The TolB Protein Interacts with the Porins of *Escherichia coli*

ALAIN RIGAL, EMMANUELLE BOUVERET, ROLAND LLOUBES, CLAUDE LAZDUNSKI,
AND HELENE BENEDETTI*

Laboratoire d'Ingénierie et de Dynamique des Macromolécules, Institut de Biologie Structurale et Microbiologie,
Centre National de la Recherche Scientifique, 13402 Marseille Cedex 20, France

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TolB is a periplasmic protein of the cell envelope Tol complex. It is partially membrane associated through an interaction with the outer membrane lipoprotein PAL (peptidoglycan-associated lipoprotein), which also belongs to the Tol system. The interaction of TolB with outer membrane porins of *Escherichia coli* was investigated with a purified TolB derivative harboring a six-histidine tag. TolB interacted with the trimeric porins OmpF, OmpC, PhoE, and LamB but not with their denatured monomeric forms or OmpA. These interactions took place both in the presence and in the absence of lipopolysaccharide. TolA, an inner membrane component of the Tol system, also interacts with the trimeric porins via its central periplasmic domain (R. Déroutie, M. Gavioli, H. Bénédetti, A. Prilipov, C. Lazdunski, and R. Llobès, EMBO J. 15:6408–6415, 1996). In the presence of the purified central domain of TolA (TolAIIHis), the TolB-porin complexes disappeared to form TolAIIHis-porin complexes. These results suggest that the interactions of TolA and TolB with porins might take place in vivo and might be concomitant events participating in porin assembly. They also suggest that the Tol system as a whole may be involved in porin assembly in the outer membrane.

The Tol system is a multiprotein complex of the *Escherichia coli* cell envelope. It is composed of three inner membrane proteins (TolA, TolQ, and TolR), two periplasmic proteins (TolB and Orf2), and a lipoprotein of the outer membrane (PAL [peptidoglycan-associated lipoprotein]). These proteins are encoded by clustered genes from two operons (24, 25). The inner membrane components of the complex interact with each other, inside the membrane, via their transmembrane domains (8, 17). TolB is partially associated with the outer membrane (13) through a specific interaction with PAL (3). Moreover, Tol proteins are thought to form a complex which has a specific stoichiometry and which may be mostly located in the contact sites between the inner and outer membranes of bacteria (12).

The exact physiological function of the Tol system is unknown. Nevertheless, these proteins are thought to be important for cell envelope integrity, since both *tol* and *pal* mutants are hypersensitive to drugs and detergents and release periplasmic proteins into the extracellular medium (6, 10). This role is probably important, as homologs of the Tol proteins are found in several gram-negative bacteria, including *Haemophilus influenzae* (7), *Brucella abortus* (22), *Pseudomonas putida* (21), and *Pseudomonas aeruginosa* (18). The central domain of TolA specifically interacts with trimeric porins such as OmpC, OmpF, LamB, and PhoE (9). Such interactions have been proposed to be involved in the process of assembly of outer membrane trimeric porins and to be relevant to the function of the Tol system.

In this study, we have investigated the interaction between trimeric porins and TolB. Like TolA, TolB interacted with the trimeric porins OmpF, OmpC, PhoE, and LamB, and no interaction was detected with OmpA or with the monomeric forms of the porins. Furthermore, our results indicated that OmpF could not interact with TolA and TolB at the same time

and that TolA displaced TolB from a TolB-OmpF complex to form a TolA-OmpF complex. These results are consistent with the involvement of TolB in the assembly of outer membrane trimeric porins and with the hypothesis that this involvement may be the specific function of the Tol system as a whole.

MATERIALS AND METHODS

Strains and plasmids. The *E. coli* strains and plasmids used in this study are described in Table 1.

