Insights into the Role of P-Bodies and Stress Granules in Protein Quality Control

Regina Nostramo, Siyuan Xing, Bo Zhang, and Paul K. Herman[1]
Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210

ABSTRACT The eukaryotic cell is highly compartmentalized, and contains a variety of both membrane-bound and membraneless organelles. The latter include the cytoplasmic ribonucleoprotein (RNP) granules, known as the processing body (P-body) and the stress granule. These RNP structures are thought to be involved in the storage of particular mRNAs during periods of stress. Here, we find that a mutant lacking both P-bodies and stress granules exhibits phenotypes suggesting that these structures also have a role in the maintenance of protein homeostasis. In particular, there was an increased occurrence of specific protein quality control (PQC) compartments in this mutant, an observation that is consistent with there being an elevated level of protein misfolding. These compartments normally house soluble misfolded proteins and allow the cell to sequester these polypeptides away from the remaining cellular milieu. Moreover, specific proteins that are normally targeted to both P-bodies and stress granules were found to instead associate with these PQC compartments in this granuleless mutant. This observation is interesting as our data indicate that this association occurs specifically in cells that have been subjected to an elevated level of proteotoxic stress. Altogether, the results here are consistent with P-bodies and stress granules having a role in normal protein homeostasis in eukaryotic cells.

KEYWORDS processing bodies; stress granules; protein quality control compartments; protein homeostasis

THE eukaryotic cell is segregated into distinct functional domains by the presence of a variety of both membrane-bound and membraneless organelles. The former have been extensively studied and include structures like the mitochondria, nucleus, and endoplasmic reticulum. The latter differ fundamentally from these well-characterized compartments in that they lack a limiting membrane (Hyman *et al.* 2014). However, it is likely that they serve similar purposes in the cell. For example, macromolecules participating in a related process can be concentrated in these structures so as to increase reaction efficiencies (Jin *et al.* 2017). Although several of these membraneless compartments, like the nucleolus and centrosome, have been studied for decades, most have been identified more recently. Many of these structures have been found to exhibit liquid-like properties in the cell and to assemble (and disassemble) rapidly in response to specific environmental signals (Brangwynne *et al.* 2009, 2011; Weber and Brangwynne 2012; Hyman *et al.* 2014; Banani *et al.* 2017). These structures have also been conserved evolutionarily, suggesting that they are likely to have important functions within the eukaryotic cell.

One family of these membraneless compartments includes cytoplasmic granules, like the processing body (P-body) and the stress granule (SG) (Anderson and Kedersha 2002; Buchan 2014; Luo *et al.* 2018). These structures contain distinct sets of mRNAs and proteins that rapidly coalesce at discrete sites in the cytoplasm in response to specific stress conditions (Thomas *et al.* 2011). The concentration of these molecules is thought to result in a type of phase transition that produces ribonucleoprotein (RNP) granules in a liquid- or gel-like state (Hyman *et al.* 2014; Banani *et al.* 2017; Boeynaems *et al.* 2018). SGs contain proteins important for translation and appear to serve as cytoplasmic repositories for mRNAs that will be translated following the resolution of the ongoing stress (Anderson and Kedersha 2008, 2009; Buchan and Parker 2009). In contrast, the biological activities of the P-body are less clear. These granules were initially identified by the presence of a number of proteins involved in mRNA processing (Bashkirov *et al.* 1997; Ingelfinger *et al.* 2002; van Dijk *et al.* 2002; Eystathioy *et al.* 2003; Sheth and Parker 2003; Cougot *et al.* 2004). This includes the 5′–3′ RNA exonuclease Xrn1 and components of the primary mRNA

doi: https://doi.org/10.1534/genetics.119.302376
Manuscript received May 29, 2019; accepted for publication July 3, 2019; published Early Online July 8, 2019.
[1]Corresponding author: Room 105, Department of Molecular Genetics, The Ohio State University, 484 W. 12th Ave., Columbus, OH 43210. E-mail: herman.81@osu.edu

decapping complex, Dcp1 and Dcp2. These early observations led researchers to propose that P-bodies were sites of mRNA decay (Eulalio *et al.* 2007a; Balagopal and Parker 2009). However, more recent work suggests that mRNA turnover is not occurring within these cytoplasmic foci. For example, no significant defects in mRNA decay were detected in yeast and mammalian cells that lack P-body foci (Stoecklin *et al.* 2006; Decker *et al.* 2007; Eulalio *et al.* 2007b). In addition, studies with particular decapping mutants indicate that mRNA decay might even be suppressed within P-body foci (Huch *et al.* 2016; Huch and Nissan 2017). Finally, there is experimental evidence of mRNAs being stored long-term within P-body granules (Arribere *et al.* 2011; Zid and O'Shea 2014; Hubstenberger *et al.* 2017; Standart and Weil 2018). As a result, the biological functions associated with the P-body remain to be determined.

P-bodies and SGs have also been found to contain a number of signaling molecules that play key roles in the regulation of cell growth and survival (Arimoto *et al.* 2008; Tudisca *et al.* 2010; Kozubowski *et al.* 2011; Takahara and Maeda 2012; Mitchell *et al.* 2013; Thedieck *et al.* 2013; Wippich *et al.* 2013; Shah *et al.* 2014). These constituents include many important protein kinases and phosphatases. This association has been shown in several instances to influence the levels or activity of the recruited signaling molecule. For example, the presence in SGs of regulators of a stress-responsive MAPK pathway determines whether cells undergo apoptosis in response to certain stresses (Arimoto *et al.* 2008). In addition, the recruitment of the budding yeast CK1 protein kinase Hrr25 to the P-body has been shown to be important for the efficient completion of meiosis (Zhang *et al.* 2018). As a result of these types of studies, it has been suggested that these RNP granules might play a role in the reprograming of specific signaling networks in response to stress (Kedersha *et al.* 2013; Shah *et al.* 2014). Therefore, it is essential that we identify the proteins targeted to P-bodies and SGs, and characterize the physiological consequences of this localization.

Recent work indicates that misfolded proteins are also compartmentalized within the eukaryotic cell (Miller *et al.* 2015b; Sontag *et al.* 2017). In particular, several model misfolded polypeptides have been found to be sequestered within distinct protein quality control (PQC) compartments (Kaganovich *et al.* 2008; Specht *et al.* 2011; Escusa-Toret *et al.* 2013; Miller *et al.* 2015a). This segregation presumably removes misfolded proteins from the remaining cellular milieu and thereby prevents unproductive interactions that might otherwise occur. The recognition of this phenomenon represents a major advance in our understanding of the eukaryotic PQC machinery. In *Saccharomyces cerevisiae*, soluble misfolded proteins have been shown to be targeted to at least two different compartments. The first was originally called the juxtanuclear quality control compartment (JUNQ) because of its proximity to the nucleus (Kaganovich *et al.* 2008). However, more recent work suggests that this structure is within the nucleus and has renamed it the intranuclear quality control compartment (INQ) (Miller *et al.* 2015a). The second compartment is characterized by the cytoplasmic foci that form when cells are subjected to particular types of proteotoxic stress. These foci are known by a variety of names including stress foci, quality control bodies (Q-bodies), and cytoQ (Kaganovich *et al.* 2008; Specht *et al.* 2011; Spokoini *et al.* 2012; Escusa-Toret *et al.* 2013; Miller *et al.* 2015a). The precise nature of the relationship between cytoQ and INQ/JUNQ is not yet clear, but at least one study has suggested that a failure to clear the proteins in the former can result in their subsequent accumulation within the latter (Escusa-Toret *et al.* 2013). The misfolded proteins targeted to these structures are thought to be either refolded or directed to the proteasome for degradation (Sontag *et al.* 2017).

Determining the biological activities associated with P-bodies and SGs remains a key area of interest in this research field. One approach to this issue has been to characterize the cellular defects associated with the loss of one or the other of these granules. For example, yeast mutants defective for P-body formation were found to exhibit a diminished ability to survive during extended periods of quiescence (Ramachandran *et al.* 2011; Shah *et al.* 2013). An alternative approach aims to define the biological consequences of losing the granule association of one or more of the proteins known to be present within the P-body, and/or the SG. Studies with the Hrr25 kinase have demonstrated that its association with the P-body serves to shield this protein from degradation within the proteasome (Zhang *et al.* 2016). Hrr25 variants that fail to localize to these foci are rapidly turned over under conditions that induce P-body formation. Here, we combine these two approaches, and examine the fate of four protein kinases in a yeast mutant that lacks both P-body and SG foci. These enzymes are unusual in that they can associate with both of these granules in the wild-type cell. Interestingly, our data indicate that these four kinases are targeted to INQ/JUNQ in this granuleless mutant, and altogether suggest a potential role for P-bodies and SGs in the control of protein homeostasis in the eukaryotic cell.

Materials and Methods

Yeast strains and growth conditions

Standard methods were employed to generate the yeast strains used in this study. The BY4741 strain was the wild type for all experiments in this report. The *ubp3*Δ and *edc3*Δ strains were obtained from the yeast knockout collection (Open Biosystems). The *edc3*Δ *lsm4*Δ*C* strain was generated by replacing the 3′-end of the *LSM4* gene that encodes the C-terminal half of the Lsm4 protein with the *LEU2* gene, as described previously (Decker *et al.* 2007). The *edc3*Δ *lsm4*Δ*C* *ubp3*Δ (ELUΔ) strain was generated by disrupting the *UBP3* locus in the *edc3*Δ *lsm4*Δ*C* strain by replacing its coding sequence with the *NAT* gene.

Standard *Escherichia coli* and yeast growth conditions were used throughout. The rich yeast medium, Yeast extract–Peptone–Adenine–Dextrose (YPAD), and the minimal media, yeast minimal (YM) and synthetic complete (SC), have been described previously (Kaiser *et al.* 1994; Chang *et al.* 2004;

Ramachandran and Herman 2010). Unless otherwise noted, all SC media was supplemented with 1% glucose and 1% sorbitol (SCDS), to ensure that P-bodies and SGs remained distinct entities (Shah *et al.* 2016). Cells containing the pESC-URA-Ubc9^{WT} or pESC-URA-Ubc9^{ts} plasmids were grown in SC media containing 2% galactose to induce expression from the relevant *UBC9* locus (Kaganovich *et al.* 2008). Cell cultures were grown with agitation at 30° for the indicated number of days. To induce misfolded protein foci formation, cells were incubated at 37° for 90 min in the presence of 100 μM of the proteasome inhibitor MG132 (or diluent, DMSO).

Plasmid construction

The plasmids used in this study are listed in Table 1. All genes are expressed from their endogenous promoters, unless otherwise indicated. All GFP-LucDM-NLS constructs were generated from the plasmid p303-ADH-GFP-LuciDM-NLS, kindly provided by the Bukau laboratory (NLS, nuclear localization signal). To generate ADHpro-GFP-LucDM-NLS (pPHY4814, CEN, and HIS3) and ADHpro-GFP-LucDM-NLS (pPHY4816, CEN, and URA3), the ADHpro-GFP-LuciDM-NLS fragment was cut from p303-ADH-GFP-LuciDM-NLS, and inserted into pRS413 and pRS416, respectively, between *Sac*I and *Xho*I. To generate a nuclear-localized VHL (von Hippel-Lindau), the NLS from this luciferase construct was PCR amplified, and inserted between the N-terminal GFP tag and VHL coding sequence. The GPDpro-Hsp42-Cherry plasmid was a generous gift from the Kaganovich laboratory. The pESC-URA-GFP-UBC9WT and *ts* plasmids were purchased from Addgene (#20368, #20369). Kinase-defective mutants were generated using the GeneArt site-directed mutagenesis system (Life Technologies).

