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Restoring *de novo* Coenzyme Q biosynthesis in *Caenorhabditis elegans coq-3* mutants yields profound rescue compared to exogenous Coenzyme Q supplementation

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Abstract

Coenzyme Q (ubiquinone or Q) is an essential lipid component of the mitochondrial electron transport chain. In *Caenorhabditis elegans* Q biosynthesis involves at least nine steps, including the hydroxylation of the hydroquinone ring by CLK-1 and two *O*-methylation steps mediated by COQ-3. We characterize two *C. elegans coq-3* deletion mutants, and show that while each has defects in Q synthesis, their phenotypes are distinct. First generation homozygous *coq-3(ok506)* mutants are fertile when fed the standard lab diet of Q-replete OP50 *E. coli*, but their second generation homozygous progeny do not reproduce. In contrast, the *coq-3(qm188)* deletion mutant remains sterile when fed Q-replete OP50. Quantitative PCR analyses suggest that the longer *qm188* deletion may alter expression of the flanking *nuo-3* and *gdi-1* genes, located 5′ and 3′, respectively of *coq-3* within an operon. We surmise that variable expression of *nuo-3*, a subunit of complex I, or of *gdi-1*, a guanine nucleotide dissociation inhibitor, may act in combination with defects in Q biosynthesis to produce a more severe phenotype. The phenotypes of both *coq-3* mutants are more drastic as compared to the *C. elegans clk-1* mutants. When fed OP50, *clk-1* mutants reproduce for many generations, but show reduced fertility, slow behaviors, and enhanced life span. The *coq-3* and *clk-1* mutants all show arrested development and are sterile when fed the Q-deficient *E. coli* strain GD1 (harboring a mutation in the *ubiG* gene). However, unlike *clk-1* mutant worms, neither *coq-3* mutant strain responded to dietary supplementation with purified exogenous Q_{10}. Here we show that the Q_9 content can be determined in lipid extracts from just 200 individual worms, enabling the determination of Q content in the *coq-3* mutants unable to reproduce. An extra-chromosomal array expressing wild-type *C. elegans coq-3* rescued fertility of both *coq-3* mutants and partially restored steady-state levels of COQ-3 polypeptide and Q_9

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content, indicating that primary defect in both is limited to *coq-3*. The limited response of the *coq-3* mutants to dietary supplementation with Q provides a powerful model to probe the effectiveness of exogenous Q supplementation as compared to restoration of *de novo* Q biosynthesis.

Keywords

dietary supplements; fertility; methyltransferase; mitochondria; operon; ubiquinone

1. Introduction

1.1 Coenzyme Q biosynthesis and function

Coenzyme Q (ubiquinone or Q)[1] is a lipid component of the mitochondrial electron transport chain. The redox activity of the benzoquinone ring allows Q to accept electrons and protons from complex I, or complex II, and ferry them to complex III. Q also functions as an electron acceptor in fatty acid beta-oxidation and in pyrimidine synthesis (Nowicka and Kruk, 2010). Additionally, the reduced or hydroquinone form of Q (QH_2) functions as a potent lipid soluble antioxidant in the plasma membrane and elsewhere (Turunen et al., 2004).

Q biosynthetic pathways described in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are good models for Q biosynthesis in animals (Tran and Clarke, 2007; Kawamukai, 2009). Figure 1 gives an overview of Q biosynthesis in eukaryotes. The polyisoprene tail produced by COQ-1 (or by Pdss1 and Pdss2 in humans) defines the number of isoprene units in the polyisoprene tail, with *S. cerevisiae* containing six (Q_6), *Escherichia coli* eight (Q_8), *Caenorhabditis elegans* nine (Q_9), and humans ten (Q_{10}) isoprene units. In *S. cerevisiae*, the redox active quinone head group is derived from either para-hydroxybenzoate (pHB) or from para-aminobenzoic acid (pABA) (Marbois et al., 2010; Pierrel et al., 2010). COQ-2, a polyprenyl-pHB transferase attaches the polyisoprenyl tail to either pHB or pABA. The polyisoprenylated ring is then subjected to various modifications, including decarboxylation, three hydroxylations, one *C*-methylation, two *O*-methylation steps, and in the case of Q biosynthesis from pABA, removal of the ring nitrogen substituent.

1.2 Q deficiencies and Q supplementation

Given the central role Q plays in metabolism, it is not surprising that disorders involving its biosynthesis lead to severe phenotypes and disorders. *E. coli* and yeast bearing mutations in the Q biosynthetic pathway are unable to respire or grow on non-fermentable carbon sources (Tran and Clarke, 2007). The complete lack of Q in mice resulting from knock-out mutations produces embryonic lethality (Levavasseur et al., 2001; Peng et al., 2008; Takahashi et al., 2008). Human patients with primary Q disorders present a variety of symptoms, including kidney disease, ataxia and cognitive delay (Quinzii and Hirano, 2010; Rahman et al., 2012).

Attempts to correct these Q deficiencies have met with mixed success. The response of Q-deficient patients to supplementation with Q_{10} is quite variable, ranging from little response to a dramatic recovery from symptoms; however, the patients fail to display full recovery

[1]**Abbreviations:** AdoMet, *S*-adenosyl-L-methionine; CGC, Caenorhabditis Genetics Center; DMQ_9, demethoxy-ubiquinone; HPLC-MS/MS, high-performance liquid chromatography tandem mass spectroscopy; LB, Luria-Bertani medium; Mit, phenotype resulting from decreased mitochondrial function; MRM, multiple reaction monitoring; pABA, *para*-aminobenzoic acid; pHB, *para*-hydroxybenzoic acid; Qn, coenzyme Q with *n* number of isoprene units in the tail; RQ_9, rhodoquinone-9; SMM, succinate minimal medium

(Quinzii and Hirano, 2010; Rahman et al., 2012). Mice suffering from nephritic syndrome due to mutation in the COQ-1 homologue PDSS2 show a limited improvement in proteinuria and renal physiology in response to dietary supplementation with Q_{10} (Saiki et al., 2008b).

1.3 C. elegans as a model for understanding the role of Q in development, behavior and aging and in probing the roles of dietary Q

The nematode *C. elegans* serves as a useful model for understanding the consequences of Q deficiency in a multi-cellular animal, and is ideal for the screening of pharmacological therapies. *C. elegans* produces large numbers of progeny, has a rapid life cycle, is genetically amenable, and responds to dietary manipulations (Brenner, 1974).

One of the best-studied long-lived Mit mutants (Mit refers to decreased mitochondrial function) is the Q-deficient *C. elegans clk-1(qm30)* mutant (Wong et al., 1995). The *clk-1* gene (an ortholog of yeast *COQ7*) encodes a carboxylate-bridged diiron hydroxylase required for production of the fully substituted benzoquinone ring of Q (Stenmark et al., 2001; Behan and Lippard, 2010). When fed the standard Q-replete diet of OP50 *E. coli*, the homozygous *C. elegans clk-1* mutant reproduces for many generations, but shows reduced fertility and slow behaviors, while living longer than wild-type N2 worms (Wong et al., 1995). Importantly, these phenotypes of the *clk-1* mutant depend on an exogenous source of Q; when fed a Q-less diet, such as the GD1 strain of *E. coli* harboring a disruption in the bacterial *ubiG O*-methyltransferase, the *clk-1* mutant fails to develop and is sterile (Jonassen et al., 2001). Feeding *clk-1* mutants the Q-less *E. coli* diet supplemented with exogenous Q_{10} restores fertility and development (Saiki et al., 2008a). *C. elegans clk-1* mutants lack the endogenously synthesized Q_9, and instead accumulate the intermediate demethoxy-ubiquinone (DMQ_9), produce small amounts of rhodoquinone-9 (RQ_9, an amino-prenylated quinone) and assimilate exogenously supplied Q_8 from their *E. coli* diet (Jonassen et al., 2001; Miyadera et al., 2001). Mitochondria isolated from the *clk-1* mutant show defects in complex I to III respiratory chain activity, while complex II to III activity was similar to wild-type mitochondria (Kayser et al., 2004). Recent studies indicate that DMQ_9 present in the *clk-1* mutant may scavenge ROS more effectively (Yang et al., 2009) and inhibit complex I from donating electrons to exogenously supplied Q (Yang et al., 2011). Despite the continued presence of DMQ_9, even small amounts of endogenously synthesized Q_9 (produced by genetic tRNA suppressors), may be sufficient to rescue many of the *clk-1* mutant phenotypes (Branicky et al., 2006). These results are consistent with the idea that the presence of DMQ_9 may contribute to the unique phenotype of the *clk-1* mutant (Hihi et al., 2002), since it has the mildest phenotype when compared to the other Q-deficient *C. elegans coq* mutants studied so far.

