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Saccharomyces cerevisiae coq10 null mutants are responsive to antimycin A

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Abstract

Deletion of *COQ10* in *Saccharomyces cerevisiae* elicits a respiratory defect characterized by the absence of cytochrome *c* reduction, which is correctable by the addition of exogenous diffusible coenzyme Q_2. Unlike other *coq* mutants with hampered coenzyme Q_6 (Q_6) synthesis, *coq10* mutants have near wild-type concentrations of Q_6. In the present study, we use Q-cycle inhibitors of the coenzyme QH_2-cytochrome *c* reductase (*bc*1 complex) to assess electron transfer properties of *coq10* cells. Our results show that *coq10* mutants respond to antimycin A, indicating an active Q cycle in these mutants, even though they are unable to transport electrons through cytochrome *c* and are not responsive to myxothiazol. EPR spectroscopic analysis also suggests that wild type and *coq10* mitochondria accumulate similar amounts of Q_6 semiquinone, despite a lower steady state level of *bc*1 complex in the *coq10* cells. Confirming the reduced respiratory chain state in *coq10* cells, we found that the expression of the *Aspergillus fumigatus* alternative oxidase in these cells leads to a decrease in antimycin-dependent H_2O_2 release and improves their respiratory growth.

Keywords

Saccharomyces cerevisiae; mitochondria; coenzyme Q

Introduction

Coenzyme Q (ubiquinone) is an essential electron carrier of the mitochondrial respiratory chain whose main function is to transfer electrons from the NADH- and succinate-coenzyme Q reductases to the *bc*1 complex [1]. Electron transfer in the *bc*1 complex occurs through the “Q-cycle” [2–4], in which electrons from reduced coenzyme Q (QH_2) follow a branched path to the iron-sulfur protein and to cytochrome b_L [4].

Biosynthesis of coenzyme Q in eukaryotes occurs in mitochondria. In *Saccharomyces cerevisiae*, the benzene ring of coenzyme Q_6 (Q_6) has a polyprenyl side chain with 6 isoprenoid units [5]. The size of the isoprenoid chain varies among species and affects coenzyme Q diffusion through cell membranes [6]. On the other hand, at least nine yeast

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nuclear genes [7–9] have been shown to be involved in the synthesis of Q_6. *COQ10*, however, is not involved in the synthesis of Q_6 but, interestingly, respective mutants have Q_6 respiratory deficiency [10–12]. All products of *COQ* genes, including Coq10p, are located in the mitochondrial inner membrane [1]. There are genetic and physical evidences that enzymes of Q_6 biosynthesis, but not Coq10p, are part of a multi-subunit complex [13–15].

Coq10p is a member of the START domain super family [10,12]. Members of this family were shown to bind lipophilic compounds such as cholesterol [16]. When over-expressed in yeast, purified Coq10p contains bound Q_6 [10,11]. The inability of Q_6 in *coq10* mutants to promote electron transfer to the *bc*1 complex suggests that Coq10p might function in the delivery of Q_6 to its proper site in the respiratory chain. A direct role of Coq10p in electron transfer is not completely excluded, although it appears unlikely based on stoichiometric considerations [10]. The present studies were undertaken to assess the respiratory functionality of Q_6 in the *coq10* mutants which are defective in the reduction of cytochrome c. Using *bc*1 complex inhibitors, we observed that *coq10* mitochondria were responsive to antimycin A but not to myxothiazol, indicating an active Q-cycle, and a defective transfer of QH_2 to the *bc*1 Rieske protein. EPR spectroscopic analysis also suggests that wild type and *coq10* mitochondria have similar amounts of Q_6 semiquinone, even with a lower steady state level of *bc*1 complex. On the other hand, the expression of *Aspergillus fumigatus* alternative oxidase [17], which transports electrons directly from QH_2 to oxygen, reduced the H_2O_2 release in *coq10* cells and improved their respiratory growth.

