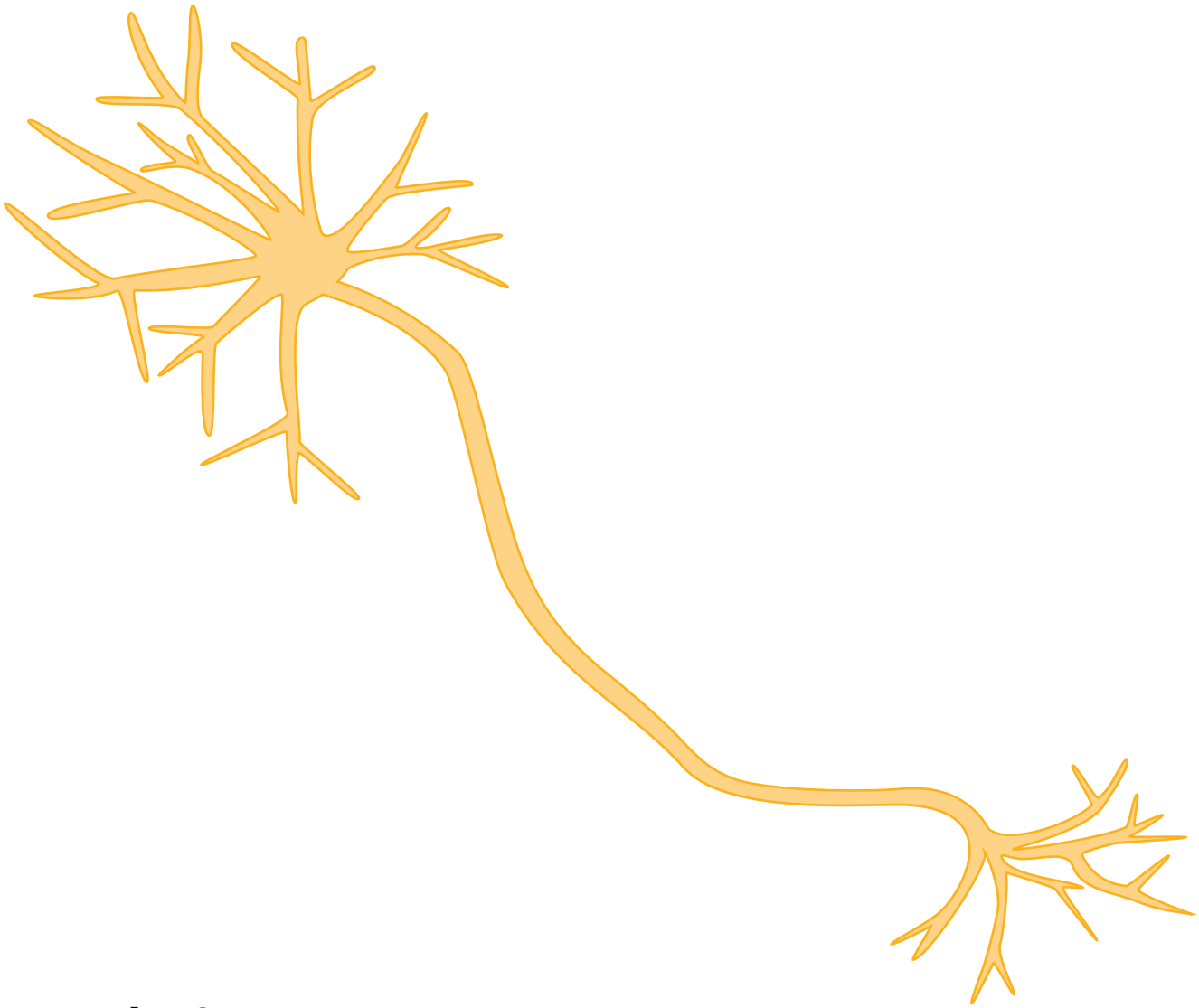
RNP granules: Elucidating the steps of neuronal local translation



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Writing assignment 2022
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Abstract

In order to compartmentalizing biochemical processes within the crowded intracellular environment, the cell makes use of organelles. Aside from the well-known membrane-bound organelles, a novel type of organelle is formed by liquid-liquid phase separation into biomolecular condensates. RNP granules are a type of condensates that are enriched in RNA and RNA-binding proteins that play a crucial role in RNA metabolism. They facilitate local translation, which is especially important in highly polarised cells such as neurons where long distances must be bridged. There are several steps in local translation, including RNP granule assembly, transport and mRNA handling and translation. In this review, we will discuss current research on RNP granule assembly and how this can be regulated by post translational modifications. We will discuss mechanisms of their active transport throughout the axon and dendrites by interacting with motor proteins or hitchhiking onto membrane-bound organelles. Then, we will address recent findings on how RNP granules allow for local protein synthesis and if disassembly is essential for mRNA translation. Finally, we summarize the implications of RNP granule components in neurodegenerative diseases and possible mechanisms towards neurodegeneration.

Introduction

Cells need to carry out various biochemical processes within its crowded intracellular environment. These processes are facilitated by compartmentalization into morphologically and functionally distinct domains. Well known cellular compartments are organelles such as the endoplasmic reticulum (ER), the Golgi, mitochondria and lysosomes. The common characteristic of these organelles is that they are surrounded by a lipid bilayer forming a membrane around their content.

Interestingly, a quickly increasing amount of work has revealed another type of organelle which is characterized by a lack of membrane, hence the name membrane-less organelle. Membrane-less organelles refer to biomolecular condensates that form by self-assembly through liquid-liquid phase separation. A phase boundary is created, concentrating molecules within the cell without the need of a membrane and thereby creating a specialized compartment (Boeynaems et al., 2018; Tauber, Tauber and Parker, 2020).

There is a wide variety of such condensates in their composition, physical properties, localization and function. By sequestering specific proteins and/or RNAs these organelles are suggested to store, transport and regulate their content and are suggested to be involved in many important tasks such as cell division, gene transcription and RNA metabolism (Banani et al., 2017; Boeynaems et al., 2018).

