Degradation of Cytochrome Oxidase Subunits in Mutants of Yeast Lacking Cytochrome c and Suppression of the Degradation by Mutation of yme1*

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We have confirmed by spectral analysis that cytochrome oxidase is not present in strains of the yeast Saccharomyces cerevisiae lacking the mitochondrial-encoded subunits I, II, and III of cytochrome oxidase. Furthermore, pulse-chase experiments demonstrated that subunit II is rapidly degraded in vivo. This degradation can be at least partially suppressed by disruption of the nuclear gene YME1, which encodes a putative ATP-Zn²⁺-dependent protease. We suggest that the cytochrome oxidase subunits are not properly assembled in the absence of cytochrome c, and that Yme1 and possibly other proteases degrade the unassembled mitochondrial-encoded subunits of cytochrome oxidase.

Eukaryotic cytochrome oxidase, also denoted cytochrome c oxidase and cytochrome a₃, is located in the inner membrane of mitochondria, where it catalyzes the transfer of electrons from cytochrome c to molecular oxygen (1–3). Cytochrome oxidase is a multisubunit complex consisting of 3 mitochondrial-encoded subunits, which form the functional core, and 8 to 10 nuclear-encoded subunits (4). In particular, cytochrome oxidase from Saccharomyces cerevisiae consists of a total of 11 subunits: I, II, and III, encoded by the mitochondrial genes COX1, COX2, and COX3, respectively, and IV, Va, Vb, VI, V1a, V1b, VII, VIIa, and VIII, encoded by the nuclear genes COX4, COX5a, COX5b, COX6, COX13, COX12, COX7, COX9, and COX8, respectively (5). (The cytochrome oxidase subunits I, II, etc., are also denoted Cox1, Cox1, etc.) Post-translational assembly of all the subunits to a functional complex requires at least the gene products of COX10 and COX11, which are involved in heme biosynthesis (6, 7), and PET117 (8), PET191 (8), and SCO1 (9), whose functions are unknown. As discussed below, lack of assembly due to the deletion of COX1, COX2, COX3, COX4, COX10, COX11, or SCO1 leads to degradation of individual unassembled subunits (6, 7, 9–11).

S. cerevisiae contains two isoforms of cytochrome c, iso-1- and iso-2-cytochrome c, which are encoded by the nuclear genes, CYC1 and CYC7, respectively (12, 13). Strains completely deficient in cytochrome c can be produced either by cyc1– cyc7 double mutations (13) or by mutation of the CYC3 gene that encodes heme lyase, which catalyzes the covalent attachment of the heme group (14, 15). Spectral examination of intact cells revealed that such mutants lacking cytochrome c are also deficient in cytochrome oxidase, presumably as a secondary effect of the cytochrome c deficiency (13, 15, 16). Also, cytochrome oxidase is exceedingly sensitive to glucose repression in mutants having trace amounts of cytochrome c, as found with certain “leaky” cyc3 mutations (14), and in mutants having low levels of function, as found with certain cyc1– missense mutations. The lack or diminished levels of cytochrome oxidase was also observed in mutants of Neurospora crassa deficient in cytochrome c (17–19).

In this study, we have used immunological procedures to demonstrate that cytochrome c-deficient cyc1– cyc7– mutants lack the mitochondrial-encoded cytochrome oxidase subunits I, II, and III and have reduced amounts of the nuclear-encoded subunits IV, V, VI, and V1a. In addition, pulse-chase experiments demonstrated that subunit II is rapidly degraded in vivo. Furthermore, a number of genes encoding, or presumably encoding, mitochondrial proteases were disrupted, and the levels of the cytochrome oxidase subunits were examined. If these proteases are responsible for the degradation of the cytochrome oxidase subunits, the disruptions should act as suppressors in cytochrome c-deficient strains.

