RESEARCH HIGHLIGHT

RNA helicases regulate RNA condensates

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Stress granules are cytoplasmic membraneless RNA condensates for storing non-translating mRNAs, which are induced in response to various forms of stress. In a recent issue of *Cell*, Tauber et al. show that RNA helicases, particularly eIF4A, act to limit the recruitment of non-translating mRNAs to stress granules by disrupting RNA-RNA interactions, which provides a prototype for regulated phase separation in RNA condensates.

Eukaryotic cells contain membraneless granules in both the nucleus (i.e. nucleolus, speckles, paraspeckles, Cajal bodies, etc.) and the cytoplasm (i.e. P-bodies and stress granules).¹ A commonality is that all these granules are composed of various RNAs and RNA-binding proteins (RBPs) and all are dynamically regulated under physiological and/or pathological conditions. Taking P-bodies (PBs) and stress granules (SGs) as examples, PBs are detectable in most normally growing cells whereas SGs are inducible in response to diverse forms of stress.² These two types of ribonucleoprotein (RNP) granules have overlapping constituents, but each has distinct RBPs to scaffold their formation. The proteomes in PBs and SGs suggest that PBs may function to facilitate RNA decay because of the enrichment with RNA decapping and deadenylation enzymes whereas SGs may store non-translating mRNAs as evidenced by enriched translating initiation factors, but not 60S ribosomes. Because specific mRNAs appear able to recycle from these granules to polysomes when translation is de-repressed,³ it has been generally thought that these granules are part of the translational control mechanism in the cell.²

Past research has been focused on the contribution of proteins, especially those containing intrinsically disordered regions (IDRs), to RNA granule formation via weak, multivalent interactions.⁴ However, it has been increasingly appreciated that RNA-RNA interactions also play a major role in this process.⁵ Remarkably, purified total RNA is able to form granule-like structures that share a related transcriptome to that detected in isolated SGs.^{6,7} Therefore, the summation of protein-protein, protein-RNA, and RNA-RNA interactions appears to drive the formation of this and likely all other types of RNP granules. Under this conceptual framework, a key question is how RNA transport in and out of these granules may be regulated.

In a recent issue of *Cell*,⁸ Roy Parker and colleagues demonstrate that several RNA helicases, particularly eIF4A, regulate SG formation in a manner independent of translational repression. This explains why eIF4A is almost 10-fold higher than other translation initiation factors in eukaryotic cells. They elegantly demonstrate that RNA can be recruited to the surface of existing granules, at least in part through RNA-RNA interactions (as in the case with purified RNA in vitro). The question is how the partition of RNPs between the free cytosolic population and SGs may be

regulated, thus preventing all RNPs from clapping into such “aggregates”. Natural candidates are RNA helicases and indeed several DEAD-box RNA helicases are highly enriched in SGs from multiple previous proteomic analyses. Focusing on the most abundant one (eIF4A) in the cell types under investigation, they show that this helicase limits the recruitment of specific RNAs to SGs (Fig. 1). Additional less abundant RNA helicases have also been implicated in the process, suggesting their collective contribution to the regulation. Importantly, this eIF4A-regulated SG formation can be separated from its role in translational control, as demonstrated on translation-repressed cells. Moreover, this function is linked to the ATP-dependent RNA binding of eIF4A, but not its ATPase activity because a specific point mutation that blocks its ability to bind RNA, but not that in its ATPase center, obligates its function in limiting RNA partitioning into SGs. This suggests that eIF4A disrupts RNA-RNA interactions during SG formation by acting as an RNA-binding competitor. As ATP hydrolysis converts eIF4A from high affinity to low affinity for RNA, its ATPase function may thus provide a regulatory function through ATP hydrolysis for eIF4A to participate in multiple rounds of action between non-translating RNAs and SGs. As pointed out by the authors, this mechanism appears analogous to the action of the protein chaperone Hsp70 in handling protein aggregates.⁹

This series of discoveries has wide important implications beyond the simple concept that RNA helicases unwind RNA helices to prevent RNA from forming aggregates and/or release them from existing aggregates.

