ROS-based lethality of *Caenorhabditis elegans* mitochondrial electron transport mutants grown on *Escherichia coli* siderophore iron release mutants

J. Amaranath Govindan[a,b], Elamparithi Jayamani[a,b], and Gary Ruvkun[a,b,1]

[a]Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114; and [b]Department of Genetics, Harvard Medical School, Boston, MA 02115

Contributed by Gary Ruvkun, September 5, 2019 (sent for review July 23, 2019; reviewed by Siu Sylvia Lee and Alexander van der Bliek)

***Caenorhabditis elegans* consumes bacteria, which can supply essential vitamins and cofactors, especially for mitochondrial functions that have a bacterial ancestry. Therefore, we screened the Keio *Escherichia coli* knockout library for mutations that induce the *C. elegans hsp-6* mitochondrial damage response gene, and identified 45 *E. coli* mutations that induce *hsp-6::gfp*. We tested whether any of these *E. coli* mutations that stress the *C. elegans* mitochondrion genetically interact with *C. elegans* mutations in mitochondrial functions. Surprisingly, 4 *E. coli* mutations that disrupt the import or removal of iron from the bacterial siderophore enterobactin were lethal in combination with a collection of *C. elegans* mutations that disrupt particular iron–sulfur proteins of the electron transport chain. Bacterial mutations that fail to synthesize enterobactin are not synthetic lethal with these *C. elegans* mitochondrial mutants; it is the enterobactin–iron complex that is lethal in combination with the *C. elegans* mitochondrial mutations. Antioxidants suppress this inviability, suggesting that reactive oxygen species (ROS) are produced by the mutant mitochondria in combination with the bacterial enterobactin–iron complex.**

mitochondria | siderophore | free radical

Caenorhabditis elegans, like many nematode species, consumes bacteria, which supplies many nutritional needs. *C. elegans* in the laboratory consumes *Escherichia coli*, but in its natural habitat of rotting fruit, feeds on a diet of hundreds of bacterial species (1, 2). These diverse bacteria supply micronutrients to the animal such as vitamins and cofactors. The bacterial supply of such cofactors is so dependable that *C. elegans* is a heme auxotroph fully dependent on the bacteria it consumes to acquire this cofactor for many mitochondrial proteins in the electron transport chain (ETC) (3). Because many of the more than 1,000 eukaryotic nuclearly encoded proteins that localize to the mitochondria share a common ancestor with bacteria, we reasoned that other bacterial gene pathways that animals may depend upon may function for mitochondrial biogenesis or function. For example, *E. coli* mutations in the ETC cytochrome *bo* terminal oxidase A gene *cyoA* cause induction of a *C. elegans* mitochondrial unfolded protein response gene, *hsp-6*, in wild-type *C. elegans* (4). Other bacterial biosynthetic pathways on which the mitochondrion may still depend could be discovered using an animal reporter gene for mitochondrial dysfunction and a comprehensive bacterial collection of mutations. Therefore, we conducted a genome-wide screen for *E. coli* mutations that induce the *C. elegans* mitochondrial unfolded protein response (UPR^{mt}) or affect the viability or growth of *C. elegans*. In this screen, we identified mutations in 45 *E. coli* gene disruptions, out of more than 4,000 gene disruptions tested, that strongly activate the mitochondrial unfolded protein response gene, *hsp-6*; 24 of these 45 mutations also slow *C. elegans* growth. Because each of the 45 *E. coli* mutations induce a mitochondrial stress response, we tested for genetic interactions between each of these *E. coli* mutant strains and a set of *C. elegans* mitochondrial mutant strains. We found that 4 *E. coli* mutations that affect the import of the iron siderophore enterobactin into the cytoplasm of *E. coli* or the retrieval of iron in the bacterial cytoplasm from the imported enterobactin bound to iron showed a dramatic synthetic lethality with a variety of mutations in *C. elegans* mitochondrial ETC components, which when grown on wild-type bacteria are viable. Because Fe(III) is insoluble in aerobic environments, bacteria produce siderophores to retrieve Fe(III). Enterobactin binds Fe(III) with a K_m of 10^{-39} M; the enterobactin bound to Fe(III) is then retrieved from outside of the cell or periplasm by specific *E. coli* enterobactin::Fe receptors and the tightly bound Fe(III) is removed from enterobactin in the bacterial cytoplasm by a dedicated enterobactin esterase fes (5). *E. coli* mutations that disrupt enterobactin biosynthesis genes were not synthetic lethal with *C. elegans* mitochondrial mutants. In fact, *E. coli* double mutants defective for both enterobactin synthesis and enterobactin import, or for both enterobactin synthesis and Fe(III) removal, were no longer synthetic lethal with the *C. elegans* mitochondrial mutants. Thus, the production of enterobactin and a failure to remove the covalently bound iron from enterobactin is required for the toxic interaction between siderophore uptake or esterase mutant *E. coli* and *C. elegans* mitochondrial mutants. Antioxidants such as ascorbic acid or resveratrol strongly suppressed the inviability of *C. elegans* mitochondrial mutants grown on the *E. coli* enterobactin siderophore utilization or import mutants. We hypothesize that reactive oxygen species (ROS) produced by *C. elegans* mitochondrial mutations when combined with ferric-chelated enterobactin synergistically produce dramatically increased ROS and lethality. This toxicity

Significance

The animal mitochondrion has a bacterial origin and depends on vitamins and other biochemicals produced by bacteria. In a genetic search for mitochondrial biochemical dependencies when the animal *Caenorhabditis elegans* is feeding on *Escherichia coli* as its sole nutritional source, we identified 45 *E. coli* mutations that disrupt mitochondrial function. Four of these *E. coli* mutations that disrupt the transport and removal of iron from an iron retrieval cofactor were lethal in combination with *C. elegans* mitochondrial mutations. Antioxidants strongly suppressed this inviability, suggesting a reactive oxygen toxicity. Iron retrieval cofactors produced by one bacterial species are often hijacked by other bacterial species. The toxicity of this iron retrieval cofactor may be a selected additional function of this biochemical.

Author contributions: J.A.G. and G.R. designed research; J.A.G. and E.J. performed research; J.A.G. analyzed data; and J.A.G. and G.R. wrote the paper.

Reviewers: S.S.L., Cornell University; and A.v.d.B., University of California, Los Angeles.

The authors declare no competing interest.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

[1]To whom correspondence may be addressed. Email: ruvkun@molbio.mgh.harvard.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1912628116/-/DCSupplemental.

First published October 7, 2019.

of a siderophore bound to Fe(III) and variations in the ETC between organisms may be an evolutionarily selected feature of siderophores that selects against their expropriation by other microbes.

Results

To systematically identify bacterial mutations that may affect mitochondrial function, we fed wild-type *C. elegans* carrying a mitochondrial unfolded protein response chaperone gene *hsp-6::gfp* with individual bacterial mutant strains from the *E. coli* Keio collection library and screened for *E. coli* mutations that induce the mitochondrial unfolded protein response (UPR^{mt}) and/or slow *C. elegans* development. The Keio library is ~4,000 single-gene in-frame insertions of a kanamycin resistance cassette in the *E. coli* K12 BW25113 background (6). *C. elegans hsp-6* encodes a mitochondrial matrix HSP70 chaperone that is specifically up-regulated by mutations or drugs that impair mitochondrial function and is a standard marker for the UPR^{mt} (7). We inoculated each of the 4,000 Keio collection *E. coli* strains individually on the surface of an agar NGM *C. elegans*/*E. coli* growth agar, added about 30 larval stage 1 (L1) wild-type *C. elegans* carrying *hsp-6::GFP* animals, and screened for induction of *hsp-6::GFP* 48 h later using a dissecting scope fluorescence microscope. We also screened the same wells for slower than normal growth of the *C. elegans* strain. We identified 45 mutant *E. coli* strains that robustly induced *hsp-6::gfp* (Dataset S1 and *SI Appendix*, Figs. S1 and S2) mainly in the intestine. In the same screening wells observed the next day, we screened the same *E. coli* mutants again for a delay in *C. elegans* rate of development from L1 to adulthood. Twenty-four of these 45 *E. coli* mutants also slowed wild-type *C. elegans* growth (Table 1). All 24 of the *E. coli* mutants that cause growth delay also were retrieved in the set of 45 *E. coli* mutants that activated *C. elegans hsp-6::gfp*, suggesting that mitochondrial dysfunction is a major axis of bacterial/host nutritional interaction (Table 1). Surprisingly, none of the ~4,000 viable *E. coli* Keio mutants screened failed to supply some essential micronutrient or macronutrient or produced a toxin that disallowed wild-type *C. elegans* growth. Thus, 45 *E. coli* gene disruptions out of 4,000 tested induce a *C. elegans* mitochondrial stress response, and one-half of these 45 *E. coli* mutations also slowed wild-type *C. elegans* growth. However, none of the 4,000 Keio mutant collection was missing any essential bacterial factor (such as heme) for *C. elegans* growth. Our screen would have easily detected such a lethal interaction. There are 303 *E. coli* Keio gene disruptions that are inviable and thus not easily tested for *C. elegans* genetic interactions.