Antibodies and proteins. Purified OmpF and LamB porins were generous gifts from Jurg Rosenbush. They were stored in 50 mM sodium phosphate buffer (pH 8) containing 100 mM NaCl and 1% *n*-octyl-polyoxyethylene (OPOE). Purification of TolAΔA (TolA with its membrane anchor deleted) and TolAIIHis (the α-helical central domain of TolA tagged with six histidines) was previously described (8, 9). TolBHis (TolB harboring a six-histidine tag) was purified from the lysate of W3110(pTolBHis)-transformed cells obtained as described by Bouveret et al. (4). The cleared lysate was applied to a 5-ml column of chelating Sepharose-agarose beads charged with Ni²⁺ and equilibrated with buffer A (50 mM sodium phosphate [pH 8], 100 mM NaCl, 5 mM imidazole). The column was washed with buffer A, and elution was performed with a linear gradient (starting buffer, buffer A; final buffer, 50 mM sodium phosphate [pH 8] containing 100 mM NaCl and 400 mM imidazole). Fractions were analyzed for the presence and purity of TolBHis by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and Western blotting with polyclonal anti-TolB/PAL antiserum (4). The protein was found to elute between 180 and 250 mM imidazole and was about 98% pure. TolBHis protein purified in this way was concentrated and dialyzed against 50 mM potassium phosphate buffer (pH 8). About half of the TolBHis was lost during these steps through aggregation and adsorption onto plastic tubes. The final concentration of TolBHis was 1 mg/ml.

Antibodies directed against TolBHis and OmpF were raised in rabbits with purified proteins. Four injections of 300 μg of protein mixed with Freund's adjuvant were given over a period of 40 days. Antibodies directed against OmpF also recognized PhoE, OmpC, and OmpA but not LamB. Antibodies directed against TolA have been described elsewhere (8).

Preparation of membrane extracts enriched with porins. Membrane extracts enriched with OmpA and with lipopolysaccharide (LPS)-associated OmpC, OmpF, and PhoE were prepared from BL21(DE3)Δ cells transformed with plasmids pOmpA, pOmpC, pOmpF, and pPhoE, respectively. The pellet of cells was resuspended in 20 mM Tris buffer (pH 8) (optical density at 600 nm [OD₆₀₀], 15 U · ml⁻¹), and cells were lysed by sonication. Membranes were recovered after 30 min of centrifugation at 105,000 × *g*. The membrane pellet was incubated for 1 h at 60°C in 20 mM Tris buffer (pH 8) containing 1% Triton X-100 (OD₆₀₀, 45 U · ml⁻¹). The mixture was centrifuged at 105,000 × *g* for 30 min, and the pellet was incubated for 1 h at 37°C in 20 mM Tris buffer (pH 8) containing 0.125% OPOE (OD₆₀₀, 45 U · ml⁻¹). The mixture was centrifuged at 105,000 × *g* for 30 min, and the pellet was solubilized for 1 h at 37°C in 20 mM Tris buffer (pH 8) containing 3% OPOE (OD₆₀₀, 45 U · ml⁻¹). The supernatant obtained

* Corresponding author. Mailing address: Laboratoire d'Ingénierie et de Dynamique des Macromolécules, Institut de Biologie Structurale et Microbiologie, Centre National de la Recherche Scientifique, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France. Phone: (0)4 91 16 45 54. Fax: (0)4 91 71 21 24. E-mail: benedetti@ibsm.cnrs-mrs.fr.

TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Genetic description	Source or reference
Strains		
W3110	Wild type	2
BL21(DE3) Δ	<i>ompF</i> (<i>E. coli</i> B) <i>ompA</i>	A. Prilipov
	<i>ompC lamB</i>	
1292	<i>supE hsdS met gal lacY</i>	W. Wood
	<i>fluA</i>	
JC7782	1292 <i>tolA</i>	J. C. Lazzaroni
JC7752	1292 <i>tolB-pal</i>	J. C. Lazzaroni
Plasmids		
pOmpA	<i>ompA</i> Ap ^r	A. Prilipov
pOmpC	<i>ompC</i> Ap ^r	A. Prilipov
pOmpF	<i>ompF</i> Ap ^r	A. Prilipov
pPhoE	<i>phoE</i> Ap ^r	A. Prilipov
pTolBHis	<i>tolB</i> Cm ^r	4
pMuIII	<i>tolB</i> Tc ^r	13

after centrifugation for 30 min at $105,000 \times g$ was found to be enriched in OmpA or in the different LPS-associated porins.

