Coenzyme Q protects *Caenorhabditis elegans* GABA neurons from calcium-dependent degeneration

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Mitochondria are key regulators of cell viability and provide essential functions that protect against neurodegenerative disease. To develop a model for mitochondrial-dependent neurodegeneration in *Caenorhabditis elegans*, we used RNA interference (RNAi) and genetic ablation to knock down expression of enzymes in the Coenzyme Q (CoQ) biosynthetic pathway. CoQ is a required component of the ATP-producing electron transport chain in mitochondria. We found that reduced levels of CoQ result in a progressive uncoordinated (Unc) phenotype that is correlated with the appearance of degenerating GABA neurons. Both the Unc and degenerative phenotypes emerge during late larval development and progress in adults. Neuron classes in motor and sensory circuits that use other neurotransmitters (dopamine, acetylcholine, glutamate, serotonin) and body muscle cells were less sensitive to CoQ depletion. Our results indicate that the mechanism of GABA neuron degeneration is calcium-dependent and requires activation of the apoptotic gene, *ced-4* (Apaf-1). A molecular cascade involving mitochondrial-initiated cell death is also consistent with our finding that GABA neuron degeneration requires the mitochondrial fission gene, *drp-1*. We conclude that the cell selectivity and developmental progression of CoQ deficiency in *C. elegans* indicate that this model may be useful for delineating the role of mitochondrial dysfunction in neurodegenerative disease.

cell death | disease | mitochondria | neurodegeneration | necrosis

Mitochondrial dysfunction, selective neuronal vulnerability, and progressive onset are unifying features of major human neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) (1). Genetic mutations linked to these diseases have been shown to disrupt the electron transport chain (ETC) (2), a mitochondrial pathway that accounts for the majority of cellular energy production (3). Chemical inhibitors of the ETC can also produce symptoms reminiscent of neurodegenerative diseases (4). Despite the broad disruption of mitochondrial function in these cases, specific neuron classes are preferentially targeted. The molecular basis for this selectivity is largely unknown (5).

The key role of mitochondrial fragmentation in both necrosis and apoptosis (6) parallels emerging evidence that excessive mitochondrial fission may contribute to neurodegenerative disease (7). Inhibition of the fission-promoting dynamin GTPase, Drp-1, for example, blocks mitochondrial fragmentation and death induced by overexpression of the mutant Huntingtin protein (mtHtt) in cultured cells (8). The progressive onset of these diseases is correlated with an age-dependent decline in mitochondrial function, but the mechanistic link between diminished mitochondrial activity and neurodegeneration is poorly understood.

Although the cell death pathways of necrosis and apoptosis feature distinct biochemical and morphological components, recent studies have revealed "aponecrotic" cell killing pathways that include elements of both mechanisms (9). Additionally, apoptosis and necrosis share common molecular triggers, such as oxidative stress and energy depletion, two hallmarks of mitochondrial dysfunction (10, 11). These findings suggest that apoptotic and necrotic pathways may represent related cell death responses and can be activated by mitochondrial impairment.

Coenzyme Q (CoQ) transfers electrons from complexes I and II to complex III in the mitochondrial ETC and fulfills a critical role in mitochondrial ATP production (12). CoQ also limits the production of reactive oxygen species that can damage cellular processes. Human patients with CoQ deficiency exhibit diverse symptoms ranging in severity from infantile multiorgan disease (13, 14) to discrete late onset cerebellar ataxia (15, 16). Although the essential role of CoQ in mitochondrial energy production and cell survival are likely to account for these deficits, the molecular basis for this heterogeneity is unclear, as are the specific cellular pathologies arising from CoQ deficiency (17).

We developed a model of CoQ deficiency for the purpose of delineating the molecular events that contribute to cell death arising from mitochondrial dysfunction. Because of its short life span, anatomic simplicity, and genetic tractability, the nematode *Caenorhabditis elegans* is particularly amenable to this approach. We used RNA interference (RNAi) and genetic ablation to knock down genes in the CoQ synthesis pathway (*coq-1*, *coq-2, coq-3*) to mimic CoQ deficiency. These treatments result in age-dependent loss of motor coordination that is correlated with progressive degeneration of GABA neurons. Our results show that CoQ deficiency in *C. elegans* results in a selective cell death pathway that includes features of both apoptotic and necrotic processes and, therefore, suggest that this experimental model may be useful for delineating the mechanism of neuron cell death in human CoQ deficiency and in related neurodegenerative diseases that are also linked to defective mitochondrial function.