To verify the *C. elegans* UPR^{mt} induction, we showed that the 45 mutant *E. coli* strains that activate *hsp-6::gfp*, also activate *hsp-60::gfp*, another marker for the *C. elegans* mitochondrial UPR^{mt} (Dataset S1). ATFS-1 is a transcription factor required for the activation of UPR^{mt} genes, such as *hsp-6* and *hsp-60*. We tested each of the Keio *E. coli* mutants on a *C. elegans atfs-1(tm4525); hsp-60::GFP C. elegans* strain and found that all 45 bacterial mutants failed to induce the *hsp-60*::GFP UPR^{mt} in the *atfs-1(tm4525)* background (Dataset S1). Thus, these *E. coli* mutants activate the *C. elegans* UPR^{mt} via the expected ATFS-1 transcription factor. The *C. elegans* stress response induced by the 45 *E. coli* Keio mutants is specific for mitochondria because none of the 45 *E. coli* mutants caused activation of *hsp-4::gfp* (Dataset S1), a reporter of the unfolded protein response in the endoplasmic reticulum (UPR^{er}) (8). Similarly, the 45 *E. coli* Keio mutants did not activate *pgp-5::gfp* (Dataset S1), a reporter of a translational stress response (9).

Because each of these 45 *E. coli* mutants induce the *C. elegans* mitochondrial-unfolded response, we examined whether any of these *E. coli* mutants strongly interact with *C. elegans* mutations in nuclear-encoded mitochondrial proteins. *C. elegans* RNA interference screens have shown that many mitochondrial gene inactivations are lethal (10). However, viable reduction-of-function mutants have been characterized (11). We tested the viable mitochondrial membrane protease *spg-7(ad2249)* mutant (12) and the viable electron transport point mutations *nduf-7(et19)*, *isp-1(qm150)*, *clk-1(qm30)*, *gas-1(fc21)*, *nduf-2.2(ok2397)*, *nuo-6(qm200)*, *mev-1(kn1)*, and *ucr-2.3(pk732)* for synthetic lethal interactions with the 45 *E. coli* mutations that induce *C. elegans hsp-6::gfp*. Dramatically, 4 of the 45 Keio *E. coli* gene disruptions that induce the *C. elegans hsp-6* mitochondrial response gene in wild-type *C. elegans* caused a dramatic developmental arrest in *spg-7(ad2249), nduf-7(et19)*, *isp-1(qm150)*, or *clk-1(qm30) C. elegans* mutants: *Δfes::kan*, *ΔfepD::kan*, *ΔfepG::kan*, *ΔfepC::kan* mutants (Table 1 and *SI Appendix*, Table S1). For example, *Δfes::kan* feeding induces a highly penetrant and dramatic developmental arrest with *C. elegans spg-7(ad2249)*, while wild-type *C. elegans* fed on *Δfes::kan* grow normally (Fig. 1*A*).

Each of the 4 *E. coli* gene disruptions that cause a synthetic arrest with *C. elegans* mitochondrial mutant strains mediates steps in the retrieval of iron from the *E. coli* siderophore enterobactin. Enterobactin is a high-affinity iron siderophore produced by many Gram-negative bacteria, including *E. coli* (5, 13). *E. coli* produce enterobactin under iron deficiency via the proteins encoded by the *entCDEBAH* operon (Fig. 1 *B* and *C*). Enterobactin is secreted into the environment, the soil or in a cell, where it binds to iron, forming a complex that is imported into *E. coli* through the transporters located in the bacterial outer membrane (13) (Fig. 1*C*). The ferric enterobactin complex is transported into the cytoplasm via the *fepDGC* ATP-binding cassette transporter (14, 15). FepB encodes a periplasmic protein that binds ferric enterobactin and directs it to inner membrane transporters, FepD and FepG, which pump it into the bacterial cytoplasm. FepC assists FepD and FepG in transporting ferric enterobactin from the periplasm into the cytoplasm (Fig. 1*C*) (16). Fes encodes enterobactin esterase that catalyzes the hydrolysis of both enterobactin and ferric enterobactin. In the *E. coli* cytoplasm, Fes hydrolyses enterobactin to release iron and linearized 2,3-dihydroxy-*N*-benzoyl-L-serine trimer as well as the dimer and monomer (Fig. 1*C*) (17–19). Genetic disruption of *E. coli fes* causes accumulation of the unhydrolyzed ferric enterobactin in the bacterial cytoplasm (20, 21), whereas genetic disruption fepDGC causes accumulation of the ferric enterobactin in the *E. coli* periplasm where the cytoplasmic Fes esterase cannot release iron from its nearly covalent bond in ferric enterobactin (14, 15) (*SI Appendix*, Fig. S3 *A* and *B*). This complex enterobactin synthesis and import biology is not parochial to *E. coli*: Siderophores related to enterobactin are used across bacterial phylogeny, and the hijacking of iron-loaded siderophores produced by one species and taken up by another bacterial species is a key feature of the iron competition landscape (22, 23).

We analyzed the synthetic lethal interaction between *E. coli* enterobactin iron release mutations and *C. elegans* mitochondrial mutations in more detail. Based on the morphological features, animal size measurements, and the germline development stage, *Δfes::kan* feeding in *spg-7(ad2249)* mutant causes an L1 arrest (*SI Appendix*, Fig. S4 *A*–*C*). *C. elegans nduf-7(et19)*, *isp-1(qm150)*, and *clk-1(qm30)* mutant animals grown on the *E. coli Δfes::kan* mutant also dramatically arrest as L1 larvae (*SI Appendix*, Table S1 and Fig. S5). *nduf-7(et19)* is a partial loss-of-function mutation in *nduf-7* (NADH-ubiquinone oxidoreductase Fe-S), a subunit of the mitochondrial ETC complex I (11, 24). *nduf-7(et19)* mutant animals strongly activate the UPR^{mt} and have a reduced respiration rate and longer life span (24). *isp-1* encodes a Rieske iron sulfur protein that functions in the cytochrome *b*-c1 complex III subunit of the mitochondrial respiratory chain (11). *isp-1(qm150)* mutants have lower oxygen consumption, decreased ROS production, and increased life span (25). *clk-1* encodes an ortholog of the highly conserved demethoxyubiquinone hydroxylase that is necessary for ubiquinone biosynthesis (11). Mutations in *C. elegans clk-1* cause slow development, reduced respiration, and increased life span when grown on an *E. coli* strain that is competent to produce

Table 1. Quantification of developmental delay phenotype in wild-type background and *spg-7(ad2249)* mutant background

