Humanized, Transgenic Worms to Study CYP2E1-Induced Toxicity

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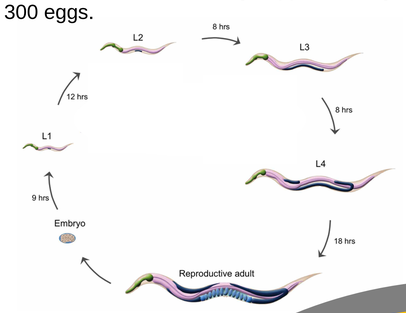
C. elegans as a toxicological model

Caenorhabditis elegans is an attractive model for toxicology:

- Short reproductive cycle (Fig. 1) and high fecundity
- Transparent bodies allow fluorescent reporters to be visualized in live animals
- Genetically tractable – many tools available including CRISPR/Cas9 and RNAi feeding for knockdown of genes
- Although worms have 84 P450 genes, total P450 expression is very low[1], allowing for low background in transgenic animals
- Biology of many cellular processes well-conserved, allowing for translation of basic biological findings
- All cells are known in the worm[2]; 1/3 of somatic cells are neurons[3]

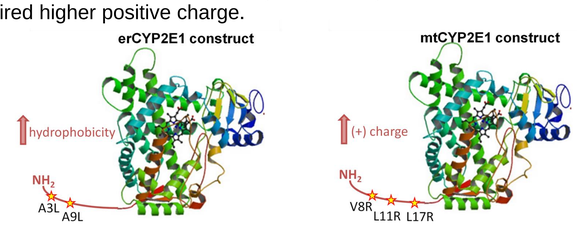
Fig. 1: *C. elegans* life cycle. Embryos develop into reproductive adults in ~55h. Each reproductive adult lays approximately 300 eggs.



Building a humanized CYP2E1 worm

- Cytochrome P450 2E1 (CYP2E1) metabolizes small hydrophobic compounds such as ethanol, acetaminophen, and trichloroethylene
- Metabolism results in detoxification or, paradoxically, bioactivation[4]
- CYP2E1 localizes to endoplasmic reticulum and mitochondria[5], and may exert different toxicities depending on its localization[6,7]
- Localization can be forced to each organelle with point mutations to targeting signal (Fig. 2)[8]

Fig. 2: CYP2E1 constructs. Targeting to endoplasmic reticulum was achieved by point mutations that increase hydrophobicity, while mitochondrial targeting required higher positive charge.



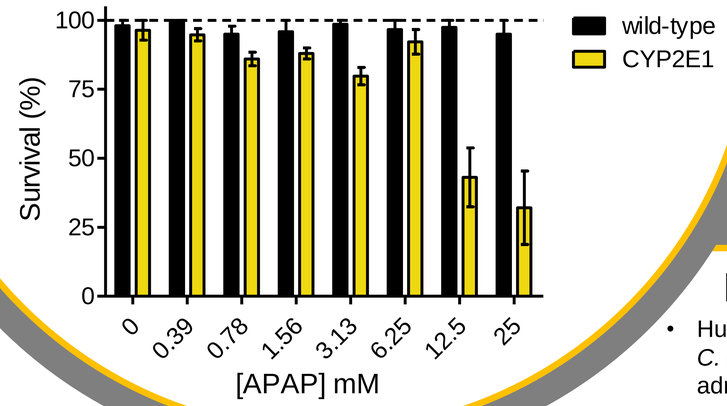
Creating a humanized transgenic nematode

1. Cloned vector:
   a) Optimized codons for expression in *C. elegans* and added introns
   b) Added point mutations for retention in ER or transport to mitochondria (Fig. 2)
   c) Amplified ubiquitous promoter (*eft-3*) and 3'-UTR (*unc-54*) from genomic DNA
   d) Ligated promoter, gene, and UTR into 95.75 Fire Vector
2. Injected DNA into worms (gonadal microinjection) and obtained stable extrachromosomal lines
3. Integrated gene into genomic DNA using gamma irradiation and isolated independent strains

CYP2E1 increases APAP lethality

- N2 (wild-type) animals were completely resistant to acetaminophen (APAP) toxicity (Fig. 5, black bars)
- CYP2E1-expressing animals demonstrated lethal toxicity at high APAP concentrations (Fig. 5, yellow bars)

Fig. 5: Lethality after acetaminophen (APAP) exposure. For experiments, 8-day old (post-reproductive) adult nematodes were exposed to acetaminophen in liquid for 48 hours in complete K+ medium containing cholesterol, $MgSO_4$, and $CaCl_2$ with UV-inactivated bacteria to avoid bacterial metabolism of the drug. Following exposure, lethality was determined by response to a harsh touch with a platinum wire. Data represents 6 experiments from 2 biological replicates.



CYP2E1 confers protection from APAP-induced growth delay

- In unexposed animals, CYP2E1 expression alone resulted in a slight growth delay (Fig. 6),
- At both 24 and 48 hours, CYP2E1 was protective against APAP-induced growth delay compared to wild-type (Fig. 7),
- APAP growth effects were most dramatic at 10 and 25 mM (Fig. 7)

Fig. 6: Larval growth. For experiments, L1 larvae grown in liquid for 48 hours. Worm length and area were measured using WormSizer[9]. N=200 worms each for two biological replicates.

