Several β-barrel-type channels are involved in the translocation or assembly of outer membrane proteins of bacteria or endosymbiotically derived organelles. Here we analyzed the functional units of the β-barrel polypeptide transporter Toc75 (translocon in outer envelope of chloroplasts) of the outer envelope of chloroplasts and of a protein, alr2269, from Nostoc PCC7120 with homology to Toc75, both proteins having a similar domain organization. We demonstrated that the N-terminal region functions as a recognition and complex assembly unit, whereas the C terminus forms the β-barrel-type pore. The pore region is, in turn, modulated by the N terminus of the proteins. The protein from Nostoc PCC7120, which shares a common ancestor with Toc75, is able to recognize precursor proteins destined for chloroplasts. In contrast, the recognition of peripheral translocon subunits by Toc75 is a novel feature acquired through evolution.

β-barrel-type channels are involved in the translocation of polypeptides (1), the assembly of proteins in the outer membrane of endosymbiotic organelles (2–4), or in the assembly of proteins in the outer membrane of bacteria (5, 6). These proteins belong to one class, which can be termed polypeptide-transporting β-barrel channels (2, 4, 7). Four proteins are in the focus of recent investigation, namely the bacterial outer membrane proteins Omp85 and ShlB, the mitochondrial Omp85 homologue (4). This protein facilitates the assembly of proteins into the outer membrane of mitochondria. Tob55/Sam50 is found in a larger complex with Mas37 (3, 12) and Tob38/Sam35 (13–15).

The fourth investigated β-barrel-type polypeptide transporter is the 75-kDa subunit of the translocon of the outer envelope of chloroplasts, Toc75. Toc75 forms a complex with Toc34, Toc64, and Toc159 (16). In contrast to the other identified polypeptide transporters, such as Omp85, the translocation of proteins through Toc75 requires the action of assisting proteins, such as Toc159 (17), but still Toc75 seems to contain a preprotein-binding site as determined by electrophysiological measurements (1). Topological modeling of Toc75 from Pisum sativum (18) or Toc75-V from Arabidopsis thaliana (19) suggests a β-barrel-type structure. Previously, it was proposed that Toc75 might have evolved from the ShlB (20, 21) or from the Omp85 class (5). This relationship to prokaryotic proteins is in line with the theory that chloroplasts have evolved from the ancestor of cyanobacteria (22, 23).

Here we identified the Nostoc PCC7120 homologue of Toc75/Omp85. To understand the evolutionary relationship between Toc75 and alr2269, we investigated the specific properties of the two proteins. We here present experimental evidence that the N-terminal domain of β-barrel-type polypeptide transporters is involved in the recognition of substrates and complex assembly, whereas the C-terminal domain assembles the pore-forming β-barrel.

MATERIALS AND METHODS

Construction Generation, Expression, and in Vitro Translation—Translation or expression of Toc34ATM, pSSU1, mSSU, pOED3, or pOE23 was described previously (24, 25). psToc75 cDNA (1) was used as the template for the generation of psA, psB, psC, and psD (Fig. 1) by PCR. The alr2269 cDNA was amplified from genomic Nostoc PCC7120 DNA. Constructs were generated by PCR, cloned into pTrcHis2 TOPO® TA (Invitrogen), and controlled by sequencing. BL21 cells (Novagen) transformed with plasmids were incubated in LB medium at 37 °C, and expression was induced by the addition of 1 mM isopropyl 1-thio-β-D-galactopyranoside. Cells were harvested 2 h after induction and lysed using a French press (at 40 megapascals) in 50 mM NaPi, pH 8.0, 100 mM NaCl, and 2 mM β-mercaptoethanol (buffer A). Full-length proteins and C-terminal constructs (Fig. 1D) were accumulated in inclusion bodies. After centrifugation for 15 min at 25,000 × g at 4 °C, the pellet was resuspended in 50 mM NaPi, pH 8, 150 mM NaCl, 10 mM β-mer-

