The thylakoid membrane protein ALB3 associates with the cpSecY-translocase in *Arabidopsis thaliana*

Eva KLOSTERMANN*, Imke DROSTE GEN. HELLING*, Jean-Pierre CARDE† and Danja SCHÜNEMANN*¹

*Institut für Biologie III der RWTH Aachen, Worringerweg 1, 52074 Aachen, Germany, and †Biologie Cellulaire et Moléculaire du Développement des Plantes, Université Bordeaux, Avenue Edouard Bourleaux, 33883 Villenave d'Ornon Cedex, France

The integration of light-harvesting chlorophyll proteins (LHCPs) into the thylakoid membrane requires the integral thylakoid membrane protein ALB3, a homologue of the bacterial cytoplasmic membrane protein YidC. In bacteria, YidC is associated with the SecY-translocase and facilitates the integration of Sec-dependent proteins into the plasma membrane. In addition, it is also involved in the insertion of Sec-independent proteins. In the present study we demonstrate, in *Arabidopsis thaliana*, that most ALB3 is a constituent of an oligomeric complex of approx. 180 kDa. In addition, we detected ALB3 in several higher-molecular-mass complexes (up to 700 kDa). Furthermore, we show that most ALB3 co-fractionates with cpSecY during gel-filtration analysis and blue native gel electrophoresis, suggesting

an association of ALB3 with the cpSecY complex. A direct interaction of ALB3 with the cpSecY complex was demonstrated by co-immunoprecipitation experiments using digitonin-solubilized thylakoid membrane proteins and anti-cpSecY or anti-ALB3 antibodies. This result was further confirmed by electron microscopic co-immunolocalization of ALB3 and cpSecY. In addition, an association of ALB3 with the cpSecY complex was demonstrated directly by cross-linking experiments using the chemical cross-linker disuccinimidyl suberate.

Key words: chloroplast, light-harvesting chlorophyll protein (LHCP), protein–protein interaction, protein translocation.

INTRODUCTION

The integral membrane proteins YidC, Oxa1p and ALB3 belong to an evolutionarily conserved protein family, and mediate protein insertion into the inner membrane of bacteria and mitochondria and the thylakoid membrane of chloroplasts respectively. In *Escherichia coli*, most inner-membrane proteins are targeted co-translationally to the cytoplasmic membrane using the signal recognition particle (SRP) and the SRP receptor FtsY. The insertion of these proteins into the inner membrane is mediated via the SecY-translocase. Recently, the inner-membrane protein YidC was identified as a novel component of the SecY-translocase [1]. Several studies indicate that YidC facilitates the lateral diffusion of the transmembrane domains of Sec-dependent proteins from the translocase into the lipid bilayer [2–5]. A subset of *E. coli* inner-membrane proteins are inserted into the membrane in a Sec-independent manner, and recent studies have shown that YidC is essential for the insertion of these proteins [6–8]. Taken together, these results indicate that YidC can function in concert with the SecY-translocase, and can also function independently of the SecY-translocase.

The mitochondrial homologue of YidC is Oxa1p. Oxa1p is involved in the insertion of mitochondrially encoded inner-membrane proteins, and also mediates the re-insertion of nuclear-encoded inner-membrane proteins that have been completely imported into the matrix [9–12]. Interestingly, yeast mitochondria do not contain proteins with homology with the subunits of the bacterial-type Sec translocase, indicating a Sec-independent function of Oxa1p [13].

In chloroplasts, the thylakoid membrane protein ALB3 was identified as a homologue of YidC and Oxa1p [14]. It was shown that ALB3 is required for the membrane insertion of members of the light-harvesting chlorophyll protein (LHCP) family, which

are targeted post-translationally to the thylakoid membrane using the chloroplast SRP (cpSRP) and cpFtsY [15,16]. In addition, it was demonstrated that the integration of LHCP into the thylakoid membrane *in vitro* could not be inhibited by preincubation of the membrane with antibodies directed against cpSecY, the chloroplast homologue of bacterial SecY [17]. Taken together, these results indicate that LHCP integration is SRP- and ALB3-dependent, but cpSecY-independent.

