

1 REVIEW

2 Stress-related biomolecular condensates in plants

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25 **Short title:** Biomolecular condensates and stress
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27 **One-sentence summary:** This review describes the mechanism, regulation, composition and
28 properties of stress-related biomolecular condensates in plants.
29

30 Abstract

31 Biomolecular condensates are membraneless organelle-like structures that can concentrate molecules
32 and often form through liquid-liquid phase separation. Biomolecular condensate assembly is tightly
33 regulated by developmental and environmental cues. Although research on biomolecular condensates
34 has intensified in the past 10 years, our current understanding of the molecular mechanisms and
35 components underlying their formation remains in its infancy, especially in plants. However, recent
36 studies have shown that the formation of biomolecular condensates may be central to plant
37 acclimation to stress conditions. Here, we describe the mechanism, regulation, and properties of
38 stress-related condensates in plants, focusing on stress granules and processing bodies, two of the
39 most well-characterized biomolecular condensates. In this regard, we showcase the proteomes of
40 stress granules and processing bodies, in an attempt to suggest methods for elucidating the
41 composition and function of biomolecular condensates. Finally, we discuss how biomolecular

condensates modulate stress responses and how they might be used as targets for biotechnological efforts to improve stress tolerance.

Introduction

Intracellular compartmentalization is integral to cellular function. In addition to conventional membrane-bound organelles, two- or three-dimensional compartments composed of multiple proteins, RNA molecules and small-molecule ligands but lacking delineating lipid membranes offer an additional mechanism for intracellular organization (Gomes & Shorter, 2019). Historically, these membraneless compartments have been termed ribonucleoprotein (RNP) granules, cellular bodies, membraneless bodies, or simply cellular aggregates. Recently, the unifying term biomolecular condensates has been coined to describe their capacity to spatially concentrate biomolecules (Banani et al., 2017).

The driving force for biomolecular condensate formation in many cases is biophysical in nature and is known as liquid-liquid phase separation (LLPS), whereby a solution separates into two (or more) phases (Pappu, 2020, Emenecker *et al.*, 2020). The first direct evidence demonstrating LLPS in cells was provided for P-granules in germ cells of the nematode *Caenorhabditis elegans*. P granules show liquid-like properties, such as fusion with one another and spontaneous exchange of their components with the cytoplasm (Brangwynne *et al.*, 2009). After this seminal example, a considerable number of follow-up studies showed that many intracellular bodies exhibit similar behavior, including Lewy bodies, stress granules (SGs), processing bodies (PBs), frososomes, purinosomes, bacterial ribonucleoprotein bodies (BR-bodies), and FLOE1 granules (Dorone *et al.*, 2021, Hardenberg *et al.*, 2021, Cohan & Pappu, 2020, Pedley *et al.*, 2022). Notably, the formation of phase-separated condensates has been reported in the nucleus, cytoplasm, membranes, and chloroplasts (in plants) and has been implicated in a plethora of cellular programs that include gene expression, mRNA biogenesis, cell signaling, and metabolism (Londono Velez *et al.*, 2022, Fare *et al.*, 2021, Alberti & Hyman, 2021). However, even though the number of studies on condensates has increased substantially in recent years, the mechanisms regulating their assembly remain largely unclear.

An emerging theme is that biomolecular condensates are major players during stress. In fact, the formation of stress-induced condensates has been described in response to a wide variety of stresses, indicating that their assembly is a common pathway invoked upon stress perception (Glauninger *et al.*, 2022). The compartmentalization of proteins into stress-induced condensates is assumed to be an early event during stress response and exerts a cytoprotective role. In this context, the formation of SGs, one of the best-characterized stress-induced condensates in all eukaryotes, is involved in post-transcriptional regulation and translational control in response to stress (Youn *et al.*, 2019). In addition to SGs, other condensates can also increase in number and/or size under stress, including

PBs, plant small interfering RNA (siRNA) bodies, or yeast G-bodies (Fuller *et al.*, 2020, Martinez-Perez *et al.*, 2017). Despite growing interest in understanding the functional relevance of these assemblies, the composition of condensates and more importantly, the mechanisms regulating their formation remain largely unknown. In plants, knowledge of stress-related condensates is still scarce. Nonetheless, recent studies have started to shed light on the molecular composition of a subset of stress-related condensates.

In this review, we focus on representative cytoplasmic stress-related condensates to provide a state-of-the-art overview of the mechanisms and regulation of phase-separated condensates and summarize the current knowledge of their composition and organization. Then, we focus on a few selected examples of LLPS-formed condensates with important functions in stress signaling and acclimation. Special attention is paid to the unknowns in plant biology, and why the field is lagging behind non-plant models. We further suggest research directions for elucidating the physiological roles of biomolecular condensates and review methodologies by which these could be realized.

PRINCIPLES GOVERNING THE PHASE SEPARATION OF CONDENSATES

Biomolecular condensates are assembled in many instances via LLPS, which leads to the formation of a dense phase with a high concentration of biomolecules surrounded by a dilute phase (Millar *et al.*, 2023). Phase separation is promoted by an increase in the concentration of biomolecules and mediated by changes in the intracellular environment (e.g., temperature, redox state, pH, etc.), as summarized in Figure 1. It has been proposed that LLPS might have been proposed to serve as a mechanism for the organization of biomolecules to regulate key biochemical functions (Fare *et al.*, 2021, Musacchio, 2022). Therefore, not surprisingly, the assembly of biomolecular condensates is tightly regulated, and its misregulation has been related to diseases such as cancer, neurodegeneration, or ageing-associated disorders (Chung *et al.*, 2023, Conti & Oppikofer, 2022, Spann *et al.*, 2019). Understanding the general principles governing the phase separation of condensates and how they are organized and structured is critical to better understanding their role in cell fate decisions and physiology.

Multivalency-driven phase separation

Biomolecular LLPS relies on multivalency, meaning that the components of condensates can undergo multiple and simultaneous inter- or intramolecular interactions between homotypic or heterotypic molecules (Li *et al.*, 2012, Han *et al.*, 2012). The multivalency and affinity in biomolecular condensate formation can be relatively well-explained by the stickers-and-spacers model, in which biomolecular condensates form by reversible sticker-sticker interactions (Abyzov *et al.*, 2022). While the stickers are responsible for the interactions driving condensation and thus biomolecular condensate formation, the intervening spacers connect the stickers and provide necessary flexibility

(Figure 1). Stickers can be made of folded domains, intrinsically disordered regions (IDRs), including low complexity domains (LCDs), as well as short linear amino-acid motifs (SLiMs) (Mittag & Parker, 2018). Many proteins with a high propensity to form condensates are enriched in IDRs and as such, they have gained significant attention as drivers of LLPS (Figure 1) (Banani *et al.*, 2017, Musacchio, 2022). For example, a prion-like domain (PLD), a form of LCD, in FLOWERING TIME CONTROL A (FCA) of *Arabidopsis thaliana* can form nuclear condensates, showing the importance of intrinsic disorder in LLPS of plant condensates (Fang *et al.*, 2019b).

Recent studies have pointed to a particularly important role for charge-charge, dipole-dipole, charge- π , π - π and hydrogen bonds in enabling IDRs to phase separate (Figure 1) (Li *et al.*, 2018, Murthy *et al.*, 2019, Krainer *et al.*, 2021). Charge- π and π - π are types of noncovalent interaction involving aromatic rings (Meyer *et al.*, 2003). Hence, tyrosine (aromatic) and arginine (charged) residues were shown to be necessary for the LLPS of a number of proteins including Fused to sarcoma (FUS), the RNA helicase LAF-1, heterogeneous nuclear ribonucleoprotein A1, and Dead-box helicase 4 in mammalian cells (Wang *et al.*, 2018, Nott *et al.*, 2015, Vernon *et al.*, 2018, Schuster *et al.*, 2020). Although the mechanisms governing LLPS are still poorly studied in plants, it was reported that a tyrosine residue array situated in an LCD region of *Arabidopsis* RNA-BINDING GLYCINE-RICH PROTEIN D2 (RBGD2) and RBGD4 promotes their temperature-dependent LLPS during SG formation, demonstrating unsurprising conservation for the role of π systems in biomolecular condensation across kingdoms (Zhu *et al.*, 2022).

Regulation of condensate assembly

Although studied mostly in yeast (*Saccharomyces cerevisiae*) and mammalian cells, the best-understood model of stress-induced biomolecular condensation is that of SGs, as these condensates form in response to exogenous stimuli and are not constitutively present in the cell. SGs are RNA-protein condensates with biphasic organization, comprising stable cores surrounded by a more dynamic shell (Wheeler *et al.*, 2016). The assembly of SGs is likely a multistep, highly controlled program that can be briefly described by three consecutive steps: first, the formation of a dense stable SG core via LLPS (nucleation); second, the growth of the core by the recruitment of additional SG components – so-called clients (growth); third, accumulation of proteins into a peripheral shell (shell assembly) (Figure 2) (Banani *et al.*, 2017, Markmiller *et al.*, 2018, Cirillo *et al.*, 2020, Kosmacz *et al.*, 2019). An important question is to what extent other types of biomolecular condensates, especially in plants, form through the same sequence of events as those described for yeast and mammalian SGs. Several recent findings in mammalian systems support the idea that PBs, a type of cytosolic biomolecular condensates functionally linked to SGs and mainly involved in mRNA degradation, may implicate a similar principle of multi-step assembly. Indeed, several findings have shown that PBs

contain densely populated sub-domains, including a relatively stable core, pointing to the existence of a differential organization within PBs (Souquere *et al.*, 2009, Hubstenberger *et al.*, 2017). In addition, typical PB core proteins can phase separate in vitro, suggesting that the nucleation step might be involved in the PB biogenesis in vivo (Schutz *et al.*, 2017, Luo *et al.*, 2018). However, unlike SGs, PBs exist at a basal level under unstressed (normal) conditions and are strongly induced in response to stress, indicating that the pathways of PB and SG assembly as a whole must be different.