Fluorescence microscopy

Cells expressing fluorescent protein fusion constructs were grown as indicated, collected by centrifugation, and spotted onto agarose pads on microscope slides as described (Shah *et al.* 2013; Zhang *et al.* 2016). Cells were imaged with a ×100/1.45 numerical aperture Plan-Apo objective lens on a Nikon Eclipse Ti inverted microscope (Nikon, Garden City, NY) with an Andor Zyla digital camera and the appropriate Nikon HC filter sets. The scale bar in all figures represents 5 μm. Merged images were created with the ImageJ (National Institutes of Health) software package. All images in a given figure panel were processed in an identical manner with respect to image size and intensity. For quantification of the percent of cells containing foci, the data represent a minimum of three replicates with ≥ 100 cells examined in each. The error bars on the graphs depict SEs. For quantification of the percent colocalization, only cells containing both types of foci were counted, and the data represent two or more independent experiments with a minimum of 100 cells being analyzed in each replicate.

Protein analysis

Protein samples were prepared for western blotting by a glass bead lysis method, separated on a 10% SDS-polyacrylamide gel, and transferred to nitrocellulose membranes as described previously (Budovskaya *et al.* 2002, 2004). The membranes were probed with the appropriate primary and secondary antibodies, and immunoreactive bands were detected using the Supersignal chemiluminescent substrate (Pierce Chemical, Rockford, IL). The samples were normalized with respect to total cell equivalents to avoid the fluctuations in the levels of specific proteins that have been observed upon the entry into stationary phase. The primary antibodies used were: anti-mCherry (mCh), Biovision (5993) and anti-GFP, Roche (11841460001). The secondary anti-rabbit and anti-mouse HRP-linked antibodies were from GE Life Sciences (NA934V and NA931V, respectively).

Stationary-phase viability assays

Cells were grown in YM-glucose minimal medium for the indicated number of days, collected by centrifugation, and resuspended in water at a concentration of five OD_{600} units/ml (Chang *et al.* 2004; Ramachandran *et al.* 2011). Fivefold serial dilutions of these cell suspensions were then spotted onto plates containing the same medium and incubated at 30° for 3 days.

Data availability

Strains and plasmids are available on request. The authors affirm that all data necessary for confirming the conclusions of this article are presented within the article, figures, and tables.

Results

Generation of a strain defective for the formation of both P-bodies and SGs

To generate a strain deficient for both P-body and SG formation, we combined mutations that affect each granule individually. Yeast mutants containing a deletion of *EDC3*, *edc3*Δ, and a truncation of *LSM4*, *lsm4*Δ*C*, have been found to be specifically defective for P-body formation (Figure 1A) (Decker *et al.* 2007). *EDC3* encodes the decapping enhancer, Edc3, and *LSM4* encodes an Sm-like protein, Lsm4, that is a constituent of two heptameric complexes in eukaryotic cells (Parker 2012; Didychuk *et al.* 2018). One of the latter is cytoplasmic and involved in mRNA decay, and the second is nuclear and part of the U6 small nuclear RNP (Didychuk *et al.* 2018). The *lsm4*Δ*C* allele encodes a truncated form of Lsm4 that lacks a C-terminal domain predicted to be intrinsically disordered (Decker *et al.* 2007; Reijns *et al.* 2008). These mutations were combined with a deletion of the *UBP3* locus that encodes a ubiquitin protease that we have shown is required for SG formation (Figure 1A) (Nostramo and Herman 2016; Nostramo *et al.* 2016). This triple mutant, *edc3*Δ *lsm4*Δ*C ubp3*Δ, is referred to as the ELUΔ strain for the remainder of this report.

We used fluorescence microscopy to assess the localization of a number of P-body and SG reporters in the ELUΔ strain. The P-body markers examined were Dcp1, Dcp2, Hrr25, the scaffolding protein Pat1, and the RNA helicase Dhh1 (Eulalio

Table 1 Plasmids used in this study

Plasmid name	Relevant details	Source
pPHY3486	*DCP2-GFP* in pRS413 (*CEN*, *URA3*)	Laboratory collection
pPHY3648	*PAT1-GFP* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY3661	*PAB1-GFP* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY3736	*HRR25-GFP* in pRS416 *(CEN*, *URA3)*	Laboratory collection
pPHY3778	*CDC28-GFP* in pRS406	Laboratory collection
pPHY3782	*PBP1-mCh* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY3966	*HOG1-mCh* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY4030	*NUP49-mCh* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY4081	*KIN28-mCh* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY4067	*FPK1-mCh* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY4076	pESC-URA-GFP-UBC9WT	Addgene
pPHY4077	pESC-URA-GFP-UBC9ts	Addgene
pPHY4083	*KSS1-mCh* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY4106	*EDC3-mCh* in pRS403	Laboratory collection
pPHY4130	*FRK1-mCh* in pRS416 (*CEN*, *URA3*)	Laboratory collection
pPHY4211	*PBP1-GFP* in pRS406	Laboratory collection
pPHY4711	GPDpro-*CBK1-mCh* in pRS416 (*CEN*, *URA3*)	This study
pPHY4712	GPDpro-*CBK1-mCh* in pRS413 (*CEN*, *HIS3*)	This study
pPHY4720	*FUS3-mCh* in pRS413 (*CEN*, *HIS3*)	This study
pPHY4721	*PBS2-mCh* in pRS413 (*CEN*, *HIS3*)	This study
pPHY4841	*PBS2-K389M-mCh* (PBS2-KD-mCh) in pRS413 (*CEN*, *HIS3*)	This study
pPHY4776	*DCP1-mCh* in pRS413 (*CEN*, *HIS3*)	This study
pPHY4777	*DHH1-GFP* in pRS413 (*CEN*, *HIS3*)	This study
pPHY4787	ADHpro-GFP-LucDM-NLS in pRS303 (*CEN*, *HIS3*)	Bukau laboratory
pPHY4813	ADH2pro-GFP-VHL in pRS416 (*CEN*, *URA3*)	This study
pPHY4814	ADHpro-GFP-LucDM-NLS in pRS413 (*CEN*, *HIS3*)	This study
pPHY4816	ADHpro-GFP-LucDM-NLS in pRS416 (*CEN*, *URA3*)	This study
pPHY4884	*CDC28-mCh* in pR406 (*CEN*, *URA3*)	This study
pPHY4901	GPDpro-VHL-mCh in pRS416 (*CEN*, *URA3*)	This study
pPHY4903	GPDpro-GFP-NLS-VHL in pRS416 (*CEN*, *URA3*)	This study
pPHY4904	GPDpro-HSP42-Cherry in pRS316 (*CEN*, *URA3*) (provided by Ravid lab; pTR1163)	Kaganovich laboratory (DK160)
pPHY4934	*PBS2-CFP* in pRS413 (*CEN*, *HIS3*)	This study
pPHY4941	GPDpro-PBS2-GFP-Full length (1–668) in pRS416 (*CEN*, *URA3*)	This study
pPHY4942	GPDpro-PBS2-GFP-1-185 in pRS416 (*CEN*, *URA3*)	This study
pPHY4943	GPDpro-PBS2-GFP-1-320 in pRS416 (*CEN*, *URA3*)	This study
pPHY4944	GPDpro-PBS2-GFP-183-320 in pRS416 (*CEN*, *URA3*)	This study
pPHY4945	GPDpro-PBS2-GFP-183-668 in pRS416 (*CEN*, *URA3*)	This study
pPHY4946	GPDpro-PBS2-GFP-320-668 in pRS416 (CEN, URA3)	This study

et al. 2007a; Balagopal and Parker 2009; Zhang *et al.* 2016). The SG reporters were the poly(A)-binding protein, Pab1, and the Pab1-binding protein Pbp1 (Kedersha *et al.* 2005; Buchan and Parker 2009; Buchan *et al.* 2011). These tagged reporters were typically present as a diffuse fluorescence throughout the cytoplasm in wild-type cells during the log phase of growth (Figure 1A). As noted previously, Hrr25 was also present at the bud neck and the spindle pole body at this time (Kafadar *et al.* 2003; Lusk *et al.* 2007; Shah *et al.* 2014). Each reporter was subsequently found to be concentrated in its appropriate granule after 1 day of growth in the supplemented minimal medium (Figure 1, A and B). In contrast, none of the reporters were found in cytoplasmic foci in ELUΔ cells (Figure 1, A and B). These results were not due to diminished levels of the reporters as western analysis indicated that near-normal levels of each tagged protein were present in the ELUΔ strain (Figure 1C). It should also be noted that each reporter was properly localized to its respective granule in the mutant defective for the assembly of the other. P-body reporters were properly localized in *ubp3*Δ cells and SG reporters in the *edc3*Δ *lsm4*Δ*C* mutant (Figure 1A). These results are consistent with our previous work indicating that the assembly of each of these granules is independent of the other (Shah *et al.* 2013). Altogether, these results suggested that both P-body and SG formation are defective in the ELUΔ strain.

The ELUΔ strain exhibits a diminished chronological life span

We and others have shown previously that granules, in addition to P-bodies and SGs, are induced as yeast cells begin to enter stationary phase (Narayanaswamy *et al.* 2009; Shah *et al.* 2014; Rabouille and Alberti 2017). Here, we tested whether three of these novel structures were able to form normally in the ELUΔ strain. Each of these foci were identified originally by the presence of a specific set of protein kinase constituents. The Novel 1 granules contain Frk1 and Kin28, Novel 2 contain Fpk1 and Kss1, and Novel 3 contain