These findings raised the question of whether ALB3 exists in a monomeric form or as a constituent of an oligomeric complex independent of the cpSecY complex, or whether at least a part of ALB3 is associated with the cpSecY complex. In the present work we demonstrate, in *Arabidopsis thaliana*, that ALB3 is a component of higher-molecular-mass complexes, and is at least partially associated with the cpSecY complex.

EXPERIMENTAL

Miscellaneous

Arabidopsis thaliana (ecotype Columbia) was grown in a growth chamber in a cycle of 9 h light at 22 °C/15 h dark at 18 °C. The light intensity was 100 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Thylakoid preparation and solubilization of digitonin (Calbiochem; high purity) were carried out as described previously [18].

Antibodies

Full-length ALB3 cDNA was obtained from 2.5 μg of total RNA (*A. thaliana*) by reverse transcription–PCR (TitanTM One Tube RT-PCR System; Roche Molecular Biochemicals) and cloned into pGEM-T Easy (Promega). Anti-ALB3 anti-

Abbreviations used: DSS, disuccinimidyl suberate; LHCP, light-harvesting chlorophyll protein; SRP, signal recognition particle; the prefix cp denotes chloroplast.

¹ To whom correspondence should be addressed (e-mail schuenemann@bio3.rwth-aachen.de).

bodies were raised using the procedure described by Moore et al. [15]. A 50-amino-acid segment of ALB3 [15] was amplified by PCR and cloned as a glutathione S-transferase fusion protein into the plasmid pGEX4T-3 (Amersham Biosciences). The fusion protein was expressed in *E. coli* strain BL21 and used as antigen for polyclonal antibody production in the rabbit (SEQLAB, Göttingen, Germany).

The production and specificity of antibodies against cpSecY have been described previously [18].

Gel-filtration analysis

Thylakoid membrane proteins were solubilized in 10 mM Hepes/KOH, pH 8.0, 200 mM NaCl and 1 mM PMSF containing either 1.5% digitonin or 1.5% dodecyl β -D-maltoside. Solubilized proteins (200 μ l; 1 mg of chlorophyll/ml) were then separated on a Superose 6 HR column (Amersham Biosciences) in 20 mM Hepes/KOH, pH 8.0, and 200 mM NaCl containing 0.1% digitonin or 0.1% dodecyl β -D-maltoside at a flow rate of 0.3 or 0.4 ml/min respectively. Fractions of 0.6 ml, 0.8 ml or 0.3 ml were collected. Aliquots of 20 μ l of the fractions were separated on an SDS/12%-polyacrylamide gel, and ALB3 and cpSecY were detected by immunoblot analysis using anti-ALB3 or anti-cpSecY antibodies respectively.

Co-immunoprecipitation

Thylakoid membrane proteins solubilized in 10 mM Hepes/KOH, pH 8.0, 200 mM NaCl, 1.5% digitonin and 1 mM PMSF were incubated overnight (500 μ l; 1 mg of chlorophyll/ml) at 4 °C with 200 μ l of anti-ALB3 serum or preimmune serum, or 1.6 mg of anti-cpSecY IgG cross-linked to 10 mg of Protein A-Sepharose beads. The beads were transferred into Wizard minicolumns (Promega) and washed with 4 ml of 20 mM Hepes/KOH, pH 8.0, 0.5 M NaCl and 0.5% digitonin. Excess fluid was removed by centrifugation in a Microfuge, and the proteins were eluted with 30 μ l of 8 M urea in sample buffer.

Blue native gel electrophoresis

Blue native gel electrophoresis was carried out as described in [19] with the following modifications. Thylakoid membranes were resuspended in 50 mM BisTris/HCl, pH 7.0, at 2 mg of chlorophyll/ml, and then solubilized by the slow addition of an equal volume of buffer containing 400 mM NaCl, 20 mM Hepes/KOH, pH 7.0, 2 mM PMSF and 2% digitonin. After incubation on ice for 30 min, samples were centrifuged at 20000 *g* for 15 min at 4 °C. The supernatant was supplemented with 0.1 vol. of a Coomassie Blue solution [5% Coomassie Blue G 250 (Serva) in 50 mM BisTris/HCl, pH 7.0, and 750 mM ϵ -aminocaproic acid] and 0.33 vol. of 50% glycerol. The samples (50 μ l, containing 41 μ g of chlorophyll) were then loaded directly on to blue native 6–17% (w/v) polyacrylamide gradient gels. Electrophoresis was carried out at 15 mA in the cold-room (4 °C). The cathode buffer contained 0.02% dye, and was replaced by buffer lacking dye after approximately one-third of the electrophoresis run. Gels were preblotted twice (200 mA, 10 min, 4 °C) and finally blotted to PVDF membranes at 150 mA for 15 h at 4 °C.

