Supporting Information

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SI Materials and Methods

Phylogenetic Analysis. For consensus tree construction, sequences were aligned and curated using the phylogeny.fr Web service at www.phylogeny.fr (1). Sequence alignments were performed with T-Coffee (v6.85) (2) using the following pair-wise alignment methods: the 10 best local alignments (Lalign_pair) and an accurate global alignment (slow_pair). After alignment, the sequences were curated and ambiguous regions (i.e., containing gaps and/or poorly aligned) were removed with Gblocks (v0.91b) (3) using the following parameters: minimum length of a block after gap cleaning, 5; no gap positions were allowed in the final alignment; all segments with contiguous nonconserved positions bigger than 8 were rejected; minimum number of sequences for a flank position, 85%. The curated alignment containing 126 sites was analyzed locally using MrBayes (v3.2.1 x64) (4) using the following parameters: preset aamodelpr = mixed; lset rates = gamma; mcmp ngen = 500000, samplefreq = 100, printfreq = 1000, diagnfreq = 5000. At the end of the run after 500,000 generations, the SD of split frequencies was 0.007. A relative burn in fraction of 0.25 was used when calculating the consensus tree. Trees were visualized with FigTree v1.4.0 (tree.bio.ed.ac.uk), and final figures were created in InkScape (inkscape.org).

Ferrochelatase Cloning into *Escherichia coli* and Expression of Ferrochelatase Genes. For amplifications of 5′ and 3′ cDNA ends (RACE) from RNA preparations of *Brugia malayi, Onchocerca volvulus, Dirofilaria immitis*, and *Acanthocheilonema viteae* worms, gene-specific degenerate forward and reverse primers were designed based upon alignments of diverse ferrochelatase (*FeCH*) gene and EST sequences available in the National Center for Biotechnology Information and European Molecular Biology Laboratory–European Bioinformatics Institute databases. These primers were used in combination with the universal RACE (Rapid Amplification of cDNA Ends) primer provided by the SMARTer RACE cDNA amplification kit (Clontech) for amplification from the species-specific cDNA RACE pools (5′ or 3′). Subsequent PCR amplification and DNA sequencing between the 3′ and 5′ ends enabled acquisition of complete coding sequences.

Except for the *FeCH* gene from *E. coli* (*EcFeCH*), all cloned gene constructs were codon optimized to provide maximal gene expression. All *FeCH* gene constructs were transformed into *E. coli* containing the RIL plasmid (Agilent) to increase gene expression. The various *FeCH* genes were also cloned into the pTrcHisA vector (Invitrogen) for subsequent expression of proteins with N-terminal 6XHis-tags in *E. coli.* For recombinant FeCH expression in *E. coli,* the *FeCH* genes from eukaryotes were cloned with truncations at their N termini to remove the potential mitochondrial targeting domains. For FeCH from *B. malayi* (BmFeCH), the N-terminal 36 amino acids were eliminated; for FeCH from *A. viteae* (AvFeCH), 38 amino acids; and for FeCH from *H. sapiens* (HsFeCH), 62 amino acids were removed. For comparative purposes, a full-length *BmFeCH* gene was also cloned and expressed.

FeCH Enzyme Assays. N-terminal 6XHis-tagged BmFeCH, AvFeCH, wBmFeCH, and HsFeCH recombinant proteins were purified for enzyme studies. When appropriate, the inhibitor NMMP (*N*-methyl mesoporphyrin; Frontier Scientific) was freshly prepared as a 5-mM stock solution in 50% ethanol containing 0.02 M NaOH. The enzyme reactions were terminated by addition of acetone. After removing the precipitated proteins by centrifugation, the formation of the end product of the reactions, Zinc (II) protoporphyrin IX (Zn-PPIX), was measured using a Perkin-Elmer LS50B spectrofluorimeter (excitation 420 nM; emission 587 nm).

Ex Vivo *B. malayi* and *A. viteae* Motility and Viability Tests. *B. malayi* adult worms isolated from control or tetracycline treated jirds were cultured in duplicate wells (two adult females or males per well, in 24-well plates, for 7 d in 2 mL of RPMI 1640 containing 10% (vol/vol) FBS (Perbio) and penicillin-streptomycin (Invitrogen; 200 U/mL; 200 μg/mL final concentration) with varying concentrations of NMMP. Worms not treated with NMMP were cultured in medium containing solvent (1% ethanol and 0.0004 M NaOH). Medium and inhibitor were changed every 2 d. Motility and viability were determined as described in the text.

For *A. viteae* experiments, one adult female or three adult males per replicate (experiment repeated twice) were cultured in RPMI 1640 with 2 mM glutamine, 25 mM Hepes (Gibco) with 10% (vol/vol) FCS (Gibco), 100 U/mL streptomycin, 100 μg/mL penicillin, and 0.25 μg/mL amphotericin B (Sigma). Medium was changed every 2 d. Worms not treated with NMMP or ivermectin were cultured in medium containing solvent only. Motility was determined as described in the text.