	Wild-type *C. elegans* animals		*spg-7(ad2249)* mutant	
	% Adults		% Adults	
E. coli genotype	Day 3	Day 4	Day 4	Day 5
BW25113 (wild type)	100 ± 0 (244)	NA	100 ± 0 (195)	ND
fes	100 ± 0 (244)	NA	0 ± 0 (169)	0 ± 0 (224), **L1-arrest**
fepG	100 ± 0 (265)	NA	0 ± 0 (185)	0 ± 0 (246), **L1-arrest**
fepD	100 ± 0 (259)	NA	0 ± 0 (183)	0 ± 0 (255), **L1-arrest**
fepC	100 ± 0 (214)	NA	0 ± 0 (186)	0 ± 0 (247), **L1-arrest**
cyoD	9.8 ± 3.7 (221)*,†	83.2 ± 9 (236)	0 ± 0 (181)	64.6 ± 6.4 (244)
yafC	100 ± 0 (240)	NA	0 ± 0 (167)	60.6 ± 8 (256)
cyoB	0 ± 0 (233)†	59 ± 6.3 (233)	0 ± 0 (188)	66.6 ± 7.2 (247)
cyoA	0 ± 0 (256)†	69.4 ± 17.2 (227)	0 ± 0 (155)	77.1 ± 5 (235)
tap	83 ± 6.4 (251)*	98.2 ± 0.8 (238)	0 ± 0 (166)	78.3 ± 20 (226)
cpxR	0 ± 0 (249)†	69.2 ± 15.4 (233)	0 ± 0 (185)	80.7 ± 13.4 (235)
stfP	0 ± 0 (255)†	64 ± 8.4 (248)	0 ± 0 (149)	74.6 ± 10.9 (250)
mdoC	0 ± 0 (247)†	72.2 ± 11.7 (229)	0 ± 0 (156)	75.1 ± 9.1 (215)
ycfk	0 ± 0 (233)†	72.9 ± 17.4 (221)	0 ± 0 (181)	78.7 ± 7.7 (208)
yejL	100 ± 0 (260)	NA	0 ± 0 (209)	76.4 ± 12.3 (260)
yejK	100 ± 0 (210)	NA	0 ± 0 (182)	80.3 ± 6.4 (228)
allA	0 ± 0 (233)†	52.8 ± 14.1 (241)	0 ± 0 (172)	81.8 ± 4.6 (236)
ybjX	0 ± 0 (197)†	56.9 ± 15.4 (230)	0 ± 0 (178)	71.5 ± 14.8 (225)
yfaE	0 ± 0 (250)†	55.1 ± 13.2 (240)	0 ± 0 (184)	82.6 ± 3.8 (236)
ybaW	0 ± 0 (246)†	63.1 ± 9.5 (255)	0 ± 0 (192)	80.1 ± 8 (237)
ycfX	0 ± 0 (240)†	62.9 ± 8.9 (257)	0 ± 0 (192)	82.4 ± 10.3 (234)
ygbM	0 ± 0 (187)†	61.4 ± 12.8 (230)	0 ± 0 (200)	69 ± 5.5 (224)
ydaN	0 ± 0 (260)†	63.1 ± 7.9 (232)	0 ± 0 (194)	79.1 ± 1.6 (220)
ybiB	0 ± 0 (243)†	57.1 ± 15.5 (236)	0 ± 0 (199)	76.7 ± 5.9 (227)
mntR	100 ± 0 (264)	NA	0 ± 0 (135)	76.9 ± 9.8 (219)
yaaJ	100 ± 0 (228)	NA	0 ± 0 (159)	66.2 ± 16.4 (231)
yfaY	0 ± 0 (253)†	61.7 ± 15.6 (245)	0 ± 0 (188)	73 ± 18 (247)
yejB	0 ± 0 (228)†	60.2 ± 3.7 (251)	0 ± 0 (172)	66.4 ± 12.2 (233)
yfiB	0 ± 0 (234)†	59.8 ± 3.6 (250)	0 ± 0 (190)	75.1 ± 8.1 (233)
tar	100 ± 0 (242)	NA	0 ± 0 (179)	73.9 ± 4 (226)
yheT	0 ± 0 (252)†	64.6 ± 4.7 (234)	0 ± 0 (154)	77.7 ± 7.5 (233)
yaaU	100 ± 0 (257)	NA	0 ± 0 (196)	80.4 ± 7.1 (251)
aroH	100 ± 0 (231)	NA	0 ± 0 (172)	76.8 ± 4 (235)
aes	0 ± 0 (232)†	63.9 ± 5.3 (239)	0 ± 0 (181)	76.3 ± 2.1 (234)
cyaA	100 ± 0 (257)	NA	0 ± 0 (165)	77.6 ± 2 (223)
hlyE	100 ± 0 (227)	NA	0 ± 0 (159)	75.1 ± 3.4 (237)
yceF	0 ± 0 (238)†	72.47 ± 3.1 (236)	0 ± 0 (193)	78.2 ± 2 (252)
wbbI	0 ± 0 (236)†	63.5 ± 8.8 (239)	0 ± 0 (183)	69.7 ± 12 (253)
ssuE	100 ± 0 (244)	NA	0 ± 0 (195)	77 ± 6.5 (232)
cspH	100 ± 0 (270)	NA	0 ± 0 (163)	71 ± 6.6 (230)
yaiF	100 ± 0 (251)	NA	0 ± 0 (198)	75.5 ± 6.4 (236)
rhsD	100 ± 0 (255)	NA	0 ± 0 (169)	82.6 ± 0.65 (236)
rhsB	100 ± 0 (265)	NA	0 ± 0 (182)	80.4 ± 5.2 (244)
ydcC	0 ± 0 (240)†	67.7 ± 15.7 (239)	0 ± 0 (183)	67.7 ± 13.2 (246)
cspD	100 ± 0 (215)	NA	0 ± 0 (137)	77.8 ± 7.4 (229)
eutD	100 ± 0 (256)	NA	0 ± 0 (165)	78.4 ± 4.6 (242)

Synchronized L1 wild-type animals were inoculated onto NGM media plates seeded with individual Keio *E. coli* mutant strains and incubated at 20 °C. The number of adult animals and the total number of animals were counted on day 3 and day 4 of feeding wild-type animals on Keio *E. coli* mutant strains. Data from 3 independent trials were collected; the average of percentage of adults and the SD are shown. The total number of animals counted in all 3 independent trials is shown in parentheses. Boldface indicates L1-arrest phenotype was observed.

*Compared to wild-type animals fed on BW25113 on day 3, unpaired *t* test, $P < 0.0001$.

†Since there were no adults or only few adults on these plates, the plates were scored on day 4 for the presence of adults. While there were no adults on day3, many of the wells had animals that reached adulthood on day 4. NA, not applicable since all of the animals were adults on day 3 itself. For columns 4 and 5, synchronized L1 *spg-7(ad2249)* mutant were inoculated onto NGM media plates seeded with individual Keio *E. coli* mutant strains and incubated at 20 °C. The number of adult animals and the total number of animals were counted on days 4 and 5 of feeding *spg-7(ad2249* animals on Keio *E. coli* mutant strains. Data from 3 independent trials were collected; the average of percentage of adults and the SD are shown. The total number of animals counted in all 3 independent trials is shown in parentheses.