Although many mechanistic details of SG assembly remain unclear, all of the proposed models converge on the view that nucleation is the key step (Glauninger *et al.*, 2022). Growing evidence suggests that stress-induced RNA-RNA, RNA-IDR, and IDR-IDR interactions initiate the nucleation step (Ditlev *et al.*, 2018, Sanders *et al.*, 2020, Protter & Parker, 2016a, Mittag & Parker, 2018). Posttranslational modifications (PTMs) of SG-associated proteins such as methylation, ubiquitination, or phosphorylation also contribute to SG nucleation (Figure 2) (Protter & Parker, 2016b). Despite a recent study in plants showing that the phosphorylation of the Arabidopsis SG component GLYCINE-RICH RNA-BINDING PROTEIN 7 (GRP7) is required for its nucleation (Xu *et al.*, 2022), the role of GRP7 in SG formation remains unclear. Although PTMs affect SG nucleation, how the two events are co-regulated upon stress perception remains an open question.

Molecular organization of biomolecular condensates

Upon formation, biomolecular condensates can increase in complexity through an increase in protein, RNA, or other molecules (including metabolites) contents, if the shell (or a similar less dense phase) is permeable to these molecules (Mitrea *et al.*, 2022). The molecular composition of condensates is tightly controlled; some components are constitutive, while others are only transiently recruited under certain conditions. The scaffold-client model can explain this differential recruitment. Scaffolds are multivalent molecules (usually proteins) stably associated with biomolecular condensates and essential for assembly, whereas clients are transiently associated with condensates and likely recruited by scaffolds (Figure 2) (Ditlev *et al.*, 2018). In contrast to clients, scaffolds are considered to be drivers of phase separation. However, classification into these two classes has some limitations, as scaffold and client proteins may switch roles (Ditlev *et al.*, 2018, Riback *et al.*, 2020). Furthermore, biomolecular condensate formation may be modular, wherein a client may be converted to a scaffold to bring in other clients. These secondary scaffolds may be important for adding accessory proteins, thereby modulating the functionalities of the biomolecular condensates.

The molecular mechanisms by which scaffolds recruit clients and how they promote phase separation are still a matter of speculation. Growing evidence suggests that multiple folded domains (e.g. the SRC homology 3 [SH3] domains in the non-catalytic region of tyrosine kinase [NcK]) or IDRs from

scaffolds contribute to generating a network of interactions between proteins or proteins and RNA, thus facilitating recruitment and LLPS (Banani *et al.*, 2017). The best such example is probably the scaffold protein Ras GTPase-activating protein-binding protein 1 (G3BP1), whose IDRs interact with RNAs to facilitate the assembly of mammalian SGs (Yang *et al.*, 2020). In contrast to mammals, only a few proteins required for the assembly of biomolecular condensates have been described in plants. A recent study revealed that a disordered region of a multivalent protein tudor staphylococcal nuclease (TSN) provides a docking platform for interaction with a large pool of other intrinsically disordered proteins (IDPs). In addition, this region was required for the recruitment of some of these IDPs to cytoplasmic foci upon stress (Gutierrez-Beltran *et al.*, 2021).

To date, the layered organization of specific condensates such as SGs is a rather well-established, albeit oversimplified notion (Protter & Parker, 2016a, Fare *et al.*, 2021). Advanced microscopy techniques have become key tools for studying the molecular organization of biomolecular condensates. For example, super-resolution microscopy revealed that mammalian G3BP1 forms a dense core surrounded by a more dilute shell (Jain *et al.*, 2016). An intriguing type of organization was observed for biomolecular condensates formed by the AUXIN RESPONSE FACTOR (ARF) family of transcription factors in the cytoplasm of *Arabidopsis* root cells (Rogg & Bartel, 2001). Using fluorescence correlation spectroscopy (FCS), ARF condensates were demonstrated to show an inverse organization, compared to SGs, with the more stable layer being at the condensate exterior, i.e. constituting the shell (Powers *et al.*, 2019). ARF sequestration into cytoplasmic condensates blocks its entry into the nucleus, thus decreasing auxin responsiveness (Powers *et al.*, 2019). Whether the stable shell of ARF condensates mediates the blockage of nuclear entry remains to be seen. This organization found in ARF condensates, however, is not unique to plants and has been observed for condensate-like structures formed in prokaryotes and known as bacterial microcompartments (Kerfeld *et al.*, 2018). Despite new technical advances, a molecular topology of multiple components inside the condensates remains elusive.

Liquid-solid properties of biomolecular condensates during stress

Non-plant biomolecular condensates can harden (i.e., become less liquid and resemble a more solid state) or increase in size over time, especially *in vitro* when the components reach equilibrium. Among other mechanisms, Ostwald ripening contributes to these changes by driving the disappearance of small condensates via their dissolution and deposition of their now released components into pre-existing larger biomolecular condensates (Dine *et al.*, 2018). The driving force for Ostwald ripening is the difference in solubility between small and large biomolecular condensates. It is thus expected that given enough time, a single biomolecular condensate would remain, akin to the separation of oil and vinegar in salad dressing where we see gradual coarsening of oil droplets. This

state, apart from Ostwald ripening, can be also driven by collisions and fusions between distinct condensates.

These events might be physiologically relevant for plant condensates as well, as Ostwald ripening may drive the formation of the eukaryotic pyrenoid in the unicellular alga *Chlamydomonas reinhardtii*, which ends up forming a single droplet (Freeman Rosenzweig *et al.*, 2017). Yet, for not completely understood reasons, most condensates do not become a singular entity in cells. During stress, however, biomolecular condensates do become larger, suggesting that the mechanisms restricting condensate sizes might be suppressed. For example, PBs and SGs increase in size during stress progression in Arabidopsis (Liu *et al.*, 2023). A lack of some scaffold proteins may also affect the size of the condensates, a phenomenon documented in TSN-deficient Arabidopsis cells (Gutierrez-Beltran *et al.*, 2015).

The presence of a tight core and a loose shell in some types of biomolecular condensates suggests that they can be viewed as ensembles of materials with varying properties. Indeed, a key feature of biomolecular condensates is that they can be both viscous (a hallmark of liquids) and elastic (as observed in solids), a phenomenon that is known as viscoelasticity (Bergeron-Sandoval & Michnick, 2018). Once the material is deformed, it may never return to its original shape. Yet, this model for condensate organization comes from the animal research field, where the material properties of biomolecular condensates have been relatively well studied, including under stress conditions. Biomolecular condensates were shown to behave more like an elastic solid or a viscous liquid, depending on various parameters including shear stress applied to a condensate, as well as its age and size (Shen *et al.*, 2020).

During the ageing of biomolecular condensates (also known as maturation or growth), the accumulation of various protein conformations causes an imbalance in intermolecular interactions (Garaizar *et al.*, 2022). These metastable conformations become more important with time, leading to the assembly of liquid-core/gel-shell (e.g., ARFs) or gel-core/liquid-shell (e.g., SGs) architectures. Importantly, changes in the architecture of biomolecular condensates can be attributed to perturbations in their turnover, allowing them to stay around longer and age (Yamasaki *et al.*, 2020), similarly to phenomena linked to chronological ageing and various human neurodegenerative diseases (Alberti & Hyman, 2016, Patel *et al.*, 2015). Interestingly, how these transitions in material properties are modulated by stresses remains elusive, but would be important to understand as these transitions may affect the residence time of key regulatory proteins in biomolecular condensates.

Biomolecular condensates and membranes

Back in 2012, the pioneering study of Rosen and coworkers showed that biomolecular condensates can interface with membranes in animal cells (Li *et al.*, 2012). Biomolecular condensates can form films on membranes that extend laterally and are characterized by smooth and circular boundaries (Yuan *et al.*, 2021). Optically, such thin films resemble membrane patches (Vequi-Suplicy *et al.*, 2010, Kusumaatmaja *et al.*, 2021). These features suggest that many membrane-bound patches may be as yet unidentified biomolecular condensates. Given that the plasma membrane is the first cellular barrier to encounter the environment and thus encounter stress, research in this direction is especially important.

In plants, an example of a condensate that wets membranes is that of the lipid transferase Sec14-HOMOLOG 8 (SFH8) (Liu *et al.*, 2022). Membranes facilitate the condensation of SFH8 by lowering the threshold concentration by 50-fold, likely through interactions with phosphatidylinositol lipids (Liu *et al.*, 2022), as has been reported for other proteins in non-plant species (Case, 2022). Many phosphatidylinositol lipids are subjected to regulation by stress, raising the question of whether biomolecular condensates might be regulated by alterations in membrane lipids. In animals, phosphatidylinositol lipids mediate the phase separation of argonaut proteins (AGOs) on the endoplasmic reticulum (ER) (Gao *et al.*, 2022). These lipids are highly responsive to stress conditions (Hou *et al.*, 2016), and it would thus be interesting to assess their potential role in biomolecular condensation in plants.

