An RNAi screen to identify factors that enhance microRNA activity after dauer

Himal Roka[1] and Xantha Karp[1,2]

CMU CENTRAL MICHIGAN UNIVERSITY

Karp Lab

Big Picture Question

How do animals develop normally after a period of arrest?

Introduction

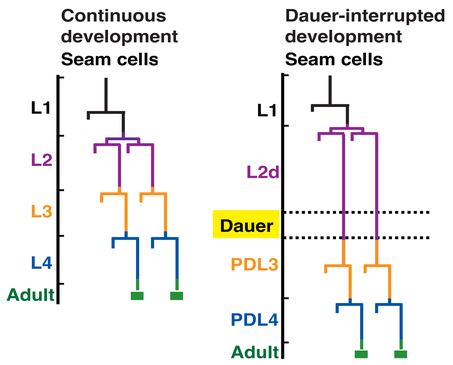


Fig. 1: Seam cell development occurs normally after dauer diapause. Dauer is a developmental arrest occurring after the second larval molt in response to adverse environments. The pattern and sequence of lateral hypodermal seam cell divisions occurs identically in continuously developing and post-dauer worms. At adulthood, seam cells differentiate and express adult-specific characteristics. Heterochronic genes specify larval vs. adult cell fate (1,2).

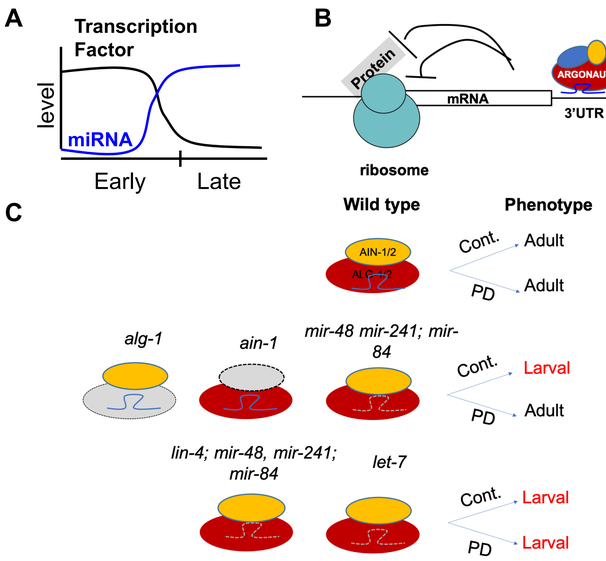


Fig. 2: MicroRNA activity is enhanced after dauer. (A) Transcription factors specifying early cell fates are downregulated by increasing levels of microRNA to allow progression to later cell fates (3). (B) Model showing components of microRNA Induced gene Silencing Complex (miRISC) which silences target genes post-transcriptionally. (C) Some mutants with compromised miRISC activity develop normally after dauer, suggesting an enhancement of miRISC activity after dauer compared to continuous development (4)

Research Question

What factors enhance post dauer microRNA activity?

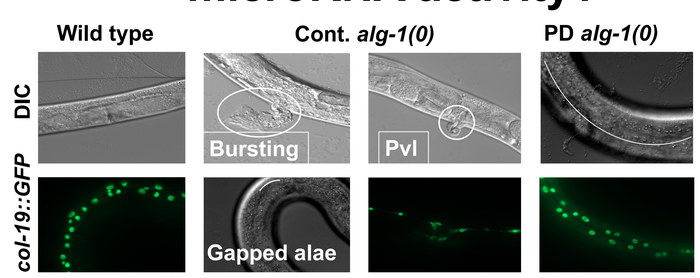
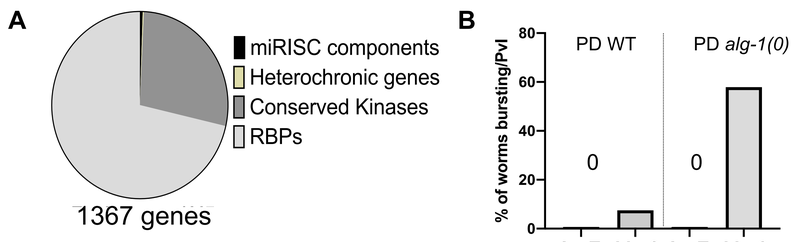


