G body dynamics and localization in *Saccharomyces cerevisiae*

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Introduction

Under prolonged periods of hypoxic stress, glycolysis enzymes, including phosphofructokinase (Pfk), and RNA, which are normally diffuse in the cytoplasm, coalesce to form nonmembrane bound structures called glycolytic bodies, G bodies.[1] The presence of G bodies is correlated with increased rates of glucose consumption. We hypothesize that glycolysis enzymes coalesce in hypoxia to compensate for the lack of respiration that would occur in normoxia. The overall mechanisms that induce G body formation and degradation are still unspecified. Glucokinase 1 (Glk1) can be copurified with mitochondria and is punctate in normoxia and hypoxia.[2] We hypothesized that Glk1 may be the site of nucleation for G body formation. To determine if Glk1 is required for G body formation, we measured the rate of of G body formation in *glk1Δ* mutants. Using Pfk2-GFP *S. cerevisiae*, we could determine and predict G body degradation rates. Measuring the fraction of cells with G bodies following reoxygenation and correcting for cell growth, we could determine that the rate of G body degradation is greater than would be expected due to cell division. From the loss of G bodies we hypothesized that G bodies can be degraded, potentially via autophagy.

If Glk1 seeds G body formation, then we would expect Glk1 and G bodies to have similar localization patterns. Given that Glk1can be copurified from the mitochondria, and that there are known structures that localize to vacuoles, we wanted to study the pattern of G bodies relative to mitochondria and vacuoles. Through the use of organelle stains and the G body marker, Pfk2-GFP, we were able to visualize G body localization patterns relative to other subcellular structures. The mitochondrial and vacuolar localization data suggest that G bodies display more frequent localization with vacuoles. To investigate the nature of G body vacuolar association, we imaged G body markers in cells with a vacuolar stain over time. Taking time series images of the cells, individual G bodies in cells were followed for 30min time scales. The time series results established that G bodies are not static and remain close to vacuoles over long timescales.

Results

Glk1 is Necessary for G body Formation

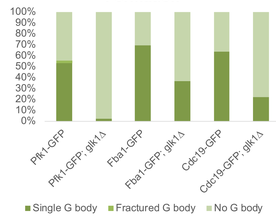

Figure 1: The effect of knocking out Glk1 in G body formation was observed using GFP tagged G body marker proteins were used as an indicator of G body formation.

G bodies are Reversible

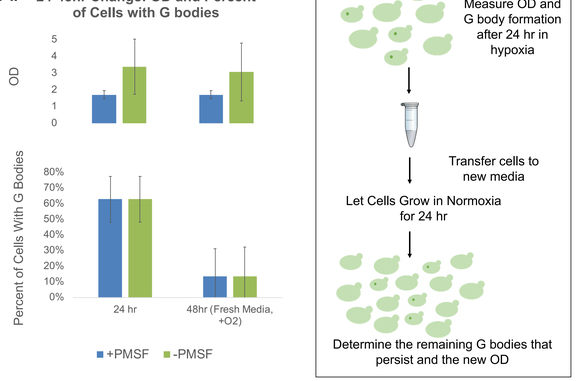

Figure 2: A. OD measurements and percentage of cells with G bodies at 24hrs vs 48hrs. Cells were first grown in hypoxia (0-24hr) and then in normoxia (24-48hr). **B.** Graphic depiction of the fold change in OD and the fraction of cells with G bodies. (*Expected: fold change if percent of cells with G bodies were diluted by cell division alone) **C.** Pfk2-GFP cells with single puncta are quantified as cells with G bodies. G bodies can persist for long periods of time in upon transferring from hypoxia to normoxia. Thus, the expected fraction of cells with G bodies after shifting to normoxia is the inverse of the fold increase in OD.

