Developmental timing of epidermal seam cells in *Caenorhabditis elegans*

Glydel Ann Lopez, Tamar Resnick, Ann Rougvie

Introduction

Much is now known about how microRNAs downregulate gene expression by binding to sequences in the 3' untranslated region of messenger RNAs. Less is understood about the regulation of miRNA gene expression. In this study, we are identifying the gene regulatory elements that temporally and spatially control *mir-48/241* expression in the nematode *Caenorhabditis elegans. mir-48/241* encodes two microRNAs that time the development of epidermal "seam" cells. In absence of *mir-48/241* expression, epidermal seam cell fates can be inappropriately timed[1] (Figure 1). A consequence of this is that affected adult seam cells fail to make alae, a cuticular ridge that runs the lateral length of the animal and is easily scored. Previous works identified a region 2kB upstream of *mir-241* that contains a putative transcription factor binding site. Here, we define this site by generating more precise and smaller deletions within this region using CRISPR/Cas9 genomic editing technology and an ssODN to direct repair upon break.

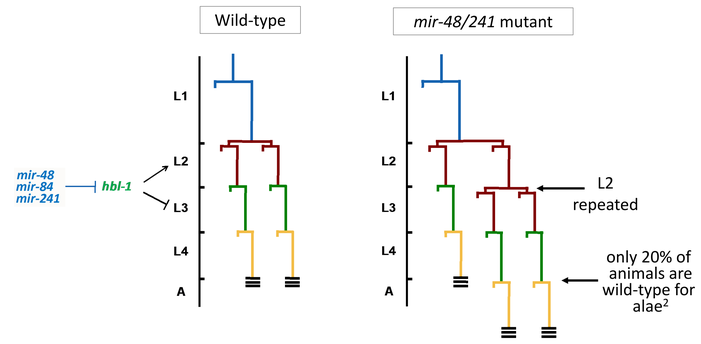


Figure 1. Lineage of epidermal seam cells in wild-type (left) compared to a *mir-48/241* mutant (right). The L2 lineage can be repeated in *mir-48/241* mutant, causing a phenotype of partial alae synthesis at the L4 molt.

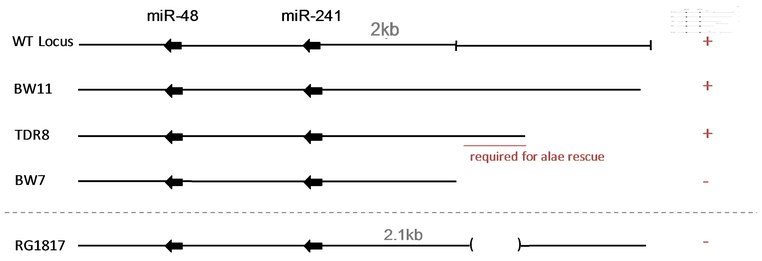


Figure 2. Data from previous rescue experiment work. BW11/TDR8/BW7 are transgenic rescue constructs that identify a 753 base pair (bp) region required for adult alae formation (T. Resnick). RG1817 is a 535bp deletion in the native *mir-48/241* locus, generated in this study, that resulted in an alae phenotype. A (-) indicates partial alae formation.

Methods

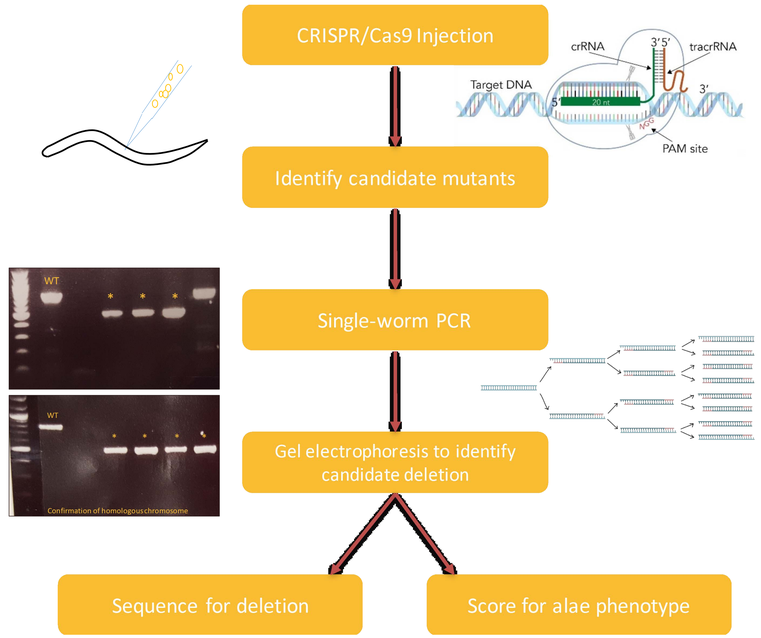


Figure 3. Schematics of generating animals with proper deletion. Plasmids containing CRISPR/Cas9 and sgRNA and an ssODN (HDR template) were injected into the gonad of young adults. Animals were homozygosed and screened for the expected deletions and any phenotypes.

