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A New Lens for RNA Localization: Liquid-Liquid Phase Separation

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Abstract

RNA localization mechanisms have been intensively studied and include localized protection of mRNA from degradation, diffusion-coupled local entrapment of mRNA, and directed transport of mRNAs along the cytoskeleton. While it is well understood how cells utilize these three mechanisms to organize mRNAs within the cytoplasm, a newly appreciated mechanism of RNA localization has emerged in recent years in which mRNAs phase-separate and form liquid-like droplets. mRNAs both contribute to condensation of proteins into liquid-like structures and are themselves regulated by being incorporated into membraneless organelles. This ability to condense into droplets is in many instances contributing to previously appreciated mRNA localization phenomena. Here we review how phase separation enables mRNAs to selectively and efficiently colocalize and be coregulated, allowing control of gene expression in time and space.

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INTRODUCTION

The subcellular localization of RNAs in time and space is found across the tree of life, from *Escherichia coli* to human cells. Evidence from genome-wide screens and the study of specific transcripts indicate that patterned localization of messenger RNAs (mRNAs) is evolutionarily conserved, ubiquitous, and important for biological function (66, 103, 116). RNA localization has been shown to contribute to body patterning in development, establishment of polarity, mitosis, organelle inheritance, cell migration, and local translation (16). But why do cells localize their RNA molecules rather than the proteins encoded by the mRNAs? Given that newly translated protein can theoretically move tens of micrometers in a matter of seconds, it is not immediately clear why mRNAs are found to be localized in such diverse contexts. One condition where diffusion may be limiting is in exceptionally large, polarized cells, such as neurons or the long hyphae of filamentous fungi. In these conditions, it is clear that active positioning of mRNAs may be critical to distribute the proteome over long distances. However, mRNAs have been found to be asymmetrically localized in even the modest-sized cells of the budding yeast *Saccharomyces cerevisiae*, where the morphology of these cells with a very small conduit between mother and bud may constrain diffusion of some cytosolic proteins (97). Thus, RNA localization may be regulated to enable cells to cope with limitations due to specific geometry and diffusion.

mRNA localization promotes coordinated control of gene expression in space and time, which makes especially large cells better able to link translation to specific cues. For example, cells can cotranslate protein products that will act together or coassemble (e.g., tubulin) (22). Having the mRNAs colocalized provides the cell with proteins in the same area, increasing efficiency of assembly of complex, larger, multiprotein structures. Finally, many types of cells use RNA localization as a stress response mechanism, halting translation of housekeeping proteins in order to divert resources to respond to stress. Thus, there are many potential contexts where there is an advantage to controlling transcript position, as well as abundance and timing of production.

HOW RNAs COME TOGETHER

In many contexts, the first step of positioning mRNAs at specific sites is the coordinated coassembly of mRNAs into complexes that have historically been called granules. Granules in the germline of

animals have long been described as allowing specific partitioning of mRNAs during development, in neurons as promoting local gene expression in dendrites, and in the cytoplasm of cells under stress as preserving the transcriptome until better conditions return. It is now increasingly clear that a pervasive mechanism by which collections of mRNAs gather into granules is via a process of condensation that exploits liquid-liquid phase separation (LLPS) to form droplets (3, 45). Condensation has many potential advantages for coordinating cohorts of RNAs. A liquid droplet can be transient because posttranslational regulation can control its formation and dissolution. The droplet interior provides a distinct molecular environment from surrounding cytoplasm or nucleoplasm, wherein biochemistry can occur. Based on either material or chemical properties, droplets can be selective and either retain or exclude specific molecular species. Below we describe the current thinking on the role of RNA in driving phase separation, how specific groups of RNAs coassemble in droplets, and how cells utilize this mode of assembly to regulate mRNAs.

mRNAs LOCALIZE TO AND PROMOTE THE FORMATION OF LIQUID-LIKE CELL COMPARTMENTS

Ribonucleoprotein (RNP) granules are membraneless compartmentalized bodies that package mRNAs and proteins together to regulate the localization, translation, and decay of mRNAs (12). Recent research has shown that these granules form through concentration-dependent phase separation to subcompartmentalize the nucleoplasm and cytosol (8, 33, 71, 94, 101, 124), and many such compartments have been discovered and shown to be involved in diverse cellular processes in eukaryotic cells and some bacterial cells (9, 32, 51, 67, 68, 82, 91, 99, 102, 104). Control of condensation can restrict diffusion of cytosolic components to retain them in a particular region of the cell and is thought to promote or prevent biochemical reactions by sequestering components in the condensed state. Phase separation is a simple but powerful mechanism to control the spatial localization and processing of molecules, without relying on the intensive construction and maintenance of membrane boundaries. Furthermore, posttranslational modification and dynamic changes to specific mRNA and protein composition could control alterations of the material state of these assemblies. This potential for lability may be a critical intrinsic feature of biological condensates in promoting adaptive responses to fluctuating environments (81, 87, 120).

