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Endogenous Expression and Localization of Peroxisome Marker DAAO-1 in *Caenorhabditis elegans*

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Abstract

Peroxisomes coordinate fatty acid β-oxidation and cellular redox homeostasis. D-amino-acid oxidase (DAAO) is a key peroxisomal matrix enzyme, yet the endogenous subcellular localization of *C. elegans* DAAO-1 remains uncharacterized. Here, we generated an endogenous mScarlet::daao-1 knock-in strain via CRISPR/Cas9. Confocal microscopy reveals that mScarlet::DAAO-1 localizes exclusively to discrete puncta throughout the intestinal tract from L1 larvae to adulthood. These puncta are spatially distinct from autofluorescent gut granules within the same cells and tissues. This knock-in strain provides a reliable, endogenously tagged red fluorescent peroxisome marker for investigating peroxisome dynamics, metabolic regulation, and organelle contact sites *in vivo*.

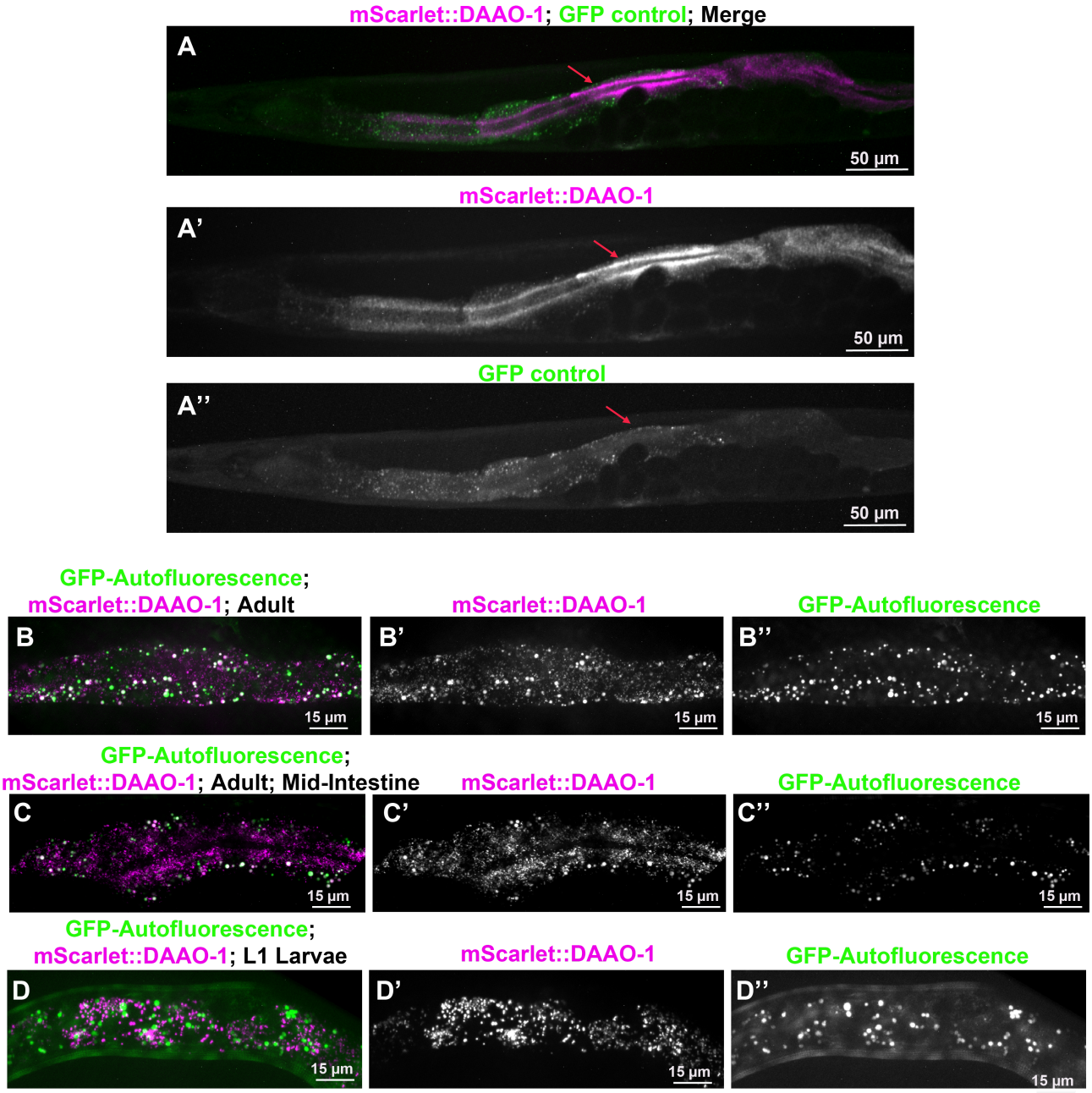


Figure 1. Endogenous mScarlet::DAAO-1 localizes to peroxisome-like puncta throughout the intestine:

