Genetic Evidence for a Multi-subunit Complex in Coenzyme Q Biosynthesis in Yeast and the Role of the Coq1 Hexaprenyl Diphosphate Synthase*

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Coenzyme Q (Q) is a lipid that functions as an electron carrier in the mitochondrial respiratory chain in eukaryotes. There are eight complementation groups of Q-deficient Saccharomyces cerevisiae mutants designated coq1-coq8. Here we provide genetic evidence that several of the Coq polypeptides interact with one another. Deletions in any of the COQ genes affect the steady-state expression of Coq3p, Coq4p, and Coq6p. Antibodies that recognize Coq1p, a hexaprenyl diphosphate synthase, were generated and used to determine that Coq1p is peripherally associated with the inner membrane on the matrix side. Yeast Δcoq1 mutants harboring diverse Coq1 orthologs from prokaryotic species produce distinct sizes of polyprenyl diphosphate and hence distinct isoforms of Q including Q7, Q8, Q9, or Q10 (Okada, K., Kainou, T., Matsuda, H., and Kawamura, M. (1998) FEBS Lett. 431, 241–244). We find that steady-state levels of Coq3p, Coq4p, and Coq6p are rescued in some cases to near wild-type levels by the presence of these diverse Coq1 orthologs in the Δcoq1 mutant. These data suggest that the lipid product of Coq1p or a Q-intermediate derived from polyprenyl diphosphate is involved in stabilizing the Coq3, Coq4, and Coq6 polypeptides. Anchors Q to membranes, and the length of this tail varies among different organisms. Saccharomyces cerevisiae synthesizes Q8 bearing six isoprene units, Escherichia coli synthesizes Q6. C. elegans synthesizes Q9, and humans synthesize Q10. The proposed Q biosynthetic pathway in S. cerevisiae and E. coli is shown in Fig. 1. In yeast, there are eight COQ genes required for Q biosynthesis and mutations or deletions in any of these genes result in the loss of Q production and failure to grow on non-fermentable carbon sources (12). The yeast COQ1 gene encodes a hexaprenyl diphosphate synthase (13) responsible for determining the tail length of Q (14). Coq2p, the polyprenyl diphosphate transferase, then attaches this tail to 4-hydroxybenzoic acid to make 3-hexaprenyl-4-hydroxybenzoic acid (HHB, Fig. 1) (15). Coq2p is promiscuous and is capable of utilizing polyprenyl tails of different lengths (16), and polyprenyl diphosphate synthases from different organisms are capable of rescuing coq1 null mutants for Q synthesis and growth on non-fermentable carbon sources (17).

Yeast strains harboring a mutation in the coq3, coq4, coq5, coq6, coq7, or coq8 gene each accumulate the same intermediate, 3-hexaprenyl-4-hydroxybenzoic acid (18). Deletions in any of the COQ genes reduce CoQ3 protein levels and enzymatic activity (19). This interdependence of Coq3p and the presence of other COQ genes suggest that the proteins involved in Q biosynthesis might form a polypeptide complex for proper activity. In this work, steady-state levels of the other Coq proteins are examined to determine whether they too are affected in coq null strains. The role of Coq1p in a possible protein complex is also explored.

EXPERIMENTAL PROCEDURES

Strains and Growth Media—All of the yeast strains used in this study are listed in Table I. Growth media for yeast were prepared as described previously (20) and included YPD (1% yeast extract, 2% peptone, 2% dextrose), YPGal (1% yeast extract, 2% peptone, 2% galactose, 0.1% dextrose), and SD-Leu (0.18% yeast nitrogen base without amino acids, 2% dextrose), and YPG (1% yeast extract, 2% peptone, 3% glycerol), described previously (20) and included YPD (1% yeast extract, 2% peptone, 2% galactose, 0.1% dextrose), and SD-Leu (0.18% yeast nitrogen base without amino acids, 2% dextrose, 0.14% NaH2PO4, 0.5% (NH4)2SO4, and a complete supplement of amino acids minus leucine). The complete supplement was modified as described previously (21). Growth media components were purchased from Difco, Fisher, or Sigma. 2% agar was added for solid media.