Results

Knockdown of *coq-1* Results in Age-Dependent Loss of Coordinated Movement. COQ-1 catalyzes the first step in Coenzyme Q synthesis, the assembly of the lipophillic polyisoprenoid tail (18). RNAi knockdown of *coq-1* is reported to induce uncoordinated (Unc) and Egg-laying defective (Egl) phenotypes, but the mechanism of these effects has not been studied (19). We replicated this experiment, using the RNAi "feeding" method to expose an RNAi-hypersensitive strain, *eri-1(mg366)* (20), to bacteria expressing *coq-1* double-stranded RNA (dsRNA). Loss of motor coordination first appeared at the L4 larval stage as a kink in the normal sinu-

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soidal wave that drives locomotion. Movement then gradually declined in adults, often culminating in paralysis (Fig. 1*A* and Movie S1). Quantification of movement loss with a swimming assay (21) verified the developmental progression of the Unc phenotype (Fig. 1*B*).

RNAi or Genetic Depletion of CoQ Induces Age-Dependent Degeneration of GABA Neurons. Observation of GABA motor neurons in the ventral nerve cord, labeled with the *unc-25::GFP* reporter (Fig. 2*A*), suggested a possible explanation for the loss in motor coordination. These GABA neurons degenerate in *coq-1* knockdown animals, and cell bodies, viewed under differential interference contrast (DIC), demonstrated the swollen appearance characteristic of necrotic cell death (Fig. 2*C*). GABAergic processes in the ventral nerve cord, circumferential commissures and GABAergic processes in the dorsal cord appear discontinuous with apparent breaks (Fig. 2*B*). These morphological defects first appeared in late larval development and progressed as animals aged (Fig. 2*D*), thereby mirroring the age-dependent pattern of the Unc phenotype.

We also validated the *coq-1* RNAi results by examining *coq-1 (ok749)* mutant animals (Fig. S1). The *ok749* deletion removes a C-terminal region comprising ≈70% of the coding sequence and is therefore a likely null allele. Prior studies of *coq-1(ok749)* reported paralysis and early larval lethality (22). We observed that homozygous *coq-1* animals develop to at least the third larval stage with a few adult escapers. This finding is likely to mean that the *coq-1(ok749)* mutation results in a maternal effect lethal phenotype in which the first generation of viable offspring progress through larval development with maternally provided CoQ (23). Homozygous *coq-1* mutants appear Unc as L3 larvae. Degeneration of GABA neurons is initially observed in young adults (Fig. S1*B*) (8/13 adult animals; Fig. S1*C*); a swollen ventral cord cholinergic motor neuron was observed in a single animal at this stage ($n = 20$). Older *coq-1(ok749)* animals show extensive swelling and degeneration of other tissues as reported (22).

We tested deletion mutants for *coq-2* and *coq-3* to ask whether genetic ablation of other CoQ biosynthetic genes phenocopies *coq-1* knockdown (24). GABA neurons of *coq-2(ok1066)* and *coq-3(ok506)* animals also show the swollen morphology (Fig. 2 *F–H*) and progressive degeneration of *coq-1*-deficient animals (Fig. 2*E*). The similar phenotypes displayed by *coq-2* and *coq-3* mutants substantiate the idea that the movement and degenerative defects result from reduced CoQ synthesis, rather than off-target effects or additional roles of the COQ-1 enzyme.

Treatment with exogenous CoQ_{10} has successfully slowed disease progression of some cases of human CoQ deficiency (25, 26). To verify that CoQ deficiency was the cause of the degenerative phenotype in *C. elegans*, we incubated *coq-1* RNAi-treated animals with CoQ_{10}. We found that supplemental Coenzyme Q_{10} rescued degeneration in a dose-dependent manner, with an EC_{50} of 72 mg/mL (Fig. S2). A requirement for high CoQ doses for efficacy has also been observed in human patients and may reflect poor uptake of the drug by neurons (27).

The similar degenerative pattern and age-dependence shown by the *coq-1*, *coq-2*, and *coq-3* knockout animals validates the specificity of the *coq-1* RNAi treatment. Hence, for most subsequent experiments, we used RNAi knockdown of *coq-1* to bypass the developmental delay of the CoQ pathway mutants and to produce large numbers of viable adult animals with CoQ-deficient phenotype.