Coprecipitation experiments. TolB protein was immobilized on Ni-nitrilotriacetic acid (NTA)-agarose beads (QUIAGEN) which had been incubated with purified TolBHis (10 μ g) or W3110(pTolBHis)-transformed cell lysates (100 μ l, corresponding to an OD₆₀₀ of about $180 \text{ U} \cdot \text{ml}^{-1}$). The beads were washed three times with 50 mM sodium phosphate buffer (pH 8) containing 100 mM NaCl and 20 mM imidazole to eliminate nonspecific binding. They were then equilibrated in buffer C (50 mM sodium phosphate buffer [pH 8] containing 100 mM NaCl) containing 0.1 or 1% SDS, 1% *n*-octyl- β -D-glucopyranoside (OG), or 1% *n*-dodecyl- β -D-maltoside (LM). Either pure trimeric or monomeric OmpF (5 μ g) or membrane extracts enriched with LPS-associated OmpF (10 μ l) were added to each mixture for 1 h of incubation at room temperature. The beads were washed three times with buffer C, resuspended in sample buffer (14), and heated for 20 min at 96°C. Eluates were analyzed by SDS-PAGE and Western blotting with the antibodies raised against OmpF.

To test the effect of TolA Δ (8) on the TolB-OmpF interaction, TolA Δ (1 μ g) was added to the beads after the formation of the TolB-OmpF complex in buffer C containing 0.1% SDS. Following 1 h of incubation at room temperature, the supernatant was recovered, and the beads (pellet) were washed three times in buffer C containing 0.1% SDS and then resuspended in sample buffer. The eluate and the supernatant were analyzed by SDS-PAGE and Western blotting with the antibodies directed against OmpF.

Miscellaneous. SDS-PAGE were performed as described by Laemmli (14), except that the sample buffer used for the detection of protein complexes contained only 0.1% SDS and no heating was performed before sample loading. Proteins were electrotransferred onto nitrocellulose membranes as described by Towbin et al. (23) but for a longer period of time (3 h). Western blot analysis was performed as described by Gorvel et al. (11). Standard methods were used for cell transformation (19). For preparation of cell lysates, the cells were grown to an OD₆₀₀ of $1 \text{ U} \cdot \text{ml}^{-1}$, harvested, and resuspended in 10 mM sodium phosphate buffer (pH 6.8) containing 1% SDS to a final OD₆₀₀ of $10 \text{ U} \cdot \text{ml}^{-1}$. Lysates were obtained by five successive freezing-thawing cycles followed by centrifugation for 30 min at $18,000 \times g$.

RESULTS

Purified TolB and the trimeric form of OmpF form a high-molecular-weight complex. Purified TolB (TolBHis) was a derivative harboring a six-histidine tag at the N terminus of the protein. First, we tested the formation of a TolBHis-OmpF complex in the presence of SDS, since this detergent is required for the formation of the TolA-OmpF complex (9). TolBHis was incubated either with purified trimers or with heat-denatured monomers of OmpF in the presence of 0.1% SDS. The formation of the TolBHis-OmpF complex was analyzed by an SDS-PAGE mobility shift assay as described by Drouiche et al. (9). A high-molecular-weight complex was observed only when TolBHis was incubated with the trimeric form of OmpF (Fig. 1). Its migration was consistent with a complex having 1:1 stoichiometry. When TolBHis was present in molar excess over the purified OmpF trimer, OmpF was

found entirely in the high-molecular-weight complex. Similarly, when the purified OmpF trimer was present in molar excess over TolBHis, all of TolBHis was shifted into the complex (Fig. 1).

