Characterization of COX19, a Widely Distributed Gene Required for Expression of Mitochondrial Cytochrome Oxidase

Marina P. Nobrega, Simone C. B. Bandeira, John Beers, and Alexander Tzagoloff

From the Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, Brazil 12244-000 and the Department of Biological Sciences Columbia University, New York, New York 10027

Received for publication, July 22, 2002, and in revised form, August 2, 2002
Published, JBC Papers in Press, August 8, 2002, DOI 10.1074/jbc.M207348200

COX19, a nuclear gene of Saccharomyces cerevisiae, was cloned by transformation of a respiratory-deficient mutant from complementation group G188 of a pet mutant collection. The gene codes for an 11-kDa protein (Cox19p) required for expression of cytochrome oxidase. Because cox19 mutants are able to synthesize the mitochondrial and nuclear genes products of cytochrome oxidase, Cox19p probably functions post-translationally during assembly of the enzyme. Cox19p is present in the cytoplasm and mitochondria, where it exists as a soluble intermembrane protein. This dual location is similar to what was previously reported for Cox17p, a low molecular weight copper protein thought to be required for maturation of the CuA center of subunit 2 of cytochrome oxidase. The similarity in their subcellular distribution, combined with the presence of four cysteines in Cox19p that align with a subset of the cysteines in Cox17p, suggests that like the latter, Cox19p may function in metal transport to mitochondria.

Cytochrome oxidase (COX) is a heteroligomeric complex of the mitochondrial respiratory chain. In eukaryotes, COX is derived from mitochondrial and nuclear genes (1). Mitochondria generally code for three subunits of the catalytic core that contains the copper and heme prosthetic groups of the complex. The remaining 9–10 subunits are products of nuclear genes. They are translated on cytoplasmic ribosomes and transported to different compartments of the organelle by the TOM (protein transport machinery of outer membrane) and TIM (protein transport machinery of inner membrane) translocases of the outer and inner membrane, respectively (2, 3). Even though the precise functions of these subunits are not known, mutations in most of them block assembly of the complex (1). This set of proteins may also contribute to the overall stability of the complex.

Much of the current information about COX assembly has come from studies of Saccharomyces cerevisiae mutants. Approximately three dozen yeast genes have been identified that, when mutated, elicit a specific deficit in COX (4, 5). Some of the genes code for accessory proteins that operate during different stages of assembly and affect a broad range of events required for expression of fully active COX. These include maturation of the mitochondrial encoded transcript for subunits 1, 2, and 3 (6, 7), translation of the resultant mRNAs (8–10), and membrane insertion and post-translational processing of subunit 2 (11, 12). Nuclear gene products also play important roles in mitochondrial copper homeostasis (13, 14) and in biosynthesis of heme A (15, 16), both of which are required for the electron transferring activities of the enzyme. Despite recent progress, there remain a significant number of COX-specific genes whose functions remain unknown. Nor is it certain that all of the COX-specific genes have been identified.

In this paper we report a new gene, COX19, that shares the hallmarks of other COX-specific genes. Mutations in COX19 elicit a deficiency in COX but not in other complexes of the respiratory chain. The presence of COX19 homologues in other eukaryotic genomes including that of man, indicates that the product of this gene is likely to participate in some fundamental aspect of COX assembly in mitochondria. Cox19p has been localized in the intermembrane space of mitochondria, although a fraction of the protein is also detected in the cytosolic fraction. In this respect, Cox19p is similar to Cox17p, which has been implicated in transport of copper to mitochondrial (13). The biochemical properties of cox19 mutants are consistent with a post-translational block in COX assembly. The primary structure of the Cox19p and its subcellular localization further suggest that it is likely to be involved in metal transport.

MATERIALS AND METHODS

Strains and Growth Media—The genotypes and sources of the yeast strains used in this study are listed in Table I. The compositions of the media used for growth of yeast are described in the text.

Cloning of COX19—E708/U1 (MATa ur3-1, cox19-1), obtained from a cross of the original mutant E708 (MATa met6 cox19-1) to W303-1A, was transformed with a yeast genomic library by the method of Schiestl and Gietz (18). The library was constructed by ligation of partial Sau3A fragments of nuclear DNA (averaging 7–10 kb) to the BamHI site of Yep34 (19). Transformation of 5 x 10^6 cells with the library (25 μg DNA) yielded ~60,000 uracil-independent clones, of which 20 grew on nonfermentable carbon sources. The respiratory competence cosegregated with the uracil independence, indicating that both phenotypes depend on a segregating plasmid. Plasmid DNA isolated from the transformant E708/U1/T1 was amplified in Escherichia coli and used to subclone COX19.