GABA Neurons Are Preferentially Sensitive to CoQ Depletion-Induced Degeneration. Having observed that CoQ deficiency results in GABA neuron degeneration, we asked whether nearby cholinergic motor neurons in the ventral nerve cord were similarly affected. The results of this analysis revealed that GABA neurons are significantly more sensitive to CoQ depletion (Fig. 2 *I* and *J* and Fig. S3). For example, in *coq-2(ok1066)* adults in which >35% of GABA neurons have degenerated, <5% of ventral cord cholinergic motor neurons are affected (Fig. 2*K*).

We next used cell-specific GFP reporters to evaluate the sensitivity of additional neuron subtypes to CoQ depletion by *coq-1* RNAi. Populations tested were cholinergic (*acr-2::GFP*), serotonergic (*tph-1::GFP*), glutamatergic (*eat-4::GFP*), and dopaminergic (*dat-1::GFP*) neurons. Although all animals displayed age-dependent Unc phenotype with *coq-1* RNAi, none of these neuron classes showed signs of degeneration comparable with that observed for GABA neurons at a similar developmental stage (Fig. S4 *A–E*). We considered the possibility that this differential effect could be due to the relative insensitivity of these neuron classes to RNAi. This explanation is made less likely, however, by the finding that all of these neuronal types were equally vulnerable to RNAi against GFP (Fig. S4*G*).

Like neurons, muscle is also a highly metabolic tissue, and muscle degeneration is seen in some cases of CoQ deficiency (28). Therefore, we used a GFP-labeled myosin heavy chain protein (MYO-3::GFP) (29) to examine muscle structure in the *coq-1* RNAi knockdown animals. We detected no morphological abnormalities in body wall muscle (Fig. S4*F*), verifying that the Unc phenotype associated with this RNAi construct is not the result of muscle degeneration. We also examined vulval muscle, reasoning that defects in these muscles might explain the Egl phenotype. However, we observed no structural differences between vulval muscles in control versus *coq-1* RNAi-treated animals.

Although our results do not preclude the possibility that non-GABAergic neurons or muscle are functionally affected by *coq-1* RNAi, the earliest degenerative phenotype was exclusively observed in GABA neurons. In fact, functional deficits in other neurons or muscle are likely because the movement defects of mutants in which GABA neurons are selectively disabled are less severe than the paralyzed adult phenotype that results from RNAi ablation of *coq-1* (30). Ultimately, *coq-1* RNAi-treated adults and become completely paralyzed and show widespread cell swelling and tissue necrosis after 5 d of RNAi treatment; non-GABA neuron cell types were similarly affected in older *coq-2* and *coq-3* adults.

***coq-1* RNAi-Mediated Degeneration Is Ca^{2+}-Dependent.** Calcium is a key effector of neurodegenerative diseases involving mitochondrial dysfunction (31). Calreticulin (*crt-1*) functions in the ER lumen to maintain Ca^{2+} levels for ready release on appropriate

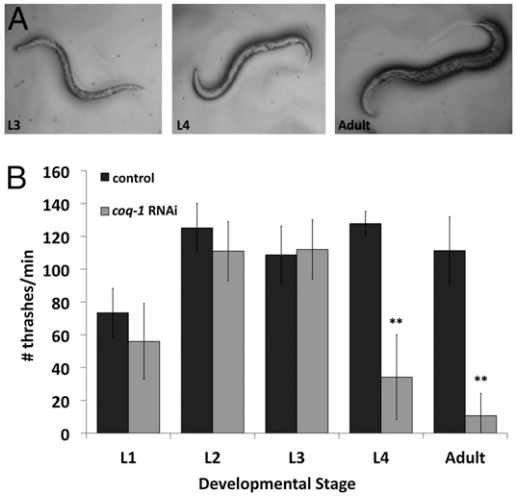

Fig. 1. RNAi knockdown of *coq-1* results in progressive loss of motor coordination. (*A*) Examples of the abnormal postures adopted by L4 larvae and adults after exposure to *coq-1* RNAi. (*B*) Swimming assay quantifying movement defects (bars represent avg ± SD, $^{**}P < 0.005$, $n = 10$).

