

# Depletion of UNK-1 affects head locomotive behaviors in *Caenorhabditis elegans*

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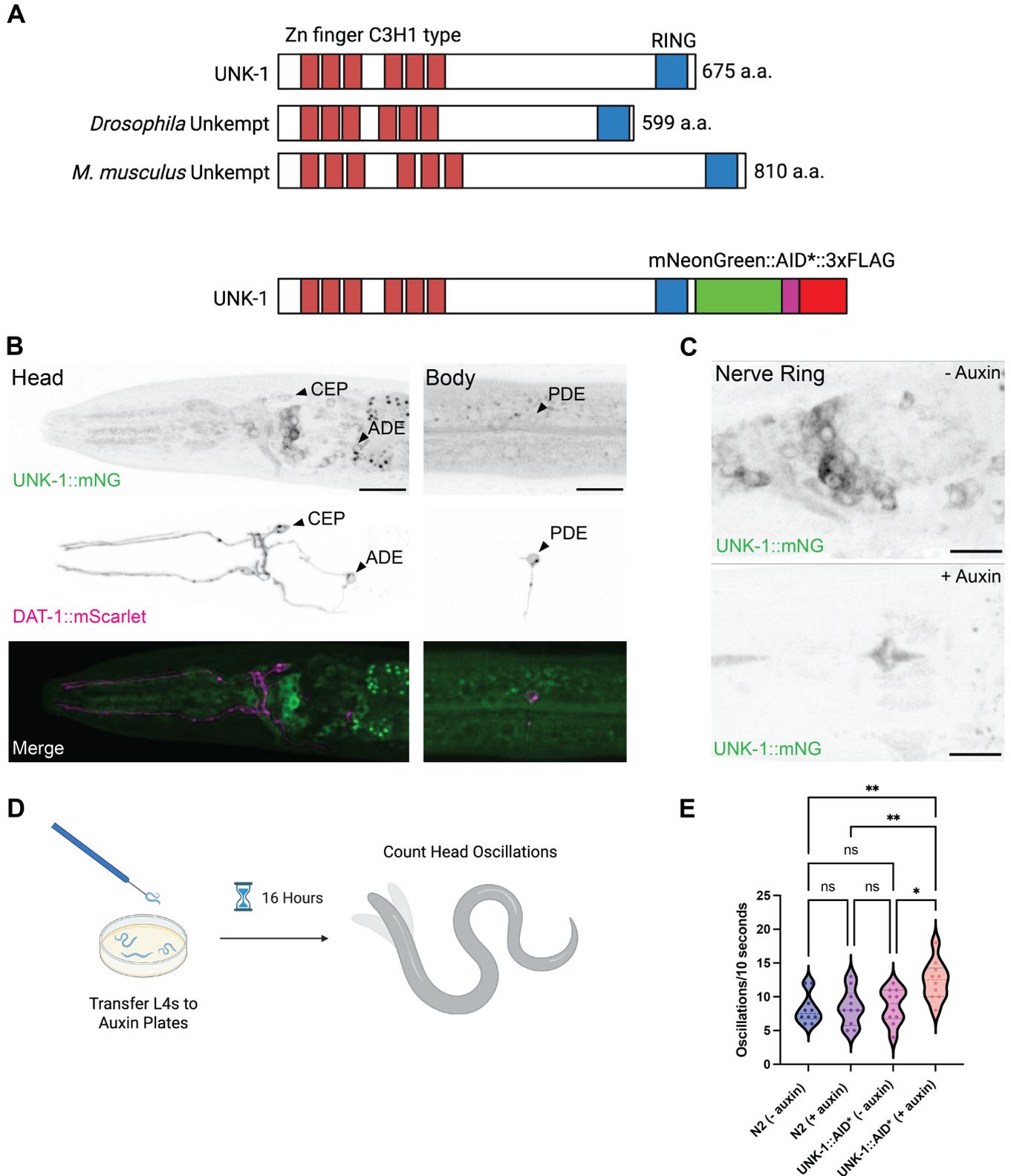
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## Abstract

During development, transcription factors execute genetic programs to define and shape the nervous system. We previously reported the transcription factor [EGL-43](#)/Mecom regulates expression of numerous genes important for neuronal development in *C. elegans*. Here we characterized the function of one [EGL-43](#) target, [UNK-1](#)/Unkempt, a conserved RNA-binding protein. Using genome editing, we introduced an auxin-inducible degron tag and mNeonGreen into the [unk-1](#) locus. We observed cytoplasmic expression of [UNK-1](#) in many head neurons. Depletion of [UNK-1](#) resulted in increased head oscillations, a phenotype typically associated with defects in neurotransmitter synthesis. Our study links [UNK-1](#) to head motor control in *C. elegans*.



