Protein Export across the Inner Membrane of Mitochondria

THE NATURE OF TRANSLOCATED DOMAINS DETERMINES THE DEPENDENCE ON THE Oxa1 TRANSLOCASE

Received for publication, September 22, 2003, and in revised form, October 30, 2003
Published, JBC Papers in Press, October 30, 2003, DOI 10.1074/jbc.M310468200

Johannes M. Herrmann‡§ and Nathalie Bonnefoy¶

From the ‡Institut für Physiologische Chemie, Universität München, Butenandtstrasse 5, München 81377, Germany and the ¶Centre de Génétique Moléculaire du CNRS, Avenue de la Terrasse, Gif-sur-Yvette 91198, France

The biogenesis of mitochondria requires the insertion of both nuclear and mitochondrially encoded proteins into the inner membrane. The inner membrane protein Oxa1 plays an important role in this process. Translocation of the terminal intermembrane space domains of subunit 2 of the cytochrome oxidase complex, Cox2, strictly depends on Oxa1. In contrast, other Oxa1 substrates can be inserted independently of Oxa1 function, although at reduced efficiency. A Saccharomyces cerevisiae mutant containing a large deletion in its mitochondrial genome allowed us to analyze the insertion process of a fusion protein of cytochrome b and Cox2. In this mutant, the N-terminal domain of Cox2 is synthesized as a hairpin loop that is flanked by hydrophobic transmembrane segments on both sides. Both genetic and biochemical evidences indicate that translocation of this region across the inner membrane still requires Oxa1 function. Thus, the position of intermembrane space domains within protein sequences does not appear to determine their dependence on the Oxa1 translocase. Our observations rather suggest that the dependence on Oxa1 correlates with the net charge of the domain that has to be translocated across the lipid bilayer.

The inner membrane of mitochondria contains a large number and variety of polypeptides. Some of these inner membrane proteins are encoded by the mitochondrial genome and have to be inserted into the lipid bilayer after synthesis in the matrix. The vast majority of inner membrane proteins, however, is synthesized in the cytosol and inserted after import into the mitochondria. The inner membrane protein Oxa1 was shown to play an important role in the insertion process of both mitochondrially and nuclear encoded proteins into the inner membrane (1–4).

Oxa1 belongs to a large protein family with members in mitochondria, bacteria, and chloroplasts (5–7). In all these systems, Oxa1 homologs have been shown to be crucial components of membrane insertion reactions. Although the precise molecular role of this protein family is still not clear, Oxa1 proteins are believed to serve as translocation factors that facilitate the transport of hydrophilic protein domains from one side of the membrane to the other.

In yeast Saccharomyces cerevisiae, seven inner membrane proteins are encoded by the mitochondrial genome. At least four of these proteins transiently interact with Oxa1 during membrane integration. These proteins are subunits 1, 2, and 3 of the cytochrome bc1 complex (Cox1, 2, and 3) and cytochrome b of the bc1 complex (3). Whereas Oxa1 function was shown to be absolutely essential for membrane integration of Cox2, other polypeptides such as Cox1, Cox3, cytochrome b, and subunit 6 of the F0F1-ATPase (Atp6) insert into the inner membrane in the absence of Oxa1, although at reduced levels (3). The ability to bypass Oxa1-mediated insertion requires the presence of the inner membrane protein Mba1, a component in a second, Oxa1-independent insertion pathway (8, 9).

The reason for the strict Oxa1 dependence of Cox2 is unclear. Cox2 spans the inner membrane twice and exposes its hydrophilic N and C termini into the intermembrane space. In contrast, the intermembrane space domains of the other mitochondrially encoded substrates of Oxa1 form hairpin loops, i.e. stretches flanked on both sides by transmembrane domains: Cox1, cytochrome b, and the precursor form of Atp6 expose four, six, and three loop domains to the intermembrane space, respectively; Cox3 contains three intermembrane space loops and a C-terminal domain of few hydrophobic residues most likely completely buried in the membrane and therefore not exposed to the intermembrane space (10–12). Oxa1 might be generally dispensable for the insertion of loop domains but strictly required for the export of terminal sequences explaining why the export of both Cox2 tails is blocked completely when Oxa1 function is compromised (1). The so far characterized nuclear encoded Oxa1 substrates likewise contain N terminal domains that have to be exported across the inner membrane.