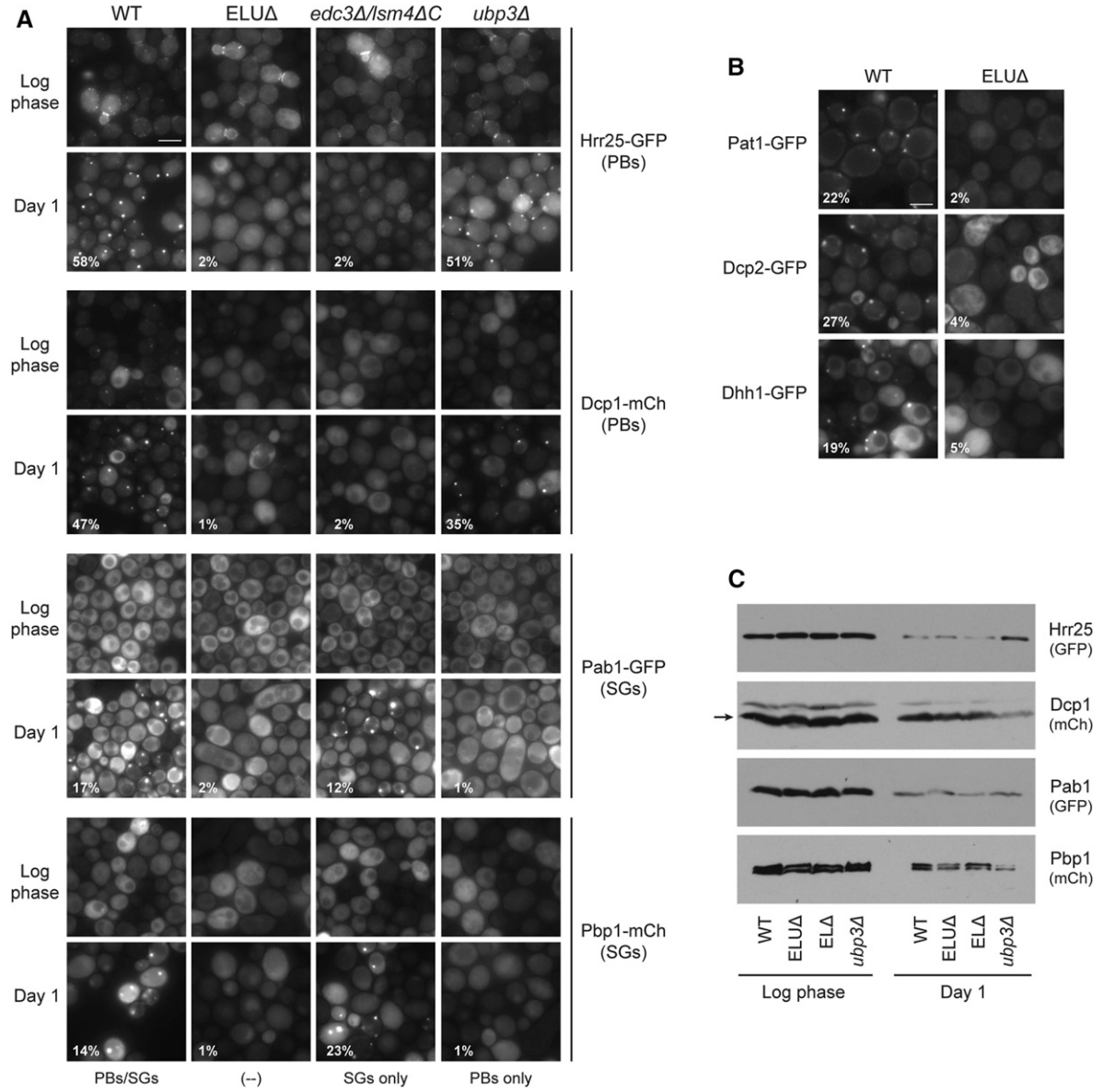

Figure 1 The ELUΔ strain is deficient for the formation of both P-bodies (PBs) and stress granules (SGs). (A and B) The localization of PB and SG proteins in strains defective for the assembly of PBs (*edc3*Δ *lsm4*Δ*C*, ELΔ), SGs (*ubp3*Δ), or both (ELUΔ). The wild-type (WT) and indicated deletion strains were grown in SC media containing 1% glucose and 1% sorbitol at 30°, and analyzed by fluorescence microscopy during log phase and after day 1 of growth. The strains expressed the indicated GFP- or mCh-tagged reporters for PBs (Hrr25 and Dcp1) or SGs (Pab1 and Pbp1). The fraction of cells with foci is indicated for each mutant at day 1. Bar, 5 μm. (B) The localization of the additional PB markers—Pat1, Dcp2, and Dhh1—was assessed in WT and ELUΔ cells on day 1. Bar, 5 μm. (C) Cell extracts were prepared from log phase and day 1 cultures of the strains described in (A), and the levels of the indicated tagged proteins were assessed by western blotting. The Dcp1 protein band is indicated by the arrow.

Hog1 (Shah *et al.* 2014). For each, we found that the fraction of cells containing foci, and the kinetics of foci formation, were similar in ELUΔ and wild-type cells (Figure 2A). Therefore, although the mutations present in the ELUΔ strain disrupt P-body and SG formation, they did not affect the assembly of at least these three cytoplasmic granules in yeast cells.

Previous work from our laboratory has indicated that mutants defective for P-body or SG formation exhibit a diminished ability to survive long-term in stationary phase (Ramachandran *et al.* 2011; Shah *et al.* 2013; Nostramo *et al.* 2016). Stationary phase is a quiescent growth phase that yeast cells enter when deprived of certain essential nutrients (Werner-Washburne *et al.* 1993; Herman 2002; Gray *et al.* 2004). The time that a yeast strain is viable in stationary phase has been deemed its chronological life span (CLS) (Longo *et al.* 2012). Here, we tested whether the ELUΔ strain might also display a decreased CLS. For this study, the indicated strains were grown in minimal medium for 7 or 14 days, and the number of cells surviving at these times was determined. We found that the CLS of the ELUΔ strain was less than that associated with either the *ubp3*Δ or the

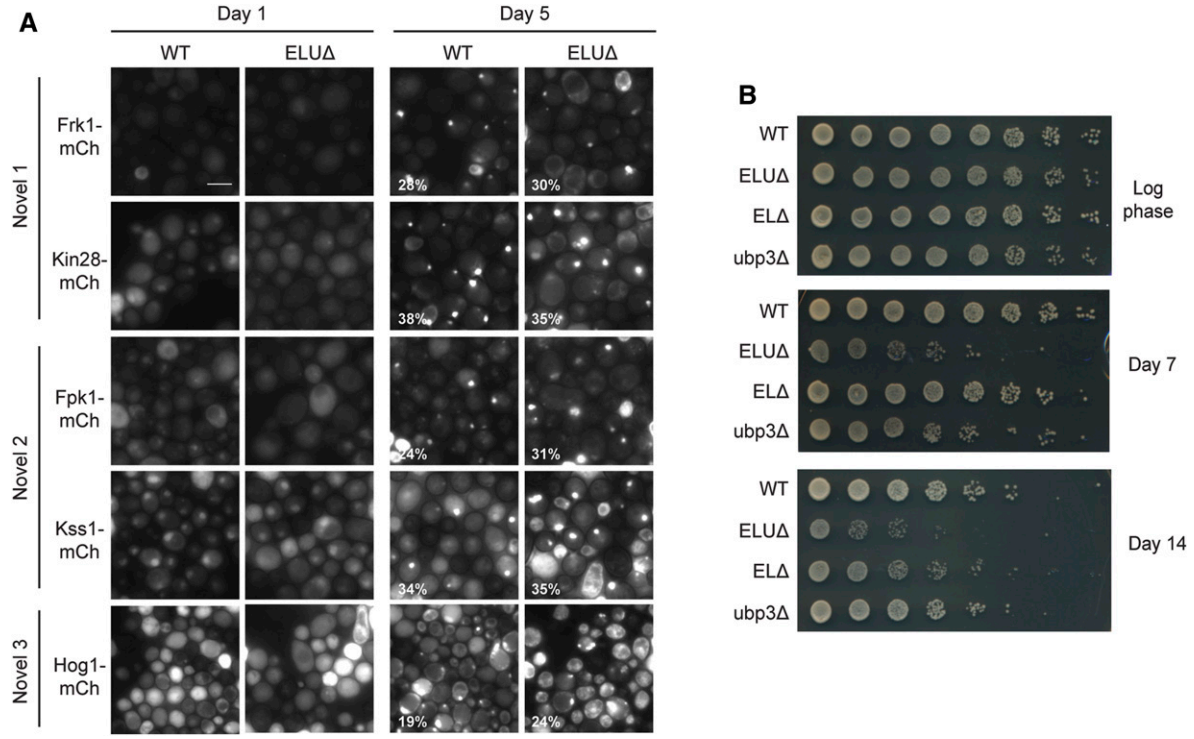

Figure 2 The ELUΔ strain exhibits a diminished chronological life span. (A) Wild-type (WT) and ELUΔ cells expressing mCherry (mCh)-tagged markers for three previously identified protein granules—termed Novel 1, Novel 2, and Novel 3—were grown in SCDS medium at 30°, and analyzed for foci formation on days 1 and 5. The fraction of cells with foci is indicated for the day 5 time point. Bar, 5 μm. (B) WT, *edc3*Δ *lsm4*Δ*C* (ELΔ), *ubp3*Δ, and ELUΔ cells were grown for up to 14 days in minimal medium and serial dilutions of the cultures were plated to minimal medium at the indicated times. The plates were examined after 2–3 days at 30° to assess cell survival and thus the chronological life span of each strain.

*edc3*Δ *lsm4*Δ*C* mutants (Figure 2B). It should be noted that an independent study found that an *edc3*Δ *lsm4*Δ*C* strain exhibited an extended CLS (Huch *et al.* 2016). However, we did not observe this here or elsewhere with other mutants defective for P-body formation (Ramachandran *et al.* 2011; Shah *et al.* 2013). Moreover, a recent study found that P-body formation is partially restored in the *edc3*Δ *lsm4*Δ*C* strain following the entry into stationary phase (Rao and Parker 2017). The presence of P-bodies at this time could explain the modest CLS defect observed for this mutant. In all, the results here are consistent with the absence of both granules having a more significant effect on the CLS than the loss of either single RNP structure alone.

Kinases that localize to both P-bodies and SGs are present in novel foci in the ELUΔ strain

One of the primary reasons for generating the ELUΔ strain was to test how the absence of these RNP granules would affect the localization of proteins that are associated with both of these structures. In a previous study, we identified four protein kinases that were localized to both P-bodies and SGs (Figure 3, A and B) (Shah *et al.* 2014). These four enzymes are the cyclin-dependent protein kinase Cdc28, the mitogen-activated protein kinase Fus3, the Ndr/LATS family member Cbk1, and the MAPK kinase Pbs2. Therefore, we assessed the localization of each kinase in wild-type, *edc3*Δ *lsm4*Δ*C*, *ubp3*Δ, and ELUΔ cells at the indicated times. Complete results are shown for Cdc28 but similar observations were made with each of the four protein kinases. Cdc28 was found to be in foci in all strains after 3 or 6 days of growth in the supplemented minimal medium (Figure 3C). This was expected for the wild type, and the two mutants that are specifically defective for the formation of either P-bodies (*edc3*Δ *lsm4*Δ*C*) or SGs (*ubp3*Δ). However, we also found that Cdc28 was still in foci in the ELUΔ strain that lacks both of these RNP structures (Figure 3C). The fraction of ELUΔ cells with these Cdc28 foci was very similar to that observed for the wild-type strain although the kinetics of foci appearance were delayed in this mutant. Similar results were seen with each of the other three kinases: Cbk1, Fus3, and Pbs2; all were present in foci in ELUΔ cells as they approach stationary phase (Figure 3D). Moreover, these foci appear to be the same structures as we observed strong colocalization between Cdc28 and Pbs2 in the ELUΔ mutant (Figure 3E). Therefore, these four protein kinases remain associated with foci even though ELUΔ cells appear to lack both P-bodies and SGs.

A potential explanation for the above observations is that the foci in ELUΔ cells might still be P-body or SG-related structures that lack the constituents examined here. If so, we would expect these kinases to be directed to foci by the same underlying machinery in wild-type and ELUΔ cells. To test this possibility, we determined the sequence elements within Pbs2 that were important for foci localization. The Pbs2 protein consists of three distinct domains: an