A framework for understanding LLPS of biological molecules is provided by the mature field of polymer chemistry, where it has been well established that weak multivalent interactions between long polymers can promote de-mixing (10, 47, 115). Increasing evidence suggests that liquid-like RNP granules can also form through weak, multivalent interactions between RNA-binding proteins (RBPs) and bound RNAs (**Figure 1**). Many RBPs have intrinsically disordered domains (IDDs) as well as an RNA-binding domain (RBD)—in many cases, multiple RBDs. Multivalency is contributed by several different kinds of interactions at both protein and RNA levels. IDDs come in many forms and frequently are low-complexity sequences. It is thought that IDDs promote transient interactions including cation-π, electrostatic, or hydrophobic interactions, linking proteins together into a dynamic network in which each one has multiple points of potential interaction with other proteins (10, 43). The actual molecular grammar in RBP-protein interactions that gives specificity and ensures a particular combination of RBPs interact is just beginning to be understood.

Another source of multivalency derives from the RNA sequences. In cases where specific binding sites for an RBP on an RNA are known, they are often found in multiple copies interspersed across the sequence (19, 31). Additionally, the same mRNA can harbor multiple binding sites for different RBPs, providing a way to recruit different combinations of RBPs to the same RNP complex (10, 72, 80). In these situations, a given mRNA has the potential to bind multiple RBPs,

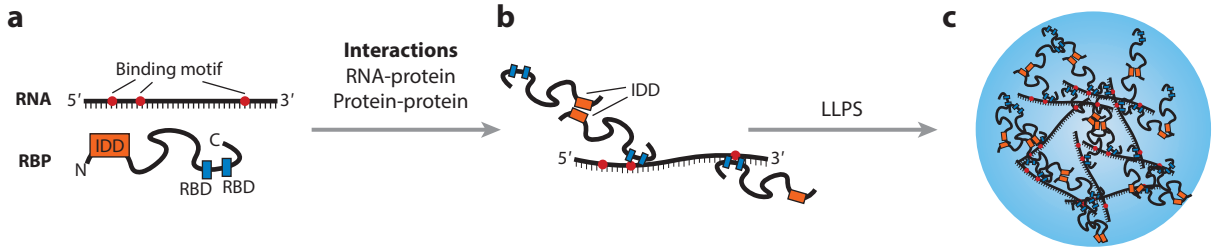

Figure 1

Multivalent interactions between RNAs and RNA-binding proteins (RBPs) promote liquid-liquid phase separation (LLPS). (*a*) RNA molecules can contain multiple RBP binding motifs (*red dots*). These binding motifs are recognized by the RNA-binding domains (RBDs; *blue rectangles*) on RBPs. Many RBPs also contain intrinsically disordered domains (IDDs; *orange rectangles*), which promote protein-protein interactions. (*b*) RNA molecules can act as scaffolds, binding multiple RBPs at once. In turn, the bound RBPs can also form protein-protein interactions with other protein molecules, relying on their IDDs. (*c*) Promoted by transient multivalent interactions between a multitude of RNA and protein molecules, LLPS can occur, forming complex RNA-protein droplets or granules in the cell.

and if an RBP has multiple RNA-binding motifs, an interconnected polymer network can readily emerge (**Figure 1**). The most common RBD is the RNA-recognition motif (RRM), a highly structured domain consisting of approximately 90 amino acids with 8 conserved aromatic residues that mediate binding through interaction with certain RNA bases (75). In other cases, mRNAs are bound via a series of RGG sequences on the RBP and multivalency is common because, in most situations, there are multiple RGG sequences clustered together in the primary sequence of an RBP. Notably, the RGG can also promote protein-protein interactions, providing yet another layer of multivalency to promote phase separation (110). Thus, the combination of both RNA-binding motifs and IDDs on RBPs and RNA-sequence features predicts that phase separation could be an emergent property of these molecules combining together. It is likely that multivalent interactions at both the level of proteins and the level of RNAs are a fundamental first step to the self-assembly of most RNPs in cells.

