Characterization of the evolutionarily conserved role of Escherichia coli YidC in membrane protein biogenesis

van Bloois, D.W.

2008

Chapter 8

Summary and discussion

Introduction

The targeting and assembly process of eukaryotic and bacterial membrane proteins are remarkably well conserved. In both eukaryotic and bacterial cells targeting of nascent membrane proteins to the membrane embedded Sec-translocon requires the signal recognition particle (SRP) and its cognate receptor (FtsY in bacteria and SRα in eukaryotes) (Luirink and Sinning, 2004). The Sec-translocon (Sec61 in eukaryotes and SecYEG in bacteria) inserts membrane proteins into the endoplasmic reticulum membrane or the bacterial inner membrane (Osborne et al., 2005). In *E. coli,* the Sec-translocon cooperates with YidC during membrane insertion of most inner membrane proteins (IMPs) analyzed thus far. In contrast, some IMPs are directly inserted by YidC independently of the translocon. YidC is a member of the YidC/Oxa1/Alb3 protein family that constitutes a class of conserved proteins involved in the biogenesis of organellar and bacterial membrane proteins (Kiefer and Kuhn, 2007). The experiments described in chapter 2-7 were aimed at understanding the evolutionary conserved role of YidC in the biogenesis of IMPs.

Central role of YidC in IMP biogenesis

YidC appears largely dispensable for insertion of Sec-dependent IMPs *per se* both *in vivo* and *in vitro* implying a later role of YidC in the biogenesis of IMPs (Fröderberg et al., 2003; Urbanus et al., 2001; van der Laan et al., 2004b). Consistently, YidC has recently been shown to function in folding of the polytopic IMP LacY (Nagamori et al., 2004). Moreover, *in vitro* cross-link studies suggest that YidC operates downstream of the Sec-translocon for several complex IMPs to assist the transfer and assembly of TMs into the lipid bilayer (Beck et al., 2001; Houben et al., 2004; Urbanus et al., 2001). TRAM, a protein with a YidC-like topology and located near the Sec61 translocon in the endoplasmic reticulum membrane, appears to have an analogous function. TRAM can be cross-linked to TMs of nascent membrane proteins similar to YidC (Heinrich et al., 2000). However, TRAM appears to interact specifically with less hydrophobic TMs. This specificity has not been observed for *E.coli* YidC, which may indicate that TRAM and YidC differ at least in their requirements for substrate recognition (Houben et al., 2004). YidC may act as an integral membrane chaperone for Sec-dependent IMPs. More specifically, YidC may function as a holdase by interacting transiently with TMs during insertion. This retention would allow sufficient time for folding and assembly of a newly synthesized IMP in the local environment of YidC near the translocon.

Adding to the complexity of IMP integration, YidC was shown to function upstream of the Sec-translocon for one studied IMP, CyoA (see below). In this case, YidC is required and sufficient for membrane assembly of the N-terminal part of the protein, whereas translocation of the large C-terminal periplasmic domain requires the Sec-translocon, including SecA (Celebi et al., 2006; du Plessis et al., 2006; chapter 3).

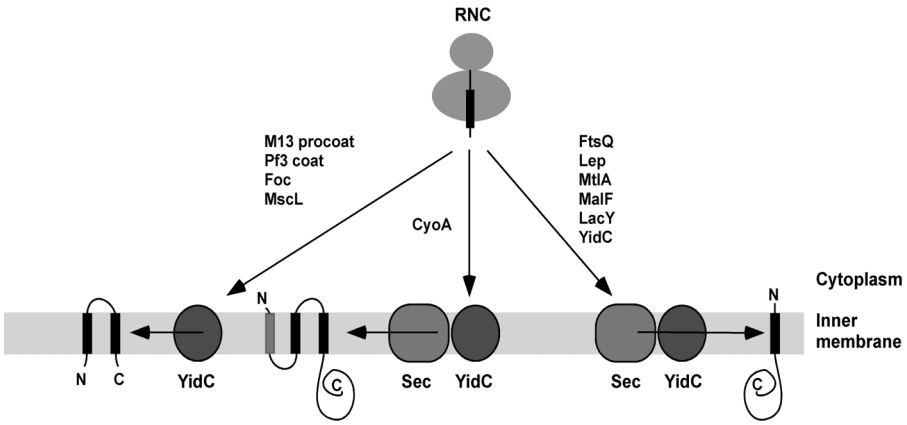

Fig.1. Model for the central role of YidC in the biogenesis of *E. coli* IMPs analyzed thus far. See text for details.The signal sequence of CyoA is represented by a solid gray bar; TMs are represented by solid black bars.

