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Over-expression of COQ10 in *Saccharomyces cerevisiae* inhibits mitochondrial respiration

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ABSTRACT

COQ10 deletion in *Saccharomyces cerevisiae* elicits a defect in mitochondrial respiration correctable by addition of coenzyme Q_2. Rescue of respiration by Q_2 is a characteristic of mutants blocked in coenzyme Q_6 synthesis. Unlike Q_6 deficient mutants, mitochondria of the *coq10* null mutant have wild-type concentrations of Q_6. The physiological significance of earlier observations that purified Coq10p contains bound Q_6 was examined in the present study by testing the *in vivo* effect of over-expression of Coq10p on respiration. Mitochondria with elevated levels of Coq10p display reduced respiration in the *bc*1 span of the electron transport chain, which can be restored with exogenous Q_2. This suggests that *in vivo* binding of Q_6 by excess Coq10p reduces the pool of this redox carrier available for its normal function in providing electrons to the bc1 complex. This is confirmed by observing that extra Coq8p relieves the inhibitory effect of excess Coq10p. Coq8p is a putative kinase, and a high-copy suppressor of the *coq10* null mutant. As shown here, when over-produced in *coq* mutants, Coq8p counteracts turnover of Coq3p and Coq4p subunits of the Q-biosynthetic complex. This can account for the observed rescue by *COQ8* of the respiratory defect in strains over-producing Coq10p.

1. Introduction

Coenzyme Q (ubiquinone) is an essential electron carrier of the mitochondrial respiratory chain. Its main function is to transfer electrons from the NADH- and succinate-coenzyme Q reductases to the *bc*1 complex [1]. Biosynthesis of coenzyme Q in eukaryotes occurs in mitochondria. The benzene ring of coenzyme Q has a polyprenyl side chain with six isoprenoid units (Q_6) in *Saccharomyces cerevisiae* and 10 units (Q_{10}) in humans [2]. Nine yeast nuclear genes (*COQ1–9*) have been shown to be involved in Q_6 synthesis starting with the conjugation of the polyprenyl chain with 4-hydroxybenzoate (4-HB) [3–10]. Recent evidence indicates that para-aminobenzoic acid (pABA) is an alternative Q_6 precursor capable of competing with 4-HB for the prenylation reaction catalyzed by Coq2p [11,12]. Accordingly, mitochondrial ferredoxin [13], and ferredoxin reductase [14] are also required for Q_6 synthesis from pABA [11,12]. *COQ* gene products are located in the mitochondrial inner membrane [15]. Yeast mutants harboring deletions in *coq3–coq9* genes accumulate the intermediate 3-hexaprenyl-4-hydroxy benzoic acid (HHB) and their respiratory deficiency is corrected by coenzyme Q_2 addition to mitochondria and partially by Q_6 to whole cells [10,16,17]. These common features are consistent with conversion of HHB to Q_6 by a multi-subunit Q-biosynthetic complex composed of Coq3p–Coq7p and Coq9p [5,15,18,19]; it has also been suggested that three other genes Coq2p, Coq8p and Co10p are part of another complex [18]. Coq2p is the transferase that adds the polyprenyl side chain to HB [6] while Coq8p has been proposed to be a protein kinase that regulates the pathway by phosphorylating Coq3p [18,20].

Unlike the Q biosynthetic mutants, *coq10* null mutant have wild type level of Q_6 but like the former, its respiratory deficiency is rescued by Q_2 and Q_6 [21]. Over-expression of *COQ8* also partially suppresses the respiratory defect of *coq10* null mutants, probably as a result of having two times more Q_6 in mitochondria [21]. Recently we shown that *coq10* mutants are responsive to antimycin, indicating an active Q-cycle [22] however, they did not respond to myxothiazol and are unable to transfers electrons through cytochrome *c*, suggesting that Coq10p might function in the delivery of Q_6 to its proper site in the bc1 complex [21,22].