The nuclear gene PIM1 encodes an ATP-dependent protease located in the mitochondrial matrix (20, 21). Because Pim1 is in the mitochondrial matrix and cytochrome oxidase is in the intermembrane space, and because pim1Δ mutants become pim1Δ mutants were not expected to be the protease acting on the cytochrome oxidase subunits. On the other hand, YTA10 (22), also denoted AGF3 (23), encodes a protease that acts on incompletely synthesized polypeptides in the mitochondrial inner membrane (24). YME1 (25) and RCA1 (26), also denoted YTA11 and YTA12, respectively (22), encode putative proteases related to Afg3 and represent members of a family of ATPases similar to proposed proteolytic complexes in Escherichia coli and yeast. However, afg3Δ and rca1Δ disruptions are deficient in the cytochrome oxidase subunits I, II, and III.

Only the yme1Δ disruption, but not pim1Δ, afg3Δ, or rca1Δ, increased the level of the cytochrome oxidase subunits II and III in cytochrome c-deficient strains. These results suggest that the cytochrome oxidase subunits are not properly assembled in the absence of cytochrome c, and that Yme1 and possibly other proteases degrade the unassembled subunits I, II, and III.

This degradation of cytochrome oxidase subunits in strains lacking cytochrome c at least superficially resembles the degradation of labile forms of cytochrome c in strains lacking either cytochrome c₁ or cytochrome oxidase, its physiological partners (27).

**MATERIALS AND METHODS**

Genetic Nomenclature and Yeast Strains—The symbols CYC1 and CYC7 denote the wild-type nuclear genes encoding, respectively, iso-1-cytochrome c and iso-2-cytochrome c. The cyc1–1011 symbol corresponds to a frameshift-TAA mutation at codon 85, resulting in a complete deficiency of iso-1-cytochrome c function; whereas cyc7–67 denotes
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a 400-base pair deletion, resulting in a complete deficiency of iso-2-cytochrome c function. The cyc-1-1011 and cyc-7-67 symbols are abbreviated in this paper as cyc-1 and cyc-7, respectively. Thus, cyc-1 cyc-7Δ strains are completely deficient in cytochrome c. YME1Δ and yme1Δ denote, respectively, the wild-type and disrupted gene encoding a putative mitochondrial Zn2+–ATP-dependent protease. Similar genetic symbols are used to denote the other alleles of the putative proteases. rΔ denotes the normal mitochondrial genome, whereas ρΔ denotes mutants completely or partially deficient in mitochondrial DNA and thus are unable to carry out mitochondrial protein synthesis, resulting in deficiencies in cytochrome oxidase subunits I, II, and III, as well as cytochrome b and other mitochondrial-encoded proteins.

An isogenic series of S. cerevisiae strains, listed in Table I, was prepared from the related strains B-8123 (MATa cyc-1011 cyc-7-67 ura3-52 lys5-10) or B-8514 (MATa CYC1 cyc-7-67 ura3-52 lys5-10) (28). All strains of the series lacked iso-2-cytochrome c because of the cyc-7-67 mutation, and each strain contained either the CYC1Δ or cyc-1-1011 allele, the YME1Δ or yme1Δ allele and were either rΔ or ρΔ. The yme1Δ strains, as well as other disruptions, were prepared as described previously (27); the ρΔ strains were prepared by growth in the presence of ethidium bromide (29). The results of these experiments are not shown in detail. The levels of the cytochromes aα3, b, cα, and cβ were quantitatively estimated by absorbance recordings of intact cells at −196 °C, with an Aviv Model 14 spectrophotometer as described previously (32).

Antibodies—Monoclonal antibodies to CoxI and CoxII were obtained from Molecular Probes, Inc. Monoclonal antibodies to CoxII (4B12-A5), CoxIV (IA12-A12), CoxV (1A3E-F5), CoxVI (11D11-H6), and CoxVIα (6F10), previously described (33), were generously provided by Dr. R. Capaldi (University of Oregon, Eugene). A polyclonal antibody to CoxI was generously provided by Dr. T. Mason (University of Massachusetts, Amherst).

