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Membrane Biogenesis of Subunit II of Cytochrome *bo* Oxidase: Contrasting Requirements for Insertion of N-terminal and C-terminal Domains

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The membrane assembly of the respiratory complexes requires the membrane insertases Oxa1 in mitochondria and YidC in bacteria. Oxa1 is responsible for the insertion of the mitochondrial cytochrome *c* oxidase subunit II (CoxII). Here, we investigated whether YidC, the bacterial Oxa1 homolog, plays a crucial role in the assembly of the bacterial subunit II (CyoA) of cytochrome *bo* oxidase. CyoA spans the membrane twice and is made with a cleavable signal peptide. We find that translocation of the short N-terminal domain of CyoA is YidC-dependent. In contrast, both the SecA/SecYEG complex and YidC are required for translocation of the large C-terminal domain. By studying the N-terminal domain of CyoA alone, we find that translocation is unaffected when SecE is depleted, suggesting that the YidC insertase on its own catalyzes membrane insertion of the N-terminal region of CyoA. Strikingly, we find that the translocation of the N-terminal domain is a prerequisite for translocation of the C-terminal domain in the full-length CyoA protein because translocation of the large C-terminal domain alone in a truncated CyoA derivative was observed in the absence of YidC. This work shows that the distinct domains of CyoA have different translocation requirements (YidC only and Sec/YidC) and confirms that the membrane biogenesis of subunit II of cytochrome oxidase in bacteria and mitochondria have conserved features.

Keywords: YidC; Oxa1; membrane protein insertion; CyoA; membrane insertase

Introduction

The Oxa1/YidC/Alb3 family of proteins plays a key role in the membrane protein biogenesis of respiratory and energy transduction complexes.[1–3] This pathway was first discovered in mitochondria, where it was shown that mutations in the *oxa1* gene led to a defect in the assembly of cytochrome *c* oxidase[4,5] and the oligomycin-sensitive ATP synthase.[6] Oxa1 is believed to play an important role in the protein transport of the nuclear encoded ATPase-su9 and the mitochondrially encoded subunit II of the cytochrome *c* oxidase (CoxII). In *Neurospora crassa*, Oxa1 has been reported to be required for the insertion of an artificial ATPase Su9 chimeric protein (Su9 is homologous to subunit c of the bacterial F_1F_o ATP synthase) from the mitochondrial matrix side after the protein is imported from the cytoplasm.[7] For the mitochondrially encoded CoxII protein, the function of Oxa1 is required for generating the N-out and C-out topology where the two ends of the protein face the mitochondrial intramembrane space.[8,9] In addition, the mitochondrial Cox18/Oxa2, a second member of the Oxa1/YidC family, is required for translocation of the large C-terminal region of CoxII from the matrix.[10,11]

YidC, the bacterial Oxa1 homolog, was shown to function in membrane protein insertion in *Escherichia coli*.[12,13] YidC is an essential inner membrane protein for the cell[12] and plays a direct role in the insertion of proteins into the inner membrane.[12,14,15] It is absolutely essential for the membrane insertion of the Sec-independent M13 procoat[12] and Pf3 coat protein.[14]

Abbreviations used: CoxII, cytochrome *c* oxidase subunit II; pmf, proton motive force; SRP, signal recognition particle; SP, signal peptidase; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

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Why is YidC essential for bacteria to grow? One reason could be that several energy transducing respiratory complexes that are necessary for the cell depend on YidC for membrane protein assembly.[16] Indeed, the membrane levels of the subunit II (CyoA) of cytochrome *bo* oxidase[16] and subunit a[17] and c[16,17] of the F_1F_o ATP synthase are markedly reduced when YidC is depleted from cellular membranes. Concomitantly, YidC depletion results in reduction in the proton motive force (pmf) and ATP levels in *E. coli*.[16] It was recently shown that the membrane insertion of subunit c occurs by the YidC pathway and does not require the Sec components.[18–20]