***C. elegans* Strains, Constructs, and Transgenesis.** A general backbone for all *C. elegans* transgenic constructs was prepared by modifying the GFP expression vector pPD95.75 (gift of Andrew Fire Department of Genetics, Stanford University, Palo Alto, CA). A 3.5-kb sequence of the *sur-5* promoter contained in plasmid pTG96 (5) was cloned into the multiple cloning site of pPD95.75 using the SphI site at the 5′ end of the sequence and by inserting an NheI site at the 3′ end of the *sur-5* promoter sequence upstream of the endogenous XmaI site of pPD95.75. *psur5BmFeCH-GFP* was generated by cloning the *BmFeCH* cDNA between the *sur-5* promoter and in frame with the C-terminal GFP sequence using the NheI and XmaI sites. *psur5NTER-BmFeCH-GFP* was generated using PCR by removing 84 bp of the 5′ sequence of *psur5BmFeCH-GFP* starting from the ATG initiation codon. An ATG codon located downstream of the deleted sequence served as initiation codon. This construct resulted in an N-terminally truncated version of BmFeCH (BmFeCHΔ28::GFP) lacking the first 28 amino acids encoded by exon 1 and encompassing the predicted mitochondrial targeting domain. All constructs were verified by restriction digestion and DNA sequencing before injection.

Constructs were injected into *unc-119(ed3)* worms according to standard protocols (6). Plasmid pDP#MM016b, carrying a *unc-119(+)* rescue construct (7) was used as a cotransformation marker. Injection mixtures had the following composition: plasmid of interest 10–30 ng/μL; coinjection marker 10–30 ng/μL; Low Molecular Weight DNA Ladder (New England Biolabs) as a filler to give a final 100 ng DNA/μL concentration.

Axenic Growth of *C. elegans*. Worms were grown in axenic basal medium [ABM: 3% (wt/vol) dry yeast extract, 3% (wt/vol) soy peptone (Sigma; cat. no. P0521), 1% (wt/vol) dextrose, cholesterol 10 mg/L] in the absence of a heme source. A porphyrin source was added in the form of hemin chloride or PPIX at a final concentration of 10 mg/L, equivalent to 15.3 μM hemin chloride and 17.8 μM PPIX (hemin stock solution: 1 mg/mL in 0.1 M NaOH; PPIX stock solution: 1 mg/mL in 0.3 M ammonium acetate; the latter was added after chilling the culture media to minimize precipitation).

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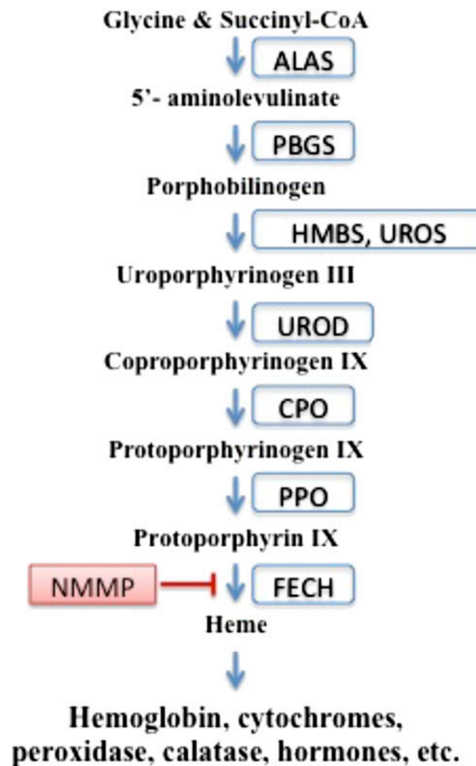

Fig. S1. Heme biosynthetic pathway in animals, fungi, and α-proteobacteria (plants, algae, archaebacteria, and other eubacteria initiate from glutamate). Enzyme names are boxed and the FeCH inhibitor NMMP is shown on the left. ALAS, δ-aminolevulinic acid synthase; CPO, coproporphyrinogen oxidase; FeCH, ferrochelatase HMBS, hydroxymethylbilane synthase; PBGS, porphobilinogen synthase; PPO, protoporphyrinogen oxidase; UROD, uroporphyrinogen decarboxylase; UROS, uroporphyrinogen III synthase. The first step and last three steps occur in mitochondria,