Coq10p is a member of the START domain super family [21–25]. This class of proteins has been shown to bind lipophilic compounds such as cholesterol [26]. When over-expressed in yeast, purified Coq10p contains Q_6 [21,23]. The amount of bound Q_6, however,

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is considerably less than the protein on a molar basis, rendering the physiological significance of the bound Q_6 questionable. To address this question we studied binding of Q_6 by Coq10p *in vivo* by measuring the effect of over-expression of the protein on NADH oxidase and NADH-cytochrome *c* reductase activity. Indeed, Coq10p over-expression was previously observed to inhibit the growth of *Schizosaccharomyces pombe* [24]. We present evidences here that high levels of Coq10p compete for the large pool of Q_6 in mitochondria of respiratory competent yeast, thereby preventing it from functioning in electron transport. We also show that the adverse effect of Coq10p on respiration can be reversed *in vitro* by addition of Q_2 to mitochondria and *in vivo* by over-expressing *COQ8* to raise the mitochondrial concentration of Q_6. These results indicate that Coq10p binds Q_6 *in vivo* and that this property is essential for a normal functioning of the electron transport chain.

2. Materials and methods

2.1. Yeast strains and growth media

The genotypes and sources of the yeast strains used in this study are listed in Table 1. The compositions of YPD, YPEG and minimal glucose medium have been described elsewhere [21].

2.2. Plasmid and strains constructions for COQ10 expression

COQ10 was amplified from pCOQ10/ST3 [21] with the primers 5′-ggcagatctatataatggttttgataataaggccc and 5′-gggaagcttcggag agccttctttagaag. The 626 bp fragment was digested with BglII and HindIII and fused to the *GAL10, GPD1,* or *TEF1* promoters in YIp351-GAL, YIp352-GPD1, and YIp352-TEF1, respectively. The later two promoters were transferred from p4XXprom [28] to YIp351 or YIp352 [29]. The resultant pCOQ10/ST24, pCOQ10/ST38 and pCOQ10/ST39 plasmids containing, respectively, the *GAL10-COQ10*, *TEF1-COQ10* and *GPD1-COQ10* fusions, were linearized and integrated at the chromosomal *LEU2* or *URA3* locus in the strain aW303ΔCOQ10 by the one-step gene insertion method [30]. Transformants containing *GAL10-COQ10* were also transformed with pMA210, a high-copy plasmid containing *GAL4* [31].

2.3. Plasmid and strains constructions for COQ8 expression

COQ8 was amplified from pCOQ8/T5 [21] with the primers 5′-ggcagatctatggttacaaatatggtgaa and 5′-ggcctgcagagcggggaagtatttaaac. The 2514 bp fragment was digested with BglII and PstI and fused to the *TEF1* promoter. The resultant pTEF1-COQ8 was integrated at the *URA3* locus in the strain aW303ΔCOQ8 [30]. A hybrid gene expressing a C-terminally HA tagged *COQ8* was constructed after PCR amplification with the primers: 5′-ggggaattccgttaca aatatggtgaaatt, 5′ggcactagttcaagcgtagtctgggacgtcgt-atgggtaaactttat aggcaaaaat. The resultant fragment was digested with BamHI and SpeI and replaced into pCOQ8/T5, pICOQ8/T5, pTEF1-COQ8 plasmids.

2.4. O_2 consumption

Oxygen consumption in mitochondria and spheroplasts was monitored on a computer-interfaced Clark-type electrode at 30 °C with 1 μM of NADH as substrate in the presence of 400 μg/ml mitochondrial protein and 0.002% digitonin. Cytochrome *c* oxidase was blocked with 1 mM KCN in the NADH-cytochrome *c* reductase assay [32].

2.5. Miscellaneous procedures

Total mitochondrial proteins were separated by polyacrylamide gel electrophoresis in the buffer system of Laemmli [33] and Western blots were treated with antibodies against Coq10p [21] followed by a second reaction with anti-rabbit IgG conjugated to a horseradish peroxidase (Sigma). The antibody–antigen complexes were visualized by the SuperSignal chemiluminescent substrate kit (Pierce). Densitometric traces of the X-ray films were performed using 1DscanEX software (Scananlytics).

