

The role of stress-activated RNA–protein granules in surviving adversity

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ABSTRACT

Severe environmental stress can trigger a plethora of physiological changes and, in the process, significant cytoplasmic reorganization. Stress-activated RNA–protein granules have been implicated in this cellular overhaul by sequestering pre-existing mRNAs and influencing their fates during and after stress acclimation. While the composition and dynamics of stress-activated granule formation have been well studied, their function and impact on RNA-cargo has remained murky. Several recent studies challenge the view that these granules degrade and silence mRNAs present at the onset of stress and instead suggest new roles for these structures in mRNA storage, transit, and inheritance. Here we discuss recent evidence for revised models of stress-activated granule functions and the role of these granules in stress survival and recovery.

Keywords: P-body; stress granule; environmental stress; RNA fates

INTRODUCTION

All cells must respond to diverse environmental stresses in order to survive, thrive, and compete in nature. A successful stress response often requires rapid physiological changes customized to specific conditions to maintain a functional cellular state, but also to prepare for the resumption of normal processes once cells acclimate to the new conditions. A key component of these responses is the reorganization of gene expression in a way that rapidly alters cellular processes and states but also prepares for stress abatement. Many stress responses invoke major alterations to the transcriptome and translome through changes in nascent transcription, regulated turnover of specific transcripts, and controlled translation. In response to severe stress, translation is globally attenuated while critical, stress-responsive transcripts are selectively translated (for review, see Advani and Ivanov 2019). mRNA decay dynamics can also be regulated in a stress-specific manner, further influencing protein production. Pre-existing transcripts not prioritized for translation can be either stabilized in a non-translating state or targeted for degradation. In yeast, changes in decay are sometimes counterintuitively related to changes in mRNA abundance, where induced mRNAs display accelerated turnover while repressed RNAs show prolonged half-lives (Shalem et al. 2008; Miller et al. 2011). Many studies have

probed how cells regulate the dynamics of mRNA synthesis, decay, and translation on a systems-wide level during an acute stress response and as cells acclimate to environmental stress.

Less obvious is the role of stress-activated changes in RNA localization within the cell and how those changes can affect functions and fates, especially during stress. It is now appreciated that many mRNAs are not randomly suspended throughout the cytoplasm but rather localize to specific sites within the cell (Aw et al. 2016; Fazal et al. 2019). Some RNAs are localized to organelles like mitochondria and the ER, and mechanisms of their delivery are beginning to become clear (Lerner et al. 2003; Gadir et al. 2011; Kaewsapsak et al. 2017; for review, see Chin and Lécuyer 2017). Many other mRNAs are localized to membrane-less structures including phase-separated mRNA–protein (mRNP) granules.

mRNP granules are components of eukaryotic stress-responsive cellular reorganization observed from yeast to plants to humans. Among the most widely studied stress-induced mRNP granules are processing bodies (P-bodies) and stress granules (SGs). Both form during acute stress, but they are distinguished from one another by protein composition as well as formation dynamics (Jain et al. 2016; Kershaw et al. 2020; for review, see Youn et al.

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2019). There are also nuclear-localized stress-induced RNP granules which are thought to assist with global reprogramming of gene expression, although they have received less study to date (for review, see Staněk and Fox 2017). For example, mammalian nuclear stress bodies are induced by heat shock and are thought to play a role in stress-responsive reprogramming of gene expression through trapping of transcription and splicing factors (for review, see Biamonti and Vourc'h 2010). Mammalian paraspeckles are similarly implicated in RNA retention and protein sequestration (for review, see Pisani and Baron 2019). These were recently reported to be dependent on prior SG formation in some stress contexts (An et al. 2019; for review, see McCluggage and Fox 2021) and contain many of the same protein components as SGs (An et al. 2019). There are likely other yet-to-be-described stress-induced mRNP granules, and examples are already mounting of noncanonical and hybrid granules that contain both P-body and SG components (Aronov et al. 2015; Shah et al. 2016; Pizzinga et al. 2019). Although biophysical features of P-body and SG formation are emerging, a major unanswered question is the role and impact these granules play with regard to the RNAs they contain. Although granule functions were originally assumed to match the functions of their constituent proteins, this assumption is called into question by recent work. Here we review what is known about the fates of granule-associated mRNAs, proposed functions of stress-induced mRNP granules, and their physiological consequences that enable a successful stress response and recovery (topics in the review text are summarized in Fig. 1).

