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Deciphering the RNA Landscape of RNA Granules

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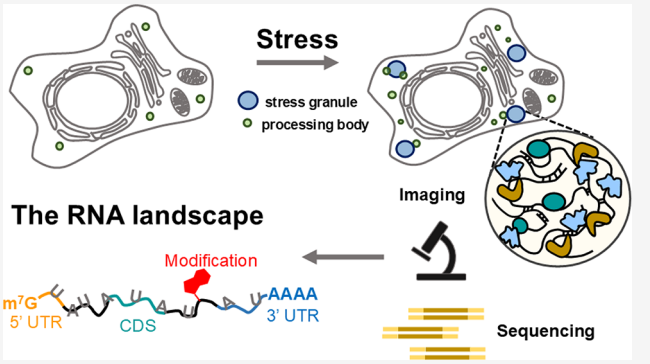
ABSTRACT: RNA granules, including stress granules (SGs) and processing bodies (PBs), are membraneless organelles that orchestrate RNA localization, metabolism, and translational control in response to cellular stress. This Perspective examines the RNA landscape within SGs and PBs, highlighting recent insights into how these compartments shape RNA fate. We review current methodologies for probing granule-associated RNAs, including high-resolution imaging, transcriptomics, and sequencing-based approaches. In particular, we spotlight emerging photoactivated proximity labeling techniques that offer unprecedented spatiotemporal resolution for mapping RNA interactions in living cells. Looking ahead, we propose combining multiomic approaches to better define the roles of RNAs within granules. Finally, we emphasize the importance of investigating RNA granules in neuronal contexts, where dysregulated RNA condensation is intimately linked to neurodegenerative diseases. Together, these approaches promise to elucidate the molecular logic by which RNA granules govern post-transcriptional gene regulation and cellular adaptation to stress.



INTRODUCTION

RNA granules are dynamic, nonmembrane-bound structures that play vital roles in RNA metabolism within eukaryotic cells. They form through the selective aggregation of RNAs and RNA-binding proteins into distinct types, such as stress granules, processing bodies, transport granules, and germ granules.[1] By concentrating specific biomolecules, RNA granules support critical processes including mRNA storage, transport, localization, and translational control, enabling cells to rapidly adapt to environmental and physiological changes.[2−4] For instance, cytosolic germ granules contain proteins and RNAs that are essential for germ cell development. In response to developmental cues, these granules dynamically regulate the localization, storage, modification, and degradation of specific mRNAs, thereby facilitating germ cells to progress through developmental checkpoints and commit to their specialized fate.[5,6]

The assembly of RNA granules is driven primarily by liquid−liquid phase separation (LLPS), a process in which multivalent interactions among proteins and RNAs give rise to dynamic, condensed droplets. Many proteins involved in LLPS feature intrinsically disordered regions (IDRs), prion-like domains, and other aggregation-prone sequences that facilitate transient, yet specific interactions.[2] In addition, RNA contributes to LLPS through its unique physicochemical properties: its exposed phosphate backbones and nucleobases promote electrostatic interactions and base stacking, which are essential for initiating and maintaining phase separation.[7] Moreover, specific sequence motifs, such as polypyrimidine or polyguanosine tracts, can alter RNA secondary structures, thereby modulating RNA−protein interaction strength and influencing granule dynamics and stability.[8] Together, these RNA-specific characteristics underscore the critical role of RNA in both the assembly and the structural organization of granules.

Despite significant advances in our understanding of RNA granules, key questions remain unanswered. In particular, the mechanisms governing the selective localization of RNAs to specific granules, the precise mRNA modifications occurring within these structures, and the impact of an RNA's translation state on its granule localization are still under investigation. Resolving these issues is essential for a comprehensive understanding of how RNA granules coordinate mRNA metabolism and mediate cellular stress responses. In the following sections, we focus on two key granule types, stress granules and processing bodies, discussing their RNA composition, functional roles, current investigative methods, outstanding questions, and future research directions.