Interestingly, *in vivo* depletion of YidC almost completely blocks the insertion of a subset of Sec-independent IMPs that include MscL, the F_1F_o-ATPase subunit F_oc and small phage coat proteins (Samuelson et al., 2000; van der Laan et al., 2004a; Yi et al., 2004; chapter 2), suggesting that YidC also operates completely independent of the Sec-translocon, as a separate insertase and translocase. Recently, *in vitro* reconstitution experiments confirmed the potential of YidC to function Sec-independently (Serek et al., 2004; van der Laan et al., 2004a). In this case, YidC functions as a insertase independently of other components possibly by providing a platform from which the TM can equilibrate with the hydrophobic interior of the membrane.

In conclusion, YidC is a central component in the biogenesis of *E. coli* IMPs as indicated by its ability to function: (i) downstream of the Sec-translocon during the biogenesis of Sec-dependent IMPs, (ii) upstream of the translocon during the biogenesis of CyoA and possibly other IMPs and (iii) independently of the translocon during the membrane assembly of Sec-independent IMPs (Fig.1).

Conservation of membrane assembly pathways

Mitochondria of animals, plants and fungi harbor two distant homologues of YidC, Oxa1 and Cox18/Oxa2 (Funes et al., 2004). Both Oxa1 and Cox18/Oxa2 function in the assembly of respiratory chain complexes, but are apparently non-redundant since mutants in one component cannot be complemented by expression of the other (Funes et al., 2004). Cox18/Oxa2 fulfills a specific function, presumably in the translocation of the C-terminal domain of Cox2 (Fiumera et al., 2007; Funes et al., 2004; He and Fox, 1997; Saracco and Fox, 2002). Oxa1 was originally identified as a factor involved in the biogenesis of respiratory chain complexes. In the absence of Oxa1, the activities of the cytochrome

bc1 complex, the cytochrome *c* oxidase and the F_1F_o-ATPase complex are dramatically reduced (Altamura et al., 1996; Bonnefoy et al., 1994). Using mitochondria isolated from *oxa1* yeast mutants, Oxa1 was shown to be involved in the insertion of proteins (in particular subunits of respiratory complexes) into the inner membrane from the mitochondrial matrix (Hell et al., 1997; Hell et al., 1998; Hell et al., 2001; Herrmann et al., 1997). The presence of Oxa1 homologues in bacteria and chloroplasts suggested that similar pathways are operational in bacteria and chloroplasts. Oxa1 operates independently of Sec-like proteins, as mitochondria of higher eukaryotes lack homologues of the Sec-translocon (Glick and von Heijne, 1996) and therefore it appeared likely that the Sec-independent "YidC" only pathway of *E. coli* is similar to the Oxa1 pathway of mitochondria.

Substrates of the Oxa1 pathway include in particular mitochondrially encoded subunits of respiratory complexes. In *S. cerevisiae*, Oxa1 catalyzes the insertion of cytochrome *b* of the *bc1* complex, Cox1, Cox2 and Cox3 of the cytochrome *c* oxidase and Atp6, Atp8 and Atp9 of the F_1F_o-ATPase complex. Consequently, in the absence of Oxa1 insertion of most of these proteins is affected although not dramatically (Hell et al., 2001) with the exception of Cox2p, which displays the strictest dependency on Oxa1 for membrane insertion (He and Fox, 1997; Hell et al., 1997). Interestingly, Atp9 and Cox2p are homologous to *E. coli* F_oc and CyoA. F_oc and CyoA are membrane embedded subunits of the F_1F_o-ATPase and cytochrome *o* oxidase complex, respectively. F_oc is a small double spanning IMP with short translocated termini. CyoA is initially is synthesized with a lipoprotein type signal sequence and mature CyoA spans the membrane twice with two translocated termini; a lipid-modified N-terminus and a large C-terminus. Strikingly, the protein levels of F_oc and CyoA are atypically sensitive to depletion of YidC, suggesting that these endogenous IMPs may be substrates of the YidC only pathway (van der Laan et al., 2003).