Incubation of TolBHis with the LPS-associated trimeric form of OmpF in the presence of 0.1% SDS also resulted in the formation of high-molecular-weight complexes with the same multiband pattern as that observed for trimeric porins associated with LPS (Fig. 1).

The amounts of the high-molecular-weight complexes formed were not affected when TolBHis and OmpF trimers were not preincubated (data not shown). The formation of TolBHis-OmpF complexes was not affected by the presence of 1% SDS but was sensitive to 96°C heating, as for TolA-OmpF complexes (data not shown).

The high-molecular-weight complexes formed in the presence of the OmpF trimers (associated or not associated with LPS) contained both the OmpF and the TolBHis proteins, as shown by Western blotting with antibodies directed against either protein (Fig. 2).

TolBHis interacts with *E. coli* trimeric porins. Membrane extracts enriched with different porins were prepared from cells expressing specifically each porin from a plasmid and were incubated with TolBHis in the presence of 0.1% SDS. TolBHis-porin complexes were detected by an SDS-PAGE mobility shift assay after Coomassie blue staining (Fig. 3) or Western blotting (data not shown).

TolBHis interacted with the trimeric porins PhoE, OmpC, and LamB but did not interact with OmpA (Fig. 3). No preincubation was required to observe the TolBHis-porin complexes (data not shown).

Coprecipitation of porins with tagged TolB. Another alternative for demonstrating TolBHis-porin interactions was to test whether TolBHis immobilized on Ni-NTA beads was able to coprecipitate the porins. For these experiments, immobilized TolBHis was obtained by incubating the Ni-NTA beads either with cell extracts containing TolBHis or with purified TolBHis. Purified OmpF trimers (associated or not associated

FIG. 1. TolBHis interacts with the trimeric form of OmpF. The formation of a TolBHis-OmpF complex was analyzed by SDS-PAGE mobility shift assays followed by Coomassie blue staining. The amounts of purified proteins used are indicated. OmpF LPS was 10 μ l of membrane extract enriched with LPS-associated OmpF. Monomeric OmpF (OmpF M) was obtained by heating the same amount of OmpF LPS for 20 min at 96°C. Molecular weight markers are indicated on the left (in thousands).

FIG. 2. Western blotting analysis of the interaction of OmpF porins with TolBHis. Purified OmpF (4 μ g) or membrane extract enriched with LPS-associated OmpF (OmpF LPS) (10 μ l) was mixed, in the presence of 0.1% SDS, with TolBHis (4 μ g) or with the same volume of 50 mM phosphate buffer (pH 8). The formation of TolBHis-OmpF complexes was analyzed by SDS-PAGE mobility shift assays followed by Western blotting and immunodetection with anti-TolB or anti-OmpF antibodies. Molecular weight markers are indicated on the left (in thousands).

with LPS) were found to coprecipitate with immobilized TolBHis in the presence of 0.1% SDS, whereas denatured monomers did not bind to TolBHis (Fig. 4A). As a control, we verified that OmpF trimers did not interact with the Ni-NTA beads alone. Similar results were obtained with 1% SDS (data not shown). OmpF trimers bound nonspecifically to Ni-NTA

FIG. 3. TolBHis interacts with the trimeric forms of OmpC, PhoE, and LamB but not OmpA. Purified LamB (2 μ g) or membrane extracts enriched with OmpA or LPS-associated OmpC and PhoE (10 μ l of each) were mixed, in the presence of 0.1% SDS, with TolBHis (4 μ g) or used directly as controls. The formation of TolBHis-porin complexes was analyzed by SDS-PAGE mobility shift assays followed by Coomassie blue staining. Molecular weight markers are indicated on the left (in thousands), and the positions of the porins are indicated on the right.

