The Carboxyl-terminal End of Cox1 Is Required for Feedback Assembly Regulation of Cox1 Synthesis in Saccharomyces cerevisiae Mitochondria*

Miguel Shingu-Vázquez†, Yolanda Camacho-Villasana‡, Luisa Sandoval-Romero†, Christine A. Butler§, Thomas D. Fox*, and Xochitl Pérez-Martinez†

From the †Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México D.F. 04510, México, the ‡Departamento de Morfología Celular y Molecular, Instituto Nacional de Rehabilitación, México D.F. 14389, México, and the §Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853

The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1 and Figs. S1 and S2.

Cytochrome c oxidase (CcO) is the terminal electron acceptor of the mitochondrial respiratory chain. It transfers electrons from cytochrome c to oxygen, with a coupled translocation of protons from the matrix to the intermembrane space. In the yeast Saccharomyces cerevisiae, this enzyme is composed of 11 subunits, three of which, Cox1, Cox2, and Cox3, are encoded by the mitochondrial genome, synthesized by organelar ribosomes, and integrated into the inner membrane from the matrix side. Assembly of this enzyme is very complex. It involves not only coordinated assembly of nuclear and mitochondrial encoded subunits, but the addition of metallic prosthetic groups like heme α and copper centers. For this process, more than 30 factors are necessary, although the functions of these proteins are just starting to be elucidated (1, 2).

The yeast model is widely used to study the mechanisms of CcO biogenesis, as several pathogenic mutations affecting CcO assembly have been described in human genes having yeast homologues. The majority of these encephalomyopathies are associated with mutations in nuclear genes encoding CcO assembly factors (3). In recent years some mutations associated with Leigh syndrome have been found in genes affecting expression of mitochondrial genes. This is the case for LRPPRC, a human protein distantly related to the yeast translational activator Pet309 (4–6), and TACO1, a gene specifically required for Cox1 synthesis (7).

Cox1 is the largest subunit of the CcO and spans the mitochondrial inner membrane 12 times (8). Cox1 contains the heme α and heme α3-Cu A centers for oxygen reduction. Cox1 is present from the first assembly intermediate, and the rest of the subunits and cofactors are thought to be added in a sequential order (9, 10). Partial assembly of Cox1 is associated with peroxide sensitivity due to pro-oxidant intermediates containing unassembled heme α3 (11). Hence, stoichiometry of Cox1 in the inner membrane has to be highly regulated. For this, many factors have been identified that control Cox1 biogenesis. Pet309 and Mss51 are specific translational activators that function through the COXI mRNA 5′-UTR (untranslated region) (4, 12). In addition, Mss51 physically interacts with Cox1, suggesting that it has a central role in coordinating the synthesis and assembly of this subunit (13, 14). Cox1 and Mss51 form a high molecular complex with Cox14. Next, Coa1 could insert into this complex (15). Although Mss51 and Coa1 are proposed to be liberated from this complex at early steps (16, 17), Cox14 might remain associated to the assembling CcO until the formation of supercomplexes (16).

The current model proposes that Mss51 limits translational activation of the COXI mRNA, and is sequestered from this function by its incorporation into assembly-intermediate complexes containing newly synthesized Cox1 and Cox14. In CcO assembly mutants, Mss51 is trapped in these complexes and thus unavailable for efficient COXI mRNA translational activation (12, 14).

Synthesis of the largest cytochrome c oxidase (CcO) subunit, Cox1, on yeast mitochondrial ribosomes is coupled to assembly of CcO. The translational activator Mss51 is sequestered in early assembly intermediate complexes by an interaction with Cox14 that depends on the presence of newly synthesized Cox1. If CcO assembly is prevented, the level of Mss51 available for translational activation is reduced. We deleted the C-terminal 11 or 15 residues of Cox1 by site-directed mutagenesis of mtDNA. Although these deletions did not prevent respiratory growth of yeast, they eliminated the assembly-feedback control of Cox1 synthesis. Furthermore, these deletions reduced the strength of the Mss51-Cox14 interaction as detected by co-immunoprecipitation, confirming the importance of the Cox1 C-terminal residues for Mss51 sequestration. We surveyed a panel of mutations that block CcO assembly for the strength of their effect on Cox1 synthesis, both by pulse labeling and expression of the ARG8m reporter fused to COXI. Deletion of the nuclear gene encoding Cox6, one of the first subunits to be added to assembling CcO, caused the most severe reduction in Cox1 synthesis. Deletion of the C-terminal 15 amino acids of Cox1 increased Cox1 synthesis in the presence of each of these mutations, except pet54. Our data suggest a novel activity of Pet54 required for normal synthesis of Cox1 that is independent of the Cox1 C-terminal end.

