Biochemical and Biophysical Research Communications 452 (2014) 920–927

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Promotion of growth by Coenzyme Q_{10} is linked to gene expression in *C. elegans*

Alexandra Fischer [a], Petra Niklowitz [b], Thomas Menke [b], Frank Döring [a,*]

[a] Institute of Human Nutrition and Food Science, Division of Molecular Prevention, Christian-Albrechts-University of Kiel, Heinrich-Hecht-Platz 10, 24118 Kiel, Germany
[b] Children's Hospital of Datteln, University of Witten/Herdecke, Dr.-Friedrich-Steiner Str. 5, 45711 Datteln, Germany

ARTICLE INFO

Article history:
Received 28 August 2014
Available online 16 September 2014

Keywords:
Coenzyme Q
Growth
C. elegans
Gene expression
Ubiquinol supplement

ABSTRACT

Coenzyme Q (CoQ, ubiquinone) is an essential component of the respiratory chain, a cofactor of pyrimidine biosynthesis and acts as an antioxidant in extra mitochondrial membranes. More recently CoQ has been identified as a modulator of apoptosis, inflammation and gene expression. CoQ deficient *Caenorhabditis elegans clk-1* mutants show several phenotypes including a delayed postembryonic growth. Using wild type and two *clk-1* mutants, here we established an experimental set-up to study the consequences of endogenous CoQ deficiency or exogenous CoQ supply on gene expression and growth. We found that a deficiency of endogenous CoQ synthesis down-regulates a cluster of genes that are important for growth (i.e., RNA polymerase II, eukaryotic initiation factor) and up-regulates oxidation reactions (i.e., cytochrome P450, superoxide dismutase) and protein interactions (i.e., F-Box proteins). Exogenous CoQ supply partially restores the expression of these genes as well as the growth retardation of CoQ deficient *clk-1* mutants. On the other hand exogenous CoQ supply does not alter the expression of a further sub-set of genes. These genes are involved in metabolism (i.e., succinate dehydrogenase complex), cell signalling or synthesis of lectins. Thus, our work provides a comprehensive overview of genes which can be modulated in their expression by endogenous or exogenous CoQ. As growth retardation in CoQ deficiency is linked to the gene expression profile we suggest that CoQ promotes growth via gene expression.

1. Introduction

Coenzyme Q (CoQ, ubiquinone) acts as a lipid component in the respiratory chain. The redox activity of the benzoquinone ring allows CoQ to accept and transfer electrons from complex I or complex II to complex III [1]. CoQ also functions as an electron acceptor in fatty acid beta-oxidation, as a cofactor of pyrimidine biosynthesis [2] and uncoupling proteins [3] and as an antioxidants in extra mitochondrial membranes [4]. More recently, CoQ has been identified as a modulator of gene expression [5–7], inflammation [8–10] and apoptosis [11,12]. The polyisoprene tail defines the number of isoprene units, whereby in humans CoQ_{10} is the predominant form of endogenous ubiquinone. *Escherichia coli* produces eight isoprene units (CoQ_8) and *Caenorhabditis elegans* nine (CoQ_9). Intracellular synthesis is the major source of CoQ, however it can also be acquired through diet and dietary supplements [13].

C. elegans clk-1 mutants lack a mitochondrial hydroxylase which is necessary for synthesis of ubiquinone [14]. These mutants accumulate demethoxyubiquinone ($DMCoQ_9$), whereby they exhibit essentially normal respiration rates and ATP levels [15]. However *clk-1* mutants have an extended live span and show reduced defecation rate, locomotor activity and growth [16]. These phenotypes depend at least in part on exogenous sources of CoQ. When fed a CoQ deficient diet, e.g., the GD1 strain of *E. coli*, *clk-1* mutants stop growing during the L2 larval stage [17] and after about 1 week they will eventually develop into sterile adults [18]. Still, there is an ongoing debate whether supplementation of CoQ is sufficient to rescue the alterations observed in *clk-1* mutants. Here we systematically investigated the effect of endogenous CoQ deficiency and CoQ supplementation on *clk-1* phenotypes and genome-wide gene expression.

Abbreviations: CoQ, Coenzyme Q, ubiquinone; DMCoQ, demethoxyubiquinone; ES, enrichment score; Ext, extinction; TOF, time of flight.

* Corresponding author. Fax: +49 (0)431 8805658.
E-mail addresses: fischer@molprev.uni-kiel.de (A. Fischer), Forschungslabor@kinderklinik-datteln.de (P. Niklowitz), t.menke@kinderklinik-datteln.de (T. Menke), sek@molprev.uni-kiel.de (F. Döring).

http://dx.doi.org/10.1016/j.bbrc.2014.09.016
0006-291X/

2. Materials and methods

2.1. Strains, diets and CoQ_{10} supplementation

Bristol N2 as wild type strain and the mutant strains *clk-1* (*qm30*, *MQ130*) and *clk-1* (*e2519*, *CB4876*) were cultured on *E. coli* GD1 (ubiG delete) lawns [19] on NGM agar plates with 100 μg/ml ampicillin. For CoQ_{10} supplementation experiments, 30 μg/ml aqueous solution of ubiquinol-10 (ubiquinol-10, Kaneka Corporation, PEG-60 hydrogenated castor oil, glycerol, water) or corresponding vehicle (no ubiquinol-10) were added to the plates.

For all expression profile experiments, 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). For growth experiments, worms were allowed to develop for 5 days on different GD1 plates with increasing ubiquinol-10 supplementations (0, 5, 30, 100 μg/ml).

