Invertebrate Models for Coenzyme Q₁₀ Deficiency

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Key Words

Caenorhabditis elegans · Coenzyme Q deficiency ·
Drosophila melanogaster

Abstract

The human syndrome of coenzyme Q (CoQ) deficiency is a heterogeneous mitochondrial disease characterized by a diminution of CoQ content in cells and tissues that affects all the electron transport processes CoQ is responsible for, like the electron transference in mitochondria for respiration and ATP production and the antioxidant capacity that it exerts in membranes and lipoproteins. Supplementation with external CoQ is the main attempt to address these pathologies, but quite variable results have been obtained ranging from little response to a dramatic recovery. Here, we present the importance of modeling human CoQ deficiencies in animal models to understand the genetics and the pathology of this disease, although the election of an organism is crucial and can sometimes be controversial. Bacteria and yeast harboring mutations that lead to CoQ deficiency are unable to grow if they have to respire but develop without any problems on media with fermentable carbon sources. The complete lack of CoQ in mammals causes embryonic lethality, whereas other mutations produce tissue-specific diseases as in humans. However, working with transgenic mammals is time and cost intensive, with no assurance of obtaining re-

sults. *Caenorhabditis elegans* and *Drosophila melanogaster* have been used for years as organisms to study embryonic development, biogenesis, degenerative pathologies, and aging because of the genetic facilities and the speed of working with these animal models. In this review, we summarize several attempts to model reliable human CoQ deficiencies in invertebrates, focusing on mutant phenotypes pretty similar to those observed in human patients.

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Overview of Coenzyme Q Function and Biosynthesis

Coenzyme Q (CoQ) is the only lipid-soluble redox compound that is synthesized by all aerobic organisms studied to date, and it is essential for ATP production by the mitochondrial oxidative phosphorylation system. The redox activity of the benzoquinone ring allows CoQ to both flow electrons from mitochondrial complexes I and II to complex III and transport protons across the inner membrane [Albert et al., 2008]. CoQ is also the electron acceptor of several dehydrogenases, including those in fatty acid β -oxidation and in pyrimidine nucleotide synthesis [Lopez-Lluch et al., 2010; Nowicka and Kruk, 2010]. Additionally, CoQ functions as a potent lipid soluble antioxidant in the plasma membrane and elsewhere [Lopez-Lluch et al., 2010].

Fig. 1. Pathway for CoQ biosynthesis in eukaryotes. Proteins involved in the biosynthetic pathway of CoQ are named as COQs (in capital letters). The isoprenyl tail proceeds from the mevalonate pathway and takes part in extramitochondrial membranes. COQ1 is the last enzyme of the pathway and defines the numbers (n) of isoprene units in the polyprenyl tail which is 6 for *S. cerevisiae*, 8 for *E. coli*, 9 in *C. elegans*, and 10 in humans. The redox-active head of CoQ (pHB, para-hydroxybenzoate) is synthesized from tyrosine in the cytosol and linked to the polyprenylated tail by

COQ2 in mitochondria. The rest of the proteins are located inside this organelle and are responsible for modifications in the redox-active head of CoQ. COQ6 and COQ7 are hydroxylases, COQ3 and COQ5 are methylases, COQ4, COQ8, COQ9, and COQ10 are proteins with unknown function. DMQ (2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinol) is an intermediate compound of the biosynthetic pathway that is accumulated without the presence of an active COQ7 hydroxylase. CoQ is finally synthesized as ubiquinol (the reduced form of CoQ).

CoQ biosynthesis depends on a highly conserved multi-enzyme complex [Bentinger et al., 2010] that involves at least 10 COQ genes, and mutations in any of these genes cause primary CoQ₁₀ deficiencies in human beings [Rahman et al., 2001]. Figure 1 represents the biosynthetic pathway of CoQ in eukaryotes, and table 1 compares the genes of yeast, humans, and invertebrates.