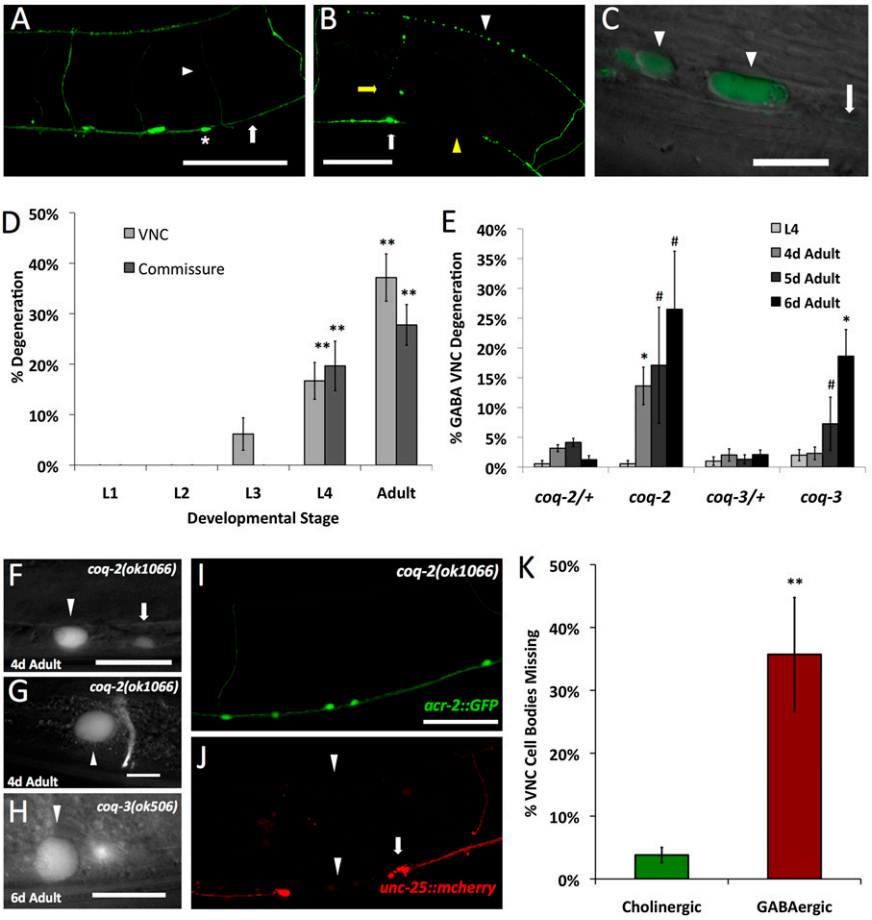

Fig. 2. CoQ depletion results in GABA neuron degeneration. (*A*) Wild-type adult GABA neuron cell bodies (asterisks), processes in the ventral nerve cord (VNC) (arrow), and dorsally projecting commissures (arrowhead) labeled with *unc-25::GFP*. (*B*) Adult *coq-1* RNAi-treated worm showing signs of degeneration (white arrowhead, dorsal process beading; white arrow, degenerating GABA cell body; yellow arrow, degenerating commissure; yellow arrowhead, VNC process break). (Scale bars: 50 μm.) (*C*) *unc-25::GFP*-labeled GABA neurons appear swollen in *coq-1* RNAi-treated adults. Shown is the ventral view of GABAergic cell bodies (arrowheads) and VNC (arrow). (Scale bar: 10 μm.) (*D*) Quantification of progressive degeneration of GABA neuron VNC processes and commissures ($n \geq 3$, error bars = SEM; $^{**}P < 0.001$ vs. empty vector-treated animals). No degeneration was observed in empty vector-treated animals. (*E*) Progressive GABA neuron degeneration in *coq-2(ok1066)* and *coq-3(ok506)* mutants. GABA neuron degeneration was not observed in L1–L3 larval stages but was detectable in L4 larvae and pronounced in adults (day, d). $^{\#}P < 0.04$ and $^{*}P < 0.001$ vs. heterozygous controls, $n \geq 20$ for each developmental stage. (*F*) Enlarged cell size of degenerating GABA neuron (arrowhead) compared with an unaffected GABA neuron cell body (arrow). (*F–H*) Swollen D-class (*F* and *H*) and RME GABA neurons (*G*) in *coq-2(ok1066)* (*F* and *G*) and *coq-3(ok506)* (*H*) mutants visualized with *unc-25::GFP*. (Scale bars: 10 μm.) Cholinergic neurons (*acr-2::GFP*) (*I*) are unaffected, whereas GABA neurons (*unc-25::mcherry)* (*J*) show degenerating processes (arrowheads) and cell bodies (arrows) in a single *coq-2 (ok1066)* animal. (Scale bar: 30 μm.) (*K*) VNC *acr-2::GFP*-cholinergic neurons were counted from VA2–VA11 (30 total) and VNC *unc-25::mcherry*-GABA neurons from VD3–VD11 (13 total). Fraction of missing cholinergic vs. GABA neurons in *coq-2(ok1066)*. $^{**}P < 0.001$, $n = 16$. Anterior left, ventral down in *A–C* and *F–J*.

stimuli (32). In *C. elegans*, *crt-1* mutants block necrotic degeneration of motor neurons (33). We tested a *crt-1*-null mutant (*bz30*) in our paradigm and found that it prevented the progressive degeneration of GABA neurons in *coq-1* RNAi-treated animals (Fig. 3*A*). The calcium chelating agent EGTA (0.5 mM) was similarly protective (Fig. 3*A*). Taken together, these results demonstrate that Ca^{2+} signaling is important for *coq-1* RNAi-mediated degeneration of GABA neurons.