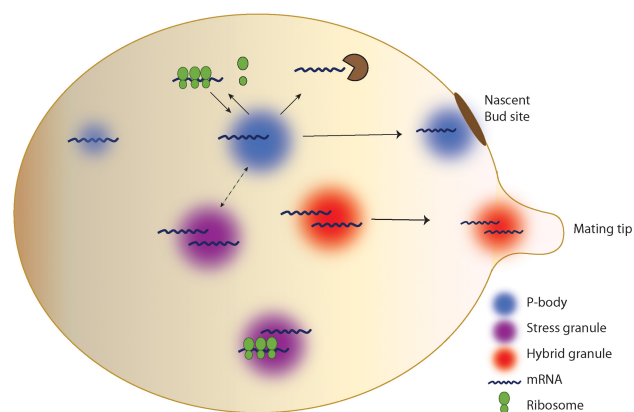


FIGURE 1. A summary of the roles of cytosolic stress-activated mRNP granules. A model for functions discussed in the text as depicted in a yeast cell. mRNAs can exit the translating pool to associate with granules. Granule-released mRNAs can be subsequently degraded or reassociated with polysomes. P-bodies, SG and hybrid granules are linked to mRNA and protein cargo trafficking and delivery in the cell, including to the nascent bud site and mating-tip projection. Hybrid granules contain mixed P-body and SG components. See text for details and references.

P-body and SG composition and structure

Distinct from membrane-bound organelles, mRNP granules are biomolecular condensates that form through liquid–liquid phase separation (LLPS). LLPS is a physical process that occurs in supersaturated solutions to produce concentrated droplets or granules suspended within a more-fluid medium, ultimately producing an emulsion (for review, see Boeynaems et al. 2018). Membrane-less organelles are ascribed a role in intracellular organization and can enable spatiotemporal biomolecular control to regulate cellular reactions (Hondele et al. 2019; for review, see Banani et al. 2017). Many of the protein components in mRNP granules contain intrinsically disordered regions, which are often critical for granule association (Protter et al. 2018; Boncella et al. 2020; for review, see Protter and Parker 2016). Phase-separated membrane-less compartments can both form and dissociate in response to specific signals. P-bodies appear to be constitutive, though much smaller in size and number during times of regular growth, and grow in size and abundance upon stress or inhibition of protein kinase A signaling in yeast (Ramachandran et al. 2011; Paige et al. 2019; Barraza et al. 2021). SGs are induced upon stress and their formation is linked to eIF2 α phosphorylation in mammalian cells (Kedersha et al. 1999), yet in other eukaryotic organisms SGs assemble in both an eIF2 α -dependent and -independent manner (Kramer et al. 2008; Grousl et al. 2009; Kato et al. 2011; Nilsson and Sunnerhagen 2011; Shah et al. 2013). It has even been proposed that granule formation itself can be a stress sensor, as some proteins rapidly phase-separate upon environmental shifts like rising temperature, pH and osmotic shifts (Wallace et al. 2015; Riback et al. 2017; Iserman et al. 2020; Watanabe et al. 2021; for review, see Yoo et al. 2019). While P-bodies and SGs share important similarities across model organisms, there are stress- and species-specific differences in composition, assembly, and induction dynamics, which are discussed in several comprehensive articles (Buchan et al. 2011; Thomas et al. 2011; Aulas et al. 2017; Markmiller et al. 2018; for review, see Guzikowski et al. 2019).