(A–A″) Representative images of an adult hermaphrodite expressing endogenous mScarlet::DAAO-1 alongside the autofluorescence GFP channel control. (A) The merged image of mScarlet::DAAO-1 channel alone showing intestinal enrichment (A′, red arrows), and the autofluorescence GFP channel alone (A″, red arrow). Scale bars, 50 µm. (B–B″) High-magnification micrographs of the adult intestine showing merged channels (B), mScarlet::DAAO-1 puncta (B′), and autofluorescent gut granules in the GFP channel (B″). Scale bars, 15 µm. (C–C″) Mid-intestinal region of an adult displaying discrete mScarlet::DAAO-1 puncta that do not colocalize with autofluorescent gut granules. Merged view (C), mScarlet channel (C′), and GFP channel (C″). Scale bars, 15 µm. (D–D″) Intestinal cells of an L1 larva showing the early

emergence of discrete mScarlet::DAAO-1 puncta distinct from larger autofluorescent granules. Merged view (D), mScarlet channel (D′), and GFP channel (D″). Scale bars, 15 µm.

Description

Description:

Peroxisomes are ubiquitous organelles that are bound by a single membrane. They are primarily characterized by two coupled metabolic activities: the β-oxidizing of very-long-chain branched-chain fatty acids that exceed mitochondrial processing capacity, and the generation and subsequent decomposition of hydrogen peroxide (H_2O_2) by catalase (Lodhi and Semenkovich, 2014). In this capacity, peroxisomes serve as a cell-autonomous counterpart to hepatic detoxification machinery, degrading reactive oxygen species and toxic metabolites before they accumulate. Because peroxisomal matrix enzymes are synthesized in the cytosol and imported post-translationally via dedeciated targeting signals, fluorescently tagging an endogenous matrix enzyme enables direct visualization of its subcellular distribution and provides a faithful marker for peroxisome morphology *in vivo*.

D-amino-acid oxidase (DAAO) is a prototypical peroxisomal matrix enzyme that oxidatively deaminates neutral and basic D-amino acids, including D-alanine, D-serine, and D-asparagine, to their corresponding α-keto acids, ammonia, and H_2O_2, thereby directly coupling peroxisomal redox handling to its enzymatic activity. In *C. elegans*, the D-amino acid oxidase family comprises DAAO-1 alongside three D-aspartate oxidases (DDO-1, DDO-2, and DDO-3) (Katane et al., 2006). A previous transcriptional *Pdaao-1::GFP* reporter demonstrated that *daao-1* expression is restricted to the intestine, initiating in the embryo and persisting throughout all larval and adult stages (Saitoh et al., 2012). Furthermore, *daao-1* mutants exhibit decreased hatching and reduced brood size, aligning with a proposed function for DAAO-1 in catabolizing dietary D-amino acids—particularly D-alanine acquired from a bacterial diet—within the intestine, analogous to the role of DAO in the mammalian liver and kidney (Saitoh et al., 2012). Nevertheless, this transcriptional reporter could not resolve the subcellular localization of DAAO-1, leaving its peroxisomal targeting in *C. elegans* unconfirmed prior to this work.

Beyond amino acid catabolism, peroxisomes collaborate closely with lipid droplets (LDs): both organelles coordinate lipid flux to support the synthesis of plasmalogens (ether phospholipids), a class of lipids enriched in neuronal membranes, and to drive fatty-acid β-oxidation (Lodhi and Semenkovich, 2014). In *C. elegans*, concerted regulation of peroxisome abundance and lipid droplets in the intestine has been connected to lipid homeostasis and lifespan regulation, highlighting that peroxisome distribution and abundance are tightly coupled to organismal physiology rather than occurring incidentally (Papsdorf et al., 2023). In addition, peroxisomes orchestrate metabolic flexibility and longevity via an interorganelle cascade. Inhibition of peroxisomal protein PRX-11 promotes longevity in *C. elegans* via enhancements to mitochondria, among others (Sharma et al., 2026; Flora et al., 2025). Because the intestine is the primary hub responsible for digesting ingested bacteria, synthesizing neutral lipids, and shuttling fatty acids to mitochondria for oxidation, establishing an endogenously tagged peroxisomal enzyme in this tissue provides a physiologically relevant and accessible marker. Such a tool is valuable beyond the study of DAAO-1 biology, serving as a vital resource for researchers examining organelle contact sites, peroxisome dynamics, or lipid metabolism in *C. elegans*.

