The Petite Phenotype Resulting from a Truncated Copy of Subunit 6 Results from Loss of Assembly of the Cytochrome bc₁ Complex and Can Be Suppressed by Overexpression of Subunit 9

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Disruption of the gene for subunit 6 of the yeast cytochrome bc₁ complex (QCR6) causes a temperature-sensitive petite phenotype in contrast to deletion of the coding region of QCR6, which shows no growth defect. Mitochondria from the petite strain carrying the disruption allele were devoid of ubiquinol-cytochrome c oxidoreductase activity but retained cytochrome c oxidase and oligomycin-sensitive ATPase activities.

Optical spectra of cytochromes in mitochondrial membranes from the petite strain lacked a cytochrome b absorption band and had a reduced amount of cytochrome c₁. Analysis of mitochondrial translation products showed normal synthesis of cytochrome b. Western analysis of mitochondrial membranes from this disruption strain indicates core protein 1 of the cytochrome bc₁ complex is present in normal amounts, while cytochrome c₁, the Rieske iron-sulfur protein, subunit 6, and subunit 7 were absent or present in very low amounts. Taken together, these findings indicate a loss of assembly of the cytochrome bc₁ complex.

High copy suppressors of the disruption strain were selected. Two separate families of suppressors were found. The first contained overlapping clones of a second gene distinct from QCR6. These plasmids contained QCR9, the gene which codes for subunit 9 of the yeast cytochrome bc₁ complex. Suppression of the QCR6 disruption strain by overexpression of QCR9 indicates a critical interaction between these two proteins in the assembly of the cytochrome bc₁ complex.

The cytochrome bc₁ complex is an oligomeric membrane protein complex of the energy-transducing systems in respiring and photosynthetic organisms (1). The bc₁ complex transfers electrons from ubiquinol to cytochrome c and links proton translocation to this electron transfer by a protonotive Q cycle mechanism (1). In yeast the bc₁ complex consists of 9 subunits that range in size from 7.2 to 44 kDa (2). The bc₁ complex consists of other eukaryotes also contain 9-11 subunits (3). Only three of the subunits, Rieske iron-sulfur protein, cytochrome c₁, and cytochrome b, have obvious electron transfer functions in the complex and are the only subunits in the purified bc₁ complexes from Paracoccus denitrificans (4) and Rhodospirillum rubrum (5). The functions of the supernumery polypeptides in eukaryotes are generally unknown.

Subunit 6 of the yeast cytochrome bc₁ complex, an unusually acidic protein that has been conserved from yeast to humans (6, 7), is involved in the binding of cytochrome c in cooperation with cytochrome c₁ (8). Recently we showed that subunit 6 regulates half of the sites' activity of the dimeric bc₁ complex, possibly in response to membrane potential (9).

QCR₆: the nuclear gene encoding subunit 6, was cloned and characterized by van Loon and co-workers (6). Several groups have shown that null mutations of subunit 6 have no detectable effect on growth of the yeast on nonfermentable carbon sources (9, 10–12). We report here the characterization of a unique mutant of subunit 6 created by a fortuitous disruption of the gene. This mutation causes the yeast to be a temperature-sensitive petite. We describe the biochemical characterization of the defect in this strain and show that the petite phenotype results from failure to assemble the cytochrome bc₁ complex. In addition we have selected for high copy plasmid suppressors of the qcr₆ petite phenotype and discovered an apparent interaction between subunits 6 and 9 of the bc₁ complex.

EXPERIMENTAL PROCEDURES

Materials—L-Amino acids, uracil, adenine sulfate, galactose, ampicillin, raffinose, lysozyme, indoleacrylic acid, and ATP were obtained from Sigma. Phenol was purchased from Mallinckrodt Chemical Works. Yeast extract, peptone, Tryptone, and yeast nitrogen base without amino acids were from Difco. Restriction enzymes and T4-DNA ligase were purchased from New England Biolabs or Bethesda Research Laboratories. Nylon membranes were purchased from ICN K & K Laboratories Inc. Nitrocellulose was from Schleicher & Schuell.

Escherichia coli strain DH5-α (ψ80dlacZΔM15, endA1, recA1, hsdR17 (rK- mK+), supE44, thi-1, λ-, gyrA96, relA1, (laczya- argF8)U169, F' (13)) was obtained from Neil Howell, University of Texas Medical Branch, Galveston, TX. The yeast YEPl3 genomic library was a gift of Mike Douglas, University of North Carolina, Chapel Hill. This library contains 5–8-kb partially digested SacI fragments of yeast genomic DNA inserted in the BamHI site of YEP13 (14).