Here, we investigate the membrane biogenesis of subunit II (CyoA) of the cytochrome *bo* oxidase in *E. coli*. Like subunit II of the mitochondrial cytochrome *c* oxidase, the bacterial CyoA spans the membrane twice and has a cleavable signal peptide. Here we show that insertion of the N-terminal domain does not require the Sec machinery while translocation of the large C-terminal domain of CyoA is Sec-dependent. YidC is required for translocation of both termini of full-length CyoA across the inner membrane similar to the Oxa1 requirement observed for translocation of subunit II of cytochrome *c* oxidase across the inner membrane in mitochondria.[8,9] The signal recognition particle (SRP) facilitates the targeting of CyoA to the bacterial inner membrane. Intriguingly, the YidC requirement for translocation of the large C-terminal domain is not observed in a truncated mutant of CyoA missing the N-terminal domain. This suggests that export of the N-terminal domain must occur before insertion of the C-terminal domain and that there is a strict N-to-C-terminal directionality in the membrane biogenesis of the full-length CyoA. Taken together, the present study demonstrates that CyoA has distinct requirements for membrane insertion of its N and C-terminal domains and that the requirement for the YidC/Oxa1 component in membrane insertion of subunit II of cytochrome oxidase is evolutionarily conserved.

Results

Since the subunit II of the mitochondrial cytochrome *c* oxidase requires Oxa1 for its insertion into the mitochondrial inner membrane from the matrix side,[8,9] we examined whether the membrane insertion of CyoA, subunit II of cytochrome *bo* oxidase, depends on YidC. This would demonstrate that the substrates for the Oxa1/YidC membrane insertases use an evolutionarily conserved pathway. The CyoA protein in *E. coli* is a lipoprotein that spans the membrane twice, where the N-terminal cysteine residue is modified with a phosphoglycerol.[21] It is synthesized in a precursor form with a signal peptide (residues −1 to −24) that is cleaved by lipoprotein signal peptidase (SP). The mature CyoA protein (residues 1 to 291) has a small 26 residue N-terminal segment and a large ~200 residue hydrophilic region exposed to the periplasm (Figure 1). We used the arabinose-dependent YidC depletion strain (JS7131) to examine the YidC dependence. This strain has the *yidC* gene under the control of the *araBAD* promoter with the chromosomal *yidC* gene disrupted.[12] To deplete YidC, the strain is grown in glucose medium for several generations. JS7131 cells expressing pre-CyoA were pulsed with [^{35}S]methionine for 30 s and chased with unlabeled methionine for the indicated times (Figure 2(a)) to examine the kinetics of processing, which reflect the translocation of the N-terminal domain. Membrane insertion and processing occurs rapidly and only the mature form of CyoA is

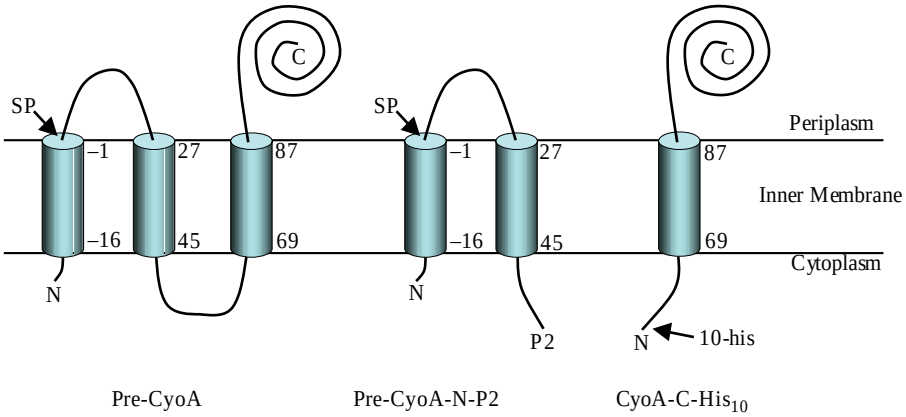

Figure 1. Membrane topology of wild-type pre-CyoA and its truncated derivatives. CyoA is synthesized in a precursor form (pre-CyoA) with a signal sequence (residues −1 to −24), which is cleaved by signal peptidase II after insertion into the membrane. The mature CyoA spans the membrane twice with a small N-terminal and large C-terminal periplasmic domain. In one construct (not shown in Figure), an "in frame" His_{10}-tag was introduced into the cytoplasmic domain after residue 50 of the full-length mature CyoA. The N-terminal derivative, CyoA-N-P2, has the Lep P2 domain fused to residue 50 in order to study the insertion of the N-terminal domain of CyoA separate from the C-terminal domain. To study the insertion of the C-terminal domain, separate from the N-terminal domain, the construct CyoA-C-His_{10} was made. This construct has residues before amino acid 42 of CyoA deleted and a His_{10}-tag introduced after residue 50.