Immunolocalization by electron microscopy

Immunolocalization experiments were performed with plastids from *Arabidopsis* leaves as described by Hutin et al. [20], with the following modifications. For double-labelling experiments, the samples were incubated with a mixture of chicken anti-ALB3 antibodies and rabbit anti-cpSecY antibodies. The

secondary rabbit anti-chicken antibodies and the secondary goat anti-rabbit antibodies were conjugated to 5 nm and 10 nm gold particles respectively.

Cross-linking and immunoprecipitation

Thylakoid membranes were resuspended in 1 ml of 20 mM Hepes/KOH, pH 8.0, and 150 mM NaCl (0.5 mg of chlorophyll/ml) and incubated with the chemical cross-linker disuccinimidyl suberate (DSS; final concentration 0.5 mM) for 1.5 h at 4 °C. The stock solution of DSS was prepared in DMSO, and the control reaction contained the same amount of DMSO as the cross-linking reaction. Reactions were quenched with Tris/HCl, pH 7.5, at a final concentration of 60 mM for 15 min at 4 °C. Thylakoid membranes were sedimented by centrifugation and solubilized at 1 mg of chlorophyll/ml in 20 mM Hepes/KOH, pH 8.0, 200 mM NaCl, 1.2% Triton X-100 and 1 mM PMSF for 30 min on ice. Insoluble material was removed by centrifugation. The supernatant was incubated overnight at 4 °C with 1.6 mg of anti-cpSecY IgG or IgG against an irrelevant protein cross-linked to 10 mg of Protein A-Sepharose beads. The beads were transferred into Wizard minicolumns (Promega) and washed with 20 mM Hepes/KOH, pH 8.0, 0.2 M NaCl and 1% Triton X-100, followed by 20 mM Hepes/KOH, pH 8.0, and 0.2 M NaCl. Excess fluid was removed by centrifugation in a Microfuge, and the proteins were eluted with 30 μ l of 8 M urea in sample buffer.

RESULTS

ALB3 and cpSecY co-fractionate during gel-filtration analysis

In order to analyse the native molecular mass of ALB3 and to determine whether ALB3 associates with the cpSecY complex, we separated thylakoid membrane proteins after detergent solubilization on a gel-filtration column and examined the elution profiles of ALB3 and cpSecY. Initially the mild detergent digitonin was used for solubilization, because it has been shown previously that digitonin is the detergent of choice for the isolation of protein translocation complexes [19,21,22]. After separation of the extracted thylakoid membrane proteins on a Superose 6HR column, we determined the relative amounts of cpSecY and ALB3 in the various fractions by immunoblot analysis using antibodies against cpSecY and ALB3. Polyclonal antibodies against *Arabidopsis* ALB3 were raised using the procedure described by Moore et al. [15]. These antibodies recognized a single protein of ~ 45 kDa in *Arabidopsis* thylakoids (results not shown). As shown in Figure 1(A), most ALB3 and cpSecY eluted from the gel filtration column as higher-molecular-mass species of approx. 200 kDa. This molecular mass is close to that of ~ 180 kDa determined previously for the cpSecY complex [18]. Co-elution of cpSecY and ALB3 in a single peak (~ 160 kDa) was also observed when the thylakoid membranes were solubilized under harsher conditions using dodecyl β -D-maltoside as detergent (Figure 1B). These results demonstrate that most, if not all, ALB3 is a constituent of a higher-molecular-mass complex. In addition, these results suggest that ALB3 is a component of the cpSecY complex.