Results

Effect of antimycin A and myxothiazol on semiquinone formation in the coq10 mutant

Antimycin A and myxothiazol are well known inhibitors of the *bc*1 complex acting, respectively, at the N and P sites of the Q cycle [18–21]. Both inhibitors enhance formation of oxygen radicals from the P site [20,21]. Antimycin A binds to the N site and blocks oxidation of cytochrome b_H, resulting in a reverse flow of electrons from cytochrome b_L to coenzyme Q to form the semiquinone (see Fig. 1). Myxothiazol, on the other hand, binds to the P site and prevents the reduction of cytochrome b_L, but allows a slow reduction of the Rieske iron-sulfur protein [4,20]. An increase of myxothiazol- dependent semiquinone is thought to occur at the P site due to an incomplete inhibition of ubiquinone oxidation [20–22]. However, the existence of semiquinones at the P site is still controversial [20,23].

The functionality of the P site in a *coq10* mutant was studied by examining antimycin A or myxothiazol-dependent production of reactive oxygen species by assaying for H_2O_2 [21,22]. Yeast stains with different respiratory capacities were also used as controls. Therefore, the effect of the two inhibitors was also tested in the parental wild type strain, in a *coq2* mutant lacking Q_6 as a result of a deletion in the gene for p-hydroxybenzoate: polyprenyl transferase (that catalyzes the second step of coenzyme Q biosynthesis [24]), in a *bcs1* mutant arrested in assembly of the *bc*1 complex [25], and in wild type and *coq10* harboring the pYES2/AfAOX plasmid, expressing *Aspergillus fumigatus* alternative oxidase (AOX) under the control of the *GAL10* promoter [17]. *A. fumigattus* AOX transfers electrons directly from QH_2 to oxygen [17].

Antimycin A increased H_2O_2 release in wild type and *coq10* mitochondria. However, a clear myxothiazol-dependent increment occurred only in the wild type. (Fig. 2A). On the other hand, the spontaneously high H_2O_2 release witnessed in the *coq2* and *bcs1* mutants suggests a greater accumulation of flavin free radicals at the NADH and/or succinate dehydrogenase sites. Under conditions of Q_6 deficiency, when the oxidation of reduced Q_6 is blocked as a result of a defective *bc*1 complex or respiratory inhibitor, keeping the the FMN flavin

reduced, NADH-coenzyme Q reductase (complex I) of mammalian and other mitochondria, including those of most yeast, has been shown to produce reactive oxygen species (ROS) [26]. NADH-coenzyme Q reductase of *S. cerevisiae* also contains FMN but is evolutionarily distinct from complex I. Even so, conditions that prevent reduction of Q_6 in *S. cerevisiae* may be expected to also favor increased production of H_2O_2 through accumulation of flavin semiquinones.

We reasoned that the presence of a bypass for reduced coenzyme Q might alleviate the production of ROS in the *coq10* mitochondria, and, indeed we did observe less H_2O_2 in the mutant expressing the alternative oxidase (AOX) of *Aspergillus fumigatus*.

Indeed, the ROS production in the *coq10* mutant was enhanced by a factor of 4–6 (Fig. 2A), while in the *coq10*/AOX transformant the H_2O_2 release was only two times that observed in the wild type cells. There was also a decrement in the antimycin A-dependent release in the mutant strain expressing AOX. Antimycin A stimulation in the *coq10* mutants, however, was qualitatively different from that seen in the *coq2* or *bcs1* mutant. Antimycin A elicited a three fold increase in ROS formation in the *coq10* mutant when normalized to the rate measured in the absence of inhibitor. In agreement with a previous report [21], antimycin A increased the rate of H_2O_2 release in wild type and AOX transformants, but had no effect in the *coq2* and *bcs1* mutants over and above the rate seen without the inhibitor (Fig. 2B). The ability of antimycin A to stimulate ROS formation in the *coq10* mutant suggests that electron transfer from the low potential cytochrome b_L to Q_6 at the P site does not depend on Coq10p. Myxothiazol also increased H_2O_2 production in wild type mitochondria, although the increment over the basal rate was less pronounced (3-fold). However, in the *coq10* mutant and in the *coq10*/AOX transformant, there were no significant effects on H_2O_2 release due to the addition of myxothiazol. Overexpression of *COQ8* partially suppresses the *coq10* mutant respiratory defect [10]. Accordingly, we found that the presence of extra *COQ8* in these experiments decreased the rate of H_2O_2 release, whereas antimycin A treatment promoted H_2O_2 levels similar to the wild type strains and *coq10*+AOX transformant. On the other hand, we also observe that the *COQ8* overexpressing strain presents a mild, but statistically significant increase in H_2O_2 when in the presence of myxothiazol.

