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

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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.

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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).