Quantitation of Cytochrome Oxidase Subunits—Yeast were grown to stationary phase in 15 ml of YPD (1% yeast extract, 2% Bacto-peptone, 2% dextrose); the cells were collected by centrifugation at 5000 × g for 10 min, and the pellet was washed in 1 ml of 100% trichloroacetic acid to precipitate the cell protein. After 10 min on ice, each sample was centrifuged at 15,000 × g for 10 min, the supernatant was removed, and the pellet was resuspended in 4 ml of cold sterile distilled water. Cells were lysed essentially by the procedure of Yaffe and Schatz (34). The cells were lysed in an equal volume of 0.4 M NaOH containing 1.7% 2-mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride, 0.002% bromophenol blue. The cell debris and other mitochondrial extracts were also prepared as described (35) and solubilized in the same manner. These solubilized samples were centrifuged at 15,000 × g for 5 min, and the pellets were discarded. The levels of each of the subunits were estimated by loading various stepwise dilutions onto a 10% SDS-polyacrylamide gel (36) and comparing the intensities of the bands to those from the normal control. Protein was transferred to nitrocellulose by standard procedures (37). Nonspecific protein binding was blocked with a 2-h incubation with 5% fetal calf serum (Life Technologies, Inc.). The filters were incubated for 3 h with primary antibody at the following dilutions: CoxI (20 ng/ml), CoxI (1:2000), CoxII (0.5 μg/ml), CoxV (1:500), CoxV (1:400), CoxVI (1:200), and CoaVI (1:100). The filters were visualized using either anti-mouse anti-goat horseradish peroxidase or alkaline phosphatase development reagents (Bio-Rad).

Pulse-Chase Labeling of CdIs and Immunoprecipitation of CoxI—Yeast strains were grown as described above and resuspended in 12 ml of semisynthetic sulfate-free medium (38) lacking yeast extract and containing 2% raffinose, 0.1% glucose, and 1 mg/ml cyclohexamide. After the cells were incubated for 15 min at 30 °C, [35S]methionine (1300 Ci/mmol, Amersham) was added to a concentration of 0.125 Ci/ml, and the cells were incubated for an additional 15 min, followed by a chase in 30 μM (final concentration) methionine. Identical volumes of 2 ml of cells were taken at the indicated times, and the cells were lysed and immunoprecipitated by the same method previously described for cytochrome c (27), except that 1 ml of polyclonal antiserum to Cox1 was added to each sample. Proteins were separated as described above, the gels were dried, and CoxI was visualized by autoradiography.

RESULTS AND DISCUSSION

Cytochrome Levels in Vivo—The levels of the cytochromes aα3, b, cα, and cβ in the isogenic series of strains were determined by low temperature spectrophotometric recordings of intact cells (Fig. 1). Strain B-8514 (curve A) (CYC1Δ cyc-7Δ rΔ) shows a normal complement of cytochromes aα3, b, cα, and cβ. (The cyc-1Δ defect is normally not manifested in the presence of CYC1 because iso-2-cytochrome c contributes to only approximately 5% of the total amount of cytochrome c (16)). Strain B-8516 (curve B) (CYC1Δ cyc-7Δ rΔ yme1Δ rΔ); a cytochrome oxidase oxidase, having the yme1Δ disruption, was nearly the same as the normal strain B-8514 (curve A), except that all of the cytochromes are slightly diminished, similar to slightly repressed cells. Similarly, strain B-9621 (curve C) (cyc-1Δ cyc-7Δ ρΔ) contains a primary deficiency in cytochrome c, had no detectable amount of cytochrome oxidase, as indicated by the absence of a cytochrome aα3 peak at 602.5 nm. The cytochrome content in strain B-9614 (curve B) (CYC1Δ cyc-7Δ yme1Δ rΔ), having the yme1Δ disruption, was nearly the same as the normal strain B-8514 (curve A), except that all of the cytochromes are slightly diminished, similar to slightly repressed cells. Similarly, strain B-9621 (curve D) (cyc-1Δ cyc-7Δ yme1Δ ρΔ) was nearly the same as strain B-8123 (curve C), demonstrating that yme1Δ does not suppress the cytochrome aα3 deficiency caused by the absence of cytochrome c. The deficiencies of cytochromes aα3 and b, due to the lack of translation of the mitochondrial genes COXI, COX2, COX3, and COB1, can be seen in strains B-8516 (curve E) (CYC1Δ cyc-7Δ rΔ) and B-9622 (curve F) (cyc-1Δ cyc-7Δ rΔ).