Other Q-deficient *C. elegans coq* mutants described to date have more severe phenotypes, and are much less responsive to dietary Q supplementation when compared to *clk-1* (Gavilan et al., 2005). First generation homozygous *C. elegans coq-8* mutants have decreased fertility even when fed the Q-replete *E. coli* OP50 diet, and their second-generation progeny lacking maternal-derived stores of Q are sterile (Asencio et al., 2009). First generation *C. elegans coq-1* mutants fed OP50 are sterile and short-lived (Gavilan et al., 2005). The *coq-3(qm188)* mutant fed OP50 does not develop reproductive organs and is sterile (Hihi et al., 2002). However, it is important to note that, despite attempts to rescue with extra-chromosomal arrays containing the wild-type gene, none of the *coq* mutants have been rescued. In the absence of genetic rescue, the presence of additional genetic defects accounting for the more severe phenotypes cannot be ruled out. Here we characterize another *coq-3* mutant and show that first generation homozygous *C. elegans coq-3(ok506)* mutants retain fertility; however, only a small fraction of the second-generation mutants survive to adulthood. Despite the phenotypic disparity in these two *coq-3* mutants, we show

that restoration of de novo Q biosynthesis with the *coq-3* gene present on an extra-chromosomal array affords dramatic rescue. This study illustrates the crucial role endogenously synthesized Q plays in fertility and development.

2. Materials and methods

2.1 Culture conditions, strains and genetic analysis

2.1.1 Culture of C. elegans and E. coli—*Caenorhabditis elegans* were maintained under standard conditions at 20°C unless otherwise indicated (Brenner, 1974). Plate media for nematode growth were prepared as previously described unless stated otherwise (Brenner, 1974). *C. elegans* were fed the *E. coli* strain OP50-1 carrying an integrated streptomycin resistance gene (CGC). OP50-1 was cultured in LB medium containing 250 μg/mL of streptomycin (final concentration) and cells harvested from overnight cultures (37 °C, with shaking at 250 rpm) were seeded onto regular NGM plates. Alternatively, nematodes were fed GD1 *E. coli*, a Q-less strain harboring an insertion in the *ubiG* gene (*ubiG::Kan*, *zei::Tn10dTet*) (Hsu et al., 1996). GD1 *E. coli* were grown overnight in LB medium with kanamycin at a final concentration of 100 μg/mL. GD1 *E. coli* were seeded onto standard NGM plates containing 100 μg/mL kanamycin. NGM plates containing OP50 or GD1 were used within two weeks after being seeded.

2.1.2 C. elegans strains, construction and back-crosses—*C. elegans* strains used in this study are listed in Table 1. Males of strain VC436, *coq-3(ok506)/nT1[qIs51]*, were backcrossed to wild-type N2 hermaphrodites. Progeny were screened for the *coq-3(ok506)* allele via PCR (primers: 5′ coq-3 ok506 and 3′ coq-3 ok506, see Supplemental Table 1). Resultant *coq-3(ok506)/+* heterozygotes were mated with strain JK2906 males, which carries the *+/nT1[qIs51]* balancer, and hermaphrodites with pharyngeal GFP were PCR screened for *coq-3(ok506)/+* heterozygosity (primers: 5′ coq-3 ok506 and 3′ coq-3 ok506). This out-crossing was repeated twice, for a total of three back-crosses, generating strain CFC315 (Table 1). All further analyses involving the *coq-3(ok506)* employ this back-crossed strain. Strain MQ992 *coq-3(qm188)/dpy-4* males were mated to JK2906 *mep-1(q660) I/nT1[qIs51]* hermaphrodites. *Coq-3(qm188)/+ nT1[qIs51]* heterozygous hermaphrodites were selected via PCR (primers: 5′coq-3 Xho-I and 3′ coq-3 Kpn-I, Supplemental Table 1), generating strain CFC1005 (Table 1). All further analyses involving the *coq-3(qm188)* allele employ this strain.

2.1.3 Molecular genetic analysis of C. elegans coq-3 mutants—One hundred N2, *coq-3(ok506)* mutant or *coq-3(qm188)* mutant worms were placed in a 1.5 mL polypropylene microcentrifuge tube containing 100 μL RNase-free water and stored frozen until RNA was extracted. The frozen worm suspension was mixed with 30 μL TRIzol (Invitrogen), ground with a micro-pestle, and an additional 170 μL TRIzol was added. The mixture was incubated at room temperature for 5 min, then 40 μL chloroform (Sigma) was added and the tube vortexed for 30 s, incubated at room temperature for 5 min and centrifuged for 20 min at 13,000 *g*. The top layer was placed in new 1.5 mL polypropylene microcentrifuge tube and precipitated with isopropanol. The resulting RNAs were subjected to reverse transcription with M-MLV reverse transcriptase (Invitrogen) and PCR amplified (primers: 5′ coq-3 XhoI and 3′ coq-3 KpnI, Supplemental Table 1). The cDNAs were sequenced (UCLA GenoSeq Facility, Los Angeles, CA) and each open reading frame analyzed to identify splicing of exons and to determine the predicted amino acid sequence (Supplemental Fig. 1).

2.2 Functional complementation of the GD1 E. coli ubiG mutant—A plasmid expressing *C. elegans coq-3* (pCH*coq3ce*) was generated by inserting the wild-type *C.*

elegans coq-3 amplified from a cDNA library (Invitrogen, CA) into the ClaI and KpnI sites of pCH1 (primers: 5′ coq-3 Cla-I and 3′ coq-3 Kpn-I, Supplemental Table 1). The pCH1 plasmid is derived from the yeast shuttle vector pRS426, with the *CYC1* promoter inserted at the EcoRI site, as previously described (Hsu et al., 1996). pBluescript (pBSK; Fermentas) was used as an empty vector control. Construction of plasmids pAHG harboring the wild-type *E. coli ubiG* gene, and pCHQ3 harboring the *S. cerevisiae* wild-type *COQ3* gene was described previously (Hsu et al., 1996). GD1 *E. coli* cells were transformed with the designated plasmids and strains were cultured at 30°C with shaking (250 rpm) overnight in LB, containing ampicillin (100 μg/mL final concentration). The optical density (OD_{600nm}) of each culture was adjusted to 1.0 with sterile water; a 3 μl aliquot of each cell suspension, along with 1:10 serial dilutions were spotted onto LB agar plate medium containing 100 μg/mL ampicillin. Duplicate samples were spotted onto succinate minimal plate medium. Succinate minimal medium (SMM) contains succinate as a sole non-fermentable carbon source. SMM plate medium was prepared as previously described (Poole et al., 1989).

2.3 Rescue of coq-3 mutants with extra-chromosomal arrays

The entire coding region of the *C. elegans coq-3* gene was amplified from a *C. elegans* cDNA library (Invitrogen) with primers carrying a KpnI restriction site at the 5′ end and a NheI restriction site at the 3′ end (primers: 5′ KpnI coq3ce and 3′ NheI coq-3ce, Supplemental Table 1). The PCR fragment was cloned into TOPO vector 2.1 (Invitrogen) and excised with NheI and KpnI restriction enzymes. This fragment was ligated into Fire Lab *C. elegans* vector p4759, which carries a *let-858* promoter and *let-858* 3′UTR, at the Nhe-I and Kpn-1 restriction sites (Addgene, MA). The resulting plasmid (p4759-Coq3ce) was injected as described (Portman, 2006), into *coq-3(ok506)/+ nT1(qIs51)* heterozygotes at 10 ng/μL, together with 30 ng/μL of the *rol-6(su1006)* co-injection marker and 110 ng/μL pBSK. Worms displaying the roll phenotype, but lacking the pharyngeal GFP marker, were designated *coq-3(ok506) −/−::Ex[coq-3]* (strain CFC614) or *coq-3(qm188) −/−::Ex[coq-3]* (strain CFC530) (Table 1), and selected for further analyses.

2.4 Determination of nematode brood size

N2 or heterozygous *coq-3* mutant gravid adult nematodes were allowed to lay eggs for one day on plates containing the designated *E. coli* diet. Individual N2 worms that developed to the L4 larval stage were transferred daily to fresh plates and the number of L1 larvae produced by each individual worm was recorded (Hodgkin and Barnes, 1991). Alternatively, first generation homozygous *coq-3* mutants were moved to individual plates with the designated *E. coli* diet when their heterozygous brood-mates reached the L4 larval stage. This method of timing for the transfer of the *coq-3* mutants was necessary because of their impaired development. The number of L1 larvae produced by each individual *coq-3* mutant worm was recorded. Student's *t* test analyses were performed to verify statistical difference between groups.