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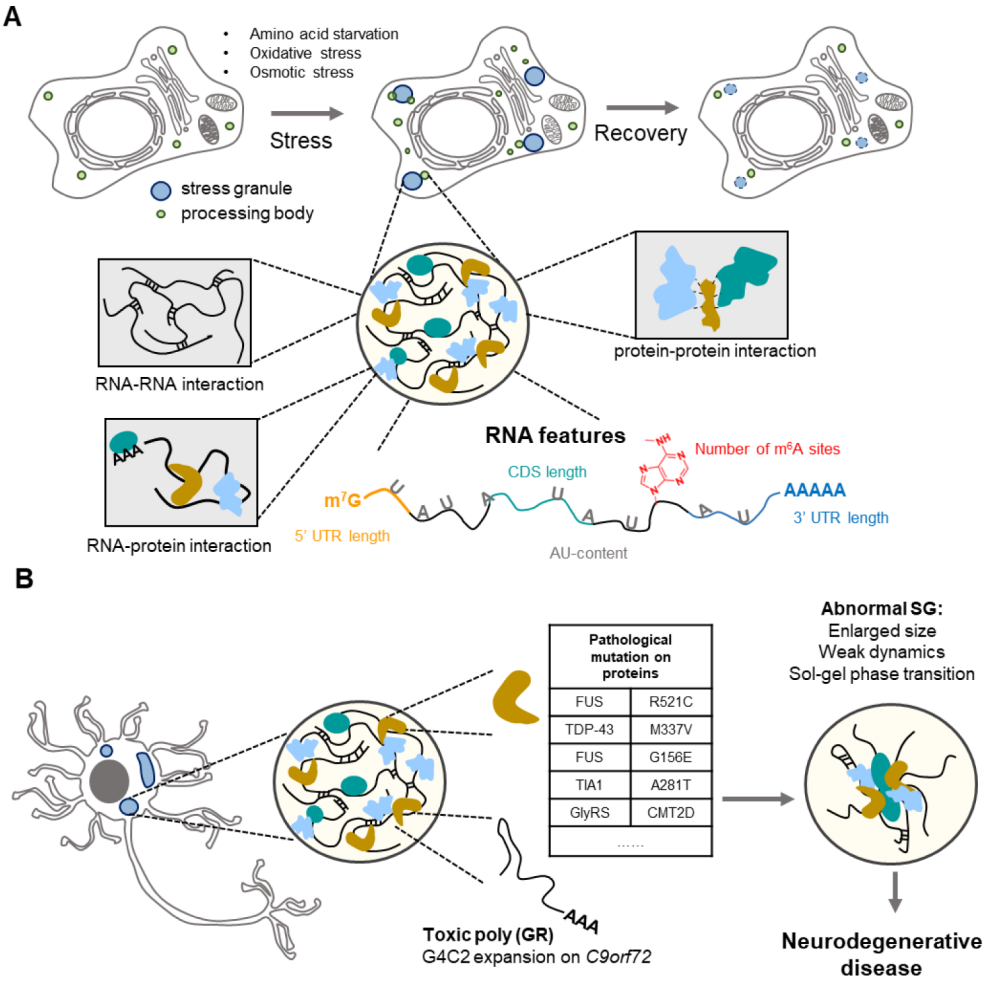

Figure 1. (A) Scheme of SG formation and recovery in mammalian cells. SGs are induced when cells are challenged with various stress, forming membraneless compartments that often localize adjacent to PBs. These dynamic structures comprise proteins and RNAs engaged in multiple interactions. Targeting to SGs is often positively correlated with RNA length, AU content, and m^{6}A modifications. (B) Scheme of abnormal SG formation in neurons under neurodegeneration disease. Mutations in SG-associated proteins can alter SG structure, leading to the formation of less dynamic aggregates. Abnormal RNA, such as G4C2 repeats on C9orf72, might also induce the formation of abnormal SGs that are related to neurodegeneration disease.