FIG. 4. OmpF trimers are coprecipitated with TolBHis immobilized on beads in the presence of SDS. TolBHis was immobilized on beads by incubation of Ni-NTA beads with purified TolBHis (10 μ g) or W3110(pTolBHis)-transformed cell lysates (100 μ l). The beads were then incubated for 1 h at room temperature with purified trimeric OmpF (OmpF) (5 μ g), membrane extracts enriched with LPS-associated trimeric OmpF (OmpF LPS) (10 μ l), or monomeric OmpF (OmpF mono) (5 μ g) in the presence of 0.1% SDS (A) or 1% OG (B). As a control, beads with no immobilized TolBHis were incubated under the same conditions with OmpF, OmpF LPS, or OmpF mono. The beads were washed three times, centrifuged, resuspended in sample buffer, and heated for 20 min at 96°C. The eluates were analyzed by SDS-PAGE and Western blotting with anti-OmpF antibodies. Lanes labeled OmpF LPS, OmpF, and OmpF mono designate the control experiments. Lanes labeled OmpF LPS loaded and OmpF loaded contained the total amounts of OmpF LPS and OmpF, respectively, added to the beads.

beads alone when the incubation was carried out with 1% OG (Fig. 4B) or 1% LM. The amount of coprecipitated OmpF seemed to be slightly higher in the presence of TolBHis (Fig. 4B), but the high background made it difficult to draw firm conclusions.

TolA displaces TolBHis in the TolBHis-OmpF complex. When TolA $\Delta\Delta$ (TolA with its membrane anchor deleted and with no six-histidine tag) was added to Ni-NTA beads chelated to the TolBHis-OmpF complex in the presence of 0.1% SDS and in amounts equal to those of TolBHis, TolA $\Delta\Delta$ was not retained on the beads (Fig. 5). Surprisingly, OmpF was no longer present on the beads. It was detected in the supernatant, with TolA $\Delta\Delta$ (Fig. 5). When smaller amounts of TolA $\Delta\Delta$ were added, a fraction of OmpF remained associated with the beads, and another fraction was detected in the supernatant with TolA $\Delta\Delta$ (data not shown). These results suggested that a TolBHis-OmpF-TolA $\Delta\Delta$ complex does not form (unless very transiently) and that TolA $\Delta\Delta$ interacts with OmpF and replaces TolBHis in the TolBHis-OmpF complex.

This finding was also assessed by an SDS-PAGE mobility shift assay. The TolAIIHis derivative (the α -helical central domain of TolA tagged with six histidines), which interacts with trimeric porins (9), was added to the preformed TolBHis-OmpF complex in amounts equal to those of TolBHis. The TolBHis-OmpF complex disappeared completely and was replaced by the TolAIIHis-OmpF complex (Fig. 6). When smaller amounts of TolAIIHis were added to TolBHis-OmpF, both complexes, TolBHis-OmpF and TolAIIHis-OmpF, were detected (data not shown). In contrast, when the TolAIIHis-OmpF complex was preformed before the addition of TolBHis, the amount of the TolAIIHis-OmpF complex did not change and no TolBHis-OmpF complex was detected (Fig. 6). Western blot analysis did not detect any TolAIIHis-TolBHis-OmpF complex (data not shown). These results are consistent with

FIG. 5. TolA $\Delta\Delta$ prevents OmpF trimers from coprecipitating with TolBHis immobilized on beads. TolBHis was immobilized on beads by incubation of Ni-NTA beads with W3110(pTolBHis)-transformed cell lysates (100 μ l). The beads were incubated for 1 h at room temperature with purified trimeric OmpF (5 μ g) in the presence of 0.1% SDS. The beads were washed three times, centrifuged, resuspended in sample buffer, and heated for 20 min at 96°C (lane 1). In a parallel experiment, the beads were incubated with OmpF for 1 h, washed three times, centrifuged, and incubated for 1 h at room temperature with TolA $\Delta\Delta$ (1 μ g). The resulting supernatant (Supt) was loaded on SDS-polyacrylamide gels after the addition of sample buffer (lane 3). The beads were washed three times, resuspended in sample buffer, and heated for 20 min at 96°C (lane 2). The presence of OmpF in the samples was analyzed by SDS-PAGE and Western blotting with anti-OmpF antibodies. Anti-TolA antibodies were used to detect TolA $\Delta\Delta$ in the supernatant (lane 3).

