RNA Stress Granules: A molecular investigation into stress resilience during aging

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Background: Aging is characterized by the accumulation of cellular damage, the physiological decline of tissue and an increased susceptibility to disease resulting from the failure to maintain homeostasis in the face of endogenous and environmental stresses [1]. A key mechanism underlying protein homeostasis (proteostasis) in response to stress is the assembly of RNA stress granules (SGs). When stress dissipates, SGs disassemble and cells return to homeostasis, thus SGs dynamic behavior offers a potential molecular mechanism that links aging and cellular stress resilience. Interestingly, changes in SG dynamics have been identified in age-dependent neurodegenerative disorders, yet SGs remain unexplored in normal aging.

SGs are non-membrane bound organelles that assemble in the cytoplasm of cells when translation initiation is inhibited or during stress (*e.g.* heat shock, osmotic pressure, oxidative stress) [2]. SG formation has been shown to increase fitness during stress [3]. During transient stress, SGs stabilize mRNA and delay the aggregation of proteins linked to neurodegeneration [4-5]. SGs preferentially sequester long, poorly translated RNAs as well as a diverse set of proteins such as nuclear pore complexes, RNA binding proteins and others varying by cell type and stressor [6-7]. SG assembly is rapid: a dense core is formed by an established network of protein-protein interactions, nucleated by G3BP1, followed by the assembly of a dynamic shell comprising RNA and RNA binding proteins that trigger liquid-liquid phase separation. After stress subsides, SGs spontaneously disassemble and allow sequestered factors to return to their functions [8]. When SGs fail to disassemble, such as during chronic stress, they disrupt, not maintain, proteostasis and facilitate protein aggregation [9]. Two previous studies found that SG components aggregate with age, but it is unknown how normal aging alters the nucleation, stability, or disassembly of SGs [10-11].

*I hypothesize that **(1)** the dynamics of SGs will be altered throughout aging and **(2)** the composition of SGs will correspondingly be altered by age.*

Aim 1: Determine the dynamics of SGs during aging in response to stress

Using a *Drosophila* model where Rasputin (RIN), the homolog of G3BP1 and the only protein required for SG formation, is endogenously tagged with GFP (RIN-GFP), I will visualize SG formation in the fly brain [12]. *Drosophila* share over 60 percent of their genome with humans, providing a translatable and practical model to study SGs throughout aging (lifespan averages 100 days). To determine if age impacts SG dynamics (*e.g.* assembly and disassembly) in fly brains, I will dissect adult fly brains and immunostain for GFP. Using a confocal microscope, I will quantify the distribution and sizes of RIN-GFP puncta in various regions of the fly brain. *Drosophila* will be dissected at five time points across aging (1, 20, 50, 80, 100 days). To capture the altered dynamics of SGs between types of stress, heat shock and oxidative stress through paraquat ingestion will be used to stress flies just before dissection. Controls will include age matched *Drosophila* that will not be subjected to stress. To distinguish between SG assembly and disassembly, flies will be dissected just after stress (assembly) or two hours after stress (disassembly). Ten to twenty flies will be studied per time point and treatment.

Expected outcomes, potential pitfalls and alternatives. Preliminary experiments show that SG assembly declines with age in flies (data not shown) thus I expect less robust assembly of new SGs for older *Drosophila.* I expect most SGs to disassemble one hour after stress for young *Drosophila.* For old *Drosophila,* I expect less dynamic SGs or slower disassembly. The assembly mechanism of SGs varies slightly by stressor. Oxidative stress has canonically been used to elicit SG formation [2]. The dynamic behavior of heat shock induced SGs could defy expectations

based on research into SGs generated by oxidative stress. Previous research shows some SG aggregation in aging [10-11]. If SG disassembly does not visibly change with age, though, changes in SG composition could alter SG dynamics in other ways. These experiments will show for the first time how aging fly brains respond to stress by assembling SGs. Future research will examine the relationship between altered SG dynamics and phenotypes of aging (*e.g.* behavior).

Aim 2: Determine the composition of SGs during aging in response to stress

SG dynamics are impacted by the composition of SGs. For example, the recruitment of protein kinases ULK1 and ULK2 and the ATPase VCP to SGs is required for SG disassembly [13]. Age-related alterations to SG composition are likely responsible for the expected decline in dynamic SG formation during aging. SGs will be isolated using immunoprecipitation (IP) of GFP tagged RIN protein/RNA complexes from the neurons of the same experimental *Drosophila* and controls described above. IPs will be optimized using IgG controls to ensure specificity of RIN-GFP complexes. Mass spectroscopy and RNA-sequencing will be used to identify the proteins and RNAs comprising SGs throughout the various stress and aging conditions. Key SG markers will be cross-referenced. The results will be analyzed using statistical models to identify which proteins and RNAs are enriched or depleted in specific conditions and age time points.

Expected outcomes, potential pitfalls and alternatives. The differential recruitment of proteins to SGs has physiological impacts on cells during the stress response and can alter SG dynamics. For example, if SGs formed during old age increasingly sequester nuclear pore complexes, proteostasis is more likely to be disturbed in aging [6]; similar logic applies to other pathways. RNAs sequestered to SGs mediate protein recruitment but do not impact global translation [8]. By linking the composition of SGs with different assembly and disassembly phenotypes, the impact of age on the mechanism of SG-supported stress resilience can be more fully understood. To follow up candidates identified by IP/mass-spec, overexpression or knockdown of genes in *Drosophila* will be used to identify the specific role of proteins in the alteration of SGs dynamics in the stress response throughout aging.

Intellectual Merit and Broader Impacts: For the past three years, I worked with *Drosophila* studying RNA biology in aging and the cellular stress response. Through my research, I optimized IP procedures to isolate SGs and prepare them for analysis by mass spectroscopy and RNA-seq. To analyze this data, I created statistical models using the R programming language. Given SGs role in stress resilience and neurodegeneration, understanding the impact of age on the dynamics of SGs is important. These findings will help generate new hypotheses about the role of the stress response in aging. Additionally, researchers are pursuing treatments to the neurodegenerative disease amyotrophic lateral sclerosis that eliminate SGs in humans. SG elimination could significantly impair the ability of neurons to survive especially during aging. The proposed experiments will provide key insights into new strategies to maintain cellular homeostasis in the human body, specifically the brain, and extend the health span as well as lifespan. As part of my research, I am responsible for leading and training a small team of undergraduates in our study of RNA biology in aging. In addition to effectively communicating my research at two international conferences and to my community, I helped review current research on the role of SGs in protein aggregation for an upcoming publication.

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