Recently, membrane wetting by DECAPPING PROTEIN 1 (DCP1), a major component of PBs, was shown to lead to PB dissolution (Liu *et al.*, 2023). DCP1 recruitment at the plasma membrane depends partially on an actin nucleating complex known as SCAR-WAVE. In turn, DCP1–SCAR/WAVE forms a condensate that promotes actin nucleation. PB dissolution decreased during heat stress, which in principle could affect the global transcriptome profile of the cell and thus stress tolerance. The link between condensation at the plasma membrane and the transcriptome merits further investigation, especially during stress. Furthermore, the above principles of condensation may allow for tight control of receptor clustering, with as yet not understood implications for stress responses, especially immune responses as in animal cells (Su *et al.*, 2016).

ESTABLISHING THE PROPERTIES OF STRESS-RELATED BIOMOLECULAR CONDENSATES IN PLANTS

Proteome

In non-plant models, characterizing condensate proteomes has aided significantly in understanding condensate dynamics, regulation, and functions. Similar attempts in plants are still in their infancy, and most of the available information concerns mass-spectrometry analysis of interactomes for SG-resident proteins in Arabidopsis. The degree of similarity among such interactomes can be used as a proxy for evaluating inherent variability among SG proteomes. Here we provide a comparative analysis of three heat stress-induced interactomes [namely of TSN2 (Gutierrez-Beltran et al., 2021), RBGD2 and RBGD4 (Zhu et al., 2022), and RNA-BINDING PROTEIN 47 (RBP47) (Kosmacz et al., 2019)] and the hypoxia-induced interactome of CALMODULIN-LIKE PROTEIN 38 (CML38) (Lokdarshi *et al.*, 2015) (Figure 3A; Supplemental Data Sets S1 and S2). Only three proteins were shared by all four interactomes: TSN1, TSN2 (in one case as a bait) and POLY-A BINDING PROTEIN 4 (PABP4). From this group, Arabidopsis TSN proteins appear to be a central hub, consistent with their scaffolding role in SGs (Gutierrez-Beltran et al., 2021, Gutierrez-Beltran et al., 2015, Maruri-Lopez *et al.*, 2021) (Figure 3B). In addition to these proteins, we observed an overlapping group comprising well-defined SG components, including RBP47, OLIGOURIDYLATE-BINDING PROTEIN 1 C (UBP1C), other PABPs, different ribosomal subunits (40 and 60S), and several translation initiation and elongation factors (eIFs, eEFs) (Figure 3B). Notably, similar to mammals and yeast, plant SG interactomes display a dense network of protein-protein interactions and are enriched for RNA-binding proteins (RBPs) (Jain et al., 2016, Marmor-Kollet *et al.*, 2020) (Figure 3C, D).

Comparison among the four plant interactomes also demonstrates that conserved core SG proteins co-exist with other cell- and stress type-specific components, suggesting that SGs are multifunctional condensates with highly heterogeneous protein contents (Figure 3B). For example, enzymes involved in protein dephosphorylation or phosphorylation, ethylene biosynthesis, the glutathione-S-transferase pathway, or glycolysis are overrepresented in some of the SG protein interactomes while missing in others. There is now growing evidence that the incorporation of enzymes in biomolecular condensates can increase their catalytic activity through concentration, conformational changes or other mechanisms (Peeples & Rosen, 2021, Mountourakis *et al.*, 2023).

SGs are functionally linked with PBs and both have been suggested to exchange proteins and RNAs (Maruri-Lopez et al., 2021, Jang *et al.*, 2020). Although PBs are constitutively present in the cell, they can increase in number and size during stress (Gutierrez-Beltran et al., 2015, Jang et al., 2020, Chicois *et al.*, 2018). In contrast to mammals, the composition of plant PBs is not well defined. We compared

published interactomes of the well-known PB components DCP1, DCP2, DCP5, and UPFRAMESHIFT 1 (UPF1) (Liu et al., 2023, Schiaffini *et al.*, 2022, Chicois et al., 2018) (Figure 3E; Supplemental Data Set S2). We determined that similar to mammals, plant PBs accumulate mRNA decapping factors (DCP1, DCP2, DCP5, PROTEIN-ASSOCIATED WITH TOPOISOMERASE 1 [PAT1], and VARICOSE [VCS]), 5'-3' processing exonucleases (XRN3 and XRN4), nonsense-mediated mRNA decay (NMD) factors (UPF1, UPF2, UPF3, and SMG7), components of the microRNA (miRNA) pathway (AGO1, AGO2, AGO5, and AGO9), and RNA helicases (RH6, RH12, and RH12) (Figure 3F). The accumulation of mRNA decay factors in PBs is in line with their canonical role in executing mRNA degradation. However, recent research suggests that thousands of mRNAs accumulate in human PBs to evade RNA decay (Hubstenberger et al., 2017). In agreement, ACETYLATION LOWERS BINDING AFFINITY 4 (ALBA4), ALBA5, and ALBA6 confer plant thermotolerance by stabilizing the mRNA of HEAT STRESS TRANSCRIPTION FACTORS (*HSEs*) in cytoplasmic biomolecular condensates, including PBs (Tong *et al.*, 2022). Intriguingly, ALBA domain-containing proteins are enriched in all four plant PB interactomes available today (Figure 3F).

To investigate how similar plant, mammalian, and yeast proteomes are, we used the eggNOG orthology database (Huerta-Cepas *et al.*, 2019). The comparison of SG proteomes revealed that ~ 15% of proteins from plants are shared by mammalian or yeast SGs (Jain et al., 2016), including well-characterized SGs core proteins such as translation associated factors (e.g., PABP2/4/8 or eIF4A), RNA-binding proteins [(e.g., the RNA-binding KH domain-containing protein HUA ENHANCER 4 [HEN4]) or ribosomal subunits (e.g., RPS2) (Figure 3G). In the case of PBs, the overlap group, which represents ~ 14% of all proteins, includes proteins involved in mRNA decay (e.g., DCP1, DCP2, DCP5 and UPF1) or RNA helicases (e.g., RH6, RH8, RH12) (Figure 3H). These results show a compositional conservation in core components between kingdoms, which is consistent with the canonical role of both condensates in RNA metabolism (Kearly *et al.*, 2022, Youn et al., 2019). However, many proteins from both SG and PB proteomes are kingdom-specific, suggesting that each condensate might play additional roles that are fully dependent on the organism.

Compared to SGs and PBs, other plant cytoplasmic stress-related condensates are even more enigmatic in terms of their protein composition and architecture. For example, plant siRNA bodies play a role in siRNA amplification during stress and typically contain SUPPRESSOR OF GENE SILENCING 3 (SGS3) and RNA-DEPENDENT RNA POLYMERASE 6 (RDR6), explaining why these bodies are also named SGS3/RDR6 bodies (Field *et al.*, 2021, Martinez de Alba *et al.*, 2015). SGS3 is an RNA-binding protein that, together with RDR6, is necessary for the synthesis of double-stranded RNA (dsRNA) templates for their subsequent processing into secondary siRNAs during stress. While the dynamics and molecular composition of siRNA bodies remain largely unknown, phase separation of both SGS3 and RDR6 proteins is important for their assembly (Kim *et al.*, 2021).

Other proteins identified in siRNA bodies include *Arabidopsis* AGO7, the m⁶A demethylase ALKBH9B, and *Nicotiana benthamiana* calmodulin-like (NbCaM) (Jouannet *et al.*, 2012, Martinez-Perez *et al.*, 2017).

Transcriptome

In addition to proteins, RNAs are found in several types of biomolecular condensates, including SGs, PBs, and siRNA bodies. Therefore, these condensates are often referred to as RNP granules. Although SGs and PBs both contain non-translating mRNAs, their fates in the two compartments were initially thought to be storage and degradation, respectively (Protter & Parker, 2016a). However, recent studies now challenge this notion. RNA immunoprecipitation followed by sequencing (RIP-seq) analysis of mammalian SG RNAs revealed a subset of translationally active mRNAs (Mateju *et al.*, 2020). Furthermore, a fluorescence-activated particle sorting assay demonstrated that mRNAs in PBs are translationally repressed, but not degraded (Hubstenberger *et al.*, 2017). Until recently, it was widely accepted that SGs and PBs are physically connected, continuously exchanging their mRNAs and proteins during stress. However, a single-mRNA imaging approach showed that, in contrast to proteins, very few mRNA molecules in fact shuttle between SGs and PBs during stress (Mateju *et al.*, 2020). In further contrast to the mammalian and yeast systems, the RNA composition and the fate and role of individual mRNAs present in plant stress-induced condensates are emerging topics. It has been suggested that the localization of heat-induced transcripts in *Arabidopsis* SGs might promote the heat-stress response (Zhu *et al.*, 2022). In agreement, the stabilization of *HSF* mRNAs in SGs and PBs was reported to facilitate thermotolerance (Tong *et al.*, 2022). More research is, however, required to unravel the RNAs within and the mechanistic role of biomolecular condensates in translation and other RNA-dependent pathways during plant stress responses.