Deletion of the COQ1 ORF—A complete deletion of the COQ1 ORF was accomplished with a PCR-targeting strategy. A 1.422-kb fragment containing LEU2 was generated from the YEp13 vector (GenBank accession number U03498) using forward primer pPG1DLF, 5’-ACAC-AAGAAGTGTCATTAGCTCAGTCTGAGTACAGTGGGACGAGA-GTCCGTGAGCGCATA-3’ and reverse primer pPG1DLR, 5’-TTCT-TTTCTTTATTTTCAACCCCTTTTTATTTGAAATTTTACAGGTGTTTCT-TATAGTAAATTTCA-3’. The 5’ end of the forward primer contained the COQ1 ORF followed by 19 nucleotides corresponding to 5579–5597 of YEp13. The reverse primer contained 45 nucleotides from +1422 to +1378 of the reverse strand of the COQ1 ORF followed by 19 residues from 7000 to 6982 of the reverse strand of YEp13. W303–1A cells were transformed

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1 The abbreviations used are: Q, coenzyme Q; ORF, open reading frame; HA, hemagglutinin antigen.

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with 1 μg of the PCR fragment as described previously (22). Transformed cells were grown on SD-Leu plates at 30 °C for 2 days. Gene disruption was verified by PCR using forward primer Coq1SeqF1, 5'-TACGGGATTAGAAGATTAAA-3', from 100 to 81 and reverse primer Coq1SeqR1, 5'-ACATATAAAAACTATTTACA-3', from 1422 to 1403 and produced a product of 1.622-kb for both the wild type and disrupted strains. The PCR product was then digested using the restriction enzyme ClaI, which digested the wild type product and produced two products of 903 and 719 bp, whereas the ClaI digestion fragments of the disrupted product were 516 and 1106 bp.

**Construction of COQ1 Plasmids—**

The pRSQ-1 plasmid (Table II) was made by PCR amplification to create a 2.21-kb fragment using forward primer pBC1–1, 5'-CGCGGGATCCCATGCAAGATTTCTTCCCTG-3', from 752 to 733 upstream of the COQ1 ORF and reverse primer JST2, 5'-GCTCTAGATTACTTTCTTCTTGTTAGTA-3', from 1422 to 1403. This fragment was then ligated into pRS316 (23) at the BamHI and XbaI sites. The single and multi-copy HA-tagged plasmids were both created by PCR using forward primer pHAC1–1, 5'-ACGCAAGCCTGACGTGTTTCAAAGGTCTGGCGC-3', from 1 to 20 of the COQ1 ORF and reverse primer pHAC1–2, 5'-ATAAGAATGCGGCAGCTTTCTTCTTGTTAGTATAC-3', from 1419 to 1400 to create a 1.456-kb fragment spanning the COQ1 ORF. This fragment was then cut with SalI and NotI and ligated into pRSHA1 and pADCL (24) to create the single copy pRSHA1–1 and multi-copy pHA1–1, respectively. The pRSHA1 plasmid was created by digesting the pADCL plasmid with BamHI and ligating a 1.5-kb fragment containing the ADH1 promoter and hemagglutin antigen (HA) epitope into pRS316 (23).

**Generation of Antisera against Coq1p—**
The 1.422-kb COQ1 ORF was PCR-amplified with forward primer pPG1F (5'-GCGGATCCGATGTTTCAAAGGTCTGCGGC-3') from 1 to 20 of the COQ1 ORF, reverse primer pPG1R (5'-GCGGATCCTTACTTTCTTCTTGTTAGTA-3') from 1422 to 1403, and Vent DNA polymerase (New England Biolabs). The product was then digested with BamHI and inserted into PET15b (Novagen) at the BamHI site to generate the plasmid PETQ1 encoding a fusion protein containing a His6 tag at the N terminus. The fusion protein was expressed in the E. coli BL21(DE3) under induction by 1 mM isopropyl β-D-1-thiogalactopyranoside. The protein was purified using nickel-affinity chromatography and sequenced (Novagen).

