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Absence of the Mitochondrial AAA Protease Yme1p Restores F₀-ATPase Subunit Accumulation in an oxa1 Deletion Mutant of Saccharomyces cerevisiae

Claire Lemaire\textsuperscript{++}, Patrice Hamel\textsuperscript{††}, Jean Velours\textsuperscript{‡‡}, and Geneviève Dujardin\textsuperscript{‡}

From the \textsuperscript{§}Centre de Génétique Moléculaire du CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France and the \textsuperscript{¶}Institut de Biochimie et Génétique Cellulaire du CNRS, Université de Bordeaux II, 1 rue Camille Saint Saëns, 33077 Bordeaux Cedex, France

The nuclear gene \textit{OXA1} encodes a protein located within the mitochondrial inner membrane that is required for the biogenesis of both cytochrome \textit{c} oxidase (Cox) and ATPase. In the absence of Oxa1p, the translocation of the mitochondrially encoded subunit Cox2p to the intermembrane space (also referred to as export) is prevented, and it has been proposed that Oxa1p could be a component of a general mitochondrial export machinery. We have examined the role of Oxa1p in light of its relationships with two mitochondrial proteases, the matrix protease Afg3p-Rca1p and the intermembrane space protease Yme1p, by analyzing the assembly and activity of the Cox and ATPase complexes in \textit{Δoxa1}, \textit{Δoxa1afg3}, and \textit{Δoxa1yme1} mutants. We show that membrane subunits of both complexes are specifically degraded in the absence of Oxa1p. Neither Afg3p nor Yme1p is responsible for the degradation of Cox subunits. However, the F₀ subunits Atp4p, Atp6p, and Atp17p are stabilized in the \textit{Δoxa1yme1} double mutant, and oligomycin-sensitive ATPase activity is restored, showing that the increased stability of the ATPase subunits allows significant translocation and assembly to occur even in the absence of Oxa1p. These results suggest that Oxa1p is not essential for the export of ATPase subunits. In addition, although respiratory function is dispensable in \textit{Saccharomyces cerevisiae}, we show that the simultaneous inactivation of \textit{AFG3} and \textit{YME1} is lethal and that the essential function does not reside in their protease activity.

In mitochondria, biogenesis of the respiratory complexes requires the expression of both the mitochondrial and nuclear genomes (1–3). The mitochondrial genome encodes only a few subunits of the respiratory complexes, whereas the other subunits and a number of proteins, which are not intrinsic components of the complexes but are required for their biogenesis, are nuclearly encoded.

The \textit{Saccharomyces cerevisiae} nuclear gene \textit{OXA1} encodes such an assembly-assisting factor that is required for the biogenesis of both cytochrome \textit{c} oxidase (Cox)\textsuperscript{1} and ATPase (4, 5).

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\textsuperscript{†} To whom correspondence should be addressed. Tel.: 33-1-69823169; Fax: 33-1-69823150; E-mail: lemaire@cgm.cnrs-gif.fr.

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\textsuperscript{1} The abbreviation used is: Cox, cytochrome \textit{c} oxidase.

Oxa1p presents five hydrophobic segments and is located within the mitochondrial inner membrane (6–8). In the absence of Oxa1p, oligomycin-sensitive ATPase activity is significantly decreased, and Cox activity is totally abolished. On non-denaturing gels, the Cox complex of \textit{Δoxa1} strains displays a higher mobility probably due to the lack of accumulation of the mitochondrially encoded subunits (9, 10). In addition, \textit{OXA1} inactivation prevents the N-terminal maturation of the precursor of Cox2p (pre-Cox2p) (11) and affects its translocation (export) from the matrix to the intermembrane space (12, 13). Defective insertion of several chimeric proteins in the inner membrane has also been observed in \textit{oxa1} mutants, and it has been proposed that Oxa1p could be a component of a general mitochondrial export machinery (14). However, the fact that the absence of Oxa1p is compensated by point mutations in the cytochrome \textit{c}₁ gene is difficult to conciliate with a unique role for Oxa1p as a mitochondrial channel (10).

Two mitochondrial proteases (Afg3p-Rca1p and Yme1p) are also involved in respiratory complex assembly in yeast (15–18). The hetero-oligomeric complex Afg3p-Rca1p acts on the matrix side of the inner membrane, whereas Yme1p is active in the intermembrane space (19, 20). Afg3p-Rca1p appears to be involved in the degradation of Cox and ATPase subunits (21), and Yme1p in the degradation of Cox2p (22–24). In addition to their proteolytic activity, these proteases seem to display a chaperone-like activity (25–28), and it has been shown that the over-expression of \textit{OXA1} can partially compensate for the inactivation of \textit{AFG3} (29).