P-bodies and SGs are formed from a coterie of proteins with well-defined functions in the cytoplasm at large. Their composition includes core sets of proteins, but each granule type can associate with accessory or client proteins that may provide distinctions or specialization (Jain et al. 2016; Kershaw et al. 2020; Xing et al. 2020; for review, see Youn et al. 2019). Core P-body proteins comprise decapping machinery, activators of decapping, exonucleases, and deadenylation machinery, while core SG proteins include translation initiation factors, ribonucleases, and translational repressors. Many of the accessory proteins are RNA-binding proteins (RBPs) that may play a role in localizing specific RNAs to mRNP granules (Jain et al. 2016; Markmiller et al. 2018; for review, see

Guzikowski et al. 2019; Youn et al. 2019). The presence and concentration of these accessory proteins vary across individual granules, and many associate with only subsets of granules within cells (Xing et al. 2020). Some studies propose a scaffold-client-like model of P-body composition, where core proteins are critical for granule assembly and structure while accessory proteins are dispensable for assembly but critical for granule function (Banani et al. 2016; Ditlev et al. 2018; Zhang et al. 2019; Xing et al. 2020).

While it is well appreciated that mRNAs are contained within stress-induced mRNP granules, less is known about how mRNAs become associated. Arresting ribosomes on transcripts with translational inhibitors, such as cycloheximide, in many cases prevents granule formation (Sheth and Parker 2003; Bounedjah et al. 2014), suggesting that mRNAs must be released from ribosomes to localize to granules. However, although ribosome-free mRNAs localize more readily to granules, those bound by ribosomes can still associate and can even be translated within granules (see more below, Lui et al. 2014; Moon et al. 2019; Pizzinga et al. 2019; Mateju et al. 2020). Several RBPs are thought to direct their mRNA targets to granules (Kurischko et al. 2011; Simpson et al. 2014; Wang et al. 2018; Ford et al. 2019), and RNA–RNA interactions can also be important (Van Treeck et al. 2018; Matheny et al. 2021). Furthermore, mRNA secondary structure and compaction can influence whether mRNAs are recruited to or excluded from membrane-less compartments, further influenced by conformational impacts of RBP binding (Langdon et al. 2018). After an RNA becomes associated with a granule, it can move bidirectionally between P-bodies and SGs, and even leave the granules to return to the cytosol (Bregues et al. 2005; Kedersha et al. 2005; Bhattacharyya et al. 2006; Zhang et al. 2011; Moon et al. 2019; Mateju et al. 2020).

PB and SG functions

The protein and RNA composition of P-bodies and SGs is emerging through proteomic and sequencing-based investigations (for example, see Jain et al. 2016; Hubstenberger et al. 2017; Khong et al. 2017; Namkoong et al. 2018; Wang et al. 2018; Kershaw et al. 2020). But elucidating the functions of these granules has lagged, in part because many of the granule components have other functions in cells. Simply ablating granule proteins through gene deletion can have other ramifications, complicating the study of granule function. Granules were originally thought to function according to the cellular roles of their protein components, but clever approaches to study those functions present new hypotheses about the importance of granules during stress. Below we highlight recent insights into P-body and SG functions in response to environmental cues.

The role of granules in modulating mRNA decay

P-bodies were initially implicated in mRNA decay, since core components have central roles in RNA degradation and early studies seemed to bolster this model. First, deletion of mRNA decapping factors in yeast significantly increases the size and number of P-bodies (Sheth and Parker 2003) and results in a build-up of P-body-associated mRNA, which originally suggested the granules as sites for mRNA decay that enlarge when decay is disrupted (Sheth and Parker 2003; Cougot et al. 2004; Fenger-Grøn et al. 2005). Additionally, degradation intermediates of a decay-resistant, polyG-tract-containing mRNA reporter, visualized by fluorescently labeled MS2 protein that binds the transcript, accumulated in P-bodies, again suggesting the granules as decay sites (Sheth and Parker 2003). However, it was later determined that this degradation-resistant reporter was not an accurate indicator of full-length RNA localization nor active RNA decay (Garcia and Parker 2015), and a debate on the potential biases of this early MS2 reporter system ensued (Garcia and Parker 2015, 2016; Haimovich et al. 2016; Heinrich et al. 2017). Not long after these seminal works were published, subsequent studies demonstrated that this connection between P-bodies and mRNA degradation is not so clear.