Cox2 is synthesized in the matrix as a precursor protein with an N-terminal extension. This presequence is proteolytically removed by the Imp1 protease in the intermembrane space after translocation across the inner membrane. Translocation of the N-terminal domain of Cox2 was found kinetically to precede export of the C terminus, and conditions that block N tail export also prevent translocation of the C terminus (13). The precise function of the presequence of Cox2 is not clear. This presequence tolerates considerable changes in its primary structure, challenging the hypothesis that this region of the protein contains important targeting information (14). The mRNA sequence that encodes the presequence, however, was shown to be essential for translation of Cox2, and deletion of the presequence-encoding region completely abolished Cox2 synthesis (14, 15). Employing a genetic suppressor screen, Fox 

5 The abbreviations used are: Cox, cytochrome oxidase; DHFR, dihydrofolate reductase; 5-FOA, 5-fluoroorotic acid.
and co-workers (14, 15) isolated a respiratory-competent yeast mutant lacking the presequence of Cox2. This mutant contains two mitochondrial genomes, which are depicted in Fig. 1, B and C: one genome in which the region encoding for the 13 residues of the presequence is missing (cox2–20 genome) and a second genome showing a large deletion (cob::cox2 genome). This large deletion fuses the COB gene for cytochrome b to that of Cox2. This results in a gene product consisting of the N-terminal 252 amino acid residues of cytochrome b and the entire mature part of Cox2. This mutant is able to grow on nonfermentable carbon sources, indicating that a functional Cox2 protein is produced. Here we report on the analysis of the insertion process of the CobCox2 fusion protein into the inner membrane of mitochondria, focusing especially on the relevance of Oxa1 function for its biogenesis. Our observations indicate that the dependence on Oxa1 is not determined primarily by the position of the exported domain within a protein but rather by its nature. We propose that Oxa1 dependence correlates with the charges that have to be translocated.

MATERIALS AND METHODS

Yeast Strains—Yeast strains used in this study were isonuclear to W303-1A (MAT a, leu2, trpl, ura3, ade2). The mitochondrial genomes were derived from that of a DT71B-10 strain (ATCC 25657). Generation of the temperature-sensitive oxa1ts strain was reported before (3). For generation of cob::cox2 strains (NYB275 and NYB276), the wild type mitochondrial genomes in W303-1A and oxa1ts cells were removed by growth on ethidium bromide, and both the cob::cox2 and cob::cox2–60 genomes (Fig. 1, C and D) were introduced by successive cytoduction (16).

For expression of Oxa1, the entire OXA1 gene was cloned into a pRS416 (URA3, CEN) expression vector (17). Yeast strains were grown in YP medium (1% yeast extract, 2% peptone) supplemented with either 2% glucose (YPD) or 2% ethanol and 2% glycerol (YPEG). For isolation of mitochondria, cells were grown in lactate medium at 30 or 24 °C (for oxa1ts mutant strains) and treated as described previously (18).

Radilabeling of Mitochondrial Translation Products—Mitochondrial translation products were radiolabeled in isolated mitochondria essentially as described before (19). Mitochondria (50 μg) were resuspended in 12 μl of labeling buffer (0.6 M sorbitol, 150 mM KCl, 15 mM potassium phosphate, 20 μM Tris-HCl, pH 7.4, 12.5 mM MgSO4, 3 mg/ml bovine serum albumin, 4 mM ATP, 0.5 mM GTP, 5 mM α-ketoglutarate, 5 mM phosphoenolpyruvate, and 12 μg/ml of all amino acids except methionine). To induce the temperature-sensitive phenotype of oxa1ts strands, mitochondria were incubated for 10 min at 37 °C or kept on ice for control. After an incubation for 2 min at 25 °C, 1 μl of [35S]methionine (1 μCi/μl) was added and the sample incubated further at 25 °C. After labeling, the reaction was diluted in 1 ml of ice-cold washing buffer (0.6 M sorbitol, 150 mM KCl, 20 mM HEPES pH 7.4). Mitochondria were reisolated by centrifugation at 16,000 × g for 4 °C for 10 min, washed again, and lysed in SDS sample buffer (20).