Ribonucleoprotein (RNP) granules are a type of biomolecular condensates that mainly consist of RNA molecules and RNA-binding proteins (RBPs) and play a role in RNA metabolism. RNP granules comprise three different types of RNA containing condensates; P-bodies, stress granules and RNA transport granules. P-bodies and RNA transport granules are present under phyiological conditions, while stress granules appear under stress conditions. Yet, given their enrichment in mRNAs, they are each suggested to play an essential role in translation, translation repression and mRNA storage.

In particular in highly polarized cells such as neurons, long distance transport of mRNA to axons or dendrites is crucial for local protein synthesis. Emerging evidence suggests that RNP granules play a key role in local protein synthesis in polarized cells (Das et al., 2021).

For the local protein synthesis, a few processes are thought to take place (Das, Singer and Yoon, 2019; Pushpalatha and Besse, 2019; Ryan and Fawzi, 2019) which we will discuss in this review. First, the RNP granule must assemble

specific RBPs and mRNAs molecules. Through many attempts in unravelling the composition of RNP granules, several key factors have been identified that drive the assembly. These include RBPs with low-complexity domains, post translational modifications (PTM) and moreover, mRNA modifications. Moreover, RNP granules are enriched in translationally inactive mRNAs and translation repressors, suggesting a site for mRNA storage. Interestingly, recent literature contradicts this idea by finding evidence for translation within RNP granules.

Secondly, RNP granules have to be transported to the site of translation either into the axon or dendrites. Emerging evidence suggests a mechanism by which RNP granule hitchhike onto other membrane-bound organelles which are tethered to motor proteins that 'walk' along microtubules.

Finally, in order to allow local protein synthesis, the mRNAs should be re-entered into translation. Despite the evidence for intragranular translation, it remains unclear whether a granule has to disassemble prior protein synthesis. Each of these steps appears to be regulated by the cell implicating an important physiological role for RNP granules. Nevertheless, due to their diffusive nature and heterogeneity, discovery of the exact mechanisms remains difficult.

Furthermore, we will discuss the role of RNP granules in neurodegenerative diseases. Owing to the increasing amount of research, an increasing number of neurodegenerative disease causing mutations have been discovered in RNP granule components. They are suggested to affect RNP granules in several manners (Boeynaems et al., 2018; Ryan and Fawzi, 2019). For instance, several disease mutations have an effect on the phase separating behaviour of RNP granules. Other disease mutations are found to impair RNP granule transport. This emphasizes the importance of further understanding the role of RNP granules specifically in neurons.

1. Granule formation and RNA regulation

Liquid-liquid phase separation drives the self-assembly of RNP granules which is established mainly through multivalent interactions. Multivalency arises when a molecule can form multiple inter- or intra- molecular interactions. Proteins involved in the assembly usually either have one or more interaction domains or an intrinsically disordered region (IDR) which are both known to create multivalency. Additionally, DNA and RNA can also undergo phase separation as they contain multiple regions that can interact with other molecules (Banani et al., 2017; Tauber, Tauber and Parker, 2020).

Characterization of the RNP granule protein composition have revealed common features such as the presence of IDRs and RNA-binding domains. This has come down to two important factors. First, many RBPs have been identified that can co-assemble into granules with several proteins and RNAs. The phase separating properties of RBPs seems to rely on PTMs, suggesting that PTMs could be a manner of the cell to regulate granule formation. Second, target mRNA that is assembled into those granules itself can modulate the phase separating properties.

1.1 How RBPs can promote RNP granule formation

As mentioned earlier, multivalency is a strong phase separation inducer and can be established through several elements. One of those elements are low-complexity domains that can be found in IDRs or prion-like domains.

Intrinsically disordered domains (IDR)

IDRs do not have a defined structure, favour certain amino acids and often have multiple repeat sequences which all contribute to the multivalency and its tendency to phase separate (Boeynaems et al., 2018). For the assembly of RNP granules, IDRs are often implicated as key modulators. Many proteins on which

RNP granule assembly relies, contain IDRs and removal of this domain would prevent their condensation.

For instance, the IDR of YTHDF2 is required for its liquid-liquid phase separation in vitro and interactions between the IDR and mRNA facilitates their co-assembly into P-bodies, stress granules and RNA transport granules in vivo (Ries et al., 2019). Similarly, removal of the low-complexity domain of *Drosophila* FMR1 prevents RNP granule formation (Zhang et al., 2022). And a broad study in neuron cells of *Drosophila Melanogaster* reveals that Ataxin2 mediates phase separation into RNP granules through two domains as a sort of switch. One domain prevents phase separation, while the IDR promotes phase separation into condensates (Singh et al., 2021).

In each of these proteins, the IDR is responsible for condensate formation. Through their multivalent properties, these domains could sequester specific proteins and RNAs to form RNP granules. However, several example exist that debate this mechanism. For instance, the *Drosophila* FMR1 proteins binds its target RNA through a KH-domain rather than its IDR (Zhang et al., 2022). Moreover, Ataxin2 does not directly bind mRNA but does so through interactions with other ribonucleoproteins in a low-complexity domain-independent manner (Singh et al., 2021). These studies show that IDRs are liquid phase separation promotors, although it remains unclear if directly interaction to RNA is always essential for this. So, despite the indications that IDRs provide a general principle on which phase separation relies, further research should be performed to explain the mechanism behind this.

Prion-like domains

Prion-like domains are a different sub-type of IDRs, yet also have minimal structure and drive phase separation into RNP granules (King, Gitler and Shorter, 2012). The prion-like domain of Sup35 phase separates into biomolecular condensates in yeast cells (Franzmann et al., 2018). Another well studied protein involved in granule formation is FUS, which has an N-terminal prion-like domain. Several studies have reported that the phase separation of FUS into RNP granules is driven by this low complexity domain both in neuronal *Xenopus* cultures and *C. elegans* neuron cells (Murakami et al., 2015; Qamar et al., 2018).

