Chloroplast YidC Homolog Albino3 Can Functionally Complement the Bacterial YidC Depletion Strain and Promote Membrane Insertion of Both Bacterial and Chloroplast Thylakoid Proteins*

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A new component of the bacterial translocation machinery, YidC, has been identified that specializes in the integration of membrane proteins. YidC is homologous to the mitochondrial Oxa1p and the chloroplast Alb3, which functions in a novel pathway for the insertion of membrane proteins from the mitochondrial matrix and chloroplast stroma, respectively. We find that Alb3 can functionally complement the Escherichia coli YidC depletion strain and promote the membrane insertion of the M13 procoat and leader peptidase that were previously shown to depend on the bacterial YidC for membrane translocation. In addition, the chloroplast Alb3 that is expressed in bacteria is essential for the insertion of chloroplast cpSecE protein into the bacterial inner membrane. Surprisingly, Alb3 is not required for the insertion of cpSecE into the thylakoid membrane. These results underscore the importance of Oxa1p homologs for membrane protein insertion in bacteria and demonstrate that the requirement for Oxa1p homologs is different in the bacterial and thylakoid membrane systems.

Two pathways have been discovered in bacteria to insert membrane proteins into the inner membrane. The Sec-dependent pathway is used for most membrane proteins, while the Sec-independent pathway is used only for a few tested membrane proteins (for review see Refs. 1–3). The cast of characters for the Sec-dependent pathway is SecA, Y, E, and G (4). SecY and SecE polypeptides are believed to form the core of the protein-conducting membrane channel (5, 6), and SecG is the translocation ATPase (7) that catalyzes translocation of long periplasmic loops (8–10). Known Sec-dependent membrane proteins are leader peptidase (Lep),1 MalF, FtsQ, and AcrB (11–15). These proteins are targeted to the membrane by the signal recognition particle pathway involving Ffh and 4.5 S RNA, and FtsY (16–18). Recently, a new component called YidC has been found to be associated with the Sec machinery. YidC appears to function in membrane protein insertion based on its interaction with the transmembrane region of FtsQ (14), leader peptidase (19, 20), and mannitol permease (21) during their integration. Until very recently, the Sec-independent pathway was believed to function without the aid of a protein machinery (22, 23). However, recent studies indicate that YidC is required for the membrane insertion of the Sec-independent procoat and pβ3 coat protein (20, 24, 25).

In mitochondria and chloroplast, YidC homologs exist that have been shown to mediate membrane protein insertion (for review see Ref. 26). The mitochondrial homolog, Oxa1p, is required for the membrane insertion of a subset of inner membrane proteins (27–29). These Oxa1p-dependent protein include proteins that are both matrix-encoded and nuclear-encoded that are imported from the cytosol into the matrix and then inserted into the inner membrane. The chloroplast homolog, Albino3 (Alb3), is required for integration of a subset of the light-harvesting chlorophyll-binding proteins (LHCP) into the thylakoid membrane (30–32).

In this paper, we test whether the chloroplast Alb3 can functionally complement a YidC depletion strain. We find that Alb3 can complement the growth defect of a YidC depletion strain and that Alb3 promotes the insertion of Lep and M13 procoat protein as well as the chloroplast SecE (cpSecE) protein into the bacterial inner membrane. Surprisingly Alb3 is not required for the membrane insertion of the cpSecE into the thylakoid membrane. These studies emphasize the importance of Oxa1p homologs for insertion of proteins into the E. coli inner membrane and reinforce the recent results that membrane insertion into chloroplast thylakoid membrane can be independent of Alb3 (31, 32).

**Experimental Procedures

Strains and Plasmids—E. coli strain JS7131 (MC1060, ΔyidC, attB:R6Kori, ParaBAD yidC+, (Spec+)) was from our laboratory stocks. pMS119, which contains the tac promoter and lacP, was used to express procoat and leader peptidase (Lep), pQS60 and pPH1 vectors, which contain the IPTG-inducible lacUV5 promoter, were used to express the light harvesting chlorophyll-binding protein (LHCP) and cpSecE, respectively. The cDNA sequence of Alb3 was amplified using the Arabidopsis thaliana plants as templates.