TolA competing with a higher affinity than TolBHis for the OmpF interaction.

TolBHis-OmpF complex formation is inhibited by crude cell extracts. In contrast to TolA-porin complexes, TolB-porin complexes were not detected in crude cell extracts (data not shown). TolB-porin complexes were not detected either when TolB was overproduced or when *tolA* (JC7782) or *pal* [JC7752(pMuII)] mutant cells were used (data not shown). When a cell lysate of wild-type cells or of *tolA* or *pal* mutant cells was added to purified TolBHis and OmpF proteins in the presence of SDS, the formation of the TolBHis-OmpF complex in vitro was inhibited (Fig. 7), suggesting that the presence of proteins other than TolA and PAL inhibited the formation of the TolBHis-OmpF complex.

DISCUSSION

TolA interacts with the trimeric forms of the major LPS-associated porins of the outer membrane (OmpF, OmpC, PhoE, and LamB) via its central domain (9). This study showed that TolBHis could also interact with the trimeric forms of these proteins but, as in the case of TolA, it did not interact with the denatured monomeric forms of the porins or with OmpA. These interactions took place both in the presence and in the absence of LPS. Two methods were used to investigate these interactions. Both used a purified TolB derivative (TolBHis) and either purified porins (LPS free) or membrane extracts. These membrane extracts were enriched with either OmpA or the LPS-associated forms of the other porins. The first method, SDS-PAGE mobility shift assays, was used to identify both the TolBHis-porin and the TolBHis-porin-LPS complexes after Coomassie blue staining or Western blotting. The second method took advantage of the six-histidine-tagged TolB derivative. In this case, the interaction between the por-

ins and TolBHis was detected due to the ability of the porins to coprecipitate with TolBHis associated with Ni-NTA beads. This interaction was specific to TolB and was not induced by the six-histidine tag. Indeed, TolAIIIHis (C-terminal domain of TolA tagged with six histidines) did not interact with the porins (9). Moreover, immunopurified TolBep, tagged with a 15-amino-acid epitope recognized by monoclonal antibody 1C11 (13), interacted with the porins in SDS-PAGE mobility shift assays (data not shown). Different data argue in favor of a specific TolB-porin interaction: first, the stoichiometry of the complex, which seems to be 1:1 (at least for OmpF and LambB); second, the replacement of TolBHis by TolAIIIHis in the TolBHis-OmpF complex; and finally, the absence of an interaction between TolBHis and OmpA. The function of the TolA-porin and TolB-porin interactions is unknown. The interactions might play a direct role in porin activity. Another hypothesis, suggested by Drouiche et al. (9) for the TolA-porin complex, is that such interactions might be involved in the assembly of LPS-associated porins in the outer membrane. The fact that both TolA and TolB interacted with the porins suggests that the Tol system as a whole may be involved in porin assembly by direct protein-protein interactions. TolB interacts with PAL. We are now testing whether PAL also interacts with the porins and whether TolB complexed to PAL still exhibits an affinity for the porins.

SDS is required for the TolA-porin interaction. SDS may modify TolA conformation or may induce a backward step in porin maturation (from stable trimers to metastable trimers), thereby furthering the TolA-porin interaction (9). Although our experiments did not allow us to demonstrate the same requirement for SDS in the TolB-porin interaction, they

FIG. 6. TolAIIHis competes with TolBHis for interaction with OmpF. The first three lanes of the gel show the migration of the purified trimeric forms of OmpF (3 μ g), TolAIIHis (3 μ g), and TolBHis (3 μ g) following SDS-PAGE and Coomassie blue staining. The fourth and fifth lanes represent experiments in which TolAIIHis (3 μ g) and TolBHis (3 μ g) were incubated for 10 min with OmpF (3 μ g) in the presence of 0.1% SDS. They indicate the molecular weights of the TolAIIHis-OmpF and TolBHis-OmpF complexes. In the sixth lane, TolBHis and OmpF were incubated for 10 min and TolAIIHis was added, followed by a further 10 min of incubation. In the seventh lane, TolAIIHis and OmpF were incubated for 10 min and TolBHis was added, followed by a further 10 min of incubation. Molecular weight markers are indicated on the left (in thousands).