2.2. HPLC analysis

Analysis of CoQ_{10} derivates was based on the method of high-pressure liquid chromatography (HPLC) with electrochemical detection and internal standardisation (ubiquinone-10, ubihydroquinone-10) and has been described elsewhere [20]. Regarding the CoQ_{10} supplementation experiments, total concentrations of CoQ_9 and CoQ_{10} were analyzed with diethoxy-ubiquinone-10 as internal standard. For quantitative protein analysis tissue residues of the homogenates were dried under argon and analyzed using a Lowry Total Protein Kit (Sigma–Aldrich).

2.3. Microscopic imaging and COPAS flow cytometric analysis

Worms were visualized using a Zeiss dissecting microscope (optical enlargement 1.5×; 4×) fitted with a digital camera as described elsewhere [21]. To sort a distinct number of worms the flow COPAS Biosort (Union Biometrica) was used. Body length (Time of Flight, TOF) and body volume (Extinction, Ext) were automatically measured from each worm as previously described [21].

2.4. Isolation of total RNA for gene expression analysis

At least 20,000 L2 worms were collected using flow cytometry and total RNA was extracted using RNeasy mini Kit (Qiagen), which included a DNA digestion step according to the manufacturer's instructions. The amount and integrity of the RNA was assessed spectrophotometrically and by Bioanalyzer 2100 (Agilent Technologies). Each sample contained at least 1 μg of total RNA per 10 μl.

2.5. Gene expression analysis

Differential gene expression and normalization of raw data were determined via an Agilent MicroArray platform (Source BioScience, ImaGenes GMbH), using a custom-designed Agilent gene expression microarray. This microarray was developed by ImaGenes/Source Bioscience and contained 61,643 oligonucleotides, which resulted in 26,843 genes. Quantile normalization was calculated using R-package [22]. Fold-changes of intensities were calculated from the arithmetic mean of gene expression values between experimental and corresponding control group. Each experiment was performed in duple- or triplicate.

For microarray analysis DAVID (Database for Annotation, Visualization an Integrated Discovery, http://www.david.abcc.ncifcrf.gov/) [23] Bioinformatics resources was used. By doing so, gene IDs of the regulated genes (fold change >1.5) were uploaded.

2.6. Statistical analysis

Data are expressed as the mean ± SD. To determine statistical significance between control and experimental group, *t*-testing using SPSS software (Version 13.0) was conducted. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. The experimental set-up aims to analyze the consequences of endogenous CoQ deficiency and exogenous CoQ supply

The experimental set-up includes the N2 wild type strain and two *clk-1* mutants (*e2519*, *qm30*). The *clk-1* mutants are characterised by absent of endogenous CoQ and accumulate about the same amount of $DMCoQ_9$ [24]. Yet for most phenotypes, *qm30* mutant worms, which exhibit most likely a null allele are more severely affected than the missense *e2519* mutant worms [25]. N2 as well as the *clk-1* mutants were fed the CoQ_8-deficient bacteria GD1, supplemented with or without CoQ_{10}. To analyze worms at a similar developmental stage, synchronized worm populations were grown until reaching the L2 stage. A comparison of body length and volume revealed no significant differences between the worms of all experimental groups (Table 1). CoQ levels in the worms were determined by HPLC-ED. As expected, *clk-1* mutants exhibited high contents of $DMCoQ_9$, whereas CoQ_9 levels were not detectable in these animals (Table 1). CoQ_{10} supplementation results in higher CoQ_{10} levels in wild type as well in *clk-1* mutant worms compared to non-supplemented worms. CoQ_8 level (data not shown) was below detection level in all groups suggesting no CoQ input from

Table 1
Body length, body volume and levels of CoQ derivates in wild type N2 worms and two *clk-1* mutant strains. CoQ_9 producing ($+CoQ_9$ synthesis) N2 (group A and B) and CoQ deficient ($-CoQ_9$ synthesis) *clk-1* worms (*e2519*, group C and D; *qm30*, group E and F) were cultivated on CoQ_8-deficient bacteria (GD1) supplemented with CoQ_{10} (+) or without CoQ_{10} (−). Animals were synchronized and grown until they reached L2 stage. Body length (time of flight, TOF) and body volume (extinction, Ext) was determined using flow cytometry. CoQ derivates (CoQ_9, demethoxy CoQ_9 ($DMCoQ_9$), CoQ_{10}) were measured via HPLC with electrochemical detection.

Group	Strain (genotype)	CoQ_9 synthesis	CoQ_{10} supplement	Body length (TOF)	Body volume (Ext)	CoQ_9 (pmol/mg protein)	$DMCoQ_9$ (pmol/mg protein)	CoQ_{10} (pmol/mg protein)
A	N2	+	–	57.3 ± 7.9	10.2 ± 1.2	872 ± 25[a]	n.d.	n.d.
B	N2	+	+	58.0 ± 5.7	10.1 ± 1.0	646 ± 20[b]	n.d.	3004 ± 93
C	*clk-1 (e2519)*	–	–	55.9 ± 9.8	9.5 ± 1.7	n.d.	1309 ± 95	n.d.
D	*clk-1 (e2519)*	–	+	64.2 ± 9.6	11.0 ± 1.6	n.d.	929 ± 80	762 ± 10
E	*clk-1 (qm30)*	–	–	52.0 ± 8.6	9.1 ± 1.7	n.d.	261 ± 15	n.d.
F	*clk-1 (qm30)*	–	+	60.2 ± 9.4	10.8 ± 1.7	n.d.	361 ± 32	1047 ± 32

n.d. = not detectable.
Data are presented as means ± SD. 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).

bacterial sources. Thus, six groups of worms varying in endogenous CoQ_9 synthesis (N2 *versus clk-1*) and exogenous supply of CoQ_{10} (±CoQ_{10}) were used in our experiments.