1 The abbreviations used are: Lep, leader peptidase; LHCP, light-harvesting chlorophyll-binding protein(s); IPTG, isopropyl-1-thio-β-
diphas thaliana cDNA library (from Arabidopsis Biological Resource Center at The Ohio State University) and cloned into pACYC184 (from New England Biolabs). The upstream region of the yidC gene including the promoter and ribosome-binding site was then cloned into the vector upstream from the alb3 start codon, yielding plasmid pACYC-Alb3. pACYC-Alb3 was expressed from pBAD24 where the yidC gene was targeted (15–55 amino acids) was replaced with a single methionine. XhoI and HpaI sites were introduced into the yidC and alb3 genes by site-directed mutagenesis, and then the two XhoI-HpaI fragments corresponding to 1–57 amino acids of YidC or Alb3 were swapped. The resulting plasmid, pACYC-H1Alb3, contains a yidC and an alb3 gene, which includes the first 57 amino acids of Alb3. The three-gene constructs, Alb3, mAlb3, and H1Alb3, were then cloned into pMS119 under the tac promoter to overexpress the plasmid-encoded Albino3 proteins. The first 57 amino acids of YidC and H1Alb3 were replaced by 1–57 amino acids from the precursor to the maltose-binding protein (preMBP), yielding two constructs: MBP-YidC and MBP-Alb3. Clones for in vitro transcription and translation have been described previously: pLHCP (33), iOE33 (34), and d23-PCm (35). PCR-based cloning was used to create mature cPsecE with a C-terminal Met. The C-terminal Met residue was added in order to visualize a protease-protected fragment of integrated cPsecE in thylakoid membranes. Using the primers PH1 and cPsecE (36) as template, the mature cPsecE coding sequence was amplified by PCR using the forward primer 5'-CGCAGGATCCACCATGGCGACGAGTAATCTG-3' and the reverse primer 5'-GGTGTGTTCAAGACTCTTCAGCATCTGACGAGTAATCTG-3', which includes the ATG codon before the stop codon. An E. coli library (from Arabidopsis Biological Resource Center at The Ohio State University) and cloned into pACYC184 (from New England Biolabs) and subsequently labeled with [35S]methionine in the presence of [35S]methionine as described (39). Translation products were diluted 3-fold and adjusted to import buffer (IB; 50 mM Hepes/KOH, pH 8.0, 0.33 M sorbitol) containing 30 mM unlabeled methionine.

Preparation of Chloroplasts, Synthases, and Stromal Extracts. Thylakoids were isolated from 10–100-day-old seedlings (Laxton's Progress). Chloroplast lyases, synthases, and stromal extracts (S.E.) were prepared as described (35, 39). Chlorophyll content was determined according to Arnon (40).

Protein Transport Assays Using Isolated Thylakoids—Standard transport assays included 50 µl of 2× thylakoids (1 mg/ml chlorophyll) in IB/10 mM MgCl2 (IBM), 50 µl of 2× S.E. (equivalent to that obtained from 2× chloroplasts), 5 mM ATP, 1 mM GTP, and 25 µl of diluted radiolabeled precursor protein. IB was added to make the final volume 150 µl. After incubation at 25 °C for 30 min, the thylakoid membranes were pelleted (3200 × g for 8 min) and resuspended in 500 µl of IB. The amount of inserted protein was assayed by adding 25 µl of thermostatin (2 mg/ml in IB/10 mM CaCl2) and incubating 40 min on ice. Thermolysin treatment was continued by addition of 100 µl of IB/50 mM EDTA. Synthesis of chloroplast synthases after chloroplast isolation was checked by incorporation of [35S]methionine (41) and resuspended in 45 µl of solubilization buffer. Otherwise, labeled membranes were resuspended in 20 µl of 10 mM EDTA and 25 µl of 2× solubilization buffer. After incubation at 70 °C for 15 min, proteins contained in 10 µl of each sample were separated by SDS-PAGE except for samples from cPsecE integration assays, which were separated by Tricine SDS-PAGE (42). Gels were dried and used to quantify the level of protein transport from phosphorimager (Molecular Dynamics Typhoon).

Assay for Antibody Inhibition of Protein Import into Thylakoids—Antibodies against Alb3, cPsecE, and Tha4 were tested for their ability to inhibit insertion into thylakoids as described in (30). Overexpressed SecE and Alb3 are from Arabidopsis. After incubation with isolated thylakoids for 1 h, the unbound antibody was removed by washing thylakoids in IBM, and standard transport assays were conducted, except that S.E. was included at twice the standard concentration.