RNA IN STRESS GRANULES

Stress granules (SGs), typically 100 nm to 1 μm in diameter, form transiently in response to cellular stress[9] (Figure 1A). Under conditions such as oxidative stress, osmotic imbalance, or viral infection, stalled protein translation causes preinitiation complexes (PICs), RNA-binding proteins (RBPs), and specific mRNAs to assemble into these liquid−liquid phase-separated condensates.[10] SG nucleation begins when core scaffolding RBPs with prion-like or IDRs, notably G3BP1 and TIA1, aggregate along with stalled PICs.[10] This multivalent assembly is regulated by stress-responsive signaling pathways: for example, amino acid deprivation inactivates mTOR, leading to 4E-BP dephosphorylation and competitive binding to eIF4E, which disrupts the eIF4E−eIF4G complex and halts translation initiation. Alternatively, oxidative stress (e.g., sodium arsenite) activates the integrated stress response, phosphorylating eIF2α at Ser51, impairing ternary complex formation and blocking methionyl-tRNA$_i^{Met}$ delivery to ribosomes.[11] Pharmacologically, cycloheximide stabilizes polysomes and prevents SG formation, while puromycin-induced polysome disassembly liberates mRNAs that drive SG assembly.[12]

SGs are thought to form a "core-shell" architecture. A stable core, comprised of G3BP1, TIA1, and translationally repressed mRNPs, nucleates SG assembly and recruits more dynamic shell components.[13] Core SG protein components with IDRs and RNA-binding domains engage in multivalent homotypic and heterotypic interactions, while RNA acts as a critical modulator of phase separation. RNA sequencing of isolated SG cores revealed a predominance of mRNAs, with subsequent FISH imaging showing that some transcripts colocalize with SGs at levels of 50−80%.[14] These mRNAs exhibit a significantly long CDS and UTR with reduced translational activity. RNAs in SG can also exhibit stress- and cell-type-dependent properties. Comparative transcriptomics under heat stress versus eIF4A inhibition reveal stress-specific RNA recruitment, highlighting context-dependent regulation of SG composition for cellular adaptation.[15]

SG RNAs also bear distinct epigenetic marks that influence their phase behavior and localization. *In vitro*, N^6-methyladenosine (m^{6}A) enhances RNA-protein phase separation. Loss of the m^{6}A writer METTL3 abolishes the selective targeting of long mRNAs to SGs, directly linking m^{6}A modification to mRNA partitioning. Likewise, m^{1}A-modified

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transcripts resume translation more rapidly after stress.[16] Beyond methylation, SG RNAs are enriched in N^4-acetylcytidine (ac^4C), and knockout of the corresponding acetyltransferase reduces the RNA content of SGs without altering their assembly kinetics.[17] Together, these findings indicate that multiple RNA modifications cooperatively promote phase separation and stress-adaptive mRNA partitioning.

RNA IN PROCESSING BODIES

Processing bodies (PBs) are abundant cytoplasmic ribonucleoprotein (RNP) granules that, despite their prevalence, have been less extensively characterized than stress granules (SGs) in terms of structure, function, and molecular composition. PBs were initially proposed as dedicated sites for mRNA decay, as they were found to contain the 5′-3′ exonuclease XRN1 and the decapping enzymes DCP1 and DCP2 in yeast.[18] These granules were later reinterpreted as multifunctional storage hubs for translationally repressed transcripts.[19] Supporting this view, PBs have been shown to enrich mRNAs with m^6A modifications in their coding sequences, marking them for degradation.[20] More recently, imaging assays demonstrated that endogenous YTHDF2-induced and chemically induced RNA decay occurs rapidly within PBs.[21,22] However, RNA sequencing of biochemically purified PBs revealed that many mRNAs within PBs are translationally repressed but not degraded,[23,24] suggesting that PBs may also function as storage sites for silenced transcripts.[25,26] Thus, the precise role of PBs remains unresolved.

In mammalian cells, PBs appear under fluorescence microscopy as discrete foci approximately 0.1−0.5 μm in diameter and number from a few up to tens per cell.[25] Their core protein machinery, including decapping activators EDC3, DCP1, and the DEAD-box helicase DDX6, features IDRs or low-complexity domains (LCDs) alongside canonical RNA-binding motifs, a combination that promotes multivalent interactions and LLPS. RNA is not merely cargo, but an architectural element within PBs. For example, the yeast *RPS28B* mRNA contains a long 3′UTR that binds the scaffold protein EDC3, thereby nucleating PB assembly via recruiting IDR-containing factors.[27]