The expression of the *GAL10/AfAOX* fusion in *coq10* cells also improved their respiratory growth when pre-incubated in media containing galactose (Fig. 2B). However the specific enzymatic activity of NADH cytochrome *c* reductase of *coq10*/AOX transformants did not change significantly (Fig. 2C). Curiously, wild type cells harboring the AOX plasmid had less NADH cytochrome *c* reductase activity when compared to the untransformed cells, but the addition of synthetic Q_2 to wild type/AOX mitochondria reestablished the enzymatic activity to wild type levels, indicating that the AOX electronic bypass is responsible for this decrement.

Detection of semiquinones by EPR spectroscopy and the steady state level of bc1 complex in the coq10 mutants

The presence of Q6 semiquinones in *coq10* mutants was checked by low temperature EPR spectroscopy of mitochondria from wild type, *coq10* and *coq1* mutants. *coq1* mitochondria are completely devoid of Q_6 whereas *coq10* organelles have near wild type levels of Q_6 [10]. Spectra were obtained from mitochondria with membrane potentials maintained at 65 mV by the addition of extramitochondrial KCl [27] and using succinate as a respiratory substrate, to minimize the contribution of flavins to the semiquinone signal at g ~2.005 [28,29]. Under these conditions, the magnitude of the g ~2.005 signal was comparable in wild type and *coq10* mutant mitochondria but was significantly lower in the *coq1* mutant (Fig. 3). Because of the absence of Q_6 in the *coq1* mutant, this signal is most likely derived

from flavin semiquinones (Fig. 3a). Semiquinone concentrations in these samples were estimated by double integration of the EPR spectrum and comparison to the standard 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy solution scanned under the same conditions. The calculated value for the wild type mitochondria was 1.3 nmoles/mg protein while the *coq10* mutant was 1.7 nmoles/mg protein. The semiquinone concentration in the *coq1* mutant was not calculated because the spectrum obtained for this mutant contained a depression close to the semiquinone signal precluding the quantification by double integration. The signals detected at g ~ 1.94, corresponding to the iron-sulfur centers, were similar in the two mutants. Approximately half of *coq10*ρ^+ cells and one fifth of *coq1*ρ^+ were converted to ρ^- and ρ^0 after cell growth for mitochondrial preparation. There are a number of cellular events that lead to mitochondrial DNA instability in yeast [30]. We can speculate that changes in the mitochondrial redox state may trigger the observed instability in these *coq* mutants. Nevertheless, this fact could also explain their lower iron-sulfur signal compared to wild type mitochondria. In order to evaluate the presence of the *bc*1 complex in the *coq10* mutant mitochondria, the steady state concentrations of some *bc*1 subunits was checked and compared to wild type mitochondria using different amounts of mitochondrial proteins for quantitative evaluation (Fig. 3B). Western analyses with subunit-specific antibodies revealed six times less cytochrome *b*, and half to two orders of magnitude decrements in the amounts of cytochrome *c1*, Rieske iron-sulfur and core 1 proteins in the *coq10* mitochondria, probably as a consequence of the *coq10* mitochondrial DNA instability. On the other hand, in a *coq2* mutant, the steady state levels of these bc1 complex proteins were one forth lower that of the wild type (not shown). Accordingly, the addition of diffusible Q_2 to the *coq10* mitochondria restored less than half of the NADH-cytochrome c reductase activity of the wild type (Fig. 2C), which is also observed in other *coq* mutants [9, 14, 24]. In agreement with this lower concentration of *bc*1 complex subunits in the *coq10* mutant, Fig. 3C shows one-dimensional BN-PAGE of wild type, *coq10* and *coq2* mutants mitochondria digitonin extracts, immuno-detected with apocytochrome *b*. The predominant signal indicates the presence of high molecular mass complexes in the wild type and in the *coq10* mitochondria digitonin extracts but with altered size in the *coq2* extract as detected before in a *coq4* point mutant [31]. These high molecular mass complexes correspond to respiratory super complexes, which in yeast should involve the association of cytochrome *c* oxidase and *bc*1 complex dimer [32]. Immuno-detection using Cox4p antibodies also revealed the same high molecular mass complexes at the same size and intensity (not shown). It is noteworthy that *coq10* mitochondria extracts revealed complexes apparently at the same size of the wild type, but much less abundant. Altogether, the EPR spectra and *bc*1 complex steady state levels suggest that even with less active *bc*1 complex in the *coq10* mitochondria, it accumulates semiquinones concentrations similar to the wild type.