The abbreviations used are: pSSU/mSSU, precursor/mature form of the small subunit of Rubisco; Rubisco, ribulose-bisphosphate carboxylase/oxygenase; MOPS, 4-morpholinepropanesulfonic acid; aa, amino acids; Ni-NTA, nickel-nitrilotriacetic acid; nS, nanosiemens; pS, picosiemens.
Domains of β-Barrier Transporters

capoethanol, 20 mM imidazole, and 5 μl urea. The solution was centrifuged at 20,000 revolutions/min for 15 min at 4 °C, the supernatant was subjected to nickel-agarose (Qiagen, Hilden, Germany), and the protein was purified according to the manual. Soluble fragments (Figs. 1D and 2A) were obtained by centrifugation of the cells for 15 min at 25,000 × g at 4 °C. After purification, proteins were dialyzed against 50 mM NaPI, pH 8.0, 100 mM NaCl. Their concentration was determined by Lowry analysis. The rapid translation system RTS IV-S, Sigma) was dissolved in 80 mM Mega-9, 10 mM MOPS/Tris (pH 7.0), and analyzed by gel electrophoresis. The concentration of proteins or peptides in the flow-through not associated with the matrix. For cross-linking analysis, wheat germ-translation 3S-labeled psSU was incubated with pseTOC75 or alr2269 constructs at 1 μg in 50 mM NaPI, pH 8.0, and 100 mM NaCl in 50 μl of bis(sulfosuccinimidyl)suberate (1 μl final, BS3; Perbio, Bonn, Germany) was added and quenched after 45 min of incubation at 4 °C by the addition of gassing (100 mM defined amount for coupling). The binding efficiency was determined using AIDA software, expressed to the percent of loaded material and normalized to the indicated binding reaction.

Liposome Swelling Assay and Transport-Specific Fractionation—Lipids for reconstitution were supplied by Nutfield Nurseries (Surrey, UK). Liposomes were prepared and reconstituted as described previously (28). Liposome swelling assay was conducted using ClustalW at the BCM search launcher server (searchlauncher.bcm.tmc.edu/ multi-align/multi-align.html) and visualized with Boxshade 3.21 (www.ch.embnet.org/software/BOX_form.html).

Electrophoretic Measurements—Mega-9 (80 μl final) was added to purified psC, alr2269 or anC. L-α-phosphatidylcholine (type IV-S, Sigma) was dissolved in 80 μl Mega-9, 10 mM MOPS/Tris (pH 7.0). Both samples were mixed (1 μg of protein/20 μl of lipids/mixture). The indicated dialysis was 2 liters of 10 mM MOPS/Tris, pH 7.0, for 2 h at 25 °C and subsequently overnight at 4 °C. Planar lipid bilayers were produced using the painting technique (29). A solution of 75 mg/ml L-α-phosphatidylcholine in n-decan was applied to a hole in a Teflon septum, separating the two 3-mL chambers. To form a stable bilayer in 20 mM KCl, the 10 mM MOPS/Tris, pH 7.0, solution level was raised and lowered 10 times. The solution in the cis chamber was changed to 250 mM KCl, 10 mM CaCl2, 10 mM MOPS/Tris, pH 7.0. Proteoliposomes were added to the cis chamber below the bilayer to allow the flow of the liposomes across the bilayer. The solution in the cis chamber was stirred to promote fusion. After fusion, the electrolytes in both compartments were changed to the final composition. Silver/AgCl electrodes were connected to the chambers through 2 m KCI-agar bridges. The electrode of the trans compartment was connected directly to the head stage of a current amplifier (GeneClamp500B, Axon Instruments, Union City, CA). The amplified currents were recorded using the pCLAMP software (Axon Instruments).

Circular Dichroism Measurements—Circular dichroism spectra were recorded in 1-nm steps for 2 s of integration time, and a slit width of 2 nm in 10 mM HEPES/KOH, pH 7.6, 100 mM NaCl on a Jasco V-560 spectrometer (Division of Instruments, SA) at 22 °C using a cuvette with a 1-mm path length. 15 spectra were recorded and averaged. The rough data were further manipulated as described previously (30).