In contrast, the results from Vijayakumar et al show that the low-complexity prion-like domain of the *Drosophila* protein Imp is not required for in vivo RNP granule formation. Instead, the prion-like domain was more important as a modulator of RNP granule physical properties and motility. Interestingly, RNA binding by the KH-domain of Imp was found to be the main driver of granule formation rather than the prion-like domain (Vijayakumar et al., 2019).

Nevertheless, prion-like domains play an essential role in the phase separation into RNP granules. Without a doubt, these domains affect the phase separating properties by adding to the multivalency of these proteins. However, similar to IDRs, it remains unclear whether the condensate forming property of prion-like domains acts independently of RNA binding. Rather, these studies suggest that RNA binding might be as important for granule formation.

Multimers

Another intrinsic property that could increase the multivalency of proteins is oligomerization. Oligomerization increases the ability of such complexes to form weak interactions with other molecules. Increasing the size of such complexes thus increases the tendency to phase separate (Banani et al., 2017). The localization for example of PGL-1 to P-bodies in *C. elegans* relies on its dimerization domains. A mutation in one of the two domains is sufficient for PGL-1 to become diffuse instead of phase separated (Aoki et al., 2021).

Collectively, these studies show that proteins domains that increase multivalency are often found to be important for the assembly of

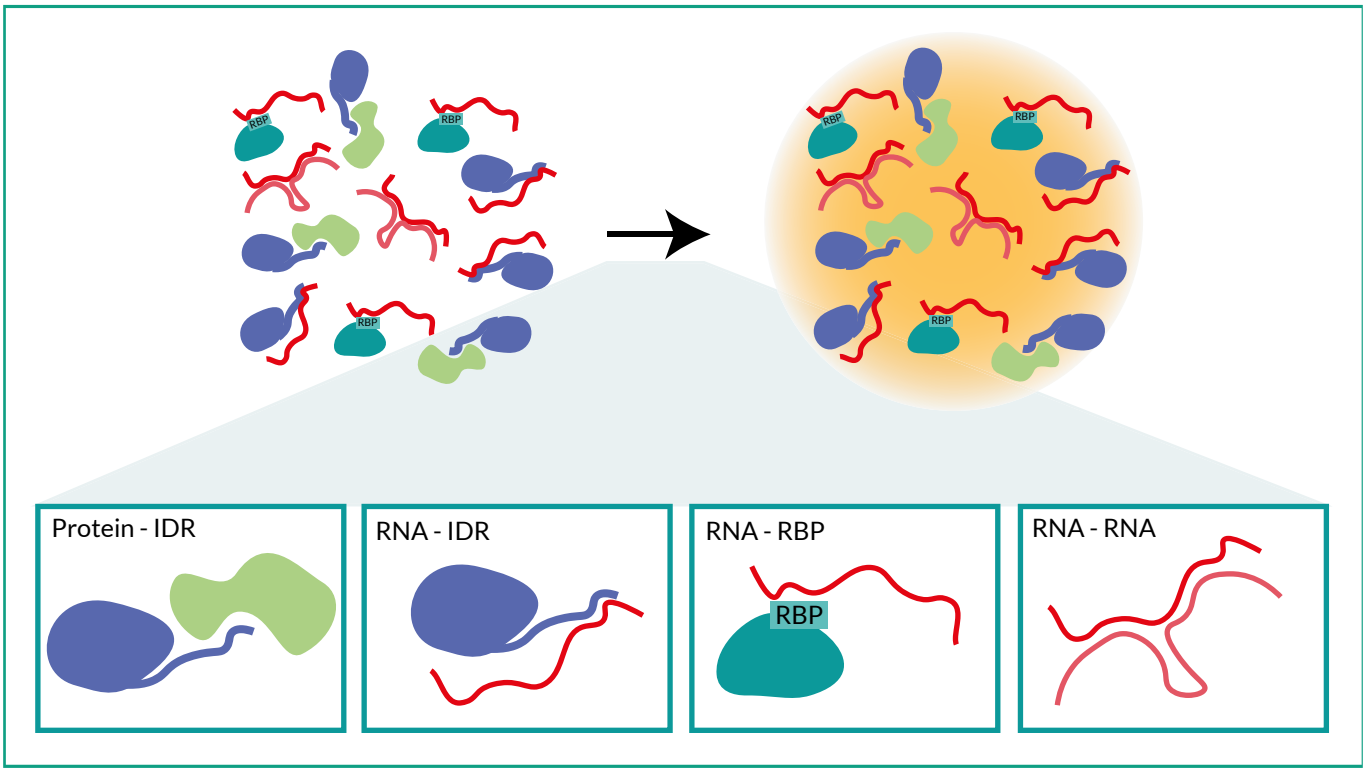

Figure 1 - Schematic representation of the interactions that drive RNP granule formation. Protein-protein, protein-RNA and RNA-RNA interactions drive phase separation. Proteins involved in the assembly often have an intrinsically disordered domain (IDR) and/or an RNA-binding domain (RBP) which can both form multivalent interactions with RNA molecules.

RNP granules. This supports the idea of self-assembly of RNP granule components through protein-protein and protein-RNA interactions into biomolecular condensates.

1.2 Post translational modifications (PTM) to regulate granule assembly

PTMs are another manner by which the phase separating tendency of a protein can be increased. However, this is not an intrinsic property of a protein but rather one that is added after translation thereby suggesting this could be a manner by which the cell regulates RNP granuels. It is the covalent attachment of a biomolecule to a specific amino acid which can both alter the multivalency, biochemical and biophysical properties of a protein (Banani et al., 2017). Owing to this, a PTM can drastically alter the phase separating behaviour of a protein and thereby direct granule formation.