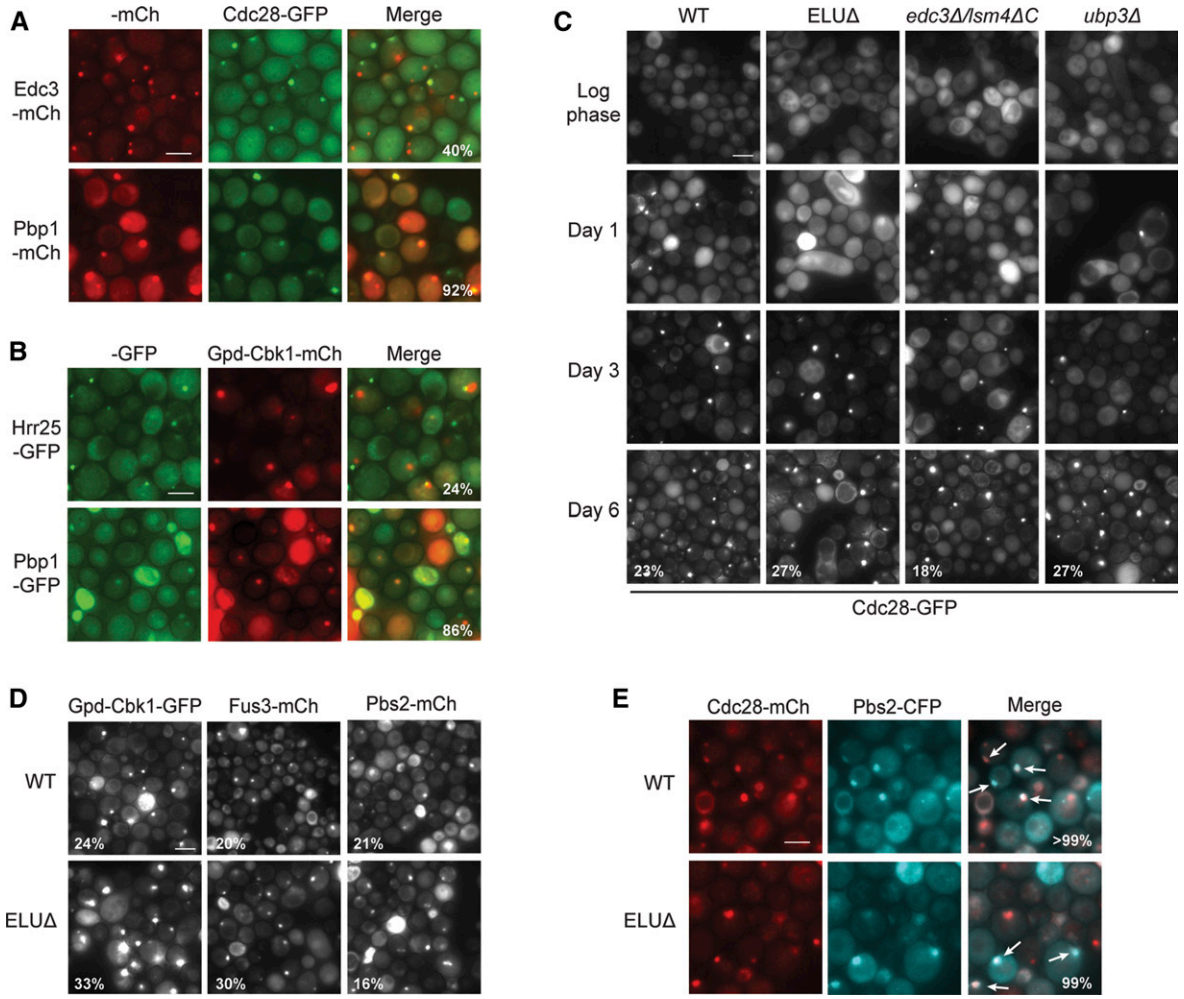

Figure 3 Protein kinases that localize to P-bodies and stress granules (SGs) are present in novel foci in the ELUΔ strain. (A and B) Cdc28 and Cbk1 were present in both P-body and SG foci. Cells expressing the indicated reporters were grown in SC medium containing 1% glucose and 1% sorbitol (SCDS) for 3–4 days at 30°. Fluorescence microscopy was then used to assess the colocalization of Cdc28-GFP [(A) 3 days] or Cbk1-mCh (mCherry) [(B) 4 days] with the P-body markers Edc3-mCh (A) and Hrr25-GFP (B), or the SG reporter Pbp1-mCh. The percent colocalization is indicated in the merged image; see *Materials and Methods* for more details. (C) Cdc28 was present in foci in the ELUΔ strain. The indicated strains were grown for ≤ 6 days in the rich medium yeast extract–peptone–adenine–dextrose and Cdc28-GFP foci formation was assessed by fluorescent microscopy. The percentage of cells with foci is indicated for each strain at day 6. (D) Cbk1, Fus3, and Pbs2 were also localized to foci in the ELUΔ strain. Wild-type (WT) and ELUΔ cells expressing the indicated protein kinase reporters were examined by fluorescence microscopy after 4 days of growth in SCDS medium at 30°. (E) Cdc28 and Pbs2 were present in the same foci in ELUΔ cells. WT and ELUΔ cells expressing Cdc28-mCh and Pbs2-CFP were grown in SCDS at 30° for 5 days before being analyzed by fluorescence microscopy. The white arrows point out foci containing both reporters and the percent colocalization is indicated. Bar, 5 μm.

N-terminal domain (NTD), a central domain that is predicted to be intrinsically disordered (IDD), and a C-terminal protein kinase domain (Figure 4A). We assessed the subcellular localization of a series of GFP-tagged truncation products of Pbs2 (Figure 4B). These studies demonstrated that the kinase domain was both necessary and sufficient for foci localization in wild-type cells (Figure 4B). Neither the NTD nor the IDD was required for Pbs2 foci formation. This kinase domain fragment was also shown to colocalize efficiently with reporters for both P-bodies and SGs (Figure 4C). Thus, the kinase domain of Pbs2 was recruited to P-bodies and SGs in wild-type cells in a manner similar to that of the full-length protein.

We next tested whether the kinase activity of Pbs2 was required for foci localization. For these studies, we generated a kinase-defective version of Pbs2 ($Pbs2^{KD}$) where a critical lysine residue within the active site, K389, was replaced with a methionine (Reiser *et al.* 2000). We found that this $Pbs2^{KD}$ variant was less efficiently targeted to foci than the wild-type protein in control cells (Figure 4, D and E). This result was consistent with Pbs2 kinase activity being important for its normal association with P-bodies and SGs. In contrast, the $Pbs2^{KD}$ variant was found to be localized to foci as well as the wild-type protein in ELUΔ cells (Figure 4, D and E). Thus, Pbs2 kinase activity was not required for foci localization in this mutant, suggesting that there are different Pbs2

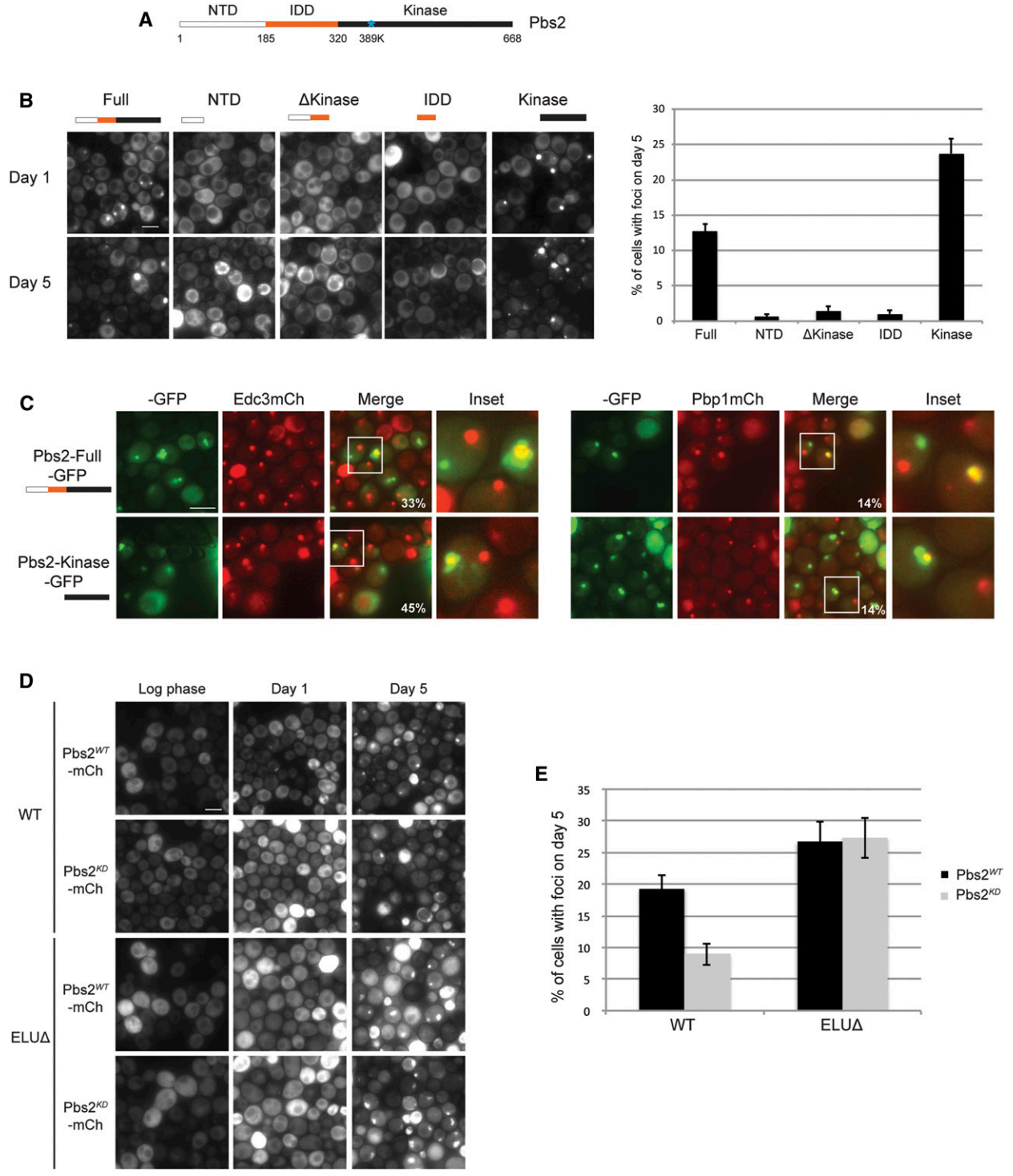

Figure 4 The kinase activity of Pbs2 is required for its localization to foci in wild-type (WT) but not ELUΔ cells. (A) The domain structure of Pbs2. The N-terminal domain (NTD) is shown in white, the central domain containing an intrinsically disordered region (IDD) is shown in orange, and the C-terminal kinase domain is in black. The blue asterisk indicates the lysine residue at position 389 within the active site that is altered in the kinase-defective variant. (B) WT cells expressing the indicated GFP-tagged Pbs2 constructs under the control of the GPD promoter were grown for 1 or 5 days, in SC medium containing 1% glucose and 1% sorbitol (SCDS) at 30°. Foci formation was analyzed by fluorescence microscopy (left) and the percentage of cells containing foci was quantified (right). (C) The Pbs2 kinase domain was localized to both P-bodies and stress granules (SGs). Fluorescence microscopy was used to assess the colocalization between GFP-tagged versions of the indicated Pbs2 constructs, and the P-body or SG reporters Edc3 and Pbp1, respectively. The strains were grown to day 5 in SCDS medium as in (B) prior to analysis. The percent colocalization is indicated in the merged images. (D and E) WT and ELUΔ cells expressing the WT Pbs2, $Pbs2^{WT}$, or the catalytically inactive variant $Pbs2^{KD}$ were grown for 1 or 5 days in SCDS medium at 30°. Foci formation was then assessed by fluorescence microscopy (D) and the percentage of cells with foci is shown in the graph (E). Bars, 5 μm.

determinants necessary for foci association in wild-type and ELUΔ cells. Altogether, these data are consistent with the possibility of the Pbs2 foci in ELUΔ cells being structures distinct from P-bodies and SGs.

INQ/JUNQ, cytoQ, SGs, and P-bodies are distinct subcellular compartments

Since P-bodies and SGs can form in conditions that also result in proteotoxic stress, we set out to test whether the four kinases discussed above might be associated with a PQC compartment in ELUΔ cells. However, before we could assess this possibility, it was important to ensure that P-body and SG reporters were not normally associated with these structures, and vice versa, under the experimental conditions used here. Therefore, we examined a variety of reporters for the INQ/JUNQ and cytoQ compartments, and several examples of these analyses are shown in Figure 5. The studies presented took advantage of a destabilized version of the luciferase enzyme (LucDM) from *Photinus pyralis* that has been shown to associate with both of these PQC compartments (Gupta *et al.* 2011; Specht *et al.* 2011). The particular GFP-LucDM-NLS reporter used here contains an NLS and was found to be predominantly targeted to INQ/JUNQ (Figure 5A) (Miller *et al.* 2015a). As a marker for cytoQ, we used Hsp42 fusion proteins that contained either an mCh or eCitrine fluorescent tag (Specht *et al.* 2011). The microscopy was performed after cells containing the appropriate reporters were shifted from 30 to 37° for 90 min in the presence of the proteasome inhibitor MG132. Both INQ/JUNQ and cytoQ were readily visible in cells after this experimental regimen. Our results confirmed previous work indicating that there is a very close association between the INQ/JUNQ compartment and the nucleus, and that cytoQ and INQ/JUNQ are distinct compartments in the cell (Figure 5A) (Miller *et al.* 2015a; Sontag *et al.* 2017). Importantly, we found no significant colocalization between markers for these two PQC compartments and reporters associated with either P-bodies or SGs; the level of colocalization was typically $< 2\%$ (Figure 5, B and C). We did note that SGs were sometimes detected in the vicinity of cytoQ (~10–20% of cells with both types of foci), but the physiological significance of this localization is not yet clear. Thus, these data indicated that P-bodies, SGs, cytoQ, and INQ/JUNQ are all separate compartments within the yeast cell.