3. Results

3.1. COQ10 over-expression impaired mitochondrial respiration

The effect of Coq10p over-production on growth and respiration was studied in strains of yeast harboring chromosomally integrated copies of *COQ10* fused to the *GAL10*, *TEF1* or *GPD1* promoters. All the strains displayed elevated concentrations of Coq10p (shown for *GAL10* and *TEF1* fusions in Fig 1A. The highest level of Coq10p, seen in the transformant with the *GAL10-COQ10* fusion, was further increased when co-transformed with a plasmid containing *GAL4* [31] (Fig 1A). Based on the amount of mitochondrial protein used in the Western analysis and the mean of densitometric traces of the X-ray films we estimate up to 300 fold increase of Coq10p in the strain transformed with *GAL10-COQ10 + GAL4* and 100 fold increase for the strain transformed with *TEF1-COQ10* fusion (Fig. 1A).

Purified Coq10p has been shown to contain Q_6, albeit in amounts considerably less than stoichiometric with the protein [21,23]. We reasoned that if binding of Q_6 by Coq10p is part of its normal function, at elevated concentrations Coq10p may sequester enough of Q_6 to affect respiration. This prediction was borne out when *COQ10* was over-expressed from the *GAL10*

Table 1
Genotypes and sources of *Saccharomyces cerevisiae* strains.

Strain	Genotype	Source
W303-1A	*MATa ade2–1, trp1–1, his3–115, leu2–3112 ura3–1 ρ+, can*^{R}	–[a]
aW303ΔCOQ1	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq1::LEU2*	[5]
aW303ΔCOQ2	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq2::HIS3*	[6]
aW303ΔCOQ3	*MATα ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq3::LEU2*	[7]
aW303ΔCOQ4	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq4::TRP1*	[8]
aW303ΔCOQ5	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq5::HIS3*	[9]
aW303ΔCOQ9	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq9::URA3*	[10]
aW303ΔCOQ10	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq10::HIS3*	[21]
aW303ΔCOQ2, ΔCOQ3	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq2::HIS3 coq3::LEU2*	This study
aW303ΔCOQ2, ΔCOQ4	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq2::HIS3 coq4::TRP1*	This study
aW303ΔCOQ2, ΔCOQ10	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 coq2::HIS3 coq10::HIS3*	This study
aW303ΔBCS1	*MATa ade2–1 his3–1,15 leu2–3112 trp1–1 ura3–1 bcs1::HIS3*	[27]

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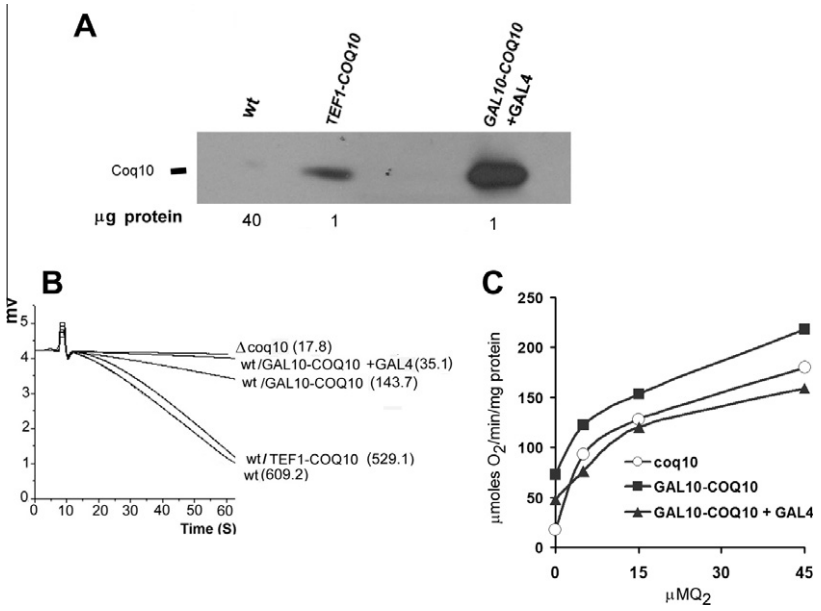

Fig. 1. NADH oxidase activity in wild type yeast over-producing Coq10p. A) Mitochondria of W303-1A (WT), and W303-1A transformed with pCOQ10/ST38 (*TEF1-COQ10*), and pCOQ10/ST24 plus pMA210 (*GAL10-COQ10 + GAL4*) were separated by SDS-PAGE on a 12% polyacrylamide gel. Because of the large variation in the levels of Coq10p in these strains, different amounts of proteins were loaded in each lane as indicated on the bottom of the panel. B) Mitochondria (400 µg protein) of the *coq10* null mutant W303ΔCOQ10 (Δcoq10) and of the strains used in A) were assayed for NADH-oxidase with a Clark electrode as described in the Materials and methods section. The numbers in parentheses are n moles of O_2 consumed/min/mg protein. C) NADH oxidase activity of mitochondria from the Δ*coq10* null mutant and of the wild type W303-1A over-producing Coq10p from *GAL10-COQ10* and *GAL10-COQ10 + GAL4* measured in the presence of different concentrations of Q_2 in the assay.