Finally, Oxa1p as well as both protease complexes appear to be conserved through evolution, showing the importance of their function. The human, \textit{Arabidopsis}, and \textit{Schizosaccharomyces pombe} \textit{OXA1} genes have been identified using functional complementation of a yeast \textit{oxa1} mutation (30–32). Human cDNAs encoding proteins highly related to Afg3p-Rca1p have also been described, and one of them encodes paraplegin, which, when mutated, is responsible for a hereditary spastic paraplegia (33, 34).

In this work, we have studied the relationships between Oxa1p and the two inner membrane AAA (ATPases associated with diverse cellular activities) proteases by analyzing the assembly and activity of the Cox and ATPase complexes in \textit{Δoxa1}, \textit{Δoxa1afg3}, and \textit{Δoxa1yme1} mutants. We show that membrane subunits of both complexes, whether mitochondrially or nuclearly encoded, are rapidly degraded in the absence of Oxa1p. Interestingly, neither Afg3p nor Yme1p is responsible for the degradation of Cox subunits, whereas F₀-ATPase subunits are stabilized in the absence of Yme1p. The increased stability of the ATPase subunits in the \textit{Δoxa1yme1} double mutant allows significant assembly to occur since oligomycin-
sensitive ATPase activity is restored. Altogether, our results suggest that Oxa1p is not essential for the export of ATPase subunits. In addition, although respiratory function is dispensable in S. cerevisiae, we show that the simultaneous inactivation of the AFG3 and YME1 genes is lethal.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—Yeast genetic methods were previously described (35). All strains are isonuclear to the wild-type strain CW04 (α ade2-1 his3-11,15 trp1-1 leu2-3,112 can1-100), except for mating type, OXA1, AFG3, and YME1 loci. CW04 and NBT1 (α oxa1::LEU2) were previously described (4). AFG3-2 (α afg3::TRP1) exhibits the Yepafg3-2 plasmid carrying the URA3 gene and the afg3::E559Q allele (21, 26). PHT7 (α yme1::URA3) and PHT9 (α yme1::KanR) were constructed by inactivating the YME1 gene using a URA3 cassette (15) or a Kan″ cassette (36). F01-6B (α afg3::HIS3) carries the afg3::HIS3 inactivation (25). The four double mutants were constructed either by gene inactivation (PHT8, α oxa1::LEU2 yme1::URA3) or by crosses, sporulation, and microdissection (F01-6C, a oxa1::LEU2 afg3::HIS3; RF0-7C, α oxa1::LEU2 afg3::HIS3; and YA1-2C, α afg3::TRP1 yme1::KanR). Strains RF0-7C and YA1-2C carry the Yepafg3-2 plasmid.

Purification and Extraction of Mitochondria—Purification of mitochondria and carbonate extraction were performed as described (6), except that the following protease inhibitors were added to each buffer: phenylmethylsulfonyl fluoride (1 mM), pepstatin (1 μM), chymostatin (10 μg/ml), antipain (10 μg/ml), and leupeptin (10 μg/ml). The mitochondrial protein concentration was determined using the Bio-Rad protein assay.

In Vivo Labeling of the Mitochondrial Translation Products and Whole Cell Extracts—Yeast cells were grown on medium containing 1% yeast extract, 1% Bacto-peatone, 2% galactose, 0.1% glucose, and 20 μg/ml adenine and harvested at mid-exponential phase. In vivo labeling of the mitochondrial translation products was performed essentially as described (37). Cells (1.8 × 10^9) were harvested and resuspended in labeling medium (40 mM K2HPO4 (pH 7.4) and 2% galactose). They were labeled for 20 min at 30 °C with [35S]methionine (130 Ci/mmol) in the presence of cycloheximide (150 μg/ml) to inhibit cytosolic protein synthesis. The reaction was stopped by addition of methionine (10 mM final concentration). Total cell proteins were extracted by alkaline lysis (38).

