Dissecting the role of the Doublesex DMD-9 transcription factor in *C. elegans*

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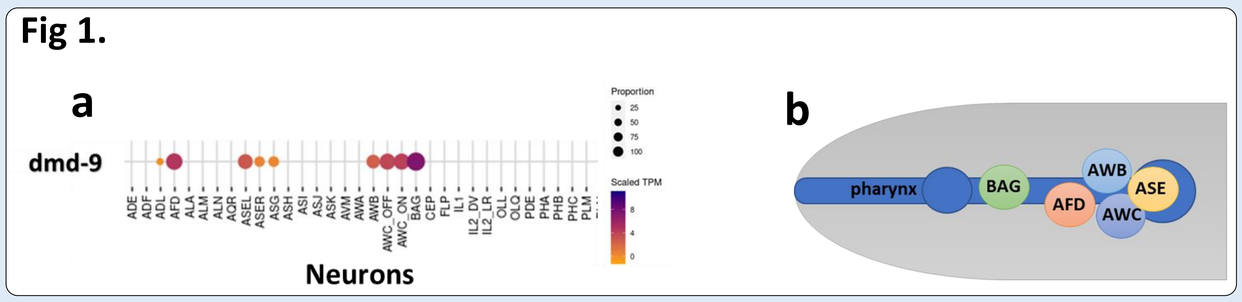
Abstract

Almost all multicellular organisms have two sexes that exhibit dimorphic molecular and morphological characteristics. Development, including sexually dimorphic development is regulated by transcription factors. In the nematode *Caenorhabditis elegans,* sexually dimorphic characteristics are regulated by transcription factors from Doublesex DNA Domain family. DMD-9 is a member of this family that shows a restricted expression pattern to a few unidentified sensory neurons. DMD-9 function is unknown. Here, I investigated the endogenous *dmd-9* expression in both sexes of *C. elegans*, hermaphrodite and male, to identify potential sexually dimorphic expression patterns. In addition, I found that as animals become sexually mature, *dmd-9* expression is lost in one of the AWCs. In addition, I identified 571 deregulated genes in *dmd-9* mutants. My future research aims to dissect the precise functions for DMD-9 in the *C. elegans* nervous system.

Introduction

DMD-9 is a member of Doublesex DNA Domain family that shows a restricted expression pattern to a few unidentified sensory neurons. DMD-9 function is unknown (Fig 1 a). The position of dmd-9 expressing neurons is shown in Fig 1 b.

Fig 1.