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† To whom correspondence should be addressed. Tel.: 52-55-5622-5662; Fax: 52-55-5622-5630; E-mail: xperez@ifc.unam.mx.

‡ The abbreviation used is: CcO, cytochrome c oxidase.
Pulse labeling of Cox1 in vivo with [35S]methionine is specifically reduced in several mutations affecting CcO assembly (for examples, see Refs. 14, 18, and 19). Lower levels of Cox1 labeling have even been documented for mutations affecting the ATP synthase (20, 21) and the CcO substrate cytochrome c (22). This reduction in Cox1 labeling is presumed to be due to decreased COX1 mRNA translation. Here, through mutagenesis of the mitochondrial COX1 gene we have found that the C-terminal domain of Cox1 is necessary for assembly-coupled translational down-regulation. Absence of Cox6, one of the first subunits to be added to the CcO, showed one of the most dramatic C-terminal end-dependent reductions of Cox1 synthesis. In addition, we report that Pet54 is a new component required for normal COX1 mRNA translation. A mutation in Pet54 seems to reduce Cox1 synthesis by a mechanism that is independent of the Cox1 C-terminal end.

EXPERIMENTAL PROCEDURES

Strains and Genetic Methods—S. cerevisiae strains used in this study, all congenic or isogenic to D273-10B (ATCC 24657), are listed under supplemental Table S1. Genetic methods and media were as previously described (23, 24). Complete fermentable media were YPD or YPGal (containing 2% glucose or 2% galactose). Non-fermentable medium was YPEG (3% glycerol, 3% ethanol). Minimal medium was synthetic complete (0.67% yeast nitrogen base, 2% glucose) lacking the indicated amino acids. The nuclear deletion constructs with KanMX4, LEU2, or URA3 cassettes were obtained by PCR. Plasmids carrying the cox1 mutations were transformed into rho0 strain NAB69 by high-velocity microprojectile bombardment (25). Transformants were selected by their ability to rescue respiratory growth when mated with a strain carrying a Cox1 D369N mutation, L45 (26). Transformants were mated with XPM10b (containing the cox1Δ:ARG8m construct) or XPM13a (containing the cox2-62 and the cox1Δ:ARG8m construct) (13). Cyto-ductants were selected for their ability to grow on YPEG as haploids or after mating to a strain with the mutation G253D (27). In all cases, correct integration of the different constructs into the mtDNA was confirmed by PCR and DNA sequencing.

Construction of the cox1 Mutant Genes—Plasmid pXPM57, containing the full-length, intronless COX1 gene was used as template for PCR amplifications. This plasmid contains 395 and 990 nucleotides of the COX1 5′-UTR and 3′-UTR, respectively, and was cloned in the XbaI-XhoI sites from pBluescript (Stratagene). All cox1 mutant plasmids were generated by the fusion PCR technique (28) using Acczyme (Bioline). The 3′ half of the COX1 coding region was amplified with primers that incorporated the mutations. These products were digested with NdeI and AflII and ligated into pXPM57 equally digested. Plasmids were sequenced to confirm the presence of the desired mutations in COX1.

Analysis of Mitochondrial Proteins—Yeast cells were grown in complete or minimal galactose media until late log phase. Crude mitochondria were obtained by disruption of cells with glass beads or by zymolase 20T treatment as described (29). Proteins were separated by SDS-PAGE on a 16% gel (30), and Western blots were probed with antibodies to HA (Roche Applied Science), c-Myc (Roche), or citrate synthase. Second-
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The Cox1 carboxyl-terminal end is required to down-regulate Cox1 synthesis in a cox2Δ mutant. A, model of the S. cerevisiae Cox1 protein, based on the crystal structure of the bovine CcO. The model was constructed using SWISS MODEL, and visualized with MacPymol. Alignment of the yeast and bovine Cox1 sequences revealed that S. cerevisiae has –23 additional residues in the C-terminal region, from Lys11 to Asn350, which are located at –52 to –29 with respect to the C-terminal end of Cox1 (Loop). Arrows indicate residues on the Cox1 C-terminal end where deletions start. The number in parentheses indicates the position of these residues with respect to the last amino acid of Cox1. B, mitochondrial translation products were labeled with [35S]methionine in the presence of cycloheximide, and proteins were analyzed as described under “Experimental Procedures.” Cells carried either the wild-type Cox1 protein, or the Cox1 protein lacking 15 (Cox1Δ15), 11 (Cox1Δ11), or 5 (Cox1Δ5) amino acids of the carboxyl-terminal end. The cox2Δ mutation (Δ) was introduced as indicated. Abbreviations are as follows: cytochrome c oxidase subunit 1, Cox1; subunit 2, Cox2; subunit 3, Cox3; cytochrome b, Cytb; subunit 6 of ATPase, Atp6; and the ribosomal protein, Var1.