Extraction of the F1, Sector F0 Subunits—F1 was released from mitochondrial membranes as described previously (39), except that the following protease inhibitors were added to the extraction buffer as in the carbonate extraction medium (see above). Mitochondria were suspended to a concentration of 5 mg/ml in extraction buffer (0.25M sucrose, 10 mM Tris-HCl, 1 mM EDTA (pH 7.5)), and 0.5 volume of chloroform was added. The two phases were vigorously mixed for 30 s and then separated by centrifugation at 4000 g for 5 min at 20 °C. The aqueous layer was centrifuged at 100,000 g for 5 min at 20 °C to recover the supernatant, which contained the F1 sector. F1-ATPase activity was then measured as described (10). For gel electrophoresis analysis, F1 was precipitated with NH42SO4 added to 60% saturation. Atp6p was extracted by organic solvents (40), except that mitochondrial protein (2 mg/ml) was incubated for 2 h at room temperature with chloroform/methanol (1:1). Cytochrome c Oxidase and ATPase Activity Measurements on Purified Mitochondria—Cytochrome c oxidase activity was estimated by measurement of cytochrome c oxidation performed spectrophotometrically at 550 nm (10). ATPase activity was measured by colorimetric determination of inorganic phosphate released from ATP (41).

Electrophoresis and Immunoblotting—Electrophoresis and electrophoreogram were performed as described previously (10), except for the analysis of the mitochondrial translation products (37). Immunodetection was carried out using the enhanced chemiluminescence method (Pierce). The anti-yeast Cox1p, Cox2p, Cox3p, and Cox4p monoclonal antibodies were purchased from Molecular Probes, Inc., whereas the anti-yeast Cox5p and Cox6p monoclonal antibodies were generous gift from R. A. Capaldi (University of Oregon) (42). The anti-yeast Cox4p monoclonal antibody was kindly provided by R. O. Poyton (University of Colorado). The anti-yeast ATPase subunit polyclonal antibodies were prepared against either the purified subunits (Atp1p, Atp2p, Atp3p, Atp4p, Atp5p, Atp16p, and Atp17p) or a synthetic peptide of the N-terminal part (Atp6p).

RESULTS

Oxa1p Is Required for the Accumulation of the Membrane Subunits of Cox and ATPase Complexes—To investigate the subunit composition of the Cox and ATPase complexes in the absence of Oxa1p, mitochondria of the wild-type and oxa1 deleted (Δoxa1) strains were treated with carbonate, and the steadystate levels of the main subunits of these two complexes were systematically analyzed in the soluble and membrane fractions by immunoblotting. For the ATPase complex, the two sectors F1 and F0 were also separately extracted using different organic solvents, and the subunits were revealed by immunoblotting or silver staining.

As previously shown (9, 10), the two membrane subunits Cox1p and Cox2p were not detectable in the absence of Oxa1p (Fig. 1A). The other membrane Cox subunits were also either not detectable (Cox6p) or poorly detectable (Cox3p and Cox5p) in the Δoxa1 mutant. The two subunits Cox4p and Cox6p, distributed between the membrane and the soluble fractions in the wild-type strain, were present mostly in the soluble fraction in the absence of Oxa1p, and their steady-state level was decreased.

The ATPase complex consists of a soluble sector (F1) and a membrane-anchored sector (F0) that assembles independently. The ATPase activity of the chloroform-extracted F1 sector was lowered ~2-fold in the Δoxa1 strain compared with the wild-type strain (4815 ± 30 versus 9870 ± 450 nmol of ATP hydrolyzed per min/mg of protein). As shown by immunoblotting, the four F1 subunits Atp1p, Atp2p, Atp3p, and Atp16p and the subunit Atp6p (oligomycin sensitivity-conferring protein subunit) were decreased by ~40% in the Δoxa1 extracts (Figs. 2A and 3A), which is consistent with the activity measurement. On the contrary, the three F0 membrane subunits Atp6p, Atp17p (subunit F), and Atp4p exhibited dramatically reduced levels in the pellet fraction of the Δoxa1 strain (Fig. 2A). Organic solvent extraction of the F0 subunits showed that Atp6p was decreased by ~40% in the Δoxa1 strain (Fig. 3B).