Energetics of Thylakoid Import—Standard transport assays lacking additional ATP were used to test energetic requirements of precursor transport. IB was substituted for S.E. as indicated in the legend to Fig. 6 to examine the requirement for soluble factors (35). The non-hydrolyzable ATP and GTP analogues AMP-PNP and GMP-PNP, were investigated for their effect on insertion by addition to 5 µM final concentration. For the assay the thylakoids were repurified over 7.5% Percoll cushions in IB/10 mM EDTA and prepared for import assays by washing two times in IB/10 mM EDTA and once in IB. The membranes were then resuspended in IB containing 1 mM dithiothreitol and used in standard transport assays. Mock protease-treated thylakoids were used in control assays.

RESULTS

Alb3 Complements the Growth Defect of a YidC Depletion Strain—To test whether the chloroplast Alb3 is a functional homolog of the E. coli YidC, we investigated whether Alb3 could complement the YidC depletion strain, JS7131. The YidC depletion strain, which is arabinose-dependent for growth, has an arabinose-inducible yidC gene permanently integrated at the attB locus of MC1060 and contains an in-frame deletion at the normal yidC locus. Complementation was assayed by testing whether Alb3 can restore growth of JS7131 on LB agar plates containing glucose.

The A. thaliana Alb3 is predicted to contain only five transmembrane segments (44) (see Fig. 1A) while E. coli YidC has six transmembrane segments with an N-terminal signal anchor domain (45). Alb3 lacks most of the N-terminal translo-

Acrylamide gel electrophoresis using a 15% poly-
and glucose plates. Only JS7131 containing the pACYC-H1Alb3 grew on both arabinose or 0.2% glucose LB plates and incubated at 37 °C overnight. The overnight culture was 1:50 back-diluted in LB with either 0.2% glucose to deplete YidC or 0.2% arabinose to express YidC. For JS7131 bearing pACYC-H1Alb3, the overnight culture was cultivated in the absence of arabinose and directly back-diluted 1:50 in LB medium with 0.2% glucose added to suppress the expression of YidC. After about 3 h of growth, cells were collected, and the cell lysate was analyzed by SDS-PAGE and subjected to Western analysis using antiserum against YidC. The upper background bands precipitated with our YidC antiserum demonstrate that an equal amount of cell lysate was loaded in each lane. B, an overnight culture of JS7131 harboring pACYC-Alb3, -mAlb3, or -H1Alb3 was grown in LB with 0.2% arabinose. The overnight culture was then back-diluted into LB medium with 0.2% glucose and grown for an additional 4 h. JS7131 harboring pMS119-Alb3, -mAlb3, or H1Alb3 was grown for 2 h, induced with 1 mM IPTG, and grown for an additional 2 h. The cell lysate was analyzed by SDS-PAGE and immunoblotted with antiserum against Alb3. The * indicates possible proteolytic fragments of H1Alb3.

We next confirmed that JS7131 expressing H1Alb3 does not express YidC when grown in glucose. Immunoblot analysis using YidC antiserum revealed that there was no detectable YidC in JS7131 expressing H1Alb3 that was grown in glucose (Fig. 2A). The top band shown in Fig. 2A is a nonspecific band that is precipitated by our YidC antiserum. As a control, we confirmed that JS7131 only synthesizes YidC in the presence of arabinose (left lane) but not in glucose (middle lane). Western blotting analysis using Alb3 antiserum was performed to detect the presence of Alb3. Fig. 2B shows that H1Alb3 is produced in glucose-grown JS7131 when expression is controlled by both the yidC promoter (lane 1) and the tac promoter (lane 4). There is some degradation of H1Alb3 (* depicts the degradation products) that can be seen when this protein is overproduced (lane 4), possibly because it cannot insert and assemble efficiently under overproducing conditions. The wild-type Alb3 (mAlb3) and Alb3 containing the targeting signal (Alb3) are not expressed in detectable amounts in JS7131 either with the yidC or tac promoter (Fig. 2B).