2.5 NovaSOL ® Q_{10} supplementation

NovaSOL® Q_{10} and NovaSOL vehicle control were provided by AQUANOVA AG (Darmstadt, Germany). N2, *coq-3(ok506)/nT1[qIs51]* and *clk-1(qm30) −/−* gravid adults were hypochlorite lysed and eggs transferred to NGM plates containing 100 μg/mL kanamycin and either 150 μg/mL NovaSOL ® Q_{10}, a water soluble micelle containing coenzyme Q_{10}, or a vehicle control micelle carrying medium chain triacylglycerides. The plates were prepared as previously described (Saiki et al., 2008a), and contained GD1 *E. coli*. N2 and *clk-1(qm30) −/−* L4 larvae were moved to individual plates with the designated diets. The *coq-2(ok1022) −/−* were selected based on size and development, and transferred to the designated diet two days post-hatching. The *coq-3(ok506) −/−* worms were selected

based on the absence of the pharyngeal GFP marker and placed on individual plates with the designated diet. Brood sizes were determined as described in section 2.4.

2.6 Morphological analyses and measurement of body size

N2 and *coq-3(ok506) −/−* gravid adults were dissolved as described and eggs were placed onto foodless NGM plates and allowed to synchronize to the L1 larval stage for 18 hours. L1 larvae were washed off plates with M9 onto NGM plates containing either OP50 or GD1 and allowed to grow for 94 hours to the second day of adulthood. Living worms were paralyzed in M9 containing 30 mM sodium azide and placed on slides containing fresh 2% agar pads. Images were taken at 40X with an ORCA ER CCB camera attached to an Axiovert 200M microscope. Body diameter measurements from the tip of the opening of the vulva directly to the outer body surface were performed with the Axiovert Software measurement tool.

2.7 Preparation of antisera to C. elegans COQ-3

Primary antibody against *C. elegans* COQ-3 protein was generated by cloning worm *coq-3* cDNA with primers containing NdeI and BamHI sites (primers: 5′ coq3ce NdeI and 3′ coq3ce BamHI, Supplemental Table 1) into the pET15b vector carrying a C-terminal His_6 tag, at the NdeI and BamHI restriction sites. The protein was expressed in BL21 cells via IPTG induction (1 mM IPTG, 3 h, at 30 °C, 250 rpm). The cells were resuspended in PBS (pH 7.4, 0.14 M NaCl, 2.7 mM KCl, 8.0 mM Na_2HPO_4, 1.5 mM KH_2PO_4) and lysed on ice with a sonicator (Fisher Sonic Dismembrator, Model 300) set at 60% maximal energy for three ten-second cycles in one minute. His-tagged COQ3 polypeptide was recovered from the lysate with Ni-NTA resin, washed two times with 8.0 M urea containing 20 mM imidazole, and eluted with 250 mM imidazole. The protein was subjected to preparative SDS-PAGE separation for further purification and stained with 0.3 M copper chloride ($CuCl_2$) for 5 min and washed in water for 3 min (Lee et al., 1987). The white band at the expected size was cut out of the gel and washed three times in wash solution (0.25 M EDTA, 0.25 M Tris, pH 9.0) with gentle agitation for 10 min per wash then two washes in PBS (pH 7.4, 0.14 M NaCl, 2.7 mM KCl, 8.0 mM Na_2HPO_4, 1.5 mM KH_2PO_4) to remove Tris. The gel slice was soaked in elution buffer (1X PBS, 1.0 mM EDTA, 0.05% SDS) overnight at 37°C overnight. Rabbits were injected with 0.5 mg of the purified antigen (Cocalico, PA). Pre-inoculated sera was compared to post-inoculated sera, and candidate sera were tested against pure COQ-3 antigen, as well as induced and non-induced *E. coli* BL21 cells containing the pET15b-*coq3ce* plasmid. Sera that identified a polypeptide of the expected 29 kDa mass were then affinity purified essentially as described (Pauli et al., 1990). In brief, antisera were incubated with purified COQ-3 blotted onto nitrocellulose membrane, washed two times with PBS, and cut into small squares. The membrane pieces were incubated with anti-COQ-3 antisera overnight at 4°C while gently shaking. The membrane pieces were pelleted and washed two times in PBS. Anti-COQ-3 antisera was recovered from the membrane by adding acidic elution buffer (0.2 M glycine, pH 2.0), and immediately neutralized by adding 1 M Tris-Cl (pH 8.0) to the eluate until the elution pH was 7.0. The eluate was stored at −80 °C until used.

2.8 Western Blot Analyses

50 N2, 100 *coq-3(ok506) −/−,* 50 *coq-3(ok506) −/−::Ex[coq-3],* 100 *coq-3(qm188) −/−,* and 50 *coq-3(qm188) −/−::Ex[coq-3]* were raised to the day-two adult stage, lysed in 2X SDS sample buffer at 95°C and subjected to 12.5% gel electrophoresis as previously described (Jonassen et al., 2002). Proteins were transferred to polyvinylidene difluoride membrane and blotted with the *C. elegans* anti-COQ-3 antibody at a 1:750 dilution. Chemiluminescent detection was performed with a horseradish peroxidase conjugated anti-rabbit IgG goat antisera and visualized with SuperSignal West Pico Chemiluminescent Substrate

(ThermoScientific, IL). Rabbit actin antisera at a 1:1000 dilution provided a loading control, as described previously (Jonassen et al., 2002).

2.9 Determination of Q_9 content in nematode lipid extracts by HPLC/MS-MS

N2, *coq-3(ok506)/nT1[qIs51], coq-3(ok506) −/−::Ex[coq-3], coq-3(qm188)/nT1[qIs51], coq-3(qm188) −/−::Ex[coq-3]* and *clk-1(qm30) −/−* worms were hypochlorite-lysed and the resulting eggs were transferred to NGM plates seeded with OP50, as described above. Two days after attaining the L4 larval stage, 200 N2, *coq-3(ok506) −/−*, *coq-3(ok506) −/−::Ex[coq-3]*, *coq-3(qm188) −/−*, *coq-3(qm188) −/−::Ex[coq-3],* and *clk-1(qm30) −/−*worms were collected and placed in 5 mL borosilicate glass tubes with PTFE-lined lids containing 200 uL water and stored at −80 °C until used. An aliquot (1.5 pmol) of diethoxy-derivative of ubiquinone-10 was used as the internal standard (Falk et al., 2011), and was added to each sample and Q_{10} standard. Lipids were extracted in 1.4 mL methanol and 1.8 mL petroleum ether (Fisher Scientific). The samples were vortexed at high speed for one min, centrifuged at 800 × *g* for one min and the top (organic) layer placed in a separate glass tube. The aqueous layer was extracted two additional times with the addition of 1.8 mL petroleum ether and the organic layers were combined in one tube. The samples were dried with an N-Evap Organomation (Organomation Associates) and resuspended in 200 μL pure ethanol. Samples were analyzed by HPLC/MS-MS to determine the content of Q_9 as described previously (Falk et al., 2011) with the following modifications. For separation of worm quinones, a constant flow rate of 500 μL/min was used the percentage of solvent B for the first 1.5 min was 0%, and increased linearly to 15% by two min. The percentage of solvent B remained unchanged for the next min and decreased linearly back to 0% by 4 min. All samples were analyzed in multiple reaction monitoring mode (MRM); MRM transitions were as follows: m/z 812.6/197.0 oxidized coenzyme Q_9 with ammonium adduct; m/z 814.6/197.0 reduced coenzyme Q_9 with ammonium adduct; m/z 908.7/225 oxidized diethoxyubiquinone$_{10}$ with ammonium adduct; and m/z 910.7/225 reduced diethoxyubiquinone$_{10}$ with ammonium adduct. Sample measurements less than the lowest standard (25 fmol) were considered below the level of detection. Values of Q_9 content were assessed for significant difference with a Student's *t*-test.