The experiments described in chapter 2 and 3 concern the membrane biogenesis pathways of F_oc and CyoA. It was shown that depletion of Ffh severely impairs membrane insertion of F_oc and CyoA *in vivo*. Furthermore, the SRP is specifically recruited by the first TM in nascent, ribosome bound F_oc and CyoA as shown by *in vitro* photo cross-linking. This suggests that the SRP is required for efficient membrane targeting of both F_oc and CyoA. Depletion of SecE did not affect insertion of F_oc. In contrast, depletion of YidC strongly inhibited insertion of F_oc. For CyoA it was shown that in the absence of translocon components translocation of the N-terminal periplasmic loop still occurs, whereas translocation of the large C-terminus is blocked. In the absence of YidC, both termini in full-length CyoA are not translocated, resulting in the accumulation of precursor CyoA. Using a CyoA derivative containing the N-terminal periplasmic loop, it was shown that YidC is critical for translocation of the N-terminal domain as indicated by the accumulation of its precursor form in the absence of YidC *in vivo*. Together, the *in vivo*

depletion studies suggest that F_oc utilizes a YidC-dependent but Sec-independent mechanism for membrane insertion. Consistently, *in vitro* photo cross-linking confirmed that the first TM of nascent F_oc is primarily in contact with YidC during membrane insertion. For CyoA the data suggest that membrane biogenesis of full-length CyoA requires both YidC and the Sec-translocon including SecA. However, translocation of the N-terminus is a prerequiste for translocation of the C-terminus explaining the crucial role of YidC in the apparently vectorial assembly of CyoA. Involvement of YidC and the Sec-translocon was recently supported by two independent studies (Celebi et al., 2006; du Plessis et al., 2006).

The data presented in chapter 2 indicate that F_oc is the first endogenous substrate of a novel membrane biogenesis pathway in which the SRP delivers the nascent IMP at YidC that catalyzes membrane insertion. A prominent role for YidC in the membrane biogenesis of F_oc is in agreement with two other studies showing that YidC is required and sufficient for insertion of F_oc *in vitro* and *in vivo* (van der Laan et al., 2004a; Yi et al., 2004). In contrast, these studies point to an SRP-independent targeting mechanism for F_oc (van der Laan et al., 2004a; Yi et al., 2004). Van der Laan et al. (2004a) used an *in vitro* translation system depleted for Ffh and FtsY to show that F_oc is still inserted into proteoliposomes reconstituted with YidC, whereas the SRP-dependent IMP FtsQ was not inserted under these conditions. Notably, immunoblot analysis of the translation lysate showed that Ffh was not fully depleted. Possibly, the very hydrophobic F_oc has a higher affinity for the SRP and is therefore able to use the limited amount of SRP present in the translation lysate more efficiently than FtsQ. Dalbey and co-workers examined the membrane biogenesis of all F_o sector subunits by an *in vivo* approach (Yi et al., 2004). Of particular interest, insertion of F_oc was not affected by depletion of Ffh, suggesting that targeting of F_oc does not require the SRP. This result is probably explained by the short depletion times used, resulting in only a minor decrease in Ffh levels that are still sufficient to facilitate the targeting of very hydrophobic IMPs such as F_oc. This is consistent with a genetic screen which identified particularly hydrophobic IMPs as subtrates of the SRP (Ulbrandt et al., 1997).

The data presented in chapter 2 were obtained by two different experimental approaches. First, Ffh is cross-linked to nascent chains of F_oc produced *in vitro* in the absence of membranes. It should be noted that cross-linking of SRP to a nascent protein appears diagnostic for SRP-mediated targeting *in vivo* as indicated by the finding that a single point (hydrophobic) substitution in the PhoE signal sequence induces SRP cross-linking *in vitro* and shunts the protein into the SRP-pathway *in vivo* (Adams et al., 2002). Moreover, the signal sequence of M13 procoat does not cross-link SRP *in vitro* unless the hydrophobicity of the core region is increased. This alteration also results in SRP mediated targeting *in vivo* (de Gier et al., 1998). Second, depletion of the SRP in three different SRP conditional strains showed a strong and reproducible effect on F_oc insertion. Combined,

the *in vitro* and *in vivo* data suggest that the SRP is required for membrane targeting of F_oc. Targeting by the SRP also explains the strictly co-translational nature of membrane insertion of F_oc as observed by van der Laan et al.(2004a).