 Altogether, these data clearly establish that there is a selective decrease in the accumulation of the membrane subunits of Cox and F0-ATPase in the absence of Oxa1p. It was previously shown that the mitochondrially encoded subunits are still synthesized in the absence of Oxa1p (4, 11). Using a PhosphorIm-
**Accumulation of ATPase subunits in mitochondrial membranes.** Western blot analysis of mitochondrial proteins was performed as described in the legend to Fig. 1. S, supernatant; P, pellet. The blots were probed with polyclonal antibodies raised against the F₁ β-subunit (Atp2p), the oligomycin sensitivity-conferring protein subunit (Atp6p), and F₁ subunits (Atp6p, Atp17p, and Atp4p). Atp6p is a mitochondrially encoded subunit, whereas the others are nuclearly encoded. A, wild type (WT); CW04) and Δoxa1 (NBT1); B, Δyme1 (PHT7) and Δoxa1Δyme1 (PHT8).

**Organic solvent extraction of the F₁-ATPase sector and of the F₀ subunit Atp9p.** The F₁ sector and Atp9p were extracted by chloroform and chloroform/methanol, respectively (see "Experimental Procedures"). Proteins were fractionated on 15% SDS-polyacrylamide gels. A, F₁ subunits were transferred to nitrocellulose, and the blots were probed with antibodies against Atp1p, Atp3p, and Atp16p. B, the Atp9p subunit of F₀ was revealed by silver staining (48). The wild-type (WT; CW04) and Δoxa1 (NBT1) strains were used.

**Stabilization of F₀-ATPase Subunits by Yme1p in an oxa1 Mutant**

Measurements of cytochrome c oxidase and ATPase activities were made on purified mitochondria. The values are averages of measurements obtained from two separate mitochondrial preparations. They are expressed as a percentage of the wild-type (WT) strain with an S.D. of ~10%. The cytochrome c oxidase activity of the wild-type strain is 770 nmol of oxidized cytochrome c/min/mg of protein. The specificity of cytochrome c oxidase activity was tested by addition of 4 μM KCN. The total ATPase activity of the wild-type strain is 1.57 μmol of Pᵢ/min/mg of protein, and the oligomycin-sensitive activity is 1.45 μmol of Pᵢ/min/mg of protein.

**Table I**

| Strain | Cytochrome c oxidase activity | ATPase activities |
|--------|-------------------------------|-------------------|
|        | %                            | Total | Oligomycin-sensitive |
| WT     | 100                          | 100   | 100                  |
| Δoxa1 | 0                            | 88    | 20                   |
| Δafg3 | 60                            | 70    | 3                    |
| Δoxa1Δafg3 | 0    | 73    | 5                    |
| Δyme1 | 62                            | 95    | 89                   |
| Δoxa1Δyme1 | 0    | 104   | 80                   |

Cox1p, Cox2p, Cox3p, Cox5p, and Cox6p were still degraded (Fig. 1B and data not shown). In addition, the extramembrane subunits, Cox4p and Cox6p, also displayed the same pattern as in the Δoxa1 single mutant (Fig. 1A and data not shown). Similarly, Cox2p remained undetected in whole cell extracts from Δoxa1Δrca1 and Δoxa1Δrca1Δafg3 strains (data not shown), suggesting that the accumulation of Cox1p, Cox3p, and Cox4p is also affected since the accumulation of these four subunits is interdependent (43). Thus, the absence of Afg3p-Rca1p or Yme1p does not seem to restore the stability of the Cox subunits.

Since it has been proposed that Afg3p-Rca1p could have a dual protease and chaperone activity (26, 27), the subunit degradation occurring in the Δoxa1Δafg3 double mutant could be due to the lack of the chaperone function of Afg3p-Rca1p. The absence of Afg3p-Rca1p leads to defects in respiratory complex assembly (17, 18, 25) that can be complemented by a proteolytically inactive variant of Afg3p in which glutamic acid 559 of the proteolytic site is changed to a glutamine (21, 27). Thus, we have constructed a strain (RF0-7C) with the double inactivation Δoxa1Δafg3Δ and a plasmid carrying the afg3-E559Q allele (21). In this strain, the chaperone activity of Afg3p should still be functional, whereas the protease activity of the Afg3-Rca1p complex is abolished. We found that Cox2p remained undetectable in whole cell extracts of RF0-7C, showing that the Cox2p degradation in the Δoxa1Δafg3 strain is not due to the absence of the chaperone activity of Afg3p-Rca1p. In conclusion, Afg3p-Rca1p is not responsible for the degradation of membrane subunits of Cox that occurs in the absence of Ox1p.