2.10 Quantitative PCR

N2, *coq-3(ok506)/nT1(qIs51)* and *coq-3(qm188)/nT1(qIs51)* gravid adults were prepared as described above onto NGM plates containing OP50. N2 worms were allowed to grow to L4 stage. *C. elegans coq-3(ok506)* and *coq-3(qm188)* homozygotes were allowed to grow an additional 24 h after their heterozygous sisters had reached L4. Worms were placed in 200 μL nuclease-free water and frozen at −80°C until use. RNA was extracted by adding 500 μL Trizol (Invitrogen), vortexed 5 min, left at room temperature for 10 min and centrifuged at 4°C for 10 min. The supernatant was transferred to a new tube and 100 μL of chloroform was added. The mix was incubated at room temperature for 2 min after a brief vortex. The upper clear phase was saved in a new tube and subjected to a DNase treatment. Total RNA (40 μg) was incubated with 1 unit of RQ1 DNase (Invitrogen), as per manufacturer's directions. The DNase treated RNA was precipitated overnight with Glycoblue (Ambion), as per manufacturer's instructions, and diluted to 500 ng/μL. Aliquots of RNA (5 μg) were subjected to cDNA synthesis using M-MLV reverse transcriptase with 0.5 μg/μL oligo(dT)$_{12–18}$, as per manufacturer's instructions (Invitrogen). Quantitative PCR reactions utilizing primers for *nuo-3*, *gdi-1*, and *cdc-42* (Supplemental Table 1) were performed in 20 μL volumes containing 1X SYBR Green I Nucleic Acid Gel Stain (Cambrex, ME), Taq polymerase prepared as previously described (Pluthero, 1993), and 0.01 nM gene-specific forward and reverse primers and cDNA. The Opticon 2 conditions used were: 50°C for 2 min, 95°C for 2 min, then 40 cycles of 95°C for 15 s, 60°C for 30 s, and finally 72°C for 30 sec. Fluorescence was monitored at 72°C for 5 min. Reactions were normalized to *cdc-42*

expression. Primer efficiencies were: 85% for *gdi-1*, 81% for *nuo-3* and 95% for *cdc-42*. Melting curves and agarose gel analyses were used to confirm the presence of a single product. The Pfaffl method was used to analyze fold-changes compared to wild-type worms (Pfaffl, 2001).

3. Results

3.1 Deletions in two C. elegans coq-3 mutants remove crucial methyltransferase motifs

The *C. elegans coq-3* gene is composed of five exons, two of which contain the four AdoMet-dependent methyltransferase motifs (Fig. 2) (Petrossian and Clarke, 2009). Sequencing of cDNAs prepared from RNA isolated from wild-type *coq-3*, *coq-3(ok506)* and *coq-3(qm188)* mutants show splicing of exons as predicted from the gene map (Fig. 2, Materials and Methods, and Supplemental Fig. 1). Thus, the transcript produced from the *coq-3(ok506)* allele contains exons 1, 2, 4 and 5. Splicing of exon 2 to exon 4 produces a frame-shift within exon 4 that eliminates the methyltransferase motifs II and III. The transcript produced from the *coq-3(qm188)* allele contains exons 1, 2, and 5, and produces an in-frame transcript that is also missing *O*-methyltransferase motifs II and III. Both *coq-3* gene deletions are predicted to behave as null alleles due to the loss of two crucial motifs.

3.2 Expression of C. elegans COQ-3 rescues the E. coli ubiG mutant

An *O*-methyltransferase enzyme is required for two steps in the biosynthesis of Q (Fig. 1) (Poon et al., 1999). *Saccharomyces cerevisiae coq3* mutants lack Q and Coq3-dependent *O*-methyltransferase activites, but Q biosynthesis and growth on non-fermentable carbon sources is restored when yeast *coq3* mutants express either *E. coli* UbiG, rat Coq3, or human Coq3 homologues, indicating strong conservation of function from *E. coli* to yeast and animals (Hsu et al., 1996; Poon et al., 1999; Jonassen et al., 2001). However, the functionality of the *C. elegans* COQ-3 in Q biosynthesis has not been assessed. Expression of either *C. elegans* COQ-3 or *S. cerevisiae* Coq3 in the GD1 *E. coli ubiG* mutant rescues growth on a non-fermentable carbon source (Fig. 3). The rescue was observed at 30°C (Fig. 3) and not at 37°C (Hsu et al., 1996) (and data not shown), indicating that the *C. elegans* COQ-3 and *S. cerevisiae* Coq3 require lower temperature for function. In contrast, expression of the *C. elegans* CLK-1 polypeptide was shown to rescue the *E. coli ubiF* mutant upon incubation at 37 °C (Adachi et al., 2003). The results indicate that *C. elegans* COQ-3 is an *O*-methyltransferase functionally homologous to the *E. coli* UbiG and *S. cerevisiae* Coq3.

3.3 Fertility in first generation homozygous coq-3(ok506) mutants depends on a diet of Q-replete E. coli, while coq-3(qm188) mutants are sterile regardless of diet

As previously reported (Hihi et al., 2002), we observed that first generation *coq-3(qm188)* mutants are sterile when fed either Q-replete or Q-less diets (Table 2). In contrast, first generation *coq-3(ok506)* mutant worms generate a significant number of larvae (118 ± 48; n =14) when fed Q-replete OP50, but are sterile when fed Q-less GD1 *E. coli* (Table 2). Similarly we observed that fertility in first generation *coq-2(ok1066)* mutant worms also depended on a diet of Q-replete OP50 *E. coli* (Table 2).

However, the diet-dependent fertility of the *coq-3(ok506)* mutant is restricted to the first generation. First generation *coq-3(ok506)* mutant worms produce progeny that arrest at various larval stages, and only a small number reach adulthood. These few adults lay an average of four eggs per worm that arrest at the L1 larval stage (data not shown).

3.4 Gonad development and size of first generation homozygous coq-3 mutant worms depends on a diet of Q-replete E. coli

The diet-dependent fertility observed in the *coq-3(ok506)* mutant suggested that the anatomical response of this mutant strain to an OP50 or GD1 diet was likely to be quite different. Fertility of N2 wild-type worms is unaffected by Q-less diets of *E. coli* (Jonassen et al., 2001; Hihi et al., 2002) (Table 2). Indeed, examination of the gonads in N2 worms fed either GD1 or OP50 *E. coli* diets reveals the expected wild-type gonad morphology, replete with oocytes and embryos (Fig. 4A and 4B). In contrast, the *coq-3(ok506)* mutants fed GD1 Q-less *E. coli* diet as hatchlings develop withered gonads that lack oocytes and embryos (Fig. 4D) and are sterile (Fig. 5B), while age matched *coq-3(ok506*) mutants fed OP50 contain oocytes and embryos. Although the gonads appear abnormal as compared to wild-type, they are nonetheless capable of generating significant numbers of progeny (Table 2 and Fig. 5A). This diet-dependent phenotype of the *coq-3(ok506*) mutant is markedly distinct from the *coq-3(qm188)* mutant worms, that fail to develop mature reproductive organs when fed the OP50 diet as hatchlings (Hihi et al., 2002), and are predominantly sterile (Fig. 5A).

The OP50 diet also has a dramatic effect on the size of *coq-3(ok506)* mutant worms. The *coq-3(ok506)* mutant worms fed OP50 as hatchlings are approximately 25% smaller than wild-type worms (Fig. 4E). The *coq-3(ok506)* mutants fed GD1 have a 50% smaller diameter than wild-type worms, and are also smaller than mutants fed OP50 (Fig. 4E). In contrast, N2 worms retain wild-type size when fed either diet.

3.5 NovaSOL ® Q_{10} rescues clk-1 mutants but not coq-2 or coq-3 mutants

NovaSOL ® Q_{10} is a water-soluble compound containing Q_{10} at its micelle core. This water-soluble formulation of Q_{10} has been previously shown to rescue *clk-1* mutant sterility when provided as a supplement to the Q-less *E. coli* GD1 diet (Saiki et al., 2008a). However, neither fertility nor development of *coq-3(ok506)* mutants were rescued when GD1 diets were supplemented with NovaSOL ® Q_{10} (Table 2). Supplementation with NovaSOL ® Q_{10} also failed to rescue the *coq-2* mutants (Table 2). These results indicate that the *clk-1* mutants are distinct in their response to NovaSOL ® Q_{10}.

3.6 Investigation of the molecular mechanism responsible for phenotypic differences of the coq-3(ok506) and coq-3(qm188) mutants

Because the *coq-3(qm188)* deletion produces an in-frame transcript (Fig. 2 and Fig. S1), we tested whether the mutation may exert a dominant negative effect. However, the brood sizes of heterozygous *coq-3(qm188)/dpy-4* worms were similar to wild-type and *dpy-4/dpy-4* worms (Table 2), indicating that the more severe phenotype of the *coq-3(qm188)* mutant cannot be explained by a dominant negative effect, consistent with a previous study (Hihi et al., 2002).

The *C. elegans coq-3* gene lies in a three-gene operon (Fig. 2). We asked whether the phenotypic differences between *coq-3(ok506)* and *coq-3(qm188)* mutants might be due to the impact of the large deletion present in the *coq-3(qm188)* mutant on the expression of neighboring genes within the operon. We performed quantitative PCR to monitor the expression of *nuo-3* and *gdi-1,* the genes flanking *coq-3* in the operon. NUO-3 is a structural subunit of the NADH-coenzyme Q oxidoreductase, and GDI-1 is a Rab-specific guanine-nucleotide dissociation inhibitor involved in membrane trafficking (WB: WBGene00000763). Although no significant differences were observed between the transcript levels of *gdi-1* or *nuo-3*, the expression of *nuo-3* in the *coq-3(qm188*) mutant was highly erratic and the expression of *gdi-1* was decreased 60% when compared to either N2 or *coq-3(ok506*) (Fig. 6). It is possible that the altered expression of either *nuo-3* or *gdi-1* in

combination with a defect in Q content could produce a synthetic sterility phenotype unique to the *coq-3(qm188)* mutant.