GENETICS

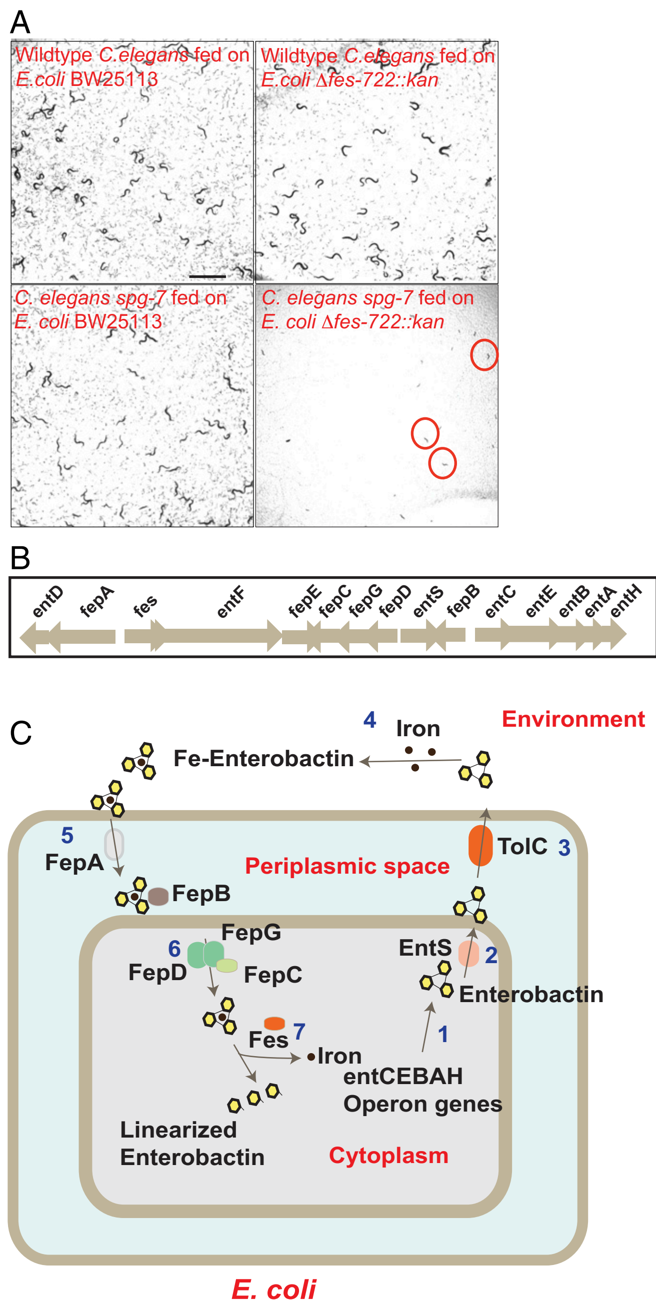


Fig. 1. *E. coli* mutations that affect enterobactin siderophore utilization or import are synthetic lethal with *C. elegans* mitochondrial mutations. (*A*) *spg-7(ad2249)* animals grown on *Δfes::kan E. coli* arrest as L1 larvae. (Scale bar, 1,000 μm.) (*B*) Operon structure of genes in the enterobactin pathway. (*C*) Diagrammatic representation of enterobactin biosynthetic pathway in *E. coli*.

bacterial ubiquinone that is identical except for a shorter isoprenoid chain that localizes this electron carrier to the membrane (26).

Not all *C. elegans* mitochondrial mutations were synthetic lethal with the *E. coli* enterobactin iron release mutations: The *C. elegans* mitochondrial mutants *gas-1(fc21)*, *nduf-2.2(ok2397)*, *nuo-6(qm200)*, *mev-1(kn1)*, and *ucr-2.3(pk732)* did not arrest when grown, for example, on the *E. coli Δfes::kan* mutant (Table 2 and *SI Appendix*, Fig. S5). Because *spg-7*, *nduf-7*, *isp-1*, and *clk-1* mediate distinct steps in mitochondrial respiratory pathways, the arrest induced by feeding mitochondrial mutants on *E. coli Δfes::kan* mutant is likely due to a toxic interaction between the mitochondrial homeostasis induction (one aspect of which is *hsp-6* expression) induced by the *spg-7*, *nduf-7*, *isp-1*, and *clk-1* mutations [but not by the *gas-1(fc21)*, *nduf-2.2(ok2397)*, *nuo-6(qm200)*, *mev-1(kn1)*, and *ucr-2.3(pk732)* mutations] and the iron toxicity caused by *E. coli* enterobactin mutants (Table 3).

To determine whether other *E. coli* genes required for enterobactin production cause the dramatic L1-synthetic larval arrest in the *spg-7 C. elegans* mitochondrial mutant, we tested each of the *E. coli* enterobactin pathway gene mutants on the *C. elegans spg-7* mutant. Feeding *C. elegans spg-7(ad2249)* mutants on *ΔentC::kan*, *ΔentD::kan*, *ΔentE::kan*, *ΔentB::kan*, *ΔentA::kan*, and *ΔentH::kan*, which are defective in the production of enterobactin, does not cause L1-larval arrest in *spg-7(ad2249)* (Table 3). This suggests that it is not lack of enterobactin or *E. coli* iron starvation that causes developmental arrest in the *C. elegans spg-7* mitochondrial mutant, but that instead it is the accumulation of iron-chelated enterobactin in the cytoplasm or periplasm of *E. coli* or iron-chelated enterobactin that is transported from *E. coli* to *C. elegans* cells with electron transport mutations that causes arrest of the *C. elegans* mitochondrial mutant. Such a model explains why it is the *E. coli* mutants that deliver ferric enterobactin, but not *E. coli* mutants that make no enterobactin that are toxic for animals with the *spg-7*, *nduf-7*, *isp-1*, and *clk-1* mitochondrial dysfunctions. Inactivation of *fes* causes accumulation of the unhydrolyzed ferric enterobactin in the *E. coli* cytoplasm (20, 21), whereas inactivation in FepDGC causes accumulation of the ferric enterobactin in the *E. coli* periplasm (14, 15), which is inaccessible for cytoplasmic Fes to act upon (*SI Appendix*, Fig. S3 *A* and *B*). As *C. elegans* consumes the Fes or Fep mutant *E. coli*, the ferric enterobactin may be unaltered by *C. elegans* esterases, which may not recognize this nearly covalent iron chelation. This ferric enterobactin may be transported into *C. elegans* cells, where if mitochondria are defective, perhaps producing ROS (*Discussion*), this ferric enterobactin is now toxic.

fes is in an *E. coli* operon with *ybdZ* and *entF*, which are also involved in enterobactin biosynthesis. Because bacterial insertion drug-resistant cassette mutations in one gene in the operon are often polar on downstream genes, we excised the Keio collection antibiotic resistance cassette insertion in *Δfes::kan* to create an in-frame nonpolar deletion allele of the single *fes* gene in the operon, which we call *Δfes*. Feeding *spg-7(ad2249)* mutant animals on *Δfes E. coli* also caused developmental arrest, suggesting that that the arrest phenotype is due to the absence of Fes protein, not polar effects on downstream genes (Fig. 2 *A* and *B*). To test whether the developmental arrest of the *C. elegans* mitochondrial mutants is due to ferric enterobactin, we inactivated enterobactin biosynthesis in the *Δfes* mutant background by constructing *Δfes entA::kan* and *Δfes entB::kan* double mutants. While 100% of *C. elegans spg-7(ad2249)* mutants arrest when grown on *Δfes* mutant *E. coli*, 0% of *spg-7(ad2249)* mutants arrest when grown the *Δfes entA::kan* or *Δfes entB::kan* double-mutant *E. coli* that does not generate enterobactin and therefore does not generate ferric enterobactin (*SI Appendix*, Fig. S3 *A* and *B* and Fig. 2 *A* and *B*). Furthermore, to test whether ferric enterobactin is required within *E. coli* to produce the arrest in *spg-7(ad2249)* mutants, we constructed *Δfes cirA782::kan*, *Δfes fepA::kan*, and *Δfes fiu::kan* double mutants (Fig. 2*B*). FepA is an outer membrane protein that binds and transports ferric enterobactin into the periplasm of *E. coli* (27, 28). Fiu is an outer membrane protein that mediates uptake of dihydroxybenzoylserine, a breakdown product of enterobactin (29). Cir is another outer membrane protein that mediates uptake of ferric enterobactin and other breakdown products of enterobactin (29). Although FepA, Fiu, and CirA mediate import of ferric enterobactin into the bacterial periplasm, FepA is the major transporter necessary for the uptake of ferric enterobactin into bacterial periplasm. While 100% of *C. elegans spg-7(ad2249)* mutants arrest when grown on *Δfes E. coli*, *Δfes cirA::kan*, or *Δfes fiu::kan* double-mutant *E. coli*, 0% of *spg-7(ad2249)* mutants arrest in the *Δfes fepA::kan* double-mutant *E. coli* (Fig. 2*B*). This result suggests that

Table 2. Table showing list of the mitochondrial mutants tested to potential interaction with Δ*fes* mutant