**FIG. 1. The biosynthetic pathway of Q.** A polyprenyl diphosphate synthase (Coq1p in *S. cerevisiae*, IspB in *E. coli*) assembles the polyprenyl diphosphate tail. After the formation of 3-polypropyl-4-hydroxybenzoic acid by the 4-hydroxybenzoic acid-polypropenyl transferase (Coq2p or UbiA), the proposed biosynthetic pathways for Q in eukaryotes and in prokaryotes are thought to diverge as shown. In yeast, n = 6 and this product is 3-hexaprenyl-4-hydroxybenzoic acid (HHB). In *E. coli*, n = 8 and gene products are identified as Ubi (and also include IspB). *S. cerevisiae* gene products are identified as Coq.

**TABLE I**

| Strain   | Genotype          | Source or reference |
|----------|-------------------|---------------------|
| W303–1A  | MATa ade2–1 his3–11 leu2–3,112 trp1–1 ura3–1 | R. Rothstein*      |
| W303–1B  | MATa ade2–1 his3–11 leu2–3,112 trp1–1 ura3–1 | R. Rothstein*      |
| W303–1A  | COQ1 W303–1A, coq1::LEU2           | This study         |
| CC303    | COQ1 W303–1B, coq1::LEU2           |                     |
| W303–1A  | COQ1 W303–1A, coq1::HIS3           |                     |
| W303–1A  | COQ2 W303–1A, coq2::HIS3           |                     |
| W303–1A  | COQ2 W303–1A, coq2::LEU2           |                     |
| W303–1A  | COQ2 W303–1A, coq2::TRP1           |                     |
| W303–1A  | COQ4 W303–1A, coq4::HIS3           |                     |
| W303–1A  | COQ4 W303–1A, coq4::LEU2           |                     |
| W303–1A  | COQ4 W303–1A, coq4::HIS3           |                     |
| W303–1A  | COQ4 W303–1A, coq4::LEU2           |                     |
| CC304.1  | COQ4 W303–1B, coq4::HIS3           |                     |

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steady-state levels of Coq3p were most affected in the differentially in each increased in this mutant (19, 28). The levels of Coq4p were also severely decreased when any of the other COQ proteins were not affected, indicating that the Coq protein gene is deleted (19), and it seemed probable that the steady-state levels of some of the other Coq polypeptides in yeast strains harboring a deletion in one of the eight COQ genes, ATP2, or COR1. Samples of mitochondria (10 μg of protein) were separated by SDS-PAGE and immunoblotted as described under “Experimental Procedures.” Nycodenz gradient-purified yeast mitochondria were isolated from parental strain W303–1A (W) or from null mutant strains generated in this genetic background or that of W303–1B. Each coq deletion strain is numbered according to its corresponding complementation group (1–8). The ATP2 and cor1 null mutants are designated as A and C, respectively.

1-thio-β-D-galactopyranoside and was purified over His-Bind resin (Novagen) and used to generate antiserum in rabbits (Cocalico).

Isolation of Mitochondria and Submitochondrial Localization of Coqlp—Yeast cultures were grown in YPGal to an 

\[ \text{OD}_{550} \text{ nm} \] of 4, and mitochondria were isolated and purified according to Yaffe (25) as described previously (26). Submitochondrial particles were generated as described previously (27).

Immunoblot Analysis—Immunoblot analysis was performed as described previously (27). Primary antibodies were used at the following concentrations: anti-Coq1p, 1:10,000; anti-Coq3p, 1:1000; anti-Coq4p, 1:1000; anti-Coq5p, 1:1000; anti-Coq6p, 1:500; anti-β-subunit of F1-ATPase, 1:10,000; anti-cytochrome c, 1:10,000; anti-cytochrome b, 1:1000; and anti-Hsp60p, 1:10,000. Goat anti-rabbit secondary antibodies conjugated to horseradish peroxidase (Calbiochem) were used at a 1:10,000 dilution.

