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Deubiquitination and the regulation of stress granule assembly

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Abstract

Stress granules (SGs) are evolutionarily-conserved ribonucleoprotein (RNP) structures that form in response to a variety of environmental and cellular cues. The presence of these RNP granules has been linked to a number of human diseases, including neurodegenerative disorders like amyotrophic lateral sclerosis (ALS) and spinocerebellar ataxia type 2 (Li et al., 2013; Nonhoff et al., 2007). Understanding how the assembly of these granules is controlled could therefore suggest possible routes of therapy for patients afflicted with these conditions. Interestingly, several reports have identified a potential role for protein deubiquitination in the assembly of these RNP granules. In particular, recent work has found that a specific deubiquitinase enzyme, Ubp3, is required for efficient SG formation in *S. cerevisiae* (Nostramo et al., 2016). This same enzyme has been linked to SGs in other organisms, including humans and the fission yeast, *Schizosaccharomyces pombe* (Takahashi et al., 2013; Wang et al., 2012). At first glance, these observations suggest that a striking degree of conservation exists for a ubiquitin-based mechanism controlling SG assembly. However, the devil is truly in the details here, as the precise nature of the involvement of this deubiquitinating enzyme seems to vary in each organism. Here, we briefly review these differences and attempt to provide an overarching model for the role of ubiquitin in SG formation.

Keywords

Stress granules; deubiquitinase enzymes; intrinsically-disordered domains; neurodegenerative disease; ubiquitin

SG formation in eukaryotic cells

In response to stress, cells undergo many molecular changes that allow them to adapt and survive under these conditions of duress. One such change is the formation of an RNP complex, known as the SG (Kedersha et al., 1999). SGs are a type of nonmembranous organelle that contains non-translating mRNAs, 40S ribosomal subunits, and translation initiation factors (Anderson and Kedersha, 2008, 2009; Malinovska et al., 2013). As a result, SGs are thought to be sites of storage for mRNAs that will be translated after the stress condition is removed. However, these granules also contain constituents that suggest other activities for these RNP structures. These latter constituents include RNA-binding proteins, transcription factors, protein kinases and other signaling molecules. One way in which stress granules have been shown to influence cell function is through the sequestration of particular

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signaling proteins. For example, the scaffolding protein, RACK1, is efficiently recruited to stress granules under particular stress conditions (Arimoto et al., 2008). As a result of this sequestration, a MAP kinase required for apoptosis, MTK1, is not activated and the cells survive this stress exposure. In other instances, cells avoid a TORC1-induced form of programmed cell death by recruiting Raptor, a key component of the TORC1 signaling complex, to these RNP granules (Thedieck et al., 2013). Finally, a recent study has found that a number of protein kinases and phosphatases are localized to a variety of cytoplasmic granules in quiescent yeast cells (Shah et al., 2014). Altogether, these observations suggest that RNP granules could serve as signaling hubs in the cytoplasm of eukaryotic cells (Kedersha et al., 2013).

The formation of SGs is triggered by a stress-induced inhibition of translation that can be either dependent or independent of the initiation factor, eIF2α (Kedersha et al., 1999; Mazroui et al., 2006). This inhibition leads to the disassembly of polysomes and the accumulation of 48S preinitiation complexes containing naked mRNA. These preinitiation complexes are then bound by mRNA-binding proteins that contain low-complexity domains that may direct the aggregation responsible for foci formation (Kedersha et al., 2013). The RNP granules that form may provide the cell with a strikingly different type of subcellular compartment. In particular, these RNP structures are highly dynamic, with components exchanging rapidly between the granule and the existing cytoplasmic pools during the period of stress. Following the restoration of normal growth conditions, the granules quickly disassemble with the RNA and protein components being rapidly dispersed back into the cytosol.

