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Review Article

Stress Granules and P-Bodies: An Insight into mRNA Translational Control and Decay

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RNA granules are higher order RNA-protein complexes formed due to protein-protein and protein-RNA interactions. Various cellular cues such as nutritional stress, oxidative stress, heat shock, unfolded proteins trigger assembly of RNA granules. Stress granules (SGs) and mRNA processing bodies (PBs) are two well-characterized, conserved RNA granule types that form the focus of this review. Other studied RNA granule types are P-granules, Neuronal granules, and GW-bodies. RNA granules (also termed as higher order messenger ribonucleoprotein complexes - mRNPs) have been proposed to play a role in post-transcriptional gene regulation specifically mRNA stability, translation repression, and transport. Both SGs and PBs contain translationally repressed mRNAs, however, processing bodies (PBs) also contain mRNAs undergoing degradation. Proteins with low complexity (LC) regions or intrinsically disordered regions (IDRs) play an important role in granule assembly in general. Mutations in RNA granule components leading to impaired assembly and disassembly of granules have been implicated in Neurodegenerative diseases and Cancers. RNA granules play a key role in determining mRNA fate in cytoplasm, hence understanding the granule assembly and disassembly processes in detail will be a major future milestone.

Keywords: RNA Granule; Ribonucleoprotein Complex; Post-Transcriptional Gene Regulation; Low Complexity Region; Intrinsically Disordered Region; Stress Granule; Processing Body; mRNA Decapping; mRNA Degradation

Fate of mRNA in Cytoplasm

Post-transcriptional gene control takes place at many different steps, such as mRNA processing and maturation, export to the cytoplasm, translation, decay, protein modifications and turnover. A mature mRNA upon arrival into cytoplasm can be a) translated, b) stored in a repressed state or c) degraded (Fig. 1). Translation and mRNA decapping/decay are closely related since the mRNA cap and tail are important for both translation and stability (Coller and Parker, 2004). The transition from translation to decay often involves a functional state in which the mRNA is stripped of ribosomes, referred to as the repressed state. A recently reported phenomenon describes decapping of mRNA while being translated, referred to as co-translational decay (Hu *et al.*, 2009). The specific conditions under which co-translational decay prevails and the subset of mRNAs targeted by this pathway are currently unclear. Translationally repressed mRNAs often localize to higher-order RNA protein complexes known as RNA granules. Repressed mRNAs can either be decapped/degraded or stored for future re-entry into translation depending on the nature of repression mRNP (Brengues *et al.*, 2005). Thus, understanding the regulation of translation by focusing on the movement of mRNAs in and out of RNA granules can provide insights into mechanisms of mRNA fate decisions.

mRNA Translation and Repression

The process of mRNA translation is initiated by the formation of eIF4F complex on the 5' cap of mRNA, which consists of the cap-binding protein eukaryotic initiation factor 4E (eIF4E), eukaryotic initiation factor 4A (eIF4A) and eukaryotic initiation factor 4G (eIF4G) (Sonenberg and Hinnebusch, 2009) (Fig. 2). eIF4G