Immunoprecipitation—For immunoprecipitations (21) mitochondria were lysed in 5 μl of 1% SDS, 20 mM HEPES pH 7.4, vortexed for 5 min, boiled for 30 s, and diluted immediately with 500 μl of ice-cold IP buffer (1% Triton X-100, 300 mM NaCl, 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, 20 mM Tris-HCl, pH 7.4). The extract was cleared by centrifugation at 16,000 × g for 4 °C for 10 min and incubated with antibodies prebound to protein A-Sepharose beads (Amersham Biosciences). After incubation for 2 h at 4 °C the beads were recovered by centrifugation at 5 × 12,000 × g, washed three times with IP buffer, one wash with 150 mM Tris-HCl, pH 7.4, and resuspended in SDS sample buffer. The antiserum was used to raise rabbits against full-length Cox2 purified from yeast or against a C-terminal peptide of Cox2 (13).

Miscellaneous—The following procedures were described before: in vitro transcription/translation of precursor proteins and import of precursor proteins into isolated mitochondria (22); enzymatic measurements of Cox activity (23) and mitochondrial subfractionation (24). Radiographic signals were quantified by densitometry using a Pharmacia ImageScanner with Image Master 1D Elite software package (Amersham Biosciences).

RESULTS

The CobCox2 Fusion Protein Is Synthesized Efficiently and Gives Rise to a Functional Cox2 Protein—Deletion of the presequence-encoding sequence of COX2 (Fig. 1B) abolishes Cox2 synthesis resulting in a respiratory deficient growth phenotype of the mutant. Torello et al. (15) isolated a suppressor of such a mutant harboring an additional truncated mitochondrial genome (Fig. 1C). On this genome, the COB and COX2 genes are fused resulting in a sequence that encodes a chimeric protein consisting of the N-terminal 252 amino acid residues of cytochrome b and residues 14–251 of Cox2 (see Fig. 1, E–G). Expression of this chimera, CobCox2, restored respiratory competence of the strain. To study the biogenesis of this fusion protein, we constructed a yeast strain (NYB275) containing two mitochondrial genomes: the cob::cox2 genome (Fig. 1B), and a genome in which the promoter and start region of the COX2 gene were deleted (cox2–60, Fig. 1D) so that Cox2 is not produced (25).

To assess the synthesis of the CobCox2 fusion protein, mitochondrial translation products were radiolabeled in isolated mitochondria by incubation with [35S]methionine, resolved by SDS-PAGE, and visualized by autoradiography (Fig. 2A). Only low amounts of mature Cox2 were found in cob::cox2 mutant mitochondria. Instead the mutant efficiently expressed a polypeptide corresponding to the amino-terminal 252 residues of cytochrome b and the entire mature part of Cox2. This stable polypeptide was also present in a precursor form together with a small amount of a mature form of the fusion protein. This indicates that mitochondrial import of precursor Cox2 is not affected in the mutant. This is in agreement with the finding that the mutant is not growth deficient on nonfermentable carbon sources, indicating that a functional Cox2 protein is produced. Here we report on the analysis of the insertion process of the CobCox2 fusion protein into the inner membrane of mitochondria, focusing especially on the relevance of Oxa1 function for its biogenesis. Our observations indicate that the dependence on Oxa1 is not determined primarily by the position of the exported domain within a protein but rather by its nature. We propose that Oxa1 dependence correlates with the charges that have to be translocated.
tein of an apparent mass of 51 kDa which was absent in the wild type. This was slightly below the calculated size of the CobCox2 fusion (55 kDa), which is most likely because of the fast migration of cytochrome b during electrophoresis (apparent mass, 30 kDa; calculated mass, 43 kDa). To show that the 51-kDa band represents the CobCox2 fusion protein, mitochondria were lyzed after the labeling reaction, and CobCox2 derivatives were isolated by immunoprecipitation. The 51-kDa band could be isolated with CobCox2-specific antibodies verifying that this species represents the CobCox2 protein. The signal of the CobCox2 protein was much stronger than that of the mature Cox2 form. This is in part because of its significantly higher content in methionine residues in the fusion protein (21 versus 6). Despite the inefficient processing of the CobCox2 fusion, Western blotting revealed almost wild type levels of endogenous mature Cox2 in the cob::cox2 mutant (Fig. 2B), explaining its respiratory-competent growth phenotype. Interestingly, no steady-state levels of CobCox2 protein could be detected by Western blotting, suggesting that this form is either completely converted to mature Cox2 over time or degraded. Expression of Cox2 from the cob::cox2 gene resulted in a fully functional Cox2 protein, and the levels of cytochrome oxidase activity reached about 65% of that in wild type mitochondria (Fig. 2C).