In fact, several recent studies provide compelling evidence against P-bodies serving as hubs of mRNA decay. Decker et al. (2007) showed in budding yeast that mRNA degradation proceeds normally when core P-body components Edc3 and Lsm4 are ablated to prevent P-body formation. Correspondingly, Huch et al. (2016) reported enhanced mRNA degradation in a similar *edc3Δ lsm4Δ* mutant yeast that does not form P-bodies. A subsequent study in human cell cultures by Horvathova et al. (2017) used dual-color imaging of mRNA ends to detect full-length transcripts and observed mRNA fragments exclusively outside of P-bodies. Similarly, live-cell imaging of yeast mRNAs using an updated MS2 system showed that mRNAs are degraded throughout the cytoplasm and not within P-bodies (Tutucci et al. 2018). Lastly, several recent studies discussed in the next section found intact, translationally repressed mRNAs within P-bodies, without evidence of truncated mRNAs or degradation intermediates (Horvathova et al. 2017; Hubstenberger et al. 2017). Consistent with these observations, Schutz et al. (2017) demonstrated that core P-body decapping proteins show reduced activity when contained within phase-separated droplets in vitro. Yet, we cannot completely rule out that some mRNA degradation may still occur at these sites, particularly for specific mRNAs. While P-bodies may not be active degradation hubs, they could still serve as hubs of other processes, since phase separation can enhance localized activity of some proteins (for review, see O'Flynn and Mittag 2020). While current evidence argues against P-bodies concentrating mRNA degradation, they may still

play a role in targeting mRNAs for degradation in the cytoplasm immediately upon P-body release.

RNP granules as sites of mRNA and protein storage

Several lines of evidence show that granule association can in fact insulate RNAs from translation and degradation, suggesting a role in mRNA storage. The mammalian studies mentioned above found, almost exclusively, intact mRNAs within P-bodies without strong evidence for degradation intermediates (Horvathova et al. 2017; Hubstenberger et al. 2017). Hubstenberger et al. further showed that the 5' ends of P-body-associated mRNAs in human epithelial cells produce a higher density of sequencing reads compared to unassociated mRNAs, suggesting that bound mRNAs are protected from the major 5' decay pathway (Hubstenberger et al. 2017). In a handful of cases, more direct evidence shows that altering mRNA localization impacts the half lives of those transcripts. For example, deletion of yeast P-body-associated RBP Mpt5 causes its target mRNA *ATP11* to fail to localize to P-bodies during glucose starvation, which correlates with a decrease in *ATP11* half-life. (Wang et al. 2018). Similarly, knockdown of human SG-associated RBP ZBP1 correlates with failed SG localization of its mRNA targets and shortened half-lives of those targets (Stöhr et al. 2006). These results suggest that P-bodies and SGs insulate cargo mRNAs from cytoplasmic degradation. Additional work is needed to determine if this trend is a universal consequence of granule localization, or if different RNAs experience distinct fates upon P-body and SG sequestration.

A remaining question is whether these mRNAs can be subsequently released from granules and returned to the translating pool, and evidence suggests they can. Early studies in yeast demonstrated that mRNA species localized to P-bodies during glucose starvation are found in the polysome fraction after glucose repletion, even in the absence of new transcription, suggesting that mRNAs can be released from P-bodies back to the translating pool (Bregues et al. 2005). Modern microscopy techniques that track the movement of individual RNAs support this notion. Moon et al. (2019) used multicolor single-molecule tracking (Morisaki et al. 2016) to distinguish translating from non-translating mRNAs via live-cell microscopy in human cells. They observed that mRNAs are translationally repressed prior to SG association, and those same transcripts can resume translation upon SG disassembly. The return of mRNAs to active translation suggests that stress-activated granules could serve as repositories to store translationally silenced mRNAs for later activation. Interestingly, work in plants demonstrates that mRNAs can be released from granules in response to environmental cues. P-bodies in *Arabidopsis thaliana* seedlings can bind up to ~1500 transcripts that are not translated in dark conditions but rapidly shift to the polysome-bound

fraction when seedlings encounter light (Jang et al. 2019). Similarly, a subpopulation of *Arabidopsis thaliana* mRNAs rapidly shifts from SGs to polysomes upon reoxygenation after hypoxic stress (Sorenson and Bailey-Serres 2014). These results suggest that stress-induced granules can protect or store mRNAs in one environment and release them back to the translating pool in another environment.

