Controlled Timelines of Gene Regulatory Networks

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Abstract

Larval development in *C. elegans* entails four molting cycles during which crucial genetic and cellular processes must be precisely synchronized to facilitate shedding and regrowth of the cuticle enveloping the body. The core genes orchestrating this developmental timer drive the periodic expression of *lin-4* miRNA throughout each molting cycle. In the larval skin, *lin-4* expression is activated by Rev-Erb ortholog NHR-85 and ROR ortholog NHR-23; its expression is inactivated by *lin-42* (Kinney et al. 2023). We set out to investigate how the timing of development is regulated in other tissues outside the larval skin, specifically the hermaphrodite gonad. Prior work implicates *lin-42* upstream of the netrin receptor gene *unc-5*, which regulates the timing of DTC turning (Tennessen et al., 2006). *lin-4* mutants have no vulvas, but the germline continues to grow. We found that *lin-42* cycles in the DTC, but NHR-23 and NHR-85 do not. We searched for different factors with which LIN-42 might act to repress *unc-5* by conducting a yeast 1-hybrid approach to find transcription factors that can bind the *unc-5* upstream regulatory region. After screening 672 genes, we found 7 hits that can bind the *unc-5* regulatory region. The screen and *in vivo* validation will continue.

Introduction

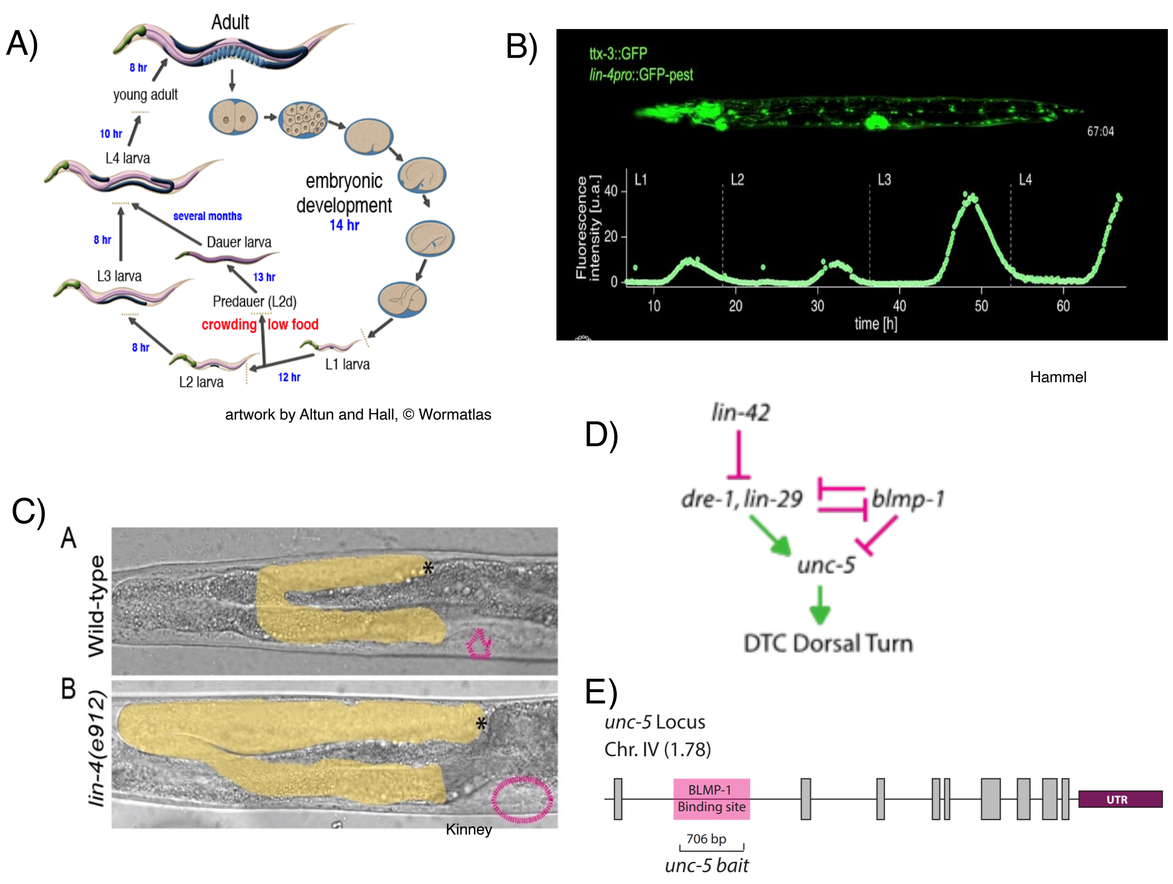

Figure 1. Overview of developmental timing and DTC migration. (A) The C. elegans life cycle undergoes four larval molts. (B) mRNA of *lin-4* turns on during each larval stage. (C) *lin-4(e912)* mutant gonads do not arrest in L1. (D) *unc-5* activation is necessary for DTC turning. There are known heterochronic factors that act in different ways. *lin-42* is known to be upstream of the *unc-5* regulatory region. (E) Image of the unc-5 locus. The pink is from BLMP-1 ChIP-seq data.