PB assembly is tightly coupled to the translational status. Puromycin, which releases ribosomes from mRNAs, robustly increases PB number by expanding the pool of nontranslating mRNPs.[25] Oxidative stress via sodium arsenite similarly enlarges and multiplies PBs, often positioning them adjacent to newly formed SGs.[12] In contrast, prolonged endoplasmic reticulum (ER) stress induced by tunicamycin has been reported to impair PB formation in certain cellular contexts, reducing their abundance.[28] Interestingly, PBs and SGs engage in continuous molecular exchange under stress. DEAD-box helicases such as DDX6, present in both granules, use ATP hydrolysis to modulate RNA entry and exit, controlling granule composition and dynamics.[29] Single-molecule imaging shows individual mRNAs associating transiently with SGs and PBs and shuttling bidirectionally on time scales of seconds to minutes.[30]

High-throughput sequencing of purified PBs demonstrates that they harbor a diverse RNA cargo, including miRNAs, long noncoding RNAs, and predominantly mRNAs.[31] In mammalian cells at steady state, PB-enriched mRNAs are strongly AU-rich and tend to encode low-abundance regulatory proteins, suggesting selective sequestration based on nucleotide composition and functional category.[23,24] In stem cells, DDX6-dependent PBs store mRNAs encoding chromatin remodelers, transcription factors, and growth regulators. Upon PB disassembly triggered by loss of DDX6 activity, these mRNAs reenter the translational pool, enabling rapid shifts in cell fate and differentiation capacity.[32] The m^6A modification further tunes PB targeting. Transcripts bearing m^6A marks recruit YTHDF2, which drives their partitioning into PBs and enforcement of a translation-repressed state.[22,33] While our understanding of PB architecture, composition, and dynamics has expanded considerably, many mechanistic details, particularly how specific RNAs and proteins are selectively partitioned and how PBs integrate into broader post-transcriptional regulatory networks, remain open questions. Recent studies have shown that SG-specific RNAs exhibit higher levels and a greater density of m^6A modification compared to PB-specific RNAs under sodium arsenite treatment. Given that m^6A binding proteins such as YTHDF1/2/3 regulate the partitioning of m^6A RNAs, their strong association with other SG components likely drives the localization of highly methylated RNAs to SGs.[34−36]

RNA GRANULES IN THE NERVOUS SYSTEM

In highly polarized neurons, RNA granules orchestrate maturation, homeostasis, and, as increasingly recognized, pathological processes.[37] These membraneless assemblies remodel dynamically over time scales from seconds to minutes in response to acute stimuli, up to weeks or months during development and aging.[37] For instance, the *Drosophila* long noncoding RNA *mimi* scaffolds neuronal granules by binding ELAV-like proteins and recruiting Staufen (Stau), FNE, and RBP9, thereby regulating RNA storage and distribution during adulthood. Loss of *mimi* granules in aging flies impairs locomotor behavior and reduces survival, underscoring their role in maintaining neuronal maturity.[38] During murine hippocampal neuron maturation, DDX6-positive granules decrease in size but increase in number,[39] whereas in aging *Drosophila* brains, the DDX6 ortholog Me31B condenses into fewer, larger, yet still dynamic foci.[40]

Transport granules are neuron-specific RNA condensates responsible for delivering RNAs from the soma to the distal neurites. By positioning RNAs near synapses, these granules help neurons coordinate local translation in response to rapid environmental stimuli.[41] Studies have shown that loss of the survival motor neuron (SMN) protein disrupts transport granule assembly, impairing the trafficking and function of β-actin mRNA.[42] Under physiological conditions, ALS/FTD-associated proteins such as TDP-43 and FUS are components of transport granules. However, disease-linked mutations promote their aggregation, which interferes with granule binding to microtubules and impairs their motility, ultimately leading to deficiencies in neuronal RNA transport.[43,44]

Functional RNA granules also operate in non-neuronal cells of the nervous system. In oligodendrocytes, MBP mRNA is packaged into transport granules containing hnRNP A2, which shuttle transcripts along microtubules to sites of myelin assembly and repress translation until arrival.[45,46] These granules harbor core translation machinery components, indicating that they serve both as transport vehicles and local translation hubs. Although studies have cataloged protein constituents extensively, the full complement of RNAs within these granules and how they regulate myelination remain to be defined.

