Original Article

Coenzyme Q regulates the expression of essential genes of the pathogen- and xenobiotic-associated defense pathway in *C. elegans*

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Coenzyme Q (CoQ) is necessary for mitochondrial energy production and modulates the expression of genes that are important for inflammatory processes, growth and detoxification reactions. A cellular surveillance-activated detoxification and defenses (cSADDs) pathway has been recently identified in *C. elegans*. The down-regulation of the components of the cSADDs pathway initiates an aversion behavior of the nematode. Here we hypothesized that CoQ regulates genes of the cSADDs pathway. To verify this we generated CoQ-deficient worms ("CoQ-free") and performed whole-genome expression profiling. We found about 30% (120 genes) of the cSADDs pathway genes were differentially regulated under CoQ-deficient condition. Remarkably, 83% of these genes were down-regulated. The majority of the CoQ-sensitive cSADDs pathway genes encode for proteins involved in larval development (enrichment score (ES) = 38.0, $p = 5.0E^{-37}$), aminoacyl-tRNA biosynthesis, proteasome function (ES 8.2, $p = 5.9E^{-31}$) and mitochondria function (ES 3.4, $p = 1.7E^{-5}$). 67% (80 genes) of these genes are categorized as lethal. Thus it is shown for the first time that CoQ regulates a substantial number of essential genes that function in the evolutionary conserved cellular surveillance-activated detoxification and defenses pathway in *C. elegans*.

***Key Words*: coenzyme Q, ubiquinol, cSADDs pathway, gene expression, proteasome**

Coenzyme Q (CoQ) is an essential lipophilic molecule of the mitochondrial respiratory chain where it is mainly known for its role in oxidative phosphorylation.[1] CoQ is also necessary for pyrimidine biosynthesis and for proper function of uncoupling proteins.[2] CoQ has been identified as a modulator of apoptosis,[3,4] inflammatory processes and gene expression.[5–12] The reduced form of CoQ, ubiquinol, serves as a potent antioxidant in mitochondria, lipid membranes and plasma lipoproteins and interacts with other lipid soluble anti-oxidants including α-tocopherol.[13–15] During the last few years, the physiological functions of CoQ-induced gene signatures have been recognized.[16–19] For example several studies *in vitro*,[8–10] in laboratory rodents as well as in humans provide evidence that ubiquinol reduces inflammatory processes and modulates lipid metabolism via gene expression.[16–18,20–22] In the model organism *C. elegans*, CoQ-dependent gene expression seems to be of importance for larval growth and detoxification reactions.[19] Recently, a xenobiotic and pathogen-associated defence pathway has been identified in *C. elegans* by the Ravkun group.[23,24] This so called *c*ellular *s*urveillance-*a*ctivated *d*etoxification and *d*efenses (cSADDs) pathway detects perturbations of the mitochondria, ribosome and proteasome and initiates an aversions behavior of the nematode. In the present study we hypothesized that CoQ-deficiency induces a gene expression signature mimicking an activation of the cSADDs pathway in *C. elegans*.

Materials and Methods

Strains, diets and CoQ_{10} supplementation. The study design has been recently described.[19] In short: Two *clk-1* mutant strains (*qm30*, *MQ130* and *e2519*, *CB4876*, *Caenorhabditis* Genetics Center, Minneapolis, MN) were cultured on *E. coli* GD1 (ubiG delete) lawns, supplemented with or without (vehicle) 30 μg/ml aqueous solution of ubiquinol-10. Aqueous solution of ubiquinol-10 (PEG-60 hydrogenated castor oil, ubiquinol-10, glycerol, water) and vehicle (no ubiquinol-10) was received from Kaneka Corporation, Japan. N2 worms served as controls. Worms were synchronized by hypochlorite treatment of gravid adults and grown at 20°C until they reached L2 stadium for either 24 h (N2 worms) or 48 h (*clk-1* mutants).

HPLC and COPAS flow cytometric analysis. Analysis of CoQ derivates was based on the method of high-pressure liquid chromatography (HPLC) with electrochemical detection and internal standardisation.[25] Total concentrations of CoQ_{10}, CoQ_9, $DMCoQ_9$ and CoQ_8 were analysed with diethoxy-ubiquinone-10 as internal standard.[19] To sort a distinct number of worms the flow COPAS Biosort (Union Biometrica, Holliston, MA) was used. Time of Flight (TOF) and optical density (Extinction, EXT) were automatically measured from each worm as previously described.[26] These parameters serve as approximations for body length (TOF) and volume (EXT) respectively of the worms.