RESULTS

Previously, the protein slr1227 from Synechocystis PCC6803 was described as a protein related to Toc75 (20, 21). More recently, it was proposed that the Nostoc gene complement is most closely related to that of the ancestor of plastids based on the analysis of Nostoc punctiforme (22). To understand the evolutionary development of the polypeptide-transporting β-barrel protein Toc75, we wanted to compare the properties of psToc75 from P. sativum and a protein of the genus Nostoc. We therefore analyzed the genome of the two species N. punctiforme and Nostoc PCC7120 for the presence of a homologue of the polypeptide transporters Omp85 from N. meningitidis (5) and psToc75 (1) (supplemental figure, panel A). We identified two proteins related to psToc75/Omp85, namely Npnu02006512 and alr2269. Sequence alignment of these two proteins revealed a sequence identity of 71% (supplemental figure). Because the two proteins identified from species N. punctiforme and Nostoc PCC7120 share such high identity, it is reasonable to use the protein encoded by the Nostoc PCC7120 gene alr2269 for further analysis (Fig. 1A). The protein encoded by alr2269 shares 19.4% identity and 29.3% similarity with psToc75 and 15.8% identity and 25.9% similarity with Omp85. The similarity in regard to the proposed topologies of the proteins is very high (Fig. 1D) (5, 19). In addition to alr2269, two further homologues to psToc75 and Omp85 can be found in the Nostoc PCC7120 proteome, namely alr4893 and alr0075 (31, 32). The two latter proteins are shorter than Omp85 with predicted molecular mass values of 72 and 54 kDa, respectively. Further, these two gene products are not as similar to psToc75 and Omp85 as alr2269 (not shown). Finally, in mass spectrometric analysis of outer membranes, only alr2269 could be detected (33). Hence, we have focused in this study on the analysis of alr2269.

The existence of the coding RNA was demonstrated by reverse transcription-mediated PCR (Fig. 1B). Immunodecoration of isolated fractions of Nostoc PCC7120 shows that the protein encoded by alr2269 is localized in the outer membrane (Fig. 1C, lane 3), but not in the thylakoid membrane (lane 1) or plasma membrane (lane 2). Topological modeling of alr2269 (Fig. 1D) revealed two dominant regions, an N-terminal mostly soluble part and a C-terminal portion mostly involved in transmembrane β-strand formation. The localization of the domains in regard to the two sides of the membrane cannot be defined, because further experimental data have to be accumulated, as has been done for Toc75 (18, 19). To test the predicted domain architecture, we constructed mutants of psToc75 and alr2269 comprising specific sections of these proteins. The first mutant (referred to as A in Fig. 1D, aa 161–470 of alr2269 and aa 149–440 of psToc75, empty box and box with bricks) was chosen in regard to the proposed polypeptide transport-associated domain (POTRA) (34). The domain does not include the previously predicted transit peptide of psToc75 ending at amino acid 131 (35). AnaB (aa 388–470) or psB (aa 365–440) (Fig. 1D, box with bricks) covers the third loop region in the topological model. The third construct (referred to as C in Fig. 1D, aa 469–833 of alr2269 and aa 439–809 of psToc75, box with crossed lines and box with transversal lines) represents the entire postulated pore-forming region (5, 19). The last mutant...
referred to as D in Fig. 1D, aa 702–833 of alr2269 and aa 678–809 of \textit{psToc75}, box with transversal lines) reflects the region with high similarity among the polypeptide transporters containing two structural motifs and eight proposed \(\beta\)-strands (31). The generated constructs of the N-terminal region (Fig. 1D, constructs A and B) were expressed in \textit{E. coli} and purified as soluble proteins to high homogeneity (Fig. 2A). The constructs C and D (in Fig. 5A) were expressed as insoluble proteins and purified under denaturing conditions. To confirm the structural content of the N-terminal constructs CD spectrosopy was performed. All N-terminal constructs showed a defined spectrum in the far UV region (Fig. 2B) (not shown for \textit{psA} and \textit{psB}), accounting for a secondary structure content (30). Using a simple approach for the estimation of the secondary structure content (30, 36), we observed that the A constructs contained both helical (50%) and \(\beta\)-sheet (20%) content, whereas for the B constructs only helical content could be determined (65%). From the obtained result, we concluded
that the soluble expressed N-terminal constructs can be used for in vitro interaction analysis in solution.