The polyisoprene tail of CoQ is produced by the COQ1 protein in yeast or by a dimeric enzyme in humans composed by a catalytic subunit (PDSS1) and a regulatory one

(PDSS2). COQ1 defines the length of the polyisoprene tail of CoQ and, because this gene differs between species, each organism presents a specific CoQ isoform, i.e. CoQ₆ for the yeast *Saccharomyces cerevisiae*, CoQ₈ for *Escherichia coli*, CoQ₉ in *Caenorhabditis elegans*, and CoQ₁₀ in humans. The subscript numbers indicate the quantity of isoprene units and therefore the length of the CoQ tail. The isoprenyl tail originates from the mevalonate pathway which takes place in the endoplasmic reticulum and is shared for cholesterol biosynthesis, t-RNA and protein

Table 1. Genes required for CoQ biosynthesis

<i>S. cerevisiae</i> ^a	<i>C. elegans</i> ^b	<i>D. melanogaster</i> ^c	Humans ^d
COQ1 (YBR003W)	<i>coq-1</i> (C24A11.9)	<i>qlless</i> (<i>cg31005</i>) <i>cg10585</i> (<i>pdss2</i>)	COQ1/PDSS1 (COQ1, subunit 1) PDSS2/DLP1 (COQ1, subunit 2)
COQ2 (YNR041C)	<i>coq-2</i> (F57B9.4)	<i>coq2</i> (<i>cg9613</i>)	COQ2
COQ3 (YOL096C)	<i>coq-3</i> (Y57G11C.11)	<i>coq3</i> (<i>cg9249</i>)	COQ3
COQ4 (YDR204W)	<i>coq-4</i> (T03F1.2)	<i>coq4</i> (<i>cg32172</i>)	COQ4
COQ5 (YML110C)	<i>coq-5</i> (ZK652.9)	<i>coq5</i> (<i>cg2453</i>)	COQ5
COQ6 (YGR255C)	<i>coq-6</i> (K07B1.2)	<i>coq6</i> (<i>cg7277</i>)	COQ6 (two isoforms: A and B)
COQ7/CAT5 (YOR125C)	<i>clk-1</i> (ZC395.2)	<i>coq7</i> (<i>cg14437</i>)	COQ7
COQ8 (YGL119W)	<i>coq-8</i> (C35D10.4)	<i>coq8</i> (<i>cg32649</i>)	COQ8/ADCK3/CABC1
COQ9 (YLR201C)		<i>coq9</i> (<i>cg30493</i>)	COQ9
COQ10 (YOL008W)	<i>coq-10</i> (R144.13)	<i>coq10</i> (<i>cg9410</i>)	COQ10A (homolog A) COQ10B (homolog B)

^a<http://browse.yeastgenome.org/fgb2/gbrowse/scgenome/>; ^bwww.wormbase.org; ^c<http://flybase.org>; ^dwww.genecards.org.

synthesis, protein glycosylation, and both geranylation and farnesylation of proteins for subcellular localization [Rauthan and Pilon, 2011].

The precursor of the redox-active head of CoQ (*p*HB, para-hydroxybenzoate) derives of tyrosine and is linked to the polyisoprene tail by the transferase COQ2 inside mitochondria. After that, the polyisoprenylated ring is subjected to various modifications in this organelle, including a decarboxylation (unknown protein), 3 hydroxylations (one carried out by COQ6 and another by COQ7; the protein responsible for the third is unknown), 1 C-methylation (done by COQ5), and 2 O-methylations (both by COQ3).

No catalytic functions have been established for COQ4, COQ8, COQ9, and COQ10, although they are suggested to be regulatory components of the biosynthetic pathway: COQ8 is supposed to be a regulatory kinase and COQ10 a chaperon for complex stability with CoQ-binding properties. See GeneCard[®] from 'The Human Gene Compendium' at www.genecards.org for a full description of the genes.