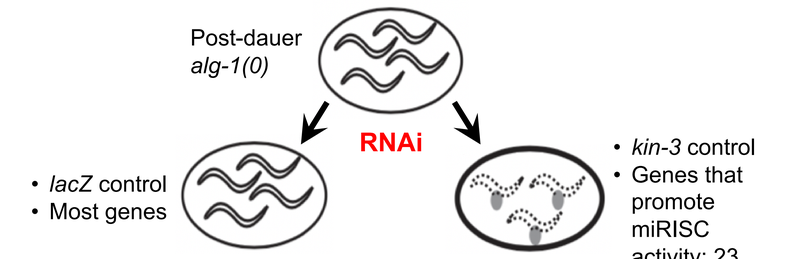
Fig. 3: The *alg-1(0)* phenotype is suppressed in post-dauer animals. *alg-1(0)* animals are viable due to the presence of *alg-2,* but display phenotypes including vulval bursting, protruding vulvas (Pvl), gaps in an adult-specific cuticular structure (alae), and reduced expression of the adult cell fate marker *col-19::gfp*. All of these phenotypes are suppressed after dauer. “Cont.” = continuous development, ”PD” = post-dauer.

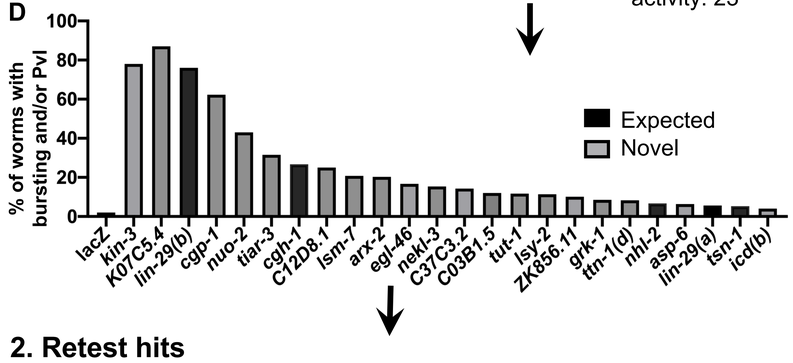
RNAi Screen



1. Primary screen

C Screen for bursting/Pvl: 276 genes screened so far





2. Retest hits

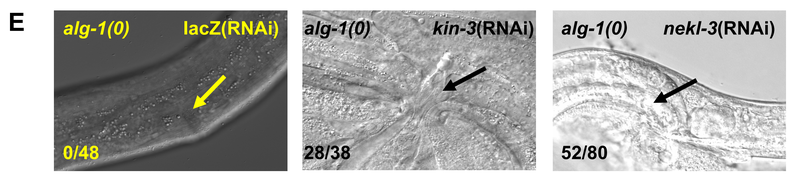


Fig. 4: *nekl-3* is a primary hit in an RNAi screen for genes that enhance *alg-1(0)* phenotypes after dauer. (A) List of genes to screen. All genes are conserved in humans (5). RBP = RNA binding protein. (B) Validating negative and positive controls for the screen. ”Wild-type” and *alg-1(0)* strains contain *daf-7(e1372)* to control dauer formation. *kin-3* encodes the catalytic domain of casein kinase 2, which phosphorylates a miRISC component to promote miRISC activity (6). (C) Strategy for the RNAi screen. Each clone is scored in duplicate (D) Hits from the primary RNAi screen. Combined data from two RNAi wells is shown. Expected genes are known heterochronic genes, miRISC components or miRISC regulators. (E) After retesting the hits from the primary screen, *nekl-3* emerged as the only strong candidate. *nekl-3* (Never in mitosis Kinase Like) encodes a serine/threonine kinase that regulates molting (7). Arrows indicate the vulva (normal or burst).

Question

Is *nekl-3* more important when miRISC is compromised?