mRNA INFLUENCES THE PHYSICAL PROPERTIES OF RNP DROPLETS

As indicated by the ability to contribute multivalent interactions, RNA is not simply a bystander but a driver of condensation. Multiple studies have shown that RNA is necessary and sufficient for the formation of RNP droplets at physiological conditions (14, 28, 62, 72, 80, 94, 95, 101, 124). Some LLPS of RBPs with RNA requires specific sequences of mRNA (62, 124), while more promiscuous RBPs can form droplets with total cellular RNA or synthetic RNAs (14, 28, 72, 80, 94, 95, 101). RNA has also been shown to be supplied by active transcription for the liquid-like state of the nucleolus (117), and while RNA can lower the critical concentration needed for an RBP with IDDs to phase-separate, high concentrations of mRNA can actually inhibit droplet formation (14, 124). Recently, high RNA concentration was shown to play a crucial role in preventing the RBP FUS from forming liquid droplets in the nuclei of mammalian cells, suggesting that RNA can exert differential effects depending on local abundance (74). Understanding how the local concentration of either total RNA or specific mRNAs controls the assembly and disassembly of liquid compartments will likely elucidate the role of LLPS as both a cause and a consequence of RNA subcellular localization.

In addition to controlling the presence or absence of RNP droplets, RNA can also influence the biophysical properties of droplets. Although these features are not yet well characterized in cells,

these physical properties could have important ramifications on the size and spatial distributions of droplets, the surface-to-volume ratios, transport within and between droplets, and prevention of mixing between droplets with distinct molecular compositions (37). Numerous studies have shown that binding of RBPs to RNA is critical for maintenance of the fluidity of the assemblies (28, 74, 124). When RNA binding is ablated, liquid droplets are observed to transition into solid-like aggregates that mimic pathological disease contexts (124); further, RNA has been shown to either increase or decrease the viscosity or surface tension of droplets depending on the concentration or RNA sequence (28, 124). In addition, differential biophysical properties have been observed between RNP granules, such as processing bodies (P-bodies) and stress granules (SGs) (12, 50, 55, 62, 118), where yeast SGs are more solid-like and P-bodies are more liquid-like. Given that RNP droplets can occupy a wide landscape of physical states, it will be critical to discern how the material states of RNP droplets influence the function of mRNAs within these structures.

High-resolution microscopy and in vitro reconstitution of RNP granules have shown that all share relatively similar spherical droplet-like shapes and dynamics consistent with LLPS, such as wetting, dripping, and fusion (45). These common material properties arise despite the fact they are formed from diverse molecular components and serve different functions. RNA control of the physical properties of droplets may be due to either intrinsic features of the RNA polymer, such as length or structure, or modulation of the proteins that are coassembled with RNAs. In fungi, different mRNA sequences are associated with different relative amounts of RBPs incorporated into droplets (64, 124). Variability in the concentration of RBPs in droplets might emerge from different affinities of the RBPs for different mRNAs or different accessibility of the binding sites for proteins on the mRNAs. These differences in RBP concentration, in turn, could readily contribute to differential biophysical properties of droplets that house different mRNAs. The degree to which these variations in biophysical states arise directly from RNA polymer features or from RNA-dependent differences in RBP recruitment remains to be understood.

RNA also appears to contribute to differences in the spatial organization within droplets. In ways that are only partially understood, RNAs generate significant substructure within the droplet complex. This has been shown for the nucleolus, where multiple phases coexist within the same nucleolar droplet complex. These multiple subcompartments of nucleoli are caused by differences in the surface tension in part contributed by the proteins interacting with RNA (33). Similarly, SGs are shown to have at least two coexisting material states, where more solid-like cores are surrounded by more fluid layers (50). In animals, where germ-line granules are large enough to resolve specific populations of RNAs, specific groups of mRNAs take residence in distinct zones within a granule, indicating that there can be sorting of specific mRNAs within a single droplet, as described in more detail below (111). Thus, RNA has the potential to influence when and where condensation occurs, which components coassemble, the emergent physical properties of droplets, and the substructures within droplets; however, many major outstanding questions remain. How is RNA regulated within liquid compartments? How are so many distinct RNA-based compartments formed and maintained as distinct entities when they share similar physical properties? What rules govern the selective incorporation or exclusion of particular RNAs into liquid droplets?

RNA SEQUENCE CONTRIBUTES TO THE MOLECULAR IDENTITY OF RNP DROPLETS

It is likely that phase separation plays an especially vital role in cytoplasmic organization in large cells, like syncytial filamentous fungi and neurons, by keeping regulators in close proximity to specific nuclei or, in the case of neurons, at synapses. In the multinucleate fungus *Ashbya gossypii*, a single RBP, Whi3, containing a long polyQ-based IDD is important for positioning mRNAs near

nuclei and at sites of polarized growth. In these cells, nuclear division occurs asynchronously, and multiple sites of polarized growth coexist in a continuous cytoplasm (2, 38, 59). These phenomena are reliant upon Whi3 undergoing liquid de-mixing with its target mRNAs to form phase-separated droplets, positioning cyclin (*CLN3*) transcripts near nuclei and formin (*BNI1*) transcripts at sites of polarized growth (67, 68, 124). *CLN3* and *BNI1* mRNAs drive Whi3 to phase-separate in vitro into droplets at physiological conditions with different biophysical properties. Whi3-*CLN3* droplets are much more viscous and display slower fusion kinetics than Whi3-*BNI1* droplets, suggesting different mRNAs can promote physically and functionally distinct droplets (124). The coexistence of two functionally distinct populations of RNP droplets make this a powerful system to study how different mRNAs are sorted into distinct droplets and how cells control when and where condensation occurs.