Summarizing the Human Syndrome of CoQ₁₀ Deficiency

Given the central role that CoQ plays in metabolism, it is not surprising that mutations involving its biosynthesis lead to severe phenotypes and disorders. Bacteria and yeast harboring mutations in any of the genes involved in the CoQ biosynthetic pathway are unable to grow on

media with non-fermentable carbon sources [Tran and Clarke, 2007]. The complete lack of CoQ in mammals, i.e. knockout mutations in mice, cause embryonic lethality [Levavasseur et al., 2001; Takahashi et al., 2008], whereas other mutations produce tissue-specific diseases in animals [Peng et al., 2008] as well as in humans [Quinzii and Hirano, 2010; Rahman et al., 2012].

Primary CoQ₁₀ deficiencies are described as heterogeneous mitochondrial diseases [Rahman et al., 2001] (OMIM 607426). Clinical presentations include encephalomyopathy with lipid storage myopathy and myoglobinuria [Sobreira et al., 1997], ataxia and cerebellar atrophy [Artuch et al., 2006], severe infantile encephalomyopathy with renal failure [Salviati et al., 2005], isolated myopathy [Horvath et al., 2006], and nephrotic syndrome [Heeringa et al., 2011]. Secondary CoQ₁₀ deficiency has also been associated with diverse mitochondrial diseases [Quinzii et al., 2006; Gempel et al., 2007; Montero et al., 2008; Haas et al., 2009; Cotan et al., 2011; Miles MV et al., 2011]. In all of these conditions, CoQ₁₀ supplementation partially improves symptoms [Montini et al., 2008; Pineda et al., 2010; Schon et al., 2010] and usually induces a return to normal growth and respiration in CoQ₁₀-deficient fibroblasts [Lopez-Martin et al., 2007; Lopez et al., 2010; Cotan et al., 2011]. Thus, adaption of somatic cells to a pathogenic mutation may affect both onset and course of CoQ₁₀ deficiency in each patient or animal model.

Supplementation with external CoQ₁₀ is the main attempt to address these pathologies. However, quite variable results have been obtained, ranging from little re-

sponse to a dramatic recovery [Quinzii and Hirano, 2010; Rahman et al., 2012]. Here, we present the importance of modeling human CoQ deficiencies in animal models to understand the genetics and the pathology of this disease.

CoQ Deficiency in the Worm *C. elegans*

C. elegans is a free-living transparent nematode (round-worm) which has been used extensively as a model organism for molecular and developmental biology [Albert et al., 2008] mainly due to the easy way to study the loss of function of a gene by silencing with RNAi, which can be done by simply feeding the worms with transgenic bacteria expressing RNA complementary to the gene of interest [Kamath et al., 2003].

Many genes take part in CoQ biosynthesis in *C. elegans* (table 1), although some of them have no catalytic activity. Interference with RNAi of these genes leads to CoQ deficiency in the nematode [Asencio et al., 2003]. In addition, inhibition of mitochondrial respiration or CoQ production causes increased expression of cell-protective genes and produces a rise of mitochondrial DNA content compared to wild-type nematodes which is related with slowing down of behavioral rates and lifespan extension [Cristina et al., 2009]. Besides its effects on biological rates and longevity, CoQ contributes to the robustness of specific developmental processes, like extracellular matrix degradation driving severe abnormalities in the hypodermis, abnormal gonads development and germ line mortality, and alteration of behavioral rates and the aging process. These and other phenotypes due to CoQ deficiency will be described below.