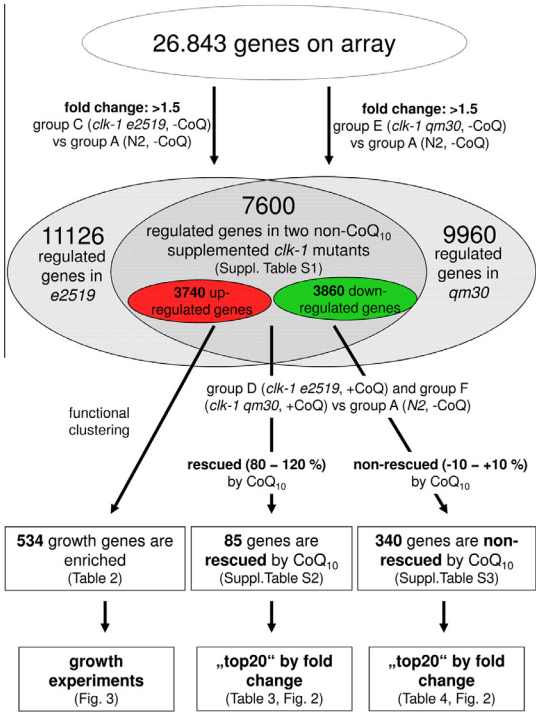


Fig. 1. Flow scheme showing the steps involved in analyzing gene expression data received from two *clk-1* mutants compared to *N2* wild type control. 7600 differentially expressed genes in non-CoQ_{10} supplemented *clk-1* strains (*e2519*, *qm30*) compared to wild type N2 were functionally clustered and adjacent growth experiments were conducted. Furthermore the rescue and the absent rescue of gene expression by CoQ_{10} supplementation were verified.

Table 2
Functional annotation clustering of differentially expressed genes (fold change > 1.5) in two *clk-1* mutant strains (*e2519*, *qm30*) compared to wild type N2. 7600 regulated genes were uploaded in DAVID (Database for Annotation, Visualization an Integrated Discovery, http://david.abcc.ncifcrf.gov/) [23] and functional annotation clustering was made for (A) down-regulated and (B) up-regulated genes.

Functional Term	Gene count*	Fold enrichment	Benjamini *p*-value
(A) Down-regulated genes			
Growth	534	11.7	1.9E−15
Proteasome	25	5.9	4.1E−12
Reproduction	176	11.5	5.7E−11
Translation	24	8.4	3.5E−9
Chaperone	23	6.6	1.6E−7
m-RNA processing	20	5.8	4.5E−7
Endoplasmic reticulum	43	4.0	5.5E−6
Pyrimidine metabolism	28	4.9	8.0E−5
(B) Up-regulated genes			
Collagen	78	14.8	1.0E−19
Oxidation/reduction	106	11.3	1.9E−17
F-Box	91	7.5	4.6E−7
Glycolysis	18	3.7	3.5E−7
Cuticle	31	4.2	6.7E−6
Phosphatase	30	4.2	4.8E−5
Xenobiotic metabolism	13	3.9	2.3E−4
Lipid glycosylation	25	3.0	7.1E−3

* Multiple entries possible.

3.2. Deficiency of endogenous CoQ synthesis causes a down-regulation of genes involved in growth and reproduction and an up-regulation of genes involved in detoxification and collagen synthesis

To identify genes which are responsive to endogenous and/or exogenous CoQ, we performed microarray-based gene expression profiling of the six experimental groups. Resulting gene expression data were analyzed according to the flow scheme in Fig. 1. First we intended to identify differentially expressed genes that are sensitive to endogenous CoQ (N2 *versus* both non-supplemented *clk-1* mutants). The resulting 7600 genes (including splice variants, fold change >1.5, Supplementary Table S1) were divided into up- and down- regulated genes and were allocated into functional clusters using DAVID bioinformatics [23]. For genes down-regulated in both *clk-1* mutants, we found an enrichment score (ES) of >11 for genes encoding proteins that are of importance for growth or reproduction, respectively (Table 2A). Genes of further clusters are involved in translation (ES 8.4), chaperone function (ES 5.9), mRNA processing (ES 5.8) and pyrimidine metabolism (ES 4.9). Genes up-regulated in the *clk-1* mutants encode proteins that are of importance for collagen synthesis (ES 14.8), oxidation/reduction reactions (ES 11.3), F-Box protein function (ES 7.5), cuticle function (ES 4.2), phosphatase function (ES 4.2) and xenobiotic metabolism (ES 3.9) (Table 2B). Consequently the gene expression analysis implies that CoQ deficient *clk-1* mutants reduce growth and reproduction but enhance detoxification reaction and collagen synthesis.

3.3. Exogenous CoQ_{10} supply restores the expression of a subset of genes that are differentially expressed in CoQ deficient clk-1 mutants

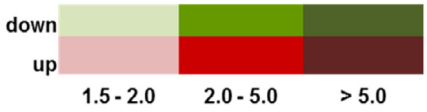
Next we raised the question whether an external CoQ_{10} supplementation of *clk-1* mutants can restore the expression of those significantly up- or down regulated genes. For this purpose the expression levels of all 7600 genes, differentially expressed in non CoQ_{10} supplemented *clk-1* worms were scored in CoQ_{10} supplemented *clk-1* mutants (group D and F) and compared to N2 wild type worms (group A). The comparison revealed that CoQ_{10} supplementation restores (80–120% of N2 control) the expression levels of 85 mainly up-regulated genes in both *clk-1* strains (Supplementary Table S2, Table 3, Fig. 2A and C). Most of these genes show low expression levels in wild type N2 (Table 3). Moreover we found 340 genes whose expression levels were not altered by CoQ_{10} supplementation (Supplementary Table S3, Table 4; Fig. 2B and D). Hence, our results show that CoQ_{10} supplementation restores the expression of a subset of genes that are differentially expressed in CoQ deficient *clk-1* mutants. The expression level of another subset of genes depends on endogenous CoQ synthesis and are essentially unaffected by CoQ_{10} supplementation.