PCR Amplification of COX19—The two reading frames located within the 700-bp HindIII fragment were amplified by PCR. The shorter reading frame in pG188/T10 was amplified with primers 5'-CAGCCTCCTGCAGGGTC and 5'-AGATATCTTCTAGATGACTGCTTGTG and cloned in pEP51 (20). The longer frame corresponding to COX19 was amplified with primers 5'-CTACATCTCGTACCTCTC- TCG and 5'-TGTTGGCGGAGATCATTATCG and cloned in pEP52 (pG188/T11).

This paper is available on line at http://www.jbc.org
Characterization of COX19

TABLE I
Genotypes and sources of S. cerevisiae strains

| Strain       | Genotype                | Source       |
|--------------|-------------------------|--------------|
| D273-10B/A1  | MATa met6 +            | (Ref. 17)    |
| W303-1A     | MATa ade2-1 his3-1,15 leu2-3,122 trp-1-1 his3-1 | R. Rothstein* |
| W303-1B     | MATa ade2-1 his3-1,15 leu2-3,122 trp-1-1 his3-1 | R. Rothstein |
| E708        | MATa met6 cox19-1       | E708 × W303-1A |
| E708/U1     | MATa ura3-1 cox19-1     | E708 × W303-1A |
| E708/LU     | MATa met6 ura3-1 leu2-3,122 cox19-1 | E708 × W303-1A |
| N559        | MATa met6 cox19-2       | (Ref. 5)     |
| W303 COX19L | MATa ade2-1 his3-1,15 leu2-3,122 trp-1-1 ura3-1 | This study   |
| aW303 COX19H| MATa ade2-1 his3-1,15 leu2-3,122 trp-1-1 ura3-1 | This study   |
| W303 COX19H/ST9 | MATa ade2-1 his3-1,15 leu2-3,122 trp-1-1 ura3-1 | This study   |
| aW303 COX19H/ST10 | MATa ade2-1 his3-1,15 leu2-3,122 trp-1-1 ura3-1 | This study   |

* R. Rothstein, Department of Human Genetics, Columbia University, New York, NY.

Table II
Respiratory and ATPase activities in wild type and cox19 mitochondria

The cox19 mutants E708 and W303ΔCOX19H are isogenic with the parental strains D273-10B/A1 and W303-1A, respectively. NADH-cytochrome c reductase and COX were measured at 23 °C, and ATPase was measured at 37 °C.

| Strain       | NADH-c red. | Cyt. oxidase | ATPase - oligomycin | ATPase + oligomycin |
|--------------|-------------|--------------|---------------------|---------------------|
| D273-10B/A1  | 4.9 ± 0.1   | 3.82 ± 0.13  | 5.88                | 0.70                |
| E708         | 3.0 ± 0.0   | 0.03 ± 0.03  | 3.7                 | 0.29                |
| W303-1A      | 4.9 ± 0.4   | 2.72 ± 0.14  | 5.64                | 0.48                |
| aW303 COX19H | 2.0 ± 0.0   | 0           | 3.55                | 0.56                |

Sciences, vol. 191, no. 23, June 14, 2001, pp. 4020-4027.

Construction of a cox19 Null Mutant—A partially deleted allele of COX19 was made by blunt ending the 0.7-kb HindIII fragment containing the entire gene with Klenow polymerase and ligating it to the HindIII site of pUC19 (21). The resultant plasmid was digested with BstZ and AccI to remove 84 nucleotides from the middle of the gene. The gapped plasmid was blunt ended and ligated to a 2-kb fragment containing the yeast LEU2 gene. This plasmid was used to substitute a 2.6-kb fragment with the partially deleted cox19::LEU2 allele, which was substituted for the wild type allele of W303-1B by the one-step gene replacement procedure (22). One of the respiratory-deficient transformants (W303ΔCOX19L) was verified by Southern analysis of total nuclear DNA to have the mutant allele.

