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A conserved START domain coenzyme Q-binding polypeptide is required for efficient Q biosynthesis, respiratory electron transport, and antioxidant function in *Saccharomyces cerevisiae*

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ABSTRACT

Coenzyme Q_n (ubiquinone or Q_n) is a redox active lipid composed of a fully substituted benzoquinone ring and a polyisoprenoid tail of *n* isoprene units. *Saccharomyces cerevisiae coq1–coq9* mutants have defects in Q biosynthesis, lack Q_6, are respiratory defective, and sensitive to stress imposed by polyunsaturated fatty acids. The hallmark phenotype of the Q-less yeast *coq* mutants is that respiration in isolated mitochondria can be rescued by the addition of Q_2, a soluble Q analog. Yeast *coq10* mutants share each of these phenotypes, with the surprising exception that they continue to produce Q_6. Structure determination of the *Caulobacter crescentus* Coq10 homolog (CC1736) revealed a steroidogenic acute regulatory protein-related lipid transfer (START) domain, a hydrophobic tunnel known to bind specific lipids in other START domain family members. Here we show that purified CC1736 binds Q_2, Q_3, Q_{10}, or demethoxy-Q_3 in an equimolar ratio, but fails to bind 3-farnesyl-4-hydroxybenzoic acid, a farnesylated analog of an early Q-intermediate. Over-expression of *C. crescentus* CC1736 or *COQ8* restores respiratory electron transport and antioxidant function of Q_6 in the yeast *coq10* null mutant. Studies with stable isotope ring precursors of Q reveal that early Q-biosynthetic intermediates accumulate in the *coq10* mutant and *de novo* Q-biosynthesis is less efficient than in the wild-type yeast or rescued *coq10* mutant. The results suggest that the Coq10 polypeptide:Q (protein:ligand) complex may serve essential functions in facilitating *de novo* Q biosynthesis and in delivering newly synthesized Q to one or more complexes of the respiratory electron transport chain.

1. Introduction

Coenzyme Q (ubiquinone or Q) is a small lipophilic electron carrier found primarily in the inner mitochondrial membrane where it plays a key role in respiratory electron transport [1]. Q consists of a polyisoprenoid 'tail' whose length is species dependent and a fully substituted redox-active benzoquinone 'head' [2]. The reduced or hydroquinone form, QH_2, also serves as a lipid soluble chain-terminating antioxidant [3]. Yeast mutants lacking Q and QH_2 are sensitive to oxidative stress induced by treatment with polyunsaturated fatty acids [4].

Abbreviations: αLnn, α-linolenic acid (C18:3, $n-3$); BCA, bicinchoninic acid; BHT, butylated hydroxytoluene; BN-PAGE, blue native-polyacrylamide gel electrophoresis; DMQ, demethoxy-Q; DOD, drop out growth medium with dextrose; FHB, farnesyl-hydroxybenzoate; HAB, hexaprenyl-4-aminobenzoic acid; HHB, hexaprenyl-4-hydroxybenzoic acid; HPLC, high performance liquid chromatography; IDMQ, 4-imino-demethoxy-Q; pABA, *para*-aminobenzoic acid; PBS, phosphate buffered saline; PUFA, polyunsaturated fatty acid; Q, coenzyme Q or ubiquinone; QH_2, coenzyme QH_2 or ubiquinol; START, steroidogenic acute regulatory-related lipid transfer; YPD, rich growth medium with dextrose; YPG, rich growth medium with glycerol; YPGal, rich growth medium with galactose

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The toxicity of polyunsaturated fatty acid autoxidation products can be abrogated by substitution of the fatty acid bis-allylic hydrogen atoms with deuterium atoms [5].