**F₁-ATPase Membrane Subunits Defective in the Δoxa1 Strain Are Stabilized in the Double Mutant Δoxa1Δyme1**

The ATPase activities of the double mutants Δoxa1Δafg3 and Δoxa1Δyme1 were compared with those of the corresponding wild-type and single mutant strains (Table I). Although the total ATPase activity was not significantly diminished in the various strains, the oligomycin-sensitive ATPase activity was strongly decreased in the Δoxa1Δafg3, and Δoxa1Δyme1 strains. Surprisingly, whereas Yme1p has not to date been reported to have a role in the degradation of ATPase subunits, we found that the inactivation of the YME1 gene in the Δoxa1 strain restored an oligomycin-sensitive ATPase activity, i.e., Δoxa1Δyme1 reached 80% of the wild-type activity (Table I). Thus, the defect in the formation of the ΔF₁,ΔF₀ complex occurring in the Δoxa1 mutant was restored in the Δoxa1Δyme1 strain since the oligomycin-sensitive ATPase activity reflects a well

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**Fig. 2.** Accumulation of ATPase subunits in mitochondrial membranes. Western blot analysis of mitochondrial proteins was performed as described in the legend to Fig. 1. S, supernatant; P, pellet. The blots were probed with polyclonal antibodies raised against the F₁ β-subunit (Atp2p), the oligomycin sensitivity-conferring protein subunit (Atp6p), and F₁ subunits (Atp6p, Atp17p, and Atp4p). Atp6p is a mitochondrially encoded subunit, whereas the others are nuclearly encoded. A, wild type (WT); CW04) and Δoxa1 (NBT1); B, Δyme1 (PHT7) and Δoxa1Δyme1 (PHT8).

**Fig. 3.** Organic solvent extraction of the F₁-ATPase sector and of the F₀ subunit Atp9p. The F₁ sector and Atp9p were extracted by chloroform and chloroform/methanol, respectively (see "Experimental Procedures"). Proteins were fractionated on 15% SDS-polyacrylamide gels. A, F₁ subunits were transferred to nitrocellulose, and the blots were probed with antibodies against Atp1p, Atp3p, and Atp16p. B, the Atp9p subunit of F₀ was revealed by silver staining (48). The wild-type (WT; CW04) and Δoxa1 (NBT1) strains were used.

**Table I Activities of Cox and ATPase complexes**
Simultaneous Inactivation of AFG3 and YME1 Is Lethal—

Since the Cox subunit stability was restored neither by YME1 nor by AFG3-RCA1 single inactivations, it was tempting to test the effect of the double inactivation. To construct the triple mutant Δoxa1Δarf3Δyme1, we crossed the Δoxa1Δarf3 double mutant (F01-6C) with the Δyme1 strain (PHT7) and dissected the ascus. However, no spore carrying the inactivation of both mutants (F01-6C) with the Δarf3 (AFG3-2), Δarf3 (AFG3-2), and Δarf3Δyme1 (YA1-2C) strains were used. The two single mutants and the double mutant carrying the Yaparf3-2 plasmid were patched on uracil-containing media without (–5FOA) or with (+5FOA) 1 mg/ml 5-fluoroorotic acid. Cells were incubated for 3 days at 28 °C. The Δyme1 (PHT9 + Yaparf3-2), Δarf3 (AFG3-2), and Δarf3Δyme1 (YA1-2C) strains were used.

The single mutation of both plasmid subunits, Atp6p, Atp17p, and Atp4p, which was strongly diminished in the Δoxa1 mutant and not affected in the Δyme1 mutant, was restored in the double mutant Δoxa1Δyme1 (Fig. 2B). On the contrary, the accumulation of these subunits was not restored in the Δoxa1Δarf3 strain carrying the Δarf3-E559Q plasmid (RF0-7C) (data not shown). Thus, the absence of Yme1p restores the F0-ATPase subunit accumulation, which is defective in the absence of Oxa1p.

Simultaneous Inactivation of AFG3 and YME1 Is Lethal—

Assembled F1F0 complex. In addition, Western blot analysis showed that the accumulation of the F0 membrane subunits was tightly linked. To test if this lethality was specifically due to the absence of Yme1p, we crossed the Δarf3Δyme1 strain (or Δarf3Δyme1 strain carrying the Δarf3-E559Q plasmid (RF0-7C) (data not shown). Thus, the absence of Yme1p restores the F1F0 complex.

The two single mutants and the double mutant carrying the Yaparf3-2 plasmid were patched on uracil-containing media without (–5FOA) or with (+5FOA) 1 mg/ml 5-fluoroorotic acid. Cells were incubated for 3 days at 28 °C. The Δyme1 (PHT9 + Yaparf3-2), Δarf3 (AFG3-2), and Δarf3Δyme1 (YA1-2C) strains were used.