The gene was also completely deleted by amplifying a plasmid containing the 0.7-kb HindIII fragment with COX19 in pUC18 (21) with the bi-directional primers: 5′-GGCAGATCTGGCGCCCACATCGGAATATC and 5′-GGCAGATCTGCGGCCCACTCGGAATATC. The product lacking the COX19 coding sequence but containing the entire sequence of the plasmid and the sequences flanking the gene was digested with BglII and ligated to a 1-kb BamHI fragment containing the yeast HIS3 gene. The null allele, recovered from this plasmid as a linear episomal plasmid YEp352 and the integrative plasmid YIp352 (20) yielding pG188/ST9 and pG188/ST10, respectively. The two plasmid constructs were used to transform the cox19 mutant aW303 ΔCOX19H. In the case of pG188/ST10, the fusion gene was integrated at the URA3 locus by transformation with the plasmid linearized at the NcoI site inside URA3. Both plasmids complemented the respiratory defect of the cox19 mutant.

In Vivo Labeling of Mitochondrial Translation Products—The parental strain D273-10B/A1 and the G188 mutant E708 were labeled with [35S]methionine in the presence of cycloheximide (24). The mitochondria were prepared, and samples representing 50 μg of protein were separated by SDS-PAGE on a linear 7.5–15% polyacrylamide gel (26).

Miscellaneous Procedures—The preparation and ligation of DNA fragments and the transformation and recovery of plasmid DNA from E. coli were performed by standard methods (27). The HindIII fragment of pG188/ST1 was sequenced by the chain termination method of Sanger et al. (28). The proteins were analyzed on 15% polyacrylamide gel unless otherwise indicated by SDS-PAGE (29). Western blots were treated with antibodies against the Cox19p and antibody-antigen complexes were visualized by a secondary reaction with Super Signal as recommended by the supplier (Pierce). The protein concentrations were determined by the method of Lowry et al. (30).

RESULTS

Phenotype of cox19 Mutants—E708 and N559 are two independent isolates previously assigned to complementation group G188 of a collection of respiratory-deficient strains of S. cerevisiae (5). Both mutants lack COX as evidenced by enzymatic assays (Table II) and the absence of the absorption bands corresponding to cytochromes a and a3 (Fig. 1A). The other complexes of the respiratory chain and oligomycin-sensitive ATPase are present in the mutants, indicating that the growth defect stems from the COX deficit.

To better understand the cause of the COX defect, the mutants were tested for their ability to express the mitochondrial and nuclear gene products. Expression of the three endogenously translated subunits was assayed by in vivo labeling of cells with [35S]methionine in the presence of cycloheximide to block cytoplasmic protein synthesis. In this assay, labeling of subunits 1, 2, and 3 of COX was comparable in the mutant and...
wild type strain (Fig. 1B). Subunit 2 was not separated from cytochrome b in the gel shown in Fig. 1B. In other experiments, however, the protein was found to be as well labeled in the mutant as in wild type yeast. The steady-state concentrations of some of the nuclear gene products was also examined. This group of COX constituents is more stable than the more hydrophobic mitochondrial translation products that are proteolytically degraded in assembly arrested mutants (1). The results of the Western analysis indicate that the levels of these proteins in the cox19 mutant are similar to those seen in other COX mutants (Fig. 1C). The lower concentration of subunit 4 is not specific to the cox19 mutant but is also observed in some other mutants (see Cox5a and Cox7 in Fig. 1C).

Cloning, Sequencing, and Identification of the Gene—The gene responsible for the COX defect of G188 mutants was cloned by transformation of E708/U1 with a plasmid library of yeast genomic DNA as described under “Materials and Methods.” Several plasmids with identical or overlapping fragments of nuclear DNA were obtained, and one (pG188/T1) was used to subclone the gene. The sequences of the end points of the insert in pG188/T1 allowed it to be mapped to the region of chromosome XII between coordinates 106668 and 114606 (Fig. 2). The gene was localized to a 0.7-kb HindIII fragment present in subclone pG188/ST1 (Fig. 2). The sequence of this region revealed the presence of two short overlapping and oppositely oriented reading frames spanning the only AccI restriction site
in the fragment. The insert in pG188/T5 starting at the AccI site (pG188/T5) did not restore respiration, suggesting that one of the reading frames was the gene.