We have found that although the different Whi3 droplet populations share the same protein and display liquid properties such as fusion, *CLN3* and *BNI1* transcripts minimally colocalize in the cytoplasm and are immiscible in vitro. Remarkably, another Whi3 target mRNA involved in cell polarity, *SPA2*, readily mixes into *BNI1* droplets and colocalizes with *BNI1* in the cell, indicating functionally related mRNAs share features that enable them to co-condense, providing evidence that the nucleotide sequence or other features of RNAs are critical for their destination (64). Specific sequences and secondary structures of mRNAs are a key determinant of whether mRNAs coassemble into the same droplets. The secondary structures of mRNAs control intermolecular mRNA hybridization between functionally related mRNAs and dictate which mRNAs are colocalized in Whi3 droplets. Whi3 mRNA targets share complementary sequences that are capable of base-pairing to promote RNA-RNA interactions that specify droplet identity. Interestingly, these sequences are exposed in the secondary structure and able to hybridize between the mRNAs involved in the same cell process, *BNI1* and *SPA2*, thus enabling them to interact. In contrast, although complementary sequences exist between the *CLN3* and *BNI1* mRNAs, they are found in highly folded regions and are thus inaccessible to hybridize (64). These data support the hypothesis that mRNA can engage in homotypic or heterotypic interactions and suggest that certain mRNA sequences and structures determine which mRNAs are together or apart in intracellular condensates (**Figure 2**). It remains to be seen how pervasive this mechanism is to promote or disfavor intermolecular interactions between different RNA species in biological condensation.

It has long been appreciated that RNA can modulate the protein-binding behavior of RBPs through certain structural contexts, where it has been shown that proteins prefer to bind certain sequences or secondary structures of RNAs (49, 106, 107). The notion that RNA-RNA intermolecular interactions play a role in RNA localization has existed for over two decades. It was first shown that the formation of the anterior pole of the *Drosophila* embryo is driven by the localization of bicoid mRNA, where it interacts with the Staufen protein to form RNP granules, delineating the morphogen gradient (34). The interaction between bicoid and Staufen is mediated by the intermolecular dimerization of bicoid mRNA molecules to recruit Staufen, and the secondary structure of bicoid's 3′ UTR is necessary for this interaction. Multiple studies have shown that the 3′ UTR is important for the proper localization of mRNAs to their correct places in a variety of cell types (76). Structured illumination microscopy of RNA granules in *Drosophila* has revealed that at least 200 mRNAs are enriched in the germplasm and that the transcripts are not randomly organized in RNA granules. Interestingly, while proteins were found to be homogeneously positioned within granules, mRNAs were found to be localized in homotypic clusters (111). It is likely in this instance that mRNA molecules interact with one another in a sequence-specific manner to give rise to clusters. We predict that RNA-RNA interactions are relevant for the sorting of specific mRNAs to diverse RNA granules and propose a model where RNA-RNA interactions are a central component of physiological RNP granule assembly.