ALB3 and cpSecY co-fractionate during blue native gel electrophoresis

Blue native gel electrophoresis has been described as a successful method for the separation of thylakoid membrane protein complexes with high resolution and sensitivity [23–25]. Therefore we sought to compare the native molecular masses of ALB3 and

Figure 1 ALB3 and cpSecY co-fractionate during gel-filtration analysis

Thylakoid membrane proteins solubilized in 1.5% digitonin (**A**) or 1.5% dodecyl β -D-maltoside (**B**) were fractionated by FPLC on a Superose 6HR column. Fractions of 0.6 ml (**A**), 0.8 ml (**B**, upper panel) or 0.3 ml (**B**, lower panel) were analysed by immunoblot analysis using antibodies against ALB3 and cpSecY (see the Experimental section for details).

Figure 2 Separation of thylakoid membrane protein complexes containing ALB3 and cpSecY by blue native gel electrophoresis

Thylakoids were solubilized in 1% digitonin and separated on 6–17% (w/v) polyacrylamide blue native gradient gels. Proteins were detected by immunoblotting using anti-ALB3 or anti-cpSecY antibodies. Immunoblots using preimmune serum of ALB3 did not show any bands (not shown). The following proteins were used as molecular mass standards: ferritin (880 and 440 kDa), β -amylase (200 kDa), BSA (132 and 66 kDa) and ovalbumin (45 kDa).

cpSecY by using this method. Thylakoid membrane proteins were solubilized in digitonin and subjected to blue native gel electrophoresis, and the distributions of ALB3 and cpSecY within the gel were detected by Western blot analysis. The two proteins showed a very similar fractionation pattern (Figure 2). In agreement with the gel-filtration analysis, ALB3 and cpSecY were found to be components of a higher-molecular-mass complex of approx. 160 kDa, and no monomeric forms of ALB3 or cpSecY were detected. However, blue native gel electrophoresis revealed that ALB3 and cpSecY were also present in a variety of oligomeric complexes with molecular masses ranging from 120 to 700 kDa (Figure 2).

Co-immunoprecipitation of ALB3 and cpSecY

We next sought to demonstrate an association of ALB3 and cpSecY by co-immunoprecipitation experiments. Antibodies raised against ALB3 or anti-cpSecY antibodies were used in immunoprecipitation experiments with digitonin-solubilized

Figure 3 Co-immunoprecipitation of ALB3 and cpSecY

Thylakoid membrane proteins (THY) were solubilized with 1.5% digitonin, and immunoprecipitation (IP) was performed using anti-ALB3 antibodies (**A**) or anti-cpSecY antibodies (**B**). Preimmune serum (PI) or antibodies against an irrelevant protein (**B**) were used as controls (see the Experimental section for details). The immunoprecipitates were subjected to immunoblot analysis with anti-ALB3 and anti-cpSecY antibodies.

Figure 4 Double immunogold labelling of ALB3 (5 nm gold particles) and cpSecY (10 nm gold particles) in thylakoid membranes of chloroplasts from *Arabidopsis*

The arrows indicate co-localization of ALB3 and cpSecY.

thylakoid membrane proteins. The precipitate was analysed for the presence of ALB3 and cpSecY by immunoblot analysis. We found that cpSecY could be immunoprecipitated together with ALB3 using anti-ALB3 antibodies (Figure 3A), and that ALB3 can be immunoprecipitated together with cpSecY using anti-cpSecY antibodies (Figure 3B). None of the proteins were precipitated by the preimmune serum (Figure 3A) or by an

Figure 5 Chemical cross-linking of ALB3 and cpSecY in intact thylakoid membranes

Lanes 1 and 2: after treatment of thylakoid membranes without (—) or with (+) the chemical cross-linker DSS, thylakoid membrane proteins were separated by SDS/PAGE and subjected to immunoblotting using antibodies against ALB3 (A) or cpSecY (B). Lanes 3–5: thylakoid membranes were treated without or with DSS as indicated, solubilized with Triton X-100 and subjected to immunoprecipitation (IP) with anti-cpSecY antibodies (lanes 3 and 4), or antibodies against an irrelevant protein (lane 5) as a control. The immunoprecipitate was analysed by Western blot (WB) using anti-ALB3 (A) or anti-cpSecY (B) antibodies. The immunoprecipitated cross-linked products are indicated (*).

antibody directed against an irrelevant protein (Figure 3B). These results demonstrate that ALB3 is at least partially associated with the cpSecY complex.