**Figure 1. Characterization of UNK-1 in *C. elegans*:**

A) Top: Protein domains of Unkempt related proteins (*C. elegans* UNK-1, *D. melanogaster* Unkempt, *M. musculus* Unkempt); Bottom: Schematic of C-terminal fusion of UNK-1 to mNeonGreen::AID\*::3xFLAG, generated using BioRender (<https://biorender.com/9wf5qlq>). B) Micrographs of L4 animals expressing endogenously labelled UNK-1::mNG (top) and DAT-1::mScarlet (Middle). UNK-1 is expressed in the cytoplasm of many sensory neurons, including all dopaminergic

neurons (CEP and ADE in the head, PDE in the body). Scale bar for all images = 20  $\mu\text{m}$ . C) Micrographs of *UNK-1::AID\** day 1 adult animals expressing *UNK-1::mNG* treated with 0 mM Auxin (top) and 4 mM Auxin (bottom) overnight. Scale bar for all images = 10  $\mu\text{m}$ . D) *UNK-1* depletion and assay schematic, generated using BioRender (<https://biorender.com/qdb7g25>). E) Quantification of head oscillation of day 1 adults during forward movement. Each dot represents a single animal,  $n = 10$  for all genotypes and treatments.  $p$  values were calculated using ordinary one-way ANOVA with Tukey's multiple comparisons tests. ns indicates not significant, \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.01$ .

## Description

Transcription factors (TFs) define multiple aspects of neuronal identity and function. Our laboratory recently discovered that the TF *EGL-43* is critical for synapse formation in dopaminergic neurons (Yee et al., 2024). *EGL-43* ChIP-seq revealed that *EGL-43* binds to promoters of many genes with a variety of cellular functions (Deng et al., 2020). Depletion of *EGL-43* decreased levels of presynaptic protein expression (Yee et al., 2024). Likewise, directly mutating the *EGL-43* binding sites within these promoters reduced corresponding protein expression. Many of the *EGL-43* target genes encode proteins with unknown functions. We sought to define the function of some of these genes. We used two criteria to select genes for further study. First, we looked for genes that were evolutionarily conserved. Second, we selected genes whose functions have not been characterized in *C. elegans*. One candidate we focused on was a gene related to *unkempt*.

Originally identified in *Drosophila* (Mohler et al., 1992), *unkempt* is highly expressed embryonically and is essential for early development as homozygous deletion causes lethality during mid-pupal development. Flies carrying a hypomorphic allele of *unkempt* survive into adulthood but exhibit an “unkempt” phenotype, characterized by roughened eyes, irregular scutellar bristles, and splayed wings (Mohler et al., 1992). In mice, the *Unkempt* gene encodes a CCCH-type zinc finger domain containing RNA-binding protein that regulates RNA stability, localization, and translation, and plays important roles in development and function of the nervous system (Murn et al., 2016; Murn et al., 2015). Intriguingly, knockout of *Unkempt* in the *mouse* nervous system results in improved memory formation and cognitive flexibility (Vinsland et al., 2021). Little is known about the function of *Unkempt* in *C. elegans*. Given the well characterized nervous system of *C. elegans*, we sought to study *Unkempt*'s function in this model system.

*C. elegans* has a single *Unkempt* ortholog called *UNK-1* (*UNKempt*-related zinc finger protein). According to WormBase release WS296, the *unk-1* locus encodes 4 unique transcripts resulting in isoforms with a shared C-terminus. To study the expression and function of all isoforms of *UNK-1*, we used CRISPR/Cas9 to generate a C-terminal fusion of mNeonGreen::*AID\**::3xFLAG to *UNK-1* (**Figure 1A**). The mNeonGreen tag allowed us to determine where *UNK-1* is expressed and its subcellular localization. The *AID* system was discovered in plants but adapted for use in *C. elegans* (Zhang et al., 2015). *AID* allows for rapid (and reversible) depletion of fusion proteins via expression of TIR1 protein, which targets proteins to the proteasome. The truncated *AID\** degron tag (Morawska and Ulrich, 2013) enabled us to rapidly degrade *UNK-1* to study its loss-of-function phenotype. Finally, the 3x FLAG tag will allow future biochemical analyses of *UNK-1*.