Gene expression and statistical analysis. Differential gene expression and normalization of raw data were determined using a custom-designed 8 × 60 K *C. elegans* Agilent gene expression microarray and the Agilent MicroArray platform (Source BioScience, ImaGenes GMbH, Berlin, Germany).[27] Fold-changes of normalized expression signals were calculated from the arithmetic mean values between *clk-1* mutans and corresponding N2 control group. Fold changes >1.5 of differential gene expression were considered as significant regulated and the significance was calculated using an unpaired *t* test with unequal variance (Welch-test). Each experiment was performed in duple- or triplicate. All data of body length, body volume and CoQ measurements are expressed as the means ± SEM. To determine statistical significance *t* testing using SPSS software (ver. 13.0) was conducted. *P* values less than 0.05 were considered statistically significant.

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Analysis of cSADDs associated overlapping genes. For analysis and interpretation of microarray data DAVID (Database for Annotation, Visualization an Integrated Discovery, http://david.abcc.ncifcrf.gov/) Bioinformatics resources were used.[28] By doing so, IDs of cSADDs associated genes showing differentially expression in both *clk-1* mutants were uploaded. For subsequent functional clustering, KEGG (Kyoto Encyclopedia for Genes and Genomes) pathway maps were utilised. The WormMart tool was applied to search for lethality phenotype of genes.[29]

Results

Generation of CoQ-deficient worms ("CoQ-free") by using both CoQ_9-deficient *clk-1* mutants and CoQ_8-deficient bacteria. *C. elegans clk-1* mutants lack a mitochondrial hydroxylase which is necessary for the endogenous synthesis of CoQ_9.[30] Thus, they are characterized by the absent of endogenous CoQ_9 but an accumulation of demethoxy-ubiquinone ($DMCoQ_9$).[31] To obtain CoQ-deficient worms we raised both *clk-1 (e2519)* and *clk-1 (qm30)* mutants on CoQ_8-deficient GD1 bacteria until L2 larval stage. Mutant worms of further experimental groups were supplemented with the reduced form of CoQ_{10}. Equally treated N2 worms served as controls. Our experimental set-up resulted in six experimental groups (A–F, Fig. 1) and has been recently described in detail.[19] In short: A comparison of body length (TOF, black bars) and volume (Ext, white bars) revealed no significant differences between all worms of the experimental groups (Fig. 1). Both *clk-1 (e2519)* and *clk-1 (qm30)* mutants (group C–F, Fig. 1) show high contents of $DMCoQ_9$, (dark grey bars) whereas CoQ_9 levels (light grey bars) are not detectable in these worms. CoQ_{10} supplementation of the worms resulted in increased CoQ_{10} levels (striped bars) in group B, D and F compared to non-supplemented worms (group A, C, E, not detectable). CoQ_8 levels (data not shown) were below detection level in all groups suggesting no substantial CoQ input from bacterial sources. Overall, by using both CoQ_9-deficient *clk-1* mutants and CoQ_8-deficient bacteria we generated CoQ-deficient worms ("CoQ-free"), a perquisite to identify genes that are either induced or suppressed by CoQ.

CoQ-deficiency induces a gene expression signature mimicking an activation of the cSADDs pathway. To identify CoQ-sensitive genes we performed a genome-wide gene expression analysis in all experimental groups as previously described.[19] Compared to wild type, 6,710 genes (7,600 including splice variants) were differentially expressed in CoQ-deficient mutants *clk-1 (e2519)* and *clk-1 (qm30)* (fold change >1.5; $p<0.05$; complete list of regulated genes was published recently).[19] 3,299 genes (3,740 including splice variants) were up-regulated, whereas 2,984 (3,984 including splice variants) were down-regulated under CoQ-deficient condition (Fig. 2). The CoQ-sensitive genes were compared to a list of 379 genes which functions in the cSADDs pathway.[23] We found that 120 genes (168 including splice variants) of the cSADDs pathway are differentially regulated in both CoQ-deficient *clk-1 (e2519)* and *clk-1 (qm30)* (Fig. 2, Supplemental Table 1*). The expression of these 120 CoQ-sensitive cSADDs pathway genes is not substantially influenced by exogenous CoQ_{10} supply (Supplemental. Table 1*). Remarkably, 83% (100 genes) of the CoQ-sensitive cSADDs pathway genes are down-regulated under CoQ-deficient condition. Next, we applied DAVID bioinformatics to allocate the 120 CoQ-

