Understanding interactions between distinct phase-separated condensates

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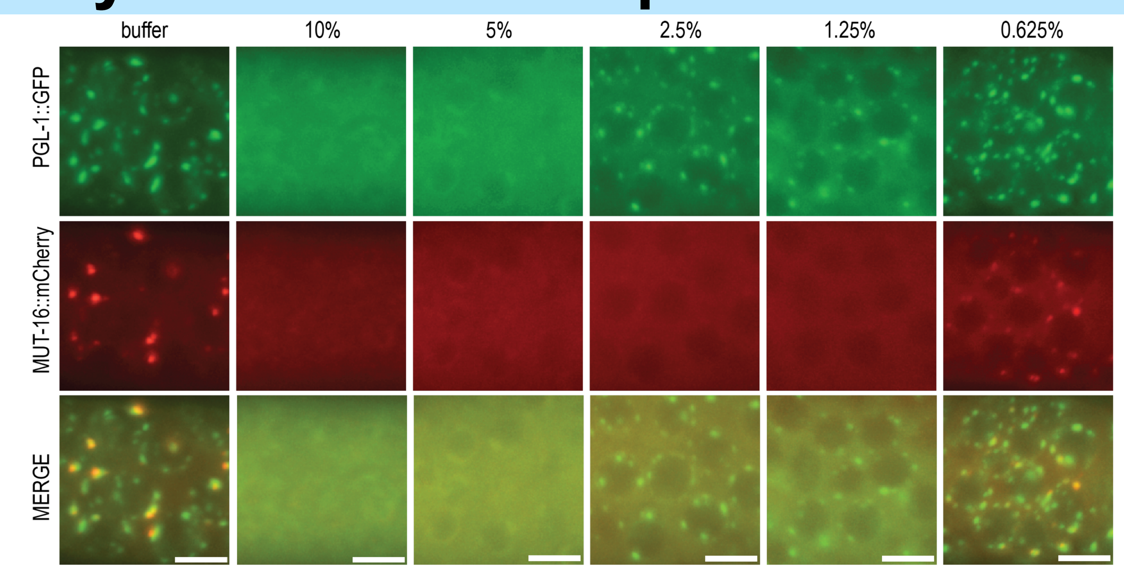
Introduction

Phase separation has emerged as a crucial cellular strategy for many biomolecular functions. Phase separation occurs when proteins, typically containing low complexity domains or intrinsically disordered regions, coalesce to create a dense and highly dynamic droplet that maintains a distinct structure within the surrounding bulk phase, similar to the immiscibility of oil droplets in water. This strategy of condensation is seemingly ubiquitous in the biological world, occurring in cells ranging from bacterial to human. Consequently, phase separation underlies many crucial cellular processes. Currently, some main goals of the field have been discovering and verifying new condensates, testing biophysical properties and conditions for condensation, and probing the specific functions of condensates. However, understanding how two distinct phases interact together to coordinate a pathway has been largely understudied.

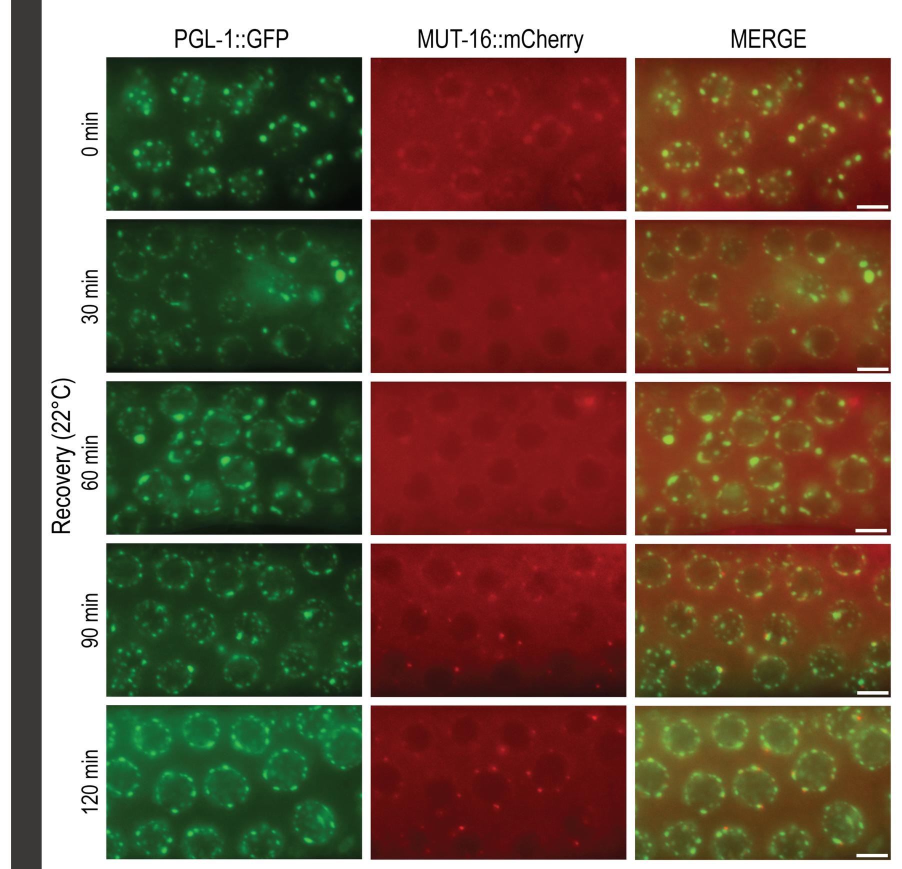
Here we utilize prominent phase-separated condensates in the *C. elegans* small interfering RNA (siRNA) pathway to probe the interactions between condensates and the potential for pathway organization and coordination. Understanding interactions between these condensates not only allows us to build a more accurate model of mRNA surveillance, but also provides basic biological insight to how phase separation coordinates cellular processes.