3.7 Extra-chromosomal arrays with wild-type coq-3 expressed from the let-858 promoter rescue fertility in the coq-3(ok506) and coq-3(qm188) mutants

Rescue of mutant phenotypes by expression of the wild-type gene allows one to determine whether the mutation under investigation is solely responsible for the observed phenotypes. Previous attempts to rescue the sterile phenotype of the *coq-3(qm188)* mutant were not successful; a genomic fragment containing the *coq-3* gene and no designated promoter failed to restore wild-type brood size, and the observed partial rescue of sterility was not maintained past the first generation (Hihi et al., 2002). In the absence of such rescue, the presence of another genetic defect accounting for the severe phenotype cannot be ruled out. Because *coq-3* lies in the middle of a three-gene operon, we utilized the *let-858* promoter to drive the expression of wild-type *coq-3*. The *let-858* promoter has been shown to drive ubiquitous expression in both somatic and reproductive tissues of the worm (Kelly et al., 1997). We show that wild-type brood sizes are restored in both *coq-3(ok506)* and *coq-3(qm188)* mutants carrying the wild-type *coq-3* gene driven by the *let-858* promoter (Fig. 5A). Remarkably, this same construct is able to rescue development and fertility in both *coq-3* mutant strains when fed the GD1 *E. coli* diet as hatchlings (Fig. 5B). The results indicate that the sole genetic defect is in fact due to the *coq-3* mutation (*ok506* or *qm188*).

3.8 Rescuing coq-3 mutants with wild-type coq-3 gene driven by a let-858 promoter partially restores COQ-3 protein levels and Q_9 content

Western analyses with antisera against *C. elegans* COQ-3 shows that both *coq-3(ok506) and coq-3(qm188)* mutants lack detectable COQ-3 protein (Fig. 7A). Steady state COQ-3 polypeptide levels are rescued in both *coq-3(ok506)* and *coq-3(qm188)* mutants carrying the wild-type *coq-3* gene driven by the *let-858* promoter (Fig. 7A). Although COQ-3 steady-state protein levels are not restored to wild type, the observation that both *coq-3* mutant strains carrying this transgene are able to generate normal levels of progeny and can develop normally on GD1 indicates that the steady state level of COQ-3 polypeptide is sufficient to rescue brood size.

We utilized HPLC-MS/MS to detect and quantify Q_9. This method allowed us to analyze the Q_9 content in lipid extracts prepared from 200 *coq-3* mutant or wild-type worms. As shown in Fig. 7B the *coq-3* mutant worms lacking the rescue construct had significantly low Q_9 content as compared to wild-type N2 worms. The residual Q_9 content present in the first generation *coq-3* mutants is likely to be derived from the maternal contribution, since Q_9 was below the level of detection in the lipid extracts prepared from *clk-1(qm30)* mutant worms. In contrast, the Q_9 content present in the *coq-3* mutants harboring the rescue construct was restored to a level that was not statistically different from that of wild-type worms (Fig. 7C).

4. Discussion

4.1 C. elegans COQ-3 functions as a conserved O-methyltransferase in Q biosynthesis Caenorhabditis elegans

COQ-3 is predicted to function as an AdoMet-dependent *O*-methyltransferase involved in coenzyme Q biosynthesis (Jonassen and Clarke, 2000). Here we show that growth of *E. coli ubiG* mutants on medium containing a non-fermentable carbon source is rescued by the expression of either *S. cerevisiae* Coq3 or *C. elegans* COQ-3. These results suggest that the worm *coq-3* gene product is involved in two steps similar to those catalyzed by *E. coli* UbiG

and S. *cerevisiae* Coq3 (Fig. 1), indicating that COQ-3 is an AdoMet-dependent *O*-methyltransferase required for synthesis of Q.

4.2 Two coq-3 null mutants are rescued by the expression of coq-3 from extra-chromosoal arrays

Previous attempts to rescue *C. elegans coq* mutants with extra-chromosomal arrays containing the wild-type gene were not successful. A segment of genomic DNA containing the *coq-3* gene without a promoter did not result in a stably rescued phenotype of the *coq-3(qm188)* mutant (Hihi et al., 2002). As the middle gene in a three-gene operon, expression of *coq-3* necessitates the presence of a promoter in the rescue construct. A similar problem may have led to the limited rescue when *coq-8* mutant worms were provided with a segment of DNA containing just the wild-type copy of the *coq-8* gene (Asencio et al., 2009), because the *C. elegans coq-8* gene is also the second gene in a three-gene operon (WB: WBGene00000763). The rescue construct developed in our study utilized the ubiquitously expressed *let-858* promoter (Kelly et al., 1997). This construct afforded dramatic rescue; the *coq-3* mutants harboring this construct were not only fertile, but produced brood sizes indistinguishable from N2 wild type on either the Q-replete OP50 and the Q-deficient GD1 *E. coli* diets. Our results indicate that the sole genetic defect in both mutants is in fact due to the *coq-3* mutation, and provides an essential foundation for interpretation of the rescue effected (or lack thereof) by dietary Q supplements.

We also were able to detect and quantify Q_9 content in lipid extracts prepared from 200 individual worms with HPLC-MS/MS (Fig. 7). This method allows us to determine the Q_9 content in mutant worms unable to reproduce. Thus, for the first time we are able to report on the changes in Q_9 content as a function of rescue. Both the first generation *coq-3*(*ok506*) and *coq-3(qm188)* mutants lack detectable levels of the COQ-3 polypeptide and exhibit similar deficiencies in Q_9 content. In the strains harboring the extra-chromosomal arrays the steady state level of the COQ-3 polypeptide and the Q_9 content while clearly increased relative to the *coq-3* mutants, were nonetheless decreased relative to wild type, indicating that even partial restoration of endogenous Q synthesis leads to profound rescue.

4.3 Two coq-3 null mutants show different phenotypes

This study shows that two *coq-3* mutants harboring deletions that remove two crucial O-methyltransferase motifs show distinct responses to the standard lab diet of OP50 *E. coli*. First generation *coq-3(ok506)* homozygous worms fed OP50 develop reproductive organs and produce a significant number of larvae. In contrast, the previously characterized *coq-3(qm188)* first generation homozygous worms are small, sterile, and fail to generate discernible reproductive tissues when fed a diet of Q-replete OP50 *E. coli* (Hihi et al., 2002). The complete restoration of fertility and development observed in both *coq-3(ok506*) and *coq-3(qm188*) mutants harboring the wild-type gene in an extra-chromosomal array indicate that loss of a functional *coq-3* gene is primarily responsible for the phenotypes.

What may account for the notably different phenotypes between these two *coq-3* mutants? We speculate that the more extensive *qm188* deletion affects the expression of the *nuo-3* and/or *gdi-1* genes, flanking genes in the operon containing *coq-3*. In *C. elegans* the presence of insertions or deletions within an operon can affect not only the expression of the gene harboring the mutation, as well as neighboring genes in the operon (Allen et al., 2011). It is particularly noteworthy that the *RNAi gdi-1* knockdown phenotype includes germ cell and gonad variation, reduced brood size and sterility (Lee et al., 2010). We suggest that the observed altered expression of *nuo-3* (encoding a subunit of complex I), or *gdi-1* (encoding a major regulator of Rab GTPase activity involved in endocytosis and exocytosis), in combination with the defect in Q biosynthesis may act synthetically to produce a more

severe phenotype in the *coq-3(qm188*) mutant as compared to the *coq-3(ok506)* mutant which contains normal expression of *nuo-3* and *gdi-1*. In this scenario, rescue of Q biosynthesis in the *coq-3(qm188)* mutant is presumed to restore or stabilize complex I (by restoring the ligand of the complex). Similarly, it is possible that restoration of energy metabolism (evident in the rescued *coq-3(qm188)* mutant) could act to augment function of GDI-1.

Indeed, the phenotype of the *coq-3(ok506*) mutant appears to be more similar to other *C. elegans coq* deletion mutants. The rescued fertility in response to an OP50 diet in *coq-3(ok506)* homozygous worms is similar to that observed in first generation *coq-2(ok1066)* mutant worms (Table 2). Although it was initially claimed that first generation homozygous *coq-2* and *coq-8* mutant worms were sterile (Gavilan et al., 2005), a subsequent study showed first generation homozygous *coq-8* mutants fed the Q-replete OP50 *E. coli* diet were fertile (Asencio et al., 2009). Nevertheless, these *C. elegans coq-2, coq-3* and *coq-8* mutants are all quite distinct from the *clk-1* mutants, because exogenous supplies of Q fail to rescue.