Cdc28 and the other protein kinases localize to the INQ/JUNQ compartment in cells exposed to a proteotoxic stress

To determine the identity of the Cdc28 compartment in ELUΔ cells, we examined the localization of a GFP-Ubc9^{ts} reporter that has been used to study protein homeostasis in eukaryotic cells (Kaganovich *et al.* 2008; Brielle *et al.* 2015). This protein is a thermolabile variant of the *S. cerevisiae* small ubiquitin-like modifier ligase Ubc9, which has been shown to misfold at temperatures $> 33°$ (Betting and Seufert 1996). Although this reporter can associate with both cytoQ and INQ/JUNQ when misfolded, it accumulates predominantly within the latter when proteasome activity is also inhibited (Kaganovich *et al.* 2008; Specht *et al.* 2011). Here, we assessed the colocalization of Cdc28 and GFP-Ubc9^{ts} in the ELUΔ mutant, and control strains that contain P-bodies and/or SGs.

As observed previously, GFP-Ubc9^{ts} puncta were readily apparent in cells incubated at 37° in the presence of the proteasome inhibitor MG132 (Figure 6A). These PQC foci were less abundant in wild-type cells grown at 30° or when cells contained the wild-type Ubc9 protein at either temperature (Figure 6A) (Kaganovich *et al.* 2008). Interestingly, we found that the Cdc28 foci in ELUΔ cells were coincident with these INQ/JUNQ structures (Figure 6B). Complete colocalization between the Cdc28-mCh and GFP-Ubc9^{ts} reporters was observed in these studies. Similar results were obtained for Fus3 and Pbs2 with the INQ/JUNQ reporter, GFP-LucDM-NLS, described above (Figure 6B). Finally, Pbs2 also exhibited significant colocalization with a third reporter for the INQ/JUNQ compartment, GFP-VHL-NLS (Figure 6B) (Kaganovich *et al.* 2008; Specht *et al.* 2011). The mammalian VHL protein is a component of the VHL tumor suppressor complex and is unstable in the absence of the ElonginBC subunits of this complex (Ivan and Kaelin 2001; McClellan *et al.* 2005). Altogether, these results demonstrated that the protein kinase colocalization was not specific to a particular INQ/JUNQ reporter. Instead, these observations indicated that this suite of four protein kinases was associated with a well-characterized PQC compartment in cells where P-body and SG assembly is abrogated.

However, this colocalization was not unique to the ELUΔ mutant as Cdc28 was also associated with the INQ/JUNQ compartment in the three isogenic control strains: *ubp3*Δ, *edc3*Δ *lsm4*Δ*C*, and the wild type. In each case, Cdc28 exhibited a significant degree of colocalization with GFP-Ubc9^{ts} under conditions that induce INQ/JUNQ formation (incubation at 37° with proteasome inhibition) (Figure 6C). The presence of INQ/JUNQ foci is consistent with an accumulation of the misfolded protein in these cells and thus of an elevated level of proteotoxic stress (Sontag *et al.* 2017). In contrast, Cdc28 was found to colocalize predominantly with the SG marker, Pbp1, when these control strains were grown at 30° (Figure 3A; see below). One interpretation of these data is that Cdc28 is sensitive to the PQC conditions in the cell and is prone to misfold, and be targeted to INQ/JUNQ, when protein homeostasis is perturbed. In this vein, it is important to reiterate that Cdc28 was present in discrete foci in the ELUΔ mutant at 30° in the absence of INQ/JUNQ reporter expression (Figure 3C). Moreover, we found that Cdc28 was also targeted to novel foci when ELUΔ cells that had been grown for 1 day at 30° were shifted to 37° for 90 min in the presence of the proteasome inhibitor MG132 (Figure 6D). This protein kinase is not normally found in foci at this stage of growth (see Figure 3C). In wild-type cells, Cdc28 was found to be localized normally to SGs and P-bodies following a similar shift to 37° with proteasome inhibition (Figure 6E). In all, these data indicated that ELUΔ cells may be subject to an elevated level of proteotoxic stress that in turn results in the association of Cdc28 with the INQ/JUNQ compartment.

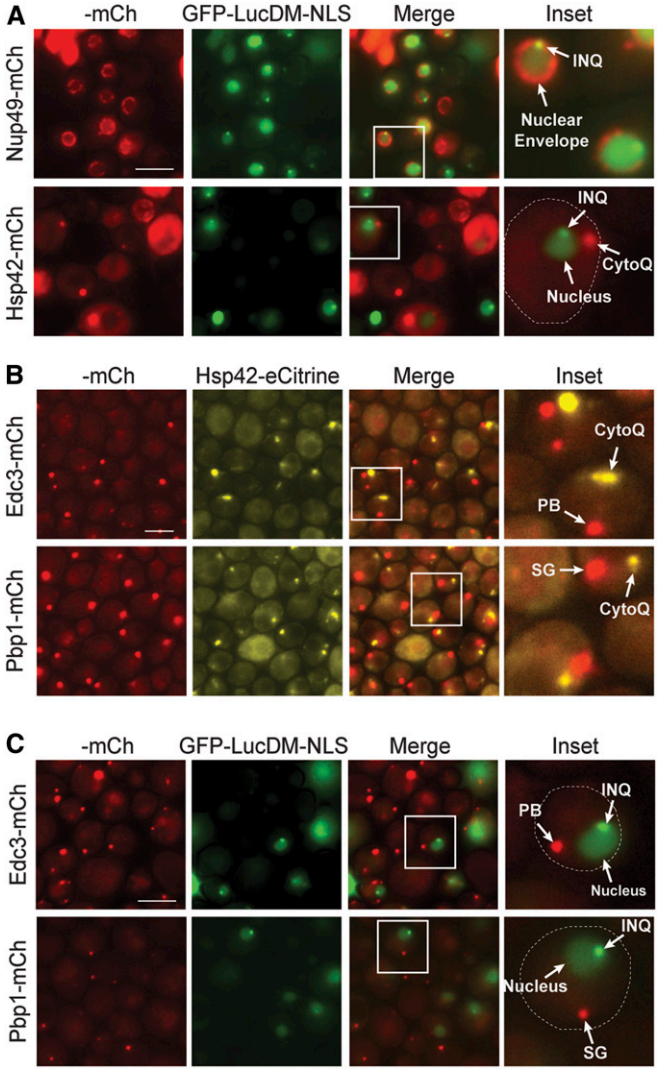

Figure 5 The protein quality control compartments, the intranuclear quality control/juxtanuclear quality control compartment (INQ/JUNQ) and CytoQ, are distinct from P-bodies (PBs) and stress granules (SGs). (A) INQ/JUNQ and CytoQ are distinct compartments in yeast cells. Wild-type cells were grown to day 1 in SC medium containing 1% glucose and 1% sorbitol (SCDS) at 30°, and then shifted to 37° for 90 min in the presence of 100 μM of the proteasome inhibitor MG132. The localization of the indicated reporters was then assessed by fluorescence microscopy. GFP-LucDM-NLS is a marker for INQ/JUNQ, Hsp42-mCherry (mCh) for CytoQ, and Nup49-mCh for the nuclear envelope. (B) PBs, SGs, and CytoQ are all distinct compartments in the yeast cytoplasm. Colocalization between the indicated reporters was assessed by fluorescence microscopy in wild-type cells after 1 day of growth in SCDS medium at 30°. Hsp42-eCitrine is a reporter for CytoQ, and Edc3-mCh and Pbp1-mCh are reporters for PBs and SGs, respectively. (C) PBs, SGs, and INQ/JUNQ are distinct compartments in the yeast cell. Wild-type cells expressing the indicated reporters were grown for 1 day in SCDS medium at 30°. The cells were then shifted to 37° for 90 min in the presence of the proteasome inhibitor MG132, prior to being examined by fluorescence microscopy. Bars, 5 μm.

Cells lacking P-bodies and SGs exhibit potential defects in protein homeostasis

The observed sequestration of misfolded proteins into specific subcellular locales provides us with a means to assess the state of protein homeostasis in living cells (Miller *et al.* 2015b; Sontag *et al.* 2017). Specifically, this insight can be gained by monitoring changes in the number and/or size of these PQC compartments. Therefore, we assessed the frequency of INQ/JUNQ foci formation in the ELUΔ mutant and the appropriate control strains. It is important to note here that a PQC defect has been detected previously in the *ubp3*Δ mutant (Oling *et al.* 2014) (see below). For these studies, we examined strains expressing the GFP-Ubc9^{ts} reporter after a 90 min incubation at 30° in the presence of the proteasome inhibitor MG132. Under these conditions, the wild-type control exhibited a low basal level of INQ/JUNQ foci formation (1.6%; Figure 7, A and B). The fraction of *edc3*Δ *lsm4*Δ*C* cells with foci was similar to the wild type (0.3%). Consistent with the afore-mentioned PQC defect, we found that ~13% of *ubp3*Δ cells contained a notable INQ/JUNQ focus. Importantly, this number was found to increase by more than twofold again in the ELUΔ strain (27.3%; Figure 7, A and B). This latter result suggested that the loss of P-bodies had a negative impact, either directly or indirectly, upon the PQC system of cells that were already lacking SG foci. At 37°, the fraction of cells with GFP-Ubc9^{ts} foci was similar for the *ubp3*Δ and ELUΔ strains (Figure 7, A and B). In a separate experiment with the wild-type Ubc9 reporter, we also observed a twofold increase in foci formation from 16.4% in the *ubp3*Δ mutant to 38.4% in the ELUΔ strain. In this latter instance, the cells were examined after a 90 min incubation at 37° in the presence of the proteasome inhibitor MG132. Collectively, these data suggested that there may be PQC defects associated with the ELUΔ strain, and that P-bodies and SGs might therefore have a role in the maintenance of normal protein homeostasis within the eukaryotic cell.