promoter, which diminished the NADH oxidase activity to approximately 20% of the wild type after correction for the rate in the mutant (Fig. 1B). An almost complete loss of NADH oxidase activity was observed when expression of *COQ10* was further increased by co-transformation of the wild type strain with *GAL10-COQ10* in combination with pMA210, a plasmid containing *GAL4* [31]. In contrast, the NADH oxidase activities of wild type transformed with the *GPD1-COQ10*, and the *TEF1-COQ10* constructs were not altered (Fig. 1 shown for *TEF1-COQ10* fusion). Even though these promoters also increase the mitochondrial concentration of Coq10p by a factor of 100, as estimated in Fig. 1A, this was still below the threshold needed for inhibition.

Restoration of NADH and succinate oxidase activity by Q_2 is a hallmark of coenzyme Q deficient mutants. Addition of Q_2 was previously found to also restore the NADH oxidase and NADH-cytochrome *c* reductase activity of mitochondria from the *coq10* null mutant [21]. To assess if the respiratory defect induced by high levels of Coq10p could be similarly reversed, NADH oxidase activity was measured as a function of Q_2 in the assay. These activity measurements confirmed that mitochondria of the *coq10* null mutant and of wild type cells over-expressing Coq10p from the *GAL10* promoter show the same response to Q_2 concentration (Fig. 1C).

Like NADH oxidase, NADH-cytochrome *c* reductase activity was also diminished in strains expressing *COQ10* from the *GAL10* promoter and was corrected by the addition of exogenous Q_2 to mitochondria (Fig. 2B). *COQ2* codes for p-hydroxybenzoate: polyprenyl transferase that catalyzes the second step of coenzyme Q biosynthesis [6]. The NADH-cytochrome *c* reductase activity of mitochondria from the *coq2* null mutant lacking Q_6, was activated by Q_2, as had been reported previously [6]. As expected, no activation of NADH-cytochrome *c* reductase activity was observed in the *bcs1* mutant in which the Rieske iron-sulfur protein fails to be incorporated into the bc1 complex [27].

Over-expression of *COQ8* increases mitochondrial Q_6 by a factor of 2 [21]. The higher concentration of Q_6 has been invoked to explain the partial suppression of the respiratory defect of *coq10* null mutants and *coq9* point mutants by *COQ8* [10,21] (see also Fig. 2B).