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Multiple lines of evidence implicate neuron-specific SGs in the pathogenesis of neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and frontotemporal dementia (FTD).[47−49] Mutations in SG-resident RBPs, including TDP-43, TIA1, and FUS, are common in ALS/FTD patients[50−52] and often map to low-complexity domains or RNA-binding regions, disrupting normal RNA interactions and phase-separation behavior. As a result, aberrant, SG-like inclusions form under basal conditions and become resistant to clearance, while altered RNA interactions further stabilize these aggregates and impart neurotoxicity.[53]

RNA itself can exacerbate pathological aggregation (Figure 1B). In ALS/FTD, expanded G4C2 repeats in *C9orf72* transcripts drive SG assembly and noncanonical translation under nonstress conditions.[54] These repeats can also produce dipeptide repeat proteins that modify SG protein dynamics and promote formation of G3BP1-containing granules with diminished fluidity.[55] Although most transcriptomic profiling has relied on immortalized cell lines, the RNA composition and functional consequences of SG formation in neurons have remained poorly defined. Addressing this gap will be critical for uncovering neuron-specific mechanisms of SG regulation and their roles in neurodegenerative disease.

DECIPHERING RNA COMPONENT AND INTERACTIONS WITHIN RNA GRANULES

Numerous studies have examined RNA composition and function in RNA granules using two main approaches: (1) high-resolution optical microscopy and (2) high-throughput sequencing of granule-associated RNAs.

Imaging-Based Methods. High-resolution microscopy visualizes RNA localization but typically captures only static snapshots (Figure 2). In fixed cells, single-molecule FISH

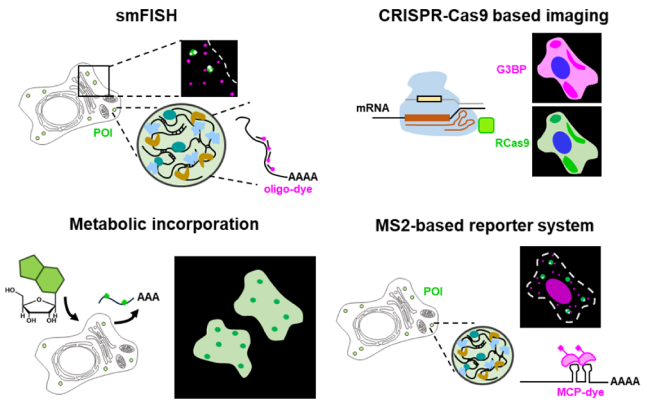

Figure 2. Scheme of imaging-based techniques for studying RNAs in SGs and PBs.

(smFISH) is widely used.[56] For example, Parker et al.[14] applied smFISH to stress granules (SGs) in human osteosarcoma cells, showing that mRNA distribution within SGs changes with different stress stimuli.[57] They later combined smFISH with super-resolution imaging to reveal that translationally repressed RNAs inside SGs are compact, whereas actively translating mRNAs are more extended, likely because of ribosome binding, implying that the translation state affects SG localization. However, smFISH cannot track the RNA dynamics over time.

In live cells, RNA tracking relies on engineered reporters (Figure 2). In the classic MS2/MCP approach,[58] RNAs bearing MS2 hairpins recruit fluorescent coat protein MCP, enabling real-time observation of RNA exchange between SGs and PBs and revealing functional RNA localization within PBs.[30,59] Combining MS2/MCP with PP7/PCP reporters demonstrated that PBs are not major sites of RNA decay, challenging prior assumptions.[30,31,59] A SunTag-MS2 reporter fused to the *ATF4* 5′UTR further showed that mRNAs can undergo initiation, elongation, and termination inside SGs, overturning the view that SG-localized RNAs are translationally silent.[60] In addition to the MS2/MCP system, the more recently developed Cas-based RNA imaging method[61,62] and fluorescent RNA technique[61,62] have also greatly facilitated our understanding of mRNA (e.g., *ACTB*) trafficking into SGs under stress. Metabolic incorporation of modified nucleosides can also enable RNA tracing in living cells.[63] For instance, overexpression of uridine-cytidine kinase enhances the incorporation of cytidine analogues into RNA, enabling researchers to monitor global RNA localization, synthesis, and turnover. This approach has shown that certain RNAs form cytoplasmic foci that colocalize with DDX6 but not with G3BP1 or the P-body marker DCP1A, suggesting that under stimulation these RNAs may aggregate into distinct granule types.[64]

Overall, imaging-based techniques provide high-resolution information about RNA localization, dynamic tracking, and turnover within granules, leading to significant breakthroughs in understanding granule function. However, these methods are limited by low throughput and the need for prior knowledge of target RNAs. Consequently, sequencing-based techniques are essential to complement imaging approaches, as they offer more comprehensive, global insights into the RNA composition in granules.