psToc75 Contains an N-terminal Preprotein-binding Domain—Previously, it was suggested that psToc75 interacts with the transit peptide of chloroplast preproteins (1). We now investigated whether the N-terminal region of psToc75 could mediate this interaction. When a matrix charged with the precursor of the subunit of the oxygen-evolving complex of 23 kDa (pOE23) was incubated with radioactive labeled psA or psB (Fig. 2C, lanes 3 and 6) or 2-fold molar excess of psA or psB (Fig. 2D, lanes 1–9), both domains of psToc75 were associated with the matrix charged with the preprotein (Fig. 2C, lane 3; 2D, lanes 3 and 9) but not with the empty matrix or a matrix charged with the mature form of the small subunit of Rubisco (Fig. 2C, lane 6; 2D, lanes 6 and 12). Interestingly, the results for the qualitative (radioactive labeled psToc75 constructs; Fig. 2C) or quantitative approach (expressed psToc75 constructs; Fig. 2D) are comparable. In addition, the two psToc75 constructs associated in a similar quantitative manner with the precursor of the 33- or 23-kDa subunit of the oxygen-evolving complex (pOE33/23) (Fig. 2D, lane 9). These results suggest that psToc75 contains a transit peptide-binding site in its N-terminal region. To confirm this notion, synthetic peptides representing the N-terminal portion of the transit sequence, the A1 peptide represents the C-terminal portion without phosphorylated serine, and the B2 peptide represents the phosphorylated C-terminal portion of the transit sequence (25, Fig. 2E). Incubation of these affinity matrices with psB revealed a physical interaction between this domain and the N-terminal portion of the transit sequence (Fig. 2F, lane 4). In contrast, psB did not bind to the C-terminal portion of the transit peptide independent of its phosphorylation state (Fig. 2F, lanes 2 and 3). Hence, we concluded that psToc75 recognizes the transit sequence of preproteins.

The Evolutionary Conservation of the N-terminal Preprotein-binding Domain—alr2269 of Nostoc PCC7120 encodes the protein with high similarity to psToc75 or Omp85 (Fig. 1A). We therefore compared the interaction of the N-terminal region of the psToc75 protein and alr2269 with preproteins destined for chloroplasts. For that, psA, psB, anaA, and anaB (Fig. 1D) were expressed, purified under non-denaturing conditions as soluble proteins (Fig. 2A, lanes 1–8), and coupled to Ni-NTA to equal molar amounts of the indicated protein domains. Utilizing these psA, psB, anaA, or anaB affinity matrices for binding studies, an interaction with psSU (Fig. 3B, lanes 3 and 5) but not with mSSU was observed (Fig. 3A, lanes 4 and 7), because mSSU was already eluted during the wash step (lanes 3 and 6). In the case of pSSU, we could not detect any protein in the final wash fraction (not shown). To confirm that the observed interaction is truly mediated by the proteins investigated, pSSU or pOE33 and the N-terminal construct of either psToc75 (psA and psB) or alr2269 (anaA and anaB) were incubated in solution, followed by immunoprecipitation using antibodies specific against psToc75 (Fig. 3C, lanes 1 and 2) or alr2269 (lanes 3 and 4). In all cases, the complexes could be immunoprecipitated using either the psToc75 (Fig. 3D, upper panel, lanes 4 and 7) or alr2269 antibodies (lower panel, lanes 4 and 7). The same antibodies did not precipitate the preproteins without the addition of psToc75 or alr2269 constructs (not shown). In line with this, no precipitation of the preproteins by the corresponding preimmune serum could be obtained (not shown). The direct interaction was further confirmed by chemical cross-linking in solution. When pSSU was incubated with the N-terminal constructs, specific cross-links were observed (Fig. 3E, lanes 1–4, triangles). Cross-links to psA or anaA migrate at 60–66 kDa, and cross-links to psB or anaB migrate at 40–45 kDa. These cross-links are in the expected size range. The cross-links are specific to the constructs added and to the presence of the transit peptide, because they are not observed while cross-linking the translation product itself (Fig. 3E, star) or using the mature form of SSU (Fig. 3E, lanes 6–9). The pSSU-paPspsB cross-links are more pronounced than pSSU-anaA/anaB cross-links (Fig. 3E, compare lanes 1 and 3; 2 and 4), suggesting that psToc75 reveals a higher affinity for the preproteins (Fig. 3, A and B). To confirm that the interaction between alr2269 and the preprotein is indeed specific and targeted to the N-terminal region, the full-length protein or the construct representing the pore-forming domain (anaC) were reconstituted into liposomes. Subsequently, the liposomes not containing proteins (Fig. 3F, lane 2), containing the full-length protein (Fig. 3F, lane 3), or the pore-forming region of alr2269 (Fig. 3F, lane 4) were incubated with 35S-labeled pSSU. Only in the presence of alr2269 was an increased interaction of the proteoliposomes with the preprotein in comparison to the empty liposomes observed (Fig. 3F, compare lanes 2 and 3). In addition, the association of 35S-labeled pSSU with alr2269 proteoliposomes (Fig. 3G, filled circles) but not with empty liposomes (Fig. 3G, triangles) or liposomes containing the pore-forming region (Fig. 3G, squares) was found to be time-dependent. This confirms the specificity of the interaction, because other outer membrane proteins not involved in the translocation do not stimulate the association of pSSU with lipid membranes (25). Hence, alr2269 contains an
The Function of the N-terminal Domain in Complex Assembly—The Toc complex contains four subunits, namely Toc75, Toc34, Toc64, and Toc159 (16). To analyze the role of the N-terminal domain of psToc75 in complex assembly, an affinity matrix coated with psA or psB was incubated with radioactive labeled Toc159, Toc64 (not shown), or the cytosolic domain of Toc34 (Fig. 4A). No direct interaction of Toc159 or Toc64 with any of the constructs could be observed (not shown). In contrast to Toc159 and Toc64, Toc34ΔTM binds to psA (Fig. 4A, upper panel, lane 3) but not to psB (upper panel, lane 5) or anaA (lower panel, lane 3). This result was confirmed by immunoprecipitation of the complex using psToc75 antibodies (not shown).