Crosstalk among stress-induced cytoplasmic biomolecular condensates

The bulk of SGs or other biomolecular condensates may exist as stable submicroscopic structures in the absence of stress in a pre-existing, standby state (Glauninger *et al.*, 2022). Considering this notion and the fact that PBs are constitutively present in the cells, we compared the available interactomes of *Arabidopsis* SG- and PB-resident proteins (TSN2 vs. DCP1 or DCP5, respectively) in the absence of stress and under heat stress to ask whether nucleation or growth of SGs and PBs engage similar proteins [Figure 4A; (Gutierrez-Beltran *et al.*, 2021, Chicois *et al.*, 2018, Liu *et al.*, 2023)]. In the absence of stress, the proteins shared by the PB- and SG-related interactomes included conserved condensate remodelers such as protein chaperones (e.g., HEAT SHOCK PROTEIN 60 [HSP60] and HSP90 and T-COMPLEX PROTEIN [CCT]) and RNA and DNA helicases (e.g., DEA-box proteins or REGULATOR OF NONSENSE TRANSCRIPTS 1 [RENT1]) (Figure 4B). This finding suggests

that SGs and PBs may use a similar set of scaffolding protein structures that do not grow further in the absence of stress.

In fact, the condensate remodelers DEAD-Box Helicase 6 (DDX6) and CCT, homologous to Arabidopsis RH6/RH8/RH12 and CCT proteins, respectively, have been described as key players in PB and SG assembly in mammalian and yeast cells (Ayache *et al.*, 2015, Di Stefano *et al.*, 2019, Jain *et al.*, 2016). In mammals, pre-existing interactions among a subset of SG- and PB-associated proteins may act hierarchically as seeding scaffolds to recruit clients (proteins and mRNAs), thereby facilitating condensate growth (Youn *et al.*, 2019). Whether plant SGs and PBs exploit a similar type of hierarchical relations in their pre-assembled state to potentiate subsequent growth and acquisition of the core-shell organization is unknown and awaits studies. Once Arabidopsis cells perceive a heat stimulus, their SG and PB proteomes become more dissimilar, with only a very few proteins being in common (Figure 4A). These observations suggest that favorable growth conditions suppress the identity of the SG and PB precursor protein complexes, whereas onset of stress facilitates their compositional and structural dichotomy.

Although SGs, PBs, and siRNA bodies have recently been recognized as major players in regulating the fate and function of cytoplasmic RNAs during plant stress responses, the interplay among these condensates remains obscure (Makinen *et al.*, 2017). As discussed above, physical interaction and material exchange among these cytoplasmic condensates depends on the environmental conditions, which may also be involved in the establishment of their identity. In this regard, siRNA bodies colocalize with SG protein markers under hypoxia and heat stress (Figure 4C) (Jouannet *et al.*, 2012, Field *et al.*, 2021), pointing to the possibility that mRNAs stalled in translation may accumulate in cytoplasmic condensates representing hybrids between SGs and siRNA bodies under abiotic stress in plants.

Whereas siRNA bodies appear to be compositionally distinct from PBs in the absence of stress, the two types of biomolecular condensates display functional interrelationship (Figure 4C) (Martinez de Alba *et al.*, 2015). Indeed, it has been proposed that mRNA decapping of non-functional RNAs in Arabidopsis PBs prevents their entry into siRNA bodies, in which they would potentially be converted into siRNAs. Recent studies have shown a tight connection between siRNA bodies and PBs during viral infection (Figure 4C). First, the RNA N6-methyladenosine demethylase AIKBH10B, which is required for viral RNA (vRNA) biogenesis, colocalized with the PB-associated proteins UPF1 and DCP1 (Li *et al.*, 2017). More recently, it was shown that the association of PB components with the cauliflower mosaic virus (CaMV) might protect vRNAs from siRNA body-dependent translational repression (Hoffmann *et al.*, 2022). Although there is increasing evidence for molecular crosstalk

among SGs, PBs, and siRNA bodies, a more exhaustive analysis is required to better understand shared and unique functions of these biomolecular condensates in plant stress biology.

BIOMOLECULAR CONDENSATES AS MEDIATORS AND REGULATORS OF STRESS RESPONSES

Condensates and gene expression

Considering that many proteins in biomolecular condensates bind RNA molecules, sequestration of these proteins within condensates may alter the translational landscape or other functions related to non-coding RNAs to favor cell survival and acclimation (Figure 5A). For example, a DEAD-box RNA helicase (Ded1p)-dependent translational switch mechanism in yeast was suggested as a mediator of acclimation to heat stress (Iserman *et al.*, 2020). In response to heat, Ded1p is targeted to SGs where it is thought to initiate the scanning of mRNAs for housekeeping genes containing a structurally complex 5' untranslated region (UTR), to silence them and in this way promote the translation of stress-response RNAs with simpler 5' UTRs (Iserman *et al.*, 2020). Interestingly, RH20 is the Arabidopsis ortholog of Ded1p, but it is unclear whether it can modulate the translation of mRNAs from housekeeping genes under stress, even though other RH proteins have been implicated in plant stress responses, presumably via their SG and/or PB localization (Chantarachot *et al.*, 2020).

Apart from the direct role of biomolecular condensates in controlling the translational landscape through RNA sequestration, they can additionally be involved in the regulation of transcription (Figure 5B). Plant GUANYLATE-BINDING PROTEIN (GBP)-LIKE GTPases (GBPLs) form biomolecular condensates in the nucleus to protect against infection and autoimmunity (Huang *et al.*, 2021). GBPL3 defense-activated condensates (GDACs) assemble when GBPL1, a pseudo-GTPase, sequesters catalytically active GBPL3 under normal conditions, but is displaced by GBPL3 LLPS when it enters the nucleus following immune cues. This altered GDAC formation impairs the recruitment of GBPL3 and salicylic acid (SA)-associated Mediator subunits to the promoters of *CALMODULIN-BINDING PROTEIN 60g* (*CBP60g*) and *SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1* (*SARD1*), which encode master transcription factors involved in immunity (Wang *et al.*, 2011).

Interestingly, some biomolecular condensates may switch function upon translocation from the nucleus to the cytoplasm. Pathogen effector-triggered immunity (ETI) often leads to programmed cell death, which is restricted by NONEXPRESSOR OF PR GENES 1 (*NPR1*), an activator of SA-mediated systemic acquired resistance (SAR). *NPR1* promotes cell survival by targeting substrates for ubiquitination and degradation through the formation in the cytoplasm of SA-induced *NPR1*-rich condensates (SINCs) (Zavaliev *et al.*, 2020). The SINCs are enriched in stress response proteins,

including nucleotide-binding leucine-rich repeat immune receptors, oxidative and DNA damage response proteins, and protein quality control machinery. The condensation of NPR1 into cytoplasmic SINC promotes the formation of a complex between NPR1 and the E3 ligase CULLIN 3 to ubiquitinate SINC-localized substrates, such as ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and the stress-related transcription factors from the WRKY family that positively affect ETI, thereby promoting survival. Importantly, this cytoplasmic function of NPR1 complements its transcriptional role in the nucleus. How and whether SINC interplay with other cytoplasmic condensates including SGs, PBs and siRNA bodies remains unknown.

Condensates and metabolism

The presence of biomolecular condensates has frequently been postulated to explain cellular features of metabolism in animal and microbial systems (Robinson *et al.*, 1987, Sweetlove & Fernie, 2013, Sweetlove & Fernie, 2018). In fact, stress-induced sequestration of enzymes into condensates was proposed to mediate or regulate biochemical reactions under adverse conditions in mammals (Figure 5C and 5D) (Peeples & Rosen, 2021). Hence, biomolecular condensates may recruit enzymes and their substrates, thereby acting as a core promoting a specific biochemical reaction (Figure 5C). Indeed, the mechanistic dissection of increased enzymatic rate in a phase-separated compartment was recently demonstrated for the SUMOylation enzyme cascade in the mammalian system. SUMOylation rates increased by up to 36-fold in molecular condensates (Peeples & Rosen, 2021) resembling the increases in reaction rates reported for other enzyme-enzyme assemblies (Zhang *et al.*, 2020). Moreover, Peeples and Rosen found that the increased SUMOylation efficiency was due to increased concentrations enhancing the mass-action, as well as through multivalent hetero- or homotypic interactions that may evoke conformational changes affecting substrate K_m . In plants, comparable direct evidence has been provided by the analyses of SG proteomes (described above), while indirect evidence comes from the evaluation of spatial allocation of metabolites in the cell by means of non-aqueous fractionation and by analysis of the metabolic fate of heavy label isotopes (Szecowka *et al.*, 2013).

Biomolecular condensates in cells are often rich in catalytically active enzymes from metabolic pathways (Figure 3B) (Ouazan-Reboul *et al.*, 2021). Their formation and disassembly are dynamically responsive to environmental conditions and stimuli. In a subset of these assemblies, metabolites may be channeled between sequential enzymes: i.e., the product of one enzyme is transferred to the next enzyme in the pathway without equilibrating with the bulk aqueous phase of the cell (Figure 5C). In such cases, the assemblies are known as metabolons. Since this finding, assemblies of consecutive enzymes have been observed in a wide variety of metabolic pathways (Shen, 2015, Sweetlove & Fernie, 2018). For example, the aggregation of enzymes of the phenylpropanoid pathway in plants

highlights substrate channeling between phenyl ammonia-lyase and cinnamate 4-hydroxylase (Rasmussen & Dixon, 1999, Achnine *et al.*, 2005). Many results from studies of this pathway [see for example those in (Crosby *et al.*, 2011, Burbulis & Winkel-Shirley, 1999)], are in keeping with a recent suggestion that the presence of enzyme-enzyme assemblies is likely important in directing flux via various branch points of a pathway (Sweetlove & Fernie, 2018). Moreover, the clear importance of phenylpropanoids in response to stress is underlined by evidence of their antioxidant roles in response to light and water stress (Nakabayashi *et al.*, 2014, Tohge *et al.*, 2016). Intriguingly, the assembly of enzymes of dhurrin metabolism is postulated to be dynamic to cope with increased demand in response to environmental stresses (Bassard *et al.*, 2017).