3.4. Exogenous CoQ_{10} supply partially restores the growth retardation of CoQ deficient clk-1 mutants

As a vast number of growth-associated genes is down-regulated in *clk-1* mutants (Table 2) we determined the postembryonic growth of these mutants in the presence and absence of exogenous CoQ_{10} supply. Worms were raised on CoQ deficient bacteria lawn, supplemented with (5.0–100 μg/ml) or without (0 μg/ml) CoQ_{10}. Worms were allowed to grow for 5 days on these dietary regimes. Subsequently we imaged the worms and determined their body length and body volume (Fig. 3). We found that the growth of N2 wild type worms was unaffected by exogenous CoQ_{10} supplementation. Contrary both *clk-1* mutant strains show an increase in body length (Fig. 3A and B) and body volume (Fig. 3A and C) in response to increasing concentration of CoQ_{10} supply. Thus CoQ_{10}

Table 3
Selected top down-regulated (A) and up-regulated (B) genes, rescued by CoQ_{10} supplementation in two *clk-1* mutants. Fold-change of mean gene expression levels was calculated between non CoQ_{10}-supplemented *clk-1* mutants *e2519* and *qm30* and corresponding N2 control. Rescue was computed as the difference between gene expression levels of CoQ_{10}-supplemented *clk-1* mutants *e2519* or *qm30* and N2 control, whereby control levels were set to 100%. A rescue of 80–120% was considered as valid and top genes given were selected according to fold change in *clk-1 e2519* worms. Both *clk-1* mutants had to fulfill these stringent criteria.

Gene ID	Gene name	Expression level* (wild type N2)	*clk-1 (e2519)* fold change	*clk-1 (qm30)* fold change
A) Down-regulated genes				
F23F12.3	F23F12.3	medium	6.8	3.4
C12D5.5	C12D5.5	medium	1.6	1.6
C34B4.6	C34B4.6	low	1.5	1.9
C30H6.9	C30H6.9	low	1.5	1.5
B) Up-regulated genes				
T28F2.8	COLlagen	low	16.3	2.4
C16C8.18	C16C8.18	low	14.4	2.9
ZK829.5	T BoX family	low	8.1	3.5
ZK265.3	ZK265.3	low	6.8	4.3
K12D9.12	irld-46, insulin/EGF-Receptor L Domain	low	4.3	3.0
K12D9.1	K12D9.1	low	4.2	3.6
C04G6.7	C04G6.7	low	3.6	7.0
F14H12.7	F14H12.7	low	3.6	3.7
T19H5.3	T19H5.3	low	3.5	2.4
R09B5.13	CaeNaCin (Caenorhabditis bacteriocin)	medium	3.3	1.5
C08E3.7	F-box A protein	low	3.3	2.7
Y47D7A.15	Y47D7A.15	low	3.1	3.1
C29F3.2	WaRThog (hedgehog-like family)	high	3.0	2.2
F58B3.9	TransThyretin-Related family domain	medium	3.0	1.8
K08F4.5	K08F4.5	low	2.8	2.2
C17C3.11	C17C3.11	low	2.7	1.5
Y55F3C.8	Serpentine Receptor, class T	low	2.7	1.5
C36A4.3	CYtochrome P450 family	high	2.7	2.3
F19F10.5	ets-7, ETS class transcription factor	low	2.6	3.6
F49F1.11	F49F1.11	low	2.6	2.2



[a] Expression level (arbitrary units, AU): 0–100 = low; 100–1000 = medium; 1000–10,000 = high; >10,000 = very high.

supplementation could at least partially rescue the growth retardation of CoQ deficient *clk-1* mutants.

4. Discussion

Mutations in the *clk-1* gene delays several processes such as the cell cycle of early embryos, the postembryonic development, the defecation rate as well as the swimming and pharyngeal pumping cycles. Mutations in the *clk-1* gene also affect reproductive features, such as germ line development and egg production [16,26]. Our results demonstrate that the sterility and growth arrest of the CoQ deficient *clk-1* worms [17] may be linked to changes in gene expression. In addition, CoQ_{10} supplementation rescues important gene expression alteration in CoQ deficient *clk-1* mutants and affects growth of worms in a concentration-dependent manner (Fig. 3). However under the conditions investigated, CoQ_{10} supplementation could not fully restore either expression of genes or body length (TOF) or body volume (Ext), resulting in the known "clock-phenotype" despite external CoQ supply. This was more obvious in *clk-1 qm30* null mutant worms. It has been further assumed that CoQ deficiency led to higher oxidative stress and that reactive oxygen species are required for collagen synthesis in *C. elegans* [27]. In accordance to this hypothesis, CoQ deficient *clk-1* mutants up-regulate several genes encoding proteins that are involved in oxidation/reduction processes and collagen synthesis. Thus, several phenotypes observed in the *clk-1* mutants are linked to gene expression suggesting an important role of CoQ in this process.