FIG. 7. TolBHis-OmpF interaction is inhibited by different cell lysates. Membrane extracts enriched with LPS-associated OmpF (10 μ l) were preincubated for 30 min with lysates (0.5 OD₆₀₀ unit) of wild-type cells (1292) (lane 1), *tolA* mutant cells (JC7782) (lane 2), or *pal* mutant cells [JC7752(pMuIII)] (lane 3) and then mixed with TolBHis (4 μ g). Lane 4, control in which membrane extracts enriched with LPS-associated OmpF (10 μ l) were mixed with TolBHis (4 μ g) without preincubation with lysate. The formation of TolBHis-OmpF complexes was analyzed by SDS-PAGE mobility shift assays followed by Western blotting and immunodetection with anti-TolB antibodies. Molecular weight markers are indicated on the right (in thousands).

showed that TolB interacts with the porins in the presence of SDS. This ability may mean that the porins are in similar conformations when they interact with TolA and TolB. Therefore, the interactions of porins with TolA and TolB *in vivo* may be concomitant events or at least closely related steps.

To clarify this point, the effect of TolA derivatives (TolAIHHis and TolA $\Delta\Delta$) on TolBHis-porin interactions was investigated with the two methods described above. The results obtained from both methods were similar. A trimeric TolA-TolBHis-porin complex was never detected, and TolA replaced TolBHis in the TolBHis-porin or TolBHis-porin-LPS complexes to form TolA-porin or TolA-porin-LPS complexes. This finding might reflect the sequence of molecular events involved in porin assembly, and it is tempting to speculate that the TolB-porin interaction occurs before the TolA-porin interaction. TolA-porin complexes were detected in crude cells extracts, whereas TolB-porin complexes were not. This finding was not a problem of antibody accessibility, since TolBHis-porin complexes formed *in vitro* were detected by antibodies directed against both proteins. The presence of TolA or PAL in the cell extracts was not responsible for the absence of TolB-porin complexes. However, the presence of other components in the extracts inhibited the formation of the complexes because purified TolBHis and OmpF trimers did not interact in the presence of *tolA* or *pal* cell lysates. These components might be periplasmic chaperones which are supposed to interact with the porins (5, 15, 20), or they might be other Tol proteins. In the presence of SDS, these components might modify OmpF trimer conformation or trap OmpF trimers in a complex so that they cannot interact with TolB. Cross-linking with formaldehyde did not make the detection of TolB-porin complexes *in vivo* possible. It is possible that formaldehyde does not cross-link TolB to porins or that the TolB-porin interaction is too transient to be detected by this method.

The interaction of TolB with trimeric porins suggests that

the whole Tol system may be involved in porin assembly. However, *tol* mutations do not prevent the process completely, since *tol* mutants are fully viable and display assembled porins, although in smaller amounts, in their outer membrane (9, 16). It is possible that the role of the Tol system in porin assembly is direct but only of a kinetic order. Another possibility is that the Tol system plays only an indirect role in porin assembly. The pleiotropic phenotypes (leakiness and hypersensitivity to drugs and detergents) of the *tol* mutants, which reflect an alteration of their outer membrane integrity, may be the cause rather than the consequence of the defect in porin assembly.

TolB, like TolA, also interacts with the N-terminal domain of different Tol-dependent colicins (4, 4a). Distinct domains of TolA are involved in interactions with colicin A (domain III) and with porins (domain II). Little is known about TolB structure, and efforts are now under way to identify the regions of the protein involved in various interactions (1).

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