In conclusion, the data presented in chapter 2 and 3 suggest that F_oc and the N-terminal region of CyoA are assembled into the membrane by the YidC only pathway. The finding that YidC is able to catalyze insertion of IMPs (domains) in the absence of translocon components strongly suggests that this pathway is similar to the mitochondrial Oxa1 pathway and confirms that the role of YidC and Oxa1 in the membrane assembly of respiratory complexes is evolutionarily conserved.

Evolutionarily conserved function of YidC

Predictions reveal significant similarities in secondary structure and topology of the YidC/Oxa1/Alb3 family members. However, a functional conservation is not immediately obvious given the poor primary sequence conservation of 15-20%. Despite this poor sequence conservation, *A. thaliana* Alb3 can functionally substitute for *E. coli* YidC in both the Sec-dependent and Sec-independent function, showing that these proteins are indeed true homologues (Jiang et al., 2002). Based on the data presented in chapter 2 and 3, it appears that YidC and Oxa1 operate in similar membrane biogenesis pathways that are predominantly used for the insertion of subunits of respiratory complexes (van der Laan et al., 2005).

The genetic complementation experiments described in chapter 4 and 5 confirm a functional correlation between YidC and its mitochondrial yeast homologues Oxa1 and Cox18. It is was shown that mature Oxa1 and mature Cox18 (fused to the N-terminal targeting domain of YidC) complement the growth defect of a YidC depletion strain when expressed in *E. coli*. Moreover, both proteins are able to suppress the PspA response upon depletion of YidC. This indicates that both Oxa1 and Cox18 support proper membrane assembly of *E. coli* respiratory chain complexes and therefore sustain the pmf. Furthermore, in the absence of YidC, Oxa1 and Cox18 facilitate the insertion of YidC-dependent/Sec-independent IMPs indicating their capacity to function as a Sec-independent insertase. Remarkably, Cox18 and Oxa1 are not in the vicinity of nascent Sec-dependent IMPs during membrane insertion as suggested by photo cross-linking experiments. Additionally, Oxa1 appeared unable to efficiently substitute for YidC in the proper folding of the Sec-dependent IMP LacY. Apparently, Oxa1 and Cox18 fail to complement the Sec-dependent function of YidC possibly because these proteins are unable to cooperate efficiently with the bacterial Sec-translocon which is notably absent in mitochondria (Glick and von Heijne, 1996). Possibly, the prokaryotic trait of Oxa1 and Cox18 to cooperate efficiently with the Sec-translocon may have been lost during the evolution of mitochondria from endosymbiotic bacteria. These data also suggest that the Sec-independent function is conserved and essential for viability probably because it directly affects the

assembly of important respiratory complexes such as the F_1F_o-ATPase and cytochrome *o* oxidase complex.

Domain swapping and complementation experiments with engineered variants of *E. coli* YidC in yeast mitochondria have shown that YidC can partially complement the function of Oxa1 if the ribosome binding, C-terminal domain of Oxa1 is appended onto YidC. However, the presence of this domain interferes with the ability to complement Cox18 (Preuss et al., 2005). This distinction is not observed in *E. coli* since both Oxa1 and Cox18 can functionally replace YidC in the YidC only pathway suggesting that both proteins share a conserved insertase-like core activity.

In conclusion, YidC, Oxa1 and Cox18 are largely exchangeable in bacteria and mitochondria, showing that these proteins share a similar function but are adapted to their specific context.

Flexible use of targeting and insertion factors

The experiments described in chapter 6 concern the identification of specific features that determine the route of targeting, insertion and translocation *in vivo* by using a collection of engineered model IMPs based on the well studied endogenous IMPs F_oc and Lep. Both F_oc and Lep span the membrane twice and have an N_{out} /C_{out} topology. Unlike F_oc, Lep contains a large translocated C-terminal domain (P2). F_oc is used as a representative of the SRP/YidC pathway (see chapter 2) and Lep is used as a representative of the SRP/Sec(A)YEG/YidC pathway (de Gier and Luirink, 2001; Facey and Kuhn, 2004).