In the present study we could further show that exogenous administration of CoQ_{10} (ubiquinol) led to higher CoQ_{10} content in wild type and *clk-1* mutant worms (Table 1). Likewise it was demonstrated, that *clk-1* mutants can assimilate exogenously supplied CoQ_8 from their *E. coli* diet [17,24] and that *C. elegans* transport exogenous CoQ into their mitochondria [28], the place where CoQ is functional. It seems likely that the exogenous CoQ is

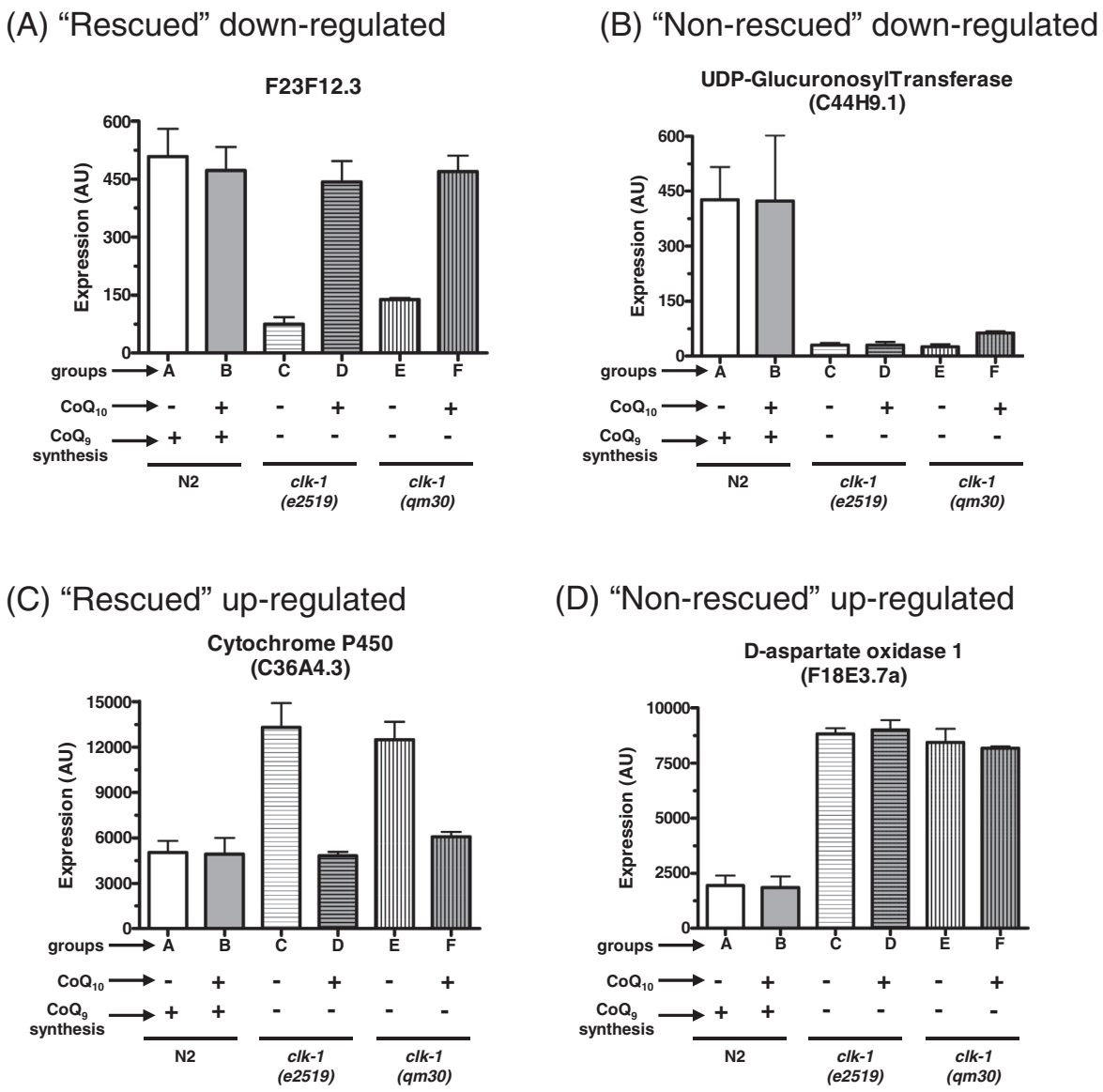


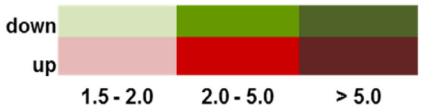
Fig. 2. Expression level (arbitrary units, AU) of selected, significant regulated genes in different experimental groups (A–F). In A and B expression level of two down-regulated (>1.5-fold) genes in two *clk-1* mutants (*e2519*, *qm30*), deficient in endogenous CoQ_9 synthesis compared to wild type N2 (+CoQ_9 synthesis) and their induction (rescue or non-rescue) by CoQ_{10} supplementation are shown. C and D gives the expression level of two up-regulated (>1.5-fold) genes in *clk-1* mutants (*e2519*, *qm30*) compared to wild type and their repression (rescue or non-rescue) by CoQ_{10} supplementation. Animals were grown on CoQ_8 deficient bacteria, supplemented with (+) or without (−) CoQ_{10}.

ingested and absorbed via the gut and is intracellular distributed. In this respect it might be interesting to analyze the underlying mechanisms of CoQ assimilation in nematodes as well as in other organisms. Beyond that the bacterial food source may play an important role to explain certain phenotypes of CoQ deficient mutants. It has been demonstrated [28] that altered bacterial metabolism, not the CoQ content itself, is responsible for the life-span extension of CoQ deficient worms. Furthermore it was evidenced [19] that worms fed the ubiquinone deficient bacterial diet (GD1) live longer due to a delay in bacterial colonization of the gut which in turn may subject the worms to less stress compared to worms fed a standard OP50 diet. Our gene expression profiles support this hypothesis. When we compared gene expression profiles of two N2 groups receiving different bacteria (OP50 *versus* GD1, data not shown) functional annotation clustering revealed an enrichment score of 4.4 for up-regulated genes (OP50) that function in oxidation/reduction processes.