and –11 from the Cox1 C terminus, VHSFNTR, are required to down-regulate Cox1 synthesis in a cox2Δ mutant.

The Last 15 Residues of Cox1 Facilitate the Interaction between Mss51 and Cox14—Assembly-mediated control of Cox1 synthesis involves sequestration of Mss51 in complexes containing Cox14 and newly synthesized Cox1. Interaction of Mss51 and Cox14 from wild-type mitochondrial extracts has been previously observed (14), and is known to be dependent upon synthesis of Cox1 (12). We therefore tested whether interactions among these components were affected by deletion of the last 15 residues of Cox1. We attached a triple Myc epitope to the C terminus of Cox14, and a triple hemagglutinin (HA) epitope to the C terminus of Mss51. The respiratory competence of the Mss51-HA, Cox14-Myc strains were comparable with wild-type levels, indicating that the tagged proteins were functional (data not shown). We first asked whether immunoprecipitation of Cox14-Myc would co-precipitate newly synthesized Cox1Δ15. Mitochondria isolated from strains containing Cox14-Myc, Mss51-HA, and either wild-type Cox1 or Cox1Δ15, were allowed to synthesize proteins in the presence of [35S]methionine. After solubilization in 1% digitonin, the mitochondria were immunoprecipitated with a Myc-specific antibody. The immunoprecipitated proteins were separated by SDS-PAGE and transferred to a PVDF membrane.

Co-precipitation of newly synthesized Cox1 and Cox1Δ15 with Cox14-Myc was equally efficient (Fig. 2A). However, probing the PVDF membrane with HA-specific antibody to detect Mss51-HA revealed that almost no Mss51-HA was co-precipitated with Cox14-Myc in the presence of the truncated Cox1Δ15, in contrast to wild-type Cox1. We were unable to analyze the interaction of unlabeled Cox1Δ15 with Cox14-Myc by Western blotting because the truncation apparently removed the epitope recognized by the Cox1-specific antibodies we tested (data not shown).

We also immunoprecipitated these mitochondrial extracts with HA-specific antibody. As previously reported, newly made Cox1 co-precipitates with Mss51-HA (13). This interaction was similarly efficient in Cox1Δ15 mitochondria (Fig. 2B). However, co-immunoprecipitation of Cox14-Myc with Mss51-HA was dramatically reduced in the presence of Cox1Δ15, compared with wild-type Cox1. Taken together, these results indicate that the ability of the truncated Cox1Δ15 protein to bridge the interaction between Cox14-Myc and Mss51-HA is compromised.

Taken together, these data indicate that the C-terminal 15 residues of Cox1 are required for normal stability of a complex (or complexes) containing Mss51, Cox14, and newly synthesized Cox1. Instability of this complex could account for the robust synthesis of Cox1Δ15 in a mutant unable to assemble CcO, because Mss51 would not be efficiently sequestered and therefore available to activate COX1 mRNA translation. To test this, we next examined the Mss51-Cox14 interaction in strains unable to assemble CcO due to a cox1Δ mutation that con-
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FIGURE 3. Interaction of Mss51 with Cox14 is not stable in COO assembly mutants lacking the C-terminal end of Cox1. Mitochondria from Cox1 or Cox1ΔC15 in the presence of either wild-type COX4 (WT) or cox4Δ (Δ) mutations were solubilized with 1% dodecyl maltoside and immunoprecipitated with a Myc-specific antibody. The immunoprecipitated (IP) fraction was analyzed by Western blot with an antibody to HA, the membrane was stripped and then reprobed with an antibody to Myc. The total fractions represent 5% of mitochondria before solubilization. Western blot with anti-Myc antibody showed a doublet, which is probably due to partial cleavage of the triple Myc epitope.