We isolated a single allele with the correct insertion, *unk-1(wy2110)*. Following 3 outcrosses, we crossed in a ubiquitously expressed TIR1 transgene and an endogenous mScarlet-tagged allele of *dat-1*, as a marker of dopaminergic neurons. This resulting strain, *TV30205 [wrdSi22; dat-1(wy2061); unk-1(wy2110)]*, is referred to as *UNK-1::AID\** throughout the text. In L4 animals *UNK-1* was observed in the cytoplasm of pharyngeal cells and many head neurons (**Figure 1B**), including weak expression in all dopaminergic neurons (CEP, ADE and PDE). This expression pattern is consistent with the known expression pattern of *EGL-43* (Yee et al., 2024) and the *unk-1* expression data from the CeNGEN neuronal gene expression database (Taylor et al., 2021).

To deplete *UNK-1* from worms, we treated wildtype *N2* or *UNK-1::AID\** L4 animals for 16h with either 0 mM or 4 mM K-NAA (auxin). Following auxin treatment, *UNK-1* expression in *UNK-1::AID\** animals was undetectable in the nerve ring of day 1 adults (**Figure 1C**). To broadly characterize the effect of loss of *UNK-1*, we recorded short videos of worm behavior of day 1 adults following auxin treatment (**Figure 1D**). Auxin treatment did not lead to obvious changes in forward or backward locomotion of either genotype (not quantified). To our surprise, we observed that auxin treatment of *UNK-1::AID\** animals, but not *N2* animals, led to a marked increase in head oscillations during forward movement (**Figure 1E**). Unfortunately due to time limitations, we were unable to assay animals carrying exclusively TIR1 or *unk-1(wy2110)*.

Head oscillations are rhythmic movements that play a key role in the worm's locomotion and environmental exploration. Acetylcholine, GABA, and tyramine are three neurotransmitters that regulate normal head oscillations (Alkema et al., 2005). In some contexts, it is advantageous for the worm to suppress head oscillations. For example, when it encounters certain fungi in the wild, uncontrolled head oscillations can trigger fungus to constrict around the worm's head, entrapping it and leading to death by strangulation (Maguire et al., 2011). The ability to suppress head oscillations allows the worm to smoothly back out

of the fungal trap and avoid capture. Since degradation of [UNK-1](#) increased head oscillations, our data suggests [UNK-1](#) is normally required to suppress this behavior.

Our study reveals an unexpected role of [UNK-1](#) in regulating head oscillations in *C. elegans*. Since [UNK-1](#) is an RNA-binding protein, future studies characterizing the RNAs it binds will be critical in furthering our understanding of [UNK-1](#) function. Given the similarity between the failure to suppress head oscillations in mutants that cannot synthesize tyramine, e.g. [tdc-1](#) (Alkema et al., 2005), [UNK-1](#) may function in this pathway, possibly by stabilizing [tdc-1](#) transcript or facilitating its translation. Future studies will involve characterization of other genetic alleles of [unk-1](#) and performing a developmental time course of [UNK-1](#) depletion.

Mutants that are defective in dopaminergic neuron identity or function have not been described to possess defects in head oscillations. Although we originally identified [unk-1](#) as a downstream target of [EGL-43](#), which is critical for dopaminergic neuron function, it is unclear if dopaminergic neurons contribute to this behavior. The RMD, SMD, RME, and RIM motor neurons innervate the *C. elegans* head muscles and have been implicated in regulating head oscillations (Alkema et al., 2005). According to the CeNGEN database [unk-1](#) is expressed in many head neurons, including the aforementioned motor neurons (Taylor et al., 2021). It will be important to thoroughly characterize the identity of each neuron expressing [UNK-1](#) through colocalization experiments using strains such as NeuroPAL (Yemini et al., 2021). Following this, we will use our new allele of [unk-1](#) to perform cell-specific degradation, as opposed to global degradation, to determine which neurons [UNK-1](#) is required in to regulate normal head oscillations.

Evidence from *Drosophila* connects Unkempt to the mTOR signaling pathway (Avet-Rochex et al., 2014; Maierbrugger et al., 2020). It will be interesting to test if mTOR signaling converges on [UNK-1](#) and if this connection is important for the head oscillation behavior. In summary, we have characterized the expression and function of [unk-1](#) in the *C. elegans* nervous system.

