Cytoplasmic mRNP granules at a glance

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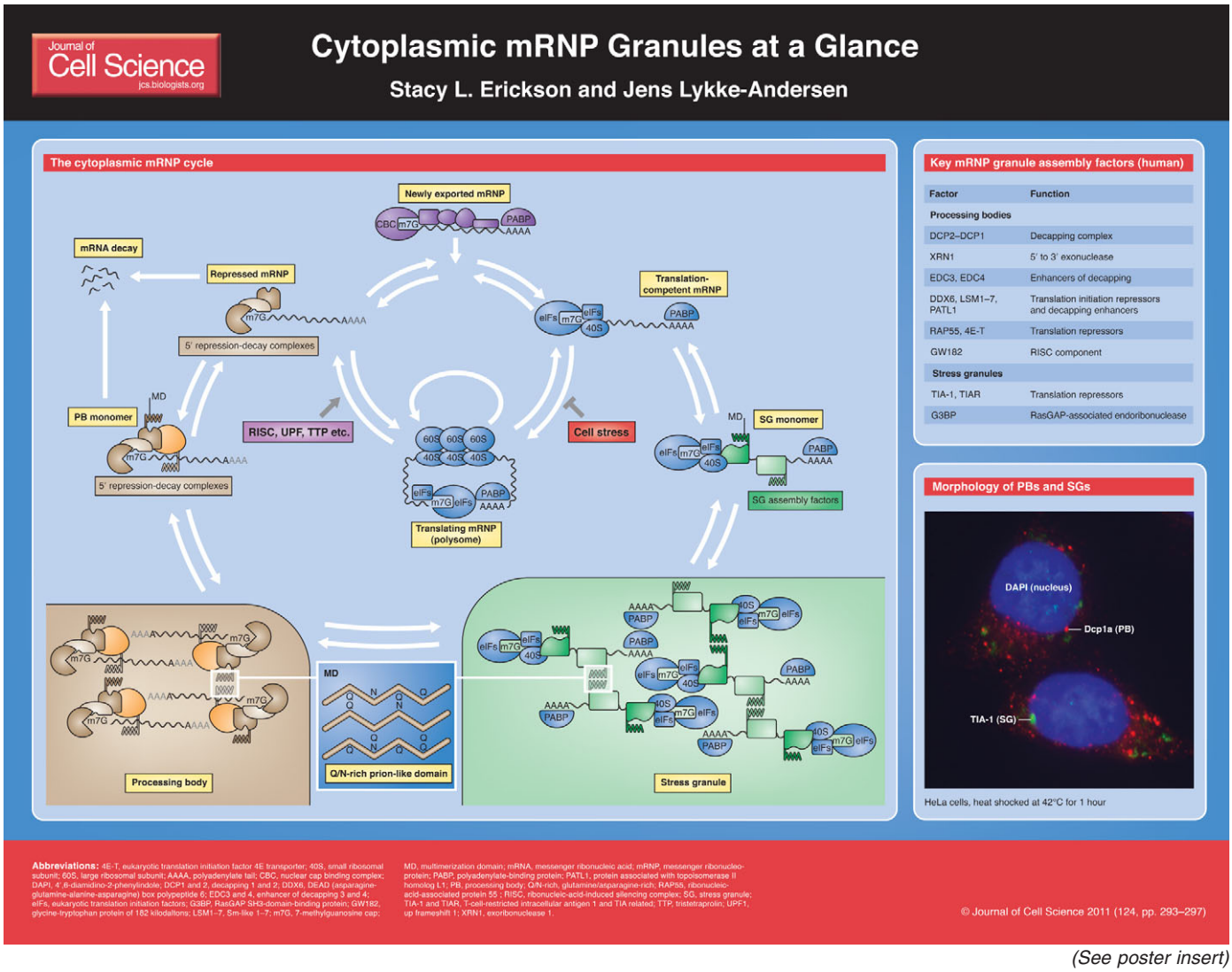
Introduction