It is important to note that others have argued that P-bodies and SGs may not have a specialized role in controlling mRNA fates during stress, and that translation and decay status of mRNAs within granules parallels the status of mRNAs outside of P-bodies and SGs (Arribere et al. 2011; Wilbertz et al. 2019). Further still, some have reported no difference in mRNA half-life when SG formation is inhibited, raising questions about the protective role of granule association (Bley et al. 2015).

While a debate remains about whether P-bodies and SGs are specialized sites of mRNA storage, there is clear evidence that granules can protect other cargo like kinases and phosphatases during stress as well as in development and meiosis (Tudisca et al. 2010; Takahara and Maeda 2012; Amen and Kaganovich 2020; Kanda et al. 2021; for review, see Zhang and Herman 2020). One study in particular showed that the yeast protein kinase Hrr25, which is critical for the completion of meiosis, is protected from proteasome degradation by localizing to P-bodies in a manner dependent on the P-body protein Dcp2. In the absence of Dcp2, active Hrr25 fails to localize to P-bodies during glucose starvation and is degraded, whereas in wild-type cells P-body-associated Hrr25 is stabilized (Zhang et al. 2016, 2018). Amen and Kaganovich demonstrated that starvation-induced SGs are critical for sequestering active Pkc1 kinase, enabling rapid Pkc1-dependent resumption of growth upon glucose repletion (Amen and Kaganovich 2020). When SG formation is disrupted, Pkc1 is degraded, which correlates with slower reinitiation of cell growth (Amen and Kaganovich 2020).

A role for P-bodies and SGs in cargo delivery and even translation

There are well-established examples of mRNP granules playing important roles in localizing mRNAs, most notably neuronal granules that are important for long-range delivery of mRNAs and localized translation in axons (for review, see Formicola et al. 2019; Pushpalatha and Besse 2019). Stress-induced granules could play a similar role. Both P-bodies and SGs can be highly mobile via interactions with cytoskeletal structures (Nadezhdina et al. 2010; for review, see Aizer and Shav-Tal 2008; Perez-Pepe et al. 2018). This controlled movement has been observed across eukaryotic model organisms, despite some mechanistic differences (Kedersha et al. 2005; Garmendia-Torres et al. 2014; Steffens et al. 2014). P-bodies in particular can

exhibit stationary localization (when associated with actin bundles) in addition to three classified movement types linked to whether or not granules are associating with microtubules: (i) freely diffusing throughout the cell, (ii) spatially confined with restricted, erratic movements or (iii) translocating rapidly on a straight-track from one region to another (Kedersha et al. 2005; Aizer et al. 2008; Garmendia-Torres et al. 2014; for review, see Aizer and Shav-Tal 2008). In yeast, rapid, long range translocation of P-bodies from mother cells to daughter cells is myosin-motor dependent (Garmendia-Torres et al. 2014). Less is known about specific SG movements, although SGs can move throughout the cytoplasm in a microtubule dependent manner, and microtubules are critical for SG assembly and disassembly (Nadezhdina et al. 2010; for review, see Bartoli et al. 2011).

Controlled granule movement along the cytoskeleton could deliver mRNAs to specific cellular sites for localized translation. Localized translation is critical for many cellular processes, including embryonic development, cell polarization, differentiation, and protein targeting to organelles (for review, see Ryder and Lerit 2018). It involves the delivery of specific mRNAs that dock to myosin motors for transit to their destination site where they are translated. Often these mRNAs are translationally silenced during transfer. A canonical example is *ASH1* in budding yeast, which is translationally silenced during transit from the mother-cell nucleus to daughter cells (for review, see Singer-Krüger and Jansen 2014).