In the yeast *Saccharomyces cerevisiae* Q_6 biosynthesis occurs in the mitochondria and is dependent on eleven known proteins, Coq1p–Coq9p, Yah1p, and Arh1p [6–8]. The yeast *coq1–coq9* mutants lack Q, are respiratory defective and unable to grow on non-fermentable carbon sources. *YAH1* and *ARH1* genes encode ferrodoxin and ferredoxin reductase, are essential for yeast viability, and play roles in iron-sulfur cluster biogenesis in addition to Q biosynthesis [7,9].

An additional protein, Coq10p, is required for Q_6 activity in the electron transport chain but is not essential for Q biosynthesis [10]. *S. cerevisiae coq10* null mutants contain wild-type levels of Q_6 but are nonetheless respiration defective. Mitochondria isolated from *coq10* mutants show greatly impaired oxidation of substrates of electron transport as measured by oxygen consumption unless supplemented with exogenous Q_2 [10]. This rescue of respiratory electron transport by addition of Q_2 is a hallmark phenotype of the *coq* mutants. Thus, while yeast *coq1–coq9* mutants are "Q-less", the *coq10* mutant contains Q_6 but its respiratory defects are nevertheless rescued when a soluble analog of Q (such as Q_2) is added. The *coq10* null mutant in the yeast *Schizosaccharomyces pombe* displays similar phenotypes as it produces endogenous Q_{10} but fails to respire as measured by oxygen consumption [11].

How Coq10p mediates Q-dependent respiratory electron transport is still mysterious. Stoichiometric considerations suggest that Coq10p is unlikely to play a direct role in shuttling Q between the respiratory complexes, because Coq10p content is three orders of magnitude less abundant than Q_6 and two orders of magnitude less abundant than other respiratory chain components, such as the bc_1 complex [10]. Yeast respiratory super complexes (as assayed by high molecular mass cytochrome *b*) were detectable but greatly decreased in the *coq10* mutant relative to that of wild-type yeast [12]. Thus, while it is possible that Coq10p transports or shuttles Q_6 or Q_{10} to the respiratory chain complexes [11], it may also serve to escort or chaperone Q to sites within the respiratory chain complexes that are critical for the Q cycle. It seems likely that Q_6 can access the P-site of the bc_1 complex without Coq10p because treatment of *coq10* mutant mitochondria with antimycin A induces H_2O_2 production [12]. While this response to antimycin suggests an active Q-cycle, the bc_1 complex is not functional since electrons are not transferred to cytochrome c_1. Treatment of *coq10* mutant mitochondria with myxothiazol failed to induce H_2O_2 production, consistent with a defect in residence and/or function of Q_6 at the bc_1 complex [12], perhaps at the N-site.

Coq10p homologs are present in a variety of organisms, from bacteria to humans [10]. Expression of the human *COQ10* homolog in *coq10* null mutants of *S. cerevisiae* and *S. pombe* restored growth on non-fermentable carbon sources [10,11]. The primary sequence of Coq10p does not share homology with any protein domains of known function and is classified as part of the aromatic-rich protein family Pfam03654 [13]. The structure of the *Caulobacter crescentus* Coq10p homolog CC1736 was determined by NMR [14] and revealed a structure similar to that of the steroidogenic acute regulatory-related lipid transfer (START) domain, which is known to bind lipids such as cholesterol or polyketides via a hydrophobic tunnel [15,16]. The START domain structure is classified as a helix-grip type, consisting of a seven-stranded anti-parallel β-sheet with a C-terminal α-helix [17]. Purification of *S. cerevisiae* Coq10p indicates that it binds endogenous Q_6, but as purified from yeast the content of Q_6 was substoichiometric [10]. Studies with *S. pombe* Coq10p indicate that this protein binds Q_{10} at an equimolar ratio of ligand to protein and that this binding depends on conserved hydrophobic amino acids [11], as shown via multiple sequence alignment (Fig. 1). Point mutation analyses and molecular modeling studies suggest that *S. cerevisiae* Coq10p likely contains a similar hydrophobic tunnel capable of binding lipid substrates [18]. One postulated function of Q binding by CC1736 and by extension Coq10p may be to chaperone Q to its proper locations in the respiratory chain complexes.