RESULTS

Steady-state Levels of Coq3, Coq4, and Coq6 Polypeptides Are Decreased in coq Null Mutant Strains—Coq3p levels are reduced when any COQ gene is deleted (19), and it seemed probable that the steady-state levels of some of the other Coq proteins were similarly affected in this mutant panel. The levels of Coq1, Coq3, Coq4, Coq5, and Coq6 polypeptides in the mitochondria were evaluated by immunoblot analysis with specific antibodies to these proteins. Fig. 2 shows that, in addition to Coq3p, the steady-state levels of Coq4p and Coq6p were also decreased by the deletion of other COQ genes. However, the levels of Coq1p and Coq5p were not affected by the deletion of other COQ genes. Mutants harboring ΔATP2 or Δcor1 were used as respiratory-deficient controls, and steady-state levels of Coq proteins were not affected, indicating that the Coq protein decrease cannot be solely attributed to the loss of respiratory electron transport. The levels of Coq6p decreased almost evenly in each coq mutant. In agreement with previous data, the steady-state levels of Coq3p were most affected in the Δcoq5 mutant (19, 28). The levels of Coq4p were also severely decreased in this Δcoq5 mutant, although Coq4p levels decreased differentially in each coq null mutant. Cytochrome c, cytochrome c1, and the β-subunit of F1-ATPase levels were not decreased by the deletion of any of the COQ genes (Fig. 2 and data not shown).

The Coq1 Polypeptide Is Peripherally Associated on the Matrix Side of the Inner Mitochondrial Membrane—The submitochondrial localization of Coq1p was examined to determine whether it colocalized with the other Coq proteins. Coq1p co-fractionates with Nycodenz-purified mitochondria as did the β-subunit of F1-ATPase (Fig. 3a). Purified mitochondria were osmotically shocked to release intermembrane space components and produce mitoplasts. These mitoplasts were subsequently sonicated to separate matrix and membrane components, and Coq1p was found to be a membrane-bound protein (Fig. 3b). To determine whether Coq1p is an inner or outer membrane protein, mitochondria and mitoplasts were subjected to treatment with proteinase K (PK, 100 μg/ml) for 30 min with or without 1% Triton X-100 (1% Triton). Coq1p was extracted from the membrane by alkaline pH treatment. Mitoplasts were incubated with 0.1 M Na2CO3, pH 11.5, on ice for 30 min. Centrifugation produced soluble (S) and insoluble (P) fractions, which were compared against Nycodenz-purified mitochondria.
distinct isoprenoid tail length (17). Therefore, the steady-state
levels of the other Coq proteins were re-examined in these
\( H9004 \)
coq1
-rescued strains to determine whether the expression of
these divergent polyprenyl diphosphate synthases and the pro-
duction of distinct \( Qn \) isoforms also restored Coq protein levels
(Fig. 5). The levels of Coq5p remain unchanged in all of the
samples. However, steady-state levels of Coq3p, Coq4p, and
Coq6p were partially restored in some of the rescued strains,
although in most cases the prokaryotic Coq1 orthologs did not
restore levels to that of wild type. The steady-state levels of
these proteins were different in each strain. Coq3p and Coq4p
levels were highest in the coq1 null strain harboring the pYD11
(Q10) plasmid, whereas Coq6p was most increased by the pYH7
(Q7) plasmid. In addition, a strain harboring a multi-copy HA-
tagged Coq1p contained increased levels of Coq3p, Coq4p, and
Coq6p as compared with the corresponding low copy HA-tagged
version. Despite a high expression of Coq1 protein from the
pHA1–1 plasmid, the rescue of the other Coq proteins was very
similar to the rescue by pRSQ1–1, a low copy plasmid that
carries \( COQ1 \) and a 752-bp promoter region upstream of the
ATG start site. The addition of \( 2M \) exogenous \( Q6 \) to the growth
medium also increased the levels of Coq3p but had little effect
on the levels of Coq4p or Coq6p.