2 Differences in hydrophobic content may underlie foci separation



Top row: 1,6-hexanediol is an aliphatic alcohol that disrupts weak hydrophobic interactions of phase-separated condensates. Updike et al. (2011) showed PGL-1::GFP foci are disrupted in concentrations of 1,6- hexanediol at or above 5%. **Middle row:** MUT-16::mCherry foci are disrupted in concentrations at or above 1.25% 1,6-hexanediol, indicating *Mutator* foci are more sensitive to perturbation of hydrophobic interactions. **Bottom row:** Image merge. All live images are from the transition zone and pachytene zone of dissected young adult gonads with distal tip to the left. Scale bars: 5µm.

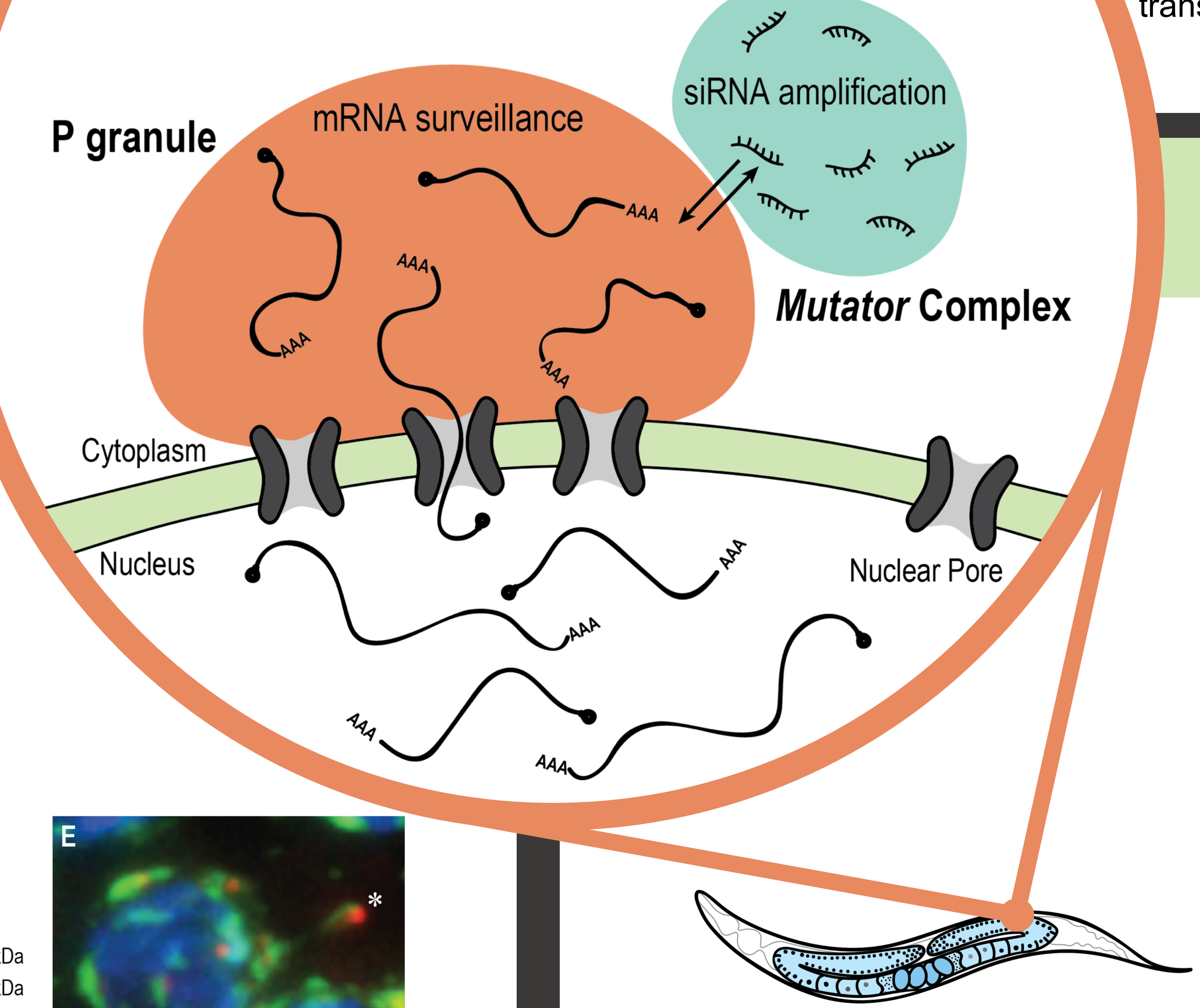
3 Foci separation is re-established after heat stress perturbation