Isogenic strains containing disruptions of PIM1, AFG3, or RCA1 had spectral curves very similar to these rΔ strains (data not presented), reflecting their rΔ phenotype caused by the disruptions (20, 26, 39).

Analysis of Cytochrome Oxidase Subunits—Western blot analysis of the isogenic strains with specific antibodies was used to estimate, relative to the normal value, the levels of the
mitochondrial-encoded subunits, I, II, and III, as illustrated in Fig. 2, and the nuclear-encoded subunits IV, V, VI, and VIa. The levels of the subunits were determined quantitatively by comparing the intensities of the bands derived from the various strains to diluted samples prepared from the normal strain B-814, similar to the procedure used by Calavetta and Capaldi. The results, presented in Table I, indicated that strain B-9614 (Cyc1′ cyc7-Δ yme1-Δ) had slightly diminished levels of all of the examined subunits, although the spectral peaks of cytochrome aa3 at 602.5 nm were nearly identical (Fig. 1). Strain B-8123 (cyc1-Δ cyc7-Δ), which contains a primary deficiency in cytochrome c, lacked the mitochondrial-encoded CoxI, CoxIII, and CoxII and had considerably decreased levels of CoxIV, CoxV, CoxVI, and CoxVIa, thus explaining the lack of cytochrome oxidase as indicated by spectral analysis (Fig. 1). Most importantly, the levels of CoxI and CoxIII, but not CoxII, were at least partially restored in strain B-9621 (cyc1-Δ cyc7-Δ yme1-Δ), thus suggesting that yme1-Δ suppresses the degradation of some of the subunits. As expected, the p+ strains B-8516 (Cyc1′ cyc7-Δ) and B-9622 (cyc1-Δ cyc7-Δ) lacked the mitochondrial-encoded subunits I, II, and III and contained diminished levels of the nuclear-encoded subunits IV, V, VI, and VIa.

In Vivo Degradation of Subunit CoxII—Pulse-chase labeling experiments were used to establish that the diminished levels of CoxII were due to degradation. The results with the B-8123 (Cyc1′ cyc7-Δ) demonstrated that deletion of yme1-Δ, which results in the loss of the cyclophilin a subunit, suppressed the degradation of some of the subunits. As expected, the p+ strains B-8514 (Cyc1′ cyc7-Δ) and B-9621 (cyc1-Δ cyc7-Δ yme1-Δ) were presented in Fig. 3. There was little or no apparent turnover of CoxII in the normal strain B-814 after 2 h. In contrast, CoxII was rapidly degraded in the cytochrome c-deficient strain B-8123, resulting in complete or almost complete degradation by 2 h. CoxII was also degraded in the related yme1-Δ strain B-9621, but at a lower rate. These pulse-chase experiments are in complete agreement with the results of the steady-state levels (Table I), indicating that the reduced levels are due to degradation.

Degradation of Unassembled Subunits and Protection by Protein-Protein Interactions—Studies by other workers indicated that deletion of any of the genes encoding components of cytochrome oxidase leads to diminution of other subunits, suggesting that the unassembled subunits are prone to degradation. Dowhan et al. (10) demonstrated that disrupting COX4, and thereby eliminating subunit IV, resulted in the loss of the cytochrome aa3 absorption peak and in the diminution of subunits I, II, III, and V, but not VI and VII. Deletion of subunits V, VI (40), and VII (41) or VIIa (42) also abolished the spectrum for cytochrome a3, although levels of other subunits were not reported. More recently, Calavetta and Capaldi (1) systematically investigated the steady state levels of subunits I, II, III, IV, V, VI, and VIa, in yeast strains specifically deleted for one or another of the mitochondrial genes COX1, COX2, COX3, or the nuclear gene COX10, which is involved in heme a synthesis. The study revealed that subunits I, II, III, and VIa were greatly diminished if any of these COX1, COX2, COX3, or COX10 genes were deleted. However, deletion of COX1 or COX2 but not COX3 decreased the levels of subunits IV and V, whereas only deletion of COX2 decreased the level of subunit VI. Deletion of COX10 also diminished the levels of subunit V, but not subunits IV and VI. Overall, these findings suggest that unassembled subunits are degraded, with the degree of degradation of each subunit probably reflecting their intrinsic stability and their association with other subunits of the complex. Thus, deletion of a single subunit could lead to a cascade of complex degradations, which is dependent on the protective associations.