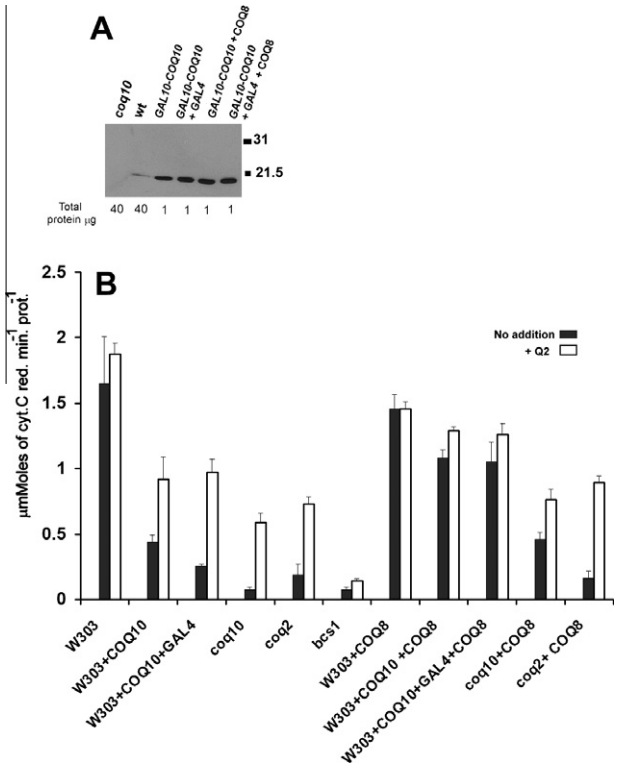

Fig. 2. *COQ8* is a high-copy suppressor of the respiratory defect of strains over-producing Coq10p. A) Immunodetection of Coq10p in mitochondria of W303-1A (WT), W303ΔCOQ10 (Δ*coq10*), and W303-1A with chromosomally integrated *GAL10-COQ10*, *GAL10-COQ10 + GAL4*, *GAL10-COQ10 +* pTEF1-COQ8 and *GAL10-COQ 10 + GAL4 +* pTEF1-COQ8. The amounts of proteins used for the Western analysis is indicated at the bottom of the panel. B) Mitochondria of W303-1A (WT), W303-1A with chromosomally integrated *GAL10-COQ10* and *GAL10-COQ10 + GAL4*), W303-1A with null mutations in *COQ10* (Δ*coq10*), *COQ2* (Δ*coq2*) and *BCS1* (Δ*bcs1*), and the same strains transformed with pTEF1-*COQ8*, were assayed for NADH-cytochrome *c* reductase activity with and without 1 µM Q_2 in the assay.

To see if the *COQ8* over-expression could also counteract the respiratory inhibition imposed by excess Coq10p, NADH-cytochrome *c* reductase activity was measured in wild type cells containing the integrated *GAL10-COQ10* constructs, and in the *coq10* and the *coq2* null mutants as positive and negative controls, respectively. The NADH-cytochrome *c* reductase activity was largely restored when the wild type strain with either *GAL10-COQ10* alone or together with *GAL4* were transformed with *COQ8* on a high-copy plasmid (Fig. 2B). The respiratory activities of the mitochondria from these cells were also increased by Q_2.

The deleterious effect of high levels of Coq10p on respiration was confirmed by the growth phenotypes of the different strains harboring *COQ10* under the control of strong promoters. Supplementation of the minimal medium WOEG with 0.1% galactose inhibited growth that correlated with the increase in the mitochondrial concentration of Coq10p (Fig. 1A and Fig. 2). The most severe impairment of growth on non-fermentable substrates was seen in the wild type co-transformed with the *GAL10-COQ10* and *GAL4*, which correlated with the Coq10p over-production. Growth of this strain was comparable to the *coq10* null mutant (Fig. 3A). *COQ8* over-expression improved growth of the wild type with the *GAL10-COQ10* fusion alone or together with *GAL4*. Transformants with the *GPD1-COQ10* and *TEF1-COQ10* fusions (not shown) grew as well as the parental wild type on minimal glucose and WOEG supplemented with 0.1% galactose. The growth properties are completely consistent with the results obtained with the measurements of NADH-oxidase and NADH-cytochrome *c* reductase. Moreover, we also check the effect of *COQ10* over-expression on different *coq* mutants. Q_6 supplementation partially rescues respiratory growth, in liquid media, of all coenzyme Q mutants [10,16,17,21]. To test the effect of the *GAL10-COQ10* fusion on the Q_6–dependent rescue of respiration, *coq5* or *coq9* mutants were transformed with the plasmid containing the *GAL10-COQ10* fusion. Following growth in rich galactose, growth of the transformants were measured in glycerol/ethanol medium supplemented with 5 μM Q_6. Rescue by Q_6 was diminished in both mutants harboring the plasmid with the *GAL10-COQ10* fusion confirming the respiratory toxicity of Coq10p excess. (Fig. 3B). On the other hand total depletion of Coq10p exacerbates the mutant phenotype of coenzyme Q biosynthesis mutants Single and double *coq2* mutants in which the second mutation was also in a gene involved in Q_6 biosynthesis (*coq3* or *coq4*) had similar generation time in rich glycerol/ethanol medium supplemented with 15 μM Q_6 (Fig. 3C). In contrast, the generation time of a *coq2/coq10* double mutant in such media was two times longer.