C. elegans clk-1 mutants lack the production of CoQ₉ and accumulate the intermediate demethoxy-ubiquinone (DMQ₉), the substrate of the COQ7 O-methyltransferase [Jonassen et al., 2001]. These worms display a pleiotropic phenotype, including slowed pharyngeal pumping and abnormalities in defecation, movement, embryogenesis, and larvae development [Wong et al., 1995]. Interestingly, adult *clk-1* mutants reproduce for many generations, live longer than wild-type N2 worms, and show a reduction of fertility and slow behaviors [Wong et al., 2005], although they fail to develop and become sterile when fed a CoQ-less diet of *E. coli* [Jonassen et al., 2001]. These investigations suggest that either dietary CoQ₈ from *E. coli* [Jonassen et al., 2001] or the intermediate DMQ₉ [Levavasseur et al., 2001] could supply the CoQ₉ role in mitochondria to drive respiration, giving the long-lived phenotype shown by *clk-1* mutants.

However, it has been demonstrated that mitochondria from *clk-1* mutants synthesize a small amount of CoQ₉ [Arroyo et al., 2006] as does the equivalent mutant yeast when grown in long-term cultures [Padilla et al., 2004]. The small amount of CoQ₆ synthesized by this mutant yeast is necessary for assembly and stability of the mitochondrial complex III to allow subsequent respiration [Santos-Ocaña et al., 2002]. Thus, either the small amount of CoQ₉ endogenously synthesized by *clk-1* mutants or the CoQ₈ coming from diet would stabilize complex III, and the accumulated intermediate DMQ₉ would support a sufficient level of respiration.

Clk-1 mutants show defects in the complexes I–III of CoQ-dependent mitochondrial activities, while activity from complex II–III remain unchanged [Kayser et al., 2004]. Recent studies indicate that DMQ₉ present in *clk-1* mutants could inhibit complex I but not complex II [Yang et al., 2011]. The kinetics of complex I shows a higher Km for CoQ than complex II [Lenaz, 1998]. As a result, the limiting amount of CoQ₉ in *clk-1* mutant affects complexes I–III activity more than II–III activity. On the other hand, the intermediate compound DMQ₉ competes with CoQ₉, for DMQ₉ being less effective than CoQ₉ in electron transfer. As a result, DMQ₉ could be a functional inhibitor of electron transport in the respiratory chain at the level of complex I [Yang et al., 2011]. However, several studies have concluded that DMQ can neither substitute the CoQ function in mitochondrial respiration [Padilla et al., 2004] nor its redox activity in the plasma membrane [Arroyo et al., 2006]. Moreover, DMQ is unable to serve as an effective antioxidant [Padilla et al., 2004]. Thus, the subsequently reduction in the electron flow of the respiratory chain through inhibition of complex I could scavenge reactive oxygen-species production, being the reason of the life extension phenotype shown by *clk-1* mutants.

Several attempts have been done to address the *clk-1* phenotype by administering exogenous CoQ, although the effects on behavioral rates, mitochondrial function, and lifespan are controversial. The administration of a water-soluble CoQ₁₀ (Aqua Q₁₀L10) restored the behavioral rates, such as the pharyngeal pumping and defecation, and reduced both mean and maximal lifespan to levels comparable to those of wild-type nematodes [Takahashi et al., 2012]. In contrast, *clk-1* mutant nematodes, fed genetically engineered bacteria that produce CoQ₁₀ instead of their own CoQ₈, exhibit a decrease in mitochondrial oxidative damage and a greater extension of lifespan, although the mitochondrial respiration rates were not improved [Yang et al., 2009]. However, the mu-

tant phenotype was not enhanced if *clk-1* nematodes were fed with CoQ₆, CoQ₇, CoQ₈, or even with CoQ₉-repleted bacteria, indicating that CoQ₁₀ is the CoQ isoform with higher antioxidant properties. A study that supports the antioxidant role of CoQ₁₀ was done in wild-type animals, demonstrating that dietary supplement of CoQ₁₀ extended the lifespan of worms by releasing oxidative stress [Ishii et al., 2004].