Oxa1 Is Required for Respiration in a cob::cox2 Background—Oxa1 is essential for the export of the N terminus of Cox2 across the inner membrane (1). In contrast, insertion of the loop-forming proteins Cox1, Cox3, and cytochrome b bypasses the need for Oxa1 to some extent (3). In the CobCox2 fusion protein, the N tail of Cox2 represents a hydrophilic sequence that is flanked on both sides by hydrophobic trans-

membrane regions. Thus, in principle this Cox2 domain now mimics a hairpin loop structure, and we asked whether Cox2 biogenesis in the cob::cox2 background was still dependent on Oxa1 function. To test this, the cob::cox2 and cox2–60 genomes were transferred into a mutant in which the nuclear copy of OXA1 was deleted and which carried a URA3 plasmid expressing Oxa1. If Oxa1 function was not required for insertion of the CobCox2 fusion protein, the plasmid would be dispensable, and counterselection against the URA3 marker on glycerol medium containing 5-fluoroorotic acid (5-FOA) should result in a respiratory competent strain. As shown in Fig. 3, the OXA1-containing plasmid was essential for growth of the Δoxa1 cob::cox2 strain on nonfermentable carbon sources, because counterselection against the plasmid on 5-FOA did not give rise to any colonies on glycerol (fifth through ninth rows). In contrast, the plasmid was dispensable in a wild type control, resulting in viable colonies on 5-FOA (second row). Thus, the cob::cox2 strain still required Oxa1 function for respiratory competence. This indicates that either the biogenesis of CobCox2 is still Oxa1-dependent or that the function of at least one other mitochondrial protein strictly depends on Oxa1 function.

CobCox2 Processing Depends on Oxa1 Function—To assess the Oxa1 dependence of the biogenesis of CobCox2 in more detail, we transferred the cob::cox2 and cox2–60 genomes into a strain harboring a temperature-sensitive oxa1 allele (26). This strain (NYB276) was unable to grow on nonfermentable carbon sources under restrictive conditions, again indicating that the cob::cox2 mutation does not bypass the requirement for Oxa1 function. The oxa1 mutation allows the irreversible inhibition of Oxa1 in isolated mitochondria (1). Upon exposure to 37°C for 10 min the Oxa1-dependent insertion of wild type Cox2 is largely blocked in this mutant, and Cox2 accumulates in its precursor form (Fig. 4A). Whereas in oxa1 deletion mitochondria Cox2 processing is completely abolished (4), some maturation still occurs in the oxa1 mitochondrial, most likely because of the residual Oxa1 activity of this mutant.

Is Oxa1 function also required for the conversion of CobCox2 into the mature Cox2 form? If the expression of the N-terminal Cox2 domain as a loop-forming structure would bypass the dependence on Oxa1 function, CobCox2 processing should not be affected in the oxa1 mutant background. To test this, wild type, OXA1 cob::cox2 or oxa1 cob::cox2 mitochondria were converted to mitoplasts by hypotonic swelling of the outer membrane and incubated for 10 min at 37°C. Then mitochondrial translation products were radiolabeled. The samples were split; one half was mock treated and the other incubated with proteinase K to digest protein sequences that are exposed to the intermembrane space. The samples were lysed, and the Cox2 species produced were isolated by immunoprecipitation (Fig. 4B). Swelling efficiency and protease protection were controlled by Western blotting with antibodies against the matrix protein Tim44 and the intermembrane space domain of the inner membrane protein Dld1 (Fig. 4B, lower panel). In wild type mitochondria (left panel), Cox2 was almost entirely present in the mature form. Both the N- and the C-terminal domains of this Cox2 species were accessible to proteinase K and, thus, had been efficiently exported into the intermembrane space. In the OXA1 cob::cox2 mitochondria, both the CobCox2 fusion protein and the mature Cox2 protein were found. Quantification of the signals and correction for the very different methionine contents of both forms revealed that 50% of the total Cox2 was present in the matured form. Protease treatment led to a complete degradation of both Cox2 species, indicating that in the presence of functional Oxa1 the fusion protein was efficiently and correctly integrated into the lipid bilayer of the inner membrane. In contrast, when Oxa1 func-