Sequencing-Based Methods. Sequencing-based approaches begin by isolating granule-associated RNAs, converting them into cDNA libraries and then applying high-throughput sequencing (Figure 3). These workflows yield an unbiased, global portrait of the subcellular transcriptome, uncovering novel sequence features, transcript-length biases, and localization signals that illuminate granule assembly and function. Three principal strategies have emerged: (1) biochemical purification, (2) base-editing tagging, and (3) proximity labeling.

During biochemical fractionation, RNA granules can be enriched by ultracentrifugation, immunoprecipitation,[13,14] or fluorescence-activated particle sorting (FAPS).[23] For example, Parker and coworkers used ultracentrifugation to isolate arsenite-induced SG cores from U-2 OS cells,[13] finding that these cores harbor mRNAs with longer sequences but reduced translation efficiency.[14] Similarly, Weil's group applied FAPS to purify GFP-LSM14A-tagged PBs from HEK293T cells, and sequencing analysis revealed that these mRNAs were translationally repressed yet largely stable.[23] While powerful, these enrichment methods may lose weakly bound components and risk contamination.

Base-editing methods label RNAs in situ, eliminating the affinity steps. TRIBE (targets of RNA-binding proteins identified by editing) fuses the ADAR enzyme to an RNA-binding protein, converting nearby adenosines to inosines, which appear as A-to-G mutations upon sequencing.[65] In *Drosophila* S2 cells, FMR1-ADAR revealed SG-associated transcripts by comparing editing before and after stress.[66]

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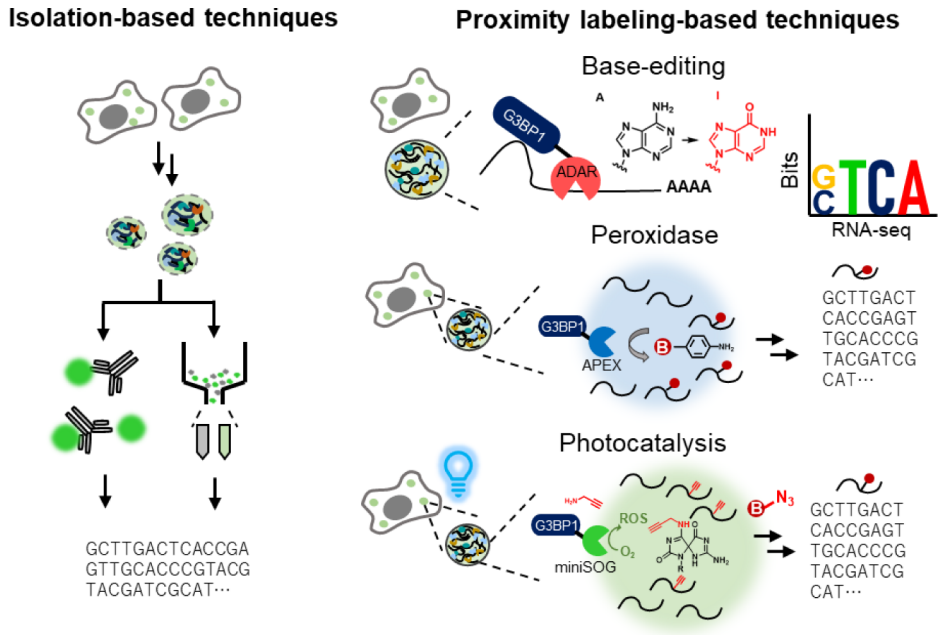

Figure 3. Scheme of sequencing-based techniques for studying RNAs in SGs and PBs.