Animals were heat shocked (HS) for 2 hours at 34°C and allowed to recover for 2 hours at 22°C. **0 min recovery**: Immediate imaging post-HS (± 5 min) revealed MUT-16::mCherry signal was diffusely colocalized with P granules. **30-60 min recovery**: No MUT-16 foci are visible, but diffuse colocalization with P granules appears reduced. **90 min recovery**: *Mutator* foci begin to appear as separate, punctate foci adjacent to P granules. **120 min recovery**: all foci appear as wild-type. All live images are from the late pachytene region of undissected young adult gonads. Scale bar: 5µm.

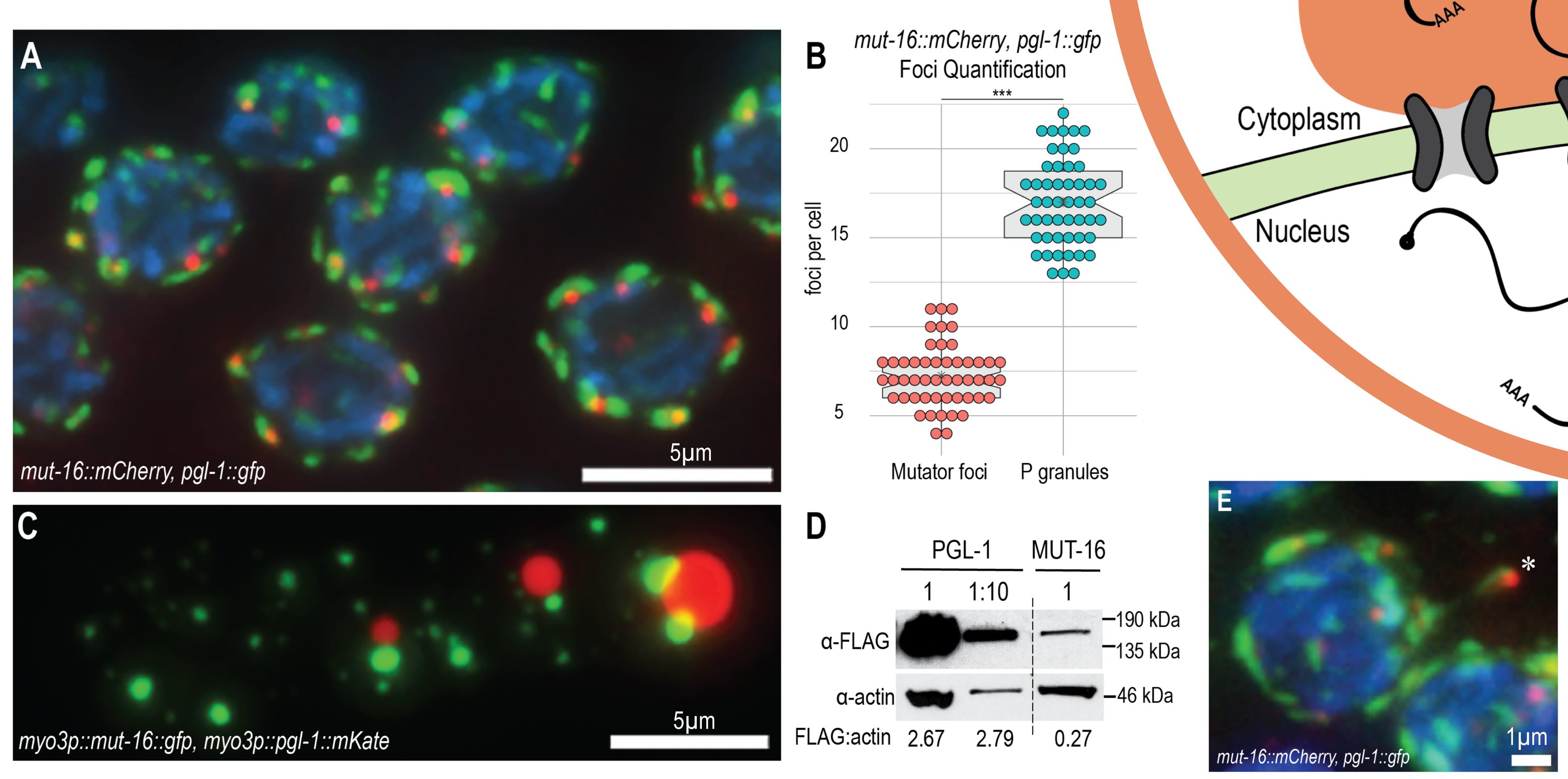
RNA regulation occurs through a series of perinuclear condensates.