Discussion

We are interested in identifying the biological activities associated with the RNP granules known as the P-body and SG. The work here extends these efforts by examining the phenotypes associated with a yeast mutant that is defective for the assembly of both of these RNP structures. These analyses provided a number of valuable insights. First, we demonstrated that it is possible to generate a mutant that is defective for the formation of P-bodies and SGs. Both types of RNP foci were largely absent from ELUΔ cells at times when these structures are typically abundant in the wild type. Second, we found that this ELUΔ mutant was viable and that it exhibited a near-normal growth rate on standard yeast media. However, this mutant did display a diminished ability to survive during the stationary phase of growth. This survival is often referred to as the CLS of this yeast. Third, we found that proteins that typically associate with both P-bodies and SGs in wild-type cells are instead found in a recently described PQC compartment in ELUΔ cells. This compartment, known as INQ or JUNQ, has been shown to house soluble misfolded proteins in cells that have been exposed to proteotoxic stress (Kaganovich *et al.* 2008). Fourth, we found that the frequency of INQ/JUNQ foci formation was elevated in ELUΔ cells relative to the wild type and other controls. The

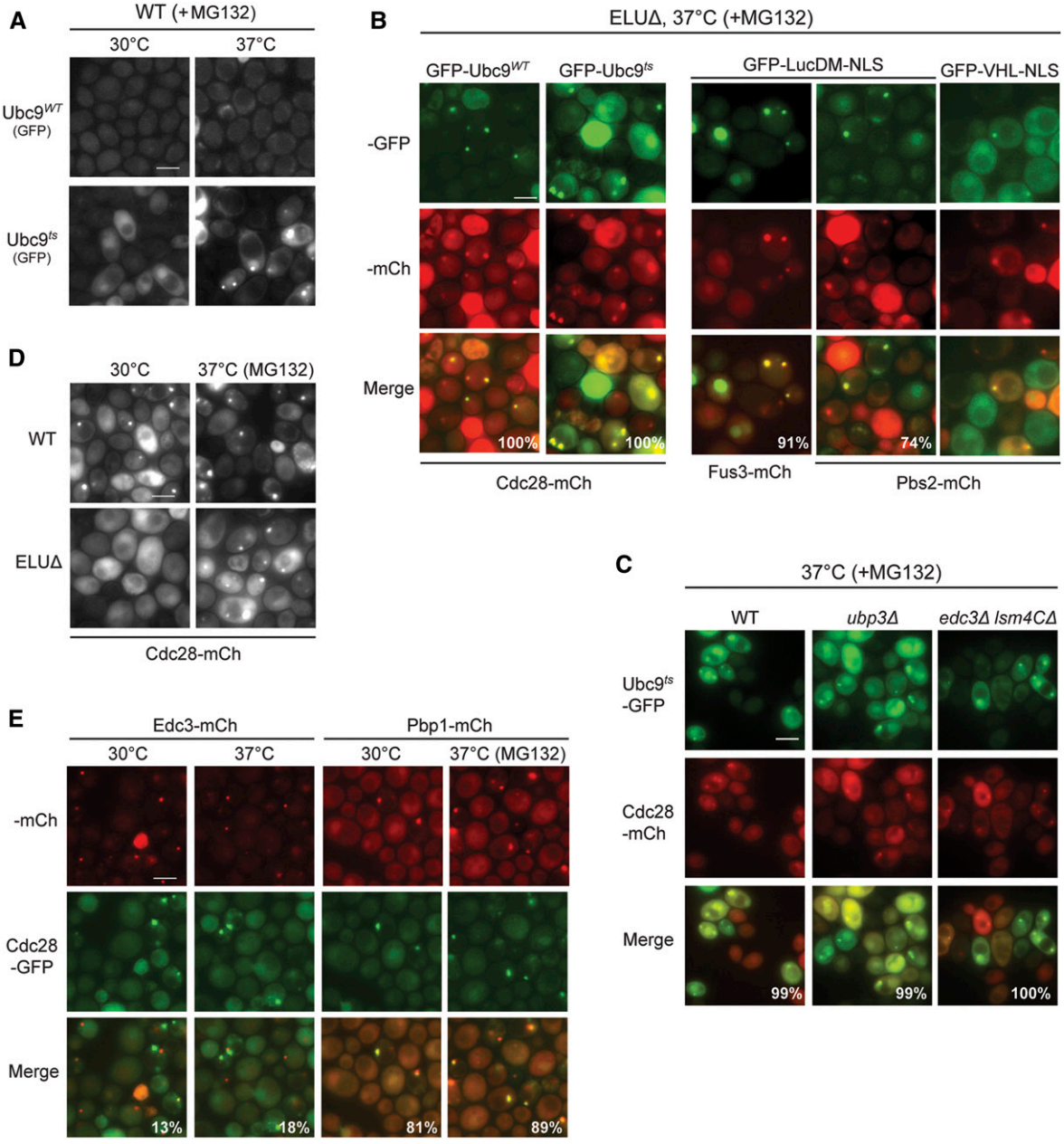

Figure 6 The protein kinases that localize to both P-bodies and stress granules (SGs) in wild-type (WT) cells are instead associated with the intranuclear quality control/juxtanuclear quality control (INQ/JUNQ) compartment in ELUΔ cells. (A) WT cells expressing the GFP-tagged, galactose-inducible Ubc9^{WT} or Ubc9^{ts} proteins were grown in SC medium containing 2% galactose at 30°, and then shifted to 37° (where indicated) in the presence of the proteasome inhibitor, MG132, for 90 min. The cells were then analyzed by fluorescence microscopy. (B) The Cdc28, Fus3, and Pbs2 enzymes are associated with the INQ/JUNQ compartment in ELUΔ cells. ELUΔ cells expressing the indicated reporters were grown as in (A) before being examined by fluorescence microscopy. The percentage of colocalization is indicated where appropriate in the merged image. GFP-Ubc9^{ts}, GFP-LucDM-NLS, and GFP-VHL-NLS are all reporters for the INQ/JUNQ compartment. See the text for additional details. (C) Cdc28 colocalization with the GFP-Ubc9^{ts} reporter was assessed in the WT, *ubp3*Δ, and *edc3*Δ *lsm4*Δ*C* control strains as described in (B). (D) WT and ELUΔ cells expressing Cdc28-mCherry (mCh) were grown at 30° for 1 day in 2% galactose and then shifted to 37° in the presence of MG132 for 90 min. (E) WT cells expressing Cdc28-GFP, and either mCh-tagged Edc3 or Pbp1, were grown for 1 day in SC medium containing 1% glucose and 1% sorbitol at 30°, and then shifted to 37° in the presence of MG132 as in (D). Cells containing both -mCh and -GFP foci were counted, and the percentage of these cells displaying foci colocalization is indicated. Bars, 5 μm.

increased prevalence of this PQC compartment is consistent with there being a defect in the maintenance of normal protein homeostasis in this mutant. Altogether, these data suggest that P-bodies and SGs may contribute to PQC in eukaryotic cells during certain periods of stress.

This potential role for P-bodies and SGs in PQC was supported by two different lines of evidence. The first arose from an analysis of four protein kinases, including Cdc28, that associate with both P-bodies and SGs in yeast cells (Shah *et al.* 2014). This localization is somewhat unusual as most

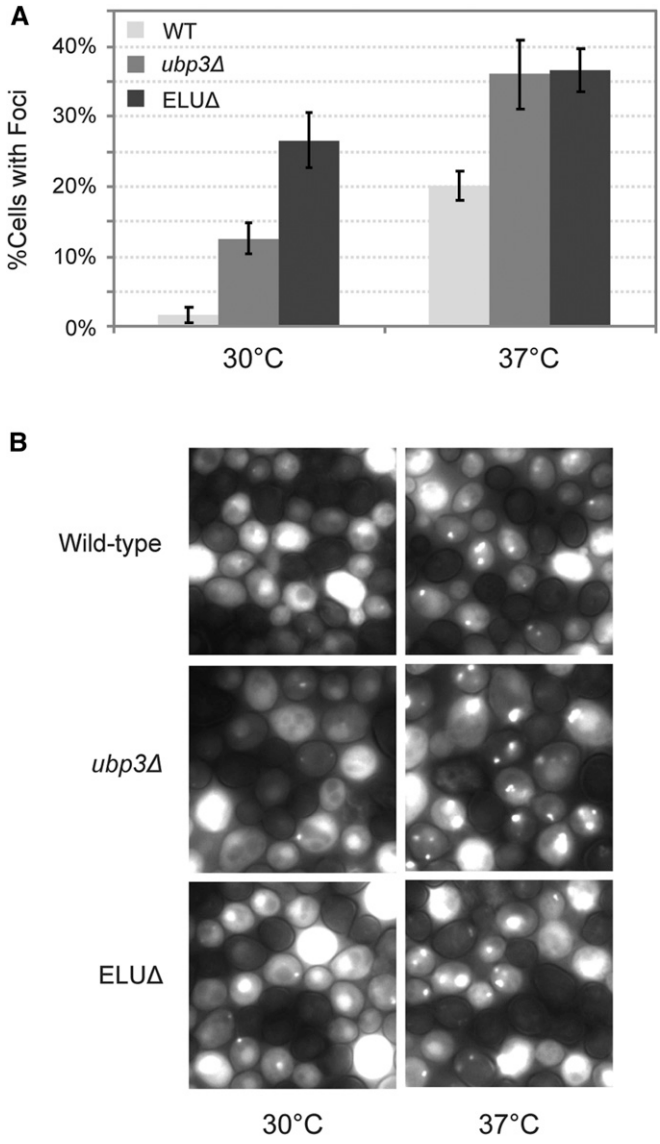

Figure 7 ELUΔ cells lacking P-bodies and stress granules (SGs) exhibit a potential defect in protein homeostasis. The frequency of intranuclear quality control/juxtanuclear quality control (INQ/JUNQ) compartment foci formation was elevated in the ELUΔ strain. Wild-type (WT), *ubp3*Δ, and ELUΔ cells expressing the GFP-tagged, galactose-inducible Ubc9^{ts} reporter were grown in 2% galactose at 30°. The proteasome inhibitor MG132 was then added to a final concentration of 100 μM, and the cells were incubated at either 30 or 37° for 90 min before being analyzed by fluorescence microscopy. A graph showing the fraction of cells with GFP-Ubc9^{ts} foci is shown in (A) and representative microscopy images are shown in (B).

constituents are found primarily in one granule or the other. Interestingly, we found that these enzymes were still located within discrete puncta in ELUΔ cells and that these structures appeared to correspond to INQ/JUNQ foci. These results are significant as Cdc28 was found to associate with this PQC compartment in wild-type cells that were subject to a proteotoxic stress (see Figure 6). One interpretation of this latter result is that these protein kinases are prone to misfold and be targeted to INQ/JUNQ when protein homeostasis is perturbed. Therefore, the localization to INQ/JUNQ in ELUΔ cells could be an indication that the PQC system is compromised in some way in this mutant.

The second line of evidence came from a study of INQ/JUNQ foci formation in particular yeast mutants. Specifically, we found that *ubp3*Δ cells lacking SGs exhibited a PQC defect that was exemplified by an increase in INQ/JUNQ foci formation relative to the wild type. This defect was further exacerbated by the introduction of mutations that disrupt P-body formation (Figure 7). These studies therefore suggest that both SGs and P-bodies might be involved in normal protein homeostasis. However, it is important to note here that a prior study suggested different underlying reasons for the PQC defects observed with the *ubp3*Δ mutant (Oling *et al.* 2014). One suggestion by Oling *et al.* was that Ubp3 might facilitate proteasomal degradation by removing the ubiquitin that adorns the misfolded proteins present within INQ/JUNQ. Ubp3 is a member of a family of ubiquitin proteases that remove ubiquitin moieties from specific sets of protein substrates (Baker *et al.* 1992). Therefore, the PQC defects observed with the *ubp3*Δ mutant could be due to the loss of SGs and/or to other activities associated with Ubp3 protease function. Although further studies are required to determine the relative contributions of each of these pathways, we do want to note here that Ubp3 catalytic activity is required for SG assembly, and that Ubp3 is physically associated with SGs in both yeast and human cells (Wang *et al.* 2012; Takahashi *et al.* 2013; Nostramo and Herman 2016; Nostramo *et al.* 2016).

The manner in which these RNP granules could influence protein homeostasis remains to be determined. One possibility suggested by the results here is that specific sets of proteins might be stabilized by their association with P-bodies and SGs during particular types of stress. A failure to associate with these RNP granules could lead to the misfolding and subsequent localization of these proteins to a PQC compartment, like INQ/JUNQ. Alternatively, the four protein kinases examined here could be misfolded prior to their association with P-bodies or SGs. In this case, these RNP granules would be functioning very much like the PQC compartments discussed here by sequestering particular sets of improperly folded polypeptides. However, it is important to note that none of the model misfolded proteins examined to date have been found at P-bodies or SGs to any significant degree (Figure 5) (Sontag *et al.* 2017). We have also found that a thermolabile version of Cdc28 was targeted to novel foci and did not associate with P-bodies when cells were shifted to an elevated temperature that induces misfolding (S. Xing and P. K. Herman, unpublished results). Finally, the effects on PQC in ELUΔ cells could be more indirect and due to a secondary consequence of losing these RNP granules, or to other defects associated with the mutations used here (Oling *et al.* 2014). Determining which of these models best explains the data here will clearly require further study.