Furthermore, the missing interaction of Toc34ΔTM to psB (Fig. 4B) points to a receptor-binding site distinct from the precursor-binding site. Supporting this notion, Toc34 was not able to compete for the interaction of radioactive labeled pSSU with psA or anaA (Fig. 4C, lanes 2 and 5), even though an association was observed (not shown). In contrast, the addition of peptides (E2 A1) (Fig. 2E) representing the transit peptide of pSSU (Fig. 4C, lanes 3 and 6) drastically reduced the recognition of the preprotein.

Besides hetero-oligomerization, β-barrel proteins of the Omp85 class were found to assemble homo-oligomeric complexes (e.g., (37)). Therefore, we investigated the ability of the N-terminal region of alr2269 to facilitate homo-oligomerization. We demonstrated that the N-terminal domain of alr2269 interacts with itself, because the radioactive labeled protein was efficiently co-eluted with the coupled protein (Fig. 4D, lane 3, anaA). However, no significant interaction of anaA with
FIG. 4. The N-terminal domain of the proteins is involved in complex formation. A, 35S-labeled Toc34TM was incubated with psA, psD (upper panel), anaA, or anaB (lower part) coupled to Ni-NTA. Flow-through (FT, lanes 2 and 4) and elution (E, lanes 3, 5) were collected, separated by SDS-PAGE, and radioactivity-visualized. 100% of the translation product is shown (TP, collected and radioactivity-visualized. 100% of the translation product (gray bars) was Toc75 or alr2269 and anaA (black bars)). The average of at least three experiments is shown. C, 35S-labeled pSSU was incubated with psA (lanes 1–3) or anaA (lanes 4–6) coupled to Ni-NTA in the presence of expressed Toc34TM (T34, lanes 2 and 5) or the transit peptide of pSSU (P, lanes 3 and 6). Bound protein was collected and visualized by phosphorimaging. 100% of the translation product is shown (TP, lane 1). D, 35S-labeled anaA was incubated with psA, psB (upper panel), anaA, or anaB (lower panel) coupled to Ni-NTA (empty). Flow-through (FT, lanes 2, 4, and 6) and elution (E, lanes 3, 5, and 7) were collected and radioactivity-visualized. 100% of the translation product is shown (TP, lane 1). E, the interaction between the fragments of psToc75 or alr2269 and anaA (black bars) or anaB (gray bars) was quantified and normalized to the interaction efficiency of psA and Toc34TM. The average of at least three experiments is shown. F, a model of interacting the regions and the recognized targets for alr2269 and psToc75 is shown.

anaB, psA, or psB could be observed (Fig. 4D, lanes 3 and 5). In line with this, only a weak interaction of anaB with any of the constructs was observed (Fig. 4E, gray bars, anaA). Summarizing, both proteins (psToc75 and alr2269) are able to recognize preproteins with a domain localized in the second half of the N terminus (Fig. 4F, bar with bricks). In addition, the more extreme N-terminal domain (Fig. 4F, open box) is involved in complex formation.