P granules (orange) capture mRNAs after export through nuclear pores (gray) and allow siRNAs produced by the *Mutator* complex (turquoise) to monitor and properly regulate mRNA transcripts in the *C. elegans* germline.



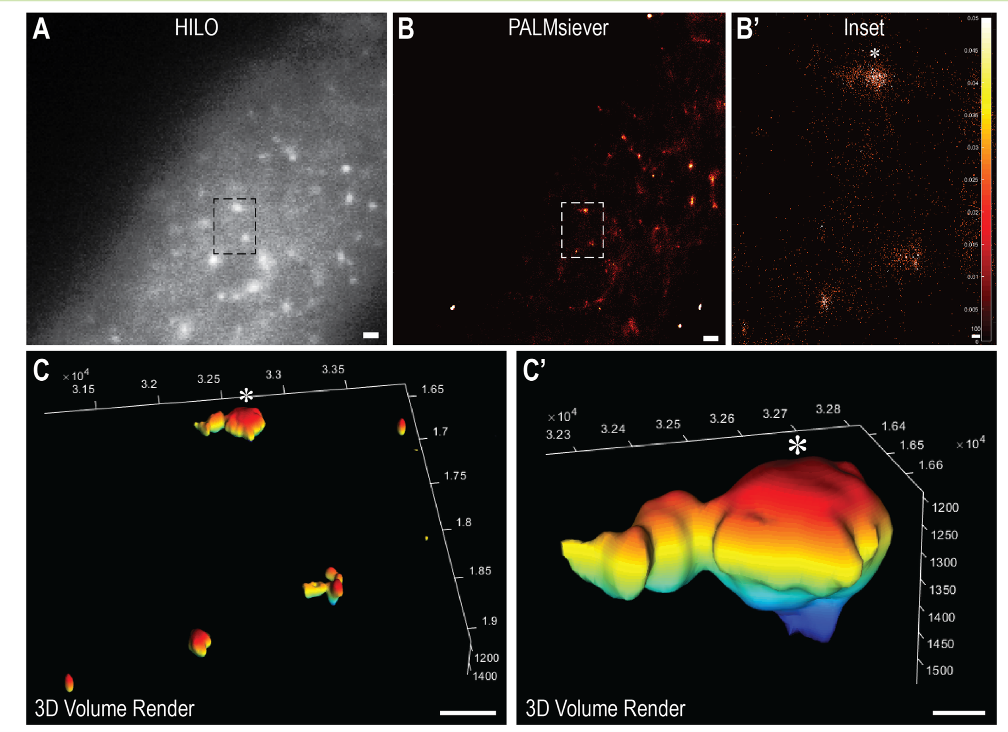
1 *Mutator* foci and P granule interaction is independent of association with nuclear pores