In summary, the work here points to a potential role for P-bodies and SGs in the maintenance of protein homeostasis. Although such an activity has not yet been ascribed to

P-bodies, a number of studies have alluded to a potential role for SGs in PQC. For example, recent work has found that Ubc9^{ts} and other misfolded proteins are associated with a small fraction of SGs in mammalian cells that have been treated with a heat stress (Mateju *et al.* 2017). However, this study suggested that the presence of these misfolded proteins alters the physical state of these RNP granules and that cells appear to have developed mechanisms to prevent this accumulation of misfolded protein. In addition, it has been reported that an extreme heat stress can trigger the formation of SG-like structures in yeast that contain aggregates of misfolded proteins (Cherkasov *et al.* 2013). Finally, particular chaperone proteins have been found to be associated with SG foci in both yeast and mammalian cells (Walters *et al.* 2015; Mateju *et al.* 2017). These observations, together with the studies here, suggest that P-bodies and SGs could play an active role in PQC during exposures to particular types of stress. This possibility is intriguing as recent work has identified links between these RNP granules and human proteinopathies, like amyotrophic lateral sclerosis, that appear to be caused by defects associated with the protein folding apparatus (Li *et al.* 2013; Aguzzi and Altmeyer 2016; Feng *et al.* 2018; Klaips *et al.* 2018). This potential connection highlights the importance of defining the precise roles that P-bodies and SGs might have in the maintenance of protein homeostasis in eukaryotic cells.

Acknowledgments

We thank Bernd Bukau, Daniel Kaganovich, and Tommer Ravid for reagents used in this study, and members of the Herman laboratory for helpful discussions and comments on the manuscript. This work was supported by grants GM-101191 and GM-128440 from the National Institutes of Health to P.K.H., and a postdoctoral fellowship from the Pelotonia Fellowship Program to R.N.

Literature Cited

Aguzzi, A., and M. Altmeyer, 2016 Phase separation: linking cellular compartmentalization to disease. Trends Cell Biol. 26: 547–558. https://doi.org/10.1016/j.tcb.2016.03.004

Anderson, P., and N. Kedersha, 2002 Stressful initiations. J. Cell Sci. 115: 3227–3234.

Anderson, P., and N. Kedersha, 2008 Stress granules: the Tao of RNA triage. Trends Biochem. Sci. 33: 141–150. https://doi.org/10.1016/j.tibs.2007.12.003

Anderson, P., and N. Kedersha, 2009 RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat. Rev. Mol. Cell Biol. 10: 430–436. https://doi.org/10.1038/nrm2694

Arimoto, K., H. Fukuda, S. Imajoh-Ohmi, H. Saito, and M. Takekawa, 2008 Formation of stress granules inhibits apoptosis by suppressing stress-responsive MAPK pathways. Nat. Cell Biol. 10: 1324–1332. https://doi.org/10.1038/ncb1791

Arribere, J. A., J. A. Doudna, and W. V. Gilbert, 2011 Reconsidering movement of eukaryotic mRNAs between polysomes and P bodies. Mol. Cell 44: 745–758. https://doi.org/10.1016/j.molcel.2011.09.019

Baker, R. T., J. W. Tobias, and A. Varshavsky, 1992 Ubiquitin-specific proteases of Saccharomyces cerevisiae. Cloning of UBP2 and UBP3, and functional analysis of the UBP gene family. J. Biol. Chem. 267: 23364–23375.

Balagopal, V., and R. Parker, 2009 Polysomes, P bodies and stress granules: states and fates of eukaryotic mRNAs. Curr. Opin. Cell Biol. 21: 403–408. https://doi.org/10.1016/j.ceb.2009.03.005

Banani, S. F., H. O. Lee, A. A. Hyman, and M. K. Rosen, 2017 Biomolecular condensates: organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol. 18: 285–298. https://doi.org/10.1038/nrm.2017.7

Bashkirov, V. I., H. Scherthan, J. A. Solinger, J. M. Buerstedde, and W. D. Heyer, 1997 A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates. J. Cell Biol. 136: 761–773. https://doi.org/10.1083/jcb.136.4.761

Betting, J., and W. Seufert, 1996 A yeast Ubc9 mutant protein with temperature-sensitive in vivo function is subject to conditional proteolysis by a ubiquitin- and proteasome-dependent pathway. J. Biol. Chem. 271: 25790–25796. https://doi.org/10.1074/jbc.271.42.25790

Boeynaems, S., S. Alberti, N. L. Fawzi, T. Mittag, M. Polymenidou *et al.*, 2018 Protein phase separation: a new phase in cell biology. Trends Cell Biol. 28: 420–435. https://doi.org/10.1016/j.tcb.2018.02.004

Brangwynne, C. P., C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege *et al.*, 2009 Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 324: 1729–1732. https://doi.org/10.1126/science.1172046

Brangwynne, C. P., T. J. Mitchison, and A. A. Hyman, 2011 Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes. Proc. Natl. Acad. Sci. USA 108: 4334–4339. https://doi.org/10.1073/pnas.1017150108

Brielle, S., R. Gura, and D. Kaganovich, 2015 Imaging stress. Cell Stress Chaperones 20: 867–874. https://doi.org/10.1007/s12192-015-0615-y

Buchan, J. R., 2014 mRNP granules. Assembly, function, and connections with disease. RNA Biol. 11: 1019–1030. https://doi.org/10.4161/15476286.2014.972208

Buchan, J. R., and R. Parker, 2009 Eukaryotic stress granules: the ins and outs of translation. Mol. Cell 36: 932–941. https://doi.org/10.1016/j.molcel.2009.11.020

Buchan, J. R., J. H. Yoon, and R. Parker, 2011 Stress-specific composition, assembly and kinetics of stress granules in Saccharomyces cerevisiae. J. Cell Sci. 124: 228–239. https://doi.org/10.1242/jcs.078444

Budovskaya, Y. V., H. Hama, D. B. DeWald, and P. K. Herman, 2002 The C terminus of the Vps34p phosphoinositide 3-kinase is necessary and sufficient for the interaction with the Vps15p protein kinase. J. Biol. Chem. 277: 287–294. https://doi.org/10.1074/jbc.M109263200

Budovskaya, Y. V., J. S. Stephan, F. Reggiori, D. J. Klionsky, and P. K. Herman, 2004 The Ras/cAMP-dependent protein kinase signaling pathway regulates an early step of the autophagy process in Saccharomyces cerevisiae. J. Biol. Chem. 279: 20663–20671. https://doi.org/10.1074/jbc.M400272200

Chang, Y. W., S. C. Howard, and P. K. Herman, 2004 The Ras/PKA signaling pathway directly targets the Srb9 protein, a component of the general RNA polymerase II transcription apparatus. Mol. Cell 15: 107–116. https://doi.org/10.1016/j.molcel.2004.05.021

Cherkasov, V., S. Hofmann, S. Druffel-Augustin, A. Mogk, J. Tyedmers *et al.*, 2013 Coordination of translational control and protein homeostasis during severe heat stress. Curr. Biol. 23: 2452–2462. https://doi.org/10.1016/j.cub.2013.09.058

Cougot, N., S. Babajko, and B. Seraphin, 2004 Cytoplasmic foci are sites of mRNA decay in human cells. J. Cell Biol. 165: 31–40. https://doi.org/10.1083/jcb.200309008

Decker, C. J., D. Teixeira, and R. Parker, 2007 Edc3p and a glutamine/asparagine-rich domain of Lsm4p function in processing body assembly in Saccharomyces cerevisiae. J. Cell Biol. 179: 437–449. https://doi.org/10.1083/jcb.200704147

Didychuk, A. L., S. E. Butcher, and D. A. Brow, 2018 The life of U6 small nuclear RNA, from cradle to grave. RNA 24: 437–460. https://doi.org/10.1261/rna.065136.117

Escusa-Toret, S., W. I. Vonk, and J. Frydman, 2013 Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. Nat. Cell Biol. 15: 1231–1243. https://doi.org/10.1038/ncb2838

Eulalio, A., I. Behm-Ansmant, and E. Izaurralde, 2007a P bodies: at the crossroads of post-transcriptional pathways. Nat. Rev. Mol. Cell Biol. 8: 9–22. https://doi.org/10.1038/nrm2080

Eulalio, A., I. Behm-Ansmant, D. Schweizer, and E. Izaurralde, 2007b P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. Mol. Cell. Biol. 27: 3970–3981. https://doi.org/10.1128/MCB.00128-07

Eystathioy, T., A. Jakymiw, E. K. Chan, B. Seraphin, N. Cougot *et al.*, 2003 The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. RNA 9: 1171–1173. https://doi.org/10.1261/rna.5810203

Feng, X., S. Luo, and B. Lu, 2018 Conformation polymorphism of polyglutamine proteins. Trends Biochem. Sci. 43: 424–435. https://doi.org/10.1016/j.tibs.2018.03.002

Gray, J. V., G. A. Petsko, G. C. Johnston, D. Ringe, R. A. Singer *et al.*, 2004 "Sleeping beauty": quiescence in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 68: 187–206. https://doi.org/10.1128/MMBR.68.2.187-206.2004

Gupta, R., P. Kasturi, A. Bracher, C. Loew, M. Zheng *et al.*, 2011 Firefly luciferase mutants as sensors of proteome stress. Nat. Methods 8: 879–884. https://doi.org/10.1038/nmeth.1697

Herman, P. K., 2002 Stationary phase in yeast. Curr. Opin. Microbiol. 5: 602–607. https://doi.org/10.1016/S1369-5274(02)00377-6

Hubstenberger, A., M. Courel, M. Bénard, S. Souquere, M. Ernoult-Lange *et al.*, 2017 P-body purification reveals the condensation of repressed mRNA regulons. Mol. Cell 68: 144–157.e5. https://doi.org/10.1016/j.molcel.2017.09.003

Huch, S., and T. Nissan, 2017 An mRNA decapping mutant deficient in P body assembly limits mRNA stabilization in response to osmotic stress. Sci. Rep. 7: 44395. https://doi.org/10.1038/srep44395

Huch, S., M. Muller, M. Muppavarapu, J. Gommlich, V. Balagopal *et al.*, 2016 The decapping activator Edc3 and the Q/N-rich domain of Lsm4 function together to enhance mRNA stability and alter mRNA decay pathway dependence in Saccharomyces cerevisiae. Biol. Open 5: 1388–1399. https://doi.org/10.1242/bio.020487

Hyman, A. A., C. A. Weber, and F. Julicher, 2014 Liquid-liquid phase separation in biology. Annu. Rev. Cell Dev. Biol. 30: 39–58. https://doi.org/10.1146/annurev-cellbio-100913-013325

Ingelfinger, D., D. J. Arndt-Jovin, R. Luhrmann, and T. Achsel, 2002 The human LSm1–7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrnl in distinct cytoplasmic foci. RNA 8: 1489–1501.