Liquid-liquid phase separation (LLPS). The findings are related to the current fever in LLPS, which is driven by weak, multivalent interactions via IDRs in proteins and RNA-RNA interactions (i.e. intra/intermolecular base-pairing, non-Watson-Crick interaction, and base-stacking), as observed with purified protein or RNA in vitro.^{10,11} The debate is how reconstituted LLPS may be related to the formation of specific membraneless organelles within cells. LLPS is characterized by (i) sphere droplet formation in a concentration-, temperature-, pH-, salt-, and crowding agent-dependent manner, (ii) exchange with external molecules and mobility within the droplet, (iii) fusion and fission of droplets, and (iv) sensitivity to interaction disruptors. Under certain conditions, liquid droplets are transitioned to immobile hydrogel or tangle. Using total RNA purified from yeast, the formation of SG-like droplets appears to fulfill all criteria for LLPS.⁶ In the current study, fluorescently-labeled mRNAs are found to be recruited to the surface of RNA homopolymer droplets to form relatively stable multiphase RNA condensates but without subsequent fusion as expected for LLPS, which may reflect the docking process of non-translating RNP to the SG core in the cell.⁸ Single molecular tracing suggests a transition from highly mobile RNPs to more rigid ones

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Fig. 1 Regulated mRNA partition in different RNA granules. A population of translating mRNA may be switched to the non-translating state or committed to specific RNA decay pathways. Under normal conditions, a fraction of non-translating RNA undergoes decapping and deadenylation, linked to the formation of P-bodies (PBs). Stress induces additional non-translating mRNAs to form stress granules (SGs). Highlighted is the function of the RNA helicase eIF4A in limiting the recruitment of non-translating mRNAs to SGs. These differential mRNA partitioning processes may involve liquid-liquid and liquid-gel phase separations, suggesting important regulatory functions of specific RNA helicases in assembly/disassembly of diverse types of membraneless condensates under physiological and/or pathological conditions.

once joined the SG core,¹² suggesting that initial docking of non-translating RNPs may utilize the LLPS mechanism, which is followed by liquid-to-gel transitions within a SG (Fig. 1).

Prototype for regulated assembly/disassembly of other RNP granules. All nuclear and cytoplasmic membraneless bodies undergo a transition from initial liquid droplets to less mobile tangle-like structures, although both may be dynamic and reversible, suggesting that a visible membraneless sphere in cells is not necessarily a purely LLPS process.¹³ LLPS has also been proposed to account for transcription activation that involves network interactions between transcription factors, enhancer-originated RNAs, and their interactions with DNA.¹⁴ This model is

attractive for envisioning weak, multivalent interactions contributed by both proteins and RNAs, together driving the assembly of transcription initiation complexes on specific gene promoters. As many RNA helicases have also been implicated in transcriptional control, it is conceivable that some specific RNA helicases may facilitate such RNP exchanges from initiation to elongation.

Implications in disease mechanism and therapy. The formation of insoluble aggregators has been linked to various diseases, particularly neurodegenerative disorders. An intensively-studied prototype is the formation of TDP-43 aggregates associated with amyotrophic lateral sclerosis (ALS) as well as other types of neurodegenerative diseases.¹⁵ It is striking to note that the most prevalent set of RBPs identified in SGs are also those involved in neurodegenerative diseases.⁷ Given age-dependent onset of most neurodegenerative diseases, which may be driven by increasing oxidative stress in aged brain, those RBP-containing granules may be viewed as pathological SGs in diseased neurons. The current findings suggest a potential venue to develop new therapeutic approaches against neurodegenerative disorders by leveraging specific regulators for SG assembly/disassembly.

ADDITIONAL INFORMATION

Competing interests: The author declares no competing interests.

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