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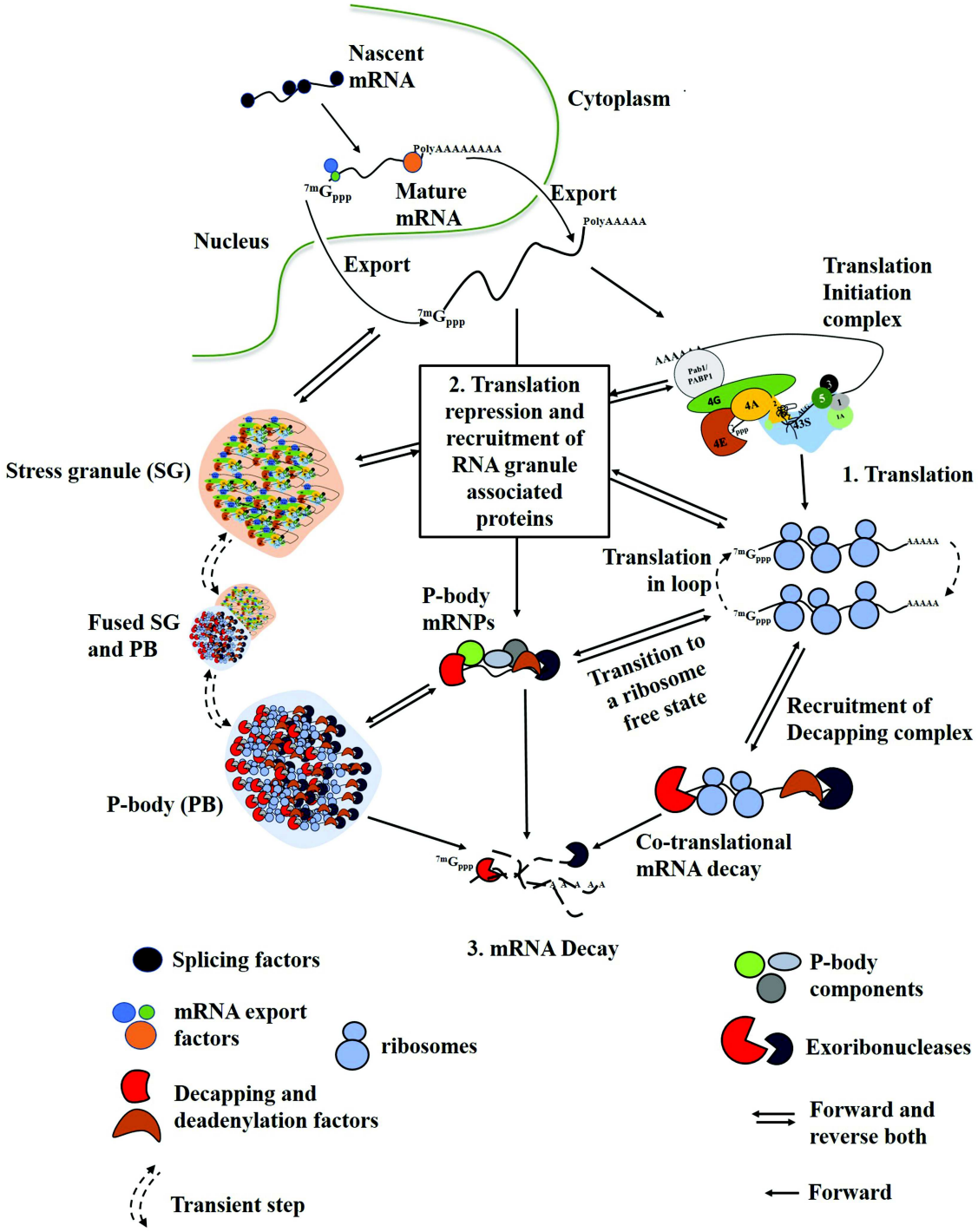

Fig. 1: mRNA life cycle showing the various fates of a mature mRNA after coming out from the nucleus. 1. mRNA can undergo translation in the cytoplasm by recruiting translation initiation factors. 2. Upon encountering stress, mRNAs are translationally repressed and can localize into RNA granules (SGs or PBs) transiently. Some resident mRNAs are degraded whereas others are stabilized and can return back to translation. 3. mRNA decay in the cytoplasm

serves as a scaffold protein interacting physically with eIF4E, to recruit eIF4A and eukaryotic initiation factor 4B (eIF4B). The mRNA is circularized by the interaction between eIF4G and poly(A) binding protein 1 (Pab1). The binding of the 43S pre-initiation complex to the cap-binding complex results in 48S complex formation, which scans for the start codon. Upon finding the start codon, 60S ribosomal subunit