Recently, stress-induced granules have been implicated in mRNA delivery in yeast. Work by Aronov et al. showed via live-cell microscopy that mating factor *MFA2* mRNA colocalizes with non-canonical P-body/SG hybrid granules and translocates with these granules to the mating-tip projection, called a shmoo (Aronov et al. 2015; Geva et al. 2020). Localized translation of the delivered mRNA produces Mfa2 mating pheromone that is secreted to attract cells of the opposite mating type. Mutants defective in P-body formation express Mfa2 pheromone throughout the cytoplasm rather than at the shmoo tip. Intriguingly, these cells are not only sterile but also defective in shmoo formation, suggesting that factors required for shmoo development may also be delivered by these non-canonical granules (Aronov et al. 2015).

There is also exciting evidence in yeast that mRNA delivery by stress-induced granules plays a role in inheritance. Garmendia-Torres et al. (2014) used a microfluidic device to show that yeast P-bodies formed during low-glucose conditions are directed from mother to the emerging bud in a myosin-dependent manner. Cells lacking the She2 protein (also involved in *ASH1* mRNA translocation [Du et al. 2008]) showed dramatically reduced P-body delivery to starved daughter cells; daughters that did inherit the mother-cell P-body grew significantly larger by the time of bud emergence than daughters who did not (Garmendia-

Torres et al. 2014). An intriguing possibility is that mRNAs are in fact translated within those granules. Several studies in yeast have shown that mRNAs encoding functionally related proteins localize to the same mRNP granules even in the absence of stress, where evidence suggests they are actively translated (Lui et al. 2014; Pizzinga et al. 2019). These granules can coalesce into P-bodies and SG upon stress. Strikingly, in response to environmental cues, those granules are translocated to the sites of polarized growth in daughter cells undergoing filamentation, in a She2/She3-dependent mechanism correlated with localized translation (Pizzinga et al. 2019). Very recently, translation within SGs was discovered using single-molecule imaging of mRNAs and nascent proteins in HeLa cells responding to arsenite stress: ATF4 transcripts localized to both the cytosolic fraction and SGs were translated, demonstrating that translational silencing is neither a requirement nor a definitive consequence of SG association (Mateju et al. 2020). This raises the possibility that SGs could deliver mRNA and protein cargo, including nascent proteins produced from sequestered transcripts.

The importance of granules during stress and environmental responses

Stress-induced mRNP granules have been observed across eukaryotic organisms and numerous types of stress. Yet, a comprehensive understanding of their function and cellular impacts is only just emerging. Myriad examples implicate stress-induced mRNP granules in promoting a successful cellular response. The ability to form SGs is associated with improved viability and decreased apoptosis in mammalian and yeast cells exposed to various stresses (Harding et al. 2000; Arimoto et al. 2008; Eisinger-Mathason et al. 2008; Hofmann et al. 2012; Kim et al. 2012; Takahara and Maeda 2012; Maharjan et al. 2017). Similarly, P-bodies have been reported to promote cell survival in response to DNA-replication stress and to enable long-term survival of quiescent cells (Shah et al. 2013; Loll-Krippléber and Brown 2017). The phenotypes described above implicate several mechanisms through which stress-induced granules could benefit stress survival and acclimation.

First, spatial reorganization of the transcriptome may help to redirect translational resources to stress-responsive transcripts (Ho et al. 2018). For example, sequestration of yeast helicase Ded1 to SG condensates upon heat shock enables the preferential translation of stress-responsive mRNAs in the cytosol at the expense of housekeeping genes that are especially dependent on Ded1 (Iserman et al. 2020). In this model, sequestering not only specific mRNAs away from translation but also important translational machinery can impact global translational outputs in the cell, thus redirecting resources to granule-free transcripts. However, formation of P-bodies and SGs is not required for global translational silencing, revealing that

any role in translational redirection is likely to be a subtle one. Surprisingly, preventing nuclear-cytoplasmic shuttling of RNA-binding decay factors decreases transcriptional efficiency, raising the possibility that sequestration of RBPs, decay factors, or proteins important for transcriptional initiation within stress-induced granules could affect transcription as well (Haimovich et al. 2013). Sequestering individual mRNAs could have wider impact, especially if those mRNAs encode regulatory proteins. Loll-Kripplé and Brown (2017) demonstrated that P-bodies induced upon DNA damage are critical for sequestering and translationally silencing *YOX1* mRNA, which encodes a transcriptional repressor that prevents a proper DNA damage response. Most RNAs that localize to granules do not show strong enrichment there, meaning that only a portion of each RNA pool is found in granules. This has raised questions about the global impact of granule function (Khong et al. 2017).