The plasmid M13mp8 (730-bp SacIqcr₆) was obtained from Les Grivell, University of Amsterdam, Amsterdam (6). pUC18-URA3#21 was obtained from J. Hill, Albert Einstein Medical College, New York.

Yeast strain W303-1B (MATa, ade2-1, his3-11,15, ura3-1, leu2-3,112, trp1-1, can1-100) was obtained from R. Rothstein, Columbia University, New York and is the parental strain of ME59. Yeast strains MES8 (MATa, ade2-1, his3-11,15, ura3-1, leu2-3,112, trp1-1, can1-100, qcr₆Δ1:LEU2) and MES9 (MATa, ade2-1, his3-11,15, ura3-1, leu2-3,112, trp1-1, can1-100, qcr₆Δ2:URA3) were previously described (9). pJY425 is (MAT a, his3-34, ura3-52, leu2-3,112).

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1 The abbreviations used are: QCR, quinol-cytochrome c reductase; kb, kilobase pair; bp, base pair; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
MES11-13A (MATa, ade2-1, ade2-26, his3-11,15, ura3-1, leu2-3,112, trpl-1, qcr6Δ:LEU2, lys1-1, met10-1) is a derivative of MEY3. Yeast strain ME55 (MAT a, adel-100, his3-519, ura3-52, leu2-3,112, qcr6Δ2:URA3) is a disruption derivative of BWG1-7A; the strain ME520 (MAT a, his3-4,4, ura3-52, leu2,112, qcr6Δ2:URA3) is a disruption derivative of the strain JT425.

Construction of qcr6Δ1-URA3 and qcr6Δ2-URA3 Strains—The plasmid M13mp8 (730 bp Sau3AI) contains a 730-bp Sau3AI fragment of the 5' half of QCR6 in an M13 sequencing vector. pUC18/BAMHI211 was constructed by placing a 1180-bp HindIII-BAMHI fragment of URA3 into the HindIII site of pUC18, a gift of J. Hill, Albert Einstein Medical College. The insert was removed from M13mp8 (730 bp Sau3AI) with SpeI and ligated into the appropriate site of the plasmid pUC18/BAMHI211. The resultant plasmid is named pMES7. The plasmid pMES7 was digested with XbaI and SpeI leaving equivalent sticky ends. The region between these two sites was removed, and the plasmid was religated to create pMES8. pMES8 contains a 210-bp internal fragment of QCR6 stretching from the SacII site to the first Sau3AI site.

The plasmid pMES8 was linearized at a unique SacI site in its 210-bp insert. Upon transformation of a ura3 strain of yeast, the plasmid integrates at the SacI site in the yeast genome by homologous recombination (17). After integration the gene for QCR6 is split with one half potentially coding for a protein truncated at the C terminus and the other half potentially coding for a protein truncated at the N terminus. This allele of QCR6 is named qcr6Δ1-URA3.

The qcr6Δ2-URA3 strain was created in the identical way except that transformants were subsequently screened for stability of the pMES8 plasmid. One such stable transformant was found that contained two tandem repeats of the pMES8 plasmid in the SacII site of QCR6. The genomic arrangement of both of these strains was confirmed by extensive Southern mapping. Both of the strains were petite when grown at 37°C.

Yeast Plasmid Recovery—Yeast plasmids were purified essentially as described by Hoffman and Winston (18). Following purification, the plasmids were transformed into E. coli strain DH5α.

Construction of a TrpE/QCR6 Fusion Vector, pMES29—The plasmid pATH2 was obtained from Alex Tzagoloff, Columbia University, and contained most of the coding region for QCR6, where membranes still contain 50% of their wild-type cytochrome c oxidase as shown in Fig. 1B. The resultant plasmid, pMES29, contained an open reading frame in the entire coding region for QCR6, where membranes still contain 50% of their wild-type cytochrome c reductase activity (9). Cytochrome c oxidase activity was diminished approximately 50% in some strains carrying the disruption (Fig. 1A), but in other strains this activity was unchanged. The disruption strain had normal amounts of oligomycin-sensitive ATPase activity (results not shown).