**DISCUSSION**

This work characterizes the mitochondrial localization of
Coq1p and establishes the interdependence of several of the
Coq polypeptides. Similar to Coq3p, Coq4p, Coq5p, and Coq6p,
Coq1p was also found to localize to the matrix side of the inner
mitochondrial membrane (Fig. 3). This agrees with a sequence
analysis that noted the mitochondrial targeting sequence of Coq1p and hypothesized this protein to be peripherally associated to the inner membrane (13). The data presented here show a decrease in the steady-state levels of Coq3p, Coq4p, and Coq6p in isolated mitochondria from coq null yeast strains. Additionally, the levels of cytochrome c, cytochrome c1, and the \( \beta \)-subunit of F1-ATPase were unchanged, indicating that the decrease in Coq proteins, subsequent loss of Q, and respiratory activity do not necessarily affect the steady-state levels of other mitochondrial proteins involved in the electron transport chain. These results suggest that previously observed decreases in cytochromes c and c1 associated with decreased mitochondrial Q levels may depend on the genetic background of the yeast strain (3, 30).

The data presented here provide further support for a Coq polypeptide multi-subunit complex. Null mutants in coq3, coq4, coq5, coq6, coq7, or coq8 fail to grow on non-fermentable carbon sources, do not produce Q, and accumulate 3-hexaprenyl-4-hydroxybenzoic acid (18). Previously, it had been shown that steady-state levels of Coq3p decrease and that Coq3 O-methyltransferase activity is also greatly reduced in these coq null strains (19). Although the steady-state levels of Coq1p and Coq5p remain unchanged in the mitochondria of the null strains, the decrease in steady-state levels of Coq3p and Coq4p was differential in nature and the coq5 null strain caused the greatest instability for both of these proteins. Although Coq5p is necessary for the stability Coq3p and Coq4p, two coq5 mutants bearing point mutations within or adjacent to methyltransferase motifs can also stabilize these proteins but a point mutant located near the C terminus cannot (28). Similarly, an E226K coq4 point mutant fails to produce Q but has a stable Coq3p (29). The differential stability of Coq3 and Coq4 polypeptides in certain point mutants compared with coq null mutants provides strong evidence of interaction among some of the Coq proteins.

Surprisingly, none of the plasmids containing \( S. \) \( c \)er\( s \)e\( v \)s\( i \)ae\( i \)e\( c \) COQ1 restored wild type levels of Coq3p, Coq4p, or Coq6p. In the case of the pRSQ1–1 plasmid, it is probable that the 752-bp sequence upstream of the ATG initiation site is insufficient as a COQ1 promoter because the levels of Coq1p are lower than they are in the wild type parental strain. In the case of the two HA-tagged plasmids, the strong ADH1 promoter drives the expression of Coq1 and there is an increase in the levels of Coq3p, Coq4p, and Coq6p in the multi-copy plasmid over the single copy. However, the multi-copy HA-tagged Coq1p may have formed aggregates in membranes and thus have reduced enzymatic activity and/or impaired interactions with other Coq proteins.