Recent findings [29] in pre-fertile *clk-1* mutants grown on CoQ containing wild-type bacteria (OP50) demonstrated an up-regulation of genes that function in cell protection and metabolism. This gene expression profile observed in *clk-1* mutants is similar to the response induced by inhibiting respiration in yeast and mammalian cells. 73 genes were identified as most significant overlapping between mitochondrial mutants (i.e., *isp-1*) and *clk-1* mutants. In our experimental set-up, only 39% of these genes were also differentially expressed in the *clk-1* mutants. Of note, our global expression profiling was done in L2 *clk-1* mutants grown without a bacterial source of CoQ. Thus our experimental set-up is useful to differentiate between the influence of endogenous and exogenous CoQ on gene expression. Indeed, we observed that genes important for collagen synthesis, detoxification and F-Box protein function are up-regulated in *clk-1* mutants and that this regulation is restored by exogenous CoQ_{10} supply. Another subset of genes differentially expressed in the *clk-1* mutants are of

Table 4
Selected top down-regulated (A) and up-regulated (B) genes, non-rescued by CoQ_{10} supplementation in two *clk-1* mutants. Fold-change of mean gene expression levels was calculated between non CoQ_{10}-supplemented *clk-1* mutants *e2519* or *qm30* and corresponding N2 control. Rescue was computed as the difference between gene expression levels of CoQ_{10}-supplemented *clk-1* mutants *e2519* and *qm30* and N2 control, whereby control levels were set to 100%. A rescue of −10% up to +10% was considered as "non-rescued" and top genes given were selected according to fold change in *clk-1 e2519* worms. Both *clk-1* mutants had to fulfill these stringent criteria.

Gene ID	Gene name	Expression level* (wild type N2)	*clk-1 (e2519)* fold change	*clk-1 (qm30)* fold change
A) Down-regulated genes				
C35D10.11	msd-4, major sperm protein domain containing	very high	5124.7	546.8
C55B6.4	C55B6.4	medium	38.1	51.1
F31E9.2	Serpentine Receptor, class G (gamma)	medium	32.4	26.8
Y57E12B.1	Y57E12B.1	medium	28.3	24.3
T13B5.5	LIPaSe related	high	27.5	31.0
W03G1.7b	Acid SphingoMyelinase	medium	26.8	11.6
C25A8.1	C25A8.1	low	26.1	30.6
W03G1.7a	Acid SphingoMyelinase	medium	21.4	10.8
K01D12.8	K01D12.8	high	20.9	7.9
B0432.12	C-type LECtin	low	20.4	12.3
F59B2.7	RAB family	medium	20.3	28.3
Y34B4A.4b	Y34B4A.4	low	16.9	26.9
Y75B8A.11	Y75B8A.11	medium	16.9	9.0
Y68A4B.1	C-type LECtin	low	16.9	14.2
F28A10.10	F28A10.10	low	16.4	17.1
T27E7.4	T27E7.4	low	16.3	14.7
C07A9.13	C07A9.13	medium	15.8	15.5
Y45F10B.12	Y45F10B.12	low	15.1	18.3
F45E6.6	SKN-1 Dependent Zygotic transcript	low	14.1	20.9
C44H9.1	UDP-GlucuronosylTransferase	medium	13.8	12.3
B) Up-regulated genes				
F54B8.15	srbc-53, serpentine receptor, class BC	low	7.5	6.3
F18E3.12	F18E3.12	high	5.6	3.7
C06B3.6.1	C06B3.6	high	4.9	2.0
F18E3.7b	D-aspartate oxidase 1	high	4.8	4.3
F57A10.2	F57A10.2	low	4.7	4.3
F18E3.7a	D-aspartate oxidase 1	high	4.6	4.1
T20D4.13	Putative uncharacterized protein	medium	4.5	5.9
T04A11.14	T04A11.14	low	4.5	2.5
Y69A2AR.5	D-amino-acid oxidase 2	medium	4.1	3.4
K09H9.5	K09H9.5	medium	3.8	4.0
M03B6.4b	M03B6.4	low	3.2	3.2
W03F9.3	W03F9.3	low	3.2	3.1
Y51B9A.6b.1	Y51B9A.6	medium	3.0	2.7
Y51B9A.6a	Y51B9A.6	medium	2.8	2.5
K09F6.6	K09F6.6	low	2.7	3.1
F36H5.2a.2	MATH domain containing	low	2.5	1.7
K02D7.5.1	RAG1-activating protein 1 homolog	medium	2.5	1.6
Y41D4B.14	Y41D4B.14	low	2.5	2.3
F55D12.6	F55D12.6	low	2.4	3.2
C10A4.1	C10A4.1	low	2.4	2.9



[a] Expression level (arbitrary units, AU): 0–100 = low; 100–1000 = medium; 1000–10,000 = high; >10,000 = very high.