SGs form in response to a wide variety of conditions, including nutrient deprivation, oxidative stress and heat shock. These granules have been observed in isolated cells and in the context of the entire organism. Examples of the latter include granule formation in the embryonic muscles of *Drosophila* following hypoxia, in rat and mouse brains following injury and in hippocampal neurons following global brain ischemia (Kayali et al., 2005; Kim et al., 2006; van der Laan et al., 2012). SGs have also been linked to numerous human diseases, including different cancers and neurodegenerative diseases, like ALS and spinocerebellar ataxia type II (Anderson et al., 2015; Li et al., 2013; Nonhoff et al., 2007). In both of the latter neurodegenerative disorders, causal mutations occur in genes encoding proteins that are found associated with stress granule foci. However it is not yet clear how these RNP granules contribute to the pathology of either disease (Li et al., 2013). Finally, evidence from recent studies indicate that stress granules are involved in the replicative and infection cycles of particular viruses (Lloyd, 2013). Accordingly, there is a growing interest in understanding the biological roles and regulation of these RNP structures.

To fully understand the role SGs play in human disease it will be important to define the mechanisms underlying their formation. This information may allow us to disrupt SG assembly and to assess the consequences of this disruption. This type of an approach has been used successfully to identify a critical role for P-bodies, a similar RNP structure, in the long-term survival of quiescent yeast cells (Ramachandran et al., 2011). With this in mind, we used a candidate approach to screen a defined set of yeast gene knockout strains for defects in SG assembly. These experiments identified an important role for the Ubp3

deubiquitinating enzyme in this assembly process (Nostramo et al., 2016). Previous work had already indicated that the mammalian ortholog of Ubp3, USP10, was required for efficient SG formation (Takahashi et al., 2013). In the following sections, we review these data and discuss potential ways that the Ubp3/USP10 deubiquitinase might influence SGs in these different organisms. Finally, we will explore the possible implications of these findings particularly as they relate to the role that protein ubiquitination might play in the regulation of SG formation.

The Ubp3/USP10 deubiquitinase and the regulation of SG assembly

A role for the Ubp3/USP10 enzyme in the regulation of SG assembly has been demonstrated in budding yeast and mammalian cells. The loss of this deubiquitinase in each organism results in both fewer and smaller SG foci in response to a number of different stimuli. In *S. cerevisiae*, a deficiency in Ubp3 greatly diminishes the ability of cells to form SGs in response to heat shock and sodium azide treatment and upon the growth arrest associated with the entry into stationary phase (Nostramo et al., 2016). In the latter case, the absence of Ubp3 results in not only a delay in SG formation, but once formed the granules are smaller, exhibit a lower fluorescence intensity and are present in a decreased fraction of cells. SGs are generally assessed by microscopy with cells expressing SG proteins tagged with appropriate fluorescent reporters, like GFP. These effects were specific to Ubp3 as no stress granule defects were observed in strains lacking any of the other fifteen Ubp deubiquitinase enzymes present in this yeast (Nostramo et al., 2016). Similarly, the loss of USP10 in mouse embryonic fibroblasts (MEFs) resulted in a diminished ability to form SGs following a treatment with arsenite (Takahashi et al., 2013). SGs were detected in fewer of the USP10-deficient cells and those foci that did form were smaller and disappeared more rapidly than in wild-type cells. Finally, SG formation was also defective in HEK293 cells that were exposed to arsenite or a heat shock (Takahashi et al., 2013).

These studies suggested that the requirement for the Ubp3/USP10 enzyme during SG assembly has been conserved from yeast to mammals. However, a deeper examination of these data reveals some potential differences in the precise role of Ubp3/USP10. In particular, whereas studies with budding yeast indicate that Ubp3 catalytic activity is needed for SG assembly, the situation is less clear in mammalian cells. The requirement in *S. cerevisiae* was demonstrated with three different strains known to possess diminished levels of Ubp3 activity. The first of these expresses a variant protein, $Ubp3^{C469A}$, in which a cysteine residue critical for catalytic activity was changed to an alanine (Cohen et al., 2003). The second strain lacks Bre5, a key protein cofactor needed for Ubp3 deubiquitinase activity (Cohen et al., 2003). Finally, the third strain expressed a distinct Ubp3 variant, $Ubp3^{LFIN\text{-}AAAA}$, where four residues critical for the interaction with Bre5 were replaced with alanines (Li et al., 2007). Each of these strains was found to exhibit significant defects in stress granule formation (Nostramo et al., 2016). However, this requirement for catalytic activity has not yet been observed with mammalian cells. Instead, a recent study found that the expression of a catalytically-defective USP10 variant could rescue, at least partially, the decrease in SG formation observed in USP10-deficient cells (Takahashi et al., 2013). Finally, to muddy the waters further, studies with *S. pombe* have found that SG formation is not affected in strains lacking Ubp3 (Wang et al., 2012). In all three organisms, the Ubp3