(A) *Mutator* foci are adjacent to, yet distinct from, P granules. **(B)** The MUT-16:PGL-1 foci ratio is ~ 7:17, indicating all *Mutator* foci are associated with P granules, but not all P granules are associated with *Mutator* foci. Foci were counted manually (n=50 cells distributed among n=5 gonads) in the late pachytene region of adult germlines. **(C)** MUT-16 and PGL-1 proteins overexpressed *via* the *myo-3* muscle-specific promoter form large foci that remain separate and adjacent in the ectopic muscle environment. Under normal expression, no foci are found in somatic tissue. **(D)** Western blot prepared from either *pgl-1::gfp::3xFLAG* or *mut-16::gfp::3xFLAG* adult animals reveals PGL-1 expression is approximately 10 fold higher than MUT-16, despite some somatic expression of MUT-16. PGL-1 was diluted 1:10 in the middle lane for quantification accuracy and FLAG:actin signal quantification was performed with ImageJ gel analysis. **(E)** A P granule and associated *Mutator* focus drips off of the nuclear periphery interacting despite loss of association with the nuclear pore environment.

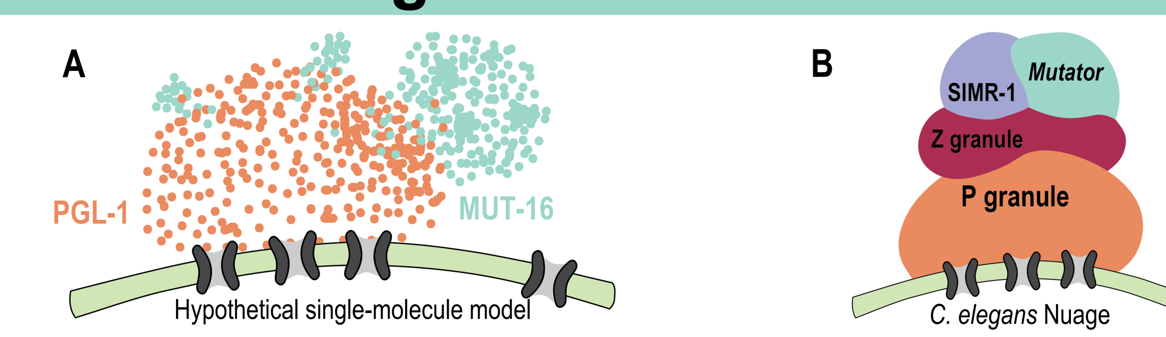


4 3D Stochastic Optical Reconstruction Microscopy (STORM) reveals *Mutator* foci in single molecule resolution

(A) Highly Inclined and Laminated Optical sheet (HILO) microscopy of MUT-16::GFP in the transition zone of fixed gonad labeled with Alexa-Fluor647 nanobooster. Scale bar: 1µm. **(B)** PALMsiever compilation of individual signals above intensity threshold in rapid-STORM analysis. Scale bar: 1µm. **(B')** Inset of dashed area in A and B. Note that MUT-16 distribution within foci is not uniform and that focus size ranges from ~100-500nm. Color bar indicates Z-axis position of individual signals calculated *via* point spread function. Scale bar: 100nm. **(C)** 3D-Volume Render of B' generated by PALMsiever. Note that the example structures are more complex than widefield imaging suggests. Scale bar: 500nm. **(C')** Detail of structure in C (asterisks) shows the complex shape of the largest focus. Scale bar: 100nm. All data is preliminary. Image collection and statistical analysis is ongoing.



5 Visualizing condensate interaction in single molecule resolution



Our ultimate goal in utilizing 3D STORM is to create a single molecule resolution model **(A)** of the interaction between phase separated condensates in *C. elegans* nuage, the collection of foci involved in RNA processing at the nuclear periphery **(B)**. The Z granule (Wan *et al.* 2018) and the SIMR foci (Manage *et al.* unpublished), add complexity to granule interaction that may be uncovered by 3D STORM.

Conclusions and Future Directions

- P granule and *Mutator* foci interactions are dictated by intrinsic protein properties and can be quickly re-established after perturbation.
- MUT-16 distribution within foci is not uniform and foci are complexly shaped, ranging in size from ~100-500nm, as seen by preliminary single molecule imaging.

We are continuing to work on this previously unseen view of phase-separated condensates.

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Let's talk!

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