Several studies have shown that PTMs such as phosphorylation, methylation and SUMOylating can control phase separation. Upon SUMOylation, CPEB3 is promoted to form biomolecular condensates. Additionally, CPEB3 localizes to P-bodies in neurons and *in vivo* data shows that the localization is directed by SUMOylation (Ford et al., 2019). Phosphorylation of the low-complexity domain of FUS inhibits condensate formation. (Murray et al., 2017). The same holds true for arginine methylation which prevents the phase separation of FUS and hnRNPA2 in neurons (Qamar et al., 2018; Ryan et al., 2018). However, the same modification can promote Lsm4 granule formation (Arribas-Layton et al., 2016). Interestingly, arginine methyltransferases are components of RNP granules and mediate the methylation of arginine residues (Scaramuzzino et al., 2013). Following these findings, arginine methylation might be a physiological mechanism to maintain RNP granules. Future research should determine whether this mechanism extends to other PTMs such as SUMOylation and

phosphorylation and whether PTMs are used as some sort of switch to regulate RNP granule formation.

1.3 How RNA itself can modulate RNP granule formation

So far, we have discussed several proteins that can control the phase separation of RNP granules determined by specific characteristics such as a low-complexity domains and PTMs. Often, direct or indirect interactions with RNAs are required for this effect. A subset of cellular RNA molecules are enriched in RNP granules which appear to be based on both structure and post transcriptional modifications. Furthermore, recent literature suggests that RNA-RNA interactions also contribute to phase separation. This suggests a role for RNA sequence and structure in RNP granule assembly.

RNA structure and sequence

RNA molecules are known to form secondary structures through which several processes can be regulated. Through this structure, RNA is able to interact with proteins in a similar manner as protein-protein interactions (Sanchez de Groot et al., 2019). For instance, the secondary structure of mRNA has been found to recruit the RBP Staufen1 (Sugimoto et al., 2015). It would then only make sense that the secondary structure of the target mRNAs could also play a role in recruitment and specificity into RNP granules. Indeed, recent evidence shows that the RNA transport granule protein Staufen2 preferentially binds the hairpin structure in the 3'UTR of Rgs4 mRNA to subsequently co-assemble into granules in neurons (Fernández□moya et al., 2021). Moreover, it was found that P-bodies in human cells preferentially recruit long but also AU-rich mRNAs while GC-rich mRNAs were excluded from the granules. (Courel et al., 2019). Singh et al supports these findings in *Drosophila* neurons as Ataxin2 preferentially binds its target mRNA in the 3'UTR, particularly in an AU-rich region (AREs) of the target mRNA (Singh et al., 2021).

Post transcriptional modifications

Similar to modification on proteins, mRNA can obtain a post transcriptional modification. Methylation of mRNAs to form m6A residues is the most common modification of mRNA in eukaryotic cells. This modification is a strong determinant in RNA metabolism such as splicing, translation and stability. The polymethylation status of mRNAs to form m6A residues is differently distributed in granules compared to the cytosol. In P-bodies, stress granules and RNA transport granules, mRNA with multiple m6A sites are enriched and barely contain any non-methylated mRNAs. The number and distribution of m6A sites in the mRNA can regulate and influence the phase separation of m6A-mRNA binding proteins such as YTHDF and FMR1 (Ries et al., 2019). In *Drosophila*, FMR1 phase separation resulted in much larger granules upon interactions with methylated mRNA than with unmodified mRNA. Interestingly, they found that methylated mRNA-FMR1 interaction and condensation increases the subsequent sequestration of unmodified mRNA (Zhang et al., 2022), indicating that RNP granules not exclusively contain methylated mRNA but later additionally recruit non-methylated mRNA molecules into these granules.

RNA-RNA interactions

Finally, emerging evidence suggests that RNP granule formation can be driven at least in part by RNA-RNA interactions. Besides base pairing, RNA molecules can also self-assemble through multivalent interactions with other RNA molecules (Tauber, Tauber and Parker, 2020). Recent work shows that the multivalency of RNA is sufficient to phase separated in absence of proteins (Jain and Vale, 2017). RNA from total RNA yeast extract would self-assemble in vitro through RNA-RNA interactions in cytosol mimicking conditions. The self-assembling RNAs overlapped with the RNAs found in stress granules suggesting that stress granules could at least in part be self-assembled through RNA-RNA interactions (Van Treeck et al., 2018). Furthermore,

disrupting the RNA-RNA interactions would inhibit G3BP from phase separating, indicating the partial dependence of granule formation on RNA-RNA interactions (Yang et al., 2020).

Collectively, these studies show that RNP granule assembly relies on protein-protein, protein-RNA and RNA-RNA interaction **(Figure 1)**. Assembly promoting proteins have characteristics that increase their multivalency, a main driver of liquid-liquid phase separation. Moreover, post translational protein modifications and post transcriptional mRNA modifications appear to determine the specificity of proteins and RNAs recruited to RNP granules. Nevertheless, from these finding it remains unclear how P-bodies, stress granules and RNA transport granules organize their distinct components. Additional studies must be performed to identify more RNP granule components to determine RNP granule type specific assembly mechanisms. This will additionally provide more RNP granule type specific markers that can be used to further investigate the heterogeneity and differences between P-bodies, stress granules and RNA transport granules.

2. Transport and interactions with the cytoskeleton and organelles

Polarized cells such as neurons rely on active transport to facilitate local protein synthesis at distal intracellular locations. Long-distance transport of untranslated mRNAs is an important step in local protein synthesis. mRNA containing RNP granules have long been observed to traffic within living neurons in a microtubule-dependent manner (Knowles et al., 1996; Das, Singer and Yoon, 2019). More recently, RNP granules are thought to be trafficked throughout neurons by motor protein dependent transport.

2.1 RNP granules interact with cytoskeleton and motor proteins

The cytoskeleton is an important cellular structure and enables many essential processes such as the correct localization of membrane-bound organelles. Moreover, the interplay between the cytoskeleton and membrane-bound organelles also plays a role in cytoskeleton organization and organelle remodeling (Koppers, Özkan and Farías, 2020). In a similar manner, RNP granules additoinaly interact with the cytoskeleton.