Superoxide anion formation and redox state of coq mutants

Leakage of electrons emanating from NADH and succinate reduce oxygen to the superoxide anion ($O_2^{\cdot -}$), which is dismutated to H_2O_2 [33]. As already noted, the H_2O_2 assays indicated substantially higher rates of superoxide production in *coq10* and in the *coq2* mutant (lacking Q_6) (Fig. 3B). Measurements of cellular glutathione, a natural ROS scavenger, were used to further assess the redox state of mutants blocked in electron transfer at the level of the *bc*1 complex. The increased oxidant production in *coq10* and *coq2* mutants was supported by their significantly greater content of oxidized glutathione relative to reduced and total glutathione (Fig. 4).

Discussion

The yeast *COQ10* gene codes for a mitochondrial inner membrane protein that binds Q_6 and is essential for respiration [10–12] Unlike *coq1-9* mutants that fail to synthesize Q_6 [7–9],

yeast *coq10* mutants have normal amounts of Q_6, but respiration is completely restored by the addition of the more diffusible Q_2 [10,12].

The ability of Coq10p to bind Q_6 suggested that one of its functions might be the delivery/exchange of Q_6 between the *bc*1 complex and the large pool of free Q_6 during electron transport [10]. This idea was supported by the homology of Coq10p to the reading frame CC1736 of *Caulobacter crescentus*, which codes for a member of the START superfamily [10, 12] implicated in the delivery of polycyclic compounds such as cholesterol. These compounds bind to a hydrophobic tunnel that is a structural hallmark of this protein family. Another possible function of Coq10p was proposed to be in the transport of Q_6 from its site of synthesis to its active sites in the *bc*1 complex, which would also require Coq10p binding to Q_6.

To better understand the function of Coq10p we tested the reducibility of Q_6 in a *coq10* null mutant in the presence of inhibitors that block Q_6 binding to the P (o) and N (i) sites of the *bc*1 complex. Reduction of Q_6 was also examined by comparing the EPR signals associated with semiquinone radicals in wild type and mutant mitochondria and by measuring their concentration of oxidized and reduced glutathione. Since glutathione is an effective scavenger of ROS, the ratio of oxidized to reduced glutathione serves as an index of redox state.

Inhibition of respiration in mammalian and yeast mitochondria with antimycin A has previously been shown to increase the rate of Q reduction to oxygen radicals [20, 21]. In agreement with these data, addition of antimycin A and myxothiazol to respiratory competent yeast mitochondria was found to stimulate oxygen radicals formation by 6 and 3-fold, respectively, as inferred by the rate of H_2O_2 released. A significant (3-fold) antimycin A-dependent increment in ROS was also observed in the *coq10* mutant. The stimulation by antimycin A was not observed in a *bc*1 mutant or in mutants lacking Q_6, and was much lower in the *coq10* mutant when myxothiazol was used. The increase in ROS production in the presence of antimycin A indicates that the mutant is capable of transferring an electron from cytochrome b_L to Q_6 at the P site. Coq10p, therefore, is not required for the accessibility of Q_6 to the b_L center at the P site. Moreover, the presence of the *Aspergillus fumigatus* alternative oxidase [17] as a bypass for reduced Q alleviates H_2O_2 release from the *coq10* mutant and even improved the respective respiratory growth. These results are also supported by EPR spectroscopy of mitochondria. The signal at g~2.005 corresponds to semiquinones and presented a lower magnitude in *coq1* mitochondria. Since this mutant lacks Q_6, the residual signal at g~2.005 is most likely contributed by flavin semiquinone. Because of the lower steady state level of *bc*1 complex in the *coq10* mitochondria, the real magnitude of the EPR signal should be larger in the mutant than in wild type cells.