It was shown that F_oc is effectively rerouted into the Sec-pathway by appending the P2 domain of Lep onto its C-terminus. This suggests that YidC is limited in its translocation capabilities, confirming a previous study in which mutant procoat and mutant Pf3 coat proteins with altered periplasmic regions were shown to strictly require the Sec-machinery in addition to YidC for efficient membrane assembly (Chen et al., 2005). Additionally, size *per se* (the number of TMs) does not seem an important constraint for YidC requirement because membrane insertion of an F_oc tandem fusion construct was still Sec-independent/YidC-dependent. This might imply that YidC is sufficient for insertion of more complex endogenous *E. coli* IMPs provided they have small translocated loops. Hydrophobicity of a TM may be more important for determining the insertion requirements. This is suggested by the finding that a Pf3 mutant with a relatively hydrophobic TM inserts readily into liposomes *in vitro* and does not require YidC *in vivo* in contrast to wild type Pf3 (Serek et al., 2004). These data show that if sufficiently hydrophobic, a TM partitions into the membrane without assistance of insertion factors pointing to a spontaneous insertion mechanism. Interestingly, a spontaneous insertion mechanism has been suggested for the endogenous *E. coli* IMP KdpD (Facey and Kuhn, 2003). These findings are reminiscent of the lack of YidC and Sec requirement of the Lep derivative (described in chapter 6) which does not contain the second TM, suggesting a similar insertion mechanism

as for the hydrophobic Pf3 coat mutant and KdpD. Alternatively, these apparent independencies may reflect a facultative use of either the Sec-machinery or YidC. Consistently, Lep H1 is cross-linked to both SecY and YidC in nascent Lep at a very early stage during membrane insertion when Lep is only 50 amino acids in length suggesting affinity for both factors (Houben et al., 2005). Furthermore, the N-terminus of Lep is translocated independent of the Sec-translocon and appears only slightly affected by YidC depletion *in vivo* (Lee et al., 1992; Samuelson et al., 2000). Finally, reconstitution experiments have shown that nascent Lep that exposes only H1 can insert into proteoliposomes that contain either YidC or SecYEG (Houben et al., 2002).

Role of YidC in quality control

Upon membrane insertion, the newly synthesized IMP is folded and often assembles with other subunits into an oligomeric complex. YidC has been implicated in the later stages of IMP biogenesis as it seems required for folding rather than insertion of LacY (Nagamori et al., 2004). It is not known whether YidC cooperates with other factors during the folding of IMPs. YidC might cooperate with components of the quality control system of the cell that remove abnormally folded or unassembled IMPs. In this respect it is interesting to note that depletion of YidC affects the stability of LacY and induces extracytoplasmic stress responses probably because depletion of YidC results in the accumulation of abnormally folded IMPs (Nagamori et al., 2004; Shimohata et al., 2007). Together, these studies point to a functional correlation between YidC and the quality control system for IMPs.

The data presented in chapter 7 suggest that YidC is physically and functionally connected with the FtsH complex, including its modulating factor HflKC. These proteins form a large complex embedded in the IM, in which the membrane protease FtsH fulfills a central role by degrading unassembled membrane proteins and is thought to possess chaperone-like properties (reviewed in Ito and Akiyama, 2005). Additionally, FtsH is required for the degradation of certain regulatory cytoplasmic proteins. The co-purification of FtsH and HflKC with YidC suggests that YidC participates in this quality control complex. Interestingly, in mitochondria homologues of FtsH and YidC have been implicated in a similar partnership (Rep et al., 1996). In this case, Oxa1 appears to function as a chaperone that protects newly synthesized proteins from degradation until they are properly folded (Lemaire et al., 2000). In *E. coli*, YidC might perform a similar function in the context of the translocon thereby selecting certain misfolded nascent IMPs after lateral exit from the translocon and presenting them to FtsH for degradation. Clearly, much more work is needed for a full understanding of the functional and structural relationship between YidC and FtsH.

