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Supporting Information

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Fig. 4. Phylogenetic tree showing relative positions of the taxa used in this study. The tree is based on a CLUSTALW multiple nucleotide sequence alignment (2,082-character) of the small subunit 18S rDNA for parasitic and free-living roundworms (Nematoda), flatworms (Platyhelminthes), and horsehair worms (Nematomorpha). Their phylogenetic positions are shown relative to free-living chordates, insects, and a priapulid worm. *F*, free-living; *P*, parasitic; (*P*), parasitic as juveniles. Taxa in boldface were assayed, or substituted for one assayed[1, 2] for heme biosynthesis enzymes, in this study. Nineteen species included designated outgroups *Xenopus laevis* X04025 and *Branchiostoma floridae* M97571. Other taxa included *Priapulus caudatus* X87984; arthropods *Scolopendra cingulata* U29493, *Panulirus argus* AY743955, and *Tenebrio molitor* X07801; flatworms *Monocelis lineata* U45961, *Echinobothrium chisholmae* AF286986, and *Schistosoma mansoni* U65657; horsehair worms *Paragordius varius* AF421772 and *Gordius aquaticus* X87985; and nematodes *Trichuris suis* AY856093, *Ascaris suum* U94367, *Panagrellus redivivus* AF083007, *Strongyloides stercoralis* AF279916, *C. elegans* X03680, *Oscheius myriophila* AF082994, *Necator americanus* AY295811[1] substituted for *Ancylostoma caninum*, *Ostertagia ostertagi* AF036598[2] substituted for *Haemonchus contortus*. Alignment having 18% parsimony-informative characters weighted by the maximum rescaled

consistency (RC) index value where characters were sampled with equal probability, and 67.5% characters were weighted as 1. The maximum-parsimony phylogram pictured is from a heuristic search employing TBR (tree bisection-reconnection) branch-swapping and ACCTRAN (accelerated transformation) character-state optimization, bootstrapped 1,000 times as implemented in PAUP*, version 4.0b4a. Tree length = 1,204, consistency index = 0.807, retention index = 0.770, rescaled consistency index = 0.621, homoplasy index = 0.193, Goloboff fit = -224.894.

Supporting Figure 5

Fig. 5. Effects of gallium protoporphyrin IX (GaPP) on worm morphology. Synchronized L1 larvae were grown in mCeHR medium supplemented with 4 μ M hemin chloride and various amounts of GaPP for 6 days. Worms were analyzed by differential interference contrast microscopy. Photomicrographs *1-5*, worms grown in 2, 6, 8, 50, and 100 μ M GaPP, respectively. Photomicrograph *6*, worms grown in mCeHR medium with 4 μ M hemin. (Bar, 100 μ m.)

Supporting Methods

Biological Materials and Strains. Worm strains used were *C. elegans* wild-type N2 strain, *Panagrellus redivivus* LKC10, and *Oscheius myriophila* DF5020. *E. coli* strains were wild-type DH10B, RP523 [δ-aminolevulinic acid dehydratase (ALAD) mutant] (1), and Delta-*vis* (ferrochelatase mutant) (2). *Saccharomyces cerevisiae* haploid strains wild-type BY4743 and ferrochelatase knock-out mutant *HEM15* were purchased as diploids from Open Biosystems (Huntsville, AL). In the *HEM15* mutant, the YOR176W open reading frame corresponding to the *HEM15* gene was replaced with a KanMX cassette.

Enzyme Assays and Heme Determinations. Free-living worms ($\approx 10^6$) cultured in mCeHR medium were washed three times in cold M9 buffer before resuspension in ice-cold 0.1 M Tris•HCl buffer, pH 8.0, containing a protease inhibitor mixture (Calbiochem protease inhibitor cocktail set II). The worm suspensions were homogenized by passage through a French pressure cell at an internal pressure up to 18,000 pounds per square inch (psi; 1 psi = 6.89 kPa) until breakdown of the worm cuticle occurred (>90%) as monitored by microscopy. The homogenate was clarified by centrifugation at 3,000 × *g* to remove cell debris, and the supernatant thus obtained was further centrifuged at 7,000 × *g* to obtain a mitochondrial-enriched pellet. This procedure provided >70% enrichment of mitochondrial membrane proteins as determined by immunoblots using ATP2 antisera. Parasitic worms were homogenized by grinding to a fine powder with a mortar and pestle in the presence of liquid N_2, and the homogenates were subsequently processed as above to obtain cytosolic- and mitochondrial-enriched fractions. Activities for ALAD (3), porphobilinogen deaminase (PBGD) (4), succinate dehydrogenase (5), and ferrochelatase (6) enzymes were determined as described previously. All samples were analyzed by using a Shimadzu UV-1601 dual-beam scanning spectrophotometer. Data are expressed as the average of triplicates, and enzyme activities are normalized to total protein concentration, as determined by the Bradford assay (BioRad). Total hemes were analyzed by recording pyridine hemochrome spectra in aqueous alkaline pyridine solutions after reduction with 5 mM sodium dithionite and oxidation with 1 mM potassium ferricyanide, as described (7). Low-temperature spectra (-191°C) of cell extracts were obtained as described previously with an optical path length of 1 mm with one sheet of wet filter paper in the reference path (8).