It is important to note that phase separation has only been formally demonstrated for a small subset of enzyme assemblies (Wunder & Mueller-Cajar, 2020); however, by analogy to yeast and mammalian they are likely. Yeast and mammalian glycolytic enzymes condense into so-called G-bodies during hypoxic stress and, like many other biomolecular condensates, these G-bodies are enriched in RNA-binding domains and mRNA (Jin *et al.*, 2017, Kohnhorst *et al.*, 2017, Fuller *et al.*, 2020). Furthermore, G-body formation correlates with increased rates of glycolysis (Jin *et al.*, 2017), although whether metabolon formation underlines this rise remains to be resolved. While these findings are intriguing, further studies will be needed to see if the same mechanisms operate in plants. This caveat notwithstanding, the above-discussed enzyme-enzyme assemblies, beyond the fact that they all contain well-characterized substrate channels, are likely responsive to either biotic or abiotic stresses. Indeed, previous reviews have pondered on the different selective pressures that variously brought about dynamic enzyme-enzyme assemblies and stable multi-enzyme complexes (Sweetlove & Fernie, 2018). The fact that (dis)assembly of such complexes in response to stress provides a flexible and energetically spartan route to adjust metabolic fluxes in response to stress is certainly a highly feasible reason for the evolution of such dynamic aggregates.

Stress-induced small molecules in biomolecular condensates

Considering the chemical and functional diversity of small molecules, it is not a surprise that they have emerged as components and regulators of biomolecular condensates in plants and animals (Klein *et al.*, 2020, Kosmacz *et al.*, 2019). A recent study reported the presence of dozens of known metabolites sequestered within Arabidopsis cytosolic SGs, including amino acids, nucleotides, and phospholipids (Kosmacz *et al.*, 2019). What drives the sequestration of the different compounds and what would be their function remains to be examined. For instance, it was speculated that proline, known for its chaperoning activities and found in both cytosolic and plastidial SGs, might contribute to the proper folding of proteins sequestered into biomolecular condensates (Kosmacz *et al.*, 2019, Dandage *et al.*, 2015, Chodasiewicz *et al.*, 2020). A different metabolite reproducibly detected in SGs

and indispensable for SG dynamics and function is adenosine triphosphate (ATP). ATP fuels the activity of RNA and protein chaperone complexes, which are essential components of the SGs in mammalian and yeast cells (Jain et al., 2016, Tauber *et al.*, 2020). Moreover, ATP is a hydrotrope that counters the formation and can also dissolve already-formed protein aggregates (Patel *et al.*, 2017). Treatments that dysregulate ATP levels interfere with SG assembly, and impede SG disassembly once they are formed (Jain et al. 2016). We are confident that, despite still being in its infancy (especially in plants), the identification and functional characterization of small molecules in biomolecular condensates will provide insight into condensate formation, dynamics, and behavior.

As already discussed, individual enzymes or entire metabolons can localize to biomolecular condensates, which might regulate (stimulate or inhibit) specific biochemical reactions (Figure 5C and D). This influence provides a direct link between metabolism, metabolite levels, and biomolecular condensates. One illustrative example with a direct relevance to stress responses comes from yeast. Using an elegant combination of genetics and cell biology, Cereghetti and colleagues showed that SGs are involved in fine-tuning ATP levels (Cereghetti *et al.*, 2021). In glucose-grown yeast cultures, stress inhibits glycolysis leading to a decrease in fructose 1,6 bisphosphate (FBP) levels. FBP is an allosteric ligand of a pyruvate kinase (*cdc19*), a glycolytic enzyme lying behind the final, ATP-producing step of the glycolytic pathway. FBP binding to *cdc19* promotes its active tetrameric structure. The decrease in FBP concentration results in tetramer disassembly. Monomeric *cdc19* is sequestered within SGs, where it is kept inactive. When the stress abates, FBP level rises, and FBP binding to *cdc19* promotes recruitment of chaperones and *cdc19* re-solubilization. In turn, once released from SGs, *cdc19* contributes to the increase in the ATP levels required for SG disassembly.

Numerous stress-induced molecules regulate condensate dynamics without necessarily being a condensate component themselves. An example is 2',3'-cyclic adenosine monophosphate (2',3'-cAMP), which is an evolutionarily conserved RNA degradation product known to accumulate in response to stress and injury (Londono Velez et al., 2022, Jackson, 2017, Yu *et al.*, 2022). 2',3'-cAMP treatment of Arabidopsis seedlings affected the levels of hundreds of transcripts, proteins, and metabolites, many of which were previously associated with plant stress responses. In addition, 2',3'-cAMP-induced SGs affected the motility of PBs (Kosmacz *et al.*, 2018, Chodasiewicz *et al.*, 2022). Although no evidence of 2',3'-cAMP being sequestered within SGs is available, 2',3'-cAMP can bind to the RNA-binding motif (RRM), present in SG core proteins such as RBP47 (Kosmacz et al., 2018). Like 2',3'-cAMP, SA is also a stress-related small molecule with an ability to promote protein condensation in plants. As described above, SA induces the condensation of cytoplasmic NPR1 and GBPL defense-activated condensates in the nucleus of Arabidopsis plants (Zavaliev et al., 2020, Kim *et al.*, 2022, Huang et al., 2021).

Another example of a metabolite that was shown to affect the dynamics and function of biomolecular condensates is S-adenosylmethionine (AdoMet). Using a combination of mutants affected in AdoMet metabolism and AdoMet supplementation experiments in yeast and human cell lines, AdoMet was demonstrated to suppress SG formation in response to acute stress and also affect the expression and recruitment of specific SG components (Begovich *et al.*, 2020). AdoMet is a co-substrate involved in methyl group transfer. Although protein-methylation does not appear to affect AdoMet function, AdoMet could theoretically work by altering RNA methylation, a hypothesis that requires testing. Intriguingly, S-adenosylmethionine synthase, an enzyme responsible for AdoMet production, is sequestered within SGs, pointing to the existence of a regulatory loop, whereby SG sequestration may contribute to the regulation of cellular AdoMet levels. A final example of a metabolite that regulates condensate formation, in this case PBs, is 5-diphosphoinositol pentakisphosphate (InsP7) (Sahu *et al.*, 2020). InsP7 was shown to inhibit the NUDT3 (Nudix Hydrolase 3)-dependent decapping of mRNAs and increases PB abundance in human cells, with this effect being environmentally and developmentally regulated.

HARNESSING BIOMOLECULAR CONDENSATES FOR GROWING RESILIENT PLANTS

Biomolecular condensates have emerged as key players in human health (Spannl *et al.*, 2019, Alberti & Hyman, 2021). Neurodegenerative diseases such as Alzheimer's disease or amyotrophic lateral sclerosis (ALS) have been linked to defects in the condensation of FUS or other prion-like RBPs (Patel *et al.*, 2015, Murakami *et al.*, 2015). Viruses such as herpes simplex or human immunodeficiency (HIV) have developed mechanisms counteracting SG formation, thus promoting their replication (Mahboubi and Stochaj, 2017). In the context of cancer, SGs are in general advantageous to tumors, as they enhance cell survival, metastasis, and tolerance to treatments (Gao *et al.*, 2019). In addition, many condensate-associated proteins aberrantly accumulate in cancer cells (Mahboubi & Stochaj, 2017, Spannl *et al.*, 2019), whereas defects in protein condensate turnover have been linked to ageing and age-related diseases (Lopez-Otin *et al.*, 2013, Alberti & Hyman, 2021). All these biomedicine-relevant findings underscore how biomolecular condensates can control cell fate through multiple and interconnected pathways, ranging from regulation of translation to the modulation of various types of cell death. That is why biomolecular condensates are considered as promising targets to improve therapeutic intervention for several diseases.

To gain insight into the biological role of biomolecular condensates, researchers have traditionally focused their efforts on the characterization of loss-of-function mutants in putative components. One of the best-studied examples in mammalian systems are G3BP1 and G3BP2, two SG proteins whose

studies have provided invaluable insight into key aspects of SG biology (Cirillo et al., 2020). For example, an initial study revealed that inhibition of SG formation in G3BP-deficient lines prevented metastasis and limited tumor invasion (Somasekharan *et al.*, 2015). A more recently report demonstrated that inhibition of SG assembly under G3BP deficiency could only occur under arsenite stress, suggesting that the core mechanisms of SG formation may differ depending on the initiation stimulus (Yang et al., 2020). Curiously, G3BP-deficient mutants are the only mutants unable to form SGs in non-plant organisms. To date, no such mutants are available in plants. However, a recent study showed that the Arabidopsis putative orthologs *G3BP-1* and *G3BP-7* were able to rescue SG formation when expressed in human cells lacking native G3BP function, pointing to some degree of conservation of SG-forming mechanisms across kingdoms (Reuper *et al.*, 2021). However, the implication of Arabidopsis G3BP proteins in SG assembly has not been fully addressed. While studies of loss-of-function mutants aid in better understanding biomolecular condensation, new complementary approaches have recently emerged. For example, an automated cell-based assay platform was used for the identification of new molecules affecting PB assembly and provided an important insight into the relationship between PB assembly and diverse intracellular programs, including organelle physiology (Martinez *et al.*, 2013). In line with this notion, a more recent study using a high-content screen identified small molecules that affect SG assembly and modulate inflammatory signaling pathways (Fang *et al.*, 2019a).