Results

G body and Glk1 Localize to Mitochondria

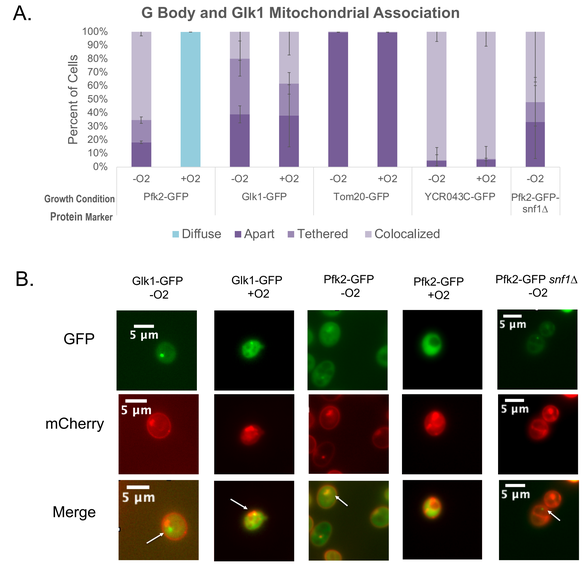

Figure 3A. GFP tagged proteins' localization to the mitochondria was observed.
- Pfk2: enzyme involved in glycolysis, G body component, Glk1: enzyme possibly required for G body formation, Tom20: mitochondrial protein (positive control), YCR943C: endoplasmic reticulum protein (negative control), Snf1: protein required for proper G body formation.

B. Colocalization images of Pfk2 and Glk1 in normoxia and hypoxia to the mitochondria

G bodies Localize to Vacuoles

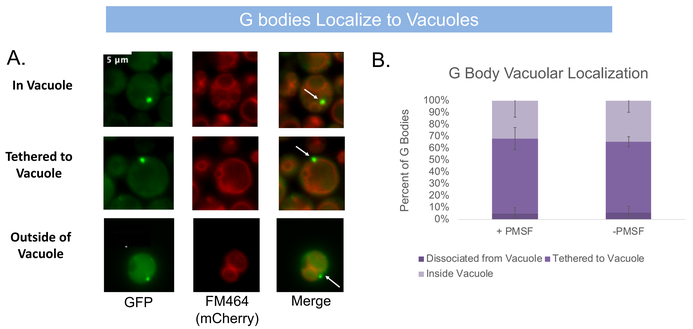

Figure 4: A. Method of quantifying Pfk2-GFP cells stained with FM464 vacuolar stain.
B. Distribution of the localization of G bodies to vacuoles with and without the administration of 1mM PMSF.

G bodies Associate With Vacuoles

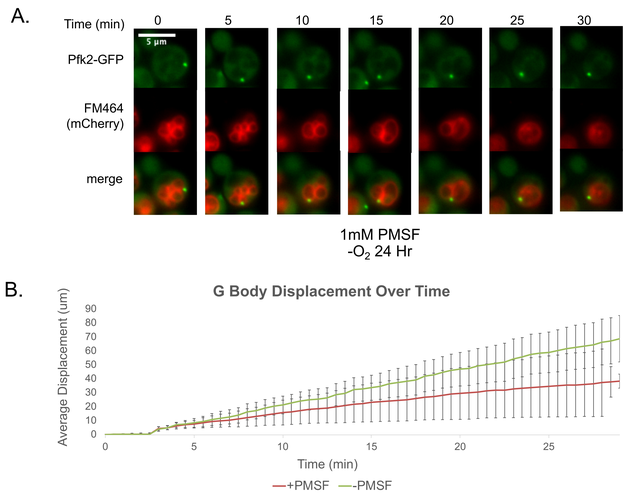

Figure 5: A. Images of PMSF treated and FM464 (vacuolar membrane stain) stained cells at different time points. **B.** The average intracellular displacement of G bodies over time. **C.** The average distance between the center of a G body and the membrane of a vacuole over time. *Figure 6B and 6C: n = 38 and n = 95 for +PMSF and –PMSF respectively

Conclusion

- Glk1 is required for G body formation
- G bodies localize to both mitochondria and vacuoles
- G bodies disappear from a population at faster rates than would be expected due to population doubling
- G bodies move around vacuoles while staying closely associated with vacuoles

Future Directions

- Engineer a Glk1p-Gfp; Pfk2p-Scarlet strain of *S. cerevisiae* to conduct colocalization experiments to determine if Glk1 is the site of G body nucleation
- Induce autophagy via nitrogen starvation to rule if autophagy is a mechanism for G body degradation

References

1. Jin M, Fuller GG, Han T, Yao Y, Alessi AF, Freeberg MA, et al. Glycolytic Enzymes Coalesce in G Bodies under Hypoxic Stress. Cell Reports. 2017 Jul 25;20(4):895–908.
2. Morgenstern M, Stiller SB, Lübbert P, Peikert CD, Dannenmaier S, Drepper F, et al. Definition of a High-Confidence Mitochondrial Proteome at Quantitative Scale. Cell Reports. 2017 Jun 27;19(13):2836–52.