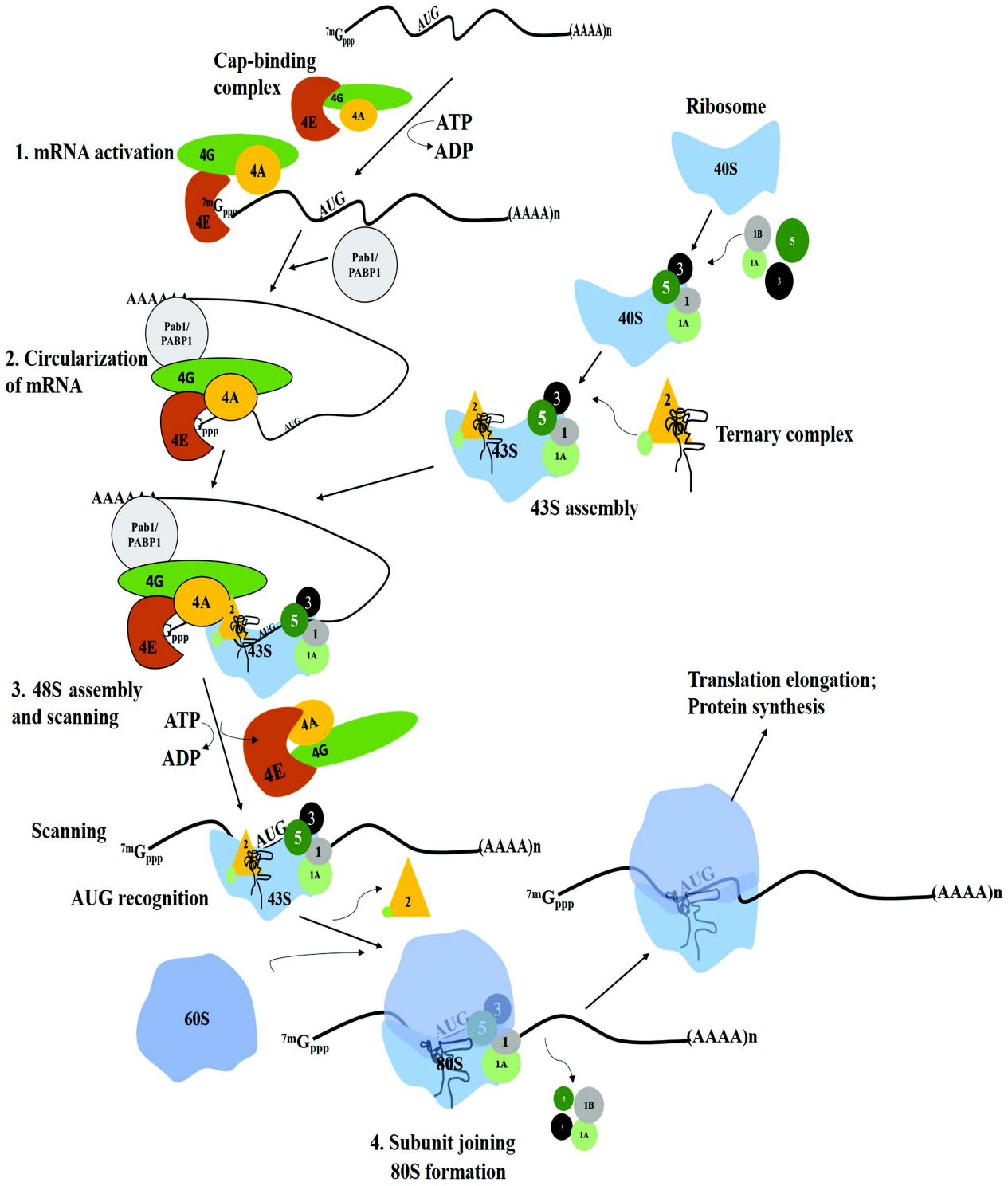

Fig. 2: Mechanism of translation initiation. 1. Activation of mRNA translation: Binding of eIF4F (eIF4E. eIF4G and eIF4A) complex to the 5' cap of the mRNA. 2. Circularization of mRNA: Binding of Pab1 to the poly(A) tail circularizes the mRNA to initiate translation. 3. Formation of 48S pre-initiation complex and scanning of mRNA: recruitment of 43S ribosomal complex to the circularized mRNA to initiate the scanning for the initiator codon (AUG). 4. Upon recognition of initiator codon the 60S ribosomal unit binds to the 48S ribosomal unit dissociating the initiation factors resulting in translation elongation

associates with the 48S complex leading to formation of 80S, marking the end of initiation. The 80S ribosomal complex is the site of protein synthesis where incoming tRNA base pairs with the mRNA codon, leading to recognition of the codon, thereby facilitating the incorporation of the tRNA bound amino acid in the growing polypeptide chain (Sonenberg and Hinnebusch, 2009). Several repression mechanisms have been reported targeting the translation initiation step. Whether this is because translation initiation is the most regulated step during translation or because elongation and termination have not been adequately studied is up for debate.

Some of the repression mechanisms targeting the initiation step are discussed here. An important regulatory mechanism for the initiation step is to disrupt the translation initiation complex (eIF4F) by eIF4E-BP (eukaryotic initiation factor 4E- binding protein), which binds to eIF4E (7-methylguanosine triphosphate cap-binding protein) and inhibits the binding of eIF4G to eIF4E (Castelli *et al.*, 2015; Hughes *et al.*, 1999). The competition between 4E-BP and eIF4G is due to the presence of the common motif Tyr-X-X-X-X-Leu-Leu/Met/Phe, where, X is any amino acids, which is important for binding to eIF4E (Richter and Sonenberg, 2005). This helps 4E-BP to repress cap-dependent translation at a global scale (Haghighat *et al.*, 1995). mTOR (mechanistic target of rapamycin) in mammalian cells during cellular growth phase phosphorylates and inactivates 4E-BP (Gingras *et al.*, 1999; Hara *et al.*, 1997). But, when mTOR signaling is inhibited, it leads to decreased phosphorylation of 4E-BP and encourages 4E-BP/eIF4E binding, resulting in translation repression (Castelli *et al.*, 2015; Showkat *et al.*, 2014).