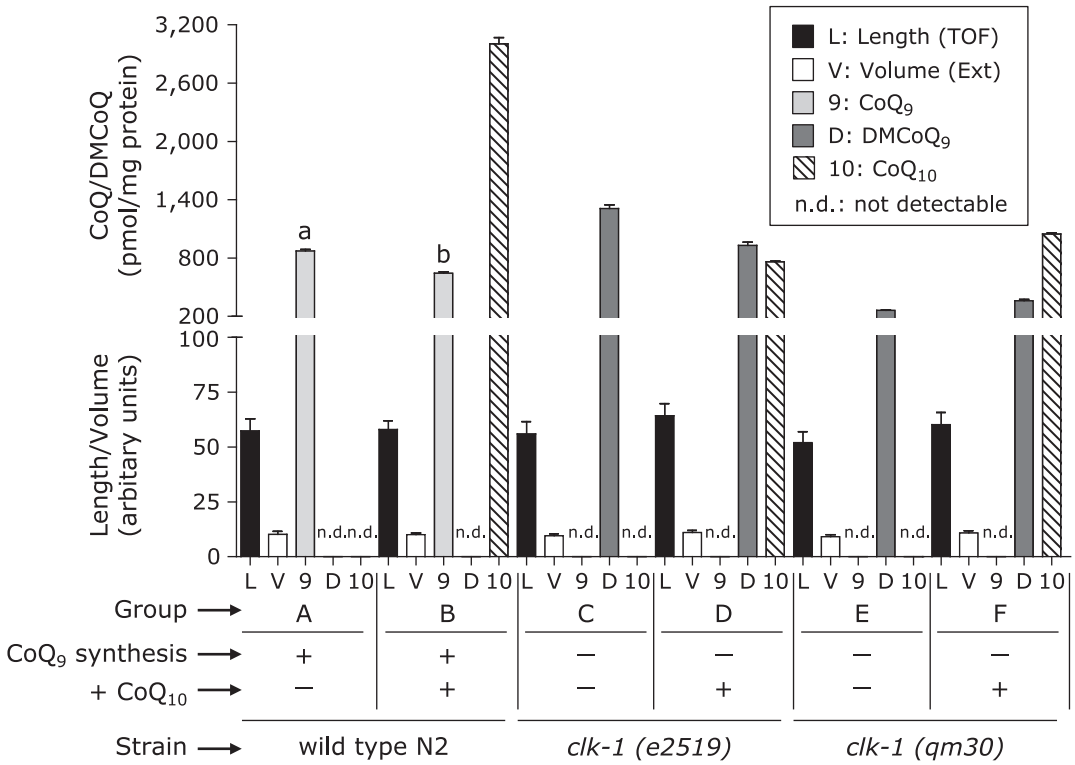

Fig. 1. Body length, body volume and concentration of CoQ derivates in CoQ_9-producing wild type N2 worms (group A and B) and two different CoQ deficient *clk-1* mutant strains (*e2519*, group C and D and *qm30*, group E and F). Worms were cultivated on CoQ_8-deficient bacteria (GD1) and supplemented with (+) CoQ_{10} or vehicle control (–CoQ_{10}). Animals were synchronized and grown until they reached L2 stage. Body length (time of flight, TOF) and body volume (extinction, Ext) was measured using flow cytometry. CoQ derivates [CoQ_9, demethoxy CoQ_9 ($DMCoQ_9$) and CoQ_{10}] were quantified using HPLC with electrochemical detection. Data are presented as means ± SEM. Values from supplemented (+CoQ_{10}) versus non-supplemented (–CoQ_{10}) animals within a strain with different superscript letters are significantly different ($p<0.05$, *t* test).