Animal strain	Description	% Larval arrest (*N*)	
		Fed on BW25113	Fed on Δ*fes-722*
Wild type		0 ± 0 (130)	0 ± 0 (115)
fzo-1(tm1133)	Mitofusin/FZO1	0 ± 0 (80)	0 ± 0 (95)
drp-1(tm1108)	Dynamin-related protein 1 (DRP1)	0 ± 0 (77)	0 ± 0 (93)
nduf-7(et19)	**NADH dehydrogenase [ubiquinone] Fe-S protein 7 (NDUF7) of complex I mutated in Leigh syndrome**	**0.7 ± 1.2 (88)**	**99.2 ± 1.2 (147)**
gas-1(fc21)	NADH dehydrogenase [ubiquinone] Fe-S protein 2 (NDUF2) of complex I	0.5 ± 0.9 (101)	0 ± 0 (119)
nduf-2.2(ok2397)	NADH dehydrogenase [ubiquinone] Fe-S protein 2 (NDUF2) of complex I	0 ± 0 (100)	0 ± 0 (120)
nuo-6(qm200)	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4 (NDUFB4/B15) of complex I	0 ± 0 (98)	0 ± 0 (99)
mev-1(kn1)	Succinate dehydrogenase subunit (SDHC-1) of complex II	0 ± 0 (96)	0 ± 0 (81)
isp-1(qm150)	**Ubiquinol-cytochrome C reductase, Rieske Fe-S protein (UQCRFS1) of complex III**	**1.2 ± 1.1 (92)**	**100 ± 0 (166)**
ucr-2.3(pk732)	Ubiquinol-cytochrome C reductase core protein 2 (UQCRC2) of complex III.	0 ± 0 (99)	0 ± 0 (95)
clk-1(qm30)	**COQ7; essential for ubiquinone biosynthesis**	**0 ± 0 (94)**	**100 ± 0 (128)**

Data from 3 independent trials were collected; the average of percentage of L1 animals and the SD are shown. The total number of animals counted in all of the 3 independent trials is shown in parentheses. Boldface indicates strains that arrested as L1-larve when fed on *E. coli* Δ*fes* mutant.

blocking uptake of ferric enterobactin from the environment into the *E. coli* can suppress the L1-larval arrest of *C. elegans* mitochondrial mutants grown on Δ*fes E. coli* mutant. Mixing experiments with Δ*fes E. coli* and wild-type *E. coli* showed that even nongrowing Δ*fes E. coli* mutant could supply the enterobactin–iron for the toxic interaction (*SI Appendix*, Tables S2 and S3).

Because the *E. coli* enterobactin uptake and iron retrieval mutants activate a mitochondrial unfolded protein response even in a wild-type *C. elegans* that grows at the normal rate (Table 1), we assessed the effect of these *E. coli* enterobactin–iron release mutants on *C. elegans* mitochondrial morphology. The mitochondria in the hypodermis of wild-type animals grown on wild-type *E. coli* BW25113 have tubular or slightly elongated morphology, but the mitochondria of wild-type animals grown on Δ*fes E. coli* are fragmented as assessed by nonyl acridine orange (NAO), a membrane potential-independent mitochondrial dye (Fig. 3*A*). We also used a transgenic strain that expresses mitochondrial outer membrane protein TOMM-20 tagged with mRFP in the body wall muscles of *C. elegans*. The mitochondrial morphology is tubular and elongated in animals grown on wild-type *E. coli* BW25113, whereas wild-type *C. elegans* grown on Δ*fes E. coli* show a more fragmented mitochondria in the body wall muscle (Fig. 3*B*). Consistent with the abnormal mitochondrial morphology, ATP levels are significantly reduced in wild-type *C. elegans* grown on Δ*fes E. coli* compared to those grown on wild-type *E. coli* BW25113 (Fig. 3 *C* and *D*). These data show that the induction of the *hsp-6* in wild-type *C. elegans* grown on the *E. coli* iron–siderophore retrieval mutants accurately reports their moderate mitochondrial defects.

Mutations in *isp-1*, *nduf-7*, and *spg-7* cause increased ROS production by the mitochondria (12, 24, 30). We found that *spg-7(ad2249)* animals that were grown on Δ*fes E. coli* mutant, but mixed with antioxidants such as ascorbic acid, *N*-acetyl cysteine (NAC), resveratrol, or trolox were completely suppressed for the arrest normally caused by *E. coli* Δ*fes* mutant fed to *C. elegans spg-7* (Fig. 3*E*). The fact that several other antioxidants were able to suppress arrest suggests that ROS is the likely cause of the toxic interaction between bacterial mutants that accumulate ferric enterobactin and the *C. elegans* mitochondrial mutants. Elevated ROS in *isp-1(qm150)* contributes to its increased longevity because the longevity increase is suppressed by NAC (30). UPR^{mt} activation in the *nduf-7(et19)* is also suppressed by NAC (24). Consistent with this observation, we found that the L1-larval arrest induced by feeding Δ*fes E. coli* to the *nduf-7(et19)* mutant is suppressible by NAC (*SI Appendix*, Table S1). When we fed *C.*

Table 3. Feeding ferric enterobactin induces developmental arrest in *C. elegans* mitochondrial mutants

Fed on	Description	% Larval arrest (*N*)	
		Wild type	*spg-7(ad2249)*
BW25113	Wild type	0 ± 0 (179)	0 ± 0 (219)
Δ*fepC726::kan*	Ferric enterobactin transport; ATP-binding protein fepC	0 ± 0 (132)	100 ± 0 (178)
Δ*fepG727::kan*	Ferric enterobactin transport system permease protein FepG	0 ± 0 (195)	100 ± 0 (196)
Δ*fepD728::kan*	Ferric enterobactin transport system permease protein FepD	0 ± 0 (198)	100 ± 0 (181)
Δ*fepB730::kan*	Ferrienterobactin-binding periplasmic protein	0 ± 0 (231)	0 ± 0 (174)
Δ*fepA721::kan*	Ferrienterobactin receptor	0 ± 0 (159)	0 ± 0 (188)
Δ*fiu-777::kan*	Catecholate siderophore receptor Fiu	0 ± 0 (188)	0 ± 0 (164)
Δ*cirA782::kan*	Colicin I receptor; postulated to participate in iron transport	0 ± 0 (201)	0 ± 0 (190)
Δ*fes-722::kan*	Enterochelin esterase	0 ± 0 (151)	100 ± 0 (205)
Δ*entF724::kan*	Enterobactin synthase component F	0 ± 0 (142)	0 ± 0 (165)
Δ*entC731::kan*	Isochorismate synthase EntC	0 ± 0 (166)	0 ± 0 (179)
Δ*entE732::kan*	Enterobactin synthase component E	0 ± 0 (202)	0 ± 0 (173)
Δ*entB733::kan*	Enterobactin synthase component B	0 ± 0 (191)	0 ± 0 (172)
Δ*entA734::kan*	2,3-Dihydro-2,3-dihydroxybenzoate dehydrogenase	0 ± 0 (183)	0 ± 0 (162)

Synchronized L1 wild type or *spg-7(ad2249)* mutant was inoculated onto NGM media plates seeded with individual Keio *E. coli* mutant strains and incubated at 20 °C. The number of L1-arrest animals and the total number of animals were counted on day 3.

A

C. elegans spg-7 fed on E. coli BW25113 | C. elegans spg-7 fed on E. coli Δfes-722 | C. elegans spg-7 fed on E. coli Δfes-722 ΔentA734

B

	% Larval arrest (N)
Fed on *E. coli*	*C. elegans spg-7*
BW25113	0±0(142)
Δfes	100±0(140)
Δfes ΔentB::kan	0±0(144)
Δfes ΔentA::kan	0±0(153)
Δfes ΔcirA::kan	100±0(134)
Δfes Δfiu::kan	100±0(136)
Δfes ΔfepA::kan	0±0(146)

Fig. 2. Enterobactin is necessary for the developmental arrest of *C. elegans* mitochondrial mutants. (*A*) *spg-7(ad2249)* animals grown on wild-type *E. coli* BW25113 develop normally, while *spg-7(ad2249)* mutant grown in *Δfes* mutant arrest as L1 larvae. In contrast, *spg-7(ad2249)* animals grown on *Δfes ΔentA::kan* double mutants that do not synthesize enterobactin develop normally. (Scale bar, 1,000 μm.) (*B*) Table showing suppression of larval arrest in animals grown on *Δfes ΔentA::kan*, *Δfes ΔentB::kan*, and *Δfes ΔfepA::kan* mutants. Data from 3 independent trials were collected; the average of percentage of adults and the SD are shown. The total number of animals counted in all 3 independent trials is shown in parentheses.

elegans spg-7(ad2249) animals the *E. coli Δfes* pregrown in the presence of transresveratrol and washed off the transresveratrol, the animals arrest, suggesting that the site of action of the antioxidants is likely within the animals (*SI Appendix*, Table S4).

