



Regulation of gametic gene expression across development via RNAi-to-RNAi cascade in *C. elegans*

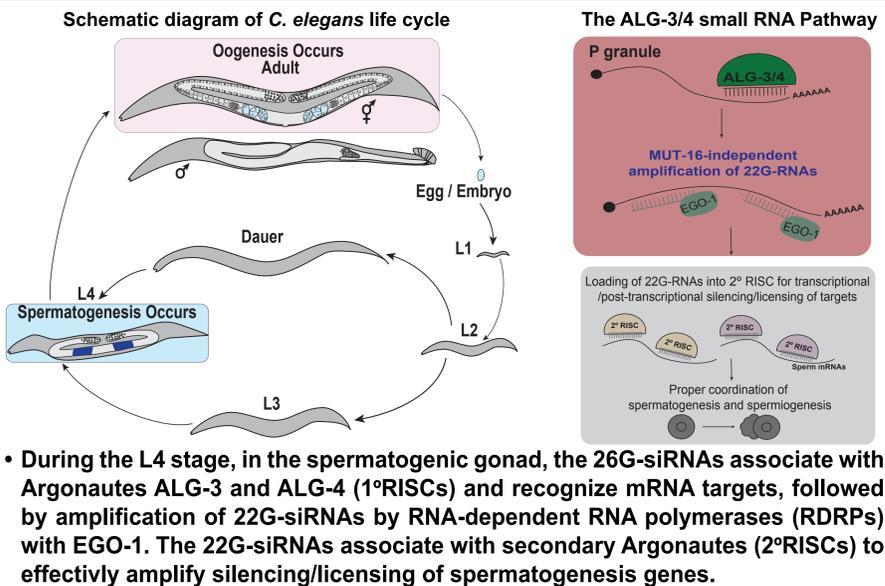
Trilotma Sen, Cara McCormick, and Alicia K. Rogers
The Department of Biology, The University of Texas at Arlington, Texas



Abstract

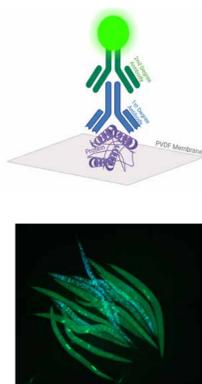
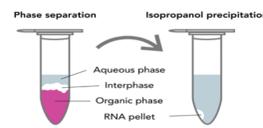
Coordination of gene regulatory networks is necessary for proper execution of cellular programs throughout development. RNA interference (RNAi) is an essential regulatory mechanism in all metazoans. Proper RNAi-mediated gene regulation requires coordination of several RNAi branches to ensure homeostasis. For example, in *Caenorhabditis elegans*, the Argonautes, ALG-3 and ALG-4, are expressed specifically during spermatogenesis (L4 stage) and bind small interfering RNAs (siRNAs) complementary to sperm-enriched genes. We find that *alg-3* and *alg-4* are regulated by siRNAs. Our work shows that gene switches are operated via these siRNAs to regulate the Argonautes' expression in a temporal manner. This RNAi-to-RNAi regulatory cascade is essential for coordinating ALG-3/4 pathway function, particularly during heat stress, to provide thermotolerant sperm-based fertility. This work provides insight into one regulatory motif used to maintain RNAi homeostasis, across developmental stages, despite environmental stressors. As RNAi pathways are evolutionarily conserved, other species likely use similar regulatory architectures to maintain RNAi homeostasis.

Background



Methods

- Brood size assay was done using synchronized *mut-16(pk710)* and Wild type worms grown on NGM plates at 20°C and 25°C.
- RNA extraction of *mut-16(pk710)* and Wild type worms at L4 and young adult stage at 20°C and 25°C using Trizol, and downstream extraction with isopropanol.
- mRNA-seq library preparation was done using Dynabeads mRNA purification kit, and libraries were prepared using NEB library prep kit. Libraries were sequenced on Illumina NextSeq500.
- Western blot analysis done on of *mut-16(pk710)* and Wild type worms at L4 and young adult stage at 20°C and 25°C. Protein extraction was in SB Buffer by incubating at 90°C. Samples were run on 12% Bis-Tris polyacrylamide gels, followed by nitrocellulose membrane transfer and downstream probing with anti-FLAG and anti-Tubulin. Secondary HRP-conjugated anti-mouse IgG was used.
- Fluorescence microscopy was done for L4s and young adults grown at 20°C and 25°C. Animals were fixed with chilled methanol and stained with DAPI solution followed by 3x washes in 1x PBST for 15 minutes. Imaging was performed on Axiomagler.M2 and processed on Fiji from Z-stacks.