Specific Apoptotic Genes Are Required for GABA Neuron Cell Death in CoQ-Depleted Animals. Ca^{2+} release from the endoplasmic reticulum can result in necrotic (34) or apoptotic (35) cell death. The cell swelling observed in *coq-1* knockdown mutants is consistent with a necrotic mechanism. To investigate whether apoptotic genes are required for GABA cell death, we tested mutants of the canonical apoptotic pathway, *egl-1*, *ced-9, ced-4,* and *ced*-3 (Fig. 3 and Fig. S5). In apoptotic cells, the BH3-only protein, EGL-1, interacts with CED-9 (Bcl-2) to release CED-4 (Apaf-1) for activation of CED-3 (caspase) that, in turn dismembers sensitive target proteins that normally maintain cellular integrity (36). Loss-of-function mutations in *egl-1* did not prevent GABA neuron degeneration after *coq-1* knockdown. A gain-of-function allele of the anti-apoptotic protein CED-9 was similarly ineffective (Fig. S5). Surprisingly, mutations in *ced-4* and *ced-3* attenuated both the *coq-1* RNAi-dependent GABA neuron degeneration and Unc phenotype (Fig. 3 *A* and *C*). These genetic results indicate that CED-4 is activated in *coq-1*–depleted GABA neurons by a pathway that is not regulated by either *egl-1* or *ced-9*. In addition to strongly suppressing degeneration of GABA ventral nerve cord (VNC) processes, the *ced-4* mutation also attenuates the elimination of GABA neuron cell bodies in *coq-1* RNAi-treated animals (Fig. S6), which suggests that GABA neuron cell death is blocked. This result is supported by our finding of comparably strong *ced-4* suppression of GABA neuron degeneration in *coq-3 (ok506)* animals (Fig. 3*B*). Curiously, we note that a strong loss-of-function allele of *ced-3* only partially rescued the uncoordinated and GABA neuron degeneration phenotypes, whereas the *ced-4* mutation affords almost complete suppression of both defects in