Because hydrogen peroxide is one of the major forms of endogenous ROS, we monitored in vivo hydrogen peroxide levels of the interaction between the *E. coli Δfes* mutations and *C. elegans*, using HyPer, a peroxide-specific sensor protein. Transgenic wild-type animals that express the HyPer sensor (*jrIs1*) (31) were grown either on *E. coli* BW25113 or *E. coli Δfes* and peroxide levels were measured. Endogenous peroxide levels were not significantly different between wild-type *C. elegans* grown *E. coli* BW25113 or *E. coli Δfes*; however, addition of exogenous peroxide results in significantly higher peroxide levels in animals grown on *E. coli Δfes* compared to animals grown on *E. coli* BW25113 (*SI Appendix*, Fig. S6). We hypothesize that the exogenous peroxide interacts with ferric enterobactin and via the Fenton reaction to generate more peroxide.

In the evolutionary "tug-of-war" for iron, animals rely on the enterobactin-binding protein Lipocalin-2 to counteract iron loss to microbes that secrete siderophores (32). Secreted lipocalin 2 sequesters the ferric enterobactin to limit bacterial growth (32). We tested whether exogenous lipocalin-2 can suppress the arrest of *spg-7(ad2249)* grown on the *E. coli Δfes* mutant. We found that *spg-7(ad2249)* animals that were grown on the *E. coli Δfes* in the presence of 0.4 μM recombinant mouse lipocalin-2 no longer arrest as L1-larvae (Fig. 3*F*).

Discussion

By screening a comprehensive collection of *E. coli* mutations, we identified 45 *E. coli* mutations that activate the *C. elegans hsp-6* mitochondrial stress response gene. Surprisingly, 4 of these *E. coli* mutations that affect the retrieval of iron from the enterobactin siderophore are synthetic lethal in combination with multiple *C. elegans* mitochondrial mutants. These *E. coli* siderophore iron retrieval mutations also caused a mild disruption of mitochondrial morphology in wild type and the induction of *C. elegans hsp-6* (Fig. 3 *A* and *B*). However, disruption of the production of *E. coli* enterobactin did not cause this synthetic lethal interaction with a *C. elegans* mitochondrial mutants and could even suppress the synthetic lethality of *E. coli* mutants defective for enterobactin siderophore utilization or import into the cytoplasm, suggesting that the production of enterobactin and binding to iron is required for the toxic interaction between the iron-loaded *E. coli* siderophore and *C. elegans* mitochondrial mutants. The bacterial literature on ferric enterobactin supports this model: fes mutants accumulate ∼50 times more intracellular ferric enterobactin than wild type (18), a fes mutant grows slowly on iron-limited media with and without addition of purified enterobactin (33), and an *E. coli* K-12 *Δfes* mutant has a growth deficit when grown on iron-rich medium and this defect was suppressed by a deletion in enterobactin synthesis gene entB (34).

We found that most but not all of the mitochondrial mutations we tested were synthetic lethal with the enterobactin iron retrieval mutants. It may be important to the genetic interaction with the *E. coli* siderophore iron release mutations whether the ROS is generated in complex I or complex III, and how accessible it might be to the iron-loaded enterobactin. The detailed cryo-EM structure of the entire animal mitochondrial ETC points to a highly intricate system of 8 proteins bearing iron sulfur cluster redox centers that pass electrons with quantum mechanical precision, and efficiently couple to movements of transmembrane segments of other coupled complex I proteins (there are 14 core proteins in complex I) to pump protons against a proton gradient (35). The many FeS clusters that mediate the movement of electrons along the many protein subunits of complex I are the likely source of ROS in the complex I mutants. Interestingly, from the set of ETC mutants that are synthetic lethal with the 4 *E. coli* siderophore iron retrieval mutants, *nduf-7* encodes an ortholog of human NDUFS7 (NADH:ubiquinone oxidoreductase core subunit S7), which has a 4Fe4S cluster. The complex III ETC mutation *isp-1* also shows a strong synergistic interaction with the *E. coli* siderophore mutations. ISP-1 is an ortholog of human UQCRFS1 (ubiquinol-cytochrome *c* reductase, Rieske iron-sulfur polypeptide 1) and has 2 iron, 2 sulfur clusters and ubiquinol-cytochrome-*c* reductase activity. Again, this is an iron sulfur center mutant protein that interacts with ubiquinone that shows such a strong synergy.

The *C. elegans spg-7* point mutation causes a mitochondrial defect and is strongly synthetically lethal with the *E. coli* siderophore Fe retrieval mutations. *spg-7* does not encode an ETC component but encodes a protease that is the ortholog of the *E. coli* FtsH protease. This protease has a small number of client proteins, which includes the iron sulfur assembly factor iscS and the iron sulfur protein lpxC and fdoH (36). *C. elegans* mitochondrial proteins from the nuclear genome that are probable clients of SPG-7 are likely to be orthologs of the *E. coli* clients of ftsH: The *C. elegans* iron sulfur assembly factor *iscu-1* is a prime candidate for causing the iron sulfur protein defect in the ETC (36).

Similarly, the *clk-1*/mammalian coenzyme Q7 mitochondrial 5-demethoxyubiquinone hydroxylase that is synthetic lethal with the *E. coli* siderophore iron retrieval mutants fails to synthesize ubiquinone with its normally 10 isoprene unit hydrophobic tail in *C. elegans*. This null allele is not lethal to *C. elegans* because *E. coli* ubiquinone is imported into *C. elegans* cells. However, the *E. coli* ubiquinone, while competent for viability, has a shorter bacterial isoprene tail and causes slow growth of *C. elegans*, probably due to inefficient transfer of electrons from complex I to *E. coli* ubiquinone or from *E. coli* ubiquinone to complex III. The most likely explanation for the synergistic lethality of the *C. elegans clk-1*

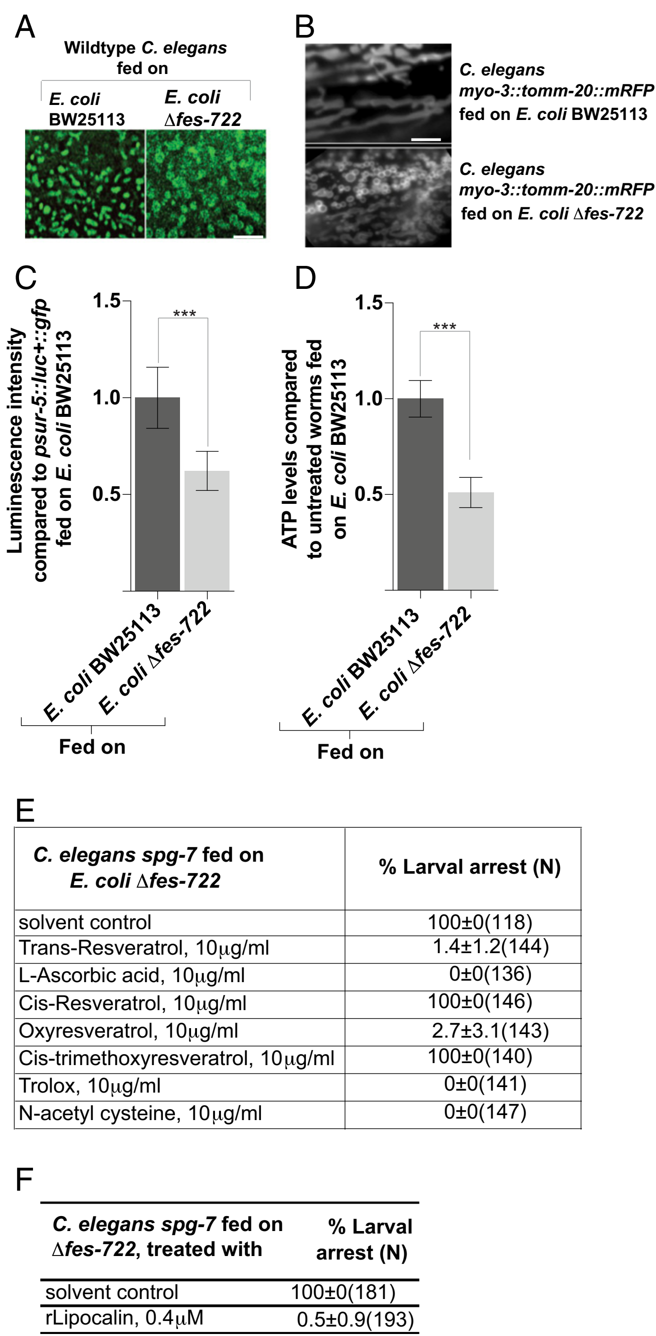
mutation with the *E. coli* siderophore mutations is that the efficiency of *E. coli* ubiquinone interaction with complex I or complex III is compromised so that ROS to synergize with the iron loaded siderophore is generated (37).