TRIBE-ID further couples ADAR editing with rapamycin-induced dimerization of G3BP1-FRB and FKBP-ADAR, confirming that SG RNAs are long, poorly translated, and short-lived.[67] By avoiding affinity purification, these approaches minimize input requirements and can operate at single-cell resolution. However, data analysis could be complicated by the inherent biases of editing enzymes.

Proximity labeling covalently tags RNAs near a protein of interest in living cells, offering high spatiotemporal precision (Figure 3). In APEX-seq, the engineered peroxidase APEX2[68,69] generates biotin radicals from biotin-phenol in the presence of H_2O_2, labeling adjacent RNAs.[15,69] Targeting APEX2 to SGs via eIF4A1 in HEK293T cells showed that longer mRNAs accumulate more slowly during heat shock.[15] Although APEX-based approaches provide direct molecular tagging, their reliance on H_2O_2 can induce cellular toxicity that perturbs SG assembly.[70,71] In addition, the labeling efficiency of APEX-seq is limited by the low reactivity of APEX-generated phenoxyl radicals toward nucleobases. To address this, several studies have employed metabolic incorporation strategies or optimized probe design to enhance radical reactivity.[72,73]

Photoactivated proximity labeling circumvents peroxide toxicity by generating reactive species through the light activation of photosensitizers. Because the lifetime and diffusion radius of these species are limited, often to the nanometer scale, this strategy achieves high spatial resolution. Using visible light also grants finer temporal control than does APEX-seq. Broadly, photoactivated methods fall into two categories: small-molecule photosensitizer-based and genetically encoded protein-based labeling. Organic dyes such as dibromofluorescein (DBF) can be tethered to protein tags (e.g., HaloTag) or targeting moieties. Under green light, DBF produces singlet oxygen that oxidizes nearby RNA.[74] However, these dyes often require long incubations and can yield a higher background from nonspecific adsorption.

Genetically encoded photocatalytic RNA labeling employs an engineered protein photocatalyst, such as miniSOG (mini singlet oxygen generator), that can be targeted to subcellular locations via protein fusion.[75] Upon blue light illumination, miniSOG generates singlet oxygen that oxidizes guanines on nearby RNAs, which then react with amine-containing probes, allowing subsequent affinity purification and sequencing analysis (i.e., chromophore-assisted proximity labeling and sequencing, CAP-seq).[76] Owning to the short lifetime (<60 μs) and limited diffusion radius (~70 nm) of singlet oxygen,[77] CAP-seq achieves high spatiotemporal resolution. When miniSOG is targeted to SGs via fusion with G3BP1, CAP-seq analysis allows kinetically resolved mapping of SG transcriptomes during stress induction and disassembly, revealing the presence of stress-imprinted nanoscale SG cores that feature AU-rich and translationally silenced RNAs.[78]

To summarize, over the past decade, high-resolution imaging and high-throughput sequencing have converged to illuminate the composition, dynamics, and function of RNA granules. Imaging methods (smFISH, MS2/MCP, SunTag, Cas-based reporters) deliver spatially precise, dynamic snapshots of individual transcripts within SGs and PBs, revealing how translation state, localization, and turnover change under stress, but they remain limited by throughput and the need to predefine RNA targets. Sequencing-based approaches, including biochemical enrichment, base-editing tagging (e.g., TRIBE), and proximity labeling (e.g., APEX-seq, CAP-seq), provide an unbiased, global census of granule-associated RNAs, uncovering length biases, localization signals, and kinetic recruitment profiles, yet each carries trade-offs in purity, enzyme bias, or cellular toxicity. Together, these methods synergize to offer both molecular detail and a transcriptome-wide scope, yielding a more complete picture of how RNA granules assemble, function, and disassemble.