Immunolocalization of ALB3 and cpSecY

Electron microscopy of double-immunogold-labelled ultrathin sections of chloroplasts from *Arabidopsis* leaves was performed to verify further an interaction of ALB3 with the cpSecY complex. ALB3 and cpSecY were visualized using secondary antibodies conjugated to 5 nm and 10 nm gold particles respectively. In all cross-reactions of different sections analysed, the gold particles were attached to the thylakoid membrane and showed a similar labelling pattern (a representative image is shown in Figure 4). Almost all observed gold particles corresponding to cpSecY (10 nm) co-localized with the gold particles corresponding to ALB3 (5 nm), whereas a significant number of free gold particles for ALB3 were detected. These observations provide further evidence that at least a portion of ALB3 interacts with the cpSecY complex.

Chemical cross-linking confirms the association of ALB3 with the cpSecY complex

In order to confirm the interaction of ALB3 and the cpSecY complex, chemical cross-linking experiments using thylakoid membranes and various cross-linkers were undertaken. SDS/PAGE of the cross-linked thylakoid membrane proteins followed by immunoblotting using anti-ALB3 and anti-cpSecY antibodies showed that the amino group-reactive homobifunctional cross-linker DSS generated cross-linked products of ALB3 (Figure 5A,

lane 2) and cpSecY (Figure 5B, lane 2) in the same molecular mass range of approx. 90 kDa. To demonstrate that these cross-linked products indeed contained both proteins, Triton X-100-solubilized thylakoid membrane proteins were immunoprecipitated with anti-cpSecY antibodies, and the precipitate was analysed for ALB3 and cpSecY by immunoblotting. It was found that DSS cross-linked ALB3 to the cpSecY complex, as both proteins were present in the immunoprecipitated cross-linked product (Figures 5A and 5B, lanes 4). The specificity of the immunoprecipitation experiment was demonstrated by control reactions performed with anti-cpSecY antibodies and a sample containing no cross-linker (Figures 5A and 5B, lanes 3) or with antibodies against an irrelevant protein and the cross-linked sample (lane 5).

DISCUSSION

Previous work with YidC, the bacterial homologue of ALB3, demonstrated that YidC is involved in the integration of inner-membrane proteins. Depending on the kind of membrane protein that is used for integration, YidC can either function in association with the Sec-translocase or operate independently of the Sec-translocase as a separate unit. Recently it was shown that the post-translational integration of LHCPs into the thylakoid membrane requires a translocase that contains ALB3, but seems to be distinct from the cpSecY translocon [15–17]. Nothing was known about a potential oligomeric organization of ALB3, or a possible connection between ALB3 and the cpSecY complex.

In the present work we have established three new and important points. First, gel-filtration analysis showed that most ALB3 is part of an oligomeric complex with a molecular mass of approx. 180 kDa, which corresponds to the native molecular mass of cpSecY. Secondly, smaller amounts of ALB3 and cpSecY are also present in various higher-molecular-mass complexes up to 700 kDa. Thirdly, ALB3 is associated with the cpSecY complex.

The direct association of ALB3 with the cpSecY translocon was clearly established by three types of experimental methods, i.e. co-immunoprecipitation experiments using anti-ALB3 or anti-cpSecY antibodies, electron microscopic co-immunolocalization and cross-linking experiments. However, the extent of the interaction between ALB3 and the cpSecY complex cannot be defined from these studies. It should be noted that the efficiency of co-immunoprecipitation of cpSecY and ALB3 was lower than that of co-immunoprecipitation of cpSecY and cpSecE, which was analysed by our group previously [18]. This suggests that, in contrast with the stable complex formed between cpSecY and cpSecE, the interaction between ALB3 and the cpSecY complex is relatively weak or transient. This conclusion is supported by the partial co-localization of ALB3 and cpSecY, as observed in the immunolocalization studies, and is also in agreement with the weak, or transient, interaction of YidC with the SecY-translocase in bacteria [1,5].