The Carboxyl-terminal End of Cox1 Regulates Cox1 Synthesis in Several Mutants Affecting COO Assembly—A wide range of mutations that affect COO assembly show reduced levels of Cox1 labeling in the presence of [35S]methionine (14, 18, 19). We tested whether labeling of the truncated variant Cox1ΔC15 would also remain unaffected when COO assembly was disrupted by mutations other than cox1Δ. Two groups of COO mutants were created. In the first group, synthesis of COO subunits was prevented by cox4Δ, cox6Δ, and cox7Δ mutations, or by COX3 mRNA translational activation mutants pet122Δ and pet54Δ. Cox6 is added to the first assembly intermediate containing Cox1, whereas Cox3, Cox4, and Cox7 are assembled later (35). In the second group, assembly chaperones were eliminated: mss2Δ (necessary for assembly of Cox2 (36)), cox11Δ, cox15Δ (involved in formation of CuB), and heme a centers in Cox1, respectively (37, 38), coa1Δ (participates in Cox1 assembly (16, 17)), pet100Δ (involved in formation of intermediates containing Cox7, Cox7a, and Cox8 (39), and pet191Δ (twins Cx4C protein necessary for full assembly of COO (40)). Cox11, Cox15, and Cox1 all seem to participate in the early stages of COO assembly (1, 15).

We constructed strains carrying each nuclear mutation with either wild-type mtDNA or the mtDNA encoding Cox1ΔC15, and carried out [35S]methionine labeling (Fig. 4, A and B). With the exception of the coa1Δ strain, which was previously demonstrated to have normal levels of Cox1 [35S]methionine labeling (16, 17), and in our hands had no respiratory growth defect in the D273-10B nuclear genetic background used here, the COO mutants showed reduced labeling of wild-type Cox1 by 42–85%. In contrast, labeling of the truncated Cox1ΔC15 protein was not reduced by most of these mutations, indicating that the C-terminal end of Cox1 regulates Cox1 synthesis independently of the stage where COO assembly is interrupted. Two mutants consistently showed the most dramatic reduction of both Cox1 and Cox1ΔC15 [35S]methionine labeling: cox6Δ and pet54Δ.

The cox6Δ mutation reduced labeling of wild-type Cox1 by 85%, and also reduced labeling of Cox1ΔC15 by 63%. Thus, the cox6Δ mutation strongly reduced Cox1 labeling, and this effect is only slightly ameliorated by the C-terminal truncation of Cox1. The pet54Δ mutation reduced labeling of wild-type Cox1 by 60%. In contrast to other COO assembly mutants, the pet54Δ mutation similarly reduced labeling of the truncated Cox1ΔC15. This was the only mutant analyzed whose Cox1 labeling was not increased by the C-terminal truncation of Cox1, suggesting that this effect might not be due simply to the lack of COO assembly. Indeed, deletion of another COX3 mRNA translational activator, Pet122, resulted in a labeling pattern similar to those of the majority of COO assembly mutants.

The more dramatic reduction of Cox1 labeling in cox6Δ and pet54Δ could be due to decreased synthesis or a more rapid degradation of newly made Cox1. To distinguish these possibilities, we analyzed expression of the mitochondrial reporter gene ARG8m, which codes for a soluble biosynthetic enzyme in the matrix, and whose activity does not depend on the presence of COO (41). ARG8m was fused in-frame to the end of the COO coding region to create the construct COOX1(1–512)::ARG8m (13). This ARG8m sequence specifies the cleavage site for the pre-Arg8 mitochondrial targeting signal, such that accumulation of mature Arg8 should not be affected by the stability of Cox1. Thus, expression of ARG8m from this construct provides a readout of COOX1 mRNA translation. Furthermore, the Cox1 moiety encoded by COOX1(1–512)::ARG8m is assembled into active COO complexes, supporting normal respiratory growth (13).

We combined some of the nuclear mutations described in Fig. 4A with the COOX1(1–512)::ARG8m construct. All the COO mutants analyzed showed reduced growth in medium lacking arginine as compared with a wild-type strain with the COOX1(1–512)::ARG8m construct (Fig. 4C). However, absence of Cox6 and Pet54 consistently showed the most dramatic reduction in Arg+ growth. [35S]Methionine labeling of the Cox1-Arg8 fusion protein in these cells was reduced in all the COO mutants, but most dramatically reduced in the cox6Δ and pet54Δ mutants (supplemental Fig. S1). These data confirm that the COO assembly defect caused by the loss of Cox6 or Pet54 reduced synthesis of the reporter fused to full-length Cox1 more than other COO mutants.