While many RBPs have been implicated in the localization of mRNA molecules in neurons, it remained unclear how these complexes were transported. Over the years, increasing evidence showed that the transport was facilitated by motor proteins such as kinesin and dynein (Baumann et al., 2012). They are important motor proteins that collaborate to transport various organelles along microtubules in anterograde and retrograde direction, respectively. One study identified KAP3 which links the RBP APC and beta-actin and beta2B-tubulin mRNA to the motor protein kinesin-2 for the transport along microtubules (Baumann et al., 2020). In rat neuronal cultures, the RBP SFPQ itself interacts with the kinesin-1 containing motor complex to ensure anterograde axonal transport of RNA (Fukuda et al., 2021).

Several studies show that neuronal P-bodies are transported in a motor-dependent manner along microtubules in dendrites (Oh et al., 2013). Furthermore, RNP granules have also been found to move bidirectionally along microtubules in the axon with a preference for anterograde direction in *Xenopus* retinal ganglion cells (Leung et al., 2018). However, they could not identify any interactions with motor proteins. Another study showed that the axonal localization of *Drosophila* Imp protein containing granules was microtubule dependent. Again, no direct interactions with motor proteins could be found (Vijayakumar et al., 2019). For RNA transport granules in dendrites however, the RBP ZBP1 bound to beta-actin mRNA would interact with kinesin-1 through PAT1 in living

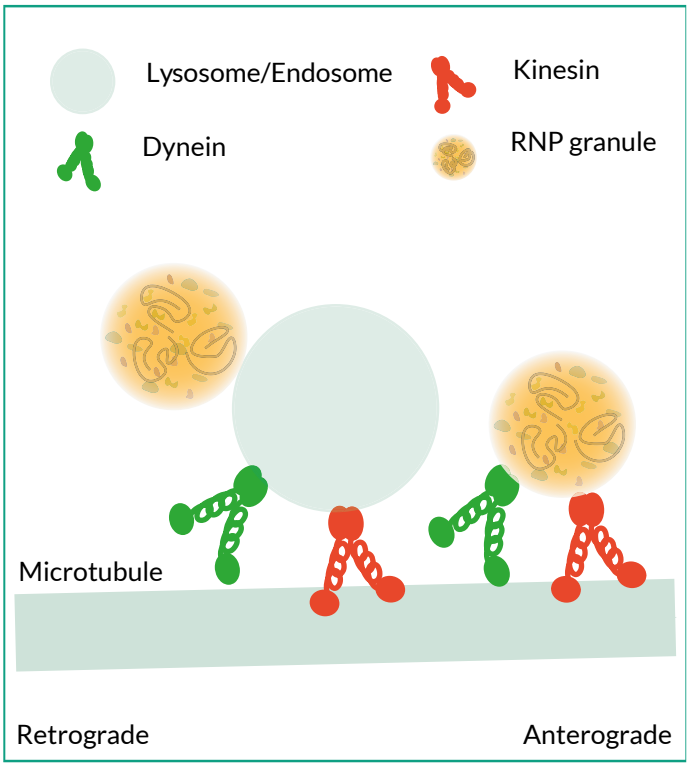

Figure 2 - Schematic of RNP granule transport. In axons and drendrites, RNP granules are actively transported along microtubules. As RNP granules move into anterograde direction for local protein synthesis, the motor protein kinesin is often implicated in RNP granule transport. However, RNP granules can also be trafficked by dynein. There are two possible mechanisms by which RNP granules travel along microtubules. One is by hitchhiking onto membrane-bound organelles such as lysosomes or endosomes. The other relies on direct interactions between RNA-binding proteins assembled into RNP granules and motor proteins.

mouse neuronal cultures (Wu et al., 2020).

These findings show that RNP granules are transported along microtubules into the axon and dendrites in a motor protein dependent manner. Interestingly, mainly kinesin motor proteins have been found that are known to walk in anterograde direction towards the edges of the cell. This is indicative for a the role of RNP granules in local protein synthesis where mRNA is delivered at the far ends of the cell after which the granules is no longer required and therefore can disassemble.

2.2 RNP granules interaction with membrane-bound organelles

Several lines of evidence show that membrane-bound organelles additionally play a role in RNP granule transport. Despite the indications that RNP granules might interact with motor proteins, direct evidences remain scarce. Instead, some granules interact with membrane-bound organelles for their transport which are in turn tethered to motor proteins. Previously, organelle hitchhiking of mRNA and RBPs had already been reported in other organisms such as the filamentous fungus *Ustilago maydis*, *Saccharomyces cerevisiae* and *Drosophila* (Baumann et al., 2014; Salogiannis and Reck-Peterson, 2017). Especially the *U. maydis* is an interesting model for polarized cells due to its long hyphal compartments similar to mammalian neurons. Ribosomes and RBPs in U. maydis associate with endosomes and mRNA localization and translation relies on endosome-dependent trafficking (Baumann et al., 2014).

This finding was supported by the work from Cioni et al who used retinal ganglion neuronal cells from the *Xenopus* (Cioni et al., 2019). They showed that RNA granules associate with late endosomes near axonal mitochondria to serve as translation platforms for axonal mitochondrial proteins. The RNA granules consist of endogenous labelled RNA although the nature of the granule is not further defined. Ribosomal proteins and other RBPs were found to closely interact with endosomes implying that these could aid in local protein synthesis of the endosome-associated mRNA. These mRNAs consist of proteins that play a role in the maintenance of axonal mitochondrial function. Nevertheless, it remains unclear how these granules become tethered to endosomes or why they associate

with late endosomes specifically.

More recently, hitchhiking of stress granules specifically onto lysosomes has also been observed in mammalian neurons (Liao et al., 2019). Additionally, they were able to identify the protein responsible for tethering the granule to the lysosome. Through direct interaction with with the lysosome and stress granule, ANXA11 tethers the two together to allow long distance axonal transport. Impairment of ANXA11 would lead to reduced delivery of actin mRNA to distal sites. These findings provide a possible mechanism for the active transport of stress granules by hitchhiking onto membrane-bound organelles who do directly interact with motor proteins. It would be interesting to uncover whether this is a general mechanism for RNP granules or if other RNP granules might directly become tethered to motor proteins as suggested by the interactions between RBPs and motor proteins. Interestingly, these findings suggest a broader function for stress granules than generally thought as to merely sequester inactive mRNA until stress is released. Here an additional role in local mRNA translation for stress granules has been suggested.