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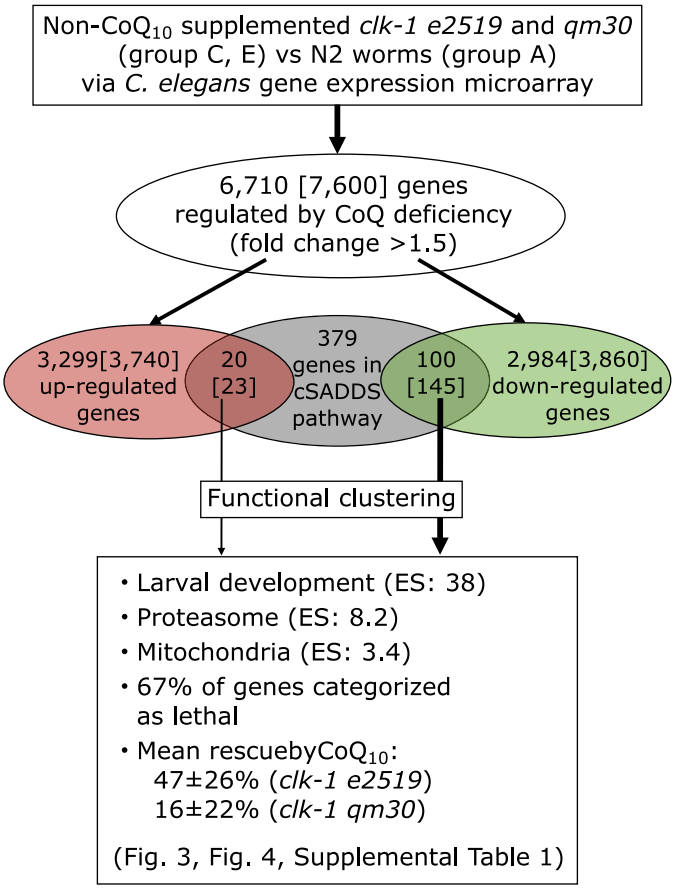

Fig. 2. Number and functions of cSADDs pathway genes that are regulated by CoQ-deficiency. The genes were functionally clustered by DAVID Bioinformatics and categorized according to lethality by the WormMart tool.[28,29] Rescue (%) by CoQ_{10} supplementation was calculated by the difference in gene expression between CoQ_{10} supplemented groups and N2 controls. Number of genes (inclusive splice variants in brackets [ ]) are shown. ES; Enrichment score.

sensitive cSADDs pathway genes into functional clusters.[28] The majority of the genes encode for proteins involved in larval development (enrichment score (ES) = 38.0, Benjamini $p = 5.0E^{-37}$), aminoacyl-tRNA biosynthesis, proteasome function (ES 8.2, $p = 5.9E^{-31}$) and mitochondria function (ES 3.4, $p = 1.7E^{-5}$) (Fig. 2, Supplemental Table 1*). 67% (80 genes) of these genes are categorized as lethal.

Discussion

Disruption of core cellular activities, including translation, respiration and protein turnover stimulate behavioral avoidance of normally attractive bacteria in *C. elegans*. Surveillance pathways overseeing these core cellular activities have been summarized to the cSADDs pathways.[23] It has been shown that the down-regulation of the components of the cSADDs pathway initiates an aversions behavior of the nematode. Based on whole-genome expression profiling of six experimental groups we identified 120 genes (32%) of the cSADDs pathway that are differentially expressed under CoQ-deficient ("CoQ-free") conditions. Out of these genes, 83% (100 genes) are down-regulated indicating that CoQ-deficiency induces a gene expression signature mimicking an activation of the cSADDs pathway. Furthermore 67% (80 genes) of the genes are categorized as essential genes and the majority of the genes encode for proteins involved in larval development, including aminoacyl-tRNA biosynthesis, proteasome function and mitochondria.