Immunoblots and Worm Fractionations. All procedures were performed at 4°C. Worms were washed extensively in M9 buffer and suspended in MESH (220 mM mannitol/2 mM EDTA/70 mM sucrose/5 mM

Hepes, pH 7.4) with a protease inhibitor mixture (Calbiochem). The worm suspensions were disrupted once by passage through a French pressure cell (<6,000 psi), followed by homogenization with 10 strokes of a Dounce homogenizer. The homogenates were centrifuged twice at 1,000 × *g* for 10 min to remove cuticle and large debris, followed by centrifugation at 100,000 × *g* to obtain pellets enriched in organelles and membranes, and supernatant fractions enriched for cytosol. The pellet was resuspended in MESH, and protein was determined by the Bradford assay (BioRad). For immunoblotting, lysates were heated at 100°C for 10 min in the presence of SDS sample buffer containing 2-mercaptoethanol and centrifuged for 5 min at 16,000 × *g* at 4°C. Proteins were separated by SDS/PAGE, transferred to nitrocellulose, and detected by either the SuperSignal West Pico or West Femto Chemiluminescence kits (Pierce) using goat anti-rabbit and anti-mouse horseradish peroxidase-conjugated secondary antibody (Pierce). Primary antibodies used in this study were rabbit polyclonal antibody against ATP2 (1:2,000) and mouse monoclonal antibodies DM-1A to α -tubulin (Sigma, 1:500).

Determination of 18 S rDNA Gene Sequence and Phylogenetic Sequence Analysis. Genomic DNA from *Trichuris suis* larvae were isolated by standard procedures involving proteinase K treatment and phenol/chloroform extractions. The 18S small subunit (SSU) rDNA gene from *T. suis* was amplified by PCR using genomic DNA as template and redundant primer mixes kindly provided by W. K. Thomas (University of New Hampshire, Durham). The PCR product thus obtained was purified and sequenced, and the DNA sequence was deposited in GenBank under accession no. AY856093. Sequences for the same segment of the SSU of the 18S rDNA were collected to illustrate taxa tested in this study with appropriate phylogenetic resolution needed, as demonstrated for some other helminths (9). The closest taxa to two that were unavailable in GenBank, *Haemonchus contortus* and *Ostertagia ostertagi*, were selected based on taxonomy and a BLAST search (Dec. 15, 2004) of the closest available large subunit 28S sequences, for which taxon representation was denser in the database. For *H. contortus*, another strongylid, *Ostertagia ostertagi* AF036598 was used (9). For *Ancylostoma caninum*, another hookworm, *Necator americanus* AY295811, was used (10). To illustrate the phylogenetic position of the studied taxa among related eukaryotes, other slowly evolving nonstudied taxa were selected (11). The tree was rooted with chordates, *Xenopus laevis* (Craniata; Vertebrata) and *Branchiostoma floridae* (Cephalochordata). Other taxa included a priapulid worm, *Priapulus caudatus* (Priapulida), the horsehair worm *Gordius aquaticus* (Nematomorpha; Gordioida), arthropods *Scolopendra cingulata* (Myriapoda), *Panulirus argus* (Crustacea), and *Tenebrio molitor* (Hexapoda, Insecta), and flatworms *Monocelis lineata* (Turbellaria) and *Echinobothrium chisholmae* (Cestoda). The beginning nucleotide of the sequences for all taxa corresponds to position 984 of the *C. elegans* rDNA gene (12). An alignment was made by using CLUSTALW (version 1.8), manually checked for the presence of conserved positions among sequences, and trimmed in GENEDOC. Phylogenetic analysis was made on a CLUSTALW multiple sequence nucleotide alignment (2,082-character) using default parameters. This was run in PAUP*, version 4.0b4a, where all characters were weighted by the maximum rescaled consistency (RC) index value and characters were sampled with equal probability. A maximum parsimony heuristic search employing TBR (tree bisection-reconnection) branch-swapping and ACCTRAN (accelerated transformation) character-state optimization, was bootstrapped 1,000 times.