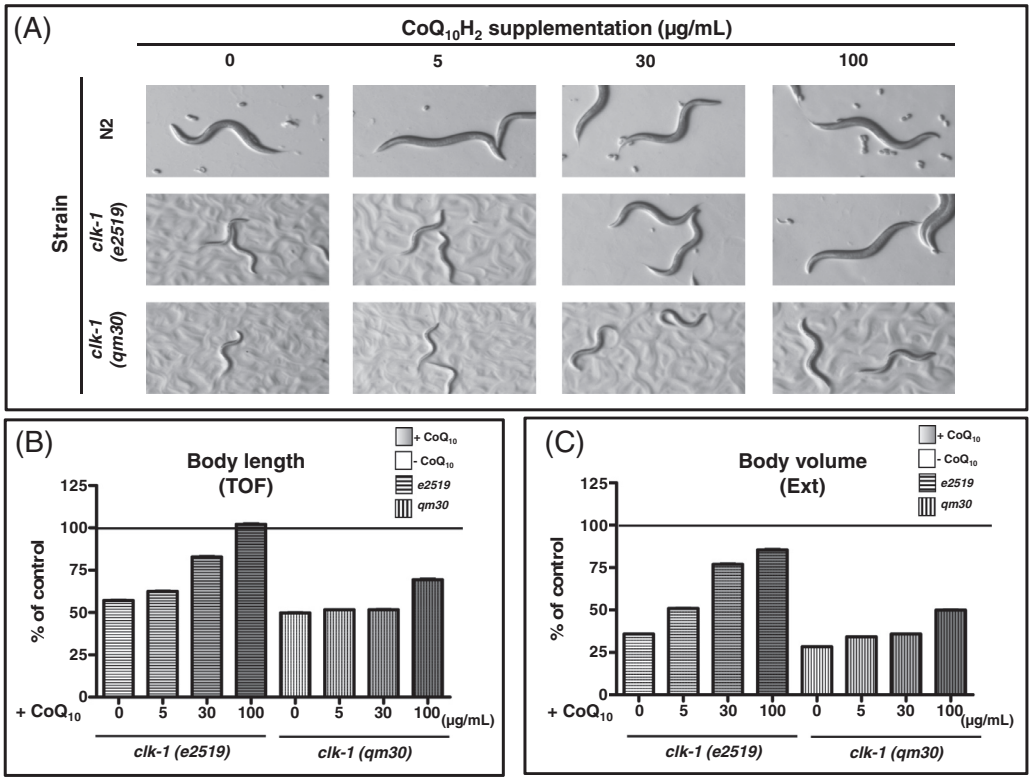


Fig. 3. Microscopic images (A), body length (time of flight, TOF, B) and body volume (extinction, Ext, C) of two *clk-1* mutants strains in dependence of increasing ubiquinol supplementation as % of *C. elegans* N2 wild type control. NGM agar plates containing CoQ_8-deficient bacteria (GD1) were supplemented with 0 µg/ml (vehicle control) 5 µg/ml, 30 µg/ml or 100 µg/ml of the reduced form of CoQ_{10} (ubiquinol). N2 (control strain), CoQ_9 synthesis deficient *clk-1* (*e2519*) and *clk-1* (*qm30*) worms were exposed to the different ubiquinol concentrations for 5 days with adjacent imaging. Body length and body volume was determined using flow cytometry-based TOF- and Ext-values and shown as % of control N2 mean values.

importance for the succinate dehydrogenase complex, the serpentine receptor function, C-type lectins, the UDP-glucuronosyltransferase and the aspartate oxidase. The expression of these genes cannot be rescued by exogenous CoQ supply.

In conclusion, our work provides a comprehensive overview of genes which can be modulated in their expression by endogenous or exogenous CoQ. As growth retardation in CoQ deficiency is linked to the gene expression profile we suggest that CoQ promotes growth via gene expression.

Acknowledgments

This work was supported by Kaneka Corporation, Japan. We thank Catherine Clarke (Department of Chemistry and Biochemistry, University of California Los Angeles) for generously providing the *E. coli* GD1 (ubiG delete) strain and the *Caenorhabditis* Genetics Center for *C. elegans* strains. We also thank Kaneka Corporation for kindly providing ubiquinol-10 and Bernd Janetzky (Technical University of Dresden, Germany) for diethoxy-ubiquinone-10.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.016.

References

[1] F. Gomez, R. Saiki, R. Chin, C. Srinivasan, C.F. Clarke, Restoring de novo coenzyme Q biosynthesis in *Caenorhabditis elegans* coq-3 mutants yields profound rescue compared to exogenous coenzyme Q supplementation, Gene 506 (2012) 106–116.
[2] B. Nowicka, J. Kruk, Occurrence, biosynthesis and function of isoprenoid quinones, Biochim. Biophys. Acta 1797 (2010) 1587–1605.
[3] M. Bentinger, M. Tekle, G. Dallner, Coenzyme Q – biosynthesis and functions, Biochem. Biophys. Res. Commun. 396 (2010) 74–79.
[4] M. Bentinger, K. Brismar, G. Dallner, The antioxidant role of coenzyme Q, Mitochondrion 7 (Suppl.) (2007) S41–S50.
[5] D.A. Groneberg, B. Kindermann, M. Althammer, M. Klapper, J. Vormann, G.P. Littarru, F. Doring, Coenzyme Q10 affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells, Int. J. Biochem. Cell Biol. 37 (2005) 1208–1218.
[6] C. Schmelzer, F. Doring, Identification of LPS-inducible genes downregulated by ubiquinone in human THP-1 monocytes, BioFactors 36 (2010) 222–228.
[7] C.K. Lee, T.D. Pugh, R.G. Klopp, J. Edwards, D.B. Allison, R. Weindruch, T.A. Prolla, The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice, Free Radical Biol. Med. 36 (2004) 1043–1057.
[8] C. Schmelzer, G. Lorenz, G. Rimbach, F. Doring, Influence of Coenzyme Q_{10} on release of pro-inflammatory chemokines in the human monocytic cell line THP-1, BioFactors 31 (2007) 211–217.
[9] C. Schmelzer, G. Lorenz, G. Rimbach, F. Doring, In vitro effects of the reduced form of Coenzyme Q(10) on secretion levels of TNF-alpha and chemokines in response to LPS in the human monocytic cell line THP-1, J. Clin. Biochem. Nutr. 44 (2009) 62–66.
[10] C. Schmelzer, G. Lorenz, I. Lindner, G. Rimbach, P. Niklowitz, T. Menke, F. Doring, Effects of coenzyme Q10 on TNF-alpha secretion in human and murine monocytic cell lines, BioFactors 31 (2007) 35–41.
[11] M.P. Barroso, C. Gomez-Diaz, J.M. Villalba, M.I. Buron, G. Lopez-Lluch, P. Navas, Plasma membrane ubiquinone controls ceramide production and prevents cell death induced by serum withdrawal, J. Bioenerg. Biomembr. 29 (1997) 259–267.
[12] R. Gonzalez, G. Ferrin, A.B. Hidalgo, I. Ranchal, P. Lopez-Cillero, M. Santos-Gonzalez, G. Lopez-Lluch, J. Briceno, M.A. Gomez, A. Poyato, J.M. Villalba, P. Navas, M. de la Mata, J. Muntane, N-acetylcysteine, coenzyme Q10 and superoxide dismutase mimetic prevent mitochondrial cell dysfunction and cell death induced by D-galactosamine in primary culture of human hepatocytes, Chem. Biol. Interact. 181 (2009) 95–106.
[13] L.K. Kwong, S. Kamzalov, I. Rebrin, A.C. Bayne, C.K. Jana, P. Morris, M.J. Forster, R.S. Sohal, Effects of coenzyme Q(10) administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat, Free Radical Biol. Med. 33 (2002) 627–638.
[14] J.J. Ewbank, T.M. Barnes, B. Lakowski, M. Lussier, H. Bussey, S. Hekimi, Structural and functional conservation of the *Caenorhabditis elegans* timing gene *clk-1*, Science 275 (1997) 980–983.