The possible myxothiazol-dependent reduction of Q_6 to the semiquinone at the P site has been proposed to result from an incomplete inhibition of electron transfer to the iron-sulfur protein [19, 20, 34]. In the strains tested, the presence of myxothiazol elevated the H_2O_2 release only in the wild type cells and in the *coq10* mutant overexpressing *COQ8*.

The Q_6 deficient mitochondria of the *coq2* mutant had a higher basal rate of ROS production than the wild type. The source of the extra ROS is probably NADH and succinate dehydrogenase-associated flavins. Similar results were reported for a Q_6 deficient *coq7* mutant, but only when the mitochondria were assayed at 42°C [35]. Since the assays in the present study were done at 30°C, the difference in ROS production may stem from the genetic background of the W303 strain used in the present study, which could engender a feebler oxidative stress response [36]. Our experiments do not distinguish between flavin and Q_6 as the source of the increased free radicals in the *bcs1* mutant. It is worth

emphasizing that even though the *coq2* and *bcs1* mutants both displayed higher basal rates of ROS production, these were not further enhanced by the addition of antimcyin A, as was the case with wild type and *coq10* mutant mitochondria.

Experimental procedures

Yeast strains and growth media

The genotypes and sources of the yeast strains used in this study are listed in Table I. The compositions of YPD, YPEG and minimal glucose medium have been described elsewhere [10].

O_2 consumption

Mitochondrial and spheroplast oxygen consumption was monitored on a computer-interfaced Clark-type electrode at 30°C with 1 mM malate/glutamate, 2% ethanol or 1 μmol of NADH as substrates in the presence of mitochondria at 400 μg/ml of protein concentration, or spheroplasts at 600 μg/ml of total cell protein. All measurements were carried out in the presence of 0.002% digitonin. In order to block cytochrome *c* oxidase respiration, 1 mM KCN was added at the end of the trace.

H_2O_2 production

H_2O_2 formation in mitochondria was monitored for 10 min at 30°C in a buffer containing 50 μM Amplex Red (Molecular Probes), 0.5 U/ml horse radish peroxidase (Sigma), 2% ethanol, 1 mM malate, 6 mM glutamate and 100 μg/ml of mitochondrial protein. Resorufin production was recorded using a fluorescence spectrophotometer at 563 nm excitation and 587 nm emission wavelengths. A calibration curve of known amounts of H_2O_2 was used to convert fluorescence to concentration of H_2O_2. Antimycin A and myxothiazol were added to a final concentration of 0.5 μg/ml and 0.5 μM, respectively.

Glutathione assays

Oxidized glutathione (GSSG), reduced glutathione (GSH), and total glutathione were determined in late stationary phase using the DTNB (5,5′-dithiobis-(2-nitrobenzoic acid) colorimetric assay [37].

EPR spectroscopy

EPR spectra were recorded at 77 K with a Bruker EMX spectrometer equipped with an ER4122 SHQ 9807 high sensitivity cavity. For these experiments, 8 mg of mitochondrial protein suspended in 0.6 M sorbitol, 10 mM Tris-Cl pH 7.5 and 1 mM EDTA, were maintained at 65 mV by incubation for 2 min with KCl (12.4 mM), valinomycin (0.1 μg/ml) and succinate (1 mM final) [27]. The samples were immediately transferred to a 1 ml disposable syringe, frozen and stored in liquid nitrogen until analysis. Spectra were acquired by extrusion of the samples from the syringe into a finger-tip Dewar flask containing liquid nitrogen and were examined at 77 K in the region of g~2.000 [38]. The spectra shown here were corrected by baseline subtractions. The spectrum of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (g=2.004), and those of known concentrations of 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy, acquired under the same conditions, were used as standards for determining the g values and semiquinone concentrations, respectively.

solutions acquired under the same conditions, was used as a standard for determining the g values.

Miscellaneous procedures

Measurements of respiratory enzymes were performed as described previously [39]. Mitochondria were prepared from yeast grown in rich media containing galactose as a carbon source [40]. Western blot quantifications were performed by 1Dscan EX software (Scanalytics, Inc.) For BN-PAGE, mitochondrial proteins were extracted with 2% final concentration of digitonin and separated on a 4–13% linear polyacrylamide gel [41]. Proteins were transferred to a PVDF membrane and probed with rabbit polyclonal antibodies against yeast cytochrome *b*. The antibody-antigen complexes were visualized using the SuperSignal chemiluminescent substrate kit (Pierce).