Membrane-bound organelles modulating RNP granules

It is widely recognized that the ER forms membrane contact sites with which membrane-bound organelles interact to modulate their dynamics and the exchange of molecules. (Shai et al., 2018; Wu, Carvalho and Voeltz, 2018). Similarly, RNP granules were found to associate with the ER in neuronal cells of *Xenopus* (Cioni et al., 2019) and later in mammalian U2OS cells (Lee et al., 2020). A large fraction of P-bodies co-localize with tubular ER in mammalian cells and maintain stable yet reversible contacts with the ER. Through these contact sites the ER is able to regulate the biogenesis of P-bodies. Increased translation activity on the ER reduces the number of P-bodies while inhibition of translation on the ER leads to increased P-body abundancy and contacts with the ER. Similar to mitochondria, early and late endosomes, the ER also mediates the fission of stress granules and P-bodies. These findings suggest that the ER might play an essential role in the maintenance of proper protein synthesis (Lee et al., 2020).

Collectively, the recent findings indicate that RNP granules are regulated by a broad network of cytoskeleton and organelle interactions. They are actively transported throughout the cell by motor proteins. However, it remains unclear if RNP granules directly interact with motor proteins or by hitchhiking onto trafficking membrane-bound organelles **(Figure 2)**. Additionally, RNP granules are continuously maintained by membrane-bound organelles in a similar manner as was already well known for membrane-bound organelles.

3. Disassembly and local protein translation

An unresolved question in the role of RNP granules in local protein translation is how mRNAs re-enter translation once it has reached its destination. This is assuming that mRNAs are repressed within RNP granules as indicated by the majority of research. However, recent studies show evidence for translation within granules contradicting this idea. Whether or not mRNAs are repressed in granules and during transport, the mechanism behind RNP granule disassembly remains unclear as only little studies touch upon this topic.

3.1 mRNA handling within granules

Regulation of mRNA translation has been broadly studied in RNP granules and the most common hypothesis is that mRNAs are repressed within them (Decker and Parker, 2012). This is shown by the presence of translation repressors in P-bodies in mammalian cells and primary mouse neuron cultures (Ayache et al., 2015; Ford et al., 2019). Furthermore, Hubstenberger et al present a new technique called

fluorescence-activated particle sorting (FAPS) which allows for the purification of endogenous P-bodies from human epithelial cells. From these purified P-bodies, a wide variety of proteins, RNAs and other molecules can be identified. They found that indeed mRNAs in P-bodies are translationally repressed (Hubstenberger et al., 2017). Another study in *C. elegans* showed that mRNAs recruited to P-bodies become translationally repressed through the combination of PGL-1 and WAGO-1 (Aoki et al., 2021).

Interestingly, opposite to what was seen before (Decker and Parker, 2012), they showed that those mRNAs are protected from 5' decay, suggesting that they can re-enter translation. Additionally, they could not find any ribosomal subunits which argues against intragranular translation (Hubstenberger et al., 2017).

Similarly, many translationally inactive mRNAs are recruited to stress granules which are thought to keep mRNAs inactive whilst stress remains present (Decker and Parker, 2012; Namkoong et al., 2018). In rat neuronal cultures, G3BP1 assembles into stress granule-like assemblies binding the 48s pre-initiation complex and thereby stalling translation (Sahoo et al., 2018). However, a recent study challenged this view by showing that translation continues even within stress granules in human cells. Although they did similarly find that translationally repressed mRNAs are preferentially recruited by stress granules. Interestingly, their results shows that from initiation to termination of translation, the entire process can occur within stress granules (Mateju et al., 2020).

Together, these findings show that RNP granules preferentially recruit translationally inactive mRNAs but could also facilitate mRNA repression. This suggests that the mRNA is prevented from translation for storage until stress conditions are releaved or until the RNP granule has reached the site for local translation. Perhaps, it is at this point that the translation machinery is allowed to translate the mRNA. However, it remains unclear how the spatio-termporal organization of the intragranular translation would be regulated.

Additionally, a novel role for stress granules is again suggested as translation was found to occur within these granules. This contradicts with the idea that stress granules disassemble when stress is released afterwhich mRNAs can re-enter translation.

Furthermore, future experiments should indicate whether these findings about intergranular translation in stress granules extends to P-bodies and RNA transport granules as well.

3.2 RNP granule disassembly

The disassembly of RNP granules is substantially less well studied and it remains unsure whether disassembly should occur prior translation. Nevertheless, once the mRNA is delivered, the stress is released or the RNA storage period is over, the RNP granule is no longer required and disassembly due to its reversible nature would be a logical next step. Some evidence shows that similar to the assembly, disassembly occurs in a regulated manner. For instance, the phosphorylation of G3BP1 inhibits its oligomerization and thereby looses its ability to assemble subsequently causing the stress-like granule to disassemble (Sahoo et al., 2018). Alternatively, when translation properties of the ER are restored after a period of stress, the ER and unfolded protein response induce P-body disassembly (Lee et al., 2020). Interestingly, another finding shows that merely the degradation of mRNA is sufficient to cause the disassembly (Zhang et al., 2022). Alternatively, Leung et al show that RNP granule anterograde movement towards axonal growth cones and subsequent local translation of beta-actin mRNA is enhanced by increasing gradients of Netrin-1. However, it is unknown whether these RNP granules had to disassemble prior translation (Leung et al., 2018).

