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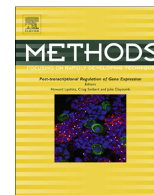

Isolation of yeast and mammalian stress granule cores

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ABSTRACT

Stress granules are dynamic, conserved RNA-protein (RNP) assemblies that form when translation is limiting; and are related to pathological aggregates in degenerative disease. Mammalian stress granules are comprised of two structures – an unstable shell and more stable cores. Herein we describe methodology for isolation of stress granule cores from both yeast and mammalian cells. The protocol consists of first enriching for stress granule cores using centrifugation and then further purifying stress granule cores using immunoprecipitation. The stress granule core isolation protocol provides a starting point for assisting future endeavors aimed at discovering conserved RNA regulatory mechanisms and potential links between RNP aggregation and degenerative disease.

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1. Description of theoretical basis and framework for the technique

Stress granules are conserved RNA-protein (RNP) assemblies that form when translation initiation is impaired [3]. Mammalian and yeast stress granules are comprised of both RNA and protein, with approximately half of proteins that localize to stress granules containing RNA-binding activity [6]. The presence of RNA is thought to be a critical stress granule scaffold as trapping mRNA in translation elongation impairs stress granule formation [2,3,7]. In addition, some proteins which localize to stress granules contain intrinsically disordered regions (IDR) which could promote physical protein-protein interactions and contribute to stress granule assembly [5,7,8,9,10,11,13]. Stress granules are comprised of a dense network of physical interactions and stress granule composition can change in response to different stressors [1,4,6]. Therefore, a broader understanding of stress granule composition is likely provide insights into RNP granule formation and RNA regulation.

Stress granules are dynamic structures which readily exchange components with the surrounding cytosol [3,8]. Mammalian stress granules are comprised of at least two phases: a dynamic phase separated shell that readily exchanges with the surrounding cytosol, and more stable RNP cores [6]. In contrast, yeast stress granules are largely comprised of a core RNP assembly, possibly with a proportionally smaller phase separated shell [6]. In both yeast and mammalian cells, stress granule cores form early during stress granule assembly suggesting these core complexes may provide the specific set of interactions necessary for seeding formation of a higher order liquid-like stress granule shell [12].

Purification of stress granules has been a major challenge in the field due to the dynamic and transient nature of stress granule shells. Recently, we established a protocol aimed at isolating the more stable stress granule core from both yeast and mammalian cells [6]. Consistent with these complexes being related to stress granules, we observe both yeast and mammalian stress granule cores are only observed under stress conditions and contain known stress granule components. Isolation of stable stress granule cores allowed for the identification of several novel members of the yeast and mammalian stress granule proteome.

Here, we provide a detailed description of the stress granule core isolation protocol for both yeast and mammalian cells. A critical step in this protocol is to first enrich for large complexes prior to affinity purification. Although, components of stress granules enrich into stress granules, the majority of these proteins remain freely distributed throughout the cytosol during stress. For example, we estimate only 18% of G3BP1 is enriched into stress granules in U-2 OS cells during arsenite stress (Fig. 1A). Similarly, we estimate the partition coefficient of PABPC1 into stress granules is ~3X lower than that of G3BP (as assessed by SIM analysis (Fig. 1B). Since we have found free stress granule components are more efficiently selected in immunopurifications (data not shown), to avoid this free pool and purify stress granules, larger stress granule assemblies must first be enriched.

In brief, the approach used is that stress granules are first isolated from stressed cultures and enriched using centrifugation.

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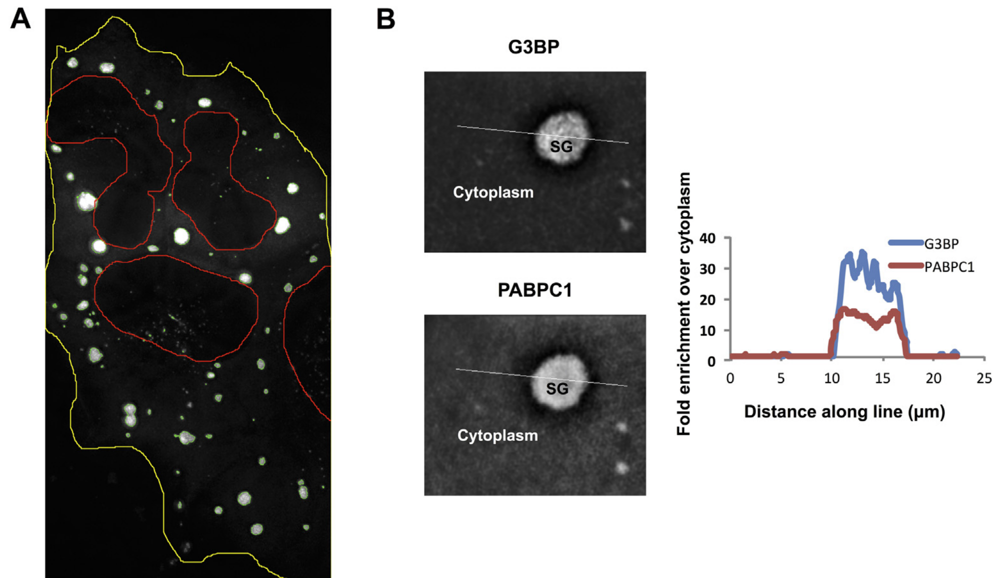