In *Drosophila*, Bruno protein represses translation of unlocalized *oskar* mRNA by binding to the Bruno recognition element (BRE) in the 3'-UTR of the mRNA (Castagnetti *et al.*, 2000; Kim-Ha *et al.*, 1995). CUP protein is recruited to *oskar* mRNA by Bruno protein recognizing the Bruno response element (BRE) within the mRNA. CUP bind to eIF4E, using the same binding site required for binding of eIF4G (Kinkelin *et al.*, 2012; Nakamura *et al.*, 2004). CUP, through its association with Bruno, binds and displaces eIF4G from eIF4E (Richter and Sonenberg, 2005), disrupting the formation of cap-binding initiation complex, resulting in translation repression of *oskar* mRNA.

In mammals, Smaug 1 (SMG) is described as an RNA-binding translational regulator involved in mRNA silencing and deadenylation (Baez and Boccaccio, 2005). SMG regulates the expression of target mRNAs by at least two distinct mechanisms: translation repression of the mRNAs is initiated by recruitment of CUP/eIF4E complex and on the other hand, recruitment of CCR4/POP2/NOT deadenylase by SMG initiates transcript destabilization and degradation (Nelson *et al.*, 2004; Smibert *et al.*, 1999).

Recently, a group of proteins containing the RGG/RG (also known as GAR-Glycine Arginine Rich) motif has been shown to repress translation by binding to eIF4G (Rajyaguru and Parker, 2012; Rajyaguru *et al.*, 2012). RGG/RG motif containing proteins function in diverse cellular processes like pre-mRNA splicing, DNA damage signaling, mRNA translation and regulation of apoptosis (Thandapani *et al.*, 2013). Some of the examples of RGG/RGX motif containing proteins involved in translation repression include; Scd6, Sbp1, FMRP, Khd1 Npl3, and Ded1.

Repression of translation at the initiation step increases the pool of non-translating mRNAs in the cytoplasm. These transcripts along with associated translation modulators (both initiation factors and repressors) localize to RNA protein complexes, which manifest itself as RNA granules (Parker and Sheth, 2007; Protter and Parker, 2016). Global translation repression in response to stress correlates with formation of RNA granules, which disassemble upon return to the normal conditions (Wheeler *et al.*, 2016).

What are RNA Granules?

RNA granules are non-membranous, microscopic cytoplasmic puncta containing translationally repressed mRNAs in association with translation repressor proteins, initiation factors and in certain cases mRNA decay factors. RNA granules are found in a variety of cells including but not limited to yeast, germ cells, embryos and neurons (Thomas *et al.*, 2011; Wheeler *et al.*, 2016). Interestingly, transcripts localized to granules can either be degraded or stored for return to translation in future (Anderson and Kedersha, 2006). Several different types of RNA granules have been reported in literature and briefly described below. For the purpose of this review, the focus will be on

discussing P-bodies and stress granules although details of other granule types have been discussed in some sections.

Stress Granule

These are non-membranous assemblies of mRNA and protein (mRNP) that form when translation is limiting (Wheeler *et al.*, 2016), which occurs during stress response (Kedersha *et al.*, 1999), by drugs blocking translation initiation (Dang *et al.*, 2006; Mokas *et al.*, 2009), upon knockdown of translation initiation factors (Mokas *et al.*, 2009) or upon overexpression of repressor proteins (Gilks *et al.*, 2004). Components of stress granule include but are not limited to poly(A) tailed mRNAs, 40S ribosomal subunit, TIA-1 (T-cell restricted intracellular antigen-1), G3BP1 (Ras-GTPase-activating protein-binding protein 1), eIF4G (scaffolding translating initiation factor), eIF4A (DEAD-box RNA helicase), Pab1 (polyA binding protein 1), eIF3 (eukaryotic initiation factor 3), eIF2 (eukaryotic initiation factor 2), ATAXIN-2 (a translation repressor), FMRP (fragile X mental retardation protein, a RGG-motif translation repressor) (Anderson and Kedersha, 2006; Kedersha *et al.*, 1999; Mazroui, 2006; Mazroui *et al.*, 2002; Nonhoff *et al.*, 2007). The presence of numerous initiation factors is consistent with the idea that stress granules mostly harbor translationally arrested mRNPs at the initiation step. In yeast, stress granules arise from pre-existing P-bodies (Buchan *et al.*, 2008).

mRNA Processing Body (P-body)

P-body was discovered as mouse XRN1 (mXRN1, a 5'-3' exoribonuclease) foci in cytoplasm (Bashkirov *et al.*, 1997). Subsequently, P-bodies have been reported in various other model organisms including yeast. Unlike stress granules, P-bodies are present during normal conditions also and are further induced upon stress (Parker and Sheth, 2007). P-bodies consist of translation repressors and mRNA decay proteins and have been implicated in translation repression and mRNA turnover. Some of the well-known components of P-bodies are Dcp1/2 (decapping complex), Edc3 (Enhancer of decapping 3), Hedls/EDC4 (Enhancer of decapping 4) and Pat1 (Protein associated with topoisomerase II homolog 1) (Fenger-Gron *et al.*, 2005; Parker and Sheth, 2007).