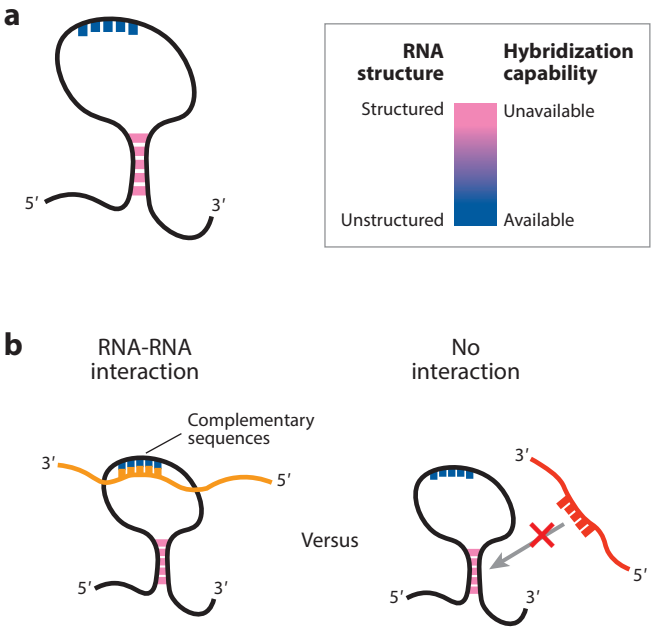

Figure 2

RNA secondary structure influences RNA-RNA interactions. RNA molecules form complex secondary structures in which intramolecular-base pairing promotes the formation of stem-loops, pseudoknots, and various other intricate structures. (*a*) The more highly structured a region of RNA is, the lower the capacity of that region to hybridize with other molecules of RNA. For example, bases found in the stem (*pink*) of a stem-loop are less likely to interact with other RNA molecules than bases found in the loop (*blue*). (*b*) Thus, sequences exposed in the loop of stem-loops of RNAs are able to base-pair with complementary sequences of other RNA molecules (*orange molecule*), promoting RNA-RNA interactions. Sequences that are hidden within the structured regions of RNA molecules are much less likely to interact with other molecules, thus preventing RNA-RNA interactions even when there are complementary sequences on other molecules (*red molecule*). Thus, secondary structure may be an additional mode of regulation of RNA-RNA interactions, promoting binding of certain RNAs together over others.

One recent study has shown that mRNA can itself form phase-separated droplets when isolated in vitro and can promote condensation within the nuclei of mammalian cells, and this is dependent upon the number of nucleotide repeat expansions that are able to form stable G-quadruplex structures. These repeat expansions serve as scaffolds for multivalent RNA-RNA interactions that allow clusters of RNAs to transition in vitro into a sol-gel (a state between solid and gel) and in cells into aggregates linked to neurological disease (48). Recently, stress granules have been shown to contain cores of interacting mRNA and protein molecules (50, 118), and these cores are formed through RNA-RNA interactions of nontranslating mRNAs (113), potentially seeding the formation of RNP granules. These few examples are likely representative of what will become a longer list of mRNA localization mechanisms that are rooted in RNA-RNA interactions.

There are many questions about how information in RNA sequence and structure influences where, when, and with what RNAs condense in cells. It will be important to determine how frequently mRNA secondary structures influence selective uptake of constituents into droplets and where in the cell and life cycle of an mRNA that RNA-RNA interactions occur. Furthermore, what is the role of RNA helicases in modulating RNA-RNA interactions and the subsequent incorporation or exclusion of particular RNAs in RNP droplets? The most important question is,

What is the function of condensing mRNAs together into either the same or distinct droplets? Possibilities include to colocalize cohorts of mRNAs for repression of translation or spatial segregation during division, to coordinate the location and time of protein production from a specific set of transcripts, and/or to modulate the activity of enzymes that can modify mRNAs within the compartment. Additionally, although this review focuses primarily on positioning mRNA, most cells have suites of noncoding RNAs of variable features that may also coordinate their position and/or function via condensation. Given the large number of distinct RNA-based condensates in the cell, these are promising and open problems that remain to be answered.

HOW DO CELLS POSITION RNA GRANULES FOR BIOLOGICAL FUNCTION?

As described above, cells can use liquid-like droplets to cluster mRNAs and proteins together. In this section, we consider the functions of granules as currently understood and how RNP droplets can be spatially positioned in the cell. Patterned localization of mRNAs has been shown to lead to the spatial control of protein translation and the response to internal and external stimuli in times of cellular stress (40, 53, 85). RNP droplet position could, in principle, be controlled in several ways, including the site where condensation occurs followed by tethering or directed transport to a destination. In the following section, we discuss what is understood about how cells use and position collections of mRNAs that are packaged into granules.

RNA Localization as a Stress Response

During the life cycle of an mRNA, following translation, transcripts can be released from ribosomes and sequestered into either SGs or P-bodies. SGs consist mostly of stalled translation preinitiation complexes along with mRNA and small ribosomal subunits. When a cell senses a stress, be it oxidative, pH, energetic, or temperature, it can form SGs to bind and sequester select mRNAs, halting normal expression and transition to translating proteins in the stress response pathways (13, 23, 88). After sensing that stress has passed, the cell releases these mRNAs to be translated or degraded, consequently leading to differential mRNA localization in the cytoplasm. In contrast, P-bodies are an assembly of translationally repressed mRNAs, proteins associated with translation initiation, and mRNA decay machinery. P-bodies not only sequester mRNA but, like neuronal RNA granules, can serve as a vessel to actively transport translationally inactive mRNAs from the nucleus to distal regions of the cell (85). Once assembled, SGs and P-bodies can persist from minutes to several hours. Each can fuse with the other, and mRNA or protein molecules that take residence inside these granules can quickly diffuse into and out of the granule (23). Recently, these two types of granules have also been shown to form through liquid de-mixing from the cytoplasm and transient multivalent interactions between numerous mRNA and protein molecules (50, 57, 62, 86, 113, 118). This fast assembly process presents a paradox as to how the structure of the granule can be maintained when its components can exchange rapidly with the cytosol. Further, how does the cell govern which proteins and mRNAs are incorporated into each granule? Moreover, are certain constituents necessary for biological function?