Because the H1Alb3 mutant was functional, but not Alb3, we asked if there was a functional requirement for the N-terminal 57 amino acids of YidC. One possibility was that the YidC region was required because it contained a hydrophobic domain that helped in the membrane translocation of the Albl3 or YidC N-terminal luminal domain. Therefore, we tested whether the first 57 amino acids of the precursor to the maltose-binding
protein containing a cleavable signal sequence could replace the first 57 amino acids within YidC or H1Alb3. Fig. 3A shows that MBP-YidC is functional as this construct complements the growth defect of the YidC depletion strain. Immunoblot analysis shows that MBP-YidC is expressed and slightly smaller than the wild-type YidC, due most likely to cleavage of the preMBP presequence by signal peptidase (Fig. 3B). This shows conclusively that the first 57 amino acids of YidC do not have a functional requirement. Notably, the construct MBP-Alb3 does not restore growth to the YidC depletion strain (Fig. 3A), most likely because the protein is not expressed as it is not detected on an immunoblot (data not shown).

The Chloroplast Alb3 Promotes Membrane Insertion of Sec-independent and Sec-dependent Membrane Proteins in E. coli—Because the E. coli YidC is essential for efficient membrane insertion of the Sec-independent M13 procot protein and the Sec-dependent leader peptidase (Lep) (20), we first tested whether H1Alb3 is required for insertion of these two proteins when YidC is depleted. Cells expressing procot were pulse-labeled with trans-[35S]methionine for 20 s and chased for 5 and 120 s. Signal peptide processing of procot is completely normal when H1Alb3 is expressed (Fig. 4A). As a control, we confirmed that processing is completely blocked when JS7131 grown in glucose (YidC-depleted cells) in the absence of H1Alb3, but is efficient when grown in arabinose (ampl YidC). In addition, H1Alb3 promotes membrane insertion of Lep (Fig. 4B). Cells expressing Lep were pulse-labeled with trans-[35S]methionine for 20 s and analyzed for protease accessibility. Cells were converted to spheroplasts and then treated with or without protease. When JS7131 is expressing H1Alb3, Lep is completely accessible to protease, indicating that the large C-terminal domain of Lep is translocated across the membrane. In the same spheroplasts, there was no lysis as Band X (46), an unidentified cytoplasmic protein, was protected from protease digestion. The efficiency of Lep insertion was just as efficient as when YidC was present (arabinose-grown cells). Translocation of the C-terminal domain of Lep was impaired in YidC-deficient JS7131 cells (glucose-grown cells, Fig. 4B) as previously reported (20). In this study where Lep was examined, the overnight culture was grown in LB medium containing both arabinose and glucose. The glucose was added to the overnight culture as it suppresses the expression of YidC, allowing a more effective depletion of YidC in the subsequent growing of JS7131 cells in glucose medium.

Alb3 Promotes the Insertion of the Chloroplast cpSecE into the E. coli Inner Membrane—The complementation and insertion data above directly links the role of Oxa1p homologs in the translocation pathways in bacteria and in the plant chloroplast. To test this idea further, we tested whether H1Alb3 can function to insert a chloroplast thylakoid membrane protein into the E. coli inner membrane. Because the chloroplast SecE has been shown to functionally substitute for SecE in E. coli (36) we tested whether this protein would require Alb3 for insertion. In this study, we used cpSecE, which lacks the chloroplast stroma-targeting signal. This protein spans the thylakoid membrane once with a short C-terminal tail in the luminal space. H1Alb3 and cpSecE-containing cells were pulse-labeled for 20 s, converted to spheroplasts, and protease-mapped. As can be seen in Fig. 5A, cpSecE was efficiently degraded by protease, indicating it inserted across the membrane when H1Alb3 was present. Likewise, cpSecE was inserted, although to a lesser extent, when JS7131 was grown in the presence of YidC (arabinose). No detectable cpSecE was inserted into the membrane when YidC was absent (glucose).

Given that H1Alb3 promoted efficient insertion of the cpSecE, we tested whether the chloroplast LHCP, which requires Alb3 for membrane insertion (30), would insert into the E. coli inner membrane. Previously, it had been shown that overexpression of LHCP in E. coli lead to its accumulation in inclusion bodies (47). Therefore, we tested whether the accumulation may have arisen because insertion was blocked due to the protein not being able to utilize YidC. H1Alb3-containing cells may then allow efficient insertion. However, we observed
no obvious membrane insertion either with H1Alb3 or YidC (Fig. 5B), showing that insertion requires other components that are lacking in E. coli. Likewise, no insertion was also found with PsbW (data not shown), a thylakoid protein shown to insert by a spontaneous pathway in chloroplast (31, 48), suggesting that in E. coli spontaneous insertion is not possible for thylakoid membrane proteins.