Characteristics of the C-terminal Region of Toc75 and alr2269—After establishing that the N-terminal domain of psToc75 is involved in preprotein recognition and complex formation, we wanted to confirm the C-terminal location of the pore-forming region as postulated by the topological model (Fig. 1D). Therefore, psC, psD, anaC, or anaD was expressed, purified, and reconstituted into liposomes of outer envelope lipid composition. The reconstitution was confirmed by silver staining of the protein content of the proteoliposomes (Fig. 5A). Only in the case of psC was a second minor proteinaceous band observed. Carbonate extraction confirmed that this protein is not reconstituted (not shown). The insertion of the constructs into liposomes was taken as a first hint that the C-terminal region might indeed be involved in pore formation. To further analyze the pore formation, we went on to establish a liposome swelling assay using proteoliposomes containing psC or psD. First, the proteoliposomes were subjected to a hypertonic NaCl medium, and the time-dependent change of the optical density as a measure of the change of the ultrastructure of the liposomes was determined (Fig. 5B). The optical density of the empty liposomes increased in a time-dependent manner (Fig. 5B, solid line) due to liposome deformation upon osmotic pressure resulting from the addition of salt (38–40). This shift could not be observed using liposomes containing psD (Fig. 5B, dashed line) or psD (gray solid line). We concluded that the reconstituted constructs were able to form pore-like structures and to exchange solutes. This behavior was not dependent on the salt concentration in the range up to 100 mM NaCl (Fig. 5C). Furthermore, reconstituting Toc34 (not shown) or the N-terminal region of psToc75, psA (Fig. 5C, white squares) did not alter the behavior of the empty liposomes, supporting the notion that the C-terminal constructs facilitate solute exchange and that the N-terminal region cannot form a pore by itself. Next, we analyzed whether the same behavior can be obtained
domains of \( \beta \)-barrel transporters.

Organelles and the bacterial outer membrane has been reported (2, 4, 31). In addition, evidence for the evolutionary conservation of some features of the proteins has been presented (37). To investigate the relationship between the proteins from prokaryotes and eukaryotes with a common ancestor, we have studied psToc75 (16) and its related protein from Nostoc PCC7120, alr2269. Previously, it was suggested that proteins of the \( \beta \)-barrel polypeptide-transporting channel family have two distinct domains (34, 42), which is also reflected by the topological model of alr2269 (Fig. 1D). In line with this, we demonstrated that the N-terminal region of psToc75 acts as a specific receptor for proteins containing an N-terminal transit sequence (Fig. 6D). This observation supports the previously postulated binding site within psToc75 (1). Furthermore, the protein not involved in the translocation of preproteins across the outer envelope was able to specifically recognize the transit sequence containing proteins (Fig. 3). Hence, the interaction between the preproteins and alr2269 revealed a prerequisite of this feature in the
common ancestor of both proteins. The interaction between the \( \beta \)-barrel polypeptide transporter and preprotein is directed toward the N-terminal region (Fig. 3F). This leads to the question of whether the pore-forming protein itself offered the first binding site for preproteins after the cyanobacterium was incorporated into the host cell. The more extreme N-terminal region of the two proteins was found to be involved in complex formation of the translocon (Fig. 4). Here, the mode of interaction differs between both proteins. The region of \( \psi \)Toc75 containing a loop facing the cytosol is involved in hetero-oligomerization by recognizing the receptor Toc34 (Figs. 4, A and B, and 6D), whereas the N terminus of alr2269 is involved in homo-oligomerization (Fig. 4). Such homo-oligomerization was also obtained for other prokaryotic proteins (37). The divergence of the amino acid sequence of the extreme N terminus (Fig. 1A) supports a different mode of complex formation. In line with this, no clear homologues of receptors of the Toc machinery were identified in cyanobacteria (16).

The proposed topological dissection of the proteins (Fig. 1D) in two functional regions suggests a pore function of the C terminus. Investigations of Shib (42) and topological modeling of \( \psi \)Toc75 (19) or Omp85 (5) support such speculation. As presented herein, the C-terminal portion of \( \psi \)Toc75 or alr2269 allowed the passage of salt or sucrose (Fig. 5). The electrophysiological properties, such as the reverse potential and the main conductance of \( \psi \)C (not shown) and Toc75 (41) or alr2269 and the C-terminal portion of the protein, were comparable (Fig. 6). This result underlined the conclusion that the C-terminal domain builds the pore and questions the two proposed membrane-inserted strands in the N-terminal domain (Fig. 1D). In contrast to the C-terminal portion, the full-length alr2269 was not able to transport sucrose (Fig. 5, E and F). Guided by this observation, we speculate that the N-terminal portion has a function in gating of the channel (Fig. 6D). This notion is supported by the obtained difference of the gating behavior of the full-length protein and the C-terminal domain. The C-terminal domain itself shows a low gating profile, whereas the full-length protein rapidly changes its conductance (Fig. 6). This result underlined the conclusion that the C-terminal domain of alr2269 is involved in homo-oligomerization (Fig. 4). Such homo-oligomerization was also obtained for other prokaryotic proteins (42).