4.4 Maternal contribution of Q_9 is necessary for viability

Previously Earls and colleagues showed that first generation *coq-1(ok749), coq-2(ok1066)* and *coq-3(ok506)* mutant worms were able to develop to at least the third larval stage with a few adult escapers, suggesting that maternal stores of Q are sufficient for development (Earls et al., 2010). Metabolic rates, life spans and fertility of first generation *clk-1* mutant homozygote were similar to their heterozygous mothers, implying that maternal Q_9 stores had a profound effect (Burgess et al., 2003). In our study, the small amounts of Q_9 present in the first generation *coq-3* mutant strain lipid extracts are most likely maternally contributed. This maternal contribution appears to be needed for viability in the first generation *coq-3* mutants. Second generation *coq-3(ok506)* homozygotes fed OP50 arrest and die at various larval stages, suggesting that Q_9 stores from the first generation homozygous mother were insufficient for viability, despite the dietary supply of *E. coli* Q_8.

4.5 Coq-3(ok506) homozygotes do not respond to exogenous Q_{10}

The use of exogenous Q to ameliorate Q deficiencies in humans is well documented (Quinzii and Hirano, 2010; Rahman et al., 2012). Similarly, certain *C. elegans* Q strains also show an improvement in phenotypes when provided with various Q formulations. Both wild-type and the oxidatively sensitive mutant *mev-1* mutant worms lived longer when the standard *E. coli* diet was supplemented with 150 μg/mL Q_{10} (Ishii et al., 2004). Degradation of GABA-ergic neurons in worms fed *coq-1(*RNAi) was rescued with exogenous Q_{10} in a dose-dependent manner (Earls et al., 2010). However, in our hands, the Q_{10} formulation used in these assays did not restore fertility in either the *clk-1* or *coq* mutant nematodes. The NovaSOL ® Q_{10} formulation used in our study was shown to rescue fertility in the *clk-1* mutants fed the GD1 diet and was also detected in the mitochondria isolated from either wild-type or *clk-1* mutants (Saiki et al., 2008a). It seems likely that the presence of rhodoquinone-9 (RQ_9) or DMQ_9 may support limited respiration or antioxidant function in the *clk-1* mutant (Jonassen et al., 2001; Yang et al., 2011). Indeed, recent studies indicate that operation of the glyoxylate cycle, which serves to replenish the four-carbon citric acid cycle components from acetylCoA, is required for the lifespan extension phenotype of the *clk-1(qm30*) mutant (Gallo et al., 2011). Such activity may enable the *clk-1* mutants to uptake and assimilate the dietary Q_{10} supplements. In contrast, here we show that the NovaSOL ® Q_{10} fails to restore fertility in either the *coq-2* or *coq-3* mutants (Table 2). The fact that we are able to successfully rescue both *coq-3* mutants with extra-chromosomal arrays expressing only COQ-3, eliminates the possibility that the lack of rescue by dietary

sources of Q can be attributed to other genetic defects, and must stem directly from the defect in an early step of Q biosynthesis.

4.6 Restoration of Q biosynthesis versus exogenous Q supplementation

The results presented suggest that restoration of *de novo* Q synthesis has a much greater impact on ameliorating the mutant phentoypes as compared to supplying Q exogenously. Previous work has shown that the respiratory defect in the yeast *coq* mutants can be rescued even when the endogenously synthesized Q content is 1–3% of normal (Tran et al., 2006; Xie et al., 2011). Indeed, Morgan and colleagues have recently suggested that small amounts of endogenous Q are capable of providing a dramatic rescue of *clk-1* mutant phenotypes (Yang et al., 2011). This is consistent with the findings that tRNA suppressors that restore only low amounts of endogenously produced Q_9 nevertheless afford dramatic rescue of brood size, slow behaviors, and long lifespan of the *clk-1* mutants (Branicky et al., 2006). Maternal supplies of Q also have extremely potent effects, as evidenced by the maternal effect in the *clk-1* mutant (Burgess et al., 2003). Similarly, the first generation fertility in the homozygous *coq-2*, *coq-3(ok506)* and *coq-8* mutants clearly depends on the maternal supply of endogenous Q. Although NovaSOL ® Q_{10} supplementation does rescue fertility of the *clk-1* mutants (Saiki et al., 2008a), the variable outcomes observed with other *coq* mutants (discussed in section 4.3) show that uptake and assimilation of exogenously supplied Q supplements remains a formidable challenge.

Subsequent therapies to combat Q deficiency in patients should focus on induction of *de novo* biosynthesis. Bentinger and colleagues have recently shown that endogenous Q levels can be raised in HepG2 cells when the growth media is supplemented with polyisoprenoid epoxides (Bentinger et al., 2008). This study also found the transcription levels of genes involved in Q biosynthesis increased under these conditions. Mice suffering from nephritic disease due to a mutation in the PDSS2 gene improve markedly if dosed with the antihyperlipidemic drug probucol (Falk et al., 2011). The endogenous Q levels in diseased mice treated with probucol are higher than in untreated mice. Surprisingly, probucol is more effective in treating the nephritic disease than high doses of exogenous Q (Falk et al., 2011). Finally, several different precursors have been shown to serve as substitutes for the pHB head group in Q, including pABA and vanillic acid (Marbois et al., 2010; Ozeir et al., 2011). The hope is that these head groups may either augment or by-pass deficiencies in Q biosynthesis in patients. Future work in *C. elegans* can help us understand the use of these compounds in Q deficiency studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Two *C. elegans coq-3* null mutants lack coenzyme Q but have distinct phenotypes.
- One null *coq-3* mutant shows limited fertility, while the other is sterile.
- Unlike *clk-1* mutants, neither *coq-3* mutant is rescued with dietary Q supplements.
- *coq-3* mutants rescued for *de novo* Q synthesis are as fertile as wild-type worms
- Augmenting *de novo* synthesis of Q is more effective than dietary therapies with Q.

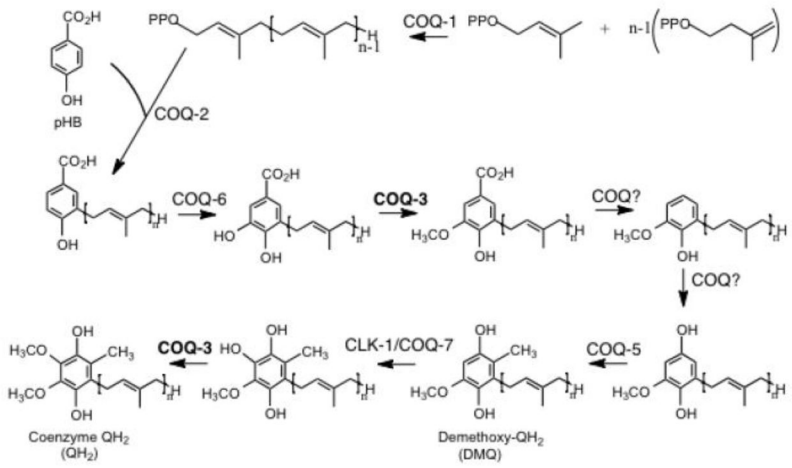

Fig. 1.

Coenzyme Q biosynthesis in *C. elegans*. The steps in *C. elegans* Q biosynthesis are proposed to be similar to that of *S. cerevisiae*. The COQ-1 polypeptide assembles the nonaprenyl-diphosphate tail, and COQ-2 condenses it with the aromatic ring precursor, pHB. Subsequent steps of ring modification include hydroxylation steps (COQ-6 and CLK-1/COQ-7), O-methylation (COQ-3), C-methylation (COQ-5). Steps not yet identified with a COQ polypeptide are indicated as COQ? In addition, catalytic functions have not yet been assigned to COQ-4. COQ-8 is a putative kinase, believed to mediate formation of a multi-subunit complex. In *S. cerevisiae*, pABA serves as an alterate ring precursor. It is not yet known whether or not *C. elegans* utilizes pABA in Q biosynthesis.

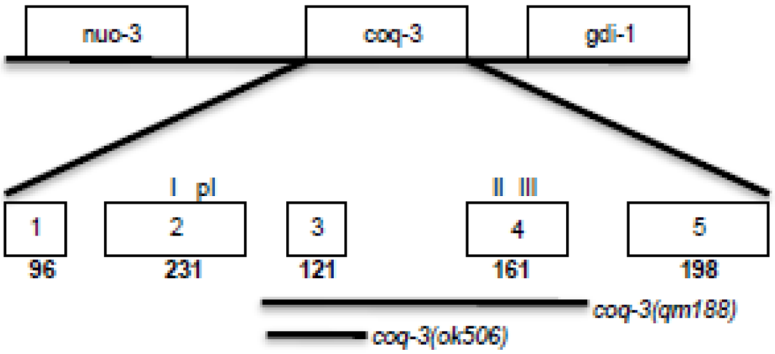

Fig. 2.