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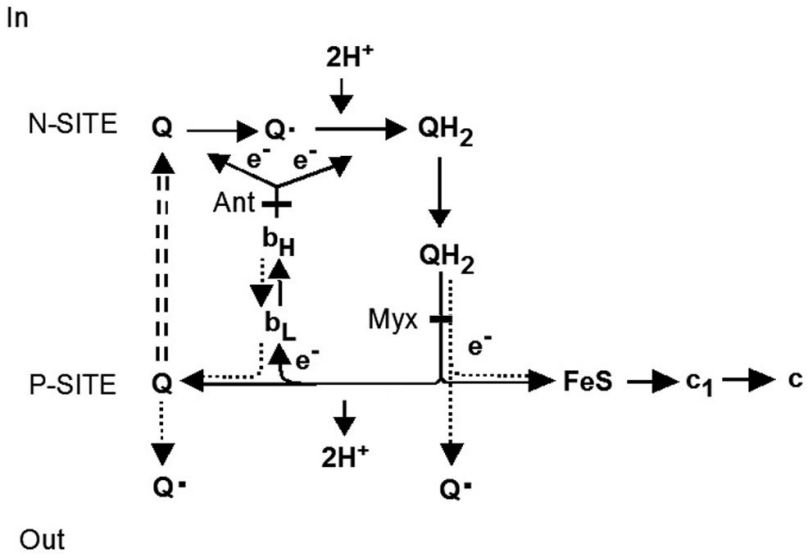

Fig. 1. Protonmotive Q cycle of electron transfer and proton translocation in the bc1 complex

The Q cycle depicted schematically is based on Trumpower et al. and Snyder et al. [4, 32] showing the pathway of electron transfer from reduced Q_6 (QH_2) to cytochrome *c*. At the P site, two electrons are transferred in a concerted manner from QH_2 to the iron-sulfur protein and to cytochrome b_L. Myxothiazol (Myx) binds to the P site and prevents electron transfer to the Rieske protein. At the N site, coenzyme Q (Q) is reduced by cytochrome b_H first to the semiquinone and then to QH_2. This step is inhibited by antimycin (Ant), which binds to the N site. The stippled arrows show the pathway of reduction of coenzyme Q to the semiquinone at the P site in the presence of antimycin A or myxothiazol. The semiquinone formed in the presence of myxothiazol is the result of a slow leak of electrons to the iron sulfur protein [21].