3.2. Coq8p over-production increases the steady state level of Coq3 and Coq4 proteins in coq selected mutants

Coq3p-Coq7p and Coq9p are part of the Q synthesis complex [9,19,20]. Although *coq8* mutants have the same phenotype as the other *coq* mutants, Coq8p is not associated with the Q synthesis complex. Coq8p has been implicated in activation of the O-methylase Coq3p [18]. When over-expressed, *COQ8* suppresses not only *coq10* [21] but also *coq9* mutants by raising the mitochondrial concentration of Q_6 [10]. To gain a better understanding of how Coq8p affects mitochondrial Q_6 levels, we analyzed the effect of Coq8p over-expression on several subunits of the Q synthesis complex in wild type and in different *coq* mutants. The abundance of Coq8p was compared in a *coq8* mutant in which the protein was expressed with a C-terminal HA tag. The respiratory defect of the *coq8* mutant was complemented with all fusion constructs indicating that the tag did not affect the function of the protein (not shown). The results of Westerns reveal a strong signal in

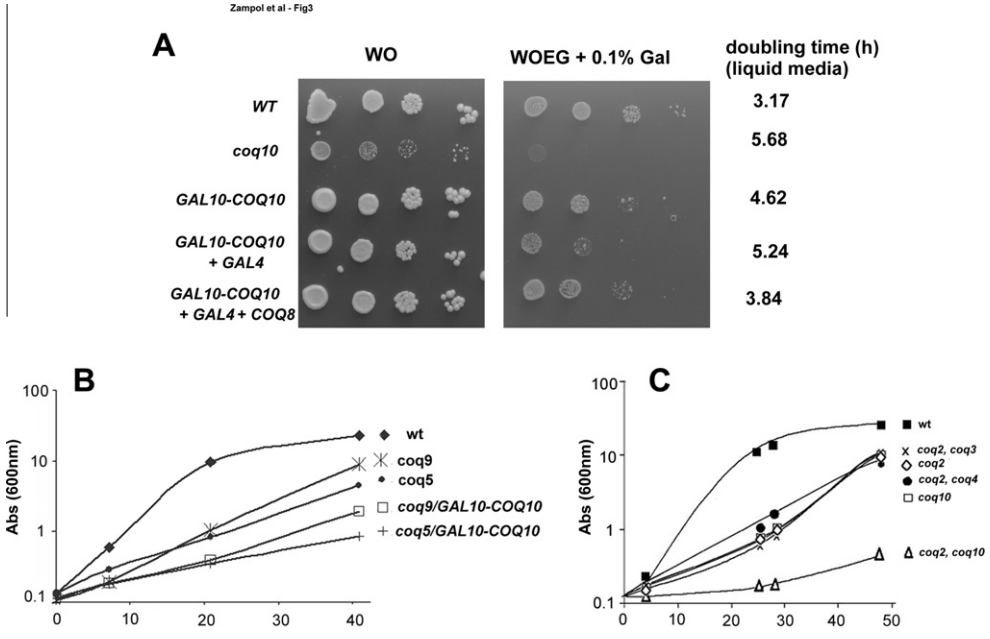

Fig. 3. Growth of strains over-producing Coq10p and Coq8p on ethanol/glycerol with limiting amounts of galactose. W303-1A (WT), the *coq10* null mutant (Δcoq10), and W303-1A expressing Coq10p from *GAL10-COQ10* alone or *GAL10-COQ10* plus *GAL4* without and with *COQ8* over-expression achieved with pTEF1-*COQ8* construct (+ COQ8) were diluted serially and spotted on minimal glucose (WO) and glycerol/ethanol (WOEG) media containing 0.1% of galactose. The plates were incubated for 2 days at 30°. Alternatively growth in liquid WOEG plus 0.1% galactose media was monitored and the doubling time of each strain calculated as indicated at the right of the panel. B) Over-expression of *COQ10* impair the respiratory growth of *coq* mutants supplemented with Q_6. Growth curve on YEPG (rich glycerol plus ethanol) supplemented with 5 μM Q_6 of the parental respiratory competent strain W303, the null mutants *coq5* and *coq9*, and the two mutants transformed with the *GAL10-COQ10* construct (*coq5/GAL10-COQ10* and *coq9/GAL10-COQ10*). C) Rescue of single and double mutants by exogenous CoQ6. The respiratory competent parental strain W303-1A (WT), the Δ*coq2 and* Δ*coq10* null mutants and the double null mutants Δ*coq2* Δ*coq3*, Δ*coq2* Δ*coq4* and Δ*coq2* Δ*coq10* were grown in liquid YPEG supplemented with 15 μM of Q_6. Growth was monitored by measuring absorbance at 600 nm during 50 h. Samples were periodically checked for contaminants. The growth curves shown are representative of three independent experiments.