The *C. elegans coq-3* gene is situated within a three-gene operon. The *coq-3* gene is flanked by *nuo-3* and *gdi-1*, and is comprised of five exons (*numbered boxes*). Four canonical S-adenosylmethionine-dependent methyltransferase motifs (*I, Post-I, II,* and *III*) are depicted above exons 2 and 4. The *coq-3(qm188)* deletion spans exon 3, intron 3, and exon 4, and generates an in-frame transcript, as detected by PCR of cDNA products, as described in Materials and Methods. The *coq-3(ok506)* deletion spans exon 3, and introduces a frame shift resulting in an early stop codon, encoding a truncated polypeptide lacking methyltransferase motifs II and III.

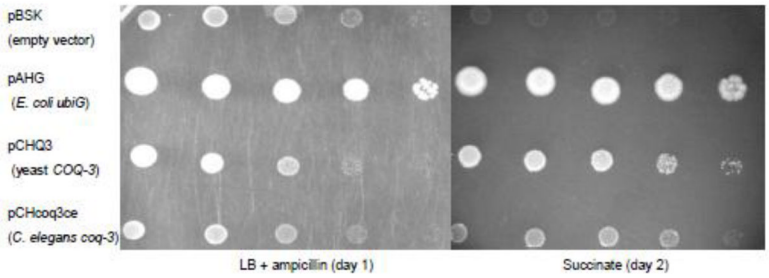

Fig. 3.

Expression of *C. elegans* COQ-3 rescues growth of the *E. coli ubiG* mutant on medium containing a non-fermentable carbon source. The *E. coli ubiG* deletion mutant GD1 was transformed with the designated plasmids: empty vector (pBSK2), *E. coli ubiG* (pAHG), *S. cerevissiae COQ3* (pCHQ3), or the *C. elegans coq-3* gene (pCH*coq3ce*). Serial dilutions were spotted onto either LB plates containing ampicillin or to succinate minimal media plates, which contain a non-fermentable carbon source. *E. coli* on LB plates were allowed to grow 1 day at 30°C and on succinate minimal media plates for 2 days at 30°C. The assay testing rescue was conducted twice with similar results each time.

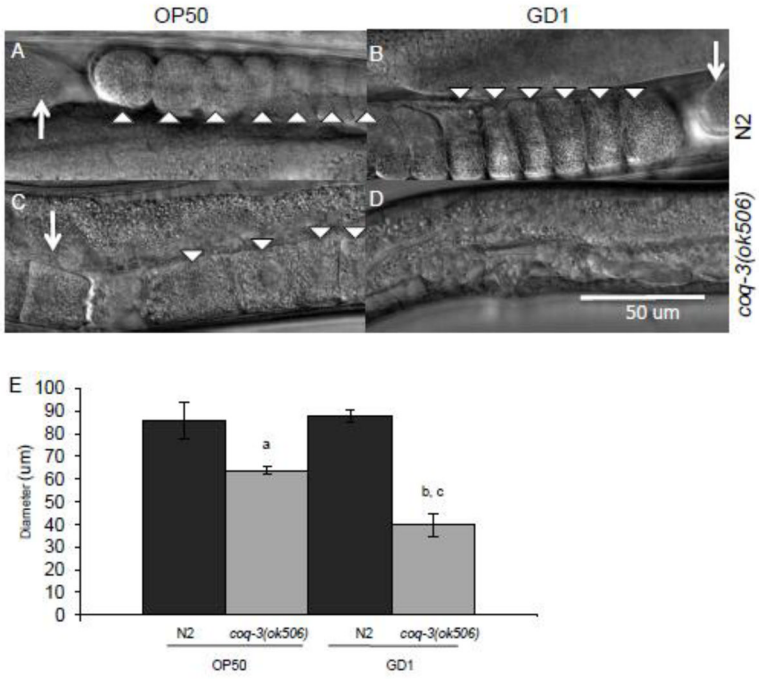

Fig. 4.

The defects in gonad development and body size of *coq-3(ok506)* mutants are corrected at least in part by dietary Q. Synchronized N2 (*A*, *B*) and *coq-3(ok506) −/−* at L1 larval stage (*C*, *D*) were transferred to NGM plates containing either OP50 (*A*, *C*) or GD1 *E. coli* (*B*, *D*), and maintained at 20°C for 70 h (corresponding to day 2 of adulthood). Oocytes are indicated by arrowheads; embryos are designated by arrows. Scale bar = 50 um. (*E*) *C. elegans coq-3(ok506) −/−* fed either OP50 or GD1 *E. coli* diets are smaller than N2 wild-type worms. Worm diameter was measured from the external tip of the vulval opening to the outer cuticle of the posterior side of the worm directly opposite the vulva. Statistical significance was determined by Student's *t* test. Number of worms imaged: three N2 fed OP50, three N2 fed GD1, five *coq-3(ok506) −/−* fed OP50, and six *coq-3(ok506) −/−* fed GD1. P-values: *a*, N2 fed OP50 vs. *coq-3(ok506) −/−* fed OP50 p = 0.003; *b*, N2 fed GD1 vs. *coq-3(ok506) −/−* fed GD1 $p = 5.6 \times 10^{-6}$; *c*, *coq-3(ok506) −/−* fed OP50 vs. *coq-3(ok506)* fed GD1 $p = 4.54 \times 10^{-5}$.

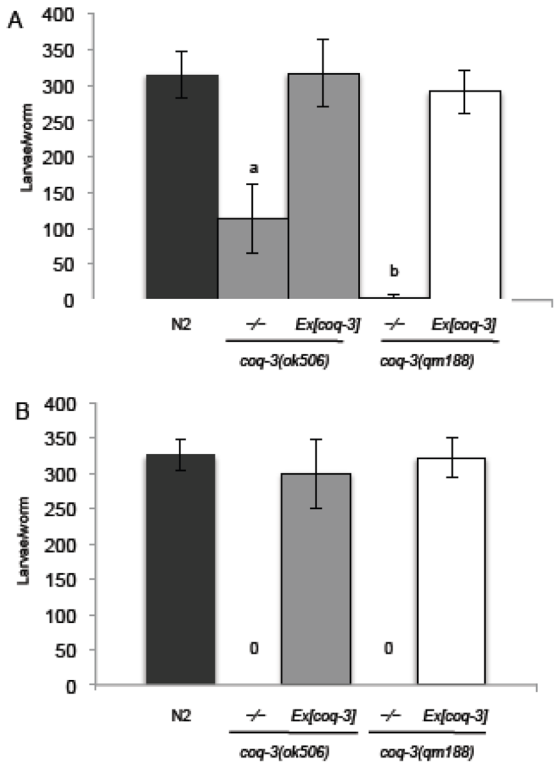

Fig. 5.

Sterility in *coq-3(qm188)* homozygotes and low fertility in *coq-3(ok506)* homozygotes are rescued when mutants harbor an extra-chromosomal array carrying wild-type *C. elegans coq-3*. The number of L1 larval progeny were determined for the designated worm strains fed either OP50 (*A*) or GD1 (*B*) *E. coli* diets as hatchlings. Larvae on plates were counted and removed. P-values: *a*, N2 fed OP50 vs. *coq-3(ok506*) fed OP50 p = 0.0004; *b*, N2 fed OP50 vs. *coq-3(ok506) −/−* fed GD1 p = 3.25×10^{-15}. Table 2 lists the numbers of worms analyzed.

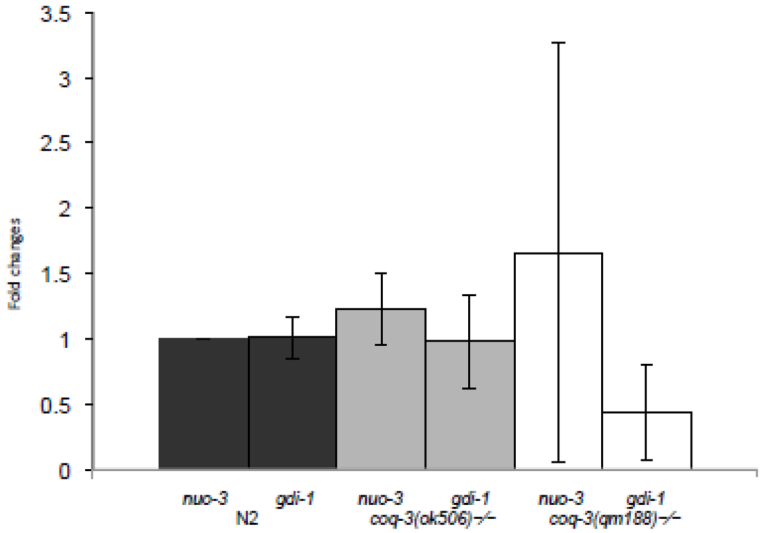

Fig. 6.