P-granules

P-granules are also called as 'germ granules' as they are present in germ-line cytoplasm and get their name from P lineage in the embryo that gives rise to germ line. P-granules are perinuclear and play an important role in germline identity, maintenance and fertility (Voronina *et al.*, 2011). Messenger RNAs and RGG-motif proteins PGL-1 and PGL-3 are the main components of these granules, however DEAD-box RNA helicases, members of the argonaute family and certain P-bodies and stress granule components are also present (Amiri *et al.*, 2001; Anderson and Kedersha, 2009; Mello *et al.*, 1996; Updike and Strome, 2010; Wang *et al.*, 2014). Cytoplasmic P-granules are dynamic and can shrink, grow and fuse like liquid droplets (Brangwynne *et al.*, 2009).

Neuronal Granules

Neuronal granules also known as transport granules are cytoplasmic mRNPs involved in transport and localization of mRNAs to distant dendritic site (Kiebler and DesGroseillers, 2000; Knowles *et al.*, 1996; Kohrmann *et al.*, 1999). Component mRNAs are maintained in a translationally arrested state until localized to prevent premature translation until properly localized. Some of the main components of these granules are mRNA, Staufen (double-stranded RNA-binding protein), FMRP (Fragile-X mental retardation protein), CPEB (Cytoplasmic polyadenylation element binding protein) and ribosomes (Kiebler and Bassell, 2006).

GW-bodies

GW-bodies, are mammalian RNA granules discovered while analyzing autoimmune serum targeting GW182 (marker protein) from motor and sensory neuropathy patients (Eystathioy *et al.*, 2002). GW182 protein is a 182 kDa protein with a Ubiquitin associated domain and RNA Recognition Motif (RRM) along with unstructured regions (Eulalio *et al.*, 2009). It has been observed that the GW-bodies are analogous to yeast P-bodies due to colocalization of hDCP1 and hLSM4 in GW-bodies (Eystathioy *et al.*, 2003). Recently it has been found that GW bodies have different dynamics and distributions in early *Drosophila* embryo as compared to P-bodies as they represent two separate pools of non-translating mRNAs (Patel *et al.*, 2016). During early cortical syncytial cycles in

Drosophila, GW bodies are mostly absent, whereas, P-bodies are present throughout the embryogenesis. Unlike P-bodies, GW-bodies are found initially in nucleus, however, increase in cytoplasmic GW-bodies results in gradual decrease in nuclear GW bodies (Patel *et al.,* 2016).

A common proposed function of all granule types described here is translation repression. RNA granules such as PBs and SGs are dynamic, can exchange mRNP components and disassemble upon removal of stress (Wheeler *et al.,* 2016). Importantly, upon release from PBs, the mRNAs can return back to translation (Brengues *et al.*, 2005) suggesting that PBs could act as fate determining sites (Mitchell and Parker, 2014). Stress granules contain a core sub-structure, which is very stable and a shell surrounding the core, which is dynamic and dissolves rapidly *in vitro* (Jain *et al.,* 2016; Wheeler *et al.,* 2016) with size approximation of about 0.1-2 μm (Anderson and Kedersha, 2009; Moser and Fritzler, 2010). The transitions between the shell and the core states are mediated by protein-protein and protein-RNA interactions (Wheeler *et al.,* 2016).

What is the Function of P-bodies and Stress Granules?

Although RNA granule formation is a conserved phenomenon from yeast to humans, the function of RNA granules is not entirely clear. RNA granules, in general, contain RNA and RNA-binding proteins involved in translation repression and/or mRNA decay. Deletion/depletion of RNA decay enzymes such as Xrn1 and Dcp2 lead to increase in P-body size and number in yeast/humans (Eulalio *et al.*, 2007). This is due to accumulation of transcripts that need to be degraded, suggesting, that P-bodies can be sites of mRNA degradation. Consistent with this idea, overexpression of DCP2 in human cells leads to disappearance of P-bodies.