Of the ETC subunits that do not interact with the *E. coli* siderophore mutations includes *gas-1* and *mev-1*. *gas-1* is orthologous to human NDUFS2 (NADH:ubiquinone oxidoreductase core subunit S2) and does not have an iron sulfur cluster. *mev-1* is an ortholog of human SDHC (succinate dehydrogenase complex subunit C) and is a heme protein. *mev-1* is a component of complex II, which does not participate in the NADH to oxygen redox/proton pumping cascade and would not be expected to generate ROS under normal redox conditions.

If the enterobactin iron retrieval *E. coli* mutants and the *C. elegans* mitochondrial electron transport mutations were simply synthetic lethal, we could not generate a molecular model for the interaction. However, the synthetic lethality was strongly suppressed by addition of free radical scavengers, pointing to a reactive oxygen-based toxicity (*SI Appendix*, Tables S1 and S4). We propose that ROS production at particular sites in the ETC is the key to the genetic interaction with *E. coli* siderophore mutations. Such localized production of ROS may not be detectable when measuring ROS levels in extracts; but the localized ROS within complex I or III could explain the inviability. Because endogenous ROS production is minimal in wild-type *C. elegans*, whereas mitochondrial mutants have increased ROS production, we propose that the triggering ROS in the *C. elegans* mitochondrial mutant animals when combined with ferric-chelated enterobactin induces a much larger ROS burst via the Fenton reaction to cause toxicity to *C. elegans*. In wild-type *C. elegans*, there may not be sufficient ROS to initiate the Fenton reaction. Nevertheless, the ferric chelated enterobactin that enters wild-type *C. elegans* does activate the mitochondrial stress response (Dataset S1 and *SI Appendix*, Fig. S2).

There is biochemical support for an interaction between *E. coli* enterobactin and *C. elegans* mitochondrial proteins: An enterobactin-biotin fusion binds to the *C. elegans* ATP synthase F1 α-subunit ATP-1 that is the ortholog of bacterial atpA (38). A 21-amino acid peptide of the ATP-1 protein binds enterobactin (38) and is highly conserved in the bacterial kingdom as well as in eukaryotes (*SI Appendix*, Fig. S7). This region of ATP synthase F1 α is cytoplasmic but could interact with the FeS clusters of the ETC, which we have found to genetically interact with *E. coli* enterobactin iron retrieval mutants.

This interaction between a bacterial siderophore iron retrieval mutations and *C. elegans* ETC components that we have discovered and the biochemical interaction between this siderophore and ATP synthase (38) may be grounded in the complex ecology of bacterial siderophores. Siderophores allow bacteria to retrieve the vanishingly small concentrations of soluble Fe(III) in the environment, including in cells (22). While many bacterial species synthesize siderophores, unrelated bacteria hijack the iron-bound siderophores from those siderophore-synthesizing species to usurp their iron, a limiting essential micronutrient. However, some bacterial species with siderophore biosynthetic pathways have evolved siderophore biosynthetic modifications to append antibiotics to the siderophore, inhibiting the growth of these siderophore-iron thieves (22). In fact, enterobactin has been engineered to carry antibiotics as a sort of Trojan horse for antibiotic delivery (23). Our data show that iron-loaded enterobactin itself is a weapon against a *C. elegans* mitochondrial mutant; this genetic interaction may be based on an evolved toxic function for iron-loaded enterobactin and other bacterial species. If *E. coli* enterobactin–Fe is retrieved by diverse bacteria, it may interact with a diversity of electron



C. elegans spg-7 fed on *E. coli Δfes-722*	% Larval arrest (N)
solvent control	100±0(118)
Trans-Resveratrol, 10μg/ml	1.4±1.2(144)
L-Ascorbic acid, 10μg/ml	0±0(136)
Cis-Resveratrol, 10μg/ml	100±0(146)
Oxyresveratrol, 10μg/ml	2.7±3.1(143)
Cis-trimethoxyresveratrol, 10μg/ml	100±0(140)
Trolox, 10μg/ml	0±0(141)
N-acetyl cysteine, 10μg/ml	0±0(147)

C. elegans spg-7 fed on *Δfes-722*, treated with	% Larval arrest (N)
solvent control	100±0(181)
rLipocalin, 0.4μM	0.5±0.9(193)

Fig. 3. Mitochondrial structure and function are disrupted in wild-type animals grown on Δ*fes* mutant *E. coli*. (*A*) Wild-type animals grown on Δ*fes* mutant *E. coli* display fragmented hypodermal mitochondrial morphology as assessed using NAO. (Scale bar, 100 μm.) (*B*) Transgenic animals expressing TOMM-20::mRFP in the muscles grown on Δ*fes* mutant *E. coli* display a fragmented mitochondrial morphology. (Scale bar, 100 μm.) (*C*) Animals grown on Δ*fes* mutant *E. coli* have decreased luciferase luminescence, a proxy for ATP levels, compared to animals grown on *E. coli* BW25113 as assessed using a strain that expresses luciferase in all tissues. Unpaired *t* test; $***P < 0.001$. Mean ± SD of $n = 10$. (*D*) ATP levels in animals grown on Δ*fes* mutant *E. coli* display are decreased compared to animals grown on *E. coli* BW25113. Unpaired *t* test; $***P < 0.001$. Mean ± SD of $n = 3$. (*E*) Antioxidant treatments with L-ascorbic acid, NAC, or transresveratrol suppress the L1-larval arrest induced by feeding Δ*fes* mutants to *C. elegans* mitochondrial mutants. *cis*-Resveratrol and *cis*-trimethoxyresveratrol fails to suppress the arrest. Data from 3 independent trials were collected; the average of percentage of adults and the SD are shown. The total number of animals counted in all 3 independent trials is shown in parentheses. (*F*) Mouse lipocalin addition suppresses the larval arrest of *spg-7(ad2249)* animals grown on Δ*fes* mutants. Data from 3 independent trials were collected; the average of percentage of adults and the SD are shown. The total number of animals counted in all 3 independent trials is shown in parentheses.

GENETICS

transport oxidants and reductants that produce ROS, or the usurping bacteria may not encode the proper enzymes (such as Fes) to remove the Fe so strongly bound to multiple catecholamines of enterobactin; this toxic role of enterobactin–Fe that we discerned in a *C. elegans* mitochondrial mutant screen may be a highly evolved siderophore role to target the diverse components of bacterial and eukaryotic mitochondrial ETCs that compete with *E. coli*.

Materials and Methods

C. elegans culture, *E. coli* cultures, staining of mitochondria, microscopy, and imaging were performed as described previously (4). Removal of kanamycin cassette was performed as described previously (6). ATP measurements were performed as described previously (31). Statistical analysis was performed with GraphPad Prism 7 software. Student's *t* test was used for analysis. Details of the genome-wide screening protocol is described in *SI Appendix, Supplemental Materials and Methods*. Details of materials and methods used are available in *SI Appendix*. Details about *E. coli* and *C. elegans* strains used are also available in *SI Appendix*.