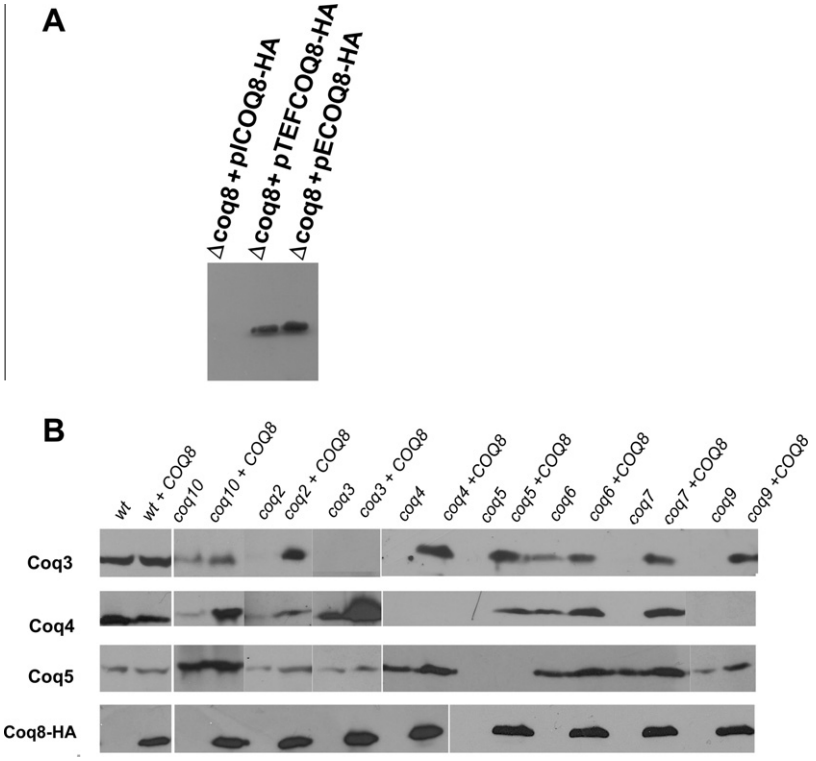

Fig. 4. Effect of Coq8p over-production in the steady state level of Coq3p, Coq4p Coq5p and Coq8p. A) Immunodetection of HA-tagged Coq8p from the modified gene on a high-copy plasmid (pECOQ8-HA) and the integrants: pICOQ8-HA and pTCOQ8-HA (TEF1-COQ8-HA fusion). B) Immunodetection of Coq3p, Coq4p and Coq5 in the W303-1A (WT) and in the indicated *coq* null mutants without and with the TEF1-*COQ8-HA* fusion. Mitochondrial proteins were separated by SDS-PAGE on 12% polyacrylamide gels and western blots were reacted with a rat monoclonal antibody against the HA tag and rabbit polyclonal antibodies against Coq3p, Coq4p and Coq5p. Antigens were visualized with the SuperSignal chemiluminescent substrate kit (Pierce Chemical Co.) after a secondary reaction with peroxidase-conjugated anti-rat and anti-rabbit IgG (Sigma).

mitochondria isolated from strains harboring the multi-copy plasmid
(pECOQ8-HA), or the TEF1-COQ8-HA fusion (pTEF1-COQ8-HA) (Fig. 4A). However we could not detect any signal for the single-copy transformant (pICOQ8-HA), perhaps because of its low expression.

In agreement with published data [15], *coq* mutants display severe reductions in Coq3p and Coq4p but not Coq5p (Fig. 4B). With the exception of the *coq9* mutant, both proteins are restored to different degrees when the mutants are transformed with pTEF1-COQ8-HA indicating that Coq8p probably stabilizes these components of the Q-biosynthesis complex (Fig. 4B). Curiously in the *coq9* mutant, extra *COQ8* did not stabilize Coq4p.The increased stability of Coq3p and Coq4p helps to explain how *COQ8* over-expression increases the mitochondrial concentration of Q_6, which was previously invoked to be responsible for rescue of respiration in the *coq10* mutant [21].