From their transcriptional birth to their degradation, cellular mRNAs are coated with proteins in messenger ribonucleoprotein (mRNP) complexes. The mRNP composition controls every aspect of the life of the mRNA, from pre-mRNA processing to mRNA localization, translation and turnover. Transitions between these events are accompanied by major mRNP remodeling and exchange of mRNP proteins. Upon entering the cytoplasm, the mRNP composition dictates whether the mRNA engages in translation, or remains translationally inactive and is subject to either storage or degradation (see Poster). In recent years, it has become clear that many translationally inactive mRNPs have the ability to assemble into cytoplasmic mRNP granules (for reviews, see Anderson and Kedersha, 2009; Arkov and Ramos, 2010; Buchan and Parker, 2009; Eulalio et al., 2007a; Franks and Lykke-Andersen, 2008; Kulkarni et al., 2010; Zeitelhofer et al., 2008). The best-characterized mRNP granules in the somatic cell cytoplasm are processing bodies (PBs) and stress granules (SGs). To outline the current understanding of cytoplasmic mRNP granules, we will discuss the protein complexes required for the assembly of mRNPs into PBs and SGs, the conditions under which assembly occurs and the potential outcomes of assembling mRNPs into large macromolecular complexes. This discussion is relevant also to other cytoplasmic mRNP granules, for example those found in germ cells and neuronal cells, and during early development (Anderson and Kedersha, 2009; Arkov and Ramos, 2010; Zeitelhofer et al., 2008), because these mRNP granules probably function in a similar manner to PBs and SGs.

Morphology and movement of PBs and SGs

PBs and SGs are highly dynamic membrane-less cytoplasmic granules of translationally repressed mRNPs and are observed in a wide variety of eukaryotes. Whereas SGs are primarily observed during cell stress, PBs are generally observed under normal growth conditions, although in human cell lines, visible PBs disappear during mitosis and quiescence (Yang et al., 2004). Under the microscope, PBs generally seem discrete and rounded, whereas



(See poster insert)

SGs can seem more diffuse (see Poster). Electron microscopy of human cells shows that PBs can range from 100 to 300 nm in diameter (Yang et al., 2004) and SGs, formed upon overexpression of the SG assembly factor TIA-1 (T-cell-restricted intracellular antigen 1), can average 100–200 nm (Gilks et al., 2004; Yang et al., 2004). Fluorescence recovery after photobleaching (FRAP) experiments revealed that many components cycle rapidly in and out of PBs and SGs, although a subset are more static (Aizer et al., 2008; Andrei et al., 2005; Eisinger-Mathason et al., 2008; Fujimura et al., 2008a; Fujimura et al., 2008b; Guil et al., 2006; Kedersha et al., 2000; Kedersha et al., 2005; Leung et al., 2006; Mollet et al., 2008). Real-time imaging of human cell lines shows that most PBs and SGs move in an apparently random manner. A subset of PBs can appear static, whereas occasionally rapid directional movement of PBs or SGs can be observed (Aizer et al., 2008; Kedersha et al., 2005; Nadezhdina et al., 2010; Yang et al., 2004). Some evidence indicates a role for the cytoskeleton in PB and SG dynamics. For example, microtubule-depolymerizing drugs can lead to impaired SG formation (Fujimura et al., 2009; Ivanov et al., 2003; Kolobova et al., 2009; Kwon et al., 2007; Loschi et al., 2009), impaired SG and PB movement, and enlarged PBs (Aizer et al., 2008; Sweet et al., 2007). By contrast, actin depolymerization does not affect SG assembly (Ivanov et al., 2003; Kwon et al., 2007) and PBs associated with actin in human cells do not appear mobile (Aizer et al., 2008). Microtubule motor proteins can also affect SG and PB dynamics. Inhibition of dynein function can lead to impaired SG formation and enlarged PBs in response to stress (Kwon et al., 2007; Loschi et al., 2009; Tsai et al., 2009), whereas depletion of kinesins can delay the disassembly of SGs and rescue the assembly defects caused by dynein depletion (Loschi et al., 2009). Although these observations suggest functional interplay between SGs and PBs and the cytoskeleton, pleiotropic effects arising from cytoskeletal manipulation make it difficult to pinpoint its importance and relevance.