Although macroscopic RNP bodies are evolutionarily conserved, the functions of these structures have not been without controversy and are not well understood. In some contexts, loss of SGs is not associated with detectable differences in RNA regulation, arguing that SGs are more of a by-product of collecting RNAs and IDD-containing RBPs (11, 24, 63, 84). In contrast, a recent study in yeast supports a functional role for the macroscopic assemblies where the ability of a key SG component, the polyA-binding protein Pab1, to phase-separate was sensitive to specific and

physiological stress inducers. Pab1 granule formation is critical for the adaptive response of the cell to certain stressors, and there is a fitness cost associated with mutations that alter granule formation (88). This study suggests that there is a physiological function of condensing proteins and RNAs into a higher-order assembly as part of stress responses. It is also strong evidence that natural selection may operate at the level of protein sequence to tune the condensation boundaries, by controlling precisely when a phase separation occurs. The adaptive role of phase separation was also recently shown for the yeast protein Sup35, where reversible phase separation of the protein in response to cellular pH changes promotes organismal fitness during stress response (35). Thus, SG formation and the proteins and mRNAs that SGs harbor could serve as a general mechanism by which cells can accurately and rapidly detect and respond to the onset of stress. But how exactly does the substructure of SGs and P-bodies emerge, and does this incorporation of proteins and mRNAs occur stochastically, or are certain mRNAs and proteins always present in SGs and P-bodies?

Recently, it was shown that 95% of the cellular mRNA content in yeast can be found within SGs and that intermolecular interactions between mRNAs may provide the nucleation event for SG formation (57, 113), but it is unclear which particular mRNAs, if any, are important for assembly. While theoretically every mRNA could be targeted to SGs in times of stress, a transcriptome-wide study in *S. cerevisiae* found that the efficiency with which transcripts are recruited to SGs varies between 1% and 95%. In fact, in the same study not one particular mRNA was found to be recruited consistently to SGs; rather, mRNAs that were not stably associated with ribosomes were preferentially recruited (57). Purification of P-bodies from mammalian cells has also revealed that P-bodies contain thousands of different mRNA molecules and that mRNAs that translate proteins expressed at low levels are more likely targeted to P-bodies (86). This suggests that there might be some selection of mRNAs based on the state of translation at the time of stress.

The efficiency and selectivity with which mRNAs can be recruited into SGs and P-bodies suggest that mRNAs can assemble differently in response to specific stressors and that SGs and P-bodies may have different assembly, disassembly, and functional kinetics depending on the type of stress. These differences likely lead to different granule compositions, such that in a given cytoplasm, there could be an array of granules that contain a diverse group of mRNAs. Further, SGs and P-bodies have different composition states depending on the type of organism they are found in: P-bodies have been found to possess more liquid-like characteristics in yeast, and SGs appear to be more solid in yeast than in mammalian cells (62). The existence of compositional differences among SGs and P-bodies raises the questions of where these differences arise and whether these differences in substructure impact their function in stress response. Are there spatial patterns as to which mRNAs end up together in the same granules or granules in the same vicinity of the cell? How exactly do granule composition and structure relate to function? Further, what determines the fate of the mRNA content after stress has subsided? Additional functional studies are required to elucidate exactly how differential compositions in RNP granules affect their assembly, selectivity, localization, and disassembly during times of cellular stress.

RNA Localization for Protein Translational Control

One well-appreciated function of mRNA localization is to enable spatially controlled production of proteins. The most common method the cell employs to localize proteins is direct targeting of the protein after translation using specific amino acid sequences, such as nuclear and mitochondrial localization sequences (46). Strikingly, however, a large-scale study of mRNAs in *Drosophila* embryogenesis reported that ~70% of mRNAs examined localized to discrete positions within the cell, and in many instances, the mRNAs colocalized with the proteins that they encoded (66). This

study suggests that the prevalence of patterned mRNA positioning and subsequently localized translation is likely underappreciated.