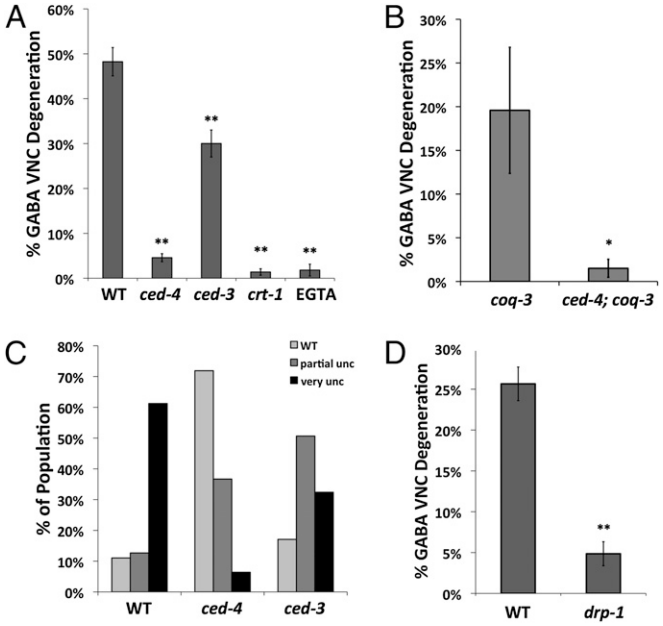

Fig. 3. Apoptotic genes and calcium and are required for *coq-1* RNAi-induced GABA neuron degeneration. (*A*) *ced-4(n1162), ced-3(n717), crt-1(bz30)*, and EGTA (0.5 mM)-treated animals were tested for *coq-1* knockdown-induced GABA neuron degeneration. All results were obtained from *coq-1* RNAi-treated animals containing *eri-1(mg366); unc-25::GFP* (*Materials and Methods*). GABA neurons were scored in adults as in Fig. 2. $n \geq 3$ experiments for each genotype. Error bars = SEM, $^{**}P < 0.0001$ vs. WT (wild-type). (*B*) *ced-4 (n1162)* suppresses GABA neuron degeneration in *coq-3(ok506)* adults, $^{*}P < 0.02$ vs. *coq-3(ok506), n* > 14. (*C*) Movement assay of *ced-4* and *ced-3* mutants treated with *coq-1* RNAi. Adults were tapped on the head and the tail and scored for movement: "partial unc" worms are uncoordinated and "very unc" worms are unable to move, *n* > 100 worms. (*D*) *drp-1* (*tm1108*) suppresses *coq-1* knockdown-induced GABA degeneration, $^{**}P < 0.001$ vs. WT, *n* > 60.

coq-1 RNAi-treated animals (Fig. 3). This observation is indicative of an additional *ced-3*-independent pathway functioning downstream of *ced-4* to evoke GABA neuron degeneration. The cytoplasmic swelling of CoQ-depleted GABA neurons in conjunction with the essential roles of CED-4/Apaf-1 and CED-3/caspase suggest that loss of CoQ synthetic activity triggers selected components of both programmed cell death and necrotic pathways.

***coq-1* Knockdown-Induced Cell Death Depends on the Mitochondrial Fission Gene *drp-1*.** Calcium has been shown to stimulate mitochondrial fission (37), which, in turn, has been linked to both apoptosis and necrosis in mammals (6, 38) and in *C. elegans* (39). We reasoned that CoQ deficiency might sensitize mitochondria to Ca^{2+}-dependent fission-related cell death. To test this idea, we used RNAi knockdown of *coq-1* in *drp-1* mutant animals (Fig. 3*D*). DRP-1 (dynamin-related protein) has been shown to function in both mitochondrial fission (39) and in a *ced-3*–dependent and *ced-9*–independent cell death pathway (40). The *drp-1* mutant blocked GABA neuron degeneration (Fig. 3*D*) and rescued the movement defect (Fig. S7), thereby implicating the fission machinery in the pathology associated with *coq-1* knockdown in *C. elegans*. Similar results were obtained in *coq-1*+*drp-1* double RNAi experiments (Fig. S8*A*). We also used double RNAi to test *fzo-1,* a gene required for mitochondrial fusion (41). In this case, *fzo-1* knockdown actually enhances the GABA neuron degeneration phenotype of *coq-1*–deficient animals as expected for a treatment that impairs mitochondrial fusion (Fig. S8 *B* and *C*). The conclusion that mitochondrial function is defective in CoQ-depleted animals is also supported by our finding that mitochondrial ATP levels are significantly reduced by *coq-1* RNAi treatment (Fig. S9). Together, these results indicate that CoQ depletion disrupts mitochondrial function and activates a cell death pathway that depends on the mitochondrial morphogenesis gene, *drp-1* (Fig. 4).

Discussion

We have shown that depletion of Coenzyme Q (CoQ) through RNAi and genetic ablation of CoQ biosynthetic genes, *coq-1*, *coq-2*, and *coq-3*, leads to the progressive loss of motor coordination and preferential degeneration of GABA neurons. The mechanism of cell death relies strongly on the function of *ced-4* (Apaf-1) and partially depends on *ced-3* (caspase) activity. The GABA neuron pathology that accompanies CoQ depletion also involves calcium signaling, possibly from ER stores, and the mitochondrial fission protein DRP-1 (Fig. 4). These results emphasize an important role for CoQ in neuron survival and link mitochondrial dysfunction to a calcium-dependent mechanism of selective neuron degeneration in *C. elegans.*

CoQ Depletion Triggers a Cell Death Pathway Featuring Elements of Both Apoptosis and Necrosis. Our findings suggest that CoQ depletion in *C. elegans* GABA neurons triggers a cell death mechanism that includes features of both apoptotic and necrotic pathways. The affected GABA neurons morphologically resemble necrotic cells (i.e., swollen soma) yet require downstream components of apoptosis, *ced-4*, and *ced-3*. In *C. elegans*, necrosis and apoptosis have been reported to function as separate pathways (42); however, our results indicate that a subset of apoptotic genes is required for a pathology (cell swelling) normally restricted to a necrotic mechanism. In the canonical apoptotic pathway, CED-9 tethers CED-4 to the outer mitochondrial membrane. EGL-1 binding to CED-9 releases CED-4 for incorporation into the apoptosome and subsequent activation of CED-3/caspase (36). Our finding that neither EGL-1 nor CED-9 affects *coq-1* RNAi-induced GABA neuron death indicates that CED-4 must be activated by an alternative mechanism in this case. Our results also suggest that CED-4 is likely to activate other downstream effectors in addition to CED-3. CED-4–dependent, CED-3–independent cell killing programs have been reported for yeast in which CED-4 is overexpressed (43) and in *C. elegans* with RNAi knockdown of the presumptive anti-apoptotic protein, ICD-1 (44). Mechanisms whereby CED-4 might activate these pathways are unknown. In the future, it will be interesting