Is Coq1p itself required for normal steady-state levels of Coq3p, Coq4p, and Coq6p, or is the lipid product of Coq1p sufficient? Previous work has demonstrated that yeast coq1 mutants are rescued by polyisoprenyl diphosphate synthases from other organisms and that the Q isoforms produced contain the polyisoprenyl tails of distinct lengths (17). In some cases, these orthologs are from organisms that do not produce Q. For example, \( H. \) \( a \)c\( c \)m\( h \)u\( p \)h\( i \)l\( i \)t\( u \)s\( i \)n\( e \)n\( z \)a\( e \)n\( e \) produces \( m \)eth\( y \)l\( e \)meth\( y \)la\( m \)on\( e \) bearing a \( h \)e\( x \)ap\( e \)n\( y \)l\( n \) tail (31), whereas \( S. \) \( u \)l\( f \)o\( l \)o\( b \)u\( s \)us\( i \)c\( i \)d\( a \)c\( i \)d\( a \)l\( d \)a\( r \)us\( i \) makes \( c \)al\( d \)e\( r \)i\( l \)e\( a \)ll\( a \)quinone with a hexaprenyl tail (32). The Coq1p ortholog taken from \( S. \) \( a \)c\( i \)d\( o \)c\( a \)l\( d \)a\( r \)us\( i \) is a geranylgeranyl diphosphate synthase that has been mutagenized and selected for the ability to produce longer prenyl products, in this case pentaprenyl diphosphate (33). Hence, it seems unlikely that these diverse polyisoprenyl diphosphate synthases from prokaryotic organisms would interact with the yeast Coq polypeptides. In fact, Coq4p is profoundly stabilized by the presence of each of the diverse Coq1 orthologs and, to our knowledge, has no homolog in prokaryotic genomes. Instead, it appears far more likely that the lipid product of these polyisoprenyl diphosphate synthases, a Q biosynthetic intermediate, or Q itself is involved in stabilizing steady-state Coq polypeptide levels.

When these Coq1p orthologs were expressed in the coq1 null mutant, the steady-state levels of Coq3p, Coq4p, and Coq6p were differentially rescued. The coq1 null strain transformed with pYD11 (Q10) restored levels of Coq3p and Coq4p to almost those of wild type, whereas the coq1 null transformed with pYH7 (Q8) showed partial restoration of Coq4p and Coq6p and very little restoration of Coq3p. What could account for the differential rescue of Coq3p, Coq4p, and Coq6p in the coq1 null mutant rescued with the prokaryotic orthologs of Coq1? It is possible that either the quantity or the tail length of the polyisoprenyl diphosphate-derived product could differentially impact the stability of the Coq polypeptides in the Q-biosynthetic complex. The amount of Q isoforms produced is known to be variable for both yeast and \( E. \) \( c \)ol\( i \) rescued with prokaryotic Coq1 orthologs (17, 34). It is possible that Coq2p may be sensitive to the prenyl tail length. Although it has been observed that the human homolog of COQ2 can likewise rescue yeast cells harboring a coq2 null mutation, the rate of Q biosynthesis

**FIG. 5. Steady-state levels of Coq1, Coq3, Coq4, Coq5, and Coq6 polypeptides in yeast strains producing distinct Q isoforms.** Samples of mitochondria (10 \( \mu \)g of protein) were separated by SDS-PAGE and immunblotted as described under “Experimental Procedures.” The wild type strain W303–1A (W) was compared with the W303–1ADcoq1 (Δ) and rescued strains producing Q isoforms with different tail lengths. W303–1ADcoq1 was transformed with Y-GGPSmut3 (Q5), pYH7 (Q8), pYE6 (Q9), pYD10 (Q9), or pYD11 (Q10), and these strains are indicated in the figure by their respective prenyl tail lengths. Alternatively, the coq1 null mutant was allowed to grow in media supplemented with 2 \( \mu \)M Q6 or was transformed with the following \( S. \) \( c \)er\( s \)e\( v \)s\( i \)ae\( i \)e\( c \) plasmids containing COQ1: a low copy plasmid, pRSQ1–1 (sc); a low copy HA-tagged COQ1, pHA1–1 (mH).
was also lower in these rescued cells compared with that of wild type (35). This could have been attributed to a lower activity in a foreign host, or perhaps the enzyme was sensitive to tail length. Similarly, Coq3p, Coq4, and Coq6p themselves may also be sensitive to prenyl length and are destabilized under these conditions. Coq3p steady-state levels could also be stabilized differentially through their interaction with the polyprenyl diphosphate-derived lipid as either substrate or product. There is precedence for the stabilization of enzymes by their substrate as in the case of methylenetetrahydrofolate reductase in E. coli (36). Whereas Coq4 has no identified catalytic function, Coq3 (a methyltransferase) and Coq6 (a flavin-dependent monooxygenase) could be stabilized through their affinity for the polyprenyl diphosphate-derived Q-intermediate.