CoQ deficiency affects gene expression of the proteasome complex. Protein homeostasis is one of the nodal points that need to be controlled to retain physiological homeostasis. The ubiquitine-proteasome system is responsible for the removal of both normal and damaged proteins with the proteasome being the major cellular protease. Progressive impairment of proteasome function during aging and cellular senescence is well documented and recently it has been shown in *C. elegans* that activation of the 20S proteasome promotes life span extension and resistance to proteotoxicity.[32,33] We found that CoQ deficiency down-regulates several cSADDs pathway genes that encode for proteins of the proteasome complex (Fig. 3) indicating reduced proteasome activity, which might potentiate derogations in CoQ deficiency. Interestingly it was recently discovered that the proteasome system also controls the rate-limiting enzyme (HMG-CoA synthase) of the mevalonate and CoQ synthesis pathway.[34]

The exogenous supply of the reduced form of CoQ_{10} (30 μg/ml ubiquinol) to CoQ-deficient worms did not substantial restore gene expression of proteasome related genes. Previously we have highlighted the influence of endogenous and exogenous CoQ on gene expression in *clk-1* mutant worms.[19] We observed that one set of genes (e.g., important for collagen synthesis) are up-regulated in *clk-1* mutants and that this regulation is restored by CoQ_{10} supplementation. Like the proteasome related genes, another sub-set of genes (e.g., C-type lectine) differentially expressed in the *clk-1* mutants could not be rescued by exogenous CoQ supply. Therefore, exogenous and endogenous CoQ might influence gene expression by different mechanisms.

CoQ deficiency affects gene expression of the aminoacyl-tRNA biosynthesis. Aminoacyl-tRNA synthetases (AARSs) catalyze the adenosine triphosphate-dependent acylation of their cognate tRNA with a specific amino acid and are therefore essential for protein translation.[35] It was shown in *C. elegans* that inactivation of AARSs rescued animals from hypoxia-induced death and the level of hypoxia resistance was inversely correlated with translation rate.[36] In CoQ deficient worms nine genes encoding for AARS, namely *qars-1*, *dars-1*, *tars-3*, *vars-2*, *kars-1*, *rars-1*, *hars-1* and *fars-1* and *3* (Fig. 4) are down-regulated. Thus, CoQ deficiency might induce a stress response pathway to protect the animals against hypoxia-induced death.

CoQ deficiency affects gene expression of pathways that surveil and defend mitochondria. Several RNAi screens were performed to identify genes that influence *C. elegans* lifespan. The screens revealed that the disruption of core cellular functions such as metabolism and translation extends lifespan.[37] *clk-1* mutants lacking a mitochondrial hydroxylase necessary for endogenous synthesis of CoQ, exhibit a respiration-defective behavioral and long lived phenotype but without having major changes in respiration.[38] A link between mitochondrial stress and ubiquitin-dependent proteolysis has been described and seems to be conserved from worm to man.[39] Worms showing both mitochondrial respiration defects and elevated levels of reactive oxygen species (ROS) levels are characterized by a limited protein turnover. In agreement with these findings, we found that CoQ deficiency down-regulates several genes that are annotated to mitochondrial function (Supplemental Table 1*) as well as to proteasome function and aminoacyl-tRNA biosynthesis (Fig. 3 and 4). This gene expression signature, induced by CoQ deficiency, might represent a general stress response pathway of animals. This, too, may contribute to lifespan extension following disruption of mitochondrial function.[37] Our hypothesis is strengthen by recent findings from the Ruvkun lab,[24] which identified in response to inhibition of mitochondrial function 45 *C. elegans* genes that are required to enhance detoxification, pathogen defense and mitochondrial repair. Seven of these genes namely *ran-4*, *gsp-2*, F40F12.7, *imb-3*, Y54E10GR.5, *unc-60*,