## Methods

### General Maintenance

Worms were cultured on standard NGM plates seeded with [OP50](#) as previously described (Brenner, 1974). [unk-1\(wy2110\)](#) animals were outcrossed to 3x to [N2](#) before they were used for experiments. Animals used for experimentation were reared at 20C and were well fed for at least 3 generations.

### Genome editing of [unk-1](#) using CRISPR/Cas9

Guides targeting [unk-1](#) were designed using the IDT Alt-R™ CRISPR-Cas9 guide RNA design tool. CRISPR injection mixes were prepared as previously described (Yee et al., 2024). Young adult [N2](#) worms were microinjected using pRF4 ([rol-6](#)) as a co-injection marker, and F1 roller progeny were singled on individual plates. F2 progeny were screened for expression of mNeonGreen and a single correct isolate was identified by Sanger sequencing. This strain, [TV30179](#), will be deposited into the CGC.

### Auxin-induced degradation of [UNK-1](#)

To prepare auxin plates, molten NGM media was supplemented with K-NAA (PhytoTech Labs) to a final concentration of 4 mM. [OP50](#) bacteria was seeded on auxin containing plates at least 2 days in advance of each experiment. L4 animals were placed on seeded auxin plates overnight (16 hours, 20C) before being subjected to microscopy or behavioral assays.

### Microscopy

To image [UNK-1](#) expression, animals were mounted on 2% agarose pads and immobilized using 10 mM levamisole. Animals were imaged using a spinning-disk system (3i) equipped with a CSU-W1 spinning-disk head (Yokogawa), 488-nm and 561-nm solid-state lasers, a C-Apochromat 40× 0.9 NA water-immersion objective, and a Prime95B camera (Photometrics).

### Head Oscillation Assays

L4 animals that were treated with 0 mM or 4 mM auxin were moved onto regular seeded NGM plates and allowed to equilibrate for 15 minutes at 20C. Animals were then recorded for 30s using an iPhone 15 Pro mounted to a Zeiss SV6 dissecting microscope. Videos were processed using Apple QuickTime Player (v.10.5) and randomly trimmed into a 10s clip. Head oscillations, defined as a complete cycle of left-to-right movement, during forward crawling were counted from each clip by playing videos at 0.25 speed. The identity of the genotypes that were tested were blinded to the experimenter. Assays were reproducible and repeated three times.

### Statistical analysis and Graphing

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All statistical analysis was performed using GraphPad Prism (version 10), using ordinary one-way ANOVA with Tukey's multiple comparison tests, comparing the mean of each column with the mean of every other column.

## Reagents

[N2](#): Wild Type

[JDW224](#): [wrdSi22](#)[*eft-3p::TIR1::F2A::mTagBFP2::AID\*::NLS::tbb-2* 3'UTR] I

[TV30179](#): [unk-1](#)([wy2110](#)[[unk-1](#)::*mNeonGreen::AID\*::3xFLAG*]) X

[TV30205](#): [wrdSi22](#)[*eft-3p::TIR1::F2A::mTagBFP2::AID\*::NLS::tbb-2* 3'UTR] I; [dat-1](#)([wy2061](#)[[dat-1](#)::*mScarlet*]) III; [unk-1](#)([wy2110](#)[[unk-1](#)::*mNeonGreen::AID\*::3xFLAG*]) X

**Acknowledgements:** We thank members of the Shen laboratory, Ev Nichols, and Aaron Gitler for critical reading of this manuscript. Biorender.com was used to generate schematics. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

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**Funding:** Funding for this study was provided by Dr. Kang Shen who is a Howard Hughes Medical Institute Investigator.

6/11/2025 - Open Access

**Author Contributions:** Lexin Gitler: investigation, writing - original draft, formal analysis. Callista Yee: conceptualization, data curation, methodology, supervision, validation, writing - original draft, writing - review editing, formal analysis.

**Reviewed By:** Anonymous

**Nomenclature Validated By:** Anonymous

**WormBase Paper ID:** WBPaper00068247

**History:** Received May 28, 2025 **Revision Received** June 5, 2025 **Accepted** June 11, 2025 **Published Online** June 11, 2025  
**Indexed** June 25, 2025

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**Citation:** Gitler L, Yee C. 2025. Depletion of UNK-1 affects head locomotive behaviors in *Caenorhabditis elegans*. microPublication Biology. [10.17912/micropub.biology.001659](https://doi.org/10.17912/micropub.biology.001659)