In yeast, Like-Sm protein 4 (Lsm4) and Enhancer of decapping 3 (Edc3) are known to function in the degradation process of mRNA. The Q/N rich prion-like domain of these proteins is required for P-body formation in yeast. Surprisingly, when the prion-like domain in these proteins is deleted, the formation of P-bodies in the cytoplasm is prevented, but interestingly, the mRNA decay rate and translation repression are not significantly affected (Decker *et al.*, 2007; Parker and Sheth, 2007; Reijns *et al.*, 2008). Similarly, the knockdown of essential P-body proteins (PATR-1, CGH-1, DCAP-2 and LSM-1) in *C. elegans* results in impairment of efficient RNA granule formation, but is ineffective in the reduction of mRNA decapping (Gallo *et al.*, 2008). It must be noted that specific transcripts were assayed in above reports to arrive to the above conclusions. It is likely that P-bodies might affect certain subset of transcripts and identifying these transcripts might be crucial to understanding the P-body function. A global analysis of translation and mRNA decay under stress conditions might reveal such details. A recent study along similar lines indicated that majority of mRNAs were degraded upon exposure to glucose stress in yeast. About 400 transcripts (out of 5590 tested) were observed to return back to translation, indicating that P-bodies could have a role in such fate decisions (Arribere *et al.*, 2011). It is unclear, if the transcript subset that returns to translation is only specific to glucose deprivation stress or other stresses in general. The transcripts that return to translation are enriched in mRNAs encoding ribosomal protein genes. It will be interesting to understand the property of mRNPs that contain such mRNAs to understand the mechanism(s) that targets them to repression/P-bodies and subsequently return to translation.

Another well-studied function of certain granules is stabilization of mRNAs. Germ granules (or P-granule) present in *C. elegans* is an example of storing translationally inactive maternal mRNAs during embryogenesis. CGH-1 (Conserved Germline Helicase 1) is a translational repressor, known to co-localize within the germ granule. The function of CGH-1 is not only restricted to repression of maternal mRNAs, but it functions to protect the maternal mRNAs from degradation or decay machinery. CGH-1 acts specifically on associated mRNAs, which are destabilized in *CGH-1* knockout worms (Boag *et al.*, 2008). Co-immunoprecipitation of repressed mRNAs in *Plasmodium berghei* along with DOZI (a CGH-1ortholog) revealed that several mRNAs that are translationally repressed in normal conditions are degraded in absence of DOZI (Mair *et al.*, 2006). This suggests that some germ granule-associated protein components protect repressed mRNAs from getting degraded. However, the contribution of

granules *per se* to this phenomenon is unclear.

Another opposite but very interesting view about RNA granule function is that resident mRNAs can undergo translation (Lui *et al.*, 2014). In this report, *PDC1* and *ENO2* transcripts in yeasts localize to RNA granules in absence of stress and undergo translation. Upon exposure to stress, these granules contribute to formation of P-bodies. It remains to be seen if translating mRNAs are present in other granule types.

The above observations clearly indicate that P-bodies/Stress granules can contribute to mRNA stability and repression. The sorting pathways in granules that determine whether a certain mRNA will be protected, degraded or repressed remain unclear. Such outcomes are likely to be governed by the composition of individual mRNPs in granule microenvironment. Understanding the assembly and disassembly of these mRNPs would be the key to understanding overall function of granules in general.

RNA Granule Assembly

RNA granule assembly has been studied and some principles have been established. It has been reported that formation of stress granules in yeast is dependent on preformed P-bodies (Buchan *et al.*, 2008). Live cell imaging using Pab1-GFP and Edc3-mCherry (stress granule and P-body markers respectively) revealed that stress granule formation is severely impaired in strains lacking core P-body components such as Edc3 and Lsm4. On the contrary, deletion of core stress granule components Pub1, Pbp1, and eIF4G1, though affected stress granule formation but showed normal P-body formation. This suggests that the formation of stress granule requires a certain threshold concentration of P-body components (Buchan *et al.*, 2008). It is very likely that certain mRNAs resident in P-bodies get relocated to a different mRNP that nucleates stress granule formation. Since stress granules lack mRNA decay factors in general, it can be hypothesized that mRNAs earmarked for return to translation are perhaps relocated and stored in stress granules.

Intrinsically disordered regions (IDRs) have been implicated in the formation and modulation of RNA granules in general (Protter and Parker, 2016). IDRs are polypeptide segment(s) in a protein, which cannot form a three-dimensional structure but can perform specific functions (van der Lee *et al.*, 2014). Hydrophobic amino acids are required to form the hydrophobic core of globular protein, but lack of bulky hydrophobic amino acids in IDRs results in an unstructured domain in protein (Romero *et al.*, 2001). Examples of low complexity sequences include repeats of Q/N (Gln-Asn), RGG/RG (Arg-Gly-Gly), YGG (Tyr-Gly-Gly), RS (Arg-Ser), PPP (Proline-rich domain), QQQ (or, polyQ), GY/GSYGS/ GYS/SYG/ SYS. These LC regions have been implicated in binding to self and other proteins leading to the formation of higher-order structures (Calabretta and Richard, 2015).