In contrast to the emerging role of biomolecular condensates in human diseases, stress-induced condensates such as SGs, PBs, or siRNA bodies have so far received limited attention in plants. Nonetheless, there is growing evidence for a direct link between protein condensation and plant stress tolerance (Londono Velez et al., 2022, Jang et al., 2020). One example is increased freezing tolerance of Arabidopsis plants with a mutation in the mRNA decapping activator SM-like protein LSM1-7 that results in fewer PBs (Perea-Resa *et al.*, 2016). A more recent study showed that the SG component Multiprotein-bridging factor 1c (MBF1c) from wheat (*Triticum aestivum*) contributed to the heat tolerance of the plant by regulating heat stress-induced mRNA translation (Tian *et al.*, 2022). Biomedical research suggests that changes in the phase separation behavior of condensate components can induce the formation of aberrant condensates, resulting in diseases (Alberti & Hyman, 2021). Although LLPS and other phase separation mechanisms are poorly understood in plants, a recent study of the heat-induced phase separation of Arabidopsis RBGD2 and RBGD4 has provided a strong argument for the importance of phase separation in plant tolerance to heat stress (Zhu et al., 2022). We therefore stand at the very beginning of the exciting path to translate basic knowledge about biomolecular condensates and phase separation to the production of resilient crops.

Conclusions/outstanding questions

Despite significant progress in studying biomolecular condensates in plants achieved in the past decade, there are still many open questions. Further delving into the compositional and structural complexity of various types of condensates would provide a better clue to the origin of their heterogeneity. Considering the dynamic interaction among different stress-related condensates, including PBs, SGs, siRNA bodies, one always wonders what is inside one condensate and not in the other at a particular moment of time. Yet, the hottest questions are: what are the key players of, and can we abolish condensate formation? To date, most of the research on biomolecular condensates in plants has been performed using one or a few condensate-localized proteins (often fused to a fluorescent reporter protein) that can be observed by microscopy. Although genetic or pharmacological manipulations can abolish or alter the microscopic localization of the reporter, this would not necessary mean that condensate assembly is abolished or altered as well. Indeed, backup or auxiliary pathways (e.g. through recruitment of alternative scaffolding factors) might kick in and achieve condensate assembly, but for some reason without recruiting the reporter under study. A deeper understanding of the mechanisms leading to condensate formation through combination of in vivo and in vitro (e.g. condensate reconstitution) approaches would allow better control over biomolecular condensation in plants, also in the context of stress responses.

Materials and Methods

Bioinformatics

To retrieve protein–protein interaction data, the STRING database (V10) was used (Szklarczyk *et al.*, 2015). Only physical protein–protein interactions were considered. The RNA-binding proteins were predicted by the RNAPred tool (Kumar *et al.*, 2011). The prediction approach was based on amino acid composition, and the threshold for the support vector machine (SVM) was 0.5. The orthology analysis was performed using eggNOG database (Huerta-Cepas *et al.*, 2019).

Supplemental data

Supplemental Data Set S1. Full list of SG-associated proteins isolated from TSN2, RBP47, RBGD2/4 and CML38 immunoprecipitations.

Supplemental Data Set S2. Full list of PB-associated proteins isolated from DCP1, DCP2, DCP5 and UPF1 immunoprecipitations.

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Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

Conceptualization, EG-B and PVB; EG-B wrote the first draft of the paper with the input of all authors; Research, EG-B and JS-M; Writing-review and editing, ARF, EG-B, JS-M, MC, PNM, and PVB; Visualization (figure preparation), EG-B, JS-M, MC, PNM, and PVB; Funding acquisition: AS, ARF, EG-B, MC, PNM, and PVB.

Figure Legends

Figure 1. Diagram of the major principles underlying biomolecular condensate formation. A certain protein concentration (dependent on various factors, such as temperature, redox state, pH), enables homotypic or heterotypic interactions between sticker domains (e.g., protein 1–protein 2 interaction on the diagram). When reaching a system-specific threshold concentration ($C_{threshold}$), the entire system undergoes phase separation into two phases. The “stickiness” (or multivalency) depends on the attraction between residues usually provided by so-called “intrinsically disordered regions” (IDRs, e.g., PrLDs or LCDs). Phase separation driven by IDR–IDR interactions can be mediated by noncovalent interactions (boxed area) that include π (aromatic ring)– π , cation (+) – π , charge (–)–charge (+), dipole (+/–)–dipole (+/–) or hydrogen bonds (H). Folded domains or nucleic acids, also mediate phase separation (e.g., protein 3 with an RNA-binding domain [RBD], in the diagram). Given enough time or at high concentrations, condensates may form filaments/aggregates with solid-like material properties.

Figure 2. Basic principles of LLPS in the assembly of SGs. SGs are believed to assemble through three major steps: (1), nucleation; (2), growth; and (3), shell assembly. Stress inhibits translation, which triggers release of mRNAs from the polysomes, which, together with RBPs, promotes

nucleation via LLPS. Next, specific recruitment of additional SG components facilitates core growth (2) and thereafter shell assembly (3). The phase-separating biomolecules (usually proteins) can be categorized as scaffold or clients. In this figure, scaffold and client components are represented as spheres (green for scaffolds and blue for clients) with attractive sites on their surface (gray patches). Each patch (valency) allows a protein to participate in one attractive protein–protein or RNA–protein interaction. In the absence of stress, SG components may exist as pre-formed protein complexes (pre-existing or standby state) serving as seeds for rapid assembly (Gutierrez-Beltran et al., 2021). Upon stress, these complexes may facilitate the recruitment of RNAs and other proteins into phase-separated condensates that become microscopically discernible fluorescent foci if properly labeled. This phase separation may be modulated by PTMs.

Figure 3. Proteomic analysis of plant SGs and PBs. A, Venn diagram showing the extent of overlap among interactomes for four different SG-associated proteins (RBD2/4, TSN2, RBP47 and CML38) under stress. B, A subset of common and specific interactors of the proteins in (A). C, Percentage of RNA-binding proteins found in the four interactomes. D, Protein–protein interaction networks among the RBD2/4, TSN2, RBP47 and CML38 interactomes. E, Venn diagram showing the extent of overlap among interactomes for four different PB-associated proteins (DCP1, DCP2, DCP5 and UPF1) under stress. F, A subset of common and specific interactors of the proteins in (E). For complete lists of Arabidopsis SG and PB proteome components see Supplemental Data Sets S1 and S2. G, H, Venn diagram showing the extent of overlap among plant, mammalian and yeast SG (G) and PB (H) proteomes.

Figure 4. Crosstalk among plant SGs, PBs and siRNA bodies. A, Venn diagrams showing the extent of overlap among DCP1, DCP5 (both for PBs) and TSN2 (for SGs) interactomes under no-stress (NS) conditions (left) and between DCP1 and TSN2 interactomes under heat stress (HS). B, A subset of common and specific interactors of DCP1, DCP5, and TSN2 at the absence of stress. C, Diagram of the relationships among SGs, PBs, and siRNA bodies under no stress conditions and upon onset of stress. For complete lists of Arabidopsis SG and PB proteome components see Supplemental Data Sets S1 and S2.

Figure 5. Biomolecular condensates play a key role in stress responses. A, The sequestration of transcription factors and regulators in condensates can regulate transcription, either promoting or inhibiting it. B, Condensates can either increase or decrease translation efficiency. C, Due to mass action, concentration of enzymes and substrates in the condensates can enhance catalysis or even allow formation of metabolons with improved efficiency. D, Condensates can inhibit enzymatic reactions and pathways in the dilute phase (e.g. cytosol) by sequestering enzymes, their ligands or substrates as well as metabolic intermediates. Inhibition of the reaction can also be achieved by

separating different components of the common pathway (e.g. enzyme and substrate) via sequestration into different types of biomolecular condensates.

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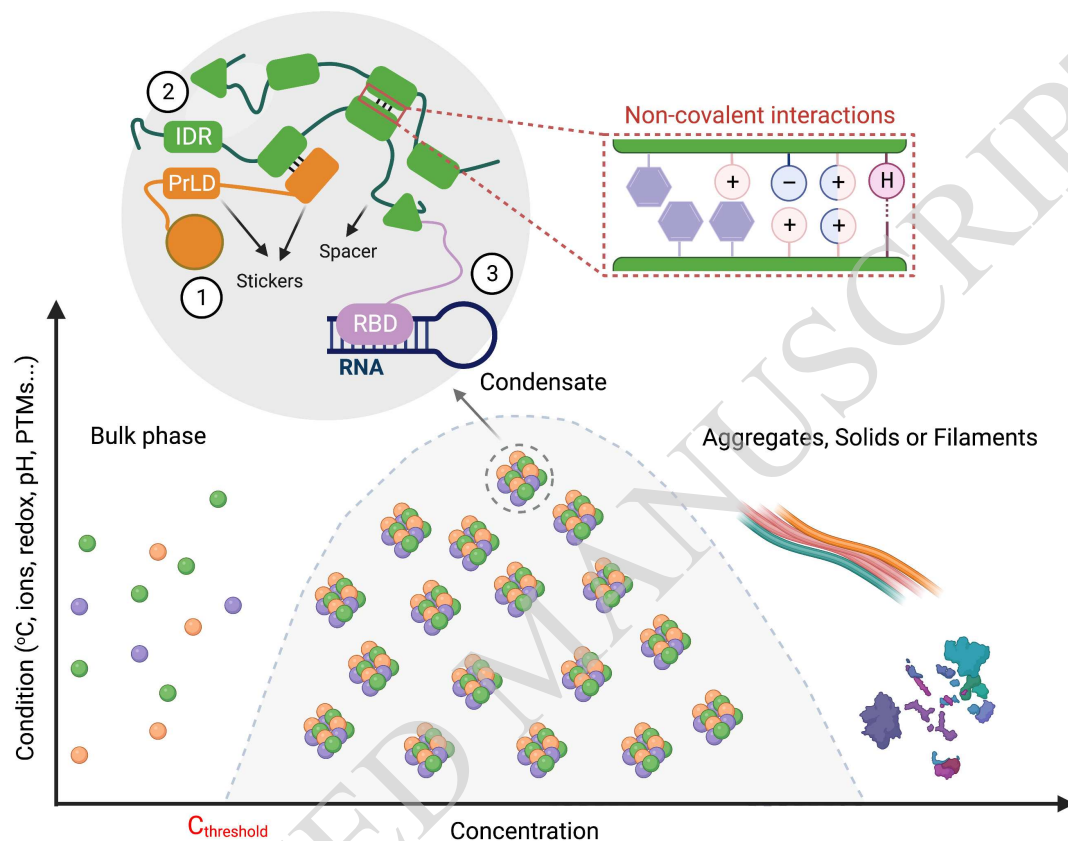