The model that stress-activated granules can protect and store mRNAs and other cargo has appealing implications for stress responses, even if only a portion of RNAs are protected. Cargo storage could shelter mRNAs and proteins for later activation, but it could also present a mechanism for delayed fate determination as cells deal with the immediate consequences of stress. Delayed decision making could serve as a mechanism for bet-hedging for a variety of future scenarios (Arribere et al. 2011; Simpson et al. 2014). An intriguing possibility is that sequestered mRNAs can be released in response to specific environmental cues, as shown for plants responding to light and oxygenation (Sorenson and Bailey-Serres 2014; Jang et al. 2019). The mechanisms regulating granule disassembly are poorly understood; however, phase separation can be exquisitely controlled by altering charged interfaces on interacting proteins, especially those with intrinsically disordered regions (Pak et al. 2016; Franzmann et al. 2018; Guo et al. 2019), raising the possibility that stress-activated kinases (perhaps including those sequestered within granules themselves) play a role in regulating granule disassembly and mRNA release (Shattuck et al. 2019). Granule-based storage could even contribute to cellular memory of past stress exposures (Berry et al. 2011; Guan et al. 2012), as recently suggested in yeast (Jiang et al. 2020).

Perhaps the most exciting recent evidence for phenotypic consequences of stress-regulated granules is suggested from yeast studies following trafficking and inheritance of granule-associated transcripts. Defects in granule formation and/or trafficking cause defects in shmoo development and polarized growth during yeast mating (Aronov et al. 2015), daughter-cell growth in low glucose conditions (Garmendia-Torres et al. 2014; Pizzinga et al. 2019), and polarized translation in daughter cells undergoing filamentation upon environmental cues (Pizzinga et al. 2019). The competitive advantage afforded to daughter or develop-

ing cells that receive the mother cell's granules could result from delivered mRNAs, proteins, or both. Cargo inheritance could lower the energy expenditure required of new daughter cells, much like maternally deposited mRNAs before the zygotic transition (whose delivery and translational silencing involves cytoplasmic granules) (for review, see Winata and Korzh 2018). Mother cells could also specialize the daughter-destined cargo according to the particular environmental assault. In fact, functionally related mRNAs, including those encoding glycolytic enzymes or translation factors, can colocalize to the same granules (Lui et al. 2014; Pizzinga et al. 2019; Morales-Polanco et al. 2021). The recent implications for active translation within granules suggest that granules may deliver not only cargo but also translational factories to daughter cells (Lui et al. 2014; Pizzinga et al. 2019; Morales-Polanco et al. 2021). This mode of transmission could also support multigenerational inheritance of prior stress exposures (Guan et al. 2012; Dodson and Kennedy 2019). Such a functional role for stress-activated granules is also appealing, because only a small portion of the mRNA pool in the cell need be bound and delivered to have an impact on the daughter cells.

CONCLUDING REMARKS

Many intriguing questions remain to be answered. While proteomic and transcriptomic studies are revealing differences in granule compositions across cell types and stress responses, how individual granules differ compositionally from one another in a single cytoplasm is not well characterized. Nor are the mechanisms with which mRNAs and proteins segregate to give granule specificity, for example, to colocalize functionally related transcripts. The environment-responsive dissolution of granules is appealing from the standpoint of stress acclimation; how this is regulated in response to environmental cues will be an exciting avenue of discovery. Finally, the true importance of granule function will continue to develop as new technologies, including single-cell phenotyping and single-molecule tracking, continue to develop. Together, this will shed light on the functions of stress-induced RNP granules and the breadth and complexity of dynamic cytoplasmic reorganization.

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