FIG. 2. The CobCox2 fusion protein is expressed and converted into a functional Cox2 protein. A, labeling of CobCox2 in isolated mitochondria. Mitochondrial translation products were radio-labeled in isolated wild type (wt) or cob::cox2 mitochondria (70 μg) for 30 min at 25°C. Mitochondria were lysed and subjected to SDS-PAGE, either directly (total, T) or after immunoprecipitation with a Cox2-specific antiserum (αCox2). For total lanes, 10% of the reactions were loaded. Positions of molecular mass standards are indicated. B, cob::cox2 mitochondria contain wild type levels of Cox2. Mitochondrial proteins (100 μg) of wild type or cob::cox2 cells were subjected to Western blotting employing antisera against aconitase (Aco1), D lactate dehydrogenase (Dld1), Cox2, and Mge1. C, cob::cox2 mitochondria contain active cytochrome oxidase. The levels of cytochrome oxidase were measured in mitochondria isolated from wild type, cob::cox2, or Δoxa1 cells. The Δoxa1 mutant served as control for a strain devoid of cytochrome oxidase activity.
Mitochondrial Protein Export

The Nature of Transferred Domains Influences Their Dependence on Oxa1—If not the final topology of a substrate protein, what determines the degree of Oxa1 dependence of an export reaction? It was shown before that the charge of transferred domains plays a crucial role in the export process (27); the export reaction becomes more efficient with increasing negative net charge of transferred protein domains (28). Both the N and the C termini of Cox2 are of highly acidic nature and contain a large number of negative charges. Therefore we asked whether charges in a transferred domain influence the dependence of the export reaction on Oxa1. For this we employed mutants of the nuclear encoded Oxa1 substrate subunit 9 (2) because the manipulation of mitochondrial DNA is difficult and, especially in the case of Cox2, also affects protein synthesis (14). The precursor protein pSu9-(1–112)-DHFR and mutants thereof (28) were synthesized in reticulocyte lysate in the presence of [35S]methionine (Fig. 5A). The preproteins were imported into wild type or oxa1ts mitochondria after a preincubation for 10 min at 37 °C. During this reaction, the precursor proteins were imported into mitochondria. After their maturation in the matrix, the proteins were allowed to insert into the inner membrane. Upon membrane insertion, the N termini of these proteins were exposed to the intermembrane space and could be degraded by the addition of protease under hypotonic swelling conditions (Fig. 5B). The signals were quantified, and the ratio of inserted to total imported protein was calculated (Fig. 5C). In the wild type Su9-(1–112)-DHFR protein, the exported domains contain two negative and one positive charge. About 60% of this protein was integrated into the membrane in wild type mitochondria which was about twice as in oxa1ts mitochondria. It was reported before that replacement of one negative charge by a positive charge (E77K mutant) results in significant lower insertion rates of the protein (28). Interestingly, this mutant form, however, completely bypassed the need for Oxa1 function, suggesting that the export of a positive charged protein domain can occur independently of Oxa1. On the contrary, when the negative net charge of the exported domain was changed to –2, the total export efficiency was increased compared with the wild type. However, this form now was even more dependent on Oxa1 function than the wild type protein. This is not because of a lower membrane potential of the oxa1ts mitochondria because the membrane potential in this mutant equals that of wild type mitochondria (1). From this we conclude that an increased negative net charge of the intermembrane space domain of Su9-(1–112)-DHFR not only causes higher insertion rates but also an increased dependence of the export reaction on Oxa1 function.

DISCUSSION

The inner membrane of mitochondria belongs to the protein-richest membranes of eukaryotic cells. Many inner membrane proteins are inserted into the inner membrane from the matrix so that intermembrane space domains have to be translocated across the membrane in an export reaction. The export of N-terminal intermembrane space domains of some of these proteins was shown to require Oxa1 function. From this it was concluded that Oxa1 represents an essential component of the N tail protein export machinery in mitochondria (2). Although internal intermembrane space domains of inner membrane proteins interact with Oxa1 during their insertion into the inner membrane, Oxa1 function appears to be not absolutely essential for their export; significant amounts of these domains are still exported in the absence of Oxa1 (3, 8). From this, it was speculated that the dependence of insertion processes on the Oxa1 translocase is determined by the location of the transferred domains within a protein sequence and that hairpin
loops might be able to bypass an Oxa1 requirement more easily.