The HindIII fragment was dissected by PCR amplification. Two constructs helped to identify the longer of the two reading frames as the gene conferring respiration to the mutant (Fig. 2). Plasmid pG188/T10 containing 112 nucleotides upstream of the ATG codon and ending at the termination codon of the shorter reading frame was ineffective in restoring respiration. Because this plasmid lacked the sequence coding for the amino-terminal 37 codons of the longer reading frame, its failure to complement pointed to the latter as the gene. This was supported by the other subclone (pG188/T11), containing the sequence starting from nucleotide 141 upstream of the initiation codon and ending at the last codon of the longer reading frame. This fragment complemented the mutant, even though it lacked the sequence coding for the first five codons of the shorter reading frame. In keeping with the previous convention for naming genes related to COX assembly, the longer reading frame located between coordinates 108675 and 108971 will henceforth be referred to as COX19 (GenBank™ accession number NP_013082).

**Phenotype of cox19 Null Mutants—**Two cox19 null alleles were introduced into chromosomal DNA of the respiratory competent strain W303. One (W303ΔCOX19L) had a partial and the other (W303ΔCOX19H) had a complete deletion of the gene. Both mutants were respiratory-deficient, had no COX activity, and displayed the absence of cytochromes $a$ and $a_3$ (Fig. 1 and Table II). Diploid cells issued from crosses of either of the two null mutants to E708 did not respire. This combined with the similarity in the phenotype of the cox19 null and point mutants constitutes strong evidence that the mutations are allelic and that COX19 complements rather than suppresses the respiratory defect of E708/U1. This was shown more directly by sequencing the mutation in E708/U1 (see below).

**Occurrence and Characteristics of Cox19p—**The COX19 product is a hydrophilic protein with a mass of 11,108 Daltons. Cox19p does not have any hydrophobic amino acid stretches of sufficient length to qualify as transmembrane domains. A noteworthy feature of the sequence is the presence of four cysteine residues. These residues align with four of the seven cysteines in Cox17p (Fig. 3A), a protein previously inferred to target cytoplasmic copper to mitochondria (13). Homologues of Cox19p are detected in a large number of...
organisms including man, mouse, fly, worm, plants, and fungi. The proteins shown in Fig. 3B as well as homologous proteins from other sources share a short conserved domain with seven identities in a span of 10 residues near the amino terminus. The four cysteines of yeast Cox19p are present in all of the homologues currently in the protein data base. The arginine at position 63, mutated in the E708, is also conserved in the other Cox19p sequences.

**Mutation in E708/U1**—To sequence the mutation in E708, total nuclear DNA from the mutant was digested with HindIII and separated on a preparative gel. Fragments in the 700-bp size range were isolated and cloned in YEp532. Screening of the plasmid library by colony hybridization yielded several positive clones, one of which was sequenced. The sequence of the HindIII fragment from the mutant disclosed a C for a G base change at the second position of codon 63. This mutation leads to the substitution of a threonine for an arginine adjacent to the carboxyl-proximal cysteine residues in the protein. To determine whether the R63T mutation is responsible for the respiratory defect of E708, the cox19 null mutant was transformed with the mutant allele. Transformants harboring the R63T allele on a multicopy plasmid grew more slowly than wild type on nonfermentable substrates (Fig. 4). The fact they also grew more slowly than the null mutant transformed with the same plasmid with the wild type gene (Fig. 4) indicates that the arginine at position 63 is important for Cox19p activity. These results further substantiate the requirement of Cox19p for expression of COX.

**Localization of Cox19p**—The hydrophilic nature of Cox19p and the presence of four cysteines that align with some of the cysteine residues of Cox17p suggested that, like Cox17p, Cox19p might be involved in metal transport. An interesting feature of Cox17p is its presence in both the cytoplasm and in the mitochondrial intermembrane space (33). This dual distribution correlates with the state of oligomerization of the protein (34). To study its subcellular location, Cox19p was expressed with a 9-residue-long hemagglutinin tag fused to the carboxyl terminus. The hybrid protein was verified to be functional as evidenced by its ability to complement a cox19 null mutant in single copy. Mouse monoclonal antibody against the HA epitope detected a mitochondrial protein that migrates between the 14- and 21-kDa markers. This protein was identified as the HA-tagged Cox19p based on its absence in wild type yeast and higher abundance in the transformant with the gene on a multicopy plasmid than the transformant with the same gene integrated in single copy into chromosomal DNA. The HA-tagged Cox19p was also detected in the post-mitochondrial supernatant fractions of the multi-copy transformants (Fig. 5A). Although Cox19p is not seen in the post-mitochondrial supernatant fraction of the single-copy transformant in Fig. 5A, in other experiments a faint signal was detected. No signal was detected in the post-mitochondrial supernatant fractions with an antibody against cytochrome b2, a mitochondrial intermembrane marker (35), even when the blot was overexposed. It is therefore unlikely that the presence of Cox19p in this fraction represents leakage from mitochondria. The mass of Cox19p (~16 kDa) estimated by SDS-PAGE, which was larger than predicted by the gene sequence, is probably an artifact of the SDS gel system. A similar discrepancy in size was observed for Cox17p (33).