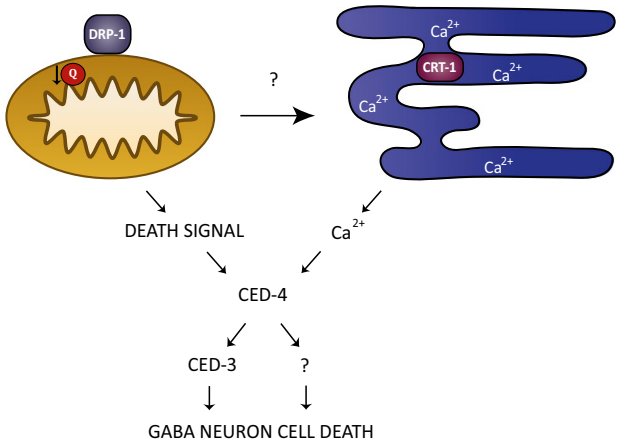

Fig. 4. Model of GABA neuron death induced by CoQ depletion. Reduced CoQ (Q) function in mitochondria activates an unknown signal that may evoke CRT-1–dependent (calreticulin) Ca^{2+} release from the endoplasmic reticulum. GABA neuron degeneration requires CED-4 and at least one additional downstream pathway that functions in parallel to CED-3 (caspase). DRP-1, a dynamin-related protein that drives mitochondrial fission, is also required for degeneration in CoQ-depleted GABA neurons.

to determine whether the downstream CED-3-independent program that is triggered in CoQ-depleted GABA neurons includes other members of the *C. elegans* caspase family (45) or novel components (46).

Mitochondrial Morphogenesis Protein, DRP-1, Is Required for the Death of CoQ-Depleted GABA Neurons. Mitochondrial fragmentation, which can trigger apoptosis, depends on the dynamin-related protein, DRP-1 (7). Previous studies have shown that *coq-1* RNAi induces mitochondrial fragmentation in *C. elegans* (47). Thus, our finding that DRP-1 is required for the execution of GABA neurons in CoQ-deficient animals (Fig. 3 and Fig. S8) is consistent with a model in which the mitochondrial fragmentation or "excessive fission" accounts for the neurodegenerative effect of CoQ depletion. Enhancement of this effect by RNAi knockdown of the mitochondrial fusion gene, *fzo-1*, also supports this idea (Fig. S8). In addition to mediating mitochondrial fusion, the mammalian homolog of FZO-1, Mfn2, promotes expression of ETC proteins (48). This finding suggests that the loss of mitochondrial energy production in *fzo-1*–deficient animals could also contribute to cell death in CoQ-depleted GABA neurons. Although mitochondrial fission has been linked to apoptosis in mammals, conflicting results have been obtained in *C. elegans* (39, 40). However, our finding that DRP-1 is required for GABA neuron degeneration in *C. elegans* parallels results with mammalian cells showing that mitochondrial fission is a necessary step in experimental models of neurodegenerative disease resulting from mitochondrial injury. For example, exposure of cortical neurons to high concentrations of the neurotransmitter NO (nitric oxide) triggers a caspase-independent death pathway that requires mitochondrial fission (46). NO-induced degeneration is accompanied by ATP depletion and production of free radicals. Both of these traits are also induced by either environmental or genetic defects that block mitochondrial ETC (5, 49, 50). We therefore speculate that CoQ depletion in *C. elegans* disrupts mitochondrial ETC function, which in turn, triggers a degenerative pathway that shares common features such as downstream signals and effector molecules that also mediate neurodegeneration in mammalian neurons.

CoQ Deficiency in *C. elegans* as a Model for Human Disease. The selective, age-dependent death of GABA neurons and loss of coordinated movement seen in CoQ-depleted worms are shared features of CoQ deficiency in humans. The most common outcome of this deficiency is cerebellar ataxia (51), although mutations that disrupt CoQ biosynthesis can also result in a more severe early-onset pathology involving multiple tissues (14, 25).