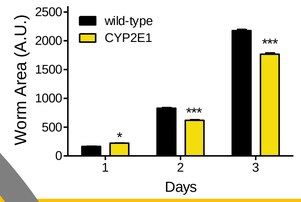
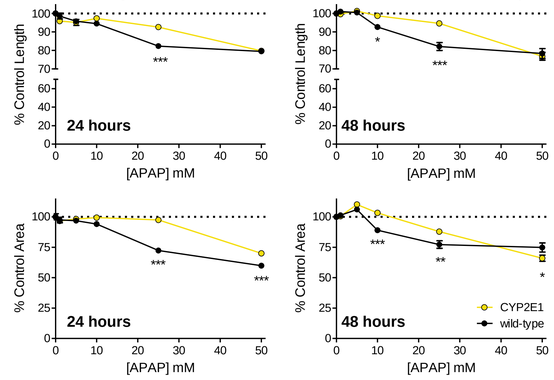


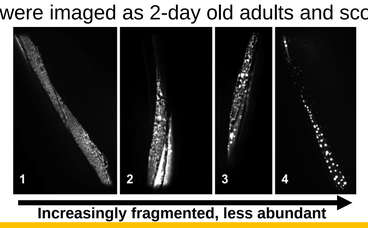
Fig. 7: Growth delay after exposure. For experiments, L1 larvae were exposed to APAP in liquid for 24 or 48 hours. Worm length and area were measured using ImageJ software plugin WormSizer[9]. N=200 worms each for 2 reps.

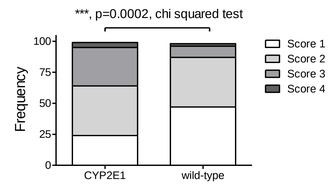


CYP2E1 expression in *C. elegans* impacts mitochondrial morphology

- CYP2E1 expression alone resulted in more fragmented, less abundant muscle mitochondria (Fig. 8).

Fig. 8: Mitochondrial morphology. For experiments, mitochondrial matrix-targeted GFP was expressed in wild-type and CYP2E1 backgrounds. Nematodes were imaged as 2-day old adults and scored blindly using in-house software.





Discussion

- Human CYP2E1 is active in transgenic nematodes, indicating compatibility with *C. elegans* redox partners, cytochrome P450 reductase (microsomes) and adrenodoxin/adrenodoxin reductase (mitochondria).
- CYP2E1 expression results in increased lethality in post-reproductive adult nematodes, which may be due to increased production of the reactive metabolite, NAPQI, by CYP2E1.
- Wild-type nematodes are sensitive to APAP-induced growth delay, likely due to a direct effect of APAP on developmental signaling. This effect is mitigated by CYP2E1, presumably through metabolism of APAP.
- CYP2E1 expression alone results in fragmentation of mitochondria, which may be due to increased ROS.

CYP2E1 is widely expressed and is metabolically active

- CYP2E1 expression was widespread (Fig. 3)
- Transgenic CYP2E1 was active in microsomes and mitochondria and absent in wild-type animals (Fig. 4A).
- 4-nitrophenol turnover by microsomal CYP2E1 showed substrate inhibition (Fig. 4B)

Fig. 3: CYP2E1 is expressed in most somatic cells. Brightfield image (top) shows worm anatomical position to demonstrate localization of expression of CYP2E1-GFP fusion (bottom).

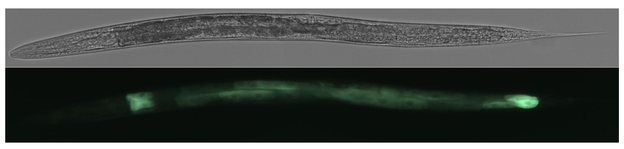
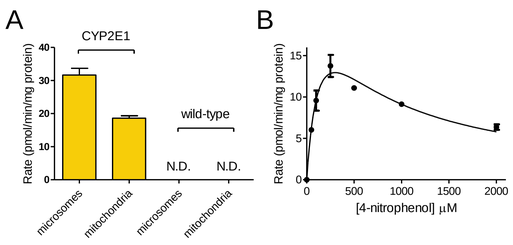


Fig. 4: Transgenic CYP2E1 is active for 4-nitrophenol oxidation. For experiments, isolated microsomes (0.5 mg/mL) or mitochondria (2 mg/mL) were incubated with 4-nitrophenol and a NADPH-regenerating system for 2 hours at 25°C. Data shown are mean and standard error from 3 experiments.



Ongoing and future experiments

- Other powerful *in vivo* reporter strains will be used, including GFP-labeled dopaminergic neurons, redox-sensitive GFP reporters, and transcriptional reporters for unfolded protein responses.
- Exposures will be expanded to include ethanol, trichloroethylene, and hexanes.
- Mitochondrial and ER-targeted CYP2E1 nematodes have been generated; similar experiments will be carried out with those constructs.

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