Figure 1. Diagram of the major principles underlying biomolecular condensate formation. A certain protein concentration (dependent on various factors, such as temperature, redox state, pH), enables homotypic or heterotypic interactions between sticker domains (e.g., protein 1–protein 2 interaction on the diagram). When reaching a system-specific threshold concentration ($C_{threshold}$), the entire system undergoes phase separation into two phases. The “stickiness” (or multivalency) depends on the attraction between residues usually provided by so-called “intrinsically disordered regions” (IDRs, e.g., PrLDs or LCDs). Phase separation driven by IDR–IDR interactions can be mediated by noncovalent interactions (boxed area) that include π (aromatic ring)– π , cation (+)– π , charge (–)–charge (+), dipole (+/–)–dipole (+/–) or hydrogen bonds (H). Folded domains or nucleic acids, also mediate phase separation (e.g., protein 3 with an RNA-binding domain [RBD], in the diagram). Given enough time or at high concentrations, condensates may form filaments/aggregates with solid-like material properties.

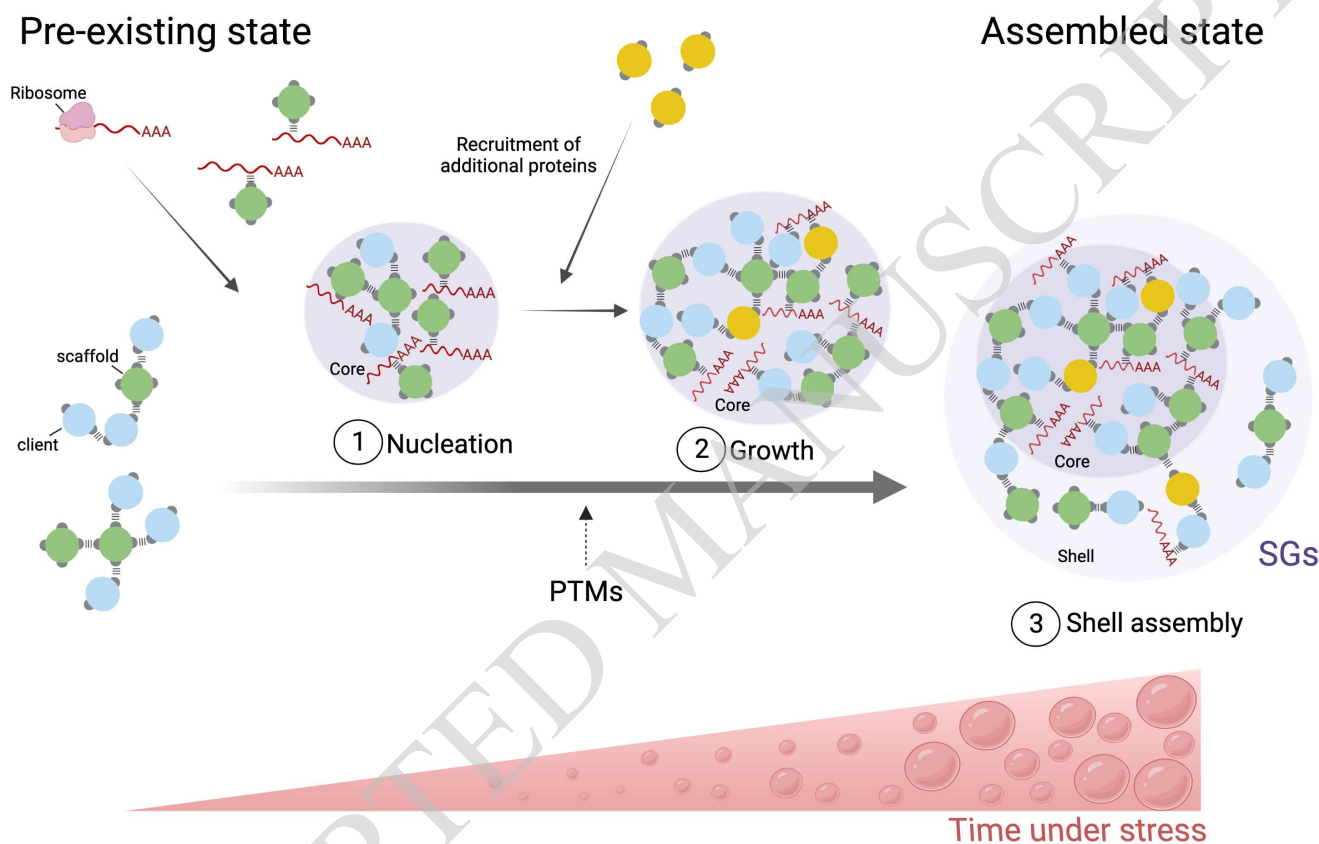


Figure 2. Basic principles of LLPS in the assembly of SGs. SGs are believed to assemble through three major steps: (1), nucleation; (2), growth; and (3), shell assembly. Stress inhibits translation, which triggers release of mRNAs from the polysomes, which, together with RBPs, promotes nucleation via LLPS. Next, specific recruitment of additional SG components facilitates core growth (2) and thereafter shell assembly (3). The phase-separating biomolecules (usually proteins) can be categorized as scaffold or clients. In this figure, scaffold and client components are represented as spheres (green for scaffolds and blue for clients) with attractive sites on their surface (gray patches). Each patch (valency) allows a protein to participate in one attractive protein–protein or RNA–protein interaction. In the absence of stress, SG components may exist as pre-formed protein complexes (pre-existing or standby state) serving as seeds for rapid assembly (Gutierrez-Beltran et al., 2021). Upon stress, these complexes may facilitate the recruitment of RNAs and other proteins into phase-separated condensates that become microscopically discernible fluorescent foci if properly labeled. This phase separation may be modulated by PTMs.