4. Discussion

The yeast *COQ10* gene codes for a mitochondrial inner membrane protein that is essential for respiration. Unlike *coq1–9* mutants that fail to synthesize Q_6 [3–10], yeast *coq10* mutants [21,25] have normal amounts of Q_6 but are defective in reducing cytochrome *c*. The respiratory block can be completely restored in isolated mitochondria by Q_2, a more diffusible substrate of the bc1 complex than Q_6 [16,18].

Coq10p is a homolog of *Caulobacter crescentus* reading frame CC1736 [23]. This bacterial protein is a member of the START superfamily implicated in the delivery of polycyclic compounds [26], which are thought to bind to a hydrophobic tunnel that is a characteristic structural feature of this protein family. This tunnel also appears to be essential for Coq10p function [25]. Coq10p was proposed to be a coenzyme Q binding protein based on the presence of Q_6 in a preparation purified from an over-expressing strain of yeast. In view of the very low amount of bound Q_6 (0.035 mol/mol protein) there was the question of whether Coq10p binds Q_6 under *in vivo* conditions.

In the present study this question was examined by measuring respiration in cells expressing different levels of Coq10p. Coq10p over-expression was previously observed to inhibit the growth of *S. pombe* [24]. Fusion of *COQ10* to strong yeast promoters such as *GAL10* raised the mitochondrial concentration of Coq10p by more than 300 fold. The over-expressing cells show a mild growth defect on minimal ethanol/glycerol media containing a low concentration of galactose for induction of the *GAL10* promoter. Although the cells grew with a longer generation time, the full effect on growth was mitigated by the only partial activation of the *GAL10-COQ10* fusion gene by the two main sources of carbon (glycerol and ethanol) in the growth medium. More direct evidence of a respiratory defect was obtained by enzymatic assays of NADH-oxidase, and NADH-cytochrome *c* reductase in isolated mitochondria of the over-producing cells grown on galactose. The deleterious effect of excess Coq10p on respiration is specific and related to a lower effective concentration of Q_6 available for electron transport. This was evident from the ability of mitochondria obtained from the cells harboring the *GAL10-COQ10* fusion gene to oxidize NADH when Q_2 was added to the assays. Similarly, significant rescue of the respiratory defect was attained by over-expression of *COQ8*, which was previously shown to double the mitochondrial concentration of Q_6. These results substantiate *in vivo* binding of the large mitochondrial pool of Q_6 by Coq10p.

Coq8p has been proposed to be a protein kinase that targets the Coq3p O-methylase [18] and functions in some aspect of the organization of this and other components of the Q-biosynthetic complex [18–20]. Mutations in *COQ* genes lead to instability of most components the complex [15]. This raised the possibility that Coq8p over-expression may reduce turnover of some components of the complex and in this manner enhance synthesis of Q_6. The mechanism by which Coq8p suppress the growth and respiratory defect of the *coq10* null mutant as well the toxicity of Coq10p over-production, was studied by comparing the steady- state levels of Coq3p, Coq4p and Coq5p in wild type, in several *coq* mutant and in the same strains transformed with pTEF1-*COQ8-HA* fusion plasmid. These immunochemical analysis disclosed a marked difference in the concentrations of Coq3p and Coq4p in *coq* mutants transformed with *COQ8-HA*. Coq5p, which been previously shown to be stable in all the *coq* mutants, was also not affected in the strains used here.

HHB, an early intermediate in Q_6 synthesis, accumulates in *coq3*-coq9 independent of where the mutational block is in the pathway [16]. This has been explained by the already mentioned high turnover of the Q-biosynthetic complex when any one of its components is mutated [15]. This circumstance has made it difficult to place some of the *COQ* gene products in the Q_6 biosynthesis pathway. The ability of extra Coq8p to stabilize components such as Coq3p and Coq4p may offer a way out of this impasse by increasing the steady-state concentrations of precursors in mutants such as *coq4* and *coq9* for which a specific role on Q synthesis is still lacking.

Human patients containing mutations in genes involved in the coenzyme Q synthesis have been described in the last few years and the present study points out that an over abundance of Coq10p can be another cause of such disorders.

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