Alb3 Is Not Required for Insertion of cpSecE into the Thylakoid—We examined also the cpSecE membrane biogenesis pathway in chloroplast to determine whether Alb3 is required. Radiolabeled cpSecE, without the targeting signal and containing an added Met residue at the C terminus, was synthesized using an in vitro system (translation products) (Fig. 6A) and added to a chloroplast lysate. cpSecE is recovered with the pelleted thylakoid fraction (−Prot) and remains quantitatively bound to the thylakoid even after extraction with alkali (−Prot/NaOH). As a control, we showed that the thylakoid-processed iOE33, which is a lumenal protein, is extracted by alkali (−Prot/NaOH, lower panel). These results with cpSecE indicate that cpSecE has integrated into the membrane. The addition of protease produces a shorter resistant fragment that is protected from the protease by the membrane (−Prot). The protease-protected fragment disappears when the integrity of the membranes is disrupted by the addition of detergent (Prot/T100). To test the requirement for Alb3, we used anti-Alb3 antibody prepared against the stroma-facing thylakoid domain of Alb3. Previously, this antibody (α-Alb3) was shown by Moest et al. to inhibit the membrane integration of LHCP while having no effect on Sec and Tat-dependent substrates (30). Fig. 6B shows that the addition of the anti-Alb3 antibody (α-Alb3) has no effect on the membrane insertion of cpSecE while inhibiting the integration of LHCP. The addition of anti-cpSecY antibody (α-cpSecY) also has no effect while strongly inhibiting the Sec-dependent substrate iOE33. Finally, the addition of anti-Tat antibody prepared against Tha4 (α-Tha4) had no effect on cpSecE insertion while inhibiting the translocation of 123-
Evolutionary Conserved Oxa1p/YidC/Alb3 Pathway

FIG. 7. cpSecE does not require stromal protein factors, nucleotides, or a ΔpH for integration into thylakoid membranes. Radiolabeled precursors shown to the left of the phosphorimage were incubated with isolated thylakoids in the presence of 1 mM GTP and the additives shown above the lanes. Stromal extract (SE) was included in all assays except where indicated (−SE). Additions include: 5 mM GMP-PNP (GMP-PNP), 5 mM AMP-PNP (AMP-PNP), 1 μM ionophore in 95% ethanol (Nigericin), and 95% ethanol for a control (Ethanol). All assays were posttreated with thermolysin to reveal characteristic degradation products (DP; see “Experimental Procedures”). cpSecE, which integrates correctly, gives rise to viable DP. Insertion of LHCP as well. The energetic studies here show that cpSecE integration into thylakoid membranes takes place by a spontaneous mechanism.

As a second test to show that Alb3 is not involved in the membrane insertion of cpSecE into the thylakoid membrane, we tested whether cpSecE could insert into protease-treated thylakoids. Transport assays were conducted with isolated thylakoids that were either mock protease-treated (MP) or protease-treated with thermolysin (P) (see “Experimental Procedures”). Following thylakoid incubation with radiolabeled proteins, all assays were posttreated with thermolysin to reveal characteristic degradation products (DP) of cpSecE and pLHCP and to eliminate all but the correctly transported mature (m) OE33 and plastocyanin (PC). Numbers beneath the lanes represent the percentage of transport relative to assays conducted with S.E. (+SE). All other notations are as in Fig. 6.

FIG. 8. Chloroplast cpSecE integrates into protease treated thylakoids. Transport assays were conducted with isolated thylakoids that were either either mock protease-treated (MP) or protease-treated with thermolysin (P) (see “Experimental Procedures”). Following thylakoid incubation with radiolabeled proteins, all assays were posttreated with thermolysin to reveal characteristic degradation products (DP) of cpSecE and pLHCP and to eliminate all but the correctly transported mature (m) OE33 and plastocyanin (PC). All other notations are as in Fig. 6.

PCm, a Tat-dependent substrate (35). The data taken together suggest that cpSecE does not require SecY, Tat, or Alb3 for its insertion into the thylakoid membrane.