Methods: Live Image Microscopy

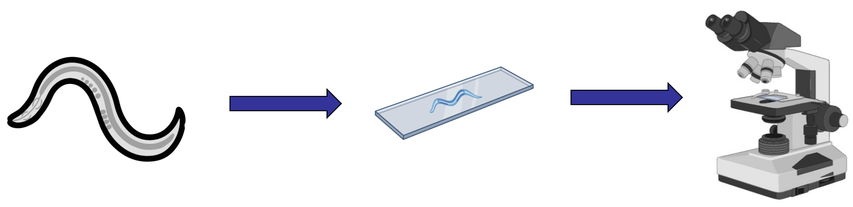

Figure 2. Overview of L4 C. *elegans* imaging. Worms between the L4 larval stage and adulthood were collected and plated onto microscope slides. Then, they were imaged under a confocal microscope. Seam cells, Hyp cells, muscle cells, DTC, and somatic gonad were the focus of the imaging.

Results: NHR-23 and NHR-85 not expressed in DTC

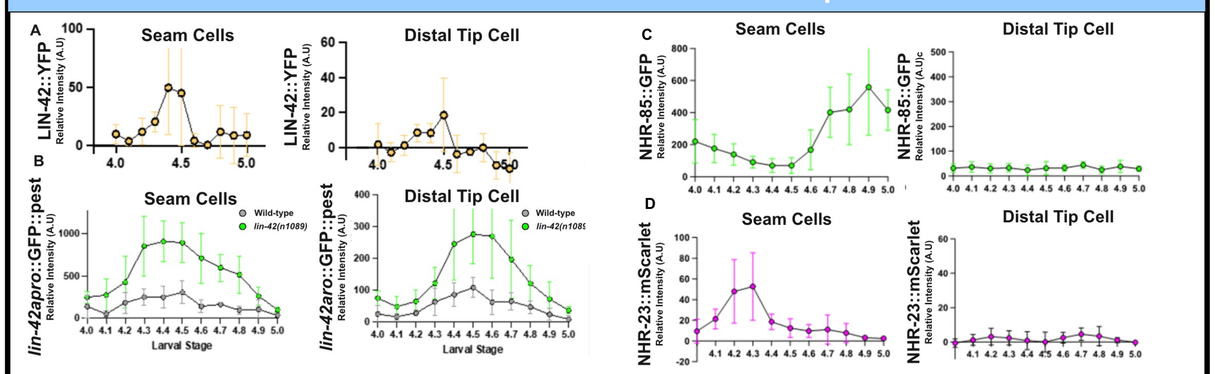

Figure 3. Measured relative intensities of seam cells and DTC. (A-B) *lin-42* is expressed in DTC at the same time as seam cells, which was around L4.5. (C-D) NHR-23 and NHR-85 have pulsatile expression in other somatic cell types, but not in the DTC. This suggests there is another factor playing a role in the activation of *unc-5*.