Fig. 1. Quantification of percentage of G3BP and PABPC1 in granules. (A) Example of the quantification of percent of G3BP in granules taken from multiple U-2 OS cells expressing G3BP-GFP. Yellow line represents cytoplasm boundary. Red line represents boundary of the nucleus. Green lines represent stress granule boundary. Fraction of total intensity of GFP (G3BP) in stress granules was determined by comparing total intensity of all granules in image to total intensity within cell boundaries using ImageJ. (B) SIM image of the same granule imaged for G3BP (top) am PABPC1. Cytoplasm and stress granule (SG) are labeled. Graph shown alongside shows normalized quantification of intensity (left to right) along with white line shown in the image. Intensity is normalized to background subtracted average intensity in the cytoplasm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Stress granules are further purified using immunoprecipitation with antibodies against known stress granule components. Together, this protocol provides a purified population of stress granule cores, which could be used for proteomic, transcriptomic, or biochemical experiments.

2. Yeast stress granule isolation protocol (cartooned in Fig. 2)

1. Grow 1.2 L culture to log phase. Typically, we use a strain with a GFP labeled component of stress granules allowing the detection of stress granules at various steps along the protocol.
2. Apply stress. Stresses that induce stress granules in yeast include sodium azide, glucose deprivation, Vanillin, or heat shock [4,6].
3. Pellet cells 4000g, 1 min at room temperature in 50 mL falcons & freeze pellet in liquid N_2.

a. Stopping point: Pellets can be stored at −80 °C. We routinely freeze down multiple cell pellets in advance of starting the stress granule core isolation protocol.

4. For each Falcon tube, re-suspend pellet in 500 µL lysis buffer on ice and transfer to 2 mL microcentrifuge tube and add glass beads (approx. 300–500 µL of glass beads). We recommend use of acid-washed glass beads (425–600 µm).
5. Lyse by vortexing tubes containing cell pellet mixed with glass beads for 2 min at 4 °C using a cell disruptor genie. Recover for 2 min on ice between vortexing cycles.
6. Repeat step 5 two additional times.
7. Poke hole in bottom of 2 mL microcentrifuge tube using a 18G ½ needle, heated to white hot over a flame. Place the microcentrifuge tube in a 15 mL Falcon tube and spin at 2000g, 2 min to collect lysate.

b. We recommend that the lysate be microscopically inspected for the presence of GFP-tagged stress granule cores to assess lysis efficiency (see note in Section 4).

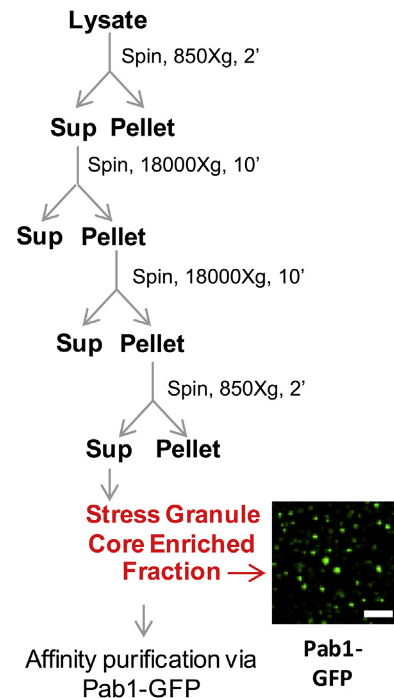

Fig. 2. Isolation of yeast stress granule cores. Scheme for preparation of stress granule core enriched fraction from crude cell lysate from yeast cells. Image shows stress granule core enriched fraction from cells carrying Pab1-GFP.

8. Transfer supernatant from 15 mL tube to a new 1.5 mL microcentrifuge tube.
9. Spin 1.5 mL microcentrifuge tube at 18,000g, 10 min at 4 °C. Following spin, discard supernatant.
10. Re-suspend pellet in 1 mL of stress granule lysis buffer.
11. Spin 1.5 mL microcentrifuge tube at 14,000g, 10 min at 4 °C. Following spin, discard supernatant.

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