The composition and function of PBs

Factors involved in PB assembly

PBs are assemblies of translationally inactive mRNPs and RNA is central to the PB structure. Accordingly, PBs dissociate upon RNase treatment of permeabilized *Drosophila* S2 cells and *Saccharomyces cerevisiae* cell extracts (Eulalio et al., 2007b; Teixeira et al., 2005). Ribosomal subunits have not been detected in PBs, suggesting that mRNPs must be free of ribosomes to assemble into a PB. This is further supported by evidence that trapping mRNPs in complex with ribosomes using translation elongation inhibitors prevents PB assembly (Cougot et al., 2004; Eulalio et al., 2007b; Teixeira et al., 2005). Conversely, conditions that inhibit mRNP association with ribosomes can enhance PB assembly (Brengues et al., 2005; Cougot et al., 2004; Eulalio et al., 2007b; Franks and Lykke-Andersen, 2007; Teixeira et al., 2005). Although the lack of associated ribosomes appears to be a precondition for the assembly of an mRNP into PBs, evidence suggests it is not sufficient; mRNPs also need to associate with protein complexes that promote PB assembly (Eulalio et al., 2007b; Franks and Lykke-Andersen, 2007).

Key factors that promote the assembly of mRNPs into PBs include decapping factors and associated proteins that repress translation initiation, and activate decapping and 5′ to 3′ decay of the mRNA (see Poster) (for reviews, see Coller and Parker, 2004; Eulalio et al., 2007a; Franks and Lykke-Andersen, 2008; Simon et al., 2006). Evidence suggests that multiple alternative complexes between these factors exist that, depending on the specific complex composition and on cell conditions, either promote the repression of translation initiation only or additionally activate 5′ to 3′ decay of target mRNAs (Decker et al., 2007; Haas et al., 2010; Pilkington and Parker, 2008; Tritschler et al., 2009; Tritschler et al., 2008; Yoon et al., 2010). Several lines of evidence suggest that recruitment of these 5′ repression-decay complexes allows mRNP assembly into PBs (Anderson and Kedersha, 2009; Balagopal and Parker, 2009; Eulalio et al., 2007a; Franks and Lykke-Andersen, 2008; Kulkarni et al., 2010). First, all of these factors are enriched in PBs. Second, PB formation is impaired upon depletion of factors that recruit the 5′ repression-decay complexes to mRNAs (Eulalio et al., 2007b; Jakymiw et al., 2005; Liu et al., 2005; Pauley et al., 2006; Sheth and Parker, 2003; Zheng et al., 2008). Third, depletion or overexpression of individual 5′ repression-decay factors affects PB formation (Anderson and Kedersha, 2009; Balagopal and Parker, 2009; Eulalio et al., 2007a; Franks and Lykke-Andersen, 2008; Kulkarni et al., 2010). The specific effects that manipulating different 5′ repression-decay factors have on PBs can vary between organisms and conditions, probably reflecting differences in factors that are rate limiting for formation of the most abundant mRNPs in the PB under the given conditions.

Recruitment of 5′ repression-decay complexes to mRNPs

5′ repression-decay complexes can be recruited to mRNPs either through their intrinsic RNA affinity or through recruitment by mRNP-specific factors (see Poster). Some decapping factors are recruited by specific RNA sequences or structures in their target mRNAs (Badis et al., 2004; Chowdhury et al., 2007; Dong et al., 2007; Li et al., 2008; Tharun and Parker, 2001). More often, 5′ repression-decay factors interact with mRNA-specific protein complexes that promote translation initiation inhibition, mRNA decay or both. These include the RNA-induced silencing complex (RISC), which silences mRNAs that are targeted by microRNAs (miRNAs) or small interfering RNAs (siRNAs) (Jakymiw et al., 2005; Liu et al., 2005; Pauley et al., 2006); the Upf complex, which targets mRNAs with premature termination codons for nonsense-mediated decay (Durand et al., 2007; Sheth and Parker, 2006); and the related proteins tristetraprolin (TTP) and butyrate response factor 1 (BRF1), which target AU-rich element (ARE)-containing mRNAs for decay (Franks and Lykke-Andersen, 2007). Evidence suggests that RISC-associated GW182 proteins promote RISC-bound mRNP assembly into PBs also independently of 5′ repression-decay factors (Behm-Ansmant et al., 2006; Eulalio et al., 2009).