enzyme was found to co-localize with stress granule foci (Nostramo et al., 2016; Takahashi et al., 2013; Wang et al., 2012). As a result, the precise nature of the Ubp3 involvement in SG formation remains to be worked out.

The key question then is how might one reconcile these apparent discrepancies. To our minds, there appear to be two potential solutions. The first takes the data at face value and posits that the presence of Ubp3 in SGs has different consequences in different eukaryotes. Whereas the deubiquitinase activity is important for SG assembly in *S. cerevisiae*, a distinct function associated with USP10 may be the critical activity in mammalian cells (Takahashi et al., 2013). The second possibility is that the deubiquitinase activity is important in each organism and that this requirement may have been missed in the experiments performed to date. For example, the expression of the catalytically-inactive USP10 was able to only partially rescue the SG defects observed in USP10-deficient MEFs (Takahashi et al., 2013). Given that this variant may have been over-expressed in this experiment, it is certainly a possibility that the deubiquitinase activity associated with USP10 is also important for SG assembly. In the case of *S. pombe*, it will be important to test whether there is a second deubiquitinase activity that is functionally redundant with Ubp3 and thus allows for SG assembly in the absence of Ubp3. In either case, it is clear that additional studies are necessary in order to resolve these questions about the importance of Ubp3 catalytic activity.

A role for deubiquitination in the regulation of SG assembly?

As a deubiquitinase, the major function of Ubp3 is to remove mono- or polyubiquitin modifications from target proteins (Reyes-Turcu et al., 2009). Identifying the Ubp3 target(s) relevant to the regulation of SG assembly is the next key step in this analysis. A preliminary screen of yeast deletion strains lacking known targets of Ubp3 failed to identify any defects in SG assembly (Nostramo et al., 2016). Futher work is therefore needed to identify this novel Ubp3 substrate. Given that Ubp3 is localized to SGs, it is likely that this target may itself be a granule protein. In fact, the simplest model is that the presence of ubiquitin on this SG protein would act to inhibit granule formation. The Ubp3-catalyzed removal of this modification could therefore initiate the assembly process. A possible mechanism for this inhibition is suggested by previous studies of protein phosphorylation within intrinsically disordered domains (IDDs) (Collins et al., 2008; Iakoucheva et al., 2004). IDDs are present in several SG proteins and these domains have been shown to be important for the presence of these proteins in foci and also in the ultimate assembly of SGs (Gilks et al., 2004). In the case of phosphorylation, this modification has been observed to introduce order, or structure, into an IDD (Bah et al., 2015; Espinoza-Fonseca et al., 2008; Galea et al., 2008; Pufall et al., 2005). Therefore, it is interesting to speculate that the addition of one or more ubiquitin residues might have a similar influence on particular IDDs and that this modification could prevent specific IDD-mediated interactions important for efficient SG assembly (Radivojac et al., 2010; Uversky et al., 2008). Clearly, a test of this possibility will require the identification of the Ubp3 substrates that are relevant for the regulation of SG assembly. Given that we know the identity of at least some of these IDD-containing proteins, it will be important to ask whether these constituents are modified by ubiquitin and if any identified modifications can be removed by Ubp3. In all, the completion of these studies should lead to

a better understanding of the mechanisms controlling SG assembly and could provide insight into potential treatments for diseases caused by altered SG dynamics.

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