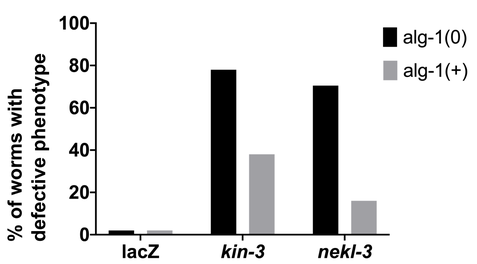


Fig. 5: RNAi of *nekl-3* in *alg-1(0)* mutant post-dauers causes a higher penetrance of bursting/Pvl phenotypes than in a wild-type background. All strains contain *daf-7(e1372)* to control dauer formation.

Question

How does *nekl-3* interact with *alg-1* during continuous development?

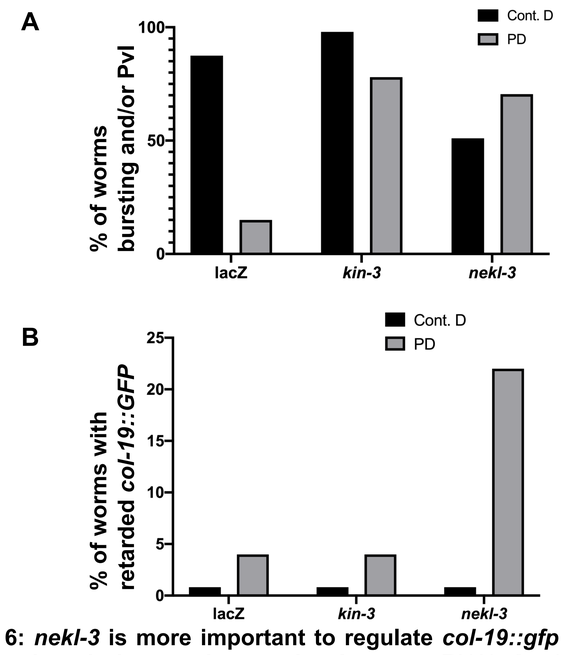


Fig. 6: *nekl-3* is more important to regulate *col-19::gfp* expression after dauer than during continuous development. RNAi of *nekl-3* and controls in *alg-1(0)* animals that developed continuously (Cont D.) or experienced dauer (PD). Post-dauer animals contained the *daf-7(e1372)* mutation whereas continuously developing animals did not. These are preliminary data from a single experiment. (A) *alg-1(0)* bursitng and Pvl phenotypes are already highly penetrant during continuous development. Surprisingly, *nekl-3(RNAi)* may have suppressed this phenotype slightly, but more experiments are needed to confirm this. (B) *alg-1(0)* adults rarely display *col-19::gfp* defects whether they develop continuously or rhtough dauer. *kin-3(RNAi)* does not enhance this phenotype. By contrast, *nekl-3(RNAi)* produces a penetrant *col-19::gfp* defect in post-dauer *alg-1(0)* animals.

Future Directions

- Test other molting regulators for enhancement of *alg-1(0)* post-dauer phenotypes
- Further characterization of *nekl-3*
  - Genetic interactions with other miRISC components
  - Genetic interactions with microRNAs and microRNA targets
  - Post-dauer expression pattern
  - Physical interactions with miRISC
- Continue primary screen

References

1. Ambros & Horvitz, 1984. Science 226: 409-416.
2. Liu & Ambros, 1991. Nature 350: 162-165.
3. Rougvie, A. E. 2001. Nat. Rev. Genet. 2, 690-701.
4. Karp & Ambros, 2012. Development 139: 2177-2186.
5. Shaya and Greenwald, 2011. Ortholist. *PLoS ONE 6*, e20085.
6. Alessi et al., 2015. PNAS 112: E7213-22.
7. Yochem et al., 2015. Dev. Bio. 398: 255–266

Acknowledgements

Thanks to Himani Galagali and Dr. John Kim (John Hopkins University) for sharing data and helpful discussions. Many thanks to thesis committee members Dr. Jennifer Schisa and Dr. Steven Gorsich (CMU) for their timely advice. Thanks to my team members Alexendra Terry, and Claudia Chabay. Thanks to WormBase for information. This project is funded by an NSF CAREER award (to XK), a CMU College of Science and Engineering research assistantship and a CMU Biology department teaching assistantship (to HR).

Affiliations:
1) Biochemistry, Cell & Molecular Biology Program, Central Michigan University
2) Department of Biology, Central Michigan University