The co-fractionation of ALB3 and cpSecY during gel-filtration analysis and blue native gel electrophoresis allows the assumption that ALB3 is largely associated with the cpSecY complex. If this is the case, it might be speculated that the chloroplast SRP pathway delivers LHCPs to the cpSecY translocon and that ALB3 mediates the lateral diffusion of LHCPs from the translocon into the bilayer. In contrast, it was shown that pretreatment of thylakoid membranes with anti-cpSecY antibodies inhibited the translocation of Sec-dependent substrates, but did not inhibit LHCP integration, suggesting that this process is Sec-independent [17]. However, it might be possible that the anti-cpSecY antibodies bind to a region of cpSecY that is not required to

mediate LHCP insertion, or prevents binding of cpSecA to the translocon. Therefore further work is necessary to elucidate the precise function of ALB3 in LHCP integration.

The oligomeric structure of the bacterial SecYEG translocase has been determined. Studies by Yahr and Wickner [26] suggested that the active form of the translocase is monomeric SecYEG. In contrast, other studies indicated that SecYEG is a conformationally dynamic structure, existing as monomers, dimers and tetramers [27,28], whereby the active translocation channel forms a dimer [29]. Detergent solubilization of thylakoid membranes releases a cpSecY complex of approx. 180 kDa. One might speculate that this complex consists of a dimer of cpSecYE and an additional ALB3, or of monomeric cpSecYE, ALB3 and other, as yet unknown, components. However, the large variety of cpSecY complexes observed during blue native gel electrophoresis indicates a dynamic system of complexes, with the assembly and disassembly of the components being adapted to the substrate protein and translocation state.

It will of interest in the future to analyse the subunit composition of the different cpSecY complexes, and the precise molecular nature and the extent of the interaction between ALB3 and the cpSecY-translocase.

We thank Alan Slusarenko for giving us the opportunity to perform the experiments described in this study in his laboratory, and for critical reading of the manuscript. We thank Laurent Nussaume for the kind donation of the chicken anti-ALB3 antibodies. This work was supported by the Deutsche Forschungsgemeinschaft (grant no. SCHU 1163/2; Emmy Noether-Program).