The Q/N-rich prion-like domain of Lsm4 is important for its accumulation within P-body. Deletion of 97 amino acids in the C-terminal prion-like region of Lsm4 resulted in defective P-body formation in *Δedc3* background (Decker *et al.*, 2007). This suggests that the prion-like domain of Lsm4 can affect the formation of microscopically visible P-bodies in Edc3 dependent manner. Ccr4 (Carbon catabolite repression 4) in yeast is involved in mRNA deadenylation and contains Q/N rich region. Ccr4 fragment (1-229) containing the Q/N rich region, but not the fragment lacking it, is prone to form P-bodies under normal conditions and shows increased localization to foci under stress (Reijns *et al.*, 2008). It was observed that the prion-like Q/N-rich region of Ccr4 is essential for its localization and accumulation within microscopically visible foci.

Stress granule protein TIA-1 (T-cell intracellular antigen-1) contains three N-terminal RNA-binding domains (RBDs) and a poly(Q/N) rich region in its C-terminal region. TIA-1 has been found to induce stress granule formation in mammalian cells (Gilks *et al.*, 2004; Kawakami *et al.*, 1992). Knockout of TIA-1 in mouse impairs the formation of stress granule whereas, the overexpression of wild-type TIA-1 induces the formation of stress granule. Interestingly, overexpression of RBDs does not lead to the formation of stress granule, but overexpression of poly(Q/N)-rich region promotes RNA granule formation. These results, suggest that poly(Q/N) rich IDRs in the C-terminal region of TIA-1 is important for the formation of stress granules (Gilks *et al.*, 2004; Kawakami *et al.*, 1992). It is interesting to note that granulophagy has been recently reported to be a stress

granule clearance mechanism (Buchan *et al.*, 2013). In yeast it depends on Cdc48 (ortholog of human Valosin containing protein). It is not currently understood if there are any specificity determinants involved in this process to ensure that only specific mRNPs resident in stress granules are transported to vacuoles.

Serine rich MEG (Maternal – effect germline defective) proteins are important P-granule components in worms. These proteins contain intrinsically disordered regions (rich in Serine and Aspargine) and recently it has been observed that serine phosphorylation of these proteins regulates P-granule dynamics (Wang *et al.*, 2014). One of these proteins, MEG3 phase separates upon binding to RNA, which contributes to P-granule asymmetry (Smith *et al.*, 2016).

Human Fragile X Mental Retardation protein (hFMRP) contains RGG motif, which is found to be the key determinant for FMRP granule formation. Cells expressing full-length FMRP form distinct and prominent cytoplasmic foci, whereas the RGG motif deletion mutant of FMRP fails to localize to cytoplasmic foci and was found to be distributed throughout the cytoplasm (Mazroui *et al.*, 2003). Similar observation was made with other domain deletions as well. This suggests that RGG motif (along with other domains) in FMRP is essential for localization to foci. The RGG-motif of PGL-3 protein, an important germ granule component, binds other mRNPs through its RGG-motif and associates with itself through self-association domain to mediate germ granule assembly (Hanazawa *et al.*, 2011).

Overall observations in literature suggest that low complexity sequences, which lead to formation of intrinsically disordered regions (IDRs) play an important role in assembly of RNA granules. It is important to note that granules form under certain specific conditions. It would be logical to assume that certain regulatory mechanisms would prevent the formation of RNA granules under normal conditions. Identifying these mechanisms and understanding disassembly upon removal of stress are indeed exciting areas of research being currently pursued in the field.