Other CoQ-deficient *C. elegans* described to date have a more severe phenotype and are much less responsive to dietary CoQ supplementation when compared to *clk-1*. First generation of *coq-1* mutants are sterile and present both morphological defects and improper development of the organs [Gavilan et al., 2005]. Also, knockdown of *coq-1* by feeding bacteria producing RNAi against this gene results in the progressive degeneration of GABA neurons and age-dependent loss of motor coordination [Earls et al., 2010]. This uncoordinated phenotype emerges during late larval development, progresses in adulthood, and proceeds with a selective cell death pathway activation where apoptotic and mitochondrial fission genes take part, which is a clear signal of initiation of a mitophagy process within these cells as in humans [Rodríguez-Hernández et al., 2009; Cotan et al., 2011]. However, neurons and body muscle cells that use other neurotransmitters, such as dopamine, acetylcholine, serotonin, or glutamate, were more resistant to CoQ depletion [Earls et al., 2010]. Similar phenotypes were described within the same work for knockdown of the genes *coq-2* and *coq-3*.

C. elegans harboring partial deletions of the *coq-3* gene show a more diverse phenotype: *coq-3(qm188)* mutants do not develop reproductive organs and subsequently become sterile [Hihi et al., 2002], whereas *coq-3(ok506)* mutants retain fertility for the first generation, although only a small fraction of the second generation survives into adulthood [Gomez et al., 2012].

An attempt to rescue the defective phenotype in these mutants was done by feeding worms with either OP50 wild-type bacteria (containing its own CoQ₈ isoform) or NovaSOL[®] Q10 (a water-soluble commercial CoQ₁₀ provided by AQUANOVA, Germany). One third of the *coq-3(ok506)* larvae developed if fed with OP50 bacteria, although no successful development was achieved in the *coq-3(qm188)* strain [Gomez et al., 2012]. Similarly to *clk-1* mutants, *coq-1*, *coq-2*, or *coq-3* mutant worms fed with a diet containing the CoQ-defective bacteria GD1 failed in larval development. However, if this diet was supplemented with NovaSOL[®] Q10, about 30% of the *clk-1* larvae completed development, although no rescue was

achieved for any *coq-1*, *coq-2*, or *coq-3* mutants [Gomez et al., 2012].

Nevertheless, a full rescue of the *coq-3* mutant phenotype was achieved by an extra-chromosomal array containing the own *C. elegans coq-3* gene despite the phenotypic disparity shown by *coq-3(qm188)* and *coq-3(ok506)* strains [Gomez et al., 2012]. Both transgenic worms showed a dramatic rescue, illustrating the crucial role the endogenous synthesized CoQ₉ isoform plays in fertility and development.

First-generation homozygous *coq-8* mutants show developmental delay, decreased fertility, and both extracellular matrix degradation and severe abnormalities in the hypodermis, suggesting that it is detached from muscle cells [Asencio et al., 2009]. Most of the embryos produced are arrested between the second and eighth cell division after fertilization, and their second-generation progenies, which lack the maternal inherited CoQ₉, become sterile because they do not properly develop gonads.

Coq-8 knockouts do not present differences in longevity in respect to the wild-type worm if fed a CoQ-rich diet with wild-type *E. coli*, although the lifespan is reduced to a half if they are fed the CoQ-deficient GD1 bacteria [Asencio et al., 2009]. However, no improvement of fertility was observed with a CoQ-rich diet, but embryo production was increased, and most of the embryos completed the development if uridine was added simultaneously to the plates containing CoQ-repleted *E. coli*, indicating that the pyrimidine nucleotide pathway necessary for DNA synthesis (and the subsequent embryo arrest after fertilization) cannot be restored by external CoQ [Asencio et al., 2009].