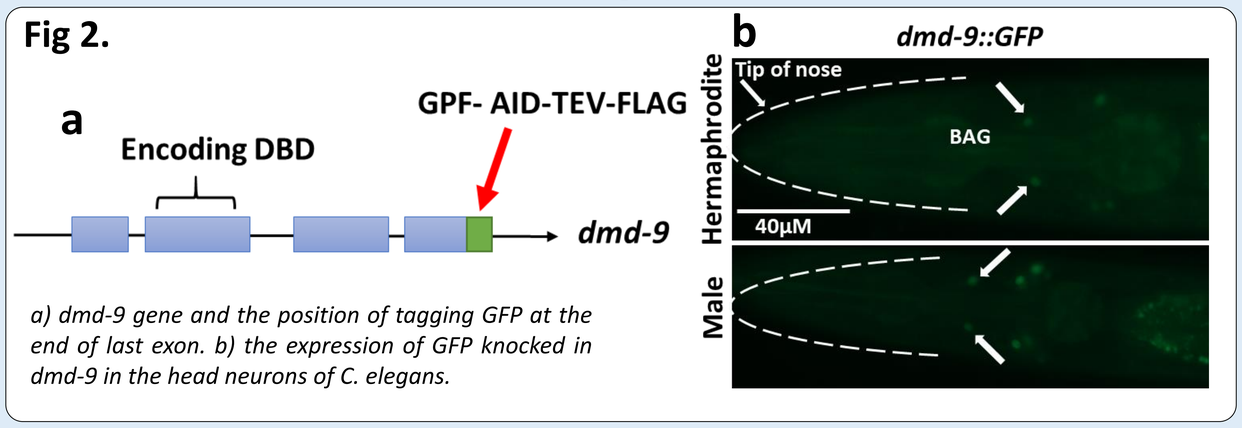


Here, I investigated the endogenous *dmd-9* expression in both sexes of *C. elegans*, hermaphrodite and male, to identify potential sexually dimorphic expression patterns. I also studied the transcriptome for deregulated genes in dmd-9 mutants.

Methods

1- To enable this analysis, I used CRISPR-Cas9 to generate an in frame GFP knock-in at the last exon of dmd-9 (Fig 2).

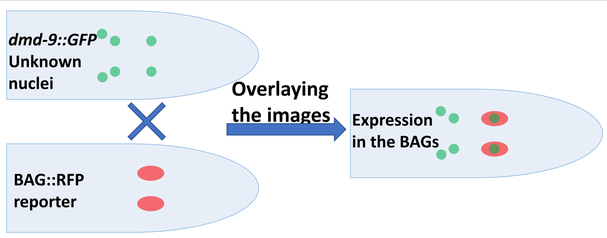
Fig 2.



a) dmd-9 gene and the position of tagging GFP at the end of last exon. b) the expression of GFP knocked in dmd-9 in the head neurons of C. elegans.

2- I co-localized dmd-9 GFP expression with known RFP reporters for the target neurons (Fig 3).

Fig 3.

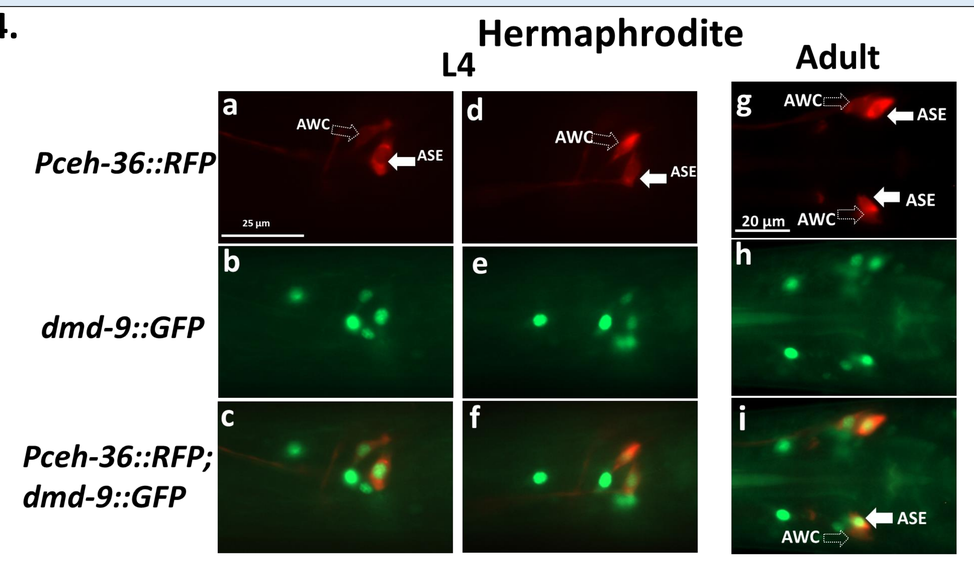


3- I performed RNA sequencing to identify alterations in the transcriptome in dmd-9 mutant animals.

Results

dmd-9 is expressed in several bilaterally-symmetric left/right pairs of sensory neurons in the head, including BAGL/R, AWBL/R, AWCL/R, and ASEL/R. AWCL/R, and ASEL/R are shown in (Fig 4).