Results

Figure 1: Temperature sensitive sterility and lower brood size in *mut-16* mutants

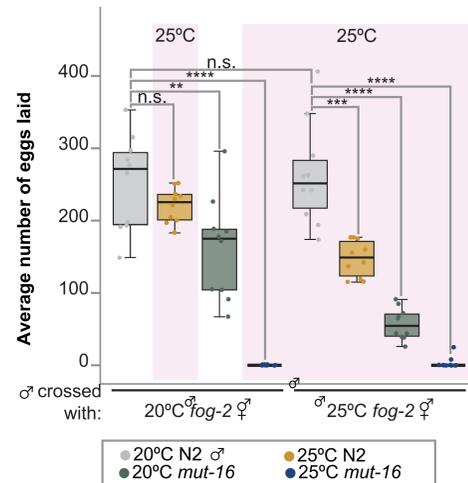


Figure 2: Sperm genes are downregulated in the germline of heat-stressed L4 *mut-16* mutants

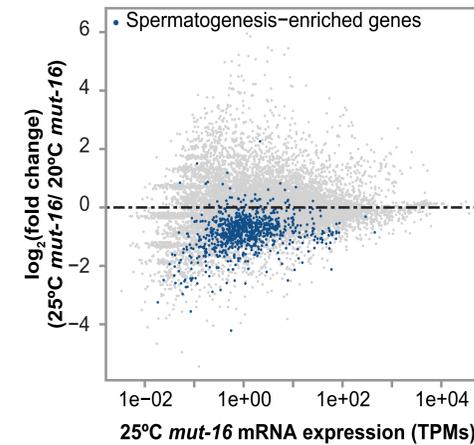


Figure 3: qRT-PCR for *alg-3* and *alg-4* mRNA expression shows downregulation in L4 *mut-16* hermaphrodites

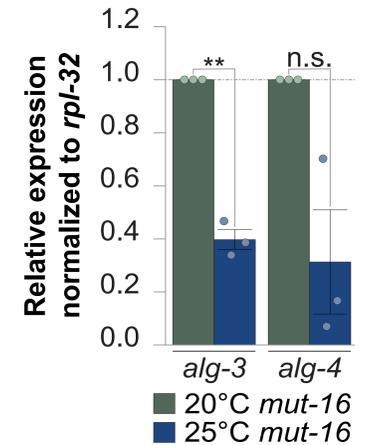


Figure 4: Western blots and Fluorescence imaging shows depleted protein levels of ALG-3 and ALG-4 in *mut-16* L4s under heat stress