ACKNOWLEDGMENTS. We thank members of the G.R. laboratory for helpful discussions. The work was supported by NIH Grant AG043184 (to G.R.). Some strains were provided by the *Caenorhabditis* Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

1. B. S. Samuel, H. Rowedder, C. Braendle, M.-A. Félix, G. Ruvkun, *Caenorhabditis elegans* responses to bacteria from its natural habitats. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E3941–E3949 (2016).
2. M.-A. Félix, C. Braendle, The natural history of *Caenorhabditis elegans*. *Curr. Biol.* **20**, R965–R969 (2010).
3. J. Sinclair, I. Hamza, Lessons from bloodless worms: Heme homeostasis in *C. elegans*. *Biometals* **28**, 481–489 (2015).
4. J. A. Govindan, E. Jayamani, X. Zhang, E. Mylonakis, G. Ruvkun, Dialogue between *E. coli* free radical pathways and the mitochondria of *C. elegans*. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 12456–12461 (2015).
5. M. A. Fischbach, H. Lin, D. R. Liu, C. T. Walsh, How pathogenic bacteria evade mammalian sabotage in the battle for iron. *Nat. Chem. Biol.* **2**, 132–138 (2006).
6. T. Baba *et al*., Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol. Syst. Biol.* **2**, 0008 (2006).
7. T. Yoneda *et al*., Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. *J. Cell Sci.* **117**, 4055–4066 (2004).
8. W. J. Kapulkin, B. G. Hiester, C. D. Link, Compensatory regulation among ER chaperones in *C. elegans*. *FEBS Lett.* **579**, 3063–3068 (2005). Correction in: *FEBS Lett.* **581**, 5952 (2007).
9. J. A. Govindan *et al*., Lipid signalling couples translational surveillance to systemic detoxification in *Caenorhabditis elegans*. *Nat. Cell Biol.* **17**, 1294–1303 (2015).
10. S. L. Rea, N. Ventura, T. E. Johnson, Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in *Caenorhabditis elegans*. *PLoS Biol.* **5**, e259 (2007).
11. A. M. van der Bliek, M. M. Sedensky, P. G. Morgan, Cell biology of the mitochondrion. *Genetics* **207**, 843–871 (2017).
12. I. O. Zubovych, S. Straud, M. G. Roth, Mitochondrial dysfunction confers resistance to multiple drugs in *Caenorhabditis elegans*. *Mol. Biol. Cell* **21**, 956–969 (2010).
13. K. N. Raymond, E. A. Dertz, S. S. Kim, Enterobactin: An archetype for microbial iron transport. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 3584–3588 (2003).
14. J. R. Pierce, C. F. Earhart, *Escherichia coli* K-12 envelope proteins specifically required for ferrienterobactin uptake. *J. Bacteriol.* **166**, 930–936 (1986).
15. C. M. Shea, M. A. McIntosh, Nucleotide sequence and genetic organization of the ferric enterobactin transport system: Homology to other periplasmic binding protein-dependent systems in *Escherichia coli*. *Mol. Microbiol.* **5**, 1415–1428 (1991).
16. M. L. Guerinot, Microbial iron transport. *Annu. Rev. Microbiol.* **48**, 743–772 (1994).
17. I. G. O'Brien, G. B. Cox, F. Gibson, Enterochelin hydrolysis and iron metabolism in *Escherichia coli*. *Biochim. Biophys. Acta* **237**, 537–549 (1971).
18. L. Langman, I. G. Young, G. E. Frost, H. Rosenberg, F. Gibson, Enterochelin system of iron transport in *Escherichia coli*: Mutations affecting ferric-enterochelin esterase. *J. Bacteriol.* **112**, 1142–1149 (1972).
19. K. T. Greenwood, R. K. Luke, Enzymatic hydrolysis of enterochelin and its iron complex in *Escherichia coli* K-12. Properties of enterochelin esterase. *Biochim. Biophys. Acta* **525**, 209–218 (1978).
20. G. Winkelmann, A. Cansier, W. Beck, G. Jung, HPLC separation of enterobactin and linear 2,3-dihydroxybenzoylserine derivatives: A study on mutants of *Escherichia coli* defective in regulation (*fur*), esterase (*fes*) and transport (*fepA*). *Biometals* **7**, 149–154 (1994).
21. T. J. Brickman, M. A. McIntosh, Overexpression and purification of ferric enterobactin esterase from *Escherichia coli*. Demonstration of enzymatic hydrolysis of enterobactin and its iron complex. *J. Biol. Chem.* **267**, 12350–12355 (1992).
22. T. C. Johnstone, E. M. Nolan, Beyond iron: Non-classical biological functions of bacterial siderophores. *Dalton Trans.* **44**, 6320–6339 (2015).
23. T. Zheng, J. L. Bullock, E. M. Nolan, Siderophore-mediated cargo delivery to the cytoplasm of *Escherichia coli* and *Pseudomonas aeruginosa*: Syntheses of monofunctionalized enterobactin scaffolds and evaluation of enterobactin-cargo conjugate uptake. *J. Am. Chem. Soc.* **134**, 18388–18400 (2012).
24. M. Rauthan, P. Ranji, R. Abukar, M. Pilon, A mutation in *Caenorhabditis elegans* NDUF-7 activates the mitochondrial stress response and prolongs lifespan via ROS and CED-4. *G3 (Bethesda)* **5**, 1639–1648 (2015).
25. J. Feng, F. Bussière, S. Hekimi, Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. *Dev. Cell* **1**, 633–644 (2001).
26. S. Felkai *et al*., CLK-1 controls respiration, behavior and aging in the nematode *Caenorhabditis elegans*. *EMBO J.* **18**, 1783–1792 (1999).
27. E. H. Fiss, P. Stanley-Samuelson, J. B. Neilands, Properties and proteolysis of ferric enterobactin outer membrane receptor in *Escherichia coli* K12. *Biochemistry* **21**, 4517–4522 (1982).
28. W. C. Hollifield, Jr, J. B. Neilands, Ferric enterobactin transport system in *Escherichia coli* K-12. Extraction, assay, and specificity of the outer membrane receptor. *Biochemistry* **17**, 1922–1928 (1978).
29. K. Hantke, Dihydroxybenzoylserine—a siderophore for *E. coli*. *FEMS Microbiol. Lett.* **55**, 5–8 (1990).
30. W. Yang, S. Hekimi, A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol.* **8**, e1000556 (2010).
31. P. Back *et al*., Exploring real-time in vivo redox biology of developing and aging *Caenorhabditis elegans*. *Free Radic. Biol. Med.* **52**, 850–859 (2012).
32. T. H. Flo *et al*., Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. *Nature* **432**, 917–921 (2004).
33. M. Zhu, M. Valdebenito, G. Winkelmann, K. Hantke, Functions of the siderophore esterases IroD and IroE in iron-salmochelin utilization. *Microbiology* **151**, 2363–2372 (2005).
34. D. Vinella, C. Albrecht, M. Cashel, R. D'Ari, Iron limitation induces SpoT-dependent accumulation of ppGpp in *Escherichia coli*. *Mol. Microbiol.* **56**, 958–970 (2005).
35. L. A. Sazanov, A giant molecular proton pump: Structure and mechanism of respiratory complex I. *Nat. Rev. Mol. Cell Biol.* **16**, 375–388 (2015).
36. K. Westphal, S. Langklotz, N. Thomanek, F. Narberhaus, A trapping approach reveals novel substrates and physiological functions of the essential protease FtsH in *Escherichia coli*. *J. Biol. Chem.* **287**, 42962–42971 (2012).
37. T. Jonassen, P. L. Larsen, C. F. Clarke, A dietary source of coenzyme Q is essential for growth of long-lived *Caenorhabditis elegans clk-1* mutants. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 421–426 (2001).
38. B. Qi, M. Han, Microbial siderophore enterobactin promotes mitochondrial iron uptake and development of the host via interaction with ATP synthase. *Cell* **175**, 571–582.e11 (2018).