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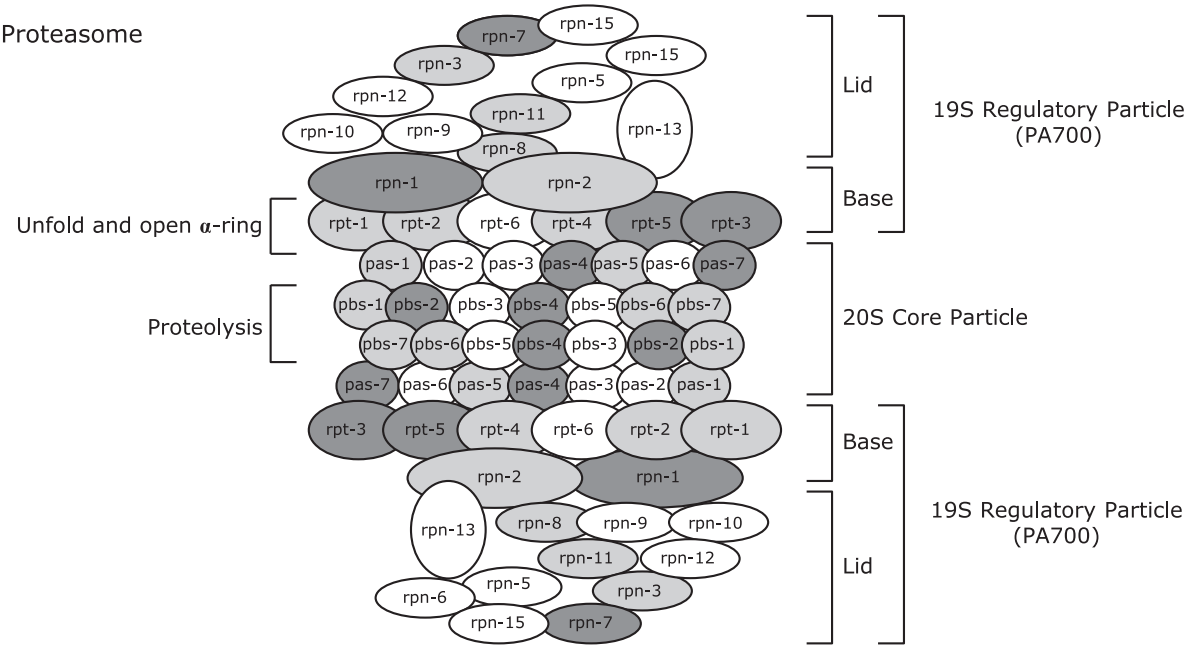
A Proteasome



B

Gene ID		Gene name	Lethal†	Mean aversion‡	*clk-1 (e2519)*		*clk-1 (qm30)*	
					Fold change§	CoQ rescue (%)¶	Fold change§	CoQ rescue (%)¶
Proteasome (ES 8.2)								
ZK945.2.2	*pas-7*	Proteasome Alpha Subunit	x	0.299	2.85	38	2.59	5
ZK945.2.1	*pas-7*	Proteasome Alpha Subunit	x	0.299	2.85	43	2.42	8
F49C12.8	*rpn-7*	proteasome Regulatory Particle	x	0.367	2.22	45	1.89	4
F56H1.4.1	*rpt-5*	proteasome Regulatory Particle	x	0.562	2.20	44	1.83	−1
C47B2.4	*pbs-2*	Proteasome Beta Subunit	x	0.370	2.18	37	1.78	−3
T20F5.2	*pbs-4*	Proteasome Beta Subunit	x	0.190	2.17	58	1.58	22
F23F12.6.1	*rpt-3*	proteasome Regulatory Particle	x	0.324	2.16	32	1.83	1
T22D1.9.1	*rpn-1*	proteasome Regulatory Particle	x	0.296	2.15	45	1.68	−6
C36B1.4.1	*pas-4*	Proteasome Alpha Subunit	x	0.220	2.07	41	1.87	5
F56H1.4.2	*rpt-5*	proteasome Regulatory Particle	x	0.562	2.02	41	1.74	−1
T22D1.9.2	*rpn-1*	proteasome Regulatory Particle	x	0.296	1.98	32	1.73	0
C52E4.4.1	*rpt-1*	proteasome Regulatory Particle	x	0.401	1.97	46	1.52	−18
C15H11.7.1	*pas-1*	Proteasome Alpha Subunit	x	0.403	1.97	55	1.65	−4
C52E4.4.2	*rpt-1*	proteasome Regulatory Particle	x	0.401	1.96	42	1.54	−12
F23F1.8a	*rpt-4*	proteasome Regulatory Particle		0.280	1.95	40	1.78	−1
C23G10.4b	*rpn-2*	proteasome Regulatory Particle		0.332	1.91	36	1.62	−8
K08D12.1.2	*pbs-1*	Proteasome Beta Subunit	x	0.235	1.91	45	1.70	−1
K08D12.1.1	*pbs-1*	Proteasome Beta Subunit	x	0.235	1.90	34	1.59	−13
C30C11.2	*rpn-3*	proteasome Regulatory Particle	x	0.288	1.90	36	1.75	1
K07D4.3.1	*rpn-11*	proteasome Regulatory Particle	x	0.640	1.90	29	1.62	−8
F29G9.5	*rpt-2*	proteasome Regulatory Particle	x	0.285	1.89	42	1.68	2
C02F5.9.1	*pbs-6*	Proteasome Beta Subunit	x	0.218	1.87	51	1.59	−4
R12E2.3.1	*rpn-8*	proteasome Regulatory Particle	x	0.385	1.82	51	1.56	0
F39H11.5.1	*pbs-7*	Proteasome Beta Subunit	x	0.260	1.79	43	1.57	−8
F23F12.6.2	*rpt-3*	proteasome Regulatory Particle	x	0.324	1.77	46	1.54	−9
F25H2.9.1	*pas-5*	Proteasome Alpha Subunit	x	0.320	1.77	48	1.53	−6
F23F1.8b.1	*rpt-4*	proteasome Regulatory Particle		0.280	1.76	45	1.57	−7
C23G10.4a.2	*rpn-2*	proteasome Regulatory Particle		0.332	1.72	36	1.54	−3