The submitochondrial localization of Cox19p was examined in mitochondria prepared from a cox19 null mutant expressing the HA-tagged Cox19p from the chromosomally integrated fu-
tion gene (aW303ΔCOX19/ST9). Mitochondria were converted to mitoplasts by incubation in hypotonic buffer, and the intermembrane proteins released by the hypotonic shock were separated from the mitoplasts by centrifugation. The different fractions were tested for susceptibility of Cox19p to proteolysis by proteinase K. The results of the fractionation indicated mitochondrial Cox19p to be located in the intermembrane space (Fig. 5B). Cox19p was protected against proteinase K when mitochondria were diluted in an isotonic buffer solution (1.2 M sorbitol, 30 mM Hepes, pH 7.5), and only a small fraction was recovered in the supernatant after sedimentation of the mitochondria. Dilution of mitochondria in 20 mM Hepes, pH 7.5, alone, caused most of the Cox19p to be released from the mitochondria. Under these conditions, both the soluble and the small fraction of Cox19p that sedimented with the mitoplasts were completely sensitive to proteinase K. The location of Cox19p in the intermembrane space was confirmed by the similarity of its distribution with that of cytochrome b$_2$ (Fig. 5B).

DISCUSSION

COX assembly is a complex process requiring the participation of a large number of nuclear gene products. In S. cerevisiae these factors target specific mitochondrially encoded subunits of the enzyme at the level of transcript processing (6, 7) and translation of the mRNA (8–10). Expression of functional COX also requires the addition of heme A and metals such as copper, zinc, and magnesium. Copper, like heme A, is an obligatory electron carrier of the enzyme (37, 38). Several genes have been implicated in maturation of the two copper centers of the enzyme (13, 14, 34, 39, 40).

In the present study we have characterized COX19, a new gene required for COX assembly. The product of this gene, Cox19p, is a small protein with a mass of 11.1 kDa. The sequence of Cox19p, deduced from the gene, indicates that it is a soluble protein. This has been confirmed by its cellular localization. A fraction of Cox19p exists as a soluble cytoplasmic protein. Another fraction has been localized to the intermembrane compartment of mitochondria and is released as a soluble protein when mitochondria are lysed by hypotonic shock. The dual localization of Cox19p in the cytoplasm and mitochondrial intermembrane space is identical to previous findings on the subcellular distribution of Cox17 (33), a small protein proposed to deliver copper to mitochondria (13).

Cox19p contains four cysteine residues whose spacing is four to seven of the seven cysteines of Cox17p. These cysteines are conserved in all of the currently known homologues of yeast Cox19p, pointing to their essentiality for function. This is also true of the arginine adjacent to the most carboxyl-proximal cysteine that is mutated in the cox19 mutant E708.

The coincidence in the size, solubility properties, and subcellular location of Cox17p and Cox19p suggests that they may also have related functions. COX17 does not rescue cox19 mutants, even when present on a high copy plasmid. Nor is COX19 a high copy suppressor of cox17 mutants. Unlike cox17 mutants that are partially rescued by addition of copper to the growth medium (13), neither copper, zinc, manganese, nor copper are able to promote growth of cox19 mutants on respiratory substrates.  

Homologues of COX19 exist in mammalian, plant, and fungal but not bacterial genomes. This feature, also shared by Cox17p, indicates that Cox19p is essential only for the biogenesis of mitochondrial but not prokaryotic COX. The absence of Cox19p homologues in bacteria that have a type oxidases is also consistent with the notion that the protein is unlikely to be directly involved in COX assembly. More likely Cox19p functions in transport of a metal or some other component essential for COX assembly to mitochondria.

Acknowledgments—We thank Taissa Lukjanenko, Sabrina L. C. Oliveira, and Erika M. Jesus, for technical help and Dr. Francisco G. Nobrega for helpful discussions and suggestions.