mRNA localization to set up local translation is likely an evolutionarily conserved mechanism to distribute gene expression to various subcellular compartments and is found across all eukaryotes, from yeast to mammalian neuronal cells and also in some prokaryotes (7, 36, 39, 40, 54, 105). Subcellular transcriptomics of neurons has revealed that distinct mRNAs are targeted to different compartments (18, 42, 106, 108, 125). This intracellular patterning, thought at first to occur only in the specialized cells where it was discovered (27, 44), likely occurs in many cell types, such as migrating fibroblasts, where subsets of mRNAs are localized to the cell protrusions (65, 78). While mRNAs are targeted to subcellular locations, their translation remains repressed during their transit from the nucleus (30, 61), where the composition of the transported RNA granule is regulated by signals that are intrinsic (42, 108, 125) and signals that are extrinsic (25, 79, 119) to the granule. Therefore, mRNAs are not just delivering genetic information from the genome to the translational machinery in the cytosol; rather, these localized mRNAs can be considered genomic bases (53) where functionally related mRNAs can be translated together according to the needs of the cell. Thus, mRNA localization for local translation provides an efficient way to coordinate gene expression (56), much like how operons in bacteria regulate related genes.

While the majority of research on local translation has been in neuronal cells (21, 90, 121), the findings are likely applicable to branching filamentous fungi, which have very similar morphologies and almost certainly undergo local translation to regulate growth and respond to environmental cues. In the pathogenic fungus *Ustilago maydis*, the transport of septin mRNAs on endosomes to the hyphal tip is essential for morphogenesis and septin cytoskeleton assembly, indicating that the septin mRNAs must be locally translated on endosomes and assembled into heteromeric complexes before reaching their destination. Based on the scale of the structures in cells, it is unclear if the septin mRNAs and RBPs associated with the endosomes are in a phase-separated state for transport; however, this is a clear example of spatial control of gene expression through mRNA transport and local translation (123).

The role of mRNA localization in bacteria is less understood and appears to vary depending on the species (15). Historically, mRNAs were not thought to localize to regions outside of the nucleoid unless actively transported by the translation complex. However, this view is now being challenged by new evidence that mRNAs can be patterned (41). Several groups have been researching how these mRNAs could be locally positioned and, through live fluorescence microscopy and smFISH to track RNA transcripts, two models of bacterial mRNA localization are emerging. In *E. coli* and *Bacillus subtilis*, certain mRNAs show a more heterogeneous localization pattern, either clustering in a translation-independent manner along the cell membrane or creating discrete foci near the poles of the cell (83, 112). In addition, evidence has emerged that some mRNAs in bacteria possess zip code sequences within the transmembrane coding sequence of membrane proteins, and that these localization elements are needed to target translationally repressed transcripts to the cell membrane (83). This suggests that mRNA targeting may be important for localized translation in bacteria, but future work is required to elucidate the precise mechanisms.

Recently much attention has been given to the active role ribosomes might play in mRNA localization for local translation. The development of a new method for tracking ribosomes and labeled mRNA has elucidated a high-resolution spatial map of mRNA localization with respect to ribosomes, and evidence for heterogeneous positioning of ribosomes has emerged (17, 122). Studies into differential subpopulations of ribosomes have shown that translating ribosomes are heterogeneous in composition at the level of core ribosomal proteins and that ribosomes can recruit certain components preferentially, raising the possibility that ribosomes may preferentially translate discrete localized pools of mRNAs (98, 100). In the gram-positive bacterium *Streptomyces*

coelicolor, rRNAs and their translated protein molecules are differentially expressed and assembled into ribosomes during development (58). Further, it has been shown that *S. cerevisiae* cells contain functionally distinct ribosome populations due to genome duplication (60). These data support the possibility that ribosome heterogeneity is a relevant consideration for spatial control of gene expression; however, it is still an open question whether an alteration in translational specificity can be attributed to ribosomes containing different rRNA or mRNA species.

FUNCTIONAL CONSEQUENCES OF ASYMMETRIC RNA LOCALIZATION IN CELL GROWTH AND DIVISION

RNA localization is also an important factor for cellular growth and morphogenesis across the tree of life, from localizing molecules in bacteria to determining sites of polarized growth in fungi and the shape of neural networks. mRNA localization, and subsequently protein localization, to one or both cell poles has been observed in a variety of bacterial cell types, but not all and not for all loci (69, 92, 96). Transporting mRNAs encoding factors for local control of gene expression for growth is most notable in filamentous fungi, where growth is achieved through apical extension of highly polarized hyphae. As hyphae elongate, the growing tip becomes a hub of cytoplasmic organization in order to orchestrate growth guidance and response to external stimuli (89). Many of the same molecular machineries are shared between fungi and yeast for the establishment of polarity, but fungi face the problem of long-term polarity maintenance, and mRNA localization has been shown to be critical for this. In the hyphal yeast *Candida albicans*, around 40 mRNAs, including *ASH1* mRNA, which is the prototypical localized mRNA in the budding yeast *S. cerevisiae*, are localized by She3p machinery to the hyphal tip to regulate hyphal growth (6, 20, 29, 73, 77). As mentioned above, transport of septin mRNAs to the hyphal tip of *U. maydis* is important for establishing polarity and critical during virulence (4, 114, 123). In *A. gossypii*, the transcripts *BNI1* and *SPA2* are localized into liquid-like RNP droplets at the growing tip to maintain polarity as well as localized within hyphae further back from the tip in order to establish new polarity sites (59, 64, 67). While mRNAs have been shown to be concentrated in fungal hyphal tips, it remains to be seen how this asymmetry is generated. In addition, evidence of local translation of these transcripts has yet to be observed, although we speculate that local translation for spatiotemporal control of polarity plays an important role in filamentous fungi, as has been shown in neuronal cells.