The energetics of cpSecE insertion was investigated by monitoring insertion into isolated thylakoids under a variety of conditions (Fig. 7). cpSecE, which inserts correctly, gives rise to degradation products (DP; upper panel) after treating the thylakoid-inserted cpSecE with protease. Unlike the SRP/Alb3-dependent LHCP and the Sec-dependent iOE33, insertion of cpSecE did not require the addition of a stroma extract (compare −S.E., and +S.E. lanes) and was unaffected by addition of GMP-PNP or AMP-PNP. GMP-PNP is an inhibitor of the SRP, and AMP-PNP almost certainly inhibits the ATPase activity of cpSecA. Although assays did not contain additional ATP, the amount of ATP in S.E. and the translation products was enough to support iOE33 transport. A slight reduction in cpSecE membrane insertion was observed when nigericin was added to collapse the pH gradient, which completely blocks the insertion of a Tat-dependent substrate t23-PCm and inhibits insertion of LHCP as well. The energetic studies here show that cpSecE inserts by an SRP-independent route, in contrast to the Alb3-dependent LHCP protein. The data also shows that cpSecE insertion is stimulated by the pH gradient.

As a second test to show that Alb3 is not involved in the membrane insertion of cpSecE into the thylakoid membrane, we tested whether cpSecE could insert into protease-treated thylakoids. Fig. 8 shows studies where mature cpSecE is added to thylakoids that were previously treated with or without thermolysin and then, after transport, were treated again with thermolysin to assay the amount of cpSecE inserted. There was only a slight reduction of cpSecE that inserts into protease-treated thylakoid (lane P) in comparison to mock protease-treated thylakoids (MP). Similar results were found for pElip2, which was previously shown to insert by a spontaneous mechanism (bottom panel) (37). These results are different from those observed for the Tat (ΔpH), Sec-, or SRP/Alb3-dependent substrates where pretreatment of thylakoids with protease (compare P with MP lanes) abolishes translocation into or across the membrane (P). Taken together, these data suggest that cpSecE integration into thylakoid membranes takes place by a spontaneous mechanism.

DISCUSSION

The Oxa1p/YidC/Alb3 family is a newly discovered group of proteins that participate in a novel transport pathway for insertion into the mitochondrial and bacterial inner membrane and the chloroplast thylakoid membrane (49). Homologs in this evolutionarily conserved pathway are found in eubacteria, archaebacteria, and in mitochondria and chloroplasts of eukaryotes (26). Almost nothing is known about the structure and function of these Oxa1p homologs.

In this report, we show that the Arabidopsis Alb3 can complement the growth defect of a YidC depletion strain, when the first 57 amino acids of YidC containing the first transmembrane segment are linked to mature Alb3 (H1Alb3). The mature Alb3 or the precursor form of Alb3 containing the stromatargeting sequence cannot substitute for YidC. Possible reasons are that they could not be expressed in E. coli since they were not detected in the Western study or that the albino3 proteins are very unstable or they fail to assemble across the membrane. We found that a functional YidC could be made when the first 57 amino acids of YidC were replaced with the maltose-binding protein preprotein region that contains a cleavable leader sequence. This indicates that there is not a functional requirement for the N-terminal YidC portion of the molecule for Albino3 or YidC itself. Most likely the YidC N-terminal portion is necessary for YidC and H1Alb3 function because this region contains an uncleaved signal that promotes translocation of the N-terminal domain.

The fact that H1Alb3 can complement the YidC depletion strain demonstrates that the chloroplast and eubacterial
Oxa1p homologs are truly functional homologs. Like YidC, the chloroplast H1Alb3 promotes the membrane insertion of the Sec-independent procoat protein and Sec-dependent Lep, suggesting that H1Alb3 may function in association with or independent of the Sec translocase as YidC does in E. coli. In addition, H1Alb3 can also promote the membrane insertion of the chloroplast thylakoid cpSecE into the E. coli inner membrane. Our studies demonstrate that Alb3 in chloroplast and YidC in bacteria function in similar and conserved ways in membrane assembly. These studies are in line with work over the last decade that has shown that, in general, transport across the bacterial inner membrane and thylakoid membrane is remarkably conserved (3, 50). Like bacteria, the chloroplast has the SecA, Y and E homologs, and there is no reason to believe that Sec-dependent insertion is drastically different. In fact, the chloroplast thylakoid cpSecE can complement the E. coli SecE-depletion strain (36). Similarly, chloroplasts have a homologous Tat pathway found in bacteria that can export proteins with metal cofactors in a folded conformation (50).