RESULTS

Activities of Respiratory Chain Complexes in a Strain with the Disruption qcr6Δ1:URA3 Allele—Mitochondrial membranes from the yeast strain carrying the qcr6Δ1:URA3 disruption were devoid of ubiquinol-cytochrome c oxidoreductase activity as shown in Fig. 1A. This differs from a deletion of the entire coding region of QCR6, where membranes still contain 50% of their wild-type cytochrome c reductase activity (9). Cytochrome c oxidase activity was diminished approximately 50% in some strains carrying the disruption (Fig. 1A), but in other strains this activity was unchanged. The disruption strain had normal amounts of oligomycin-sensitive ATPase activity (results not shown).

A strain carrying the qcr6Δ1:URA3 allele was also examined and was found to resemble the qcr6Δ1:URA3 strain in loss of ubiquinol-cytochrome c oxidoreductase activity while retaining normal levels of cytochrome c oxidase as shown in Fig. 1B.

Spectral Analysis of Cytochromes in a Strain with the Disruption qcr6Δ1:URA3 Allele—Mitochondrial membranes from the yeast strain carrying the qcr6Δ1:URA3 disruption lacked the optical spectrum characteristic of the cytochrome bc complex as shown in Fig. 2. An optical spectrum of membranes from a yeast strain carrying a wild-type QCR6 allele is shown in the upper trace of Fig. 2. The spectrum shows the typical absorption at 600-605 nm due to cytochromes a and a2. S. Chan and D. Schneider, unpublished data.
Fig. 1. Cytochrome c reductase and cytochrome c oxidase activities of different QCR6 alleles and in complemented QCR6 disruption strains. 

A, mitochondrial membranes from strains with the wild-type, QCR6, the disruption, qcr6α1:URA3, or the deletion, qcr6α1:LEU2, alleles of QCR6 were tested for both cytochrome c reductase and cytochrome c oxidase activities. Activities are in micromoles of cytochrome c reduced or oxidized per min per mg of protein. B, mitochondria from strains with the wild-type allele, QCR6, the disruption allele, qcr6α2:URA3, the disruption allele with a high copy QCR6, or a disruption allele with a high copy QCR9 were tested for cytochrome c reductase and cytochrome c oxidase activities. Activities are in micromoles of cytochrome c reduced or oxidized per min per mg of protein.

An optical spectrum of membranes from a strain carrying the qcr6α1:URA3 disruption allele is shown in the bottom trace of Fig. 2. The spectrum consists of an absorption band at approximately 552 nm attributable to cytochrome c1 and cytochrome c, though due to the large reduction in the intensity of this band, it is probably mostly due to cytochrome c. The typical absorption peak at 560–562 nm due to the b cytochromes, and a shoulder at 550–553 nm due to cytochrome c1 and cytochrome c.

Because mature cytochrome b was missing in the disruption strain, mitochondrial translation products were labeled in the presence of cycloheximide and analyzed by SDS-PAGE. Translated apocytochrome b was clearly present in normal amounts (results not shown), indicating that the defect in cytochrome b is in a post-translational event.

Analysis of Nuclear Encoded Subunits in a Strain with the Disruption qcr6α1:URA3 Allele—Because the defect in this mutant is localized to the mitochondrial cytochrome bc1 complex, we examined the nuclear gene products that were present in mitochondrial membranes of a strain carrying this disruption allele, using antibodies against the nuclear encoded subunits of the complex (23). Antibodies against subunit 6 proved difficult to obtain, and, probably due to its small size and predicted solubility, it is rapidly cleared from immunized animals. We did possess a large amount of rabbit polyclonal sera, initially raised against the low molecular weight subunits of the yeast cytochrome bc1 complex that cross-reacted with subunit 6 in purified bc1 complex. However, this sera also cross-reacted with innumerable yeast proteins. To alleviate this problem, the gene for subunit 6 was fused in frame to the E. coli TrpE gene. This allowed for the induced production of large amounts of subunit 6 antigen in E. coli, where cross-reacting yeast proteins would be absent. This E. coli-produced subunit 6 antigen was used for affinity purification of highly specific subunit 6 antibodies from rabbit polyclonal antibodies raised against the low molecular weight subunits of the yeast cytochrome bc1 complex (23).