Concluding remarks and perspectives

We have at best a rough idea about the diverse functions of YidC and the substrates that make use of this versatile protein. Clearly, high resolution structural information is required to determine how YidC fulfills its complex tasks. A single structure would be most welcome but not sufficient. YidC's tendency to dimerize and its complex interactions with the Sec-translocon, the FtsH complex and substrates suggests that YidC adopts different conformations in the cell. It will be quite a challenge to solve the structures of YidC and its binding partners caught in action. Also, more information is needed about the substrate specificity of YidC. Which IMPs make use of a specific function of YidC? Until now, only a few model IMPs have been analyzed with respect to their requirements for insertion and assembly. A more global, proteomics approach is required to define more general rules for substrate selection and handling. Finally, although the concept of "modules" for targeting, insertion, folding, assembly and degradation is attractive, more information is needed to confirm this notion to establish how the modules are connected at the molecular level.

References

Adams, H., Scotti, P.A., De Cock, H., Luirink, J., and Tommassen, J. (2002). The presence of a helix breaker in the hydrophobic core of signal sequences of secretory proteins prevents recognition by the signal-recognition particle in Escherichia coli. Eur J Biochem *269*, 5564-5571.

Altamura, N., Capitanio, N., Bonnefoy, N., Papa, S., and Dujardin, G. (1996). The Saccharomyces cerevisiae OXA1 gene is required for the correct assembly of cytochrome c oxidase and oligomycin-sensitive ATP synthase. FEBS Lett *382*, 111-115.

Beck, K., Eisner, G., Trescher, D., Dalbey, R.E., Brunner, J., and Muller, M. (2001). YidC, an assembly site for polytopic Escherichia coli membrane proteins located in immediate proximity to the SecYE translocon and lipids. EMBO Rep *2*, 709-714.

Bonnefoy, N., Chalvet, F., Hamel, P., Slonimski, P.P., and Dujardin, G. (1994). OXA1, a Saccharomyces cerevisiae nuclear gene whose sequence is conserved from prokaryotes to eukaryotes controls cytochrome oxidase biogenesis. J Mol Biol *239*, 201-212.

Celebi, N., Yi, L., Facey, S.J., Kuhn, A., and Dalbey, R.E. (2006). Membrane biogenesis of subunit II of cytochrome bo oxidase: contrasting requirements for insertion of N-terminal and C-terminal domains. J Mol Biol *357*, 1428-1436.

Chen, M., Xie, K., Yuan, J., Yi, L., Facey, S.J., Pradel, N., Wu, L.F., Kuhn, A., and Dalbey, R.E. (2005). Involvement of SecDF and YidC in the membrane insertion of M13 procoat mutants. Biochemistry *44*, 10741-10749.

de Gier, J.-W., and Luirink, J. (2001). Biogenesis of inner membrane proteins in Escherichia coli. Mol Microbiol *40*, 314-322.

de Gier, J.W., Scotti, P.A., Sääf, A., Valent, Q.A., Kuhn, A., Luirink, J., and von Heijne, G. (1998). Differential use of the signal recognition particle translocase targeting pathway for inner membrane protein assembly in Escherichia coli. Proc Natl Acad Sci U S A *95*, 14646-14651.

du Plessis, D.J., Nouwen, N., and Driessen, A.J. (2006). Subunit a of cytochrome o oxidase requires both YidC and SecYEG for membrane insertion. J Biol Chem *281*, 12248-12252.

Facey, S.J., and Kuhn, A. (2003). The sensor protein KdpD inserts into the Escherichia coli membrane independent of the Sec translocase and YidC. Eur J Biochem *270*, 1724-1734.

Facey, S.J., and Kuhn, A. (2004). Membrane integration of E. coli model membrane proteins. Biochim Biophys Acta *1694*, 55-66.

Fiumera, H.L., Broadley, S.A., and Fox, T.D. (2007). Translocation of mitochondrially synthesized Cox2 domains from the matrix to the intermembrane space. Mol Cell Biol *27*, 4664-4673.

Fröderberg, L., Houben, E., Samuelson, J.C., Chen, M., Park, S.K., Phillips, G.J., Dalbey, R., Luirink, J., and De Gier, J.W. (2003). Versatility of inner membrane protein biogenesis in Escherichia coli. Mol Microbiol *47*, 1015-1027.

Funes, S., Nargang, F.E., Neupert, W., and Herrmann, J.M. (2004). The Oxa2 protein of Neurospora crassa plays a critical role in the biogenesis of cytochrome oxidase and defines a ubiquitous subbranch of the Oxa1/YidC/Alb3 protein family. Mol Biol Cell *15*, 1853-1861.