The results presented in this paper can be explained by the protection of one or more of the cytochrome oxidase subunits by cytochrome c. Cytochrome c exhibits high affinity binding to subunit I and low affinity binding to another subunit (43). The absence of cytochrome c may destabilize CoxI, and possibly CoxI and CoxIII, thereby making these, as well as other subunits, susceptible to degradation.

The results shown in Fig. 2 and summarized in Table I demonstrated that degradation of subunits I and III, but not subunit I, in a cytochrome c-deficient strain can be partially suppressed by the yme1-Δ mutation, which prevents synthesis of the Yme1 presumptive protease. The simplest explanation of these results is that Yme1 is a protease that, by itself or in complex with other proteins, is involved in turnover of unassembled subunits of cytochrome oxidase, confirming a previous report that turnover of unassembled subunits of cytochrome oxidase requires a metal/ion-dependent factor (11). In addition, Weber et al. (2) observed that CoxI was degraded in a cox4-deficient strain, and that this degradation was suppressed by yme1-Δ. The lack of suppression by yme1-Δ of the CoxI complete deficiency (Table I), and of the possible partial deficiencies of other subunits, suggests that still other proteases may be involved in the degradation process. In fact, the turnover of CoxV was not suppressed by yme1-Δ, pm1-Δ, afg3-Δ, or rca1-Δ (results not presented).

In summary, these results indicate that cytochrome c protects cytochrome oxidase subunits from degradation, and our previous results (27) demonstrated that cytochrome oxidase or cytochrome c3 protects certain labile forms of cytochrome c from degradation, a phenomenon that was previously believed to occur only with strongly interacting components of protein complexes.