Collectively, these findings provide the first insights into the spatio-temporal organization of local protein synthesis and disassembly. However, many questions remain. For example, do RNP granules dock at specific sites and know

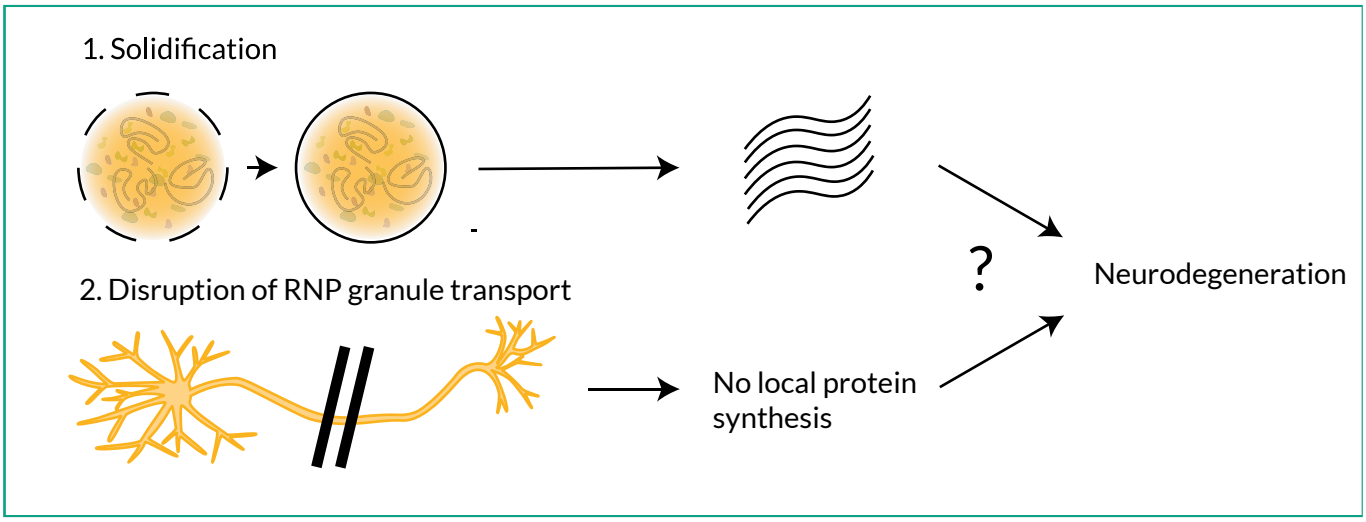

Figure 3 - How disease mutations could affect RNP granules and subsequently cause neurodegeneration. One hypothesis is that disease mutations alter the phase behaviour, leading to solidification of the condensate, promoting aberrant fibril formation and eventually cause neurodegeneration. Even though aberrant fibril formation is a hallmark of neurodegenerative disease, the pathogenic mechanism remains unclear. Another hypothesis is that disease mutations disrupt RNP granule transport afterwhich RNP granules fail to deliver mRNAs, preventing local protein synthesis. This could disrupt the local protein homeostasis and via unknown mechanisms lead to neurodegeneration.

where or when to allow translation again? Do RNP granules first have to disassemble prior translation? What are the cues for disassembly? What is the disassembly mechanism? How do all the translation components travel to the site of translation if not in RNP granules? Furthermore, from these studies it remains unclear how these findings extend to RNA transport granules.

4. RNP granules and their implications in neurodegenerative disease

As more RNP granule components become identified, more proteins emerge as key players in neurodegenerative diseases. Although it is yet unknown why RNP granule proteins aggregate, the increasing list of RNP granule components involved in neurodegenerative diseases slowly leads towards a better understanding of the mechanism towards neurodegeneration.

Occasionally, the liquid-like state of granules irreversibly transits to a solid-like state in which protein aggregation is favoured. Solidification of granules and protein aggregation are both hallmarks of neurodegenerative diseases. Many disease mutations are found to affect their phase separating behaviour and could promote solidification and subsequently aggregation. Alternatively, disease associated proteins are found to interfere with other RNP granule processes such as transport (Shin and Brangwynne, 2017; Ryan and Fawzi, 2019).

Several RNP granule components have been implicated in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Frontotemporal dementia (FTD), FUS- associated frontotemporal lobar degeneration (FTLD), Spinocerebellar ataxia (SCA), Parkinson's disease (PD), Huntington's disease (HD) and Alzheimer's disease (AD).

FUS is a well-known RNP granule components, however has also been implicated in several diseases including ALS, FTD and FTLD. It is one of the first proteins that was shown to undergo liquid phase separation and upon ALS-associated mutations it separates into more solid-like granules (Patel et al., 2015). Similarly, FTLD-associated hypomethylation of

FUS induces irreversible solid-like condensates that disrupt RNP granule function and impair novel protein synthesis in neurons (Qamar et al., 2018). Additionally, ALS-associated FUS stress granules were found to be structurally distinct and therefore could affect many downstream cellular pathways leading to neurodegeneration(An et al., 2022).

Another protein associated with ALS is Ataxin-2, however it also contributes to the progression of several other diseases such as SCA, FTD and HD (Huelsmeier et al., 2021). In Drosophila HD models it was shown that the low-complexity C-terminal of Ataxin-2 was responsible for the progression of disease (Huelsmeier et al., 2021). Furthermore, a reduction of Ataxin-2 would lead to a reduced progression of ALS suggesting that normally functioning Ataxin-2 plays a role in the disease (Becker et al., 2017).

TDP-43 is another well-known RNP granule components that promote liquid phase separation and maintains the liquid like properties of those granules. However, upon an ALS-associated mutation in TDP-43, these liquid like properties are disrupted and TDP-43 granules became more viscous and demonstrated disrupted transport (Gopal et al., 2017; Grese et al., 2021). Additionally, it was shown that TDP-43 mutations would lead to the inhibition of local protein synthesis in distal axons (Altman et al., 2021).

Another disease associated protein mutation in Annexin A11 impairs the tethering function of stress granules to lysosomes and thereby RNA transport (Liao et al., 2019). This suggests that disruption of novel protein synthesis at distal sites in neurons might bring the cell in imbalance which eventually leads to neurodegeneration. Furthermore, while glutamate was found to enhance local translation of mRNA in tau-containing RNP granules, it additionally caused the hyperphosphorylation of tau which is associated with AD (Kobayashi et al., 2017).