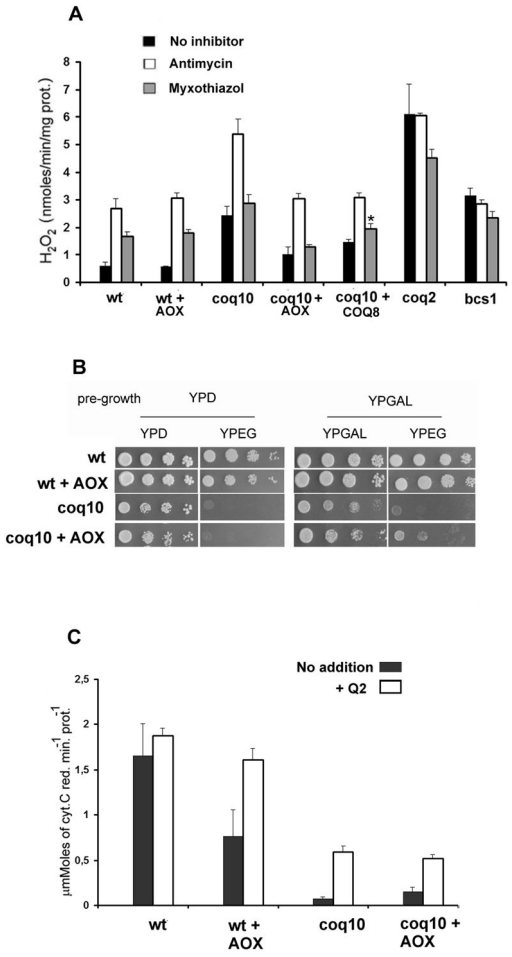

Fig. 2. Antimycin- and myxothiazol-dependent production of H_2O_2

Mitochondria were isolated from the following strains: wild type W303-1A; the *coq* mutants aW303ΔCOQ2 (*coq2*) and aW303 ΔCOQ10 (*coq10*); the *bc*1 deficient mutant aW303 ΔBCS1 (*bcs1*); wild type and *coq10* mutant transformed with pYES2-*AfAOX* (wt+AOX and *coq10* + AOX) and YEp352-COQ8 [10] (*coq10* + *COQ8*). A) Mitochondria (100 μg protein) were assayed as described in the Materials and Methods section for H_2O_2 release before and after the addition of 0.5 μg/ml antimycin A or myxothiazol at a final concentration of 0.5 μM. Both inhibitors increase the basal rate of monoelectronic reduction of oxygen, which generates the superoxide radical $O_{2\cdot}$ [21] that then dismutates to H_2O_2 [30]. The vertical bars indicate ranges of four independent experiments. * $p < 0.01$ *vs* absence of inhibitor, statistical analysis and comparison were performed using unpaired Student's t test conducted by graphPad Prism software. B) Respiratory growth properties of wild type cells, *coq10* mutants, and respective transformants with pYES2/*AfAOX* (wt+AOX, *coq10*+AOX) after pre-growth on glucose (YPD), or galactose (YPGal). C) Measurements of NADH cytochrome *c* reductase activity in isolated mitochondria from wild type cells and *coq10* mutants and respective transformants with pYES2/*AfAOX* (wt+AOX, *coq10*+AOX), with or not the addition of 1 μM of synthetic Q_2. The vertical bars indicate ranges of four independent experiments.

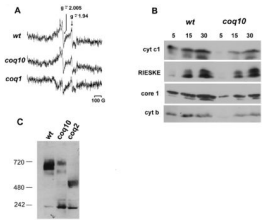

Fig. 3. Detection of semiquinone by EPR spectroscopy and *bc*1 steady state level

A) Representative low temperature EPR spectra of mitochondria isolated from W303 wild type cells (wt), *coq10* and *coq1* mutants maintained at 65 mV by the addition of KCl and succinate. The experimental conditions were as described in Materials and Methods. Spectra were obtained at a microwave power of 10 mW, modulation amplitude of 5G, time constant of 81.920 ms, and a scan rate of 5.96 G. s^{-1}. The receiver gain was 1.12×10^5. Arrows correspond to the expected signal peaks for semiquinones (g ~2.004) and iron-sulfur centers (g ~ 1.94). B) Western blot of *bc*1 complex subunit polypeptides. 5, 15 and 30 μg of mitochondrial proteins from wild type (wt) and *coq10* mutants were separated on a 12% polyacrylamide gel as indicated. The proteins were transferred to nitrocellulose and separately probed with Rieske iron-sulfur protein, core1, cytochrome *c*1, and cytochrome *b* antiserum. C) Mitochondria from wild type (wt), *coq10*, and *coq2* mutants were isolated with 2% digitonin and samples representing 250 mg of starting mitochondrial protein were analyzed by BN-PAGE, which immuno-blot was probed with cytochrome *b* antisera. Estimated molecular masses are indicated and were based on the migration of Fo-F1 ATPase dimmers and monomers [42].