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Table 2. Expect (*E*) values for heme biosynthetic enzyme orthologs in *C. elegans*

Enzymes*	Mouse	*D. melanogaster*	*S. cerevisiae*	*C. elegans*
ALAD	1×10^{-131}	1×10^{-109}	2.7×10^{-89}	0.15^{NS}
PBGD	1×10^{-127}	7×10^{-78}	1.3×10^{-57}	0.98^{NS}
UROS	1×10^{-92}	4×10^{-26}	4.8×10^{-11}	0.1^{NS}
UROD	1×10^{-148}	1×10^{-105}	3.6×10^{-94}	0.46^{NS}
CPOX	1×10^{-124}	1×10^{-123}	1.8×10^{-83}	0.77^{NS}
PPOX	1×10^{-118}	3×10^{-79}	4.7×10^{-18}	0.09^{NS}
FC	1×10^{-152}	1×10^{-112}	1.2×10^{-84}	0.3^{NS}

E values for other genetic model organisms are shown for the purpose of comparison. NS, not significant, *E*-value cut-off $\leq 1 \times 10^{-3}$.

*****Human enzyme protein sequences were used to perform BLAST searches on Nov. 14, 2004, against genome databases from *C. elegans* (Wormbase, WS134 release, Nov. 12, 2004), *D. melanogaster* (Flybase), *S. cerevisiae* (Saccharomyces Genome Database), and mouse (National Center for Biotechnology Information GenBank). ALAD, δ -aminolevulinic acid dehydratase (AY319481); PBGD, porphobilinogen deaminase (NM_000190); UROS, uroporphyrinogen III synthase (BC002573); UROD, uroporphyrinogen decarboxylase (CR456976); CPOX, coproporphyrinogen oxidase (NM_000097); PPOX, protoporphyrinogen oxidase (NM_000309); FC, ferrochelatase (BC039841).