REFERENCES

1. Barrientos, A., Barros, M. H., Valnot, I., Rotig, A., Rustin, P., and Tzagoloff, A. (2002) Gene (Amst.) 286, 53–64
2. Neupert, W. (1997) Annu. Rev. Biochem. 66, 863–917
3. Schatz, G. (1998) Nature 395, 439–440
4. McEwen, J. E., Ko, C., Kloeckner-Gruessem, B., and Poyton, R. O. (1986) J. Biol. Chem. 261, 11872–11879
5. Tzagoloff, A., and Dieckmann, C. L. (1991) J. Microbiol. Res. 54, 211–225
6. Lapetina, L., and Tzagoloff, A. (1990) EMBO J. 9, 4552–4556
7. Lapetina, L., and Tzagoloff, A. (1990) EMBO J. 9, 4552–4556
8. Mulero, J. J., and Faye, G. (1988) Mol. Biol. Cell 14, 4001–4043
9. Hell, K., Tzagoloff, A., Neupert, W., and Stuart, R. A. (2000) J. Biol. Chem. 275, 4571–4578
10. Barros, M. H., Carlson, C. G., Glerum, D. M., and Tzagoloff, A. (2001) FEBS Lett. 492, 133–138
11. Tzagoloff, A., Charlton, S. A., and Faye, G. (1988) J. Biol. Chem. 263, 1122–1131
12. Glerum, D. M., Shlom, A., and Tzagoloff, A. (1996) J. Biol. Chem. 271, 14504–14509
13. Glerum, D. M., Shlom, A., and Tzagoloff, A. (1996) J. Biol. Chem. 271, 20531–20535
14. Tzagoloff, A., Nobrega, M., Gorman, N., and Sinclair, P. (1993) Biochem. Mol. Biol. Int. 31, 593–598
15. Barros, M. H., Carlsson, C. G., Glerum, D. M., and Tzagoloff, A. (2001) FEBS Lett. 492, 133–138
16. Tzagoloff, A., Akai, A., and Foy, F. (1976) FEBS Lett. 65, 391–396
17. Schiestl, R. H., and Gietz, R. D. (1989) J. Biol. Chem. 264, 163–167
18. Yamasaki, T., Kimura, S., and Sato, A. (1988) Biochem. Biophys. Res. Commun. 155, 167–172
19. Botstein, D., and R. W. Davis. (1982) in The molecular biology of the yeast Saccharomyces cerevisiae: 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
20. Hill, J. E., Myers, A. M., Keener, T. J., and Tzagoloff, A. (1986) Yeast 2, 163–167
21. Yamasaki, T., Kimura, S., and Sato, A. (1988) Biochem. Biophys. Res. Commun. 155, 167–172
22. Rothstein, R. J. (1983) Methods Enzymol. 101, 201–211
23. Foy, F., Kojima, H., and Nakahara, H. (1974) J. Mol. Biol. 85, 205–203
24. Tzagoloff, A., Akai, A., and Needleman, R. B. (1975) J. Biol. Chem. 250, 8228–8235
25. King, E. J. (1932) Biochim. J. 26, 292–297
26. Chua, N. H., and Bennoun, P. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 2173–2179
27. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
28. Sanger, F., Nicken, S., and Coulsdon, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
29. Laemmli, U. K. (1970) Nature 227, 680–685
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
31. Deleted in proof
32. Thompson, J. D., Higgins, D. G., and Gibson T. J. (1994) Nucleic Acids Res. 22, 4673–4680
33. Boe, J., Glum, D. M., and Tzagoloff, A. (1997) J. Biol. Chem. 322, 33191–33196
34. Heaton, D. N., George, G. N., Garrison, G., and Winge, D. R. (2001) Biochemistry 40, 743–753
35. Matsuoka, G., Bohni, P. C., and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033
36. Glick, B., and Low, L. A. (1993) Methods Enzymol. 260, 213–223
37. Benett, R. H. (1997) Eu. J. Biochem. 245, 521–532
38. Yoshikawa, S., Shimizu, K.-i., and Tsukihara T. (2000) J. Inorg. Biochem. 82, 1–7
39. Hiser, L., Di Valentin, M., Hamer, A. G., and Holser, J. P. (2000) J. Biol. Chem. 275, 619–624
40. Carr, H. S., George, G. N., and Winge, D. R. (2002) J. Biol. Chem. 277, 31237–31242

2 M. P. Nobrega, S. C. B. Bandeira, J. Beers, and A. Tzagoloff, unpublished results.