To resolve the native expression and subcellular pattern of DAAO-1, we generated an endogenously tagged red fluorescent reporter by knocking mScarlet into the 5′ end of the endogenous *daao-1* locus (mScarlet::daao-1). Because gut granules exhibit prominent autofluorescence in the GFP channel in *C. elegans* intestinal cells, we used this endogenous autofluorescence as an internal landmark to delineate intestinal boundaries and assess spatial compartmentalization (Figure). In both L1 larvae and adult hermaphrodites, mScarlet::DAAO-1 localized as isolated, well-defined puncta dispersed throughout the full length of the intestine (Figure A–D), aligning with a predicted peroxisomal matrix pattern rather than diffuse cytoplasmic labeling. At higher magnification, mScarlet::DAAO-1 puncta were intermingled with, yet strictly distinguishable from, the larger autofluorescent gut granules (Figure B–D). This demonstrates that endogenous DAAO-1 displays punctate subcellular localization characteristic of peroxisomes, consistent with its predicted PTS1 motif and known orthologs (Sharma, 2026), and that DAAO-1 labels a peroxisomal compartment spatially distinct from lysosome-related gut granules within the same intestinal cells. This punctate distribution was evident in L1 larvae and persisted through adulthood, underscoring that intestinal DAAO-1 expression—and, by implication, the intestinal peroxisome population it identifies—originates early in development and is sustained throughout life. Future studies that cross endogenous *daao-1* tags with matrix-targeted peroxisomal markers (such as GFP::PTS1) or organelle-contact reporters will be useful for formally verifying structural overlap and examining dynamic organelle contacts.

In summary, we engineered an endogenous mScarlet::daao-1 knock-in in *C. elegans* and demonstrated that DAAO-1 is confined to punctate, as predicted peroxisome-like structures dispersed throughout the intestine from the L1 larval stage to adulthood, and remains clearly distinguishable from autofluorescent gut granules within the same cells. This strain offers an endogenously expressed, robust marker for peroxisomes in the *C. elegans* intestine—a tissue critical for lipid digestion, β-oxidation, and metabolic detoxification—providing a valuable tool for investigations into organelle-organelle interactions (such as peroxisome–lipid droplet contact sites), peroxisome homeostasis, and D-amino acid metabolism.

Methods

Microscopy All fluorescence imaging was performed using a spinning-disk confocal system equipped with a Nikon 60× 1.2 NA water-immersion objective, a Hamamatsu C15440 ORCA-Fusion BT Digital camera, and a Yokogawa CSU-X1 confocal scanner unit. Imaging acquisition was controlled by Nikon's NIS-Element software (Version 6.10). Image processing and channel merging were conducted using the ImageJ/FIJI Bio-Formats plugin (Fiji/ImageJ: ImageJ version 1.54f; Bio-Formats plugin: version 7.0.0) (National Institutes of Health) (Linkert et al., 2010; Schindelin et al., 2012).

Mounting *C. elegans* for imaging Animals were mounted on 7% agarose pads in M9 buffer containing 7.5 mM levamisole and covered with a coverslip. The synchronized adult animals were used for imaging in panels A–C", and L1 hermaphrodites were imaged in panels D-D".

Generation of CRISPR knock-in strains CRISPR/Cas9 editing was performed using the Bristol N2 strain as the wild type. Synthetic crRNAs and tracrRNA were purchased from Horizon Discovery. Repair templates and single-stranded DNA oligos were synthesized by Integrated DNA Technologies (IDT). Approximately 20 young adult hermaphrodites were injected with the CRISPR/Cas9 injection mix. The N-terminal tag was amplified from the plasmid pMS050, which contains *C. elegans* codon-optimized mScarlet-I-C1. We co-injected the dpy-10(cn64) roller marker with the mScarlet::DAAO-1 injection reagent (Arribere et al., 2014). The sequences for the dpy-10(cn64) crRNA and single-stranded repair template (ssODN) have been added to the Reagents section/table. F1 roller progeny of injected animals were initially selected based on visible mScarlet expression and subsequently screened by single-worm PCR across the insertion boundaries using the primers: Forward 5' tgtgtgcggaaattgcagaaa 3' and Reverse 5' TGCCACGTCACTTGTAGTGT 3'.