Ivan, M., and W. G. Kaelin, Jr., 2001 The von Hippel-Lindau tumor suppressor protein. Curr. Opin. Genet. Dev. 11: 27–34. https://doi.org/10.1016/S0959-437X(00)00152-0

Jin, M., G. G. Fuller, T. Han, Y. Yao, A. F. Alessi *et al.*, 2017 Glycolytic enzymes coalesce in G bodies under hypoxic stress. Cell Rep. 20: 895–908. https://doi.org/10.1016/j.celrep.2017.06.082

Kafadar, K. A., H. Zhu, M. Snyder, and M. S. Cyert, 2003 Negative regulation of calcineurin signaling by Hrr25p, a yeast homolog of casein kinase I. Genes Dev. 17: 2698–2708. https://doi.org/10.1101/gad.1140603

Kaganovich, D., R. Kopito, and J. Frydman, 2008 Misfolded proteins partition between two distinct quality control compartments. Nature 454: 1088–1095. https://doi.org/10.1038/nature07195

Kaiser, C., S. Michaelis, and A. Mitchell, 1994 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kedersha, N., G. Stoecklin, M. Ayodele, P. Yacono, J. Lykke-Andersen *et al.*, 2005 Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J. Cell Biol. 169: 871–884. https://doi.org/10.1083/jcb.200502088

Kedersha, N., P. Ivanov, and P. Anderson, 2013 Stress granules and cell signaling: more than just a passing phase? Trends Biochem. Sci. 38: 494–506. https://doi.org/10.1016/j.tibs.2013.07.004

Klaips, C. L., G. G. Jayaraj, and F. U. Hartl, 2018 Pathways of cellular proteostasis in aging and disease. J. Cell Biol. 217: 51–63. https://doi.org/10.1083/jcb.201709072

Kozubowski, L., E. F. Aboobakar, M. E. Cardenas, and J. Heitman, 2011 Calcineurin colocalizes with P-bodies and stress granules during thermal stress in Cryptococcus neoformans. Eukaryot. Cell 10: 1396–1402. https://doi.org/10.1128/EC.05087-11

Li, Y. R., O. D. King, J. Shorter, and A. D. Gitler, 2013 Stress granules as crucibles of ALS pathogenesis. J. Cell Biol. 201: 361–372. https://doi.org/10.1083/jcb.201302044

Longo, V. D., G. S. Shadel, M. Kaeberlein, and B. Kennedy, 2012 Replicative and chronological aging in Saccharomyces cerevisiae. Cell Metab. 16: 18–31. https://doi.org/10.1016/j.cmet.2012.06.002

Luo, Y., Z. Na, and S. A. Slavoff, 2018 P-bodies: composition, properties, and functions. Biochemistry 57: 2424–2431. https://doi.org/10.1021/acs.biochem.7b01162

Lusk, C. P., D. D. Waller, T. Makhnevych, A. Dienemann, M. Whiteway *et al.*, 2007 Nup53p is a target of two mitotic kinases, Cdk1p and Hrr25p. Traffic 8: 647–660. https://doi.org/10.1111/j.1600-0854.2007.00559.x

Mateju, D., T. M. Franzmann, A. Patel, A. Kopach, E. E. Boczek *et al.*, 2017 An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. EMBO J. 36: 1669–1687. https://doi.org/10.15252/embj.201695957

McClellan, A. J., M. D. Scott, and J. Frydman, 2005 Folding and quality control of the VHL tumor suppressor proceed through distinct chaperone pathways. Cell 121: 739–748. https://doi.org/10.1016/j.cell.2005.03.024

Miller, S. B., C. T. Ho, J. Winkler, M. Khokhrina, A. Neuner *et al.*, 2015a Compartment-specific aggregases direct distinct nuclear and cytoplasmic aggregate deposition. EMBO J. 34: 778–797. https://doi.org/10.15252/embj.201489524

Miller, S. B., A. Mogk, and B. Bukau, 2015b Spatially organized aggregation of misfolded proteins as cellular stress defense strategy. J. Mol. Biol. 427: 1564–1574. https://doi.org/10.1016/j.jmb.2015.02.006

Mitchell, S. F., S. Jain, M. She, and R. Parker, 2013 Global analysis of yeast mRNPs. Nat. Struct. Mol. Biol. 20: 127–133. https://doi.org/10.1038/nsmb.2468

Narayanaswamy, R., M. Levy, M. Tsechansky, G. M. Stovall, J. D. O'Connell *et al.*, 2009 Widespread reorganization of metabolic enzymes into reversible assemblies upon nutrient starvation. Proc. Natl. Acad. Sci. USA 106: 10147–10152. https://doi.org/10.1073/pnas.0812771106

Nostramo, R., and P. K. Herman, 2016 Deubiquitination and the regulation of stress granule assembly. Curr. Genet. 62: 503–506. https://doi.org/10.1007/s00294-016-0571-9

Nostramo, R., S. N. Varia, B. Zhang, M. M. Emerson, and P. K. Herman, 2016 The catalytic activity of the Ubp3 deubiquitinating

protease is required for efficient stress granule assembly in Saccharomyces cerevisiae. Mol. Cell. Biol. 36: 173–183.
Oling, D., F. Eisele, K. Kvint, and T. Nystrom, 2014 Opposing roles of Ubp3-dependent deubiquitination regulate replicative life span and heat resistance. EMBO J. 33: 747–761. https://doi.org/10.1002/embj.201386822
Parker, R., 2012 RNA degradation in Saccharomyces cerevisiae. Genetics 191: 671–702. https://doi.org/10.1534/genetics.111.137265
Rabouille, C., and S. Alberti, 2017 Cell adaptation upon stress: the emerging role of membrane-less compartments. Curr. Opin. Cell Biol. 47: 34–42. https://doi.org/10.1016/j.ceb.2017.02.006
Ramachandran, V., and P. K. Herman, 2010 Antagonistic interactions between the cAMP-dependent protein kinase and Tor signaling pathways modulate cell growth in Saccharomyces cerevisiae. Genetics 187: 441–454. https://doi.org/10.1534/genetics.110.123372
Ramachandran, V., K. H. Shah, and P. K. Herman, 2011 The cAMP-dependent protein kinase signaling pathway is a key regulator of P body foci formation. Mol. Cell 43: 973–981. https://doi.org/10.1016/j.molcel.2011.06.032
Rao, B. S., and R. Parker, 2017 Numerous interactions act redundantly to assemble a tunable size of P bodies in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 114: E9569–E9578. https://doi.org/10.1073/pnas.1712396114
Reijns, M. A., R. D. Alexander, M. P. Spiller, and J. D. Beggs, 2008 A role for Q/N-rich aggregation-prone regions in P-body localization. J. Cell Sci. 121: 2463–2472. https://doi.org/10.1242/jcs.024976
Reiser, V., S. M. Salah, and G. Ammerer, 2000 Polarized localization of yeast Pbs2 depends on osmostress, the membrane protein Sho1 and Cdc42. Nat. Cell Biol. 2: 620–627. https://doi.org/10.1038/35023568
Shah, K. H., B. Zhang, V. Ramachandran, and P. K. Herman, 2013 Processing body and stress granule assembly occur by independent and differentially regulated pathways in Saccharomyces cerevisiae. Genetics 193: 109–123. https://doi.org/10.1534/genetics.112.146993
Shah, K. H., R. Nostramo, B. Zhang, S. N. Varia, B. M. Klett *et al.*, 2014 Protein kinases are associated with multiple, distinct cytoplasmic granules in quiescent yeast cells. Genetics 198: 1495–1512. https://doi.org/10.1534/genetics.114.172031
Shah, K. H., S. N. Varia, L. A. Cook, and P. K. Herman, 2016 A hybrid-body containing constituents of both P-bodies and stress granules forms in response to hypoosmotic stress in Saccharomyces cerevisiae. PLoS One 11: e0158776. https://doi.org/10.1371/journal.pone.0158776
Sheth, U., and R. Parker, 2003 Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science 300: 805–808. https://doi.org/10.1126/science.1082320
Sontag, E. M., R. S. Samant, and J. Frydman, 2017 Mechanisms and functions of spatial protein quality control. Annu. Rev. Biochem. 86: 97–122. https://doi.org/10.1146/annurev-biochem-060815-014616
Specht, S., S. B. Miller, A. Mogk, and B. Bukau, 2011 Hsp42 is required for sequestration of protein aggregates into deposition sites in Saccharomyces cerevisiae. J. Cell Biol. 195: 617–629. https://doi.org/10.1083/jcb.201106037
Spokoini, R., O. Moldavski, Y. Nahmias, J. L. England, M. Schuldiner *et al.*, 2012 Confinement to organelle-associated inclusion structures mediates asymmetric inheritance of aggregated protein in budding yeast. Cell Rep. 2: 738–747. https://doi.org/10.1016/j.celrep.2012.08.024
Standart, N., and D. Weil, 2018 P-bodies: cytosolic droplets for coordinated mRNA storage. Trends Genet. 34: 612–626. https://doi.org/10.1016/j.tig.2018.05.005
Stoecklin, G., T. Mayo, and P. Anderson, 2006 ARE-mRNA degradation requires the 5′-3′ decay pathway. EMBO Rep. 7: 72–77. https://doi.org/10.1038/sj.embor.7400572
Takahara, T., and T. Maeda, 2012 Transient sequestration of TORC1 into stress granules during heat stress. Mol. Cell 47: 242–252. https://doi.org/10.1016/j.molcel.2012.05.019
Takahashi, M., M. Higuchi, H. Matsuki, M. Yoshita, T. Ohsawa *et al.*, 2013 Stress granules inhibit apoptosis by reducing reactive oxygen species production. Mol. Cell. Biol. 33: 815–829. https://doi.org/10.1128/MCB.00763-12
Thedieck, K., B. Holzwarth, M. T. Prentzell, C. Boehlke, K. Kläsener *et al.*, 2013 Inhibition of mTORC1 by astrin and stress granules prevents apoptosis in cancer cells. Cell 154: 859–874 (erratum: Cell 155: 964–966). https://doi.org/10.1016/j.cell.2013.07.031
Thomas, M. G., M. Loschi, M. A. Desbats, and G. L. Boccaccio, 2011 RNA granules: the good, the bad and the ugly. Cell. Signal. 23: 324–334. https://doi.org/10.1016/j.cellsig.2010.08.011
Tudisca, V., V. Recouvreux, S. Moreno, E. Boy-Marcotte, M. Jacquet *et al.*, 2010 Differential localization to cytoplasm, nucleus or P-bodies of yeast PKA subunits under different growth conditions. Eur. J. Cell Biol. 89: 339–348. https://doi.org/10.1016/j.ejcb.2009.08.005
van Dijk, E., N. Cougot, S. Meyer, S. Babajko, E. Wahle *et al.*, 2002 Human Dcp2: a catalytically active mRNA decapping enzyme located in specific cytoplasmic structures. EMBO J. 21: 6915–6924. https://doi.org/10.1093/emboj/cdf678
Walters, R. W., D. Muhlrad, J. Garcia, and R. Parker, 2015 Differential effects of Ydj1 and Sis1 on Hsp70-mediated clearance of stress granules in Saccharomyces cerevisiae. RNA 21: 1660–1671. https://doi.org/10.1261/rna.053116.115
Wang, C. Y., W. L. Wen, D. Nilsson, P. Sunnerhagen, T. H. Chang *et al.*, 2012 Analysis of stress granule assembly in Schizosaccharomyces pombe. RNA 18: 694–703. https://doi.org/10.1261/rna.030270.111
Weber, S. C., and C. P. Brangwynne, 2012 Getting RNA and protein in phase. Cell 149: 1188–1191. https://doi.org/10.1016/j.cell.2012.05.022
Werner-Washburne, M., E. Braun, G. C. Johnston, and R. A. Singer, 1993 Stationary phase in the yeast Saccharomyces cerevisiae. Microbiol. Rev. 57: 383–401.
Wippich, F., B. Bodenmiller, M. G. Trajkovska, S. Wanka, R. Aebersold *et al.*, 2013 Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. Cell 152: 791–805. https://doi.org/10.1016/j.cell.2013.01.033
Zhang, B., Q. Shi, S. N. Varia, S. Xing, B. M. Klett *et al.*, 2016 The activity-dependent regulation of protein kinase stability by the localization to P-bodies. Genetics 203: 1191–1202. https://doi.org/10.1534/genetics.116.187419
Zhang, B., A. Butler, Q. Shi, and P. K. Herman, 2018 P-body localization of the Hrr25/Casein Kinase 1 protein kinase is required for the completion of meiosis. Mol. Cell. Biol. 38: e00678-17. https://doi.org/10.1128/MCB.00678-17
Zid, B. M., and E. K. O'Shea, 2014 Promoter sequences direct cytoplasmic localization and translation of mRNAs during starvation in yeast. Nature 514: 117–121. https://doi.org/10.1038/nature13578

Communicating editor: O. Cohen-Fix