Finally, other mitochondrial disorders that alter the CoQ₉ level have been described in *C. elegans*, even when the disease-causing mutation does not affect any of the genes directly involved in CoQ synthesis. An example is the *mev-1* mutant which is defective in the mitochondrial complex II and presents a reduction of 25% in the content of CoQ₉ [Vasta et al., 2011]. Other mutations disturbing the mitochondrial complexes I and III, like the *gas-1* and *isp-1* mutants, respectively, do not show significant reduction of CoQ₉ compared to that of wild-type nematodes. However, all of them have altered rates of the reduced and oxidized forms of CoQ, ubiquinol and ubiquinone, respectively. As expected by deficiency in such mitochondrial respiratory complexes, both *gas-1* (complex I defective) and *mev-1* (complex II defective) mutants are unable to reduce CoQ₉ in mitochondria and present a significant increase in the amount of ubiquinone, whereas the *isp-1* (complex III defective) mutants

are unable to oxidize CoQ₉ and show higher levels of ubiquinol [Vasta et al., 2011]. So, the phenotype described by these mutants could be classified as a secondary CoQ₉ deficiency in *C. elegans*.

CoQ Deficiency in the Fruit Fly *Drosophila melanogaster*

Drosophila melanogaster is a species of Diptera in the family Drosophilidae which is known generally as the common fruit fly or vinegar fly. It has been widely used for biological research in studies of genetics, development and organogenesis [Maung and Jenny, 2011], physiology [Teleman et al., 2012], microbial pathogenesis [Limmer et al., 2011], therapeutic drug discovery in pharmacology [Pandey and Nichols, 2011], degenerative diseases [Grice et al., 2011], cardiomyopathies [Qian and Bodmer, 2012], inflammatory diseases [Roeder et al., 2012], cancer [Miles WO et al., 2011; Zhang et al., 2011], aging [Sanz et al., 2010; Biteau et al., 2011; Katewa and Kapahi, 2011], and life history evolution [Carey, 2011].

Regarding mitochondria, *Drosophila* has been used to model several human diseases [Sánchez-Martínez et al., 2006, 2012; Oliveira et al., 2010], to study the genetic base of a mitochondrial disease [Kemppainen et al., 2009; Fernández-Ayala et al., 2010], or even for developing mitochondrial gene therapy [Fernandez-Ayala et al., 2009].

However, the fruit fly is typically used because it is easy to care for, breeds quickly, lays many eggs, and its genetics is easy, because it contains only 4 pairs of chromosomes. Nowadays, *Drosophila* is getting more relevant because of the genetic resources developed such as balancer chromosomes, which come loaded with genetic markers for recessive mutation maintenance in a wild-type phenotype, and the inducible UAS/GAL4 system for space-temporal transgene expression that allows tissue-specific or developmental stage-specific gene expression [Duffy, 2002].

CoQ was detected firstly in the house fly (*Musca domestica*) presenting the isoform CoQ₉ [Lester and Crane, 1959]. Other insects, like the ladybird *Anatis ocellata*, the caterpillar of hawk moth (*Celerio euphorbiae*), and *Sphinx pinastri* present mainly CoQ₁₀ [Heller et al., 1960]. Incorporation of [⁵⁻³H]mevalonate into CoQ₉ in embryonic cells of *Drosophila* suggested that it could produce CoQ₉ [Havel et al., 1986]. Recently, it has been described that adult *Drosophila* contain both CoQ₉ and CoQ₁₀ [Cheng et al., 2011]. However, we analyzed different wild-type and mutant strains, and we found that *Drosophila* con-

tains 3 CoQ isoforms (around 5% CoQ₈, 82% CoQ₉, and 13% CoQ₁₀) with varying proportions depending on the developmental stage and age of the fly [Guerra et al., 2012].

Within the genes that take part in CoQ biosynthesis in *Drosophila*, *qless* (*cg31005*) is the orthologue of the human PDSS1 prenyl transferase that synthesizes the isoprenoid chain of CoQ (table 1). Neurons lacking *qless* up-regulate markers of mitochondrial stress and undergo caspase-dependent apoptosis [Grant et al., 2010]. Dietary supplementation with CoQ₁₀ rescued the neural phenotype of *qless* mutants.