Mechanism of PB assembly

To assemble mRNPs into PBs, PB assembly complexes must release the mRNP from polysomes and promote association with other repressed mRNPs (Anderson and Kedersha, 2009; Balagopal and Parker, 2009; Eulalio et al., 2007a; Franks and Lykke-Andersen, 2008; Kulkarni et al., 2010). Accordingly, many 5′ repression-decay factors have been implicated in repressing translation initiation (Coller and Parker, 2005; Ferraiuolo et al., 2005; Minshall and Standart, 2004; Tanaka et al., 2006; Tritschler et al., 2010). Given that ribosomes prevent mRNP assembly into PBs, ribosomes probably need to 'run off' the repressed mRNA before PB association. In *S. cerevisiae*, ribosomes can continue translation elongation even after mRNPs have undergone decapping and initiated 5′ to 3′ decay (Hu et al., 2009). Thus, the extent to which individual mRNPs accumulate in PBs is most probably determined not only by their affinity for PB assembly complexes, but also by the relative rates of ribosome run off and mRNA decay. Consistent with this, increased PB formation occurs under conditions that slow mRNA decay rates or enhance translational initiation repression (Coller and Parker, 2005; Cougot et al., 2004; Fenger-Grøn et al., 2005; Franks and Lykke-Andersen, 2007; Sheth and Parker, 2003).

Evidence indicates that, once ribosomes run off, specific multimerization domains present in PB assembly factors are important for the

mRNP to assemble into a PB. Several PB assembly factors contain prion-like glutamine and asparagine (Q/N)-rich regions (see Poster). The best-studied region is in the C terminus of *S. cerevisiae* Lsm4, a component of the 5′ to 3′ decay-stimulating Lsm1–7 complex. Deletion of this Q/N-rich domain is associated with impaired PB assembly (Decker et al., 2007; Reijns et al., 2008). Although Q/N-rich regions appear to be lacking from most metazoan Lsm4 proteins, other PB assembly factors contain Q/N-rich regions. Overexpression studies suggest that some of these might play important roles in the assembly of mRNPs into PBs, although further studies are needed to verify this (Behm-Ansmant et al., 2006; Coller and Parker, 2005; Eulalio et al., 2009; Fenger-Grøn et al., 2005; Haas et al., 2010; Jinek et al., 2008; Yu et al., 2005). Other protein domains might also be important, as exemplified by the C-terminal homodimerization domain of the decapping enhancer Edc3, which is important for PB formation in *S. cerevisiae* (Decker et al., 2007; Ling et al., 2008). Important questions for future studies include determining why, as all evidence suggests, PB assembly complexes only assemble into PBs when they are associated with RNA and what prevents polysome-associated mRNPs from assembling into PBs.