Both mitochondrial membranes and whole mitochondria were probed with the subunit-specific antibodies seen in Fig. 3. Identical results were seen with both protein sources. The membranes from strains carrying either a wild-type QCR6 allele or a deletion qcr6Δ1:LEU2 allele (9) contained similar amounts of all the subunits probed for, except for subunit 6, which was absent in the deletion strain (Fig. 3). The strain with the disruption qcr6α1:URA3 allele, despite overloading with protein, contained normal amounts of only core protein 1, while all of the other subunits were absent or present in amounts that were significantly reduced. These include subunit 6, cytochrome c1, the Rieske iron-sulfur protein, and subunit 7 (not shown). This pattern of subunit loss is similar to that seen in either a subunit 8 null mutant, a cytochrome c1 null mutant, or a cytochrome b null mutant.
If the suppression is linked to the library plasmid, then when the strain becomes a leucine auxotroph it should also become a temperature-sensitive petite. When the strain retains leucine prototrophy, it should continue to be a suppressor. This was the case for seven different strains. No derived colonies of these strains were found to be Leu<sup>+</sup> pet<sup>-</sup> or leu<sup>-</sup> Pet<sup>+</sup>, as would be expected from a chromosomal suppressor.

The high copy library plasmids were rescued from the seven suppressed strains and transformed into *E. coli* for amplification. To identify suppressor plasmids that might carry a wild-type *QCR6*, the plasmids were digested with either *Acl* or *SphI*, separated by agarose gel electrophoresis, transferred to nylon, and probed with a 715-bp *Acl* fragment encompassing *QCR6*. Three of the clones hybridized with the *QCR6* probe and gave restriction fragments of the expected size for the *QCR6* gene. However, clones 16, 20, 22, and 24 failed to hybridize with the *QCR6* probe.

**Restriction Mapping and Characterization of the Extragenic Complementing Plasmids**—The clones 16, 20, 22, and 24 all suppressed the *qcr6a2:URA3* allele but were extragenic to *QCR6*. These clones were subjected to restriction analysis and mapped. All four clones were found to have overlapping maps that stretched over 9 kb of the yeast genome as shown in Fig. 4. By eliminating nonoverlapping regions of the clones, the complementing region was confined to 2.8 kb.

The restriction map of this region was suspiciously similar to the restriction map of a gene previously cloned in this laboratory (30). This gene, *QCR9*, codes for subunit 9, the 7.2-kDa subunit, of the yeast mitochondrial cytochrome *bc* complex.

These overlapping clones were digested with either *XbaI* or *EcoRI* and *PstI*, subjected to Southern analysis, and hybridized with a 1.9-kb fragment encompassing the gene for *QCR9*. All of these overlapping clones hybridized with the *QCR9* probe, and they all gave fragments of the expected size for the *QCR9* gene. This 2.8-kb fragment includes all of the elements required for expression of *QCR9* (30).

**Partial Restoration of Cytochrome c Reductase Activity in the Complemented Strains**—To identify the effect overproducing *QCR9* has on the respiratory chain in a *qcr6a2:URA3* strain, ubiquinol-cytochrome *c* oxidoreductase and cytochrome *c* oxidase activities were measured. These activities were measured in mitochondria from the strains overproducing either *QCR6* or *QCR9* in a *qcr6a2:URA3* background and compared with those from the wild-type parent strain and a *qcr6a2:URA3* strain. Respiratory chain activities in these strains are shown in Fig. 1B. As can be seen, the high copy *QCR6* restores the cytochrome *c* reductase activity up to 30%.

**FIG. 3.** Immunological detection of cytochrome *bc* complex subunits in yeast with different *QCR6* alleles. Mitochondrial membranes from strains with different alleles of *QCR6* were subjected to Western analysis. Core protein 1 antibodies were saved as containing potential suppressors. Those with neither leucine nor uracil. Those that grew on both qcr6a2:URA3 us to learn more about the function of this subunit by looking at 10% dextrose plates and grown at 30 °C. Under these conditions there should be little selection for respiratory competence, and the unstable library plasmid should be spontaneously lost. Colonies from these plates were then tested on defined medium minus leucine and on YPG plates at 37 °C.

**FIG. 4.** Restriction map of MES20-complementing clones that failed to hybridize with *QCR6*. The inserts of those plasmids that failed to hybridize with a *QCR6* probe were mapped. The restriction maps of the four clones were found to be overlapping. The dashed line signifies that the insert is in the opposite direction of the solid lines. The position of the *QCR9* transcript is shown. E, *EcoRI*; H, *HindIII*; P, *PstI*; and X, *XbaI*. 

![Diagram of mitochondria with subunits and antibodies](image-url)

![Restriction map of MES20-complementing clones](image-url)
We previously described a novel mutation of QCR6 that causes a temperature-sensitive petite phenotype in *S. cerevisiae*. In this paper mitochondria from the petite strain carrying the qcr6Δ1:URA3 disruption allele were biochemically characterized, and suppressors of this petite strain were selected, identified, and characterized.