down 1.5–2.0 2.0–5.0 >5.0

† Defined as lethal gene in WormMart[29]
‡ Mean aversion values given by [23]
§ Fold change in *clk-1* mutant compared to matched N2 controls
¶ % of rescue in gene expression by CoQ_{10red} supplementation to control level

Fig. 3. Proteasome associated cSADDs pathway genes that are regulated by CoQ deficiency. Functional clustering of proteasome associated genes (A) was done according to KEGG (Kyoto Encyclopedia for Genes and Genomes). Lethality, defined by the WormMart tool;[29] mean aversion ratio as given and % rescue in gene expression to wild type level by CoQ_{10} supplementation of selected genes are given (B).[23]

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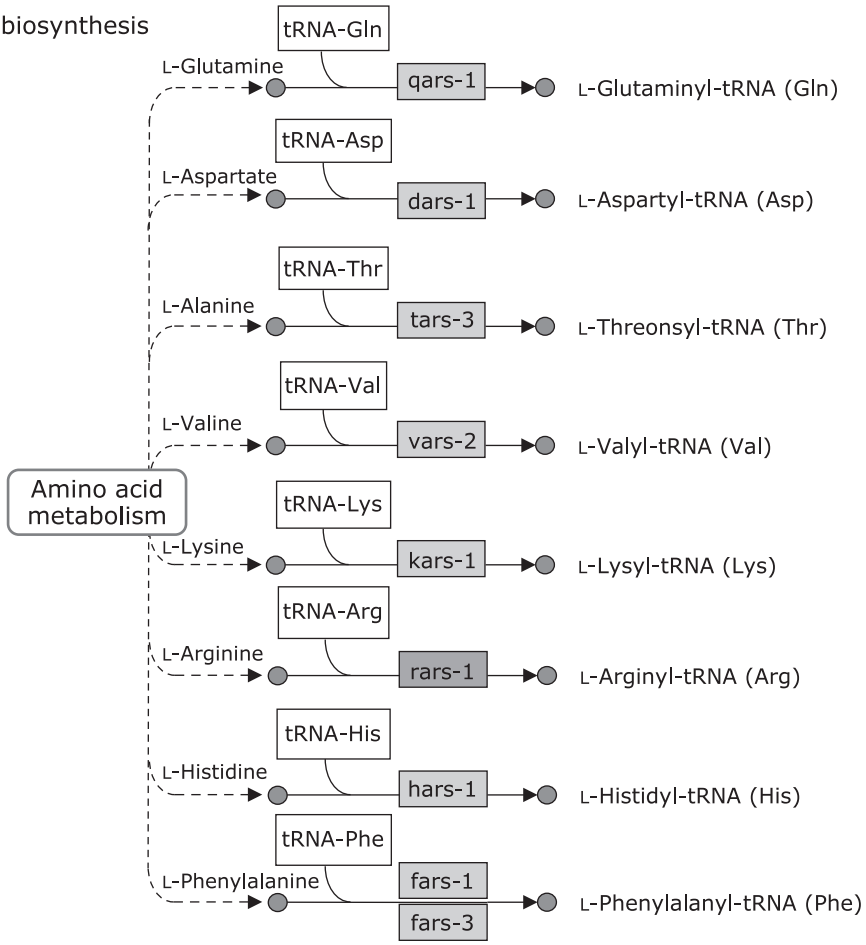
A Aminoacyl-tRNA biosynthesis