RNA localization is also critical for local control of the cell cycle in multinucleate fungi such as *Ashbya*. In *Ashbya*, as well as many filamentous ascomycete fungi, nuclei divide asynchronously despite sharing a common cytoplasm (1, 26, 38, 68). Asynchronous division requires that individual nuclei be insulated from one another, thus not impacting each other's division cycle. To achieve this, cyclin transcripts are not free to diffuse as they are bound by the RNP Whi3. The formation of RNP droplets localizes mRNAs near their source nuclei, and, we predict, locally regulates their translation (68). Although Whi3 binding to *CLN3* is essential for spatiotemporal control of the *Ashbya* nuclear division cycle, it remains to be seen exactly where that interaction occurs. And while genetic studies indicate Whi3 droplets likely control *CLN3* translation, definitive proof of this remains to be found. It is also not yet clear how common cell cycle control mRNAs are localized in the vicinity of nuclei; however, it has been seen that cyclin transcripts are also found in aggregates in immature starfish oocytes (109). It was also recently reported that in yeast the aggregation of the RBP Rim4 is necessary for the translational repression of the B-type cyclin Clb3 and the proper establishment of meiotic chromosome segregation during gametogenesis (5). Further, sequestration of mRNAs during meiosis prevents transcripts from being degraded, and the protection of mRNAs from external signals allows the delayed translation of transcripts until the end of meiosis II (52). These results indicate that cells can use mRNA localization as a

general mechanism to regulate the timing of cell division, but further work is needed to elucidate the proteins, RNAs, and signaling pathways involved in the regulation of this timing.

Prokaryotes also utilize mRNA localization through their own cell cycle. In bacteria, including *E. coli*, *Caulobacter crescentus*, and *B. subtilis*, it has been shown that mRNA can be localized to ribosomes in a cell cycle–dependent manner (70, 93, 112). Though bacteria do not contain nuclei, they do encounter unique problems during cell division. What exactly are the reasons for mRNA localization in the bacterial cell cycle? Is it more energy efficient? One could imagine cells could employ asymmetric mRNA localization to ensure each daughter cell after division gets its fair share of mRNA transcripts, setting up proper distribution and expression of the encoded proteins. Prokaryotes have their own unique cell cycle to orchestrate, and mRNA localization may be one mechanism by which cells ensure accurate cell division.

CONCLUSION

It has become increasingly clear in recent years that mRNA localization is a mechanism to compartmentalize the cytosol for the spatial and temporal control of diverse biological processes. We present an emerging mechanism that allows for the coassembly of mRNAs into condensed liquid droplets within the cytoplasm that can efficiently localize mRNAs to generate compartments and asymmetries within cells. The recent findings that mRNAs can be localized by these liquid bodies, but also that the mRNA molecule itself can lead to differential assembly, composition, and dissolution kinetics open up novel avenues to be explored in RNA research.

Although mRNA behavior is well described in living cells, the precise kinetics and mechanisms of formation, localization, and disassembly of RNP granules are still unknown. Compellingly, it has been widely speculated that the coassembly of mRNAs and proteins has evolved to exhibit these liquid properties to allow for rapid exchange of components from the cytosol. However, it remains to be seen what impact this has on the ability of these bodies to control local gene expression. One of the major outstanding questions in the field is if translation can be achieved within liquid RNPs. An answer to this question would link RNP physical state to function, which is a major gap in knowledge within the field. Further, it has yet to be elucidated if other RNA species, such as noncoding RNAs, have an effect on the coordinated regulation and localization of RNPs within the cell. Remarkably, the importance of mRNA localization in microbes is just now beginning to be appreciated; thus, future studies will likely reveal shared parallels in regulation surrounding the spatiotemporal control of mRNA localization and the impact it has on cellular function. The lability of RNP condensations makes them especially attractive as a mechanism by which microbial cells react to fluctuating environments. Microbes should continue to serve as powerful systems for studying how cells use mRNAs to physically and dynamically pattern the cytoplasm.

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