Several findings indicate that the petite phenotype is due to a lack of cytochrome *bc* complex. Mitochondria from the strain carrying the disruption qcr6Δ1:URA3 allele were devoid of ubiquinol-cytochrome *c* oxidoreductase activity but had normal amounts of cytochrome *c* oxidase and oligomycin-sensitive ATPase. Optical spectra of mitochondrial membranes from the petite strain point to a loss of both cytochromes *b* and *c*1, while cytochromes *a* and *a*3 are present. This loss of cytochrome *bc* complex is similar to what is seen as a result of mutations in either subunit 7, subunit 8, or cytochrome *c*1 (11).

Immunological probing for subunits in the petite strain indicated that most of the subunits of the *bc* complex are missing. Most of the nuclear encoded subunits of the cytochrome *bc* complex are known to be rapidly degraded in the absence of complete assembly of the complex (31). The apparently toxic hybrid protein produced from the qcr6Δ1:URA3 allele was undetectable with the subunit 6 antibodies used in this study. This may be due to a lack of cross-reactivity with the poisonous protein or the presence of the toxic protein at levels below detection. The latter possibility is consistent with the low levels of transcript from the locus responsible for the petite phenotype (data not shown).

On the basis of these findings we suggest that the deleterious derivative of subunit 6 disrupts assembly of the cytochrome *bc* complex. Since only a small number of *bc* complex is undergoing assembly at one time, a low level of toxic protein could cause such a deleterious effect. Our results are consistent with findings of Schoppink and co-workers (35) and Crivellone and co-workers (11) with findings presented here. In the model, core protein 1 (*I*) and core protein 2 (*II*) form a stable (*S*) subcomplex, while cytochrome *b* (*b*) subunits 8 and 7 (*7*) form a subcomplex that is believed to be unstable (*U*). The core protein subcomplex and cytochrome *b*-subunit 7-subunit 8 subcomplexes then associate to form a subcomplex with intermediate stability (*S/U*). A separate nucleation site originates from subunits 6 (*6*) and 9 (*9*), which combine and then associate with cytochrome *c*1 (*c1*) to form a subcomplex of intermediate stability (*S/U*). The *c1* subcomplex then associates with the *b*-core subcomplex to form a stable (*S*) cytochrome *bc* complex without iron-sulfur protein (*ISP*). Insertion of the iron-sulfur protein then completes the assembly pathway (11). We propose that the aberrant form of subunit 6 (*6*) combines with cytochrome *c*1 before subunit 9, causing the *c1* to be degraded by protease, thus preventing completion of the assembly pathway. This can be suppressed by overproducing subunit 9, which binds to the aberrant subunit 6 and sequesters it from the pathway. A normal subunit 6, either in single or multiple copies, forms a subunit 6-9 dimer, which competes with the aberrant subunit 6 for cytochrome *c*1.

Suppression of the qcr6Δ2:URA3 petite phenotype by either QCR9 or QCR6 leads to only a partial recovery of cytochrome *c* reductase activity, but this proved to be enough to permit growth on nonfermentable carbon sources. Schoppink and co-workers (35) previously showed that as little as 5% of the normal ubiquinol-cytochrome *c* oxidoreductase activity is sufficient for yeast to grow on nonfermentable carbon sources, although one might expect this activity threshold to vary among strains.

An alternative explanation for the lack of *bc* complex is that the aberrant subunit 6 removes subunit 9 from the assembly pathway, since deletion of *QCR9* also results in a temperature-sensitive petite phenotype (30). We feel this explanation is unlikely, since deletion of *QCR9* leads to a different assembly phenotype (30). However, we cannot exclude the possibility that loss of both subunit 6 and subunit 9 may lead to a phenotype similar to the qcr6Δ2:URA3 disruption allele.

Since overexpression of *QCR9* protects against the effects of the aberrant subunit 6, we suggest that subunits 6 and 9 form a subcomplex before they bind to cytochrome *c*1 (Fig. 5). Perhaps the toxic derivative of subunit 6 binds better to cytochrome *c*1 than to subunit 9, leading to rapid degradation of cytochrome *c*1. Overexpression of subunit 9 may override the otherwise weak interaction with subunit 6 and thus cause the poisonous subunit 6 to be removed from the pathway. Since subunit 6 is not required for assembly of the *bc* complex (9), the complex is assembled without it.