Cox6, together with Cox5a, are the first subunits to assemble with Cox1 (35). It has been suggested that Cox5a and Cox6 confer stability to newly synthesized Cox1 (42). To further test whether deletion of Cox6 confers a strong decrease in Cox1 synthesis we first asked whether conditions that alter Cox1 pulse labeling in COO assembly-defective mutants similarly alter expression of the COOX1(1–512)::ARG8m reporter. In cox14Δ cells, [35S]methionine labeling of Cox1 is restored to wild-type levels even in the presence of mutations affecting COO assembly (14). Consistent with this finding, double mutant cox6Δ, cox14Δ cells grew on medium lacking arginine as well as wild-type COO6, COX14 cells (Fig. 5A). Mito-
The C-terminal end of Cox1 regulates COX1 translation in many CcO mutants. A, Cox1 (−) or Cox1ΔC15 (+) cells with a deletion in the indicated genes were pulse-labeled with [35S]methionine in the presence of cycloheximide, and proteins were analyzed as described under “Experimental Procedures.” B, quantification of the Cox1 signals from A. The level of Cox1 labeling was normalized to the Cox3/Atp6 signal, and was expressed as a percentage of the wild-type, Cox1 signal (except for signals from the pet54Δ and pet122Δ mutants, which were normalized with respect to Cytb). Error bars indicate standard deviations from 3 independent experiments. We also compared the signal of the cytochrome b to the Cox3/Atp6 signal (or the signal from Cox2 to the cytochrome b in the pet54Δ and pet122Δ mutants), and in those cases no significant difference was observed (data not shown). C, translation of the mitochondrial reporter gene COX1(1–512)::ARG8m was analyzed by growth of the indicated mutants on media lacking (−Arg) or containing arginine (+Arg). In this construct the precursor of Arg8 was fused to the C-terminal end of the complete Cox1. Cells were spotted as serial dilutions and grown for 3 days at 30 °C.

FIGURE 4. The Cox1 C-terminal end regulates Cox1 synthesis in many CcO mutants. A, Cox1 (−) or Cox1ΔC15 (+) cells with a deletion in the indicated genes were pulse-labeled with [35S]methionine in the presence of cycloheximide, and proteins were analyzed as described under “Experimental Procedures.” B, quantification of the Cox1 signals from A. The level of Cox1 labeling was normalized to the Cox3/Atp6 signal, and was expressed as a percentage of the wild-type, Cox1 signal (except for signals from the pet54Δ and pet122Δ mutants, which were normalized with respect to Cytb). Error bars indicate standard deviations from 3 independent experiments. We also compared the signal of the cytochrome b to the Cox3/Atp6 signal (or the signal from Cox2 to the cytochrome b in the pet54Δ and pet122Δ mutants), and in those cases no significant difference was observed (data not shown). C, translation of the mitochondrial reporter gene COX1(1–512)::ARG8m was analyzed by growth of the indicated mutants on media lacking (−Arg) or containing arginine (+Arg). In this construct the precursor of Arg8 was fused to the C-terminal end of the complete Cox1. Cells were spotted as serial dilutions and grown for 3 days at 30 °C.

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It remains unclear whether assembly-feedback regulated synthesis of Cox1 in mitochondria occurs in other species. A few examples in mammals suggest that translation of the COX1 mRNA might be reduced by defects associated with COX assembly. It has been documented that a 15-base pair deletion in the human mitochondrial COX3 gene (46), as well as lack of cytochrome c in mouse fibroblasts (47) leads to a modest reduction of [35S]methionine labeling of Cox1. However, these studies did not clearly distinguish whether decreased Cox1 labeling was due to reduced synthesis or increased turnover.

Regulated synthesis of Cox1 in S. cerevisiae is the first identified example in mitochondria where an organelle-encoded protein has amino acid sequences that couple regulation of its own synthesis to assembly. However, a similar mechanism has been demonstrated in the chloroplast of Chlamydomonas reinhardtii. Synthesis of some organelle-encoded subunits is strongly reduced when other subunits from the photosynthetic complexes are missing (48–50). For the b6f complex, a C-terminal extension of 11 residues within cytochrome f is necessary for this regulation, possibly by stabilizing an interaction with the translational activator Tca1 when the enzyme is not assembled (51). This extension showed no obvious similarity with the Cox1 C-terminus.