The diameter of alr2269 is smaller than the pore diameter of the C-terminal portion of the protein, were comparable (Fig. 6). Therefore, the transport activity of alr2269 and anaD show reflecting activity as well (Fig. 5). Using the swelling assay in the presence of 300 mM NaCl, we obtained an adaptation for proteoliposomes containing \( \psi \)C, anaC, or alr2269 but not of the liposomes containing \( \psi \)D and anaD (not shown). This leads to the speculation that the two constructs form multimeric ensembles within the membrane, which are disrupted in the presence of high concentration of salt (Fig. 6D). Therefore, the transport activity of \( \psi \)D and anaD reflecting eight conserved \( \beta \)-strands (31) leads to the hypothesis that \( \beta \)-barrel proteins, similar to helical proteins (44), have evolved from a minimal structural unit. Such minimal structure might be represented by toxins containing four or five segments and forming a multimeric \( \beta \)-barrel channel (45) or by the smallest monomeric \( \beta \)-barrel membrane proteins containing eight \( \beta \)-strands (45). Therefore, \( \psi \)D and anaD might reflect the evolutionary base unit for the development of the \( \beta \)-barrel pore.

Acknowledgments—We thank Prof. J. Soll for antibodies and advice during manuscript preparation and Prof. S. Nussberger for helpful discussions.