The *Drosophila* homolog of COQ2 is encoded by the gene *cg9613* which is called *sbo* (small boy), because mutations cause a small larval phenotype [Liu et al., 2011]. The *sbo* null mutants are developmentally arrested at the first instar larval stage and present about half of the ATP level of wild-type control larvae. Interestingly, *sbo* heterozygous animals complete their development, show reduced levels of CoQ (19% of CoQ₉ and 65% of CoQ₁₀ compared to wild-type flies), and present lifespan extension and a delayed aging phenotype due to the loss of negative geotaxis, a parameter whose loss accompanies aging in flies [Liu et al., 2011]. However, this result seems controversial because of the shorter lifespan shown by wild-type flies (around 1 month) compared to that published by other groups [Sanz et al., 2010] where wild-type flies reach at least 2–3 months of age.

Chromosomal deletions around the *cg9613* (*sbo*, *coq2*) gene in *Drosophila* demonstrate that *cg9613* mutant flies were more susceptible to bacterial and fungal infections, while they were more resistant to viruses [Cheng et al., 2011]. The gene expression of several anti-microbial peptides, like Diptericin B (*DptB*), Defensin (*Def*) and Drosomycin (*Droso*) which are specifically involved in the defense against fungi (*Droso*), Gram-negative (*DptB* and *Def*) and Gram-positive bacteria (*Droso* and *Def*), is drastically reduced in these mutants. Supplementation of food with CoQ₁₀ restored the gene expression of these anti-microbial genes and thus the sensitivity to bacterial and fungal infections in *coq2* mutants, although the resistance to viruses was lost [Cheng et al., 2011]. These results show that CoQ is necessary for the defense against bacteria and fungi, whereas it diminishes immune response against viruses, maybe because viruses need high levels of CoQ in the host to complete their life cycle, and they fail in CoQ-deficient flies.

Another attempt to mimic the CoQ deficiency syndrome in *Drosophila* was done interfering independently with the expression of each of the *coq* genes (table 1) with

Fig. 2. UAS-GAL4 system for gene silencing with RNAi in *Drosophila*. The figure shows a parental fly to the left carrying the construct for transgenic expression of a RNAi against the *coq* gene of interest, and another parental fly to the right harboring the construct for ubiquitous gene expression of the GAL4 protein. The RNAi is under the control of a minimal promoter that needs the transcription activator GAL4 bound to the enhancer sequence

UAS to allow the transgene expression; the RNAi is unexpressed without the presence of the GAL4 protein as is the case in the parental fly to the left. The gene silencing occurs in the progeny in all tissues and during all developmental stages due to the ubiquitous expression of the GAL4 protein driven by the *daughterless* (*da*) gene promoter.

RNAi using the UAS-GAL4 system as shown in figure 2 [Fernandez-Ayala et al., 2012]. This technology needs the crossing of 2 parental flies to study gene silencing in the progeny: a parental fly carrying the RNAi against the *coq* gene of interest, which is under the control of both an Hsp (Heat-shock protein) minimal promoter and the enhancer sequence UAS that needs the transcription activator GAL4 bound to it to allow the transgene expression, and another parental fly carrying the *GAL4* gene under the control of the *daughterless* (*da*) gene promoter, a gene

that is widely expressed at low levels in all tissues during all developmental stages. For this experimental approach, flies were cultured at 3 different temperatures (29, 25, and 18°C) to generate stress conditions and different expression levels of the inducer GAL4 protein [Guerra et al., 2012].