In this regard, it is interesting that exogenous Q6 restored levels of Coq3p but had very little effect on Coq4p and Coq6p. Because Q6 is the direct product of the final methyltransferase reaction, Q itself may play a role in stabilizing steady-state levels of Coq3p.

This study provides genetic evidence for the interactions among the Coq proteins in yeast, as indicated by the effects of steady-state stability in coq null mutants. In the case of the coq1 null mutant, polyprenyl diphosphate synthases from a wide variety of prokaryotic organisms are able to restore Q synthesis. Although a direct role of Coq1p in stabilizing the Coq polypeptides cannot be excluded, the findings presented here argue for the involvement of polyprenyl diphosphate itself or a Q-intermediate derived from it in stabilizing the Coq3, Coq4, and Coq6 polypeptides. The identity of this lipid product and the nature of its interaction with Coq polypeptide Q-biosynthetic complex will require further study.

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REFERENCES
1. Brandt, U., and Trumpower, B. (1994) Crit. Rev. Biochem. Mol. Biol. 29, 165–197
2. Kagan, V. E., Nohl, H., and Quinn, P. J. (1996) Handbook of Antioxidants (Cadenas, E., and Packer, L., eds) pp. 157–201, Marcel Dekker, New York, NY
3. Santos-Ocana, C., Do, T. Q., Padilla, S., Navas, P., and Clarke, C. F. (2002) J. Biol. Chem. 277, 10973–10981
4. Gille, L., and Nohl, H. (2000) Arch. Biochem. Biophys. 375, 347–354
5. Santos-Ocana, C., Villalba, J. M., Cordoba, F., Padilla, S., Crane, F. L., Clarke, C. F., and Navas, P. (1998) J. Bioenerg. Biomembr. 30, 465–475
6. Larsen, P. L., and Clarke, C. F. (2002) Science 295, 120–123
7. Jonassen, T., Marbois, B. N., Faull, K. F., Clarke, C. F., and Larsen, P. L. (2002) J. Biol. Chem. 277, 45020–45027
8. Gerome, V., Darin, N., Chretien, D., Benit, P., DeLonlay, P., Rotig, A., Munich, A., and Rustin, P. (2002) Mol. Genet. Metab. 77, 21–30
9. Shults, C. W., Oakes, D., Kieburz, K., Beal, M. F., Haar, R., Plumb, S., Janes, J. L., Nutt, J., Shoulson, I., Carter, J., Kompoliti, K., Perlmutter, J. S., Reich, S., Stern, M., Watts, R. L., Kurlan, R., Molto, E., Harrison, M., and Lew, M. (2002) Arch. Neurol. 59, 1544–1550
10. Markovitch, R. (2003) FEMS Microbiol. Lett. 203, 131–139
11. Turunen, M., Olsson, J., and Dallner, G. (2004) Biochim. Biophys. Acta 1660, 171–199
12. Tzagoloff, A., and Dieckmann, C. L. (1990) Microbiol. Rev. 54, 211–225
13. Ashby, M. N., and Edwards, P. A. (1999) J. Biol. Chem. 265, 13157–13164
14. Okada, K., Suzuki, K., Kamiya, Y., Zhu, X., Fujisaki, S., Nishimura, Y., Nishino, T., Nakagawa, T., Kawamukai, M., and Matsuda, H. (1996) Biochim. Biophys. Acta 1302, 217–222
15. Ashby, M. N., Kutsunai, S. Y., Ackerman, S., Tzagoloff, A., and Edwards, P. A. (1992) J. Biol. Chem. 267, 4128–4136