Most of the Coq proteins in *S. cerevisiae* including Coq10p are localized to the matrix side of the inner mitochondrial membrane [8]. Blue native-PAGE and co-precipitation experiments demonstrate that several of the Coq proteins exist in a high molecular weight complex [8,19–21]. Additionally, the steady-state levels of several of these Coq proteins are interdependent as levels decrease significantly in various *coq* null mutants [20]. In contrast, steady state levels of Coq10p are not affected by other *coq* gene deletions [20]. Coq10p has not been demonstrated to interact with the other Coq proteins by co-immunoprecipitation but it was suggested to exist in an oligomeric form via sucrose gradient sedimentation [10] and was recently shown to co-migrate via BN-PAGE with Coq2p and Coq8p [22]. Coq8p contains protein kinase motifs and is required for phosphorylation of Coq3p, Coq5p, and Coq7p [23]. Although Coq8p has not been shown to exist in a macromolecular complex with other Coq proteins, it is required for the stability of several Coq proteins [20]. Over-expression of Coq8p stabilizes the steady-state levels of several Coq proteins in various *coq* null mutants including the *coq10* null [10,24,25] and increases the accumulation of later stage coenzyme Q biosynthetic intermediates [25,26]. Over-expression of Coq2p and Coq7p in the *coq10* null mutant also restores growth on non-fermentable carbon sources, however the greatest effect is observed with over-expression of Coq8p [10]. Over-expression of Coq8p leads to increased levels of endogenous Q_6 [10], which is thought to overcome the defect in Coq10p and allow for functional respiration. In contrast, severe over-expression (300-fold compared to wild-type yeast) of Coq10p in *S. cerevisiae* impairs mitochondrial respiration as observed by decreased oxygen consumption and a decreased ability to utilize non-fermentable carbon sources [24]. It was hypothesized that the respiratory defect caused by over-expression of Coq10p in *S. cerevisiae* is due to sequestering of the endogenous Q_6 by the excess Coq10p [24]. Over-expression of Coq8p suppresses the respiratory deficiency resulting from severe over-expression of Coq10p.

Here we show that expression of the *C. crescentus* CC1736 START domain polypeptide in *S. cerevisiae* restores growth of the *coq10* null mutant on non-fermentable carbon sources and functional electron transport. The content of Q_6 and Q_6 biosynthetic intermediates were also examined in the *coq10* null mutant as well as wild type and the *coq10* null over-expressing CC1736 or Coq8p. Binding studies with Q of varying tail lengths as well as Q biosynthetic intermediates were performed with purified recombinant CC1736. The results suggest that CC1736 and Coq10p bind Q and late-stage Q-intermediates, and play conserved roles in facilitating *de novo* Q synthesis and respiratory electron transport.

2. Materials and methods

2.1. Yeast strains and growth media

Yeast strains used in this study are described in Table 1. Media were prepared as described [27], and included: YPD (2% glucose, 1% yeast extract, 2% peptone), YPGal (2% galactose, 1% yeast extract, 2% peptone, 0.20% glucose), and YPG (3% glycerol, 1% yeast extract, 2% peptone). Synthetic Dextrose/Minimal medium (SDC and SD–Ura) was prepared as described [28], and consisted of all components minus uracil. Drop out dextrose medium (DOD) was prepared as described [29] except that dextrose was used in place of galactose. Plate media contained 2% bacto agar.

2.2. Construction of multicopy yeast expression vector with the CYC1 promoter and a mitochondrial leader sequence from COQ3

Plasmids used and generated in this study are listed in Table 2. A 0.5 kb BamH1-Kpn1 fragment containing the yeast *CYC1* promoter and the amino terminal mitochondrial leader sequence (residues 1 to 35) of yeast *COQ3* was isolated from pQM [30]. This fragment