B

Gene ID		Gene name	Lethal†	Mean aversion‡	*clk-1 (e2519)*		*clk-1 (qm30)*	
					Fold change§	CoQ rescue (%)¶	Fold change§	CoQ rescue (%)¶
Aminoacyl-tRNA biosynthesis, larval development (ES 38.1)								
T02G5.9b	*kars-1*	lysyl (K) tRNA Synthetase		0.560	1.89	96	2.04	36
T08B2.9b.4	*fars-1*	phenylalanyl (F) tRNA Synthetase		0.180	1.81	58	1.78	24
T02G5.9a	*kars-1*	lysyl (K) tRNA Synthetase		0.560	1.76	70	1.98	32
Y87G2A.5	*vars-2*	Valyl tRNA Synthetase	x	0.239	1.75	72	1.66	43
F22B5.9	*fars-3*	phenylalanyl (F) tRNA Synthetase	x	0.138	1.75	57	1.91	22
T08B2.9a	*fars-1*	phenylalanyl (F) tRNA Synthetase		0.180	1.72	56	1.71	30
B0464.1.2	*dars-1*	aspartyl(D) tRNA Synthetase	x	0.220	1.67	80	1.62	23
T11G6.1b	*hars-1*	Histidyl tRNA Synthetase	x	0.170	1.62	58	1.69	19
B0464.1.1	*dars-1*	aspartyl(D) tRNA Synthetase	x	0.220	1.61	67	1.62	18
F26F4.10a.2	*rars-1*	aRginyl aa-tRNA syntheTase		0.200	1.55	50	1.57	17
Y41E3.4	*qars-1*	glutamyl(E)/glutaminyl(Q) tRNA Synthetase		0.219	1.52	38	1.56	6
F26F4.10b.2	*rars-1*	aRginyl aa-tRNA syntheTase		0.200	1.51	48	1.66	21
C47D12.6b.3	*tars-1*	Threonyl tRNA Synthetase		0.526	1.49	88	1.45	19

down 1.5–2.0 2.0–5.0 >5.0

† Defined as lethal gene in WormMart[29]
‡ Mean aversion values given by [23]
§ Fold change in *clk-1* mutant compared to matched N2 controls
¶ % of rescue in gene expression by CoQ_{10red} supplementation to control level

Fig. 4. Aminoacyl-tRNA biosynthesis associated cSADDs pathway that are regulated by CoQ deficiency. Functional clustering of aminoacyl-tRNA biosynthesis associated genes (A) was done according to KEGG (Kyoto Encyclopedia for Genes and Genomes). Lethality, defined by the WormMart tool;[29] mean aversion ratio as given and % rescue in gene expression to wild type level by CoQ_{10} supplementation of selected genes are given (B).[23]

and *elo-3* were down-regulated in CoQ deficient *clk-1* mutants in the present study (Supplemental Table 1*). The authors further presented a link between ubiquinone synthesis and mitochondrial surveillance by inhibition of the mevalonate and CoQ synthesis pathway either by RNAi or statin drugs, which also disrupts mitochondrial surveillange. Likewise we have previously shown that dietary restriction reduces the level of CoQ and ubiquinol via down regulation of genes involved in the mevalonate pathway in *C. elegans*.[40]

We conclude that CoQ regulates a number of essential and conserved genes of a general response pathway which is also inducible by the perturbation of the mitochondria and other essential cellular functions.

Acknowledgments

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Abbreviations

AARSs	aminoacyl-tRNA synthetases
CoQ	coenzyme Q
cSADDs	cellular surveillance-activated detoxification and defences
DMCoQ	demethoxy-ubiquinone
ES	enrichment score
EXT	extinction
TOF	time of flight

Conflict of Interest

No potential conflicts of interest were disclosed.

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