16. Melaer, M., and Heide, L. (1994) Biochim. Biophys. Acta 1212, 93–102
17. Okada, K., Kainou, T., Matsuda, H., and Kawamukai, M. (1998) FEBS Lett. 431, 241–244
18. Poon, W. W., Do, T. Q., Marbois, B. N., and Clarke, C. F. (1997) Mol. Aspects Med. 18, S121–S127
19. Hsu, A. Y., Do, T. Q., Lee, P. T., and Clarke, C. F. (2000) Biochim. Biophys. Acta 1484, 287–297
20. Burke, D., Dawson, D., and Stevens, T. (2000) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Poon, W. W., Barkovich, R. J., Hsu, A. Y., Frankel, A., Lee, P. T., Shepherd, J. N., Myles, D. C., and Clarke, C. F. (1999) J. Biol. Chem. 274, 21665–21672
22. Gietz, R. D., Schiestl, R. H., Willems, A. R., and Woods, R. A. (1995) Methods Enzymol. 194, 627–643
23. Hsu, A. Y., Poon, W. W., Shepherd, J. A., Myles, D. C., and Clarke, C. F. (1996) Biochemistry 35, 9797–9806
24. Spain, B. H., Koo, D., Ramakrishnan, M., Dzudzor, B., and Colicelli, J. (1995) J. Biol. Chem. 270, 25435–25444
25. Yaffe, M. P. (1991) Methods Enzymol. 194, 627–643
26. Hsu, A. Y., Poon, W. W., Shepherd, J. A., Myles, D. C., and Clarke, C. F. (1996) Biochemistry 35, 9797–9806
27. Baba, S. W., Belogrudov, G. I., Lee, J. C., Lee, P. T., Trasah, J., Shepherd, J. N., and Clarke, C. F. (2004) J. Biol. Chem. 279, 10052–10059
28. Belogrudov, G. I., Do, T. Q., Jonassen, T., Hsu, A. Y., Gin, P., and Clarke, C. F. (2001) Arch. Biochem. Biophys. 392, 48–58
29. Padilla, S., Jonassen, T., Jimenez-Hidalgo, M. A., Fernandez-Ayala, D. J., Lopez-Lluch, G., Marbois, B. N., Navas, P. Clarke, C. F., and Santos-Ocana, C. (2004) J. Biol. Chem. 279, 26995–26999
30. Hollander, R., and Mannheim, W. (1975) Int. J. Syst. Bacteriol. 25, 102–107
31. Thurl, S., Witke, W., Buhrow, I., and Schafer, W. (1986) Biochim. Biophys. Acta 867, 191–197
32. Ohnuma, S., Hirooka, K., Hemmi, H., Ishida, C., Ohito, C., and Nishino, T. (1996) J. Biol. Chem. 271, 18831–18837
33. Jonassen, T., Davis, D. E., Larsen, P. L., and Clarke, C. F. (2003) J. Biol. Chem. 278, 51735–51742
34. Orszag, M., Attersee, M. A., Stransh, J., Willems, A. R., and Czernik, I. (2004) Biochim. J. 382, 519–526
35. Gunther, B. D., Sheppard, C. A., Tron, P., Barns, R., Matthews, R. G., and Ludwig, M. L. (1999) Nat. Struct. Biol. 6, 359–365
36. Do, T. Q., Schultz, J. B., and Clarke, C. F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7534–7539
37. Barkovich, R. J., Slatnick, A., Shepherd, J. A., Lee, P. T., Myles, D. C., Tzagoloff, A., and Clarke, C. F. (1997) J. Biol. Chem. 272, 9182–9188
38. Marbois, B. N., and Clarke, C. F. (1996) J. Biol. Chem. 271, 3995–3998
39. Tzagoloff, A., Wu, M. A., and Crevello, M. (1986) J. Biol. Chem. 261, 17163–17169
40. Okada, K., Kamiya, Y., Zhu, X., Suzuki, K., Tanaka, K., Nakagawa, T., Matsuda, H., and Kawamukai, M. (1997) J. Bacteriol. 179, 5992–5998
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