The C-terminal end of Cox1 contains the consensus motif SPP(P/A)/XH, where His^{553} (as numbered in the bovine sequence) is necessary for the tunneling of protons through the D channel to the heme a\_5-Cu_{B} center (52). Interestingly, this histidine is part of the VHSFNT motif and is removed by our deletion of the last 15 residues of Cox1, demonstrating that it is not essential for oxidative phosphorylation in yeast.

The hydrophilic carboxyl-terminal domain of Cox1 is less conserved overall than the transmembrane domains. However, this region seems to be crucial for COX activity. In the protist Acanthamoeba castellanii the mitochondrial COX1 gene lacks the region coding for the C-terminal end. Interestingly, a nucle-
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REFERENCES

1. Fontanesi, F., Soto, I. C., and Barrientos, A. (2008) IUBMB Life 60, 557–568

2. Herrmann, J. M., and Funes, S. (2005) Gene 354, 43–52

3. Pecina, P., Houstková, H., Hansíkova, H., Zeman, J., and Houstek, J. (2004) Physiol. Res. 53, Suppl. 1, S213–223

4. Manthey, G. M., and McEwen, J. E. (1995) EMBO J. 14, 4031–4043

5. Mootha, V. K., LePAGE, P., Miller, K., Bunkentborgh, J., Reich, M., Djéramdje, R., Delmonte, T., Villeneuve, A., Sladek, R., Xu, F., Mitchell, G. A., Morin, C., Mann, M., Hudson, T. J., Robinson, B., Rioux, J. D., and Lander, E. S. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 605–610

6. Sasarman, F., Brunel-Guittton, C., Antonicka, H., Wai, T., Shoubridge, E. A., and Consortium, L. (2010) Mol. Biol. Cell 21, 1315–1323

7. Weraarpachai, W., Antonicka, H., Sasarman, F., Seeger, J., Scharf, B., Koleda, J. E., Lo, Ch., and Morin, C. (2009) Nat. Genet. 41, 833–837

8. Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yano, R., and Toshikawa, S. (1996) Science 272, 1136–1144

9. Lemaire, C., Robineau, S., and Netter, P. (1998) Curr. Genet. 34, 138–145

10. Nijtmans, L. G., Taanman, J. W., Muijsers, A. O., Speijer, D., and Van den Dobet, C. (1998) Eur. J. Biochem. 254, 389–394

11. Khalimonchouk, O., Bird, A., and Winge, D. R. (2007) J. Biol. Chem. 282, 17442–17449

12. Perez-Martinez, X., Butler, C. A., Shingu-Vazquez, M., and Fox, T. D. (2009) Mol. Biol. Cell 20, 4371–4380

13. Perez-Martinez, X., Broadley, S. A., and Fox, T. D. (2003) EMBO J. 22, 5951–5961

14. Barrientos, A., Zambrano, A., and Tzagoloff, A. (2004) EMBO J. 23, 3472–3482

15. Khalimonchouk, O., Bestwick, M., Meunier, B., Watts, T. C., and Winge, D. R. (2010) Mol. Cell. Biol. 30, 1004–1017

16. Mick, D. U., Wagner, K., van der Laan, M., Frazier, A. E., Perschil, I., Pawlas, M., Meyer, H. E., Warscheid, B., and Rehling, P. (2007) EMBO J. 26, 4347–4358

17. Piersel, F., Bestwick, M. L., Cobine, P. A., Khalimonchouk, O., Cricco, J. A., and Winge, D. R. (2007) EMBO J. 26, 4355–4364

18. Cabral, F., Soloz, M., Rudin, Y., Schatz, G., Claviller, L., and Slnom, P. P. (1978) I. Biol. Chem. 253, 297–304

19. Carlson, C. G., Barrientos, A., Tzagoloff, A., and Glur, D. M. (2003) J. Biol. Chem. 278, 3770–3775

20. Rak, M., Tetaud, E., Godard, F., Sagot, I., Salin, B., Duvezin-Caubet, S., Slonimski, P. P., Rytká, J., and di Rago, J. P. (2007) J. Biol. Chem. 282, 10853–10864

21. Soto, I. C., Fontanesi, F., Valledor, M., Horn, D., Singh, R., and Barrientos, A. (2009) Biochim. Biophys. Acta 1793, 1776–1786