Methods: Yeast 1-Hybrid

Yeast 1-Hybrid Can Find Potential Binding to DNA

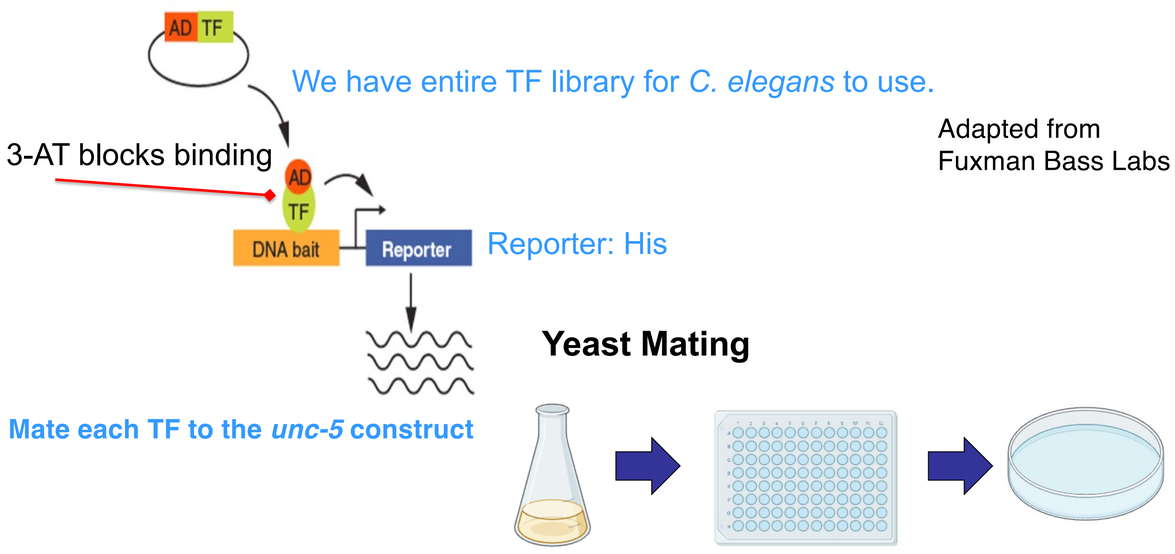

Figure 4. A combination of plasmid BAK002 (*unc-5 PMW2*) in the host yeast strain is grown overnight in YPD. The first plate of the WTF2.2 yeast library (Reece-Hoyes et al. 2011) is placed into a 96-well plate with 100 µL of the overnight culture. Allow for an overnight mating and plate onto 3-AT plates without histidine or tryptophan.

Results: Several transcription factors bind *unc-5* locus

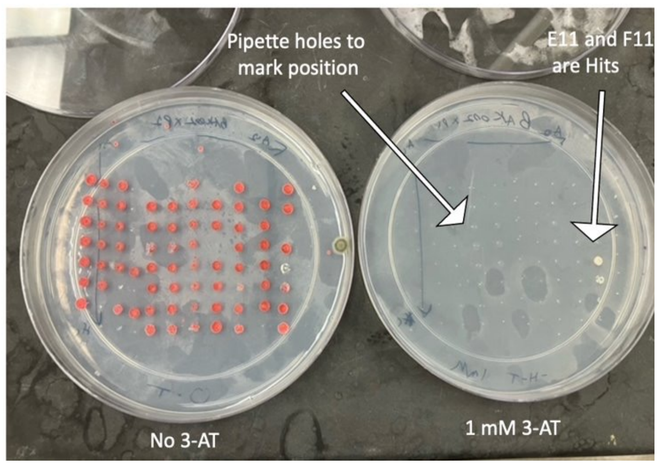

Figure 5. Yeast 1-hybrid plates. Left, control with no 3-AT inhibitor to verify expression of TF and presence of *unc-5* upstream DNA bait. Right, selection plate with 2 "hit" colonies (right arrow), in which a *C. elegans* transcription factor binds the *unc-5* upstream region.

Table 1. List of *unc-5* upstream-binding "hits"

TF Hits	Function	Expressed in DTC?	RNAi Available?
nhr-96	Nuclear hormone receptor	Yes	Yes
sma-2	TGFbeta signaling	Yes	Yes
nhr-21	Nuclear hormone receptor	Yes	Yes
hlh-11	Lipid metabolism	Yes	Yes
hlh-29	Regulates ovulation	No	Yes
ccch-3	Unknown	No	Yes
ets-7	Related to lifespan	No	Yes

Conclusion

Previous research demonstrated NHR-85 and NHR-23 had pulsatile expression at each larval molt creating an oscillatory rhythm (Kinney et al. 2023) that regulates developmental timing in some larval cells via *lin-42*. We have concluded that DTC migration regulated by *unc-5* is under a separate timing mechanism. Our results demonstrate that multiple transcription factors that are expressed in the distal tip cell are able to bind the *unc-5* upstream regulatory region. Understanding the timing mechanism of DTC turning provides more insight into developmental timing regulators in various cell and tissue types.

Future Directions

- Continue Yeast 1- Hybrid Screens on nearly all C. *elegans* TFs
- RNAi on hits to look for DTC turning defects both on their own and in combination
- RNAi on an *unc-5* reporter to see how the hits affect *unc-5* expression
- Use Yeast 2- Hybrid to assess binding between TFs and LIN-42

References and Acknowledgments

1. Kinney, Brian, Shubham Sahu, Natalia Stec, Kelly Hills-Muckey, Dexter W. Adams, Jing Wang, Matt Jaremko, Leemor Joshua-Tor, Wolfgang Keil, and Christopher M. Hammell. 2023. "A Circadian-like Gene Network Programs the Timing and Dosage of Heterochronic miRNA Transcription during C. Elegans Development." *Developmental Cell*, August, S1534-5807(23)00402-1. https://doi.org/10.1016/j.devcel.2023.08.006.
2. Reece-Hoyes, John S., Alos Diallo, Bryan Lajoie, Amanda Kent, Shaleen Shrestha, Sreenath Kadreppa, Colin Pesyna, Job Dekker, Chad L. Myers, and Albertha J. M. Walhout. 2011. "Enhanced Yeast One-Hybrid Assays for High-Throughput Gene-Centered Regulatory Network Mapping." *Nature Methods* 8 (12): 1059–64. https://doi.org/10.1038/nmeth.1748.
3. Tennessen, Jason M et al. "Novel heterochronic functions of the Caenorhabditis elegans period-related protein LIN-42." *Developmental biology* vol. 289,1 (2006): 30-43. doi:10.1016/j.ydbio.2005.09.044

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