We also examined the Alb3-dependent thylakoid protein LHCP for membrane insertion in E. coli. However, LHCP was not inserted even in cells expressing H1Alb3 (Fig. 5B). This defect in insertion may arise because E. coli lacks the novel SRP component, SRP 43, that is required for targeting in the chloroplast thylakoid system (51). Similarly, PsbW, which inserts into the thylakoid membrane by a spontaneous pathway in chloroplasts, does not insert into the inner membrane in E. coli even with H1Alb3 present (data not shown). Based on this result, it seems that E. coli cannot support spontaneous insertion of thylakoid membrane proteins. Although YidC and H1Alb3 are essential for insertion of the cpSecE and bacterial SecE into the E. coli inner membrane, Alb3 is not required for insertion of the cpSecE into thylakoids. We found that antibody against Alb3 did not inhibit the integration of cpSecE into thylakoids, even though it blocked the insertion of the Alb3-dependent LHCP (Fig. 6B). In fact, cpSecE insertion may occur by a novel pathway since antibodies that inhibit transport by the thylakoid Sec or Tat pathways had no effect on cpSecE insertion. Neither stromal factors nor nucleotide triphosphates were needed for cpSecE membrane insertion, while only the depletion of the ΔpH was found to reduce cpSecE insertion by ~45% (Fig. 7). Strikingly, cpSecE inserted with high efficiency into protease-treated thylakoids (Fig. 8), which blocked the insertion of the Alb3-dependent protein LHCP. These results bolster the observation of Robinson and co-workers where they find that to date most of the tested thylakoidal membrane proteins do not apparently require Alb3 and insert by a novel, possibly spontaneous mechanism (31, 32). Of course, we cannot rule out that under normal in vivo conditions Alb3 is involved in cpSecE assembly. Moreover, there are other Oxa1p homologs in Arabidopsis (26), one of which is predicted with high probability to reside in the chloroplast.

Given these contrasting requirements for an Oxa1p homolog in the bacterial and chloroplast system for SecE, our hypothesis is that the requirement for Alb3 changed following the endosymbiotic event that gave rise to chloroplasts. Whereas cpSecE originally depended on Alb3 in the bacterial ancestor of chloroplasts, the dependence changed when much of the ancestral genome including the cpSecE gene moved to the nucleus of the plant eukaryotic cell. As a result, it became necessary for SecE to be imported into the chloroplast and then inserted posttranslationally rather than by a cotranslational mechanism. This change may also account for some differences in the targeting and translocation pathways: SRP43 is needed in the chloroplast thylakoidal targeting system, not in the bacterial system; chloroplasts lack the Sec translocase components SecD, SecF, and SecG, which are found in bacteria (52); and, as stated above, many chloroplast thylakoid membrane proteins utilize a novel “unassisted” membrane protein insertion pathway that requires no known translocation components (31). In this context, it is predicted that cpSecE insertion in cyanobacteria will be dependent on a homologue of Alb3/YidC. Another possible reason for the different requirement for Alb3 in the E. coli inner membrane and chloroplast thylakoids is the difference in lipid composition. The thylakoid membrane is unusual in that a high percentage of the lipids are glycerolipids, whereas E. coli contains mainly phospholipids.

Taken together, the complementation studies reported here demonstrate that the chloroplast Alb3 functions in a similar way as the E. coli YidC. This is in line with other studies that have shown that the translocation machineries present in bacteria and chloroplasts such as the Sec translocase and the Tat translocase are remarkably similar (2, 50, 53). Oxa1p, Alb3, and YidC belong to the same protein family and were found to be involved in membrane protein assembly in mitochondria, chloroplast, and bacteria, respectively. The fact that Alb3 can functionally substitute for YidC further demonstrates that the Oxa1p/Alb3/YidC group represents a conserved translocation pathway in the three systems. Moreover, while YidC is required for membrane insertion of bacterial SecE in E. coli, Alb3 is not required for the insertion of cpSecE into the thylakoid membranes. This may suggest that functional changes in the Oxa1p/Alb3/YidC pathway occurred during the evolutionary process or that the substrate itself has changed from a ribosome-bound nascent protein to a ribosome-released full-length protein such that one system requires an Oxa1p homolog and the other does not.

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Chloroplast YidC Homolog Albino3 Can Functionally Complement the Bacterial YidC Depletion Strain and Promote Membrane Insertion of Both Bacterial and Chloroplast Thylakoid Proteins

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