Despite the rarity of CoQ deficiency in humans, it shares important pathologies with prevalent glutamine repeat diseases. These include the autosomal dominant Huntington's Disease (HD) and spinocerebellar ataxias. The age-related death of medium spiny GABAergic neurons of the striatum occurs in HD (52) and GABAergic Purkinje cells are significant targets for degeneration in the cerebellar ataxias (53). Recent studies have linked neuronal degeneration in HD to disrupted mitochondrial function (54). For example, exogenous CoQ is neuroprotective in mouse models of HD (55). In addition, the pathology induced by overexpression of mtHtt in mammalian cells or in C. *elegans* depends on the mitochondrial fission machinery (8); *ced-3* function is also required in *C. elegans* (56). Lastly, *coq-1* RNAi in *C. elegans* enhances the toxicity of a mutant form of human tau that has been implicated in the pathology of AD (57). It is striking that salient features of these human genetic diseases including selective neuron sensitivity, mitochondrial dysfunction, Ca^{2+} dependence, and aberrant cell death (58, 59) are observed in *C. elegans* with the depletion of CoQ. These parallels suggest that neurodegenerative mechanisms arising from mitochondrial dysfunction are conserved and, thus, can be effectively delineated by studies in *C. elegans*.

Materials and Methods

Strains and Maintenance. *C. elegans* strains (*SI Materials and Methods*) were maintained at 20 °C unless otherwise indicated (60). GFP reporters and the *ced-3(n717), ced-4(n1162), crt-1(bz30),* and *drp-1(tm1108)* mutant alleles were crossed into the RNAi-hypersensitive strain *eri-1(mg366). eri-1(mg366)* was verified by using single-worm PCR (*SI Materials and Methods*). *unc-25::GFP(juIs76)* was crossed into *coq-2(ok1066)/hT2* and *coq-3(ok506)/nT1* to mark GABA neurons, and the cholinergic neuron reporter *acr-2::GFP(juIs14)* and *unc-25::mcherry(wdEx658)* were crossed into *coq-2(ok1066)/hT2.* The *unc-25::mcherry* was generated by bombardment (61) with the plasmid pMLH41 [*punc-25::mcherry, unc-119(+)*] (*SI Materials and Methods*).

RNAi. RNAi assays were performed by feeding (62). Briefly, 3 mL of LB/ampicillin (50 mg/mL) was inoculated with 30 μL of overnight culture and grown in a 37 °C shaker. At $OD_{600} \approx 0.800$, the culture was diluted to 6 mL with LB/amp + IPTG (40 mM final concentration) and incubated at 37 °C for another 4 h. Bacteria were pelleted, dispersed in 250 mL of M9/IPTG, and spread onto 60-mm NGM plates. L4 larvae were added to plates and incubated at 20 °C for 5 d before scoring progeny. RNAi clones were sequenced to confirm inserts. EGTA (0.5 mM) was added to media before pouring plates.

Degeneration Assay. Animals were anesthetized in 0.1% tricaine/tetramisole on 2% agar pads (63). Percent VNC degeneration was determined by dividing the number of degenerating VNC intervals between GABA motor neuron cell bodies by the total number of intervals between all GABA neuron cell soma per animal (for example, if 3 VNC intervals are scored as degenerating of a total of 12 total intervals in a given animal, the percent VNC degeneration would be 3/12 or 25%). Commissures were scored similarly, with the number of degenerating commissures divided by the total number of discrete GABA neuron commissures per animal (16 total). A 63× objective was used for scoring. The observer was blinded to experimental versus control samples to avoid bias. *unc-25::GFP(juIs76)* was used to mark GABA neurons for experiments with *coq-1* RNAi, *coq-1(ok749), coq-2(ok1066),* and *coq-3(ok506). unc-25::mcherry(wdEx658)* was also used to mark GABA neurons in *coq-2(ok1066)* for results shown in Fig. 2. Swimming assays were performed as described (21). *coq-3(ok506)* and *coq-3(ok506); ced-4(n1162)* animals were scored as 7-d-old adults (Fig. 3*B*). The following mutants and treatments did not perturb GABA neuron morphology: *ced-4(n1162), ced-3(n717); crt-1(bz30); drp-1(tm1108),* CoQ10, 0.5 mM EGTA.

Microscopy. Animals were imaged in a Zeiss Axioplan compound microscope with a CCD camera (ORCA I; Hamamatsu). Confocal images were obtained on a Zeiss LSM 510 confocal microscope and on a Leica TCS SP5 confocal microscope.

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