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BmFeCH          ------------------------- MYAIQNSGVWYTAVRRMTSWIANEIPSVINEER 33
SmFeCH          ------------------------------------------------------MDANTE 6
wBmFeCH         ------------------------------------------------------------
EcFeCH          ------------------------------------------------------------
ScFeCH          ------------------------------MLSRTIRTQGSFLRRSQLTITRSFSVTFNM 30
DmFeCH          ----------------------------------------MFLHNTKFCRLASGLAGGVR 20
HsFeCH          MRSLGANMAAALRAAGVLLRDPLASSSWRVCQPWRWKSGAAAAAVTTETAQHAQGAKPQV 60

BmFeCH          SIPELKKKTGVLVVNVGTPSGYDYLPIRRFLREFLSDRRVIELPRILWWPILHFFILTTR 93
SmFeCH          HPAVGYGKLGVLLVNLGTPDGTDVTSMRRYLREFLSDRRVIEWSRLFWYPILYGIVLNTR 66
wBmFeCH         ------MKKAVVLLNLGGPD--SLSAVRPFLFNLFYDKRIINLPNP-FRFFLAKFISAKR 51
EcFeCH          ---MRQTKTGILLANLGTPDAPTPEAVKRYLKQFLSDRRVVDTSRLLWWPLLRGVILPLR 57
ScFeCH          QNAQKRSPTGIVLMNMGGPS--KVEETYDFLYQLFADNDLIPISAK-YQKTIAKYIAKFR 87
DmFeCH          NLSGQKPKTAILMLNMGGPT--HTDQVHDYLLRIMTDRDMIQLP---VQSRLGPWIAQRR 75
HsFeCH          QPQKRKPKTGILMLNMGGPE--TLGDVHDFLLRLFLDQDLMTLP---IQNKLAPFIAKRR 115
                         .::: *:* *          :* .:: *. ::  .       :   :   *

BmFeCH          PFIVGKSYKLIWNTVEDECPLQTVTRNQSAKLANRLSDQS-------------------- 133
SmFeCH          PRKVGKAYELIWNKDLNESWLRTYTRNQAALMAEAFGG---------------------- 104
wBmFeCH         ENNARKIYEQIG----GKSPILENTKMQAEALERELNRSVFCHPSSVTLGPRKENWIPVS 107
EcFeCH          SPRVAKLYASVW--MEGGSPLMVYSRQQQQALAQRLPE---------------------- 93
ScFeCH          TPKIEKQYREIG----GGSPIRKWSEYQATEVCKILDK---------------------- 121
DmFeCH          TPEVQKKYKEIG----GGSPILKWTELQGQLMCEQLDR---------------------- 109
HsFeCH          TPKIQEQYRRIG----GGSPIKIWTSKQGEGMVKLLDE---------------------- 149
                     : *  :     . . :   :  *   : . :

BmFeCH          --------IMVDWAFRYGEPSIASRIRKFEKEACDKLIIFPLFPQFSAVTNASIFDETCR 185
SmFeCH          -----QPQVVVDWAMRYGQPSIASRIEALQKAGCERILVFPLYPQYAAATTATVNDKAFE 159
wBmFeCH         RIGMTSKLTKVFICMRYWHPFANEVVKSVKQFDPDEVILLPLYPQYSTTTTLSSIENWQK 167
EcFeCH          --------MPVALGMSYGSPSLESAVDELLAEHVDHIVVLPLYPQFSCSTVGAVWDELAR 145
ScFeCH          -TCPETAPHKPYVAFRYAKPLTAETYKQMLKDGVKKAVAFSQYPHFSYSTTGSSINELWR 180
DmFeCH          -ISPETAPHKHYVGFRYVNPLTENTLAEIEKDKPERVVLFSQYPQYSCATSGSSFNSIFT 168
HsFeCH          -LSPNTAPHKYYIGFRYVHPLTEEAIEEMERDGLERAIAFTQYPQYSCSTTGSSLNAIYR 208
                              : *  *   .    .     .. : :. :*:::  *  :  :

BmFeCH          CLMK--QRRQMILSVVPPFYDNELYIKTILKHLNSALKRFRAPP---QVIIVSYHGIPLS 240
SmFeCH          ALLK--MRWQPALRTVPPYHDDPVYIDALATSINKHLATLDWEP---ELVLASFHGIPKS 214
wBmFeCH         NAKQ--YGIKCNTKIIRHHYDNQDFIEAHANLITKHYKLASEVGK--PRVLFSAHSLPLS 223
EcFeCH          ILAR--KRSIPGISFIRDYADNHDYINALANSVRASFAKHGEP----DLLLLSYHGIPQR 199
ScFeCH          QIKALDSERSISWSVIDRWPTNEGLIKAFSENITKKLQEFPQPVRDKVVLLFSAHSLPMD 240
DmFeCH          HYRSNNLPSDIKWSIIDRWGTHPLLIKTFAQRIRDELAKFVETKRNDVVILFTAHSLPLK 228
HsFeCH          YYNQVGRKPTMKWSTIDRWPTHHLLIQCFADHILKELDHFPLEKRSEVVILFSAHSLPMS 268
                               :     .   *.     :                :: : *.:*