Glick, B.S., and von Heijne, G. (1996). Saccharomyces cerevisiae mitochondria lack a bacterial-type Sec machinery. Protein Sci *5*, 2651-2652.

Hasona, A., Crowley, P.J., Levesque, C.M., Mair, R.W., Cvitkovitch, D.G., Bleiweis, A.S., and Brady, L.J. (2005). Streptococcal viability and diminished stress tolerance in mutants lacking the signal recognition particle pathway or YidC2. Proc Natl Acad Sci U S A *102*, 17466-17471.

He, S., and Fox, T.D. (1997). Membrane translocation of mitochondrially coded Cox2p: distinct requirements for export of N and C termini and dependence on the conserved protein Oxa1p. Mol Biol Cell *8*, 1449-1460.

Heinrich, S.U., Mothes, W., Brunner, J., and Rapoport, T.A. (2000). The Sec61p complex mediates the integration of a membrane protein by allowing lipid partitioning of the transmembrane domain. Cell *102*, 233-244.

Hell, K., Herrmann, J., Pratje, E., Neupert, W., and Stuart, R.A. (1997). Oxa1p mediates the export of the N- and C-termini of pCoxII from the mitochondrial matrix to the intermembrane space. FEBS Lett *418*, 367-370.

Hell, K., Herrmann, J.M., Pratje, E., Neupert, W., and Stuart, R.A. (1998). Oxa1p, an essential component of the N-tail protein export machinery in mitochondria. Proc Natl Acad of Sci of the U S A *95*, 2250-2255.

Hell, K., Neupert, W., and Stuart, R.A. (2001). Oxa1p acts as a general membrane insertion machinery for proteins encoded by mitochondrial DNA. EMBO J *20*, 1281-1288.

Herrmann, J.M., Neupert, W., and Stuart, R.A. (1997). Insertion into the mitochondrial inner membrane of a polytopic protein, the nuclear-encoded Oxa1p. EMBO J *16*, 2217-2226.

Houben, E.N., Ten Hagen-Jongman, C.M., Brunner, J., Oudega, B., and Luirink, J. (2004). The two membrane segments of leader peptidase partition one by one into the lipid bilayer via a Sec/YidC interface. EMBO Rep *5*, 970-975.

Houben, E.N., Zarivach, R., Oudega, B., and Luirink, J. (2005). Early encounters of a nascent membrane protein: specificity and timing of contacts inside and outside the ribosome. J Cell Biol *170*, 27-35.

Houben, E.N.G., Urbanus, M.L., van der Laan, M., ten Hagen-Jongman, C.M., Driessen, A.J.M., Brunner, J., Oudega, B., and Luirink, J. (2002). YidC and SecY mediate membrane insertion of a type I transmembrane domain. J Biol Chem *277*, 35880-35886.

Ito, K., and Akiyama, Y. (2005). Cellular functions, mechanism of action, and regulation of FtsH protease. Annu Rev Microbiol *59*, 211-231.

Jia, L., Dienhart, M., Schramp, M., McCauley, M., Hell, K., and Stuart, R.A. (2003). Yeast Oxa1 interacts with mitochondrial ribosomes: the importance of the C-terminal region of Oxa1. EMBO J *22*, 6438-6447.

Jiang, F., Yi, L., Moore, M., Chen, M., Rohl, T., Van Wijk, K.J., De Gier, J.W., Henry, R., and Dalbey, R.E. (2002). Chloroplast YidC homolog Albino3 can functionally

complement the bacterial YidC depletion strain and promote membrane insertion of both bacterial and chloroplast thylakoid proteins. J Biol Chem *277*, 19281-19288.

Kiefer, D., and Kuhn, A. (2007). YidC as an essential and multifunctional component in membrane protein assembly. Int Rev Cytol *259*, 113-138.

Lee, J.-I., Kuhn, A., and Dalbey, R.E. (1992). Distinct domains of an oligotopic membrane protein are Sec-dependent and Sec-independent for membrane insertion. J Biol Chem *267*, 938-943.

Lemaire, C., Hamel, P., Velours, J., and Dujardin, G. (2000). Absence of the mitochondrial AAA protease Yme1p restores F0-ATPase subunit accumulation in an oxa1 deletion mutant of Saccharomyces cerevisiae. J Biol Chem *275*, 23471-23475.