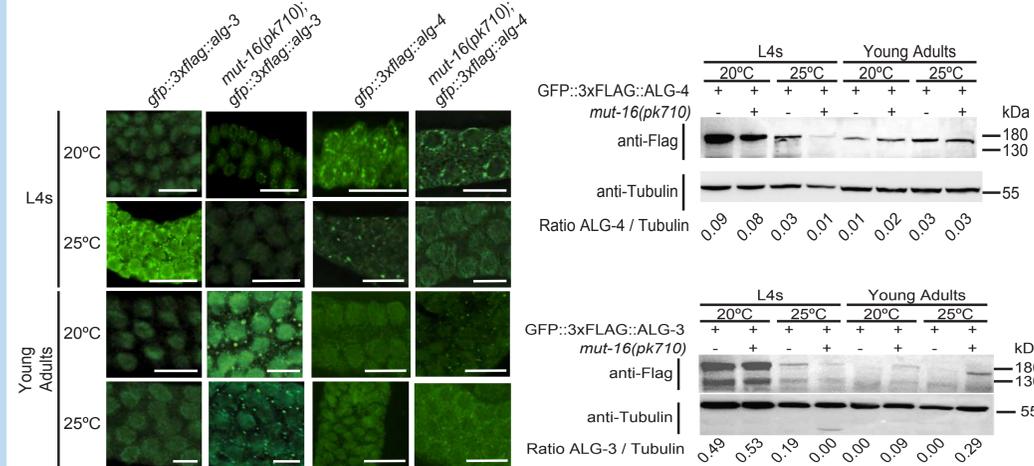
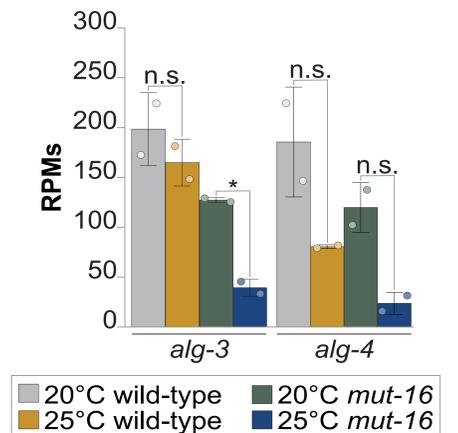
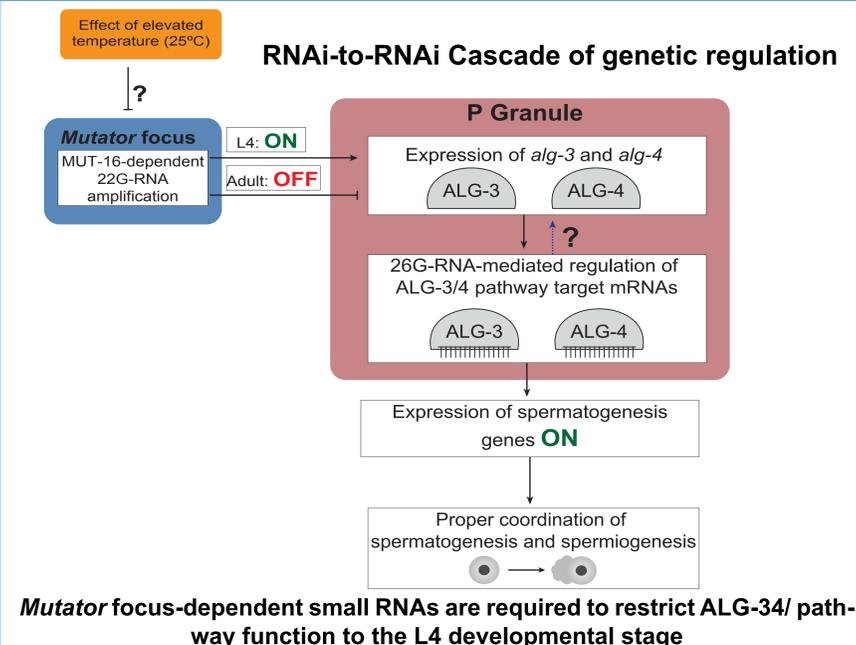


Figure 5: Small RNA reads mapping to *alg-3* and *alg-4* shows reduced small RNA levels on *alg-3* and *alg-4* loci in *mut-16* L4 hermaphrodites



Conclusion



Future Directions & Impact

- Our goal is identify how the ALG-3/4 pathway is regulated and how do these pathways control sperm development?
- To dissect the regulatory architecture of regulation of *alg-3* and *alg-4*, we will use guide RNA based feeding assay to overexpress *alg-3* and *alg-4* for our future experiments. We will also use CRISPR-directed mutagenesis to uncouple the *Mutator* complex's influence of ALG-3/4 pathway.
- Together, these studies will provide a map of molecular interactions in RNAi pathway functions deepening our understanding of genetic regulation by small RNAs.
- This research would add to our incomplete understanding of underlying factors contributing to infertility and heritable diseases.
- Such studies in model metazoans could have far-reaching translational implications for human reproductive health.

References

- Sen T., McCormick C., Rogers A.K., Small RNA-mediated genetic switches coordinate ALG-3/4 small RNA pathway function, *Nucleic Acids Research*, Volume 52, Issue 16, 9 September 2024, Pages 9431–9449, <https://doi.org/10.1093/nar/gkae586>.