Given the broad variety of RNP granule components, the number of neurodegenerative diseases associated with RNP granule components is noteworthy. This raises the question whether distinct diseases could be caused by a more general mechanism in which RNP granules are involved. One mechanism could be that disease mutations create a more aggregation prone environment where aberrant fibril formation is promoted. Another more general mechanism would be the disruption of RNP granule functioning such as local translation. If disease mutations would impair normal local protein synthesis at far ends in neuronal cells, this might disrupt local protein homeostasis causing neuronal misfunctioning and eventually neurodegeneration **(Figure 3)**. Together, the finding that various RNP granule components are associated with neurodegenerative diseases emphasizes the importance for further understanding of RNP granules.

Concluding remarks and future perspectives

During this review, we have shed light on the assembly mechanism of RNP granules and touched upon the selection mechanisms for RNP granule components. A common characteristic of proteins involved in the assembly is that they have increased multivalency and their phase separating properties are regulated by PTMs. Furthermore, RNP granules interact with various membrane-bound organelles and the cytoskeleton either directly or indirectly. Through hitchhiking onto lysosomes, RNP granules are transported into the axon. Within RNP granules, mRNA molecules are usually translationally repressed, yet could still re-enter translation. Nevertheless, it remains unknown how the local translation of these mRNA molecules will be spatio-temporally organized as the literature on disassembly and intragranular translation remains scarce.

Until date, many RNP granule related articles have failed to adequately identify the type of granules. The term granule has been loosely

used to indicate any type of RNA containing assembly. However, as more is discovered about these granules, it becomes clear that there is a wide variety of granules, some of which are cell type specific. Nowadays, most articles distinguish between P-bodies and stress granules and their composition is becoming increasingly identified. However, RNA transport granules are significantly underrepresented and as a result, far less is known about them. Furthermore, the term RNA granule is frequently used for RNP granules, as well as stress granules and P-bodies, making the name RNA transport granule exceedingly confusing. Finally, there are still articles published that utilise the term RNA granule to describe fluorescent puncta containing RNA. As a result, establishing whether results and conclusions may be transferred is challenging. In future articles, care has to be taken when discussing RNP granules since we must properly describe and identify the type of RNP granule.

Despite the increasing amount of work on RNP granules, many questions remain. For example, the exact mechanism of assembly and recruitment of additional RNP granule components remains unknown. Implications of the ER as a docking site for RNP granules might suggest a site of exchange of granule components. However, future experiments would have to uncover a mechanism and involved proteins. Further, the spatio-temporal organization of RNP granule is poorly understood. While it is clear that RNP granules are favourably transported in anterograde direction, how this is regulated remains unknown. Moreover, it is not clear what determines the site of local translation although some findings points towards the direction of local cue-induced translation. Still, the induction of local protein synthesis remains unsure as to whether disassembly is required prior translation. Finally, a more detailed list of RNP granule components is necessary in order to further unravel the differences in composition, function and localization of P-bodies, stress granules and RNA transport granules. Especially RNA transport granules have been underexposed.

Regarding the strong associations with neurodegenerative diseases, further research is needed to fully comprehend the mechanisms behind RNP granules and local protein synthesis. Most RNP granules however, are extremely dynamic and have a heterogeneous composition, limiting the possibilities of current experimental techniques. Yet, more sophisticated approaches such as proximity labelling techniques (APEX), RNP granule purification methods (FAPS) and live cell imaging of in vivo translation (Sun-tag), will allow us to address at least some of these questions in these future.

Layman's abstract

Cells need to carry out various biochemical processes within its crowded intracellular environment. In order to organize this, the cell is subdivided into multiple distinct compartments, called organelles, which are physically separated and allow exchange biomolecules in a controlled manner.

Recently, a new type of organelle has been discovered. These are called condensates and do not have a physical border. Instead, they separate themselves from their environment by transitioning into a different phase, like oil and water.

Up until now, these condensates are suggested to play important roles in various physiological processes of the cell. In this review we will focus on one specific type of condensate, called RNP granule, which is involved in the RNA metabolism. RNP granules are suggested to play a role in local mRNA translation at distal sites of the cell. mRNAs are gene transcripts that have to be translated into proteins. So, local mRNA translation leads to the local synthesis of new proteins. Especially in the far stretching neuronal cells, local RNA translation is essential to maintain.

Local RNA translation knows several steps. First, the RNP granules has to be assembled by recruiting specific mRNAs and proteins. These specific mRNAs and proteins are recruited

based on their sequence and structure. For instance, proteins with a low-complexity region are favoured. On the other hand, longer mRNAs with specific modifications are preferred. These proteins and mRNAs interact and thereby assembly into an RNP granule. Secondly, the RNP granule has to be transported from the centre of the cell towards the cell edge. The cell has various transport systems in place which have been well studied. Increasing evidence suggests that RNP granules too make use of these systems. While there are some indications that RNP granules directly interact with this transport system, recent findings show that they hitchhike onto other organelles. Finally, after transportation to the final destination, the RNP granule has to permit the mRNA to become locally translated. Yet, on this step many questions still remain. For example, it is not clear whether the RNP granule has to disassemble prior translation. Additionally, further research has to reveal how RNP granules know where to go and when they have arrived.

Nevertheless, RNP granules are a fundamental organelle for neuronal cells as they seem to be tightly regulated. It is therefore not surprising that they have also been implicated in disease. Several RNP granule associated proteins are found in neurodegenerative diseases. When mutated, these proteins cause the RNP granule to solidify and promotes the formation of pathogenic protein assemblies often found in patient brains. Another example is when a disease mutation disrupts the transport of RNP granules, which as we now know is important for local protein synthesis. In this manner, the mutation could create an imbalance in novel protein synthesis, impairing neuronal functioning and eventually cause neuronal cell death. The implications of neurodegenerative diseases emphasizes the importance to further unravel the mechanisms underlying RNP granules by the help of advanced techniques.

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