Diseases Related to RNA Granules

Recent studies have pointed towards the connection between RNA granules and certain neurodegenerative diseases known to be associated with the misregulated protein aggregation (Stoppini, 2004). TDP-43 (Transactivating Response Region DNA binding protein of 43 kDa) a principal protein involved in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia, forms ubiquitinated inclusion bodies in the neuronal cells of patients (Neumann *et al.*, 2006). TDP-43 generally localizes to the nucleus and has been implicated in regulating splicing and mRNA stability (Costessi *et al.*, 2014; Da Cruz and Cleveland, 2011; Lagier-Tourenne *et al.*, 2010). Due to mutations in the TDP-43 protein, it is transported to cytoplasm from nucleus, where it accumulates as large cytoplasmic aggregates (Kwong *et al.*, 2007). It is proposed that aberrant granule formation by TDP43 in cytoplasm is one of the reasons for disease pathology. About 44 mutations have been identified in TDP-43 protein in ALS and FTLD patients, with most of them located in C-terminal glycine-rich region (Da Cruz and Cleveland, 2011; Li *et al.*, 2013). TDP-43 consists of two RNA-recognition motifs (RRM1 and RRM2) required for the binding of TDP-43 to transcripts. It also contains an RGG-motif, which is required for the binding of TDP-43 protein to RNA-binding proteins such as, hnRNP A1, hnRNP A2/B1 (King *et al.*, 2012). This facilitates TDP-43 to undergo reversible stress granule formation (Liu-Yesucevitz *et al.*, 2010). TDP-43 can undergo disease related post-translational modifications such as hyperphosphorylation, ubiquitination and proteolytic cleavage (Lagier-Tourenne *et al.*, 2010; Romano *et al.*, 2014).

Aggregation of Fused in Sarcoma (FUS), an RGG-motif containing RNA binding protein, encoded by '*FUS*' gene has also been reported to play role in various neurodegenerative diseases like ALS (Amyotrophic Lateral Sclerosis), FTLD (Frontotemporal Lobar Degeneration), and Huntington disease. FUS is a nuclear-cytoplasmic shuttle protein, which can bind both to DNA and RNA (Deng *et al.*, 2014). Wild-type FUS can undergo liquid-liquid phase separation (LLPS), wherein one or more proteins can organize and compartmentalize their associated components. This creates a non-membrane bound organelle, which is dynamic and can rapidly exchange molecules with the cell cytoplasm. This, in turn, increases the local concentration of a particular macromolecular component within the separated phase (Patel *et al.*, 2015). Disease-causing mutations

in FUS trigger the transformation from reversible LLPS form to irreversible protein aggregates (i.e. FUS aggregates) or amyloid structures in the cell, which causes the FUS pathology (Bolognesi *et al.*, 2016; Patel *et al.*, 2015). FUS pathology has been described by FUS-immunoreactivity to inclusions in neuronal and glial cells. Inclusions are predominantly observed in the cytoplasm, but not in the nucleus (Neumann *et al.*, 2009a; Neumann *et al.*, 2009b; Vance *et al.*, 2009). More than 50 different FUS mutations are reported in patients with ALS (Deng *et al.*, 2014). It has been implicated that the serine/tyrosine/glycine/glutamine (SYGQ) rich low complexity domain (LC) domain of FUS protein is required for FUS aggregation in yeast (Fushimi *et al.*, 2011; Kato *et al.*, 2012). Tyrosine to Serine mutations in this domain prevent the recruitment of FUS into stress granules (Kato *et al.*, 2012). This contradicts the results stating SYGQ domain is not required for the recruitment of FUS mutant into stress granules (Bentmann *et al.*, 2013). It has been proposed that the mutations in the tyrosine residues in SYGQ domain could interfere with other interactions required for localization to stress granules by forming aberrant associations with other proteins (Bentmann *et al.*, 2013). Arginine methylation of FUS by arginine methyltransferases 1 and 8 (PRMT1 and PRMT8), contributes to the pathogenesis of ALS-FUS, which regulates nuclear-cytoplasmic localization of mutant FUS-related in ALS (Dormann *et al.*, 2012; Scaramuzzino *et al.*, 2013; Tradewell *et al.*, 2012). Formation of both stress granule and insoluble FUS-protein aggregates is affected by methylation of arginine residues in FUS protein (Yamaguchi and Kitajo, 2012).

Yeast has been used as the model system to identify modifiers of FUS and TDP-43 related toxicity (Couthouis *et al.*, 2011; Sun *et al.*, 2011). These studies have identified various yeast factors that modulate toxicity of above proteins. Interestingly, many of these yeast factors are conserved in humans that are RNA-binding proteins with IDRs. These proteins are also known components of RNA granules. This highlights the role of RNA granules in FUS and TDP-43 pathology. An emerging idea in the field is that interventions that may help in clearance of these pathological aggregates containing RNA might be effective in treating the disease. This continues to be an active area of research.

Concluding Remarks

To end, RNA granules are fascinating higher order structures containing mRNAs and proteins that are conserved from yeast to humans. Diverse mechanisms contribute to assembly of these structures. Interestingly, despite many studies, it is not entirely clear what is the function of RNA granules. It is also unclear how do these structures disassemble. Studies focusing on the understanding of RNA granule function and disassembly will provide insights into the regulation of various cellular processes and some neurodegenerative processes.

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