[15] S. Felkai, J.J. Ewbank, J. Lemieux, J.C. Labbe, G.G. Brown, S. Hekimi, CLK-1 controls respiration, behavior and aging in the nematode *Caenorhabditis elegans*, EMBO J. 18 (1999) 1783–1792.
[16] A. Wong, P. Boutis, S. Hekimi, Mutations in the *clk-1* gene of *Caenorhabditis elegans* affect developmental and behavioral timing, Genetics 139 (1995) 1247–1259.
[17] T. Jonassen, P.L. Larsen, C.F. Clarke, A dietary source of coenzyme Q is essential for growth of long-lived *Caenorhabditis elegans clk-1* mutants, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 421–426.
[18] J. Burgess, A.K. Hihi, C.Y. Benard, R. Branicky, S. Hekimi, Molecular mechanism of maternal rescue in the *clk-1* mutants of *Caenorhabditis elegans*, J. Biol. Chem. 278 (2003) 49555–49562.
[19] F. Gomez, G.C. Monsalve, V. Tse, R. Saiki, E. Weng, L. Lee, C. Srinivasan, A.R. Frand, C.F. Clarke, Delayed accumulation of intestinal coliform bacteria enhances life span and stress resistance in *Caenorhabditis elegans* fed respiratory deficient *E. coli*, BMC Microbiol. 12 (2012) 300.
[20] S. Onur, P. Niklowitz, A. Fischer, C.C. Metges, T. Grune, T. Menke, G. Rimbach, F. Doring, A comparative study into alterations of coenzyme Q redox status in ageing pigs, mice, and worms, BioFactors 40 (2014) 346–354.
[21] M. Klapper, M. Ehmke, D. Palgunow, M. Boehme, C. Matthaeus, G. Bergner, B. Dietzek, J. Popp, F. Doering, Fluorescence based fixative and vital staining of lipid droplets in *C. elegans* reveal fat stores using microscopic and flow cytometry approaches, J. Lipid Res. (2011).
[22] B.M. Bolstad, R.A. Irizarry, M. Astrand, T.P. Speed, A comparison of normalization methods for high density oligonucleotide array data based on variance and bias, Bioinformatics 19 (2003) 185–193.
[23] W. Huang da, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (2009) 44–57.
[24] H. Miyadera, H. Amino, A. Hiraishi, H. Taka, K. Murayama, H. Miyoshi, K. Sakamoto, N. Ishii, S. Hekimi, K. Kita, Altered quinone biosynthesis in the long-lived *clk-1* mutants of *Caenorhabditis elegans*, J. Biol. Chem. 276 (2001) 7713–7716.
[25] A.K. Hihi, H. Kebir, S. Hekimi, Sensitivity of *Caenorhabditis elegans clk-1* mutants to ubiquinone side-chain length reveals multiple ubiquinone-dependent processes, J. Biol. Chem. 278 (2003) 41013–41018.
[26] Y. Shibata, R. Branicky, I.O. Landaverde, S. Hekimi, Redox regulation of germline and vulval development in *Caenorhabditis elegans*, Science 302 (2003) 1779–1782.
[27] H. Moribe, E. Mekada, Co-occurrence of tetraspanin and ROS generators: conservation in protein cross-linking and other developmental processes, Worm 2 (2013) e23415.
[28] R. Saiki, A.L. Lunceford, T. Bixler, P. Dang, W. Lee, S. Furukawa, P.L. Larsen, C.F. Clarke, Altered bacterial metabolism, not coenzyme Q content, is responsible for the lifespan extension in *Caenorhabditis elegans* fed an *Escherichia coli* diet lacking coenzyme Q, Aging Cell 7 (2008) 291–304.
[29] D. Cristina, M. Cary, A. Lunceford, C. Clarke, C. Kenyon, A regulated response to impaired respiration slows behavioral rates and increases lifespan in *Caenorhabditis elegans*, PLoS Genet. 5 (2009) e1000450.