The amplification sequence is shown below, with red letters indicating the mScarlet sequence. tgtgtgcggaaattgcagaa
atcgtagtttttagttatttttagtaggaaaaattgatttttttaagagatttttcatgacagaaaaatttgaacaaaatgttgaaaaaaaatttttttttttttttttgataaaatttttt
ttttttttcaaaaactcaaaaaatctatatttttcagaaATGGTCTCCAAGGGAGAGGCCGTCATCAAGGAGTTCATGCG
TTTCAAGGTCCACATGGAGGGATCCATGAACGGACACGAGTTCGAGATCGAGGGAGAGGG
AGAGGGACGTCCATACGAGGGAACCCAAACCGCCAAGCTCAAGGTCACCAAGgtaagtttaaacatatatatactaacta
accctgattatttaaattttcagGGAGGACCACTCCCATTCTCCTGGGACATCCTCTCCCCACAATTCATGTACGGATCC
CGTGCCTTCATCAAGCACCCAGCCGACATCCCAGACTACTACAAGCAATCCTTCCCAGAG
GGATTCAAGTGGGAGCGTGTCATGAACTTCGAGGACGGAGGAGCCGTCACCGTCACCCAA
GACACCTCCCTCGAGGACGGAACCCTCATCTACAAGgtaagtttaaacagttcggtactaactaaccatacatatttaaa
ttttcagGTCAAGCTCCGTGGAACCAACTTCCCACCAGACGGACCAGTCATGCAAAAGAA
GACCATGGGATGGGAGGCCTCCACCGAGCGTCTCTACCCAGAGGACGGAGTCCTCAAGGG
AGACATCAAGATGGCCCTCCGTCTCAAGGACGGAGGACGTTACCTCGCCGACTTCAAGgtaagtttaaacatgattttac
taactaactaatctgatttaaattttcagACCACCTACAAGGCCAAGAAGCCAGTCCAAATGCCAGGAGCCTACAACGTC
GACCGTAAGCTCGACATCACCTCCCACAACGAGGACTACACCGTCGTCGAGCAATACGAG
CGTTCCGAGGGACGTCACTCCACCGGAGGAATGGACGAGCTCTACAAGCCTAAAATTGCT
GTACTAGGCGCAGGAATTAACGGTATAGCATCGGCTCTTGCAATTCAGGAACGACTGCCA
AATTGTGAGgttaaaattttaaaattttaaaattgaaaatattttgaaaaacaatttttttgaaacttttttcgaaaagttttaaaaaaaattcagGT
GACAATAATTGCTGAAAAATTTTCTCCGAACACTACAAGTGACGTGGCA

Candidate insertions were validated by Sanger sequencing of both the 5′ and 3′ genomic junctions. All mScarlet::DAAO-1 lines used for imaging were established as homozygous knock-in stocks before imaging.

mScarlet::DAAO-1 CRISPR information:

Guide RNA: 5' GCGCCTAGTACAGCAATTTT”

Repair template primer F1: 5' tcaaaaactcaaaaaatctatatttttcagaaATGGTCTCCAAGGGAGAGGCCGT 3'

Repair template primer R1: 5' ATTGCAAGAGCCGATGCTATACCGTTAATTCCTGCTCCAA GAACTGCGATCTTAGGCTTGTAGAGCTCGTCCATTC 3' Genotype Primer F1: 5' tgtgtgcggaaattgcagaaa 3'

Genotype Primer R1: 5' TGCCACGTCACTTGTAGTGT 3'

wt amplicon size: 400 bp mScarlet::DAAO-1 amplicon size: 1249 bp

dpy-10(cn64) CRISPR information:

Guide RNA: 5' GCTACCATAGGCACCACGAG”

Repair template: 5' CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCT ATGGTAGCGGAGCTTCACATGGCTTCAGACCAACAGCCTAT 3'

Reagents

C. elegans strains used in this study: N2 Bristol wild-type; XFB15 daao-1(xmb14[mScarlet::daao-1]) IV.

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