Flies with RNAi against *coq* genes show a decrease in CoQ levels dependent on the affected gene and intensity of the gene silencing, with stronger disease phenotypes in flies cultured at 29°C under terminal stress and with

Fig. 3. Morphological defects and improper organ development in *pdss2* silenced larvae. Silencing of *cg10585/pdss2* produces lethality during embryogenesis. Few larvae hatched from the eggs, presenting morphological defects and improper organ development, but died at the first instar L1 larva stage. The picture shows 1-day-old larvae cultured at 25°C. An egg is shown to the left to compare with the larval size. RNAi(*pdss2*)/*da-GAL4* expresses the RNAi against the *cg10585/pdss2* gene. RNAi(*pdss2*)/- carries the RNAi but does not express it because it does not carry the transcription activator GAL4.

higher *GAL4* expression [Guerra et al., 2012]. Interestingly, RNAi against the *cg10585* gene (*pdss2*, the regulatory subunit of COQ1) showed the most serious phenotype with developmental arrest just after egg hatching, and moreover, the few larvae presented morphological defects and improper development of the organs (fig. 3) as was previously described for *coq-1* mutants in the worm *C. elegans* [Gavilan et al., 2005].

Gene interference of *qlless* (*cg31005/pdss1*, the catalytic subunit of COQ1) produced lethality at an early larva stage, whereas RNAi against either *coq2*, *coq3*, *coq5*, *coq7*, *coq8*, *coq9*, or *coq10* caused lethality at later larva stages or even at pupa stages [Guerra et al., 2012]. However, when the affected gene was *coq6*, flies managed to achieve adulthood but suffered a severe CoQ deficiency.

Remarkably, the silencing of the *coq7* gene induced a severe reduction in the CoQ content at all developmental stages and the subsequent accumulation of DMQ, the intermediate compound in the CoQ biosynthesis that is the substrate of the COQ7 protein [Fernandez-Ayala et al., 2012]. Since *Drosophila* presented 3 species of CoQ (Q₈, Q₉, and Q₁₀), the interference of *coq7* accumulated DMQ for all of them. The mutant phenotype included pupa lethality at 25°C and around 20% of survival lowering the temperature to 18°C, although surviving flies presented developmental delay, reduced fertility, short lifespan, and higher reactive oxygen-species production during aging; mitochondrial preparations showed low oxygen consumption and a deficit in the electron transference of the respiratory chain. These results seem controversial to those shown by the *clk-1* mutant *C. elegans* which had been amply described above. However, it is important to

notice that *clk-1* mutants present a point mutation that affects COQ7 almost at the end of the protein, and the gene silencing described here reduced the presence of *coq7* mRNA to less than 10% [Fernandez-Ayala et al., 2012]. Additionally, DMQ was also detected when *coq3*, *coq6*, and *coq9* were silenced, supporting the idea of a multi-enzymatic complex for CoQ biosynthesis, because loss of a component of such a complex could cause its instability and malfunction [Guerra et al., 2012].

Few other studies have been done with *Drosophila*, relating CoQ with several aspects of physiology and pathology. The removal of CoQ from the diet reduced lifespan and accelerates the aging process [Palmer and Sackton, 2003]. Since the common laboratory diet includes yeast, flies fed Q-less yeast were not long lived, whereas survival was higher in adults fed the wild-type yeast strain [Palmer and Sackton, 2003]. This result was similar to that published in the same work, where feeding flies with a yeast strain deficient in the mitochondrial respiratory complex III suggested that the mitochondrial functionality of the yeast diet, but not the absence of CoQ in the diet, was responsible for life shortening.

On the other hand, CoQ supplementation fails as an effective treatment for Parkinson's disease [Faust et al., 2009]. Inhibition of *dj-1alpha* (*cg6646*), the *Drosophila* homologue of the familial Parkinson's disease gene *DJ-1*, leads to oxidative stress, mitochondrial dysfunction, and dopaminergic neurons loss. CoQ₁₀ supplementation showed no protective effect, while other drugs combining antioxidant and anti-inflammatory properties did.

Finally, either gene silencing with RNAi or deletions affecting any of the biosynthetic *coq* genes in *D. melanogaster* can be used as reliable models for human mitochondrial diseases with primary CoQ deficiency, allowing further studies on mitochondrial biogenesis during the development of a certain mitochondrial pathology but also on the therapy against neurodegeneration.

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