooth neuropathy [18], suggesting connections between SGs and diseases of the peripheral nervous system. SG and P-body formation may also have wider implications in human disease, for instance in viral infections and cancer [55–57]. However, the pathological consequences of altering the physical properties of SGs and P-bodies remain largely unknown.

Although many of the studies discussed here have shed light on the previously obscure area of membraneless organelles, it is not yet fully understood how these formations connect to the physiology or responses of cells. It is clear that the molecular crowding effects, apparent as a result of such phase transitions, can promote protein aggregation and may prove important in promoting biological functions that would otherwise be less efficient. These functions could include, for example, metabolic reactions, the formation and folding of protein complexes, the processivity of enzymes, such as nucleases and helicases, and the enhancement of weak intermolecular interactions.

Abbreviations

ALS, amyotrophic lateral sclerosis; eIF2, eukaryotic translation initiation factor 2; IDR, intrinsically disordered region; LCD, low complexity domain; LLPS, liquid–liquid phase separation; P-body, mRNA processing body; PLD, prion-like domain; RNPB, ribonucleoprotein body; SG, stress granule.

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Competing Interests

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

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