Luirink, J., and Sinning, I. (2004). SRP-mediated protein targeting: structure and function revisited. Biochim Biophys Acta *1694*, 17-35.

Nagamori, S., Smirnova, I.N., and Kaback, H.R. (2004). Role of YidC in folding of polytopic membrane proteins. J Cell Biol *165*, 53-62.

Osborne, A.R., Rapoport, T.A., and van den Berg, B. (2005). Protein translocation by the Sec61/SecY channel. Annu Rev Cell Dev Biol *21*, 529-550.

Preuss, M., Ott, M., Funes, S., Luirink, J., and Herrmann, J.M. (2005). Evolution of mitochondrial oxa proteins from bacterial YidC. Inherited and acquired functions of a conserved protein insertion machinery. J Biol Chem *280*, 13004-13011.

Rep, M., Nooy, J., Guelin, E., and Grivell, L.A. (1996). Three genes for mitochondrial proteins suppress null-mutations in both Afg3 and Rca1 when over-expressed. Curr Genet *30*, 206-211.

Samuelson, J.C., Chen, M.Y., Jiang, F.L., Moller, I., Wiedmann, M., Kuhn, A., Phillips, G.J., and Dalbey, R.E. (2000). YidC mediates membrane protein insertion in bacteria. Nature *406*, 637-641.

Saracco, S.A., and Fox, T.D. (2002). Cox18p is required for export of the mitochondrially encoded Saccharomyces cerevisiae Cox2p C-tail and interacts with Pnt1p and Mss2p in the inner membrane. Mol Biol Cell *13*, 1122-1131.

Serek, J., Bauer-Manz, G., Struhalla, G., Van Den Berg, L., Kiefer, D., Dalbey, R., and Kuhn, A. (2004). Escherichia coli YidC is a membrane insertase for Sec-independent proteins. EMBO J *23*, 294-301.

Shimohata, N., Nagamori, S., Akiyama, Y., Kaback, H.R., and Ito, K. (2007). SecY alterations that impair membrane protein folding and generate a membrane stress. J Cell Biol *176*, 307-317.

Szyrach, G., Ott, M., Bonnefoy, N., Neupert, W., and Herrmann, J.M. (2003). Ribosome binding to the Oxa1 complex facilitates co-translational protein insertion in mitochondria. EMBO J *22*, 6448-6457.

Ulbrandt, N.D., Newitt, J.A., and Bernstein, H.D. (1997). The E. coli signal recognition particle is required for the insertion of a subset of inner membrane proteins. Cell *88*, 187-196.

Urbanus, M.L., Scotti, P.A., Fröderberg, L., Saaf, A., deGier, J.W.L., Brunner, J., Samuelson, J.C., Dalbey, R.E., Oudega, B., and Luirink, J. (2001). Sec-dependent membrane protein insertion: sequential interaction of nascent FtsQ with SecY and YidC. EMBO Rep *2*, 524-529.

van der Laan, M., Bechtluft, P., Kol, S., Nouwen, N., and Driessen, A.J. (2004a). F1F0 ATP synthase subunit c is a substrate of the novel YidC pathway for membrane protein biogenesis. J Cell Biol *165*, 213-222.

van der Laan, M., Nouwen, N., and Driessen, A.J. (2004b). SecYEG proteoliposomes catalyze the Deltaphi-dependent membrane insertion of FtsQ. J Biol Chem *279*, 1659-1664.

van der Laan, M., Nouwen, N.P., and Driessen, A.J. (2005). YidC--an evolutionary conserved device for the assembly of energy-transducing membrane protein complexes. Curr Opin Microbiol *8*, 182-187.

van der Laan, M., Urbanus, M.L., Ten Hagen-Jongman, C.M., Nouwen, N., Oudega, B., Harms, N., Driessen, A.J., and Luirink, J. (2003). A conserved function of YidC in the biogenesis of respiratory chain complexes. Proc Natl Acad Sci U S A *100*, 5801-5806.

Yi, L., Celebi, N., Chen, M., and Dalbey, R.E. (2004). Sec/SRP requirements and energetics of membrane insertion of subunits a, b, and c of the Escherichia coli F1F0 ATP synthase. J Biol Chem *279*, 39260-39267.