We are using genome editing technology to engineer deletions in the endogenous *mir-48/241* gene regulatory region and monitor their phenotypic consequence on epidermal seam cell fate timing. The CRISPR-Cas9 system is used to generate double-stranded breaks in a region previously suspected to contain a *mir-48/241* epidermal seam cell enhancer sequence. Randomly sized deletions are identified following repair of these breaks by the non-homologous end joining. Precise deletions are identified following homology repair directed by the ssODN.[3]

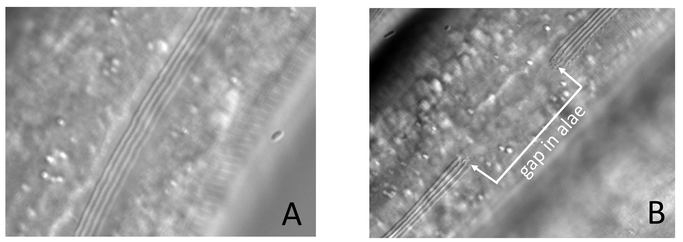
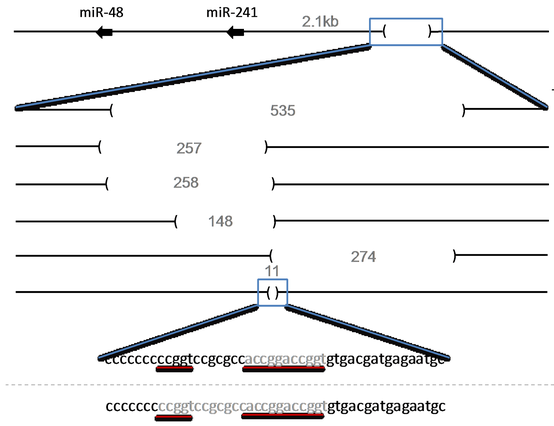


Figure 4. **A** Image of an adult cuticle with a full alae. **B**. Image of a partial alae phenotype observed in RG1842.

Results



Strain	Adult alae ?	% WT alae
RG1817	-	7
RG1839	+	100
RG1840	-	63
RG1842	-	96
RG1843	+	100
RG1847	+	100
		in progress

Figure 5. Map of the deletion created. Strains were scored for alae phenotype. Candidate repeated sequences that could serve as a transcription factor binding site are underlined in red. % is the percentage of animals with WT alae.

Conclusions

Only 7% of RG1817 animals have WT alae. This percentage compares closely to the phenotype of animals with *mir48/241* deleted.[2] This indicates that seam cell regulatory element(s) reside in the deleted region. Additional deletions suggest that the seam cell function is provided redundantly. We have identified a candidate repeated sequence that could be a transcription factor binding site. A deletion that removes these repeats is in progress.

Acknowledgements

I would like to thank Dr. Rougvie, Dr. Resnick, and Dr. Edelman for all the guidance, support and patience they provided me through this project. Thank you to the CGC staff for being so accommodating with all my worm supply requests. I am appreciative for Dr. Spike's guidance with designing ssODNs.

Bibliography

(1) Resnick, T. D., Mcculloch, K. A. and Rougvie, A. E. (2010). miRNAs give worms the time of their lives: Small RNAs and temporal control in Caenorhabditis elegans. *Developmental Dynamics* 239, 1477-1489.
(2) Abbott, A. L., Alvarez-Saavedra, E., Miska, E. A., Lau, N. C., Bartel, D. P., Horvitz, H. R. and Ambros, V. (2005). The let-7 MicroRNA Family Members mir-48, mir-84, and mir-241 Function Together to Regulate Developmental Timing in Caenorhabditis elegans. *Developmental Cell*9, 403–414.
(3) Paix, A., Wang, Y., Smith, H. E., Lee, C.-Y. S., Calidas, D., Lu, T., Smith, J., Schmidt, H., Krause, M. W. and Seydoux, G. (2014). Scalable and Versatile Genome Editing Using Linear DNAs with Microhomology to Cas9 Sites in Caenorhabditis elegans. *Genetics* 198, 1347–1356.