Concerning the Cox subunits, Cox2p is a known substrate of Yme1p (20, 23, 24), and the complex Afg3p-Rca1p has been shown to participate in the degradation of Cox1p and Cox3p (21, 27). Since pre-Cox2p export is blocked in the Δoxa1 mutant (11–13) and Yme1p is active in the intermembrane space, it is not surprising that Yme1p does not degrade pre-Cox2p in the Δoxa1 mutant. However, the fact that the inactivation of neither YME1 nor AFG3 could restore pre-Cox2p or the other Cox subunit stability suggests that either another protease is responsible for the degradation of these subunits or that they can be degraded by Yme1p and Afg3p-Rca1p. We rather favor the first hypothesis since the Cox subunits are still unstable when both Yme1p and Afg3p protease activities are blocked.

It has been shown that the assembly of Cox1p, Cox2p, Cox3p, and Cox4p is interdependent (43). For example, in a cox2 mutant, the accumulation of the three other subunits is reduced. A specific pre-Cox2p export defect could then explain the general instability of most of the other Cox subunits found in Δoxa1 strains. However, the fact that the suppressor mutations that compensate for the absence of Oxa1p fully compensate for the maturation defect of Cox2p but only partially restore Cox activity (10) suggests that Oxa1p is not required only for Cox2p export. Whether the primary function of Oxa1p in Cox biogenesis is linked to export of membrane subunits and/or their assembly still remains an open question, both processes being tightly linked.

As far as the ATPase is concerned, we found that the F0 membrane subunits are stabilized in the absence of Yme1p. Cross-linking experiments have shown that the subunits Atp6p, Atp17p, and Atp4p display accessible targets in the intermembrane space (41). However, it is difficult to determine whether they all represent true targets of Yme1p or whether only one subunit is degraded by this protease and exerts a protective effect upon the others. Nevertheless, the restoration of oligomycin-sensitive ATPase activity in the Δoxa1Δyme1 double mutant shows that the increased stability of the ATPase membrane subunits allows significant translocation and assembly to occur. This result suggests that Oxa1p is not essential for the export of the ATPase subunits.

It has been proposed that the assembly of the three mitochondrially encoded subunits of the F0 sector (Atp6p, Atp8p, and Atp9p) occurs first and is required for the subsequent assembly of Atp4p and then Atp5p (44–46). We found that Atp6p and Atp4p levels are dramatically reduced in the Δoxa1 strain, whereas Atp9p and Atp5p levels are not. For Atp9p, our results can be related to the data showing that Atp9p is associated with the F1 sector independently of the other F0 subunits (44). For Atp5p, our data suggest that its accumulation is independent of the formation of the F0 complex. In accordance with these results, we found that Atp5p was also stable in rho0 cells (data not shown), which are devoid of mitochondrial DNA and therefore of mitochondrially encoded subunits of F0.

Finally, we have shown that the simultaneous absence of Afg3p (or Rca1p) and Yme1p is lethal and that viability is restored by the introduction of the proteolytically inactive variant of Afg3p. This suggests that Afg3p-Rca1p and Yme1p have an overlapping essential function that is probably due to the chaperone-like function. Alternatively, the cell viability might require both protease and chaperone activities to be functional. Although Oxa1p partially compensates for the AFG3 inactivation and could also display a “chaperone-like” function, the lethality of the double inactivation of AFG3 and YME1 cannot be cured by overexpressing the OXA1 gene (data not shown). It has been shown that the growth of Δyme1 rho− cells is severely affected (47). However, the lethality of the double mutant Δarf3Δyme1 is probably not simply due to the accumulation of rho− mutants since the percentage of rho− mutants is ~15% in...
the Δafg3 mutant. In S. cerevisiae, respiratory function is dispensable, but the integrity of the mitochondrial compartment is essential. Thus, it is tempting to propose that Afg3p-Rca1p and Yme1p would play a role only in export and assembly of the membrane subunits of respiratory complexes and other protein complexes controlling mitochondrial compartment integrity, whereas Oxa1p would play a role indispensable, but the integrity of the mitochondrial compartment is essential. Thus, it is tempting to propose that Afg3p-Rca1p and Yme1p would play a role only in export and assembly of the membrane subunits of respiratory complexes.

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