REFERENCES

1. Hinnah, S. C., Hill, K., Wagner, R., Schlicher, T., and Soll, J. (1997) EMBO J. 16, 7351–7369
2. Paschen, S. A., Waizenegger, T., Stan, T., Preuss, M., Czerniak, M., Hell, K., Rapaport, D., and Neupert, W. (2003) Nature 426, 862–866
3. Kosjek, V., Wiedemann, N., Milenkovic, D., Lohaus, C., Meyer, H. E., Guiraud, B., Pfanner, N., and Meisinger, C. (2003) J. Biol. Chem. 278, 48520–48523
4. Gentle, I., Gabriel, K., Beech, P., Waller, R., and Lohle, T. (2004) J. Cell Biol. 164, 19–24
5. Voulhoux, R., Bos, M. P., Geurtsen, J. M., Mol, M., and Tommassen, J. (2003) Science 299, 202–205
6. Jacob-Dubuisson, F., Locht, C., and Antoine, R. (2001) Mol. Microbiol. 40, 306–313
7. Ven, M. B., Poohody, C. R., Partovi, S. M., Zhai, Y., Tseng, Y. H., and Saier, M. H. (2002) Biochim. Biophys. Acta 1562, 6–31
8. Schiebel, E., Schwarz, H., and Braun, V. (1989) J. Biol. Chem. 264, 13311–13320
9. Hertle, R., Brutsche, S., Groeger, W., Hobbie, S., Koch, W., Konninger, U., and Braun, V. (1997) Mol. Microbiol. 25, 853–865
10. Genevros, S., Steggs, L., Roholl, P., Letessier, J. J., and van der Ley, P. (2000) EMBO J. 19, 1780–1789
11. Voulhoux, R., and Tommassen, J. (2004) Res. Microbiol. 155, 129–135
12. Gratzer, T., Lohle, T., Bauer, R. E., Lamping, E., Paltauf, F., Rohrbuehle, S. D., Haucke, V., Junne, T., Schatz, G., and Horst, M. (1995) J. Cell Biol. 129, 25–34
13. Waizenegger, T., Habib, S. J., Lech, M., Mokranjac, D., Paschen, S. A., Hell, K., Neupert, W., and Rapaport, D. (2004) EMBO Rep. 5, 704–709
14. Milenkovic, D., Kosjek, V., Wiedemann, N., Lohaus, C., Meyer, H. E., Guiraud, B., Pfanner, N., and Meisinger, C. (2004) J. Biol. Chem. 279, 22781–22785
15. Ishikawa, D., Yamamoto, H., Tamura, Y., Moritoh, K., and Endo, T. (2004) J. Cell Biol. 166, 621–628
16. Soll, J., and Schleiff, E. (2004) Nat. Rev. Mol. Cell Biol. 5, 198–208
17. Schleiff, E., Jelic, M., and Soll, J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 4604–4609
18. Sveshnikova, N., Grimn, R., Soll, J., and Schleiff, E. (2000) Biol. Chem. 381, 687–693
19. Schleiff, E., Eichacker, L. A., Eckart, K., Becker, T., Miro, O., Stahl, T., and Soll, J. (2000) Protein Sci. 12, 745–759
20. Boelter, B., Soll, J., Schulz, A., Hinnah, S., and Wagner, R. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 15831–15836
21. Kreusler, J. S., Dril, J., Janssens, P., and Lievens, P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 784–789
22. Luckey, M., and Nikaido, H. (1980) J. Bacteriol. 141, 1065–1072
23. Timmis, J. N., Ayliffe, M. A., Huang, C. Y., and Martin, W. J. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 621–626
24. Schleiff, E., Metzkar, M., and Soll, J. (2002) Plant Mol. Biol. 50, 177–185
25. Becker, T., Jelic, M., Vojta, A., Radunz, A., Soll, J., and Schleiff, E. (2004) EMBO J. 23, 520–530
26. Allen, M. M. (1968) J. Physiol. 4, 1–4
27. Luckey, M., and Nikaido, H. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 167–171
28. Bevans, C. G., and Harris, A. L. (1999) J. Biol. Chem. 274, 3711–3719
29. Mueller, P., Rudin, D., Tien, R., and Westcott, W. C. (1963) J. Phys. Chem. 67, 524–535
30. Becker, T., and Turnbull, J. L. (1998) Biochemistry 37, 13043–13051
31. Moslavec, S., Miro, O., Bredekemeier, R., Soll, J., von Hasseled, A., and Schleiff, E. (2005) FEBS Lett. 572, 1367–1370
32. Moslavec, S., Bredekemeier, R., Miro, O., Granvogl, B., Eichacker, L. A., and Schleiff, E. (2005) J. Proteome Res., in press
33. Sanchez-Pulido, L., Devos, D., Genevros, S., Vicente, M., and Valencia, A.
(2003) Trends Biochem. Sci. 28, 523–526
35. Tranel, P. J., Froehlich, J., Goyal, A., and Keegstra, K. (1995) EMBO J. 14, 2436–2446
36. Greenfield, N., and Fasman, G. D. (1969) Biochemistry 8, 4108–4116
37. Surana, N. K., Grass, S., Hardy, G. G., Li, H., Thanassi, D. G., and Geme, J. W., III (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 14497–14502
38. White, G. F., Racher, K. I., Lipski, A., Hallett, F. R., and Wood, J. M. (2000) Biochem. Biophys. Acta 1468, 175–186
39. Pencer, J., White, G. F., and Hallett, F. R. (2001) Biophys. J. 81, 2716–2728
40. Hincha, D. K. (2003) Biochim. Biophys. Acta 1611, 180–186
41. Hinnah, S. C., Wagner, R., Sveshnikova, N., Harrer, R., and Soll, J. (2002) Biophys. J. 83, 899–911
42. Koenninger, U. W., Hobbie, S., Benz, R., and Braun, V. (1999) Mol. Microbiol. 35, 1212–1225
43. Smart, O. S., Breed, J., Smith, G. R., and Sansom, M. S. (1997) Biophys. J. 72, 1109–1126
44. Shimizu, T., Mitsuke, H., Noto, K., and Arai, M. (2004) J. Mol. Biol. 339, 1–15
45. Wimley, W. C. (2003) Curr. Opin. Struct. Biol. 13, 404–411
The Evolutionarily Related β-Barrel Polypeptide Transporters from *Pisum sativum* and *Nostoc* PCC7120 Contain Two Distinct Functional Domains
Franziska Ertel, Oliver Mirus, Rolf Bredemeier, Suncana Moslavac, Thomas Becker and Enrico Schleiff

*J. Biol. Chem.* 2005, 280:28281-28289.
doi: 10.1074/jbc.M503035200 originally published online June 10, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M503035200

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2005/06/21/M503035200.DC1

This article cites 44 references, 18 of which can be accessed free at
http://www.jbc.org/content/280/31/28281.full.html#ref-list-1