Release of mRNPs from PBs

The majority of mRNPs in PBs seem to be only transiently associated. FRAP experiments show that many factors rapidly cycle in and out of PBs (Aizer et al., 2008; Andrei et al., 2005; Fujimura et al., 2008b; Kedersha et al., 2005; Leung et al., 2006), and PBs rapidly disappear when new mRNPs are prevented from entering PBs upon treatment of *S. cerevisiae*, *Drosophila* or human cells with translation elongation inhibitors (Cougot et al., 2004; Eulalio et al., 2007b; Teixeira et al., 2005). Evidence suggests that the release of mRNPs from PBs involves either mRNA decay or release of the mRNP back into translation. For example, inhibiting 5′ to 3′ decay can cause enhanced PB accumulation (Cougot et al., 2004; Fenger-Grøn et al., 2005; Franks and Lykke-Andersen, 2007; Sheth and Parker, 2003). Conversely, PBs are depleted upon reactivation of translation of mRNPs that have been repressed by glucose starvation in *S. cerevisiae* (Brengues et al., 2005) and a specific miRNA-repressed mRNA in human cells has been observed to leave the PBs once repression is lifted (Bhattacharyya et al., 2006). However, some mRNPs might remain static in PBs, as suggested by the slow cycling of some PB factors (Aizer et al., 2008; Andrei et al., 2005; Kedersha et al., 2005; Leung et al., 2006). The factors that determine whether PB-associated mRNPs are targeted for mRNA decay or translational repression alone remain an important subject for future studies.

PB function

The functional consequence of the assembly of mRNPs into PBs remains unclear. Manipulations that lead to the loss of visible PBs in *S. cerevisiae*, *Drosophila* or human cells do not disrupt the various mRNA decay pathways tested (Decker et al., 2007; Eulalio et al., 2007b; Stalder and Muhlemann, 2009; Stoecklin et al., 2006). Moreover, no defect in miRNA-mediated translational repression is observed in *Drosophila* S2 cells that are depleted of visible PBs (Eulalio et al., 2007b). Although it is difficult to rule out that sub-microscopic PBs exist under such conditions, these observations suggest that mRNP assembly into macroscopic PBs is not rate limiting for the repression and turnover of these substrates. Future work to determine the protein domains that are crucial to mRNP assembly into PBs and how they are recruited to specific mRNAs might provide important clues to the significance of mRNP assembly into PBs. Currently, it can only be speculated that PBs might serve functions such as sequestering mRNA decay enzymes away from the cytoplasm to prevent promiscuous mRNA decay, concentrating decay enzymes to enhance the kinetics of currently untested mRNA decay pathways or preventing repressed mRNPs from competing for the translation machinery when decay factors are limiting. The conserved ability of eukaryotic cells to assemble mRNPs into PBs suggests that some important function must exist. Delineating this function is a key challenge for future research in the field.

The composition and function of SGs

The composition of mRNPs that assemble into SGs

In contrast to PBs, SGs contain components of the small ribosomal subunit, several translation initiation factors and poly(A)-binding protein. This suggests that mRNPs that assemble into SGs are stalled at a step in translation initiation after the recruitment of a subset of the translation initiation machinery (see Poster). Consistent with this observation, assembly of mRNPs into SGs is induced in a number of conditions that stall translation at the initiation step, including depletion of initiation factors and exposure to cell stresses or to drugs that impair translation initiation or cause the dissociation of ribosomes (for reviews, see Anderson and Kedersha, 2009; Buchan and Parker, 2009). Of note, knockdown of subunits of eukaryotic translation initiation factor eIF3 inhibits SG formation in human cells, suggesting that this factor plays an important role in SG assembly or that eIF3 depletion stalls the mRNP at a step at which it is not competent for SG assembly (Ohn et al., 2008). It is unclear whether assembly of mRNPs with translation initiation components is a requirement for their assembly into SGs. For example, some translation factors only accumulate in SGs under certain conditions (Buchan et al., 2008; Grousl et al., 2009; Hoyle et al., 2007). Thus, it is likely that mRNPs that assemble into SGs can be stalled at any one of several steps in translation initiation.

Mechanism of mRNP assembly into SGs

It is unclear whether all mRNPs that are stalled at a step of translation initiation can assemble into SGs or whether additional factors have to be independently recruited. Factors that can promote mRNP assembly into SGs include the homologous RNA-binding proteins TIA-1 and TIAR (TIA related), and the RasGAP (GTPase-activating protein)-associated endoribonuclease G3BP. All are highly abundant in SGs, and affect SG formation when depleted or overexpressed (Gilks et al., 2004; Kedersha et al., 1999; Ohn et al., 2008; Tourrière et al., 2003). Several other RNA-binding proteins can induce SG formation when overexpressed, but their role in assembly has not been established (Anderson and Kedersha, 2009; Buchan and Parker, 2009).