The normal subunit 6, either in single or multiple copies,
permits the formation of a subunit 6–subunit 9 dimer, which competes with the aberrant subunit 6 for cytochrome c. The fact that a normal subunit 6 rescues only 30% of the bc complexes when expressed at high copy number may simply reflect a lower affinity of the subunit 6–subunit 9 dimer than of the mutant subunit 6 for the binding site on cytochrome c.

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REFERENCES
1. Trumpower, B. L. (1990) J. Biol. Chem. 265, 11409–11412
2. Ljungdahl, P. O., Pennoyer, J. D., Brown, T. A., and Trumpower, B. L. (1987) Biochim. Biophys. Acta 891, 227–242
3. Engel, W. D., Schägger, H., and von Jagow, G. (1983) Z. Phys. Chem. 364, 1753–1763
4. Yang, X., and Trumpower, B. L. (1986) J. Biol. Chem. 261, 12282–12289
5. Kriauciunas, A., Yu, L., Yu, C., Wynn, R. M., and Knaff, D. B. (1989) Biochim. Biophys. Acta 976, 70–76
6. van Loon, A. P. G. M., De Groot, R. J., De Haan, M., Dekker, A., and Grivell, L. A. (1984) EMBO J. 3, 1039–1043
7. Ohta, S., Goto, K., Arni, H., and Kagawa, Y. (1987) FEBS Lett. 226, 171–175
8. Kim, C. H., Blany, C., and King, T. E. (1987) J. Biol. Chem. 262, 8103–8108
9. Schmitt, M. E., and Trumpower, B. L. (1990) J. Biol. Chem. 265, 17005–17011
10. Schoppink, P., Hemrika, W., Reynen, J., Grivell, L., and Berden, J. (1988) Eur. J. Biochem. 173, 115–122
11. Crivellone, M. D., Wu, M., and Tzagoloff, A. (1988) J. Biol. Chem. 263, 14323–14333
12. Kim, C. H., and Zitomer, R. S. (1990) FEBS Lett. 266, 78–82
13. King, P. V., and Blakesley, R. (1986) Focus 8, 1
14. Broach, J. R., Strathern, J. N., and Hicks, J. B. (1979) Gene (Amst.) 8, 121–133
15. Schmitt, M. E., Brown, T. A., and Trumpower, B. L. (1990) Nucleic Acids Res. 18, 3091–3092
16. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Botstein, D., and Davis, R. W. (1982) in The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 607–636, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Hoffman, C. S., and Winston, F. (1987) Gene (Amst.) 57, 267–272
19. Myers, A. M., Crivellone, M. D., Koerner, T. J., and Tzagoloff, A. (1987) J. Biol. Chem. 262, 16822–16829
20. Laemmli, U. K. (1970) Nature 227, 680–685
21. Olmsted, J. B. (1981) J. Biol. Chem. 256, 11955–11957
22. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
23. Ljungdahl, P. O. (1987) Mutational Analysis of the Mitochondrial Rieske Iron-Sulfur Protein of Saccharomyces cerevisiae. Ph.D. thesis, Dartmouth College
24. Needleman, R. B., and Tzagoloff, A. (1975) Anal. Biochem. 64, 545–549
25. Ljungdahl, P. O., Pennoyer, J. D., Robertson, D. E., and Trumpower, B. L. (1987) Biochim. Biophys. Acta 891, 227–242
26. Trumpower, B. L., and Edwards, C. A. (1979) J. Biol. Chem. 254, 8697–8706
27. Beckmann, J. D., Ljungdahl, P. O., Lopez, J. L., and Trumpower, B. L. (1987) J. Biol. Chem. 262, 8901–8909
28. Sherman, F., Fink, G. R., and Hicks, J. B. (1983) Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
29. Bender, A., and Pringle, J. R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9976–9980
30. Phillips, J. D., Schmitt, M. E., Brown, T. A., Beckmann, J. D., and Trumpower, B. L. (1990) J. Biol. Chem. 265, 20813–20821
31. Schoppink, P. J. (1989) Yeast Ubiquinol-cytochrome c Oxidoreductase. Ph.D. thesis, University of Amsterdam
32. Kim, C. H., Balny, C., and King, T. E. (1987) J. Biol. Chem. 262, 8103–8108
33. Schagger, H., von Jagow, G., Borchard, U., and Machleidt, W. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 307–311
34. Gonzalez-Halphen, D., Lindorfer, M. A., and Capaldi, R. A. (1988) Biochemistry 27, 7021–7031
35. Schoppink, P. J., Hemrika, W., and Berden, J. A. (1989) Biochim. Biophys. Acta 974, 192–201