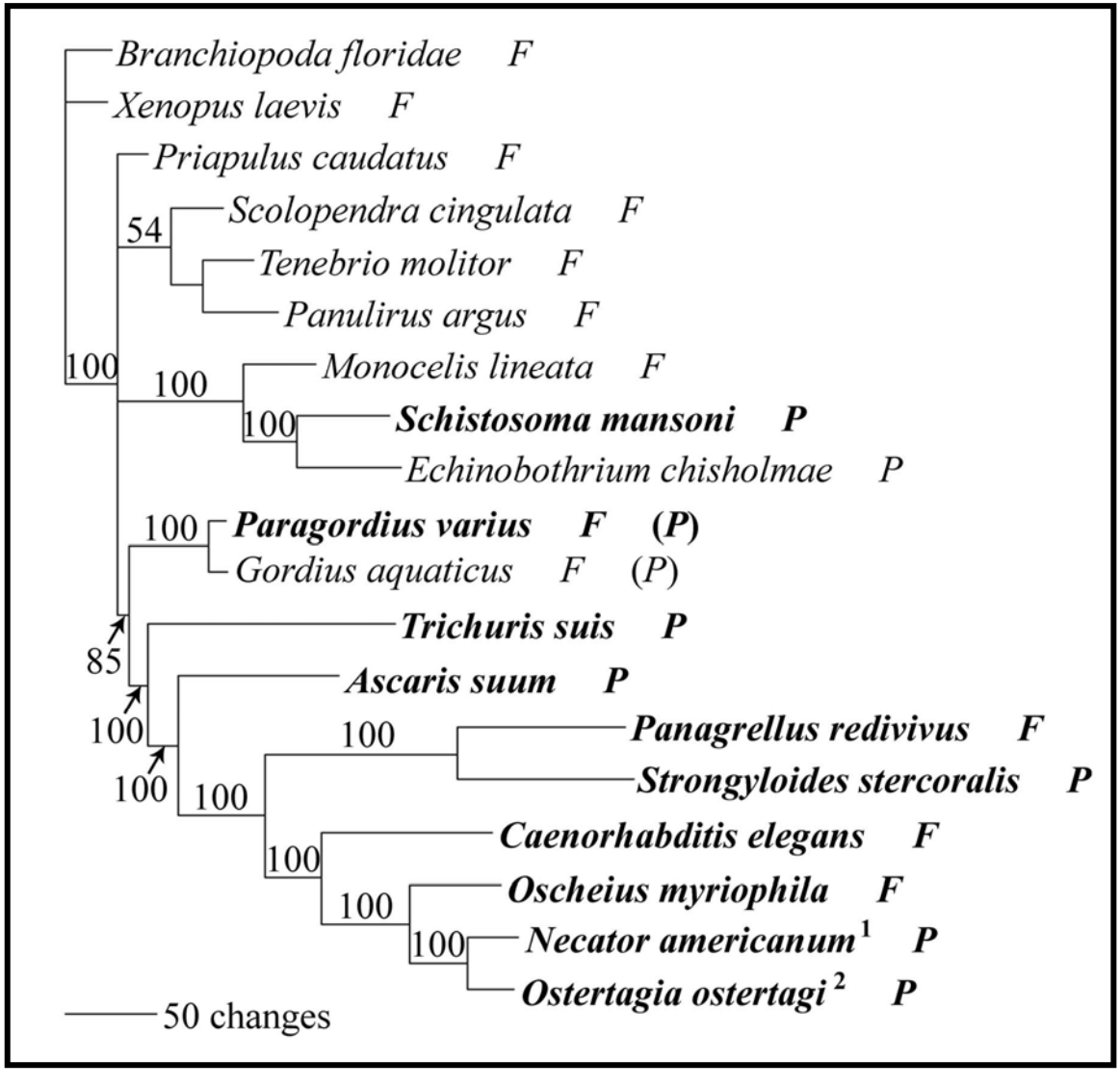

Branchiopoda floridae F
Xenopus laevis F
Priapulus caudatus F
Scolopendra cingulata F
Tenebrio molitor F
Panulirus argus F
Monocelis lineata F
Schistosoma mansoni P
Echinobothrium chisholmae P
Paragordius varius F (P)
Gordius aquaticus F (P)
Trichuris suis P
Ascaris suum P
Panagrellus redivivus F
Strongyloides stercoralis P
Caenorhabditis elegans F
Oscheius myriophila F
Necator americanum[1] P
Ostertagia ostertagi[2] P
54
100
100
100
100
100
100
85
100
100
100
100
50 changes

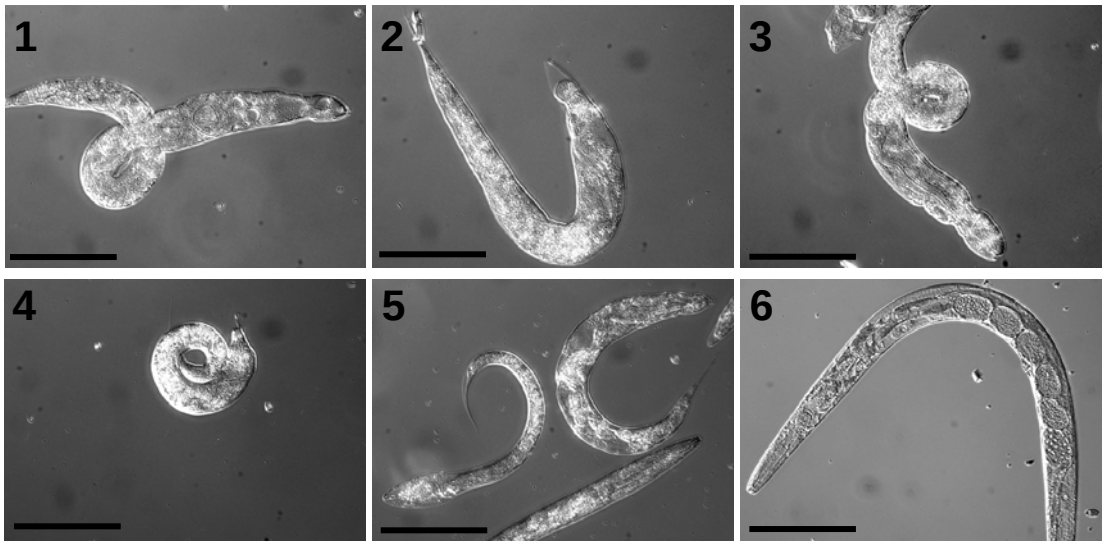
Table 3. Analysis for potential metabolic inhibition of heme biosynthetic enzyme activities in *C. elegans*

	Enzyme activity, nmol/min per mg*		
Organism	**ALAD**	**PBGD**	**FC**
C. elegans	ND	ND	ND
E. coli	1.35 ± 0.044	0.032 ± 0.002	9.52 ± 0
S. cerevisiae	0.69 ± 0.012	0.026 ± 0.002	10.59 ± 0.65
S. cerevisiae FC mutant	(-)	(-)	ND
C. elegans + *E. coli*†	0.699 ± 0.05	0.018 ± 0.002	3.95 ± 0.37
C. elegans + *S. cerevisiae*†	0.313 ± 0.001	0.015 ± 0.001	7.45 ± 0.76
C. elegans + *S. cerevisiae* FC mutant†	(-)	(-)	ND

E. coli and *S. cerevisiae* lysates were used as controls and in mixing experiments.

*****Mean values (triplicates) of product formed ± SD. ALAD, δ -aminolevulinic acid dehydratase; PBGD, porphobilinogen deaminase; FC, ferrochelatase; ND, enzyme activity not detected under assay conditions, (-), not assayed.

†Equal proportions (50%) of protein from each organism were mixed prior to assaying for enzymes. Because enzyme activity was normalized to total protein, activities are reduced by half.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