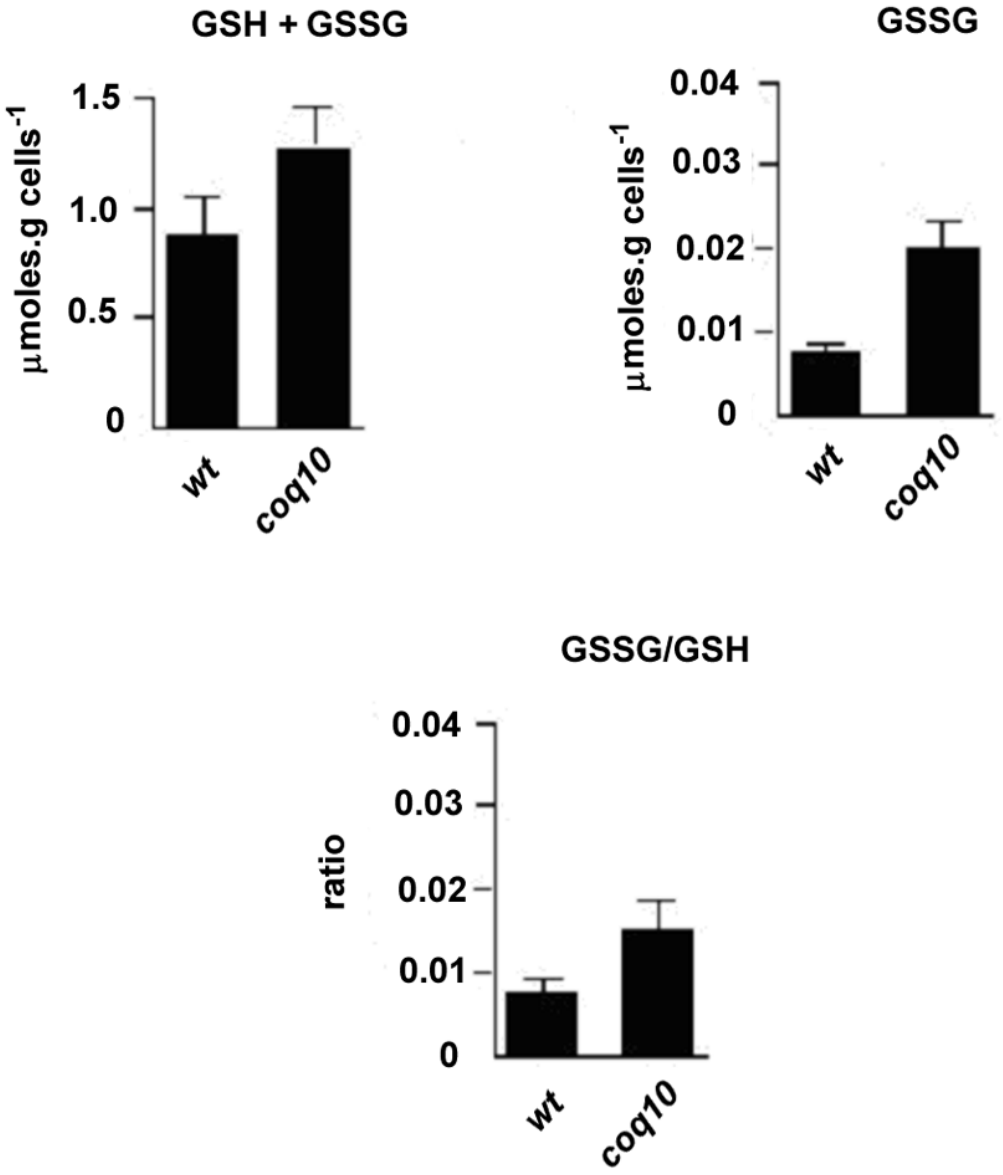

Fig. 4. Whole cell glutathione in wild type and *coq10* mutants

A) Oxidized (GSSG) and total glutathione were assayed in whole cells as previously described [33]. Briefly, total glutathione was determined with 76 μM DTNB in the presence of 0.27 mM NADPH and 0.12 U/ml glutathione reductase. GSSG was estimated by incubation of cells for 1h in the presence of 5 mM *N*-ethylmaleimide at pH to 7. The concentration of reduced glutathione (GSH) was calculated from the difference between total glutathione and GSSG and used to express the ratio GSSG/GSH. The values reported are averages of three independent measurements with the ranges indicated by the vertical bars.

Table 1

Genotypes and Sources of *Saccharomyces cerevisiae* Strains

Strain	Genotype	Source
W303-1[A]	*MAT*a *ade2-1, trp1-1, his3-115, leu2-3,112 ura3-1 ρ+, can*^{R}	Rothstein, R. Columbia University
aW303 ΔCOQ1	*MAT*a *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 coq1::LEU2*	[14]
aW303 ΔCOQ2	*MAT*a *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 coq2::HIS3*	[23]
aW303 ΔCOQ10	*MAT*a *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 coq10::HIS3*	[10]
aW303 ΔBCS1	*MAT*a *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 bcs1::HIS3*	[24]

[a]Dr. R. Rothstein, Department of Human Genetics, Columbia University, New York, NY