Transcript levels of the neighboring *nuo-3* gene are erratic in the *coq-3(qm188)* homozygote as compared to *coq-3(ok506)* or N2. N2, *coq-3(ok506)/nT1(qIs51)* and *coq-3(qm188)/nT1(qIs51)* gravid adults were treated with hypochlorite and placed on NGM plate medium containing OP50 *E. coli*. Homozygous mutants were collected 24 hours after their heterozygous sisters reached the L4 stage. N2 worms were collected at the L4 stage. Quantitative PCR was used to assay *nuo-3* and *gdi-1* mRNA levels, and the content was normalized to *cdc-42* RNA content. The Pfaffl analytical method was used to derive fold changes compared to N2 worms (Pfaffl, 2001).

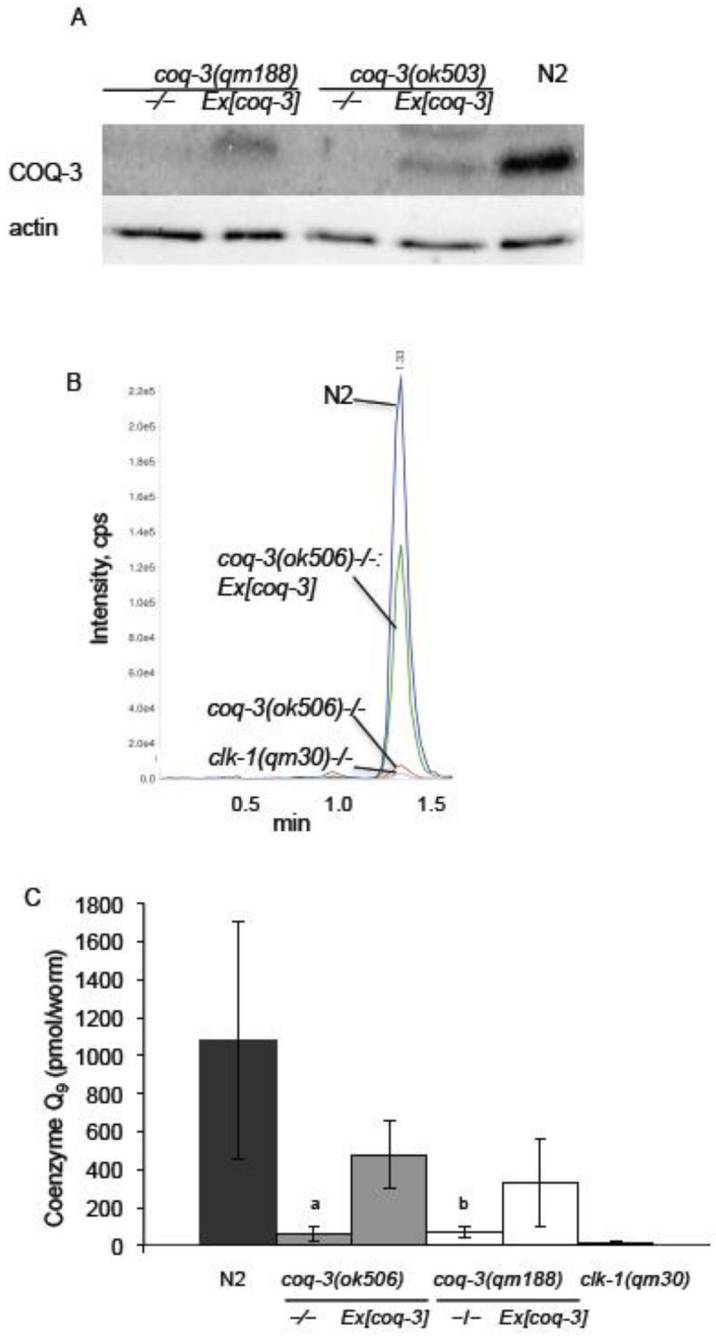

Fig. 7.

Extra-chromosomal array containing wild-type *coq-3* partially restores steady state levels of the COQ-3 polypeptide in both *coq-3(ok506) and coq-3(qm188)* deletion mutants. (*A*) Western blot showing expression of COQ-3 in *coq-3* mutant strains *coq-3(qm188) −/−* and *coq-3(ok506) −/−* expressing wild-type *coq-3* cDNA in a plasmid containing the *let-858* promoter and 3′UTR. N2, *coq-3(ok506)::Ex[coq-3]* and *coq-3(qm188)::Ex[coq-3]* lanes contain 50 two-day old adult worms each, and the *coq-3(ok506) −/−* and *coq-3(qm188) −/−* lanes contain 100 worms aged two days after their heterozygous sisters reached the L4 stage. Anti-sera to actin (rabbit) was used as loading control. (*B*) Lipids were extracted from 200 day-two adult worms and analyzed via HPLC-MS/MS as described in Materials and

Methods (section 2.9). Detection of the precursor-to-product ion transition (812.6/197.0) (Q_9 with ammonium adduct) was performed with MRM. The traces indicate arbitrary units (cps) and the scale is the same for all traces within a panel. (*C*) The areas of the peaks in panel B were quantified as described in Materials and Methods. The *coq-3* mutant worms carrying the coq-3 extra-chromosomal array show recovery of Q_9 biosynthesis. Values are presented as mean ± SD (two biological replicates analyzed in duplicate, n = 4); p-values compared to wild-type Q_9: a, *coq-3(ok506) −/−* = 0.017; b, *coq-3(qm188) −/−* = 0.012; c, *clk-1(qm30) −/−* = 0.014.

Table 1

C. elegans strains used in this study

Strain	Genotype	Source
N2	wild-type (Bristol)	CGC
VC436	*coq-3(ok506)* IV/*nT1[qIs51]* (IV;V)	CGC
MQ992	*coq-3(qm188)* IV/*dpy-4(e1166)*	CGC
CB1166	*dpy-4(e1166)/dpy-4(e1166)*	CGC
VC752	*coq-2(ok1066)* III/*hT2[bli-4(e937) let-?(q782) qIs48]* (I;III)	CGC
JK2906	*mep-1(q660)* I/*nT1[qIs51*] (IV;V)	CGC
MQ130	*clk-1(qm30)*	(Wong et al., 1995)
CFC315	*coq-3(ok506)* IV (out-crossed 3×)	This report
CFC1005	*coq-3(qm188)* IV/*nT1[qIs51]* (IV;V)	This report
CFC614	*coq-3(ok506) −/−::Ex[coq-3]*	This report
CFC530	*coq-3(qm188) −/−::Ex[coq-3]*	This report

Table 2

Determination of *C. elegans* Brood Size[a]

Genotype	Diet	Average larvae per worm, ± SD	n
N2	OP50	307 +/− 29	17
N2	GD1	310 +/− 32	15
coq-3(ok506) −/−	OP50	118 +/− 48	14*
coq-3(ok506) −/−	GD1	0	14*
coq-3(qm188) −/−	OP50	2	9*
coq-3(qm188) −/−	GD1	0	11*
coq-3(ok506)::Ex[coq-3]	OP50	317 +/− 48	17
coq-3(ok506)::Ex[coq-3]	GD1	300 +/− 49	18
coq-3(qm188)::Ex[coq-3]	OP50	291 +/− 30	15
coq-3(qm188)::Ex[coq-3]	GD1	322 +/− 29	11
coq-2(ok1066) −/−	OP50	26 +/− 19	4*
coq-2(ok1066) −/−	GD1 + NovaSOL Q_{10}[c]	0	4*
coq-2(ok1066) −/−	GD1 + Vehicle	0	4*
N2	GD1 + NovaSOL Q_{10}[c]	305 +/− 16	8
N2	GD1 + Vehicle	325 +/− 20	10
clk-1(qm30) −/−	GD1 + NovaSOL Q_{10}[c]	136 +/− 35	9**
clk-1(qm30) −/−	GD1 + Vehicle	0	20**
coq-3(ok506) −/−	GD1 + NovaSOL Q_{10}[c]	0	20**
coq-3(ok506) −/−	GD1 + Vehicle	0	20**
N2	OP50	286 +/− 56	16
coq-3(qm188)/dpy-4	OP50	254 +/− 40	20
dpy-4/dpy-4	OP50	259 +/− 70	17
coq-3(ok506) −/−	GD1 to OP50[b]	0	10

[a] Fed designated diet as hatchlings.

[b] Fed GD1 two days post hatching, transferred to OP50.

[c] NovaSOL Q10 final concentration of Q_{10} was (150 μg/mL)

* N2 fed OP50 vs. designated strain and diet, p-value <.0001.

** N2 fed GD1 + vehicle vs. designated strain and diet, p-value <.0001.