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REFERENCES

1. Hatefi, Y. (1985) Annu. Rev. Biochem. 54, 1015–1069
2. Wikstrom, M. (1989) Nature 338, 776–778
3. Babcock, G. T., and Wikstrom, M. (1992) Biochemistry 31, 301–309
4. Kadenbach, B., and Marle, P. (1983) FEBS Lett. 151, 1–11
5. Geier, B. M., Schagger, H., Orlowski, C., Link, T. A., Hagen, W. R., Brandt, U., and Von Jagow, G. (1995) Eur. J. Biochem. 227, 296–302
6. Nobrega, M. P., Nobrega, F. G., and Tzagoloff, A. (1990) J. Biol. Chem. 265, 14220–14226
7. Tzagoloff, A., Capitanio, N., Nobrega, M. P., and Gatti, D. (1990) EMBO J. 9, 2759–2764
8. McDowen, J. E., Hong, K. H., Park, S., and Preciado, G. T. (1993) Curr. Genet. 23, 9–14
9. Schulze, M. and Rodel, G. (1989) Mol. Gen. Genet. 216, 37–43
10. Dowhan, W., Bibus, C. R., and Schatz, G. (1985) EMBO J. 4, 179–184
11. Nakai, T., Mura, Y, Yasuhara, T., and Ohashi, A. (1994) J. Biochem. (Tokyo) 116, 752–758
12. Sherman, F., Stewart, J. W., Margoliash, E., Parker, J., and Campbell, W. (1966) Proc. Natl. Acad. Sci. U. S. A. 55, 1498–1504
13. Downie, J. A., Stewart, J. W., Brockman, N., Schweinger, R. A., and Sherman, F. (1977) J. Mol. Biol. 113, 364–384
14. Relly, C. and Sherman, F. (1985) Biochim. Biophys. Acta 85, 640–651
15. Dumont, M. E., Ernst, J. F., Hampe, D. M., and Sherman, F. (1987) EMBO J. 6, 235–241
16. Sherman, F., Taber, H. and Campbell, W. (1965) J. Mol. Biol. 13, 21–39
17. Bottorff, D. A., Parmaksizoglu, S., Lemire, E. G., Coffin, J. W., Bertrand, H., and Nargang, F. E. (1994) Curr. Genet. 26, 329–335
18. Drygas, M. E., Lambowitz, A. M., and Nargang, F. E. (1989) J. Biol. Chem. 264, 17897–17906
19. Nargang, F. E., Drygas, M. E., Kwon, P. L., Nicholson, D. W., and Neupert, W. (1988) J. Biol. Chem. 263, 9388–9394
20. Van Dyck, L., Pearce, D. A., and Sherman, F. (1994) J. Biol. Chem. 269, 238–242
21. Suzuki, C. K., Suda, K., Wang, N. and Schatz, G. (1994) Science 264, 273–276
22. Schnall, R., Mannhaupt, G., Stucka, R., Tauer, R., Ehnle, S., Schwarzo, C., Vetter, I., and Feldmann, H. (1994) Yeast 10, 1141–1155
23. Guerin, E., Rep, M., and Grivell, L. A. (1994) Yeast 10, 1389–1394
24. Pajic, A., Tauer, R., Feldmann, H., Neupert, W., and Langer, T. (1994) FEBS Lett. 333, 201–206
25. Thorsness, P. E., White, K. H., and Fox, T. D. (1993) Mol. Cell. Biol. 13, 5418–5426
26. Tzagoloff, A., Yue, J., Jang, J., and Paul, M. F. (1994) J. Biol. Chem. 269, 26144–26151
27. Pearce, D. A., and Sherman, F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3735–3739
28. Yamamoto, T., Moerschell, R. P., Wakem, L. P., Komar-Panicucou, S., and Sherman, F. (1992) Genetics 131, 811–819
29. Dujon, B. (1993) Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 505–635, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
30. Thorsness, P. E., and Fox, T. D. (1993) Genetics 134, 21–28
31. Weber, E. R., Rooks, R. S., Shater, K. S., Chase, J. W., and Thorsness, P. E. (1995) Genetics 140, 435–442
32. Hickey, D. R., J ayaraman, K., Goodhue, C. T., Shah, J., Clements, J. M., Tsunasawa, S., and Sherman, F. (1991) Gene (Amst.) 104, 73–81
33. Taanman, J.-W., and Capaldi, R. A. (1993) J. Biol. Chem. 268, 17854–17861
34. Yaffe, M. P., and Schatz, G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4819–4823
35. Dumont, M. E., Ernst, J. F., and Sherman, F. (1988) J. Biol. Chem. 263, 15928–15937
36. Schagger, H., and Von Jagow, G. (1987) Anal. Biochem. 168, 368–379
37. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual 2d Ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
38. Reid, G. (1983) Methods Enzymol. 97, 324–329
39. Tauer, R., Mannhaupt, G., Schnall, R., Pajic, A., Langer, T., and Feldmann, H. (1994) FEBS Lett. 335, 197–200
40. Patterson, T. E., Trueblood, C. E., Wright, R. M., and Poyton, R. O. (1987) Cytochrome Systems. Molecular Biology and Bioenergetics (Papa, S., Chance, B., and Ernster, L., eds) pp. 253–260, Plenum Press, New York
41. Agger, R., and Capaldi, R. A. (1990) J. Biol. Chem. 265, 16389–16393
42. Wright, R. M., Dircks, L. K., and Poyton, R. O. (1986) J. Biol. Chem. 261, 17183–17191
43. Cooper, C. E., Nicholls, P., and Freedman, J. A. (1991) Biochem. Cell Biol. 69, 586–607