The CobCox2 fusion protein offered the fortunate opportunity to study the insertion of a normally N-terminal intermembrane space domain as an internal hairpin loop that is flanked by hydrophobic transmembrane segments. However, we cannot predict whether the transmembrane segments that flank the

Cox2 N terminus in the CobCox2 protein are able to interact with each other to allow the simultaneous insertion of both stretches. But such a fused sequence of mitochondrial translation products is not completely artificial and even occurs in Nature: Cox2 is similarly synthesized as a fusion protein with Cox1 in mitochondria of Acanthamoeba castellanii and subsequently cleaved into two functional cytochrome oxidase subunits (29). As shown in this study, export of the Cox2 N-terminal domain in the CobCox2 fusion protein remained dependent on Oxa1 function, indicating that Oxa1 dependence is not simply determined by the location of the transferred domain within a protein sequence but rather by its specific properties. What might these special properties be? The N tail of Cox2 is a highly charged protein domain: this stretch of 26 residues contains five negative charges resulting in a highly acidic sequence with an isoelectric point of 3.4. Fig. 6 shows a comparison of the lengths and net charges of the intermembrane space domains of all mitochondrial encoded inner membrane proteins of S. cerevisiae. The N- and C-terminal domains of Cox2 show the most negative charge (−5 and −14, respectively). Proteins of lower Oxa1 dependence (Cox1, Cox3, cytochrome b, and Atp6) contain intermembrane space domains with a net charge of −2 or −3. Intermembrane space domains of proteins that efficiently insert in the absence of Oxa1 are either uncharged (Atp8) or even positive (Atp9, +1). Consis-
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**Fig. 6.** Dependence on Oxa1 of mitochondrial encoded membrane proteins correlates with the number of charges that have to be translocated across the inner membrane. Lengths and net charges of intermembrane space domains of the proteins encoded by the genome of *S. cerevisiae* and of the Cob-Cox2 joining loop were calculated based on the topologies published (10–12). Strictly Oxa1-dependent proteins are depicted in black, partially dependent proteins in gray, and proteins for which Oxa1 function might be dispensable are shown by white symbols (3).

...tently, intermembrane space domains of nuclear encoded Oxa1 substrates are also negatively charged: the N termini of Oxa1 and subunit 9 of *Neurospora crassa* have a net charge of −8 and −1, respectively. Insertion of both proteins is significantly more efficient in the presence of Oxa1 than in its absence (2). However, when the net charge of the N terminus of subunit 9 was changed to +1, Oxa1 function became dispensable. From this we propose that the content of charged residues in an inner membrane protein determines its dependence on the Oxa1 translocase.

What does Oxa1 dependence mean? Two insertion pathways into the inner membrane of mitochondria were identified: one depends on Oxa1, the second on the inner membrane protein Mba1 (8). Several inner membrane proteins can both interact with Oxa1 or with Mba1 during their membrane integration. Insertion of proteins that comprise domains of lower charge content such as cytochrome b and Cox3 was more affected in mba1 mutants than export of the highly charged Cox2 tails (8). Thus, the Mba1 pathway may be predominantly used by proteins that export less charged domains, whereas Oxa1 is primarily important for proteins with highly charged domains. The overlapping substrate range of both pathways allows the membrane insertion of proteins in the absence of either Mba1 or Oxa1. However, highly charged domains are apparently not efficiently translocated by the Mba1 pathway, explaining the correlation with their Oxa1 dependence.

Like the inner membrane of mitochondria, the inner membrane of bacteria contains at least two insertion pathways. One requires function of the Sec translocase, the other is Sec-independent (for review, see Ref. 30). Similar to our observations in mitochondria, the charge content of a transferred domain influences its sorting pathway (31, 32) and its dependence on the membrane potential (33, 34). The bacterial Oxa1 homolog YidC was shown to play a role in the export of both uncharged and charged domains; however, it was reported that a substrate with five negative charges (Procoat-Lep) depended on YidC more extensively than substrates with lower charge content (35). Thus, like its mitochondrial homolog, YidC might be especially important for the translocation of highly charged protein domains.

The defects of *oax1* deletion strains can be suppressed by the expression of inner membrane proteins in which positive residues were introduced into transmembrane domains (36). These charges were suggested to interact with negative charges in domains that have to be translocated and thereby allow their export in an Oxa1-independent manner. Such a mechanism would be unable to facilitate the export of uncharged domains, and, consistently, Mba1 was found to be essential for respiration in these suppressors (36). These observations are consistent with the proposed role of Oxa1 as a translocator for negatively charged protein sequences. The precise function of Oxa1 in the export reaction is still unclear, and future studies will have to focus on the molecular mechanism by which this conserved membrane component facilitates the insertion of proteins into lipid bilayers of mitochondria, bacteria, and chloroplasts.

**Acknowledgments**—We are very grateful to Sandra Esser for excellent technical assistance. We thank Benedikt Westermann for critical reading of the manuscript and Tom D. Fox for generously providing the *cob::cox2* mutant.

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