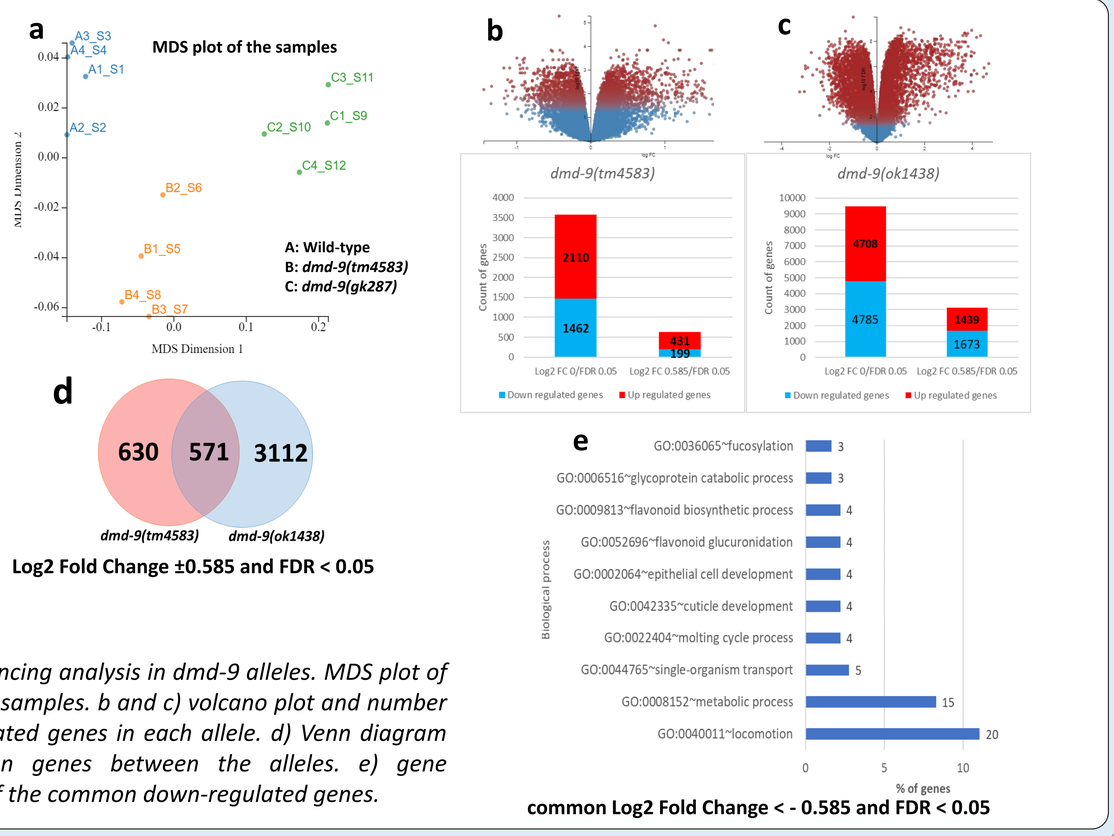
Fig 4.



Co-localizing of dmd-9::GFP with Pceh-36::RFP. a-c) the right-AWC and ASE neurons and d-f) the left-AWC and ASE neurons at immature animals (L4). g-h) dorsal view of AWC and ASE neurons in Adult animals. The arrows show the neurons.

RNA sequencing results for two alleles of *dmd-9* show deregulation of hundred genes at immature (L4) animals (Fig 5). 571 common genes are deregulated in both mutants.

Fig 5.



RNA sequencing analysis in dmd-9 alleles. MDS plot of sequenced samples. b and c) volcano plot and number of deregulated genes in each allele. d) Venn diagram of common genes between the alleles. e) gene ontology of the common down-regulated genes.

Conclusion

DMD-9 is asymmetrically expressed in AWC neurons. Mutation in dmd-9 causes deregulation of several hundred genes. a My future research aims to dissect the precise functions for DMD-9 in the *C. elegans* nervous system.