Similar to PBs, prion-like domains have been implicated in SG formation. TIA-1 and TIAR both contain a Q/N-rich domain, which, in the case of TIA-1, is required for the spontaneous induction of SGs upon overexpression and can be replaced by the prion-like domain of a heterologous protein (Gilks et al., 2004). The RNA-binding domain of TIA-1 is also required for normal SG formation (Kedersha et al., 1999), suggesting that TIA-1 is directly recruited to mRNPs.

A number of post-translational modifications affect SG assembly. For example, *O*-linked *N*-acetylglucosamine (O-GlcNac)-modified proteins have been found in SGs and depletion of components of the O-GlcNac modification pathway was shown to impair SG assembly (Ohn et al., 2008). In addition, lysine deacetylation (Kwon et al., 2007), arginine methylation (De Leeuw et al., 2007; Dolzhanskaya et al., 2006; Goulet et al., 2008; Hua and Zhou, 2004) and deubiquitylation (Ohn et al., 2008) have all been implicated in SG formation. The relationship between these post-translational modifications and their role in the formation of SG-competent mRNPs remain important points to be addressed in future investigations.

Possible functions of SGs

Similar to PBs, the significance of the assembly of mRNPs into SGs remains unclear. Although

several factors that are involved in SG formation are translational repressors, there is a lack of clear evidence that the assembly of mRNPs into SGs in itself is important for translational repression (Buchan et al., 2008; Fujimura et al., 2009; Kwon et al., 2007; Loschi et al., 2009; Mokas et al., 2009; Ohn et al., 2008). The stabilization of mRNAs that occurs during many stresses also does not seem to require visible SGs (Buchan et al., 2008). An alternative idea that has been put forth is that mRNPs that are assembled into SGs remain poised to re-enter translation as soon as stress is relieved (Buchan and Parker, 2009). Consistent with this, several studies have presented evidence that the ability to form SGs is correlated with the survival of cells exposed to stress (Buchan and Parker, 2009). Understanding the role of SG formation in mRNP function remains an important goal of future studies.

Relationship between the mRNPs in PBs and SGs

Immunofluorescence assays have revealed that PBs and SGs often, but not always, exist in close proximity, a characteristic that can be enhanced when certain SG and PB components are overexpressed (Kedersha et al., 2005). Recent studies in *S. cerevisiae* suggest that PBs can transform into SGs during glucose deprivation, suggesting that, under these conditions, mRNPs in PBs undergo a transition into the mRNPs typically found in SGs (Buchan et al., 2008). This has led to the hypothesis that mRNPs in PBs, SGs and polysomes represent different steps of a cycle from translationally repressed mRNPs to fully translated mRNPs (see Poster); the formation of PBs and SGs is thus simply a consequence of the accumulation of mRNPs at a specific step of this cycle when a transition becomes rate limiting for a considerable fraction of cellular mRNAs (Balagopal and Parker, 2009; Buchan and Parker, 2009; Franks and Lykke-Andersen, 2008). The transitions in mRNP composition that occur between these steps control whether the mRNA is translated, stored or degraded, and are thus important topics of future investigation.

Perspectives

Although several of the factors and conditions that lead to assembly of mRNPs into mRNP granules are now known, the importance of this assembly remains elusive. Because of the highly conserved nature of cytoplasmic mRNP granules in eukaryotes, there is little doubt that the ability to assemble mRNPs into mRNP granules must provide some benefit to cells. Whether this benefit is gained from the ability to form PBs, SGs or more specialized mRNP granules remains to be seen. The rules that govern the formation and disassembly of mRNP granules are likely to be highly similar among granules. Thus, extensive studies of PBs and SGs should help generate tools for investigating the biological importance of assembling mRNPs into various granules. The role of granule formation in the life of cytoplasmic mRNPs thus remains a key question for future studies.

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