REFERENCES

- Scotti, P. A., Urbanus, M. L., Brunner, J., de Gier, J. W., von Heijne, G., van der Does, C., Driessen, A. J., Oudega, B. and Lührink, J. (2000) YidC, the *Escherichia coli* homologue of mitochondrial Oxa1p, is a component of the Sec translocase. *EMBO J.* **19**, 542–549
- Houben, E. N., Scotti, P. A., Valent, Q. A., Brunner, J., de Gier, J. L., Oudega, B. and Lührink, J. (2000) Nascent Lep inserts into the *Escherichia coli* inner membrane in the vicinity of YidC, SecY and SecA. *FEBS Lett.* **476**, 229–233
- Beck, K., Eisner, G., Trescher, D., Dalbey, R. E., Brunner, J. and Müller, 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
- Urbanus, M. L., Scotti, P. A., Fröderberg, L., Sääf, A., de Gier, J. W., Brunner, J., Samuelson, J. C., Dalbey, R. E., Oudega, B. and Lührink, 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., Houben, E. N., Nouwen, N., Lührink, J. and Driessen, A. J. (2001) Reconstitution of Sec-dependent membrane protein insertion: nascent FtsQ interacts with YidC in a SecYEG-dependent manner. *EMBO Rep.* **2**, 519–523
- Samuelson, J. C., Chen, M., Jiang, F., Møller, I., Wiedmann, M., Kuhn, A., Phillips, G. J. and Dalbey, R. E. (2000) YidC mediates membrane protein insertion in bacteria. *Nature (London)* **406**, 637–641
- Samuelson, J. C., Jiang, F., Yi, L., Chen, M., de Gier, J. W., Kuhn, A. and Dalbey, R. E. (2001) Function of YidC for the insertion of M13 procoat protein in *Escherichia coli*: translocation of mutants that show differences in their membrane potential dependence and Sec requirement. *J. Biol. Chem.* **276**, 34847–34852
- Chen, M., Samuelson, J. C., Jiang, F., Müller, M., Kuhn, A. and Dalbey, R. E. (2002) Direct interaction of YidC with the Sec-independent Pf3 coat protein during its membrane protein insertion. *J. Biol. Chem.* **277**, 7670–7675
- 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. Sci. 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
- Kermorgant, M., Bonnefoy, N. and Dujardin, G. (1997) Oxa1p, which is required for cytochrome *c* oxidase and ATP synthase complex formation, is embedded in the mitochondrial inner membrane. *Curr. Genet.* **31**, 302–307
- Glick, B. S. and von Heijne, G. (1996) *Saccharomyces cerevisiae* mitochondria lack a bacterial-type sec machinery. *Protein Sci.* **5**, 2651–2652
- Sundberg, E., Slagter, J. G., Fridborg, I., Cleary, S. P., Robinson, C. and Coupland, G. (1997) ALBIN3, an *Arabidopsis* nuclear gene essential for chloroplast differentiation, encodes a chloroplast protein that shows homology to proteins present in bacterial membranes and yeast mitochondria. *Plant Cell* **9**, 717–730
- Moore, M., Harrison, M. S., Peterson, E. C. and Henry, R. (2000) Chloroplast Oxa1p homolog albino3 is required for post-translational integration of the light harvesting chlorophyll-binding protein into thylakoid membranes. *J. Biol. Chem.* **275**, 1529–1532
- Woolhead, C. A., Thompson, S. J., Moore, M., Tissier, C., Mant, A., Rodger, A., Henry, R. and Robinson, C. (2001) Distinct Albino3-dependent and -independent pathways for thylakoid membrane protein insertion. *J. Biol. Chem.* **276**, 40841–40846
- Mori, H., Summer, E. J., Ma, X. and Cline, K. (1999) Component specificity for the thylakoidal Sec and Δ pH-dependent protein transport pathways. *J. Cell Biol.* **146**, 45–56
- Schünemann, D., Amin, P., Hartmann, E. and Hoffman, N. E. (1999) Chloroplast SecY is complexed to SecE and involved in the translocation of the 33-kDa but not the 23-kDa subunit of the oxygen-evolving complex. *J. Biol. Chem.* **274**, 12177–12182
- Schägger, H. and von Jagow, G. (1991) Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Anal. Biochem.* **199**, 223–231
- Hutin, C., Havaux, M., Carde, J. P., Kloppstech, K., Meierhoff, K., Hoffman, N. and Nussaume, L. (2002) Double mutation cpSRP43 –/cpSRP54 – is necessary to abolish the cpSRP pathway required for thylakoid targeting of the light-harvesting chlorophyll proteins. *Plant J.* **29**, 531–543
- Duong, F. and Wickner, W. (1997) Distinct catalytic roles of the SecYE, SecG and SecDFyajC subunits of preprotein translocase holoenzyme. *EMBO J.* **16**, 2756–2768
- Görlich, D. and Rapoport, T. A. (1993) Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane. *Cell* **75**, 615–630
- Berghöfer, J. and Klösken, R. B. (1999) Two distinct translocation intermediates can be distinguished during protein transport by the TAT (Δ pH) pathway across the thylakoid membrane. *FEBS Lett.* **460**, 328–332
- Neff, D. and Dencher, N. A. (1999) Purification of multisubunit membrane protein complexes: isolation of chloroplast FoF1-ATP synthase, Cfo and CF1 by blue native electrophoresis. *Biochem. Biophys. Res. Commun.* **259**, 569–575
- Cline, K. and Mori, H. (2001) Thylakoid Δ pH-dependent precursor proteins bind to a cpTatC-Hcf106 complex before Tha4-dependent transport. *J. Cell Biol.* **154**, 719–729
- Yahr, T. L. and Wickner, W. T. (2000) Evaluating the oligomeric state of SecYEG in preprotein translocase. *EMBO J.* **19**, 4393–4401
- Manting, E. H., van Der Does, C., Remigy, H., Engel, A. and Driessen, A. J. (2000) SecYEG assembles into a tetramer to form the active protein translocation channel. *EMBO J.* **19**, 852–861
- Collinson, I., Breyton, C., Duong, F., Tziatzios, C., Schubert, D., Or, E., Rapoport, T. and Kühlbrandt, W. (2001) Projection structure and oligomeric properties of a bacterial core protein translocase. *EMBO J.* **20**, 2462–2471
- Bessonneau, P., Besson, V., Collinson, I. and Duong, F. (2002) The SecYEG preprotein translocation channel is a conformationally dynamic and dimeric structure. *EMBO J.* **21**, 995–1003

Received 30 April 2002/4 September 2002; accepted 9 September 2002

Published as BJ Immediate Publication 9 September 2002, DOI 10.1042/BJ20021291