BmFeCH          YQSSGDPYGFQCKYTTSLLRQRCRISNCELITTFQSRFGPAEWLKPYTEDTVIELAKRGV 300
SmFeCH          YFEKGDPYYCQCQKTARLLREKLGWPQDRLQVTFQSRFGPEEWLQPYTDATVERLAKEGV 274
wBmFeCH         VIKKGDPYALQVEETVKLIVKKLHIKDLDWSICYQSKIGPVKWLEPSTESELLRAKADGV 283
EcFeCH          YADEGDDYPQRCRTTTRELASALGMAPEKVMMTFQSRFGREPWLMPYTDETLKMLGEKGV 259
ScFeCH          VVNTGDAYPAEVAATVYNIMQKLKFKNPYRLV-WQSQVGPKPWLGAQTAE-IAEFLGPKV 298
DmFeCH          AVNRGDAYPSEIGASVHSVMQELGQTNPYSLA-WQSKVGPLAWLAPATDDAIKGYVKQGL 287
HsFeCH          VVNRGDPYPQEVSATVQKVMERLEYCNPYRLV-WQSKVGPMPWLGPQTDESIKGLCERGR 327
                  . ** *  .   :.  : .            :**:.*   ** . *   :

BmFeCH          KRIMVIAPGFFSDCLETIDELKLQLADSFRK-HGGEEYVYVPCLNDSTEAIDILESLSRK 359
SmFeCH          KRIAVINPGFVSDCLETLEEIAGQAAESFHH-NGGEKFAHIPCLNDSPEGMAVLNHVVRR 333
wBmFeCH         P-VVLLPISFVSEHSETLVELDMEYKTIIKD----GYYFRIPTLSTNSLFIKCLAGLCIN 338
EcFeCH          GHIQVMCPGFAADCLETLEEIAEQNREVFLG-AGGKKYEYIPALNATPEHIEMMANLVAA 318
ScFeCH          DGLMFIPIAFTSDHIETLHEIDLG---VIGESEYKDKFKRCESLNGNQTFIEGMADLVKS 355
DmFeCH          KNFILVPIAFVNEHIETLHELDIEYCDELAKEVGVEEIRRAATPNDHPLFIDALTNVVAD 347
HsFeCH          KNILLVPIAFTSDHIETLYELDIEYSQVLAKECGVENIRRAESLNGNPLFSKALADLVHS 387
                  . .:  .*  :  **:  *:       :               .        :  :

BmFeCH          HIQCFL---------------------------------- 365
SmFeCH          ELEGWL---------------------------------- 339
wBmFeCH         HS-------------------------------------- 340
EcFeCH          YR-------------------------------------- 320
ScFeCH          HLQSNQLYSNQLPLDFALGKSNDPVKDLSLVFGNHEST 393
DmFeCH          HLKSQQAVNPKFLMRCPMCSNPKCRESKSWYRQLCSN- 384
HsFeCH          HIQSNELCSKQLTLSCPLCVNPVCRETKSFFTSQQL-- 423
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Fig. S2. ClustalX sequence alignment of BmFeCH with FeCH orthologs from *Sinorhizobium meliloti* (SmFeCH), *Wolbachia* from *Brugia malayi* (wBmFeCH), *E. coli* (EcFeCH), *Saccharomyces cerevisiae* (ScFeCH), *Drosophila melanogaster* (DmFeCH), and *Homo sapiens* (HsFeCH). Eukaryotic N-terminal extensions are shown in bold typeface with the predicted mitochondrial targeting domains underlined. The invariant active site residues are shown in red. A C-terminal extension (green) involved in dimerization is found only in FeCH proteins of eukaryotic origin. The four cysteine residues involved in [2Fe-2S] cluster formation are shown in blue. Underneath the alignments, "*****" indicates positions that have a single fully conserved residue; ":" indicates substitution with a highly similar amino acid, whereas "." indicates substitution with a similar residue. FeCH accession numbers: *Homo sapiens* XP_008784; *Drosophila melanogaster* AAC26225; *Saccharomyces cerevisiae* NP_014819; *Escherichia coli* P23871; *Wolbachia* endosymbiont strain TRS of *Brugia malayi* AAW71307 ; *Sinorhizobium meliloti* AAL67571; *Brugia malayi*BmFeCH ADI33748.1.

Fig. S3. Growth of WT *Caenorhabditis elegans* (blue bars) and transgenic *C. elegans* IP637 carrying full-length *BmFeCH* (red bars) on axenic basal medium (ABM) and ABM supplemented with either PPIX (~18 μM) or hemin (~15 μM) in the presence or absence of the FeCH inhibitor NMMP (1 μM and 10 μM).