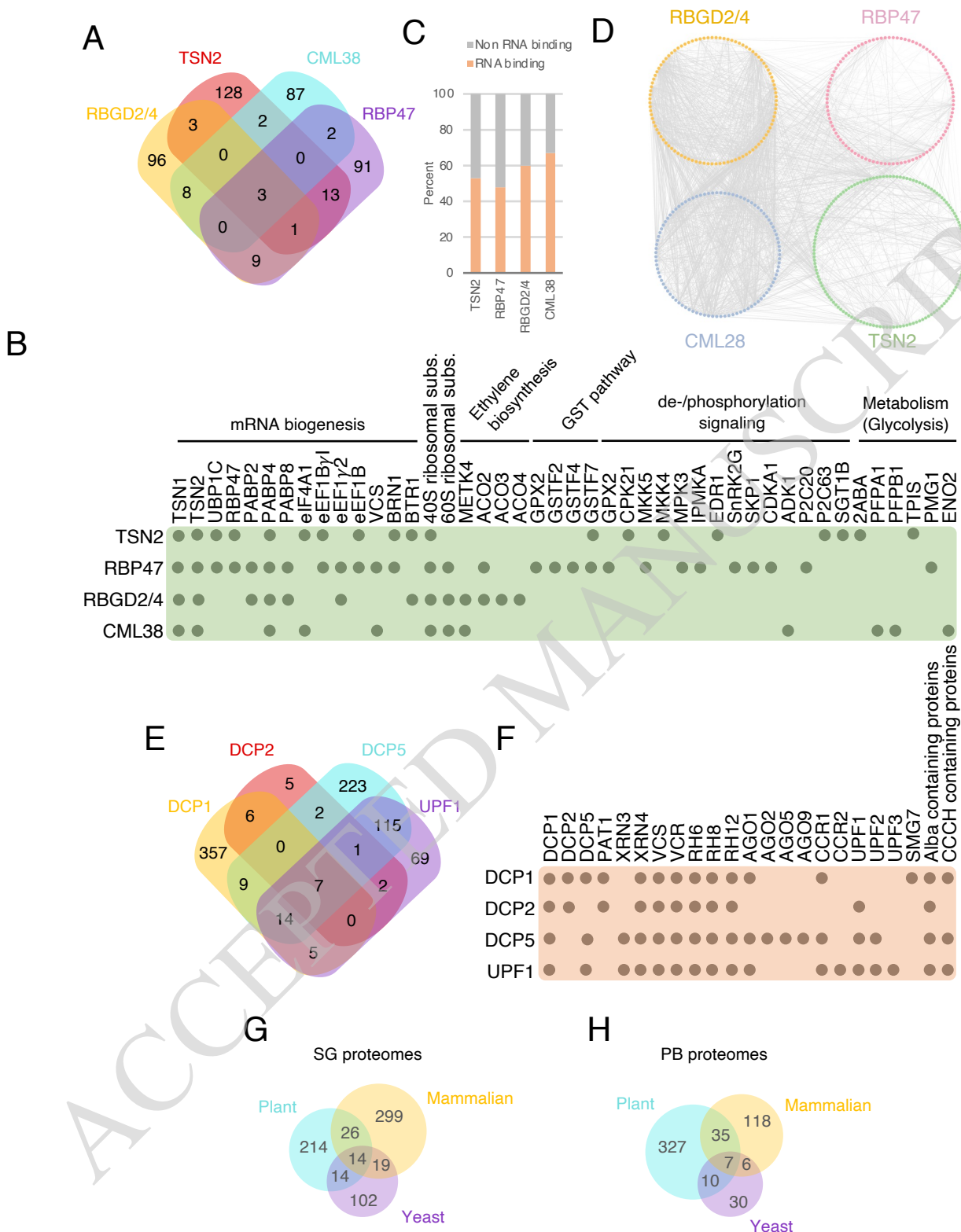


Figure 3. Proteomic analysis of plant SGs and PBs. A, Venn diagram showing the extent of overlap among interactomes for four different SG-associated proteins (RBD2/4, TSN2, RBP47 and CML38) under stress. B, A subset of common and specific interactors of the proteins in (A). C, Percentage of RNA-binding proteins found in the four interactomes. D, Protein–protein interaction networks among the RBD2/4, TSN2, RBP47 and CML38 interactomes. E, Venn diagram showing the extent of overlap among interactomes for four different PB-associated proteins (DCP1, DCP2, DCP5 and UPF1) under stress. F, A subset of common and specific interactors of the proteins in (E). For complete lists of Arabidopsis SG and PB proteome components see Supplemental Data Sets S1 and S2. G, H, Venn diagram showing the extent of overlap among plant, mammalian and yeast SG (G) and PB (H) proteomes.

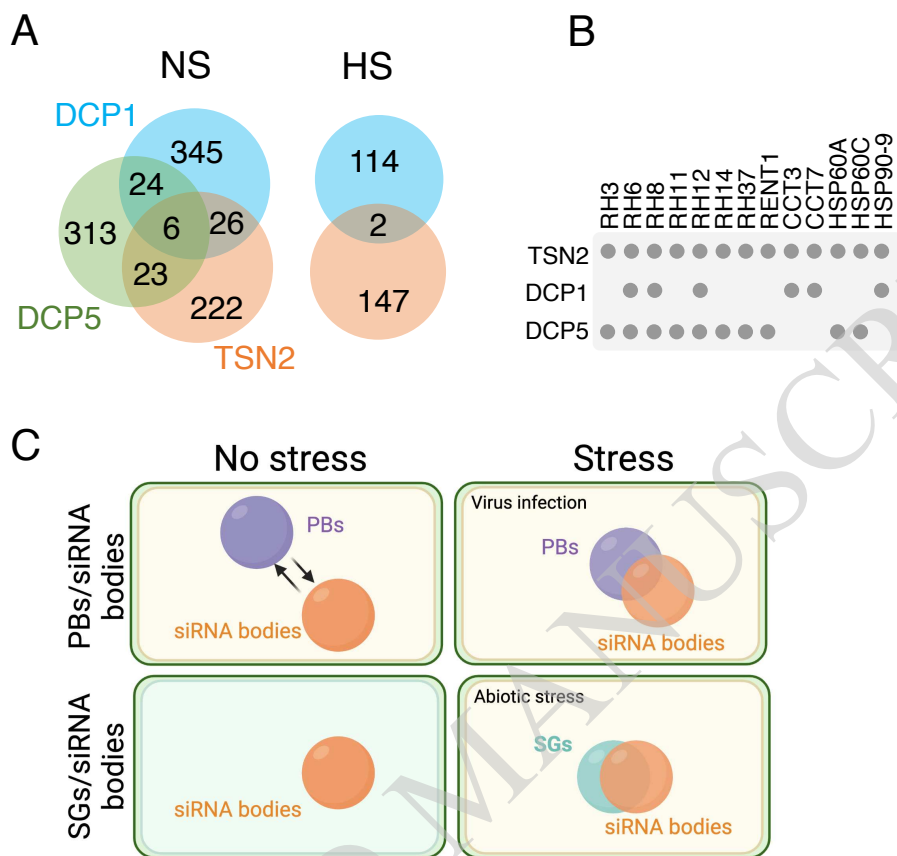


Figure 4. Crosstalk among plant SGs, PBs and siRNA bodies. A, Venn diagrams showing the extent of overlap among DCP1, DCP5 (both for PBs) and TSN2 (for SGs) interactomes under no-stress (NS) conditions (left) and between DCP1 and TSN2 interactomes under heat stress (HS). B, A subset of common and specific interactors of DCP1, DCP5, and TSN2 at the absence of stress. C, Diagram of the relationships among SGs, PBs, and siRNA bodies under no stress conditions and upon onset of stress. For complete lists of Arabidopsis SG and PB proteome components see Supplemental Data Sets S1 and S2.

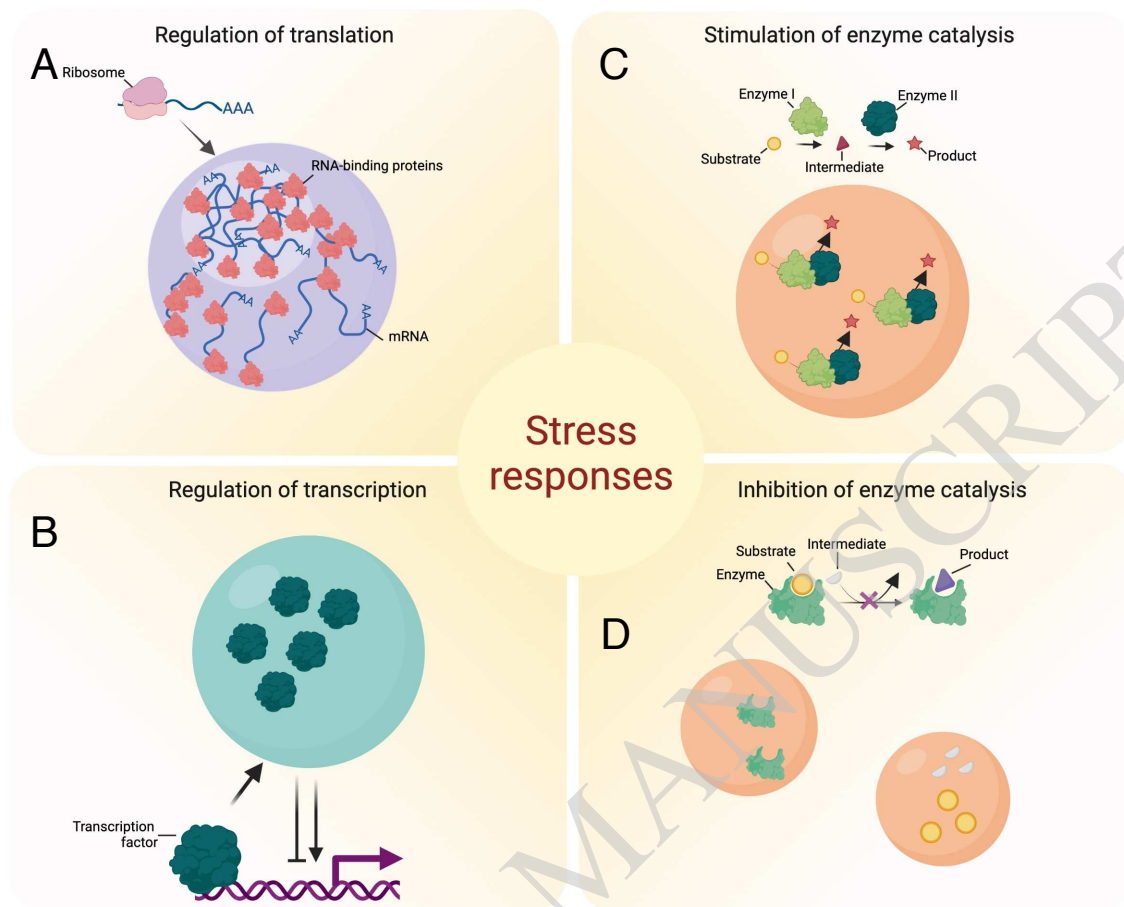


Figure 5. Biomolecular condensates play a key role in stress responses. A, The sequestration of transcription factors and regulators in condensates can regulate transcription, either promoting or inhibiting it. B, Condensates can either increase or decrease translation efficiency. C, Due to mass action, concentration of enzymes and substrates in the condensates can enhance catalysis or even allow formation of metabolons with improved efficiency. D, Condensates can inhibit enzymatic reactions and pathways in the dilute phase (e.g. cytosol) by sequestering enzymes, their ligands or substrates as well as metabolic intermediates. Inhibition of the reaction can also be achieved by separating different components of the common pathway (e.g. enzyme and substrate) via sequestration into different types of biomolecular condensates.

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