■ FUTURE PERSPECTIVES

Transcriptome-wide analyses are transforming our view of RNA granules by revealing the full complement of RNAs they harbor, how those RNAs behave, and how localization influences translation. Because RNAs both carry genetic

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Table 1. Future Directions for Studying RNA Granules

Future Directions	Aims	Techniques in Requirement
Transcriptome-wide analysis for RNA targeting mechanism	Profiling translatome	Spatiotemporally resolved ribosome profiling
	Profiling RNA modifications	Spatially resolved m^{6}A-seq
Defining principles on RNA-RBP colocalization	Multiomic profiling	Proximity labeling of both proteins and RNAs
RNA granules analysis in primary cells, tissues, *in vivo*, and clinical samples	Profiling RNA granules without genetic manipulation	Immuno-proximity labeling techniques
Precise control of RNA condensate properties	Perturbing RNA granule assembly with drugs	Chemicals targeting specific RNA condensates
	Manipulating critical domains or sites for RNA granules disassembly	AI-guided identification of RNA structures and protein domains
	Manipulating RNA granules dynamics with light	Photoswitchable or photocleavable peptides for protein control

information and regulate gene expression, mapping their spatial distribution alongside their translational activity is essential. Although SGs were long thought to be translationally silent, recent work shows that a subset of SG-resident mRNAs continues to be translated.[60] Prior imaging-based studies, however, suffered from low throughput and reporter bias toward specific motifs. Unbiased, global measurements now demonstrate that, during SG disassembly, SG-enriched transcripts preferentially reengage ribosomes, whereas mRNAs stalled in translation remain outside granules.[79] This dynamic interplay between localization and translation calls for integrated assays, combining ribosome profiling with high-resolution spatial transcriptomics across SG assembly and disassembly to reveal how granule targeting modulates translational control. The role of RNA modifications, such as m^{6}A, in regulating molecular partitioning between the cytoplasm and various RNA condensates remains poorly understood. To uncover the underlying mechanisms, effective labeling tools are needed for profiling RNA modifications and associated RNA-binding proteins (Table 1).

While SG assembly is driven by multivalent RNA−RNA and RNA−protein interactions, the principles that determine which RNAs and RBPs colocalize remain poorly defined. To close this knowledge gap, future work should integrate transcriptome-wide and proteome-wide data sets collected under diverse stress paradigms, thereby revealing compositional rules across conditions. In particular, photoactivated proximity labeling methods, which combine temporal precision with subcellular resolution, are ideally suited to map RNA-protein contacts in living cells. For example, genetically encoded miniSOG can be used under blue-light illumination to trigger reactive oxygen species that label both nearby RNAs (e.g., via CAP-seq) and proteins (e.g., via RinID,[80] PDPL,[81] or LITag),[82] enabling multiomic profiling in the same cell line.

Neurons rely on diverse RNA granules, including transport RNPs, SGs, and PBs, to regulate local mRNA translation and synaptic plasticity.[37] However, the molecular composition and functional roles of neuronal RNA granules in primary neurons, tissues, and living animals remain incompletely characterized. Aberrant dynamics of these granules, often manifesting as persistent or misassembled condensates, are hallmarks of multiple neurodegenerative diseases.[9] For example, the ALS-linked FUS R521C mutation disrupts SG assembly, mislocalizes FUS to the cytoplasm in cortical neurons, and drives apoptosis *in vivo*.[51] Similarly, chronic cellular stress in Alzheimer's models induces Tau hyperphosphorylation, which recruits SG proteins and RNAs into pathological aggregates.[83] Employing spatially resolved RNA labeling alongside proximity proteomics in neuronal disease models will reveal how granule composition shifts during pathology, link these changes to dysfunction, and uncover novel intervention targets. For clinically relevant samples, there is an urgent need for nongenetically encoded labeling techniques with high efficiency (Table 1).

Dissecting the molecular roles of specific RNAs or proteins within RNA condensates remains a major challenge. Targeted chemical approaches that disrupt specific protein domains or RNA secondary structures, which often promote granule disassembly, offer a more precise alternative to broad-spectrum LLPS-disrupting agents, such as 1,6-hexanediol (Table 1). Advances in AI and structural biology have further enabled the rational design of domain truncations or site-specific mutations that reduce the phase separation propensity of biomolecules, facilitating more targeted functional analyses. In parallel, light-inducible tools such as optoDroplets, optoGranules, and SPARK-ON allow for controlled granule assembly using light.[84−86] Complementary technologies for light-induced disassembly hold strong potential for revealing the molecular mechanisms that govern the cellular regulation of RNA condensates (Table 1).

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Notes

The authors declare no competing financial interest.

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