22. Barrientos, A., Pierre, D., Lee, J., and Tzagoloff, A. (2003) J. Biol. Chem. 278, 8881–8887

23. Burke, D., Dawson, M., and Stearns, T. (2000) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

24. Guthrie, C., and Fink, G. R., eds (2002) Guide to Yeast Genetics and Molecular and Cell Biology, Academic Press, San Diego

25. Bonnefoy, N., Remacle, C., and Fox, T. D. (2007) Methods Cell Biol. 80, 525–548

26. Meunier, B., Lemarre, P., and Colson, A. M. (1993) Eur. J. Biochem. 213, 129–135

27. Meunier, B. (2001) Biochem. J. 354, 407–412

28. Vo, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene 77, 51–59

29. Diekert, K., de Kroon, A. I., Kispal, G., and Lill, R. (2001) Methods Cell Biol. 65, 37–45

30. Laemmli, U. K. (1970) Nature 227, 680–688

31. Bonnefoy, N., Bsat, N., and Fox, T. D. (2001) Mol. Cell. Biol. 21, 2359–2372

32. Westernmann, B., Herrmann, J. M., and Neupert, W. (2001) Methods Cell Biol. 65, 429–438

33. Schwede, T., Kopp, J., Guex, N., and Peitsch, M. C. (2003) Nucleic Acids Res. 31, 3381–3385

34. Bonnefoy, N., and Fox, T. D. (2000) Mol. Gen. Genet. 262, 1036–1046

35. Horan, S., Bourges, I., Taanman, J. W., and Meunier, B. (2005) Biochem. J. 390, 703–708
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36. Broadley, S. A., Demlow, C. M., and Fox, T. D. (2001) *Mol. Cell. Biol.* 21, 7663–7672
37. Hiser, L., Di Valentin, M., Hamer, A. G., and Hosler, P. (2000) *J. Biol. Chem.* 275, 619–623
38. Barros, M. H., Carlson, C. G., Glerum, D. M., and Tzagoloff, A. (2001) *FEBS Lett.* 492, 133–138
39. Church, C., Goehring, B., Forsha, D., Wazny, P., and Poyton, R. O. (2005) *J. Biol. Chem.* 280, 1854–1863
40. Khalimonchuk, O., Rigby, K., Bestwick, M., Pierrel, F., Cobine, P. A., and Winge, D. R. (2008) *Eukaryot. Cell* 7, 1427–1431
41. Steele, D. F., Butler, C. A., and Fox, T. D. (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93, 5253–5257
42. Fontanesi, F., Jin, C., Tzagoloff, A., and Barrientos, A. (2008) *Hum. Mol. Genet.* 17, 775–788
43. Costanzo, M. C., Seaver, E. C., and Fox, T. D. (1986) *EMBO J.* 5, 3637–3641
44. Valencik, M. L., Kloecener-Gruissem, B., Poyton, R. O., and McEwen, J. E. (1989) *EMBO J.* 8, 3899–3904
45. Valencik, M. L., and McEwen, J. E. (1991) *Mol. Cell. Biol.* 11, 2399–2405
46. Hoffbuhr, K. C., Davidson, E., Filiano, B. A., Davidson, M., Kennaway, N. G., and King, M. P. (2000) *J. Biol. Chem.* 275, 13994–14003
47. Vempati, U. D., Han, X., and Moraes, C. T. (2009) *J. Biol. Chem.* 284, 4383–4391
48. Drapier, D., Rimbault, B., Vallon, O., Wollman, F. A., and Choquet, Y. (2007) *EMBO J.* 26, 3581–3591
49. Minai, L., Wostrikoff, K., Wollman, F. A., and Choquet, Y. (2006) *Plant Cell* 18, 159–175
50. Wostrikoff, K., Girard-Bascou, J., Wollman, F. A., and Choquet, Y. (2004) *EMBO J.* 23, 2696–2705
51. Choquet, Y., Zito, F., Wostrikoff, K., and Wollman, F. A. (2003) *Plant Cell* 15, 1443–1454
52. Muramoto, K., Hirata, K., Shinzawa-Itoh, K., Yoko-o, S., Yamashita, E., Aoyama, H., Tsukihara, T., and Yoshikawa, S. (2007) *Proc. Natl. Acad. Sci. U.S.A.* 104, 7881–7886
53. Gawryluk, R. M., and Gray, M. W. (2010) *Mol. Biol. Evol.* 27, 7–10