FEBS Letters 537 (2003) 42–46 FEBS 26983

Identification of key regions within the *Escherichia coli* TatAB subunits

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Received 17 December 2002; revised 20 January 2003; accepted 21 January 2003

First published online 29 January 2003

Edited by Gunnar von Heijne

Abstract The twin-arginine translocation (Tat) system catalyzes the transport of folded proteins across the bacterial plasma membrane or the chloroplast thylakoid membrane. In *Escherichia coli* and most other species, three important *tat* genes have been identified but the structure and mechanism of this system are poorly understood; the role and location of TatA are particularly unclear. In this report we have used site-specific mutagenesis to probe the significance of conserved features of the related TatA/B subunits. We find that an apparent 'hinge' region between the transmembrane (TM) span and an adjacent amphipathic region is important in both proteins, in that substitution of turn-inducing residues inhibits the export of a natural Tat substrate. Surprisingly, large-scale mutagenesis of the conserved amphipathic regions of TatA and TatB leads only to minor effects on Tat-dependent export suggesting that this particular feature is not central to the translocation mechanism. This domain is, however, critical for the translocation process and we identify Gly/Pro residues in these regions of TatA/B that are essential for efficient export.

Key words: Protein translocation; Twin-arginine translocation; TatAB subunits; *Escherichia coli*

1. Introduction

The twin-arginine translocation (Tat) system is a protein transporter found in the thylakoid membranes of photosynthetic organisms and the plasma membranes of most free-living bacteria (reviewed in [1,2]). It recognizes substrates bearing cleavable signal peptides that contain a twin-arginine motif which is usually essential for efficient translocation [3,4]. Thereafter, the substrates are transported into the thylakoid lumen or periplasm and the precursor protein is processed to the mature size. Operating in parallel with Sec-type protein transport systems, the Tat system is completely different in key mechanistic respects. Whereas Sec-type systems invariably transport proteins in a largely unfolded state (reviewed in [2]), there is now convincing evidence that the Tat system is able to transport fully-folded proteins across the thylakoid membrane and bacterial plasma membrane [5–11]. The primary substrates for the Tat system in *Escherichia coli* are a series of periplasmic redox proteins that contain FeS, molybdopterin or other complex cofactors [7,8]. These cofactors are inserted in the cytosol by complex pathways which must necessitate the subsequent export of the protein in a largely, if not completely folded state.

To date, three core *tat* genes (*tatABC*) have been identified in bacteria [9–12] and homologous genes encoding the chloroplastic Tat subunits have been characterized in plants [13–15]. Purification of a Tat complex from detergent-solubilized *E. coli* membranes [16] yields a ca. 650 kDa complex containing only TatABC, further emphasizing the involvement of only three protein constituents. However, this complex has not been shown to be active and the involvement of further Tat subunits cannot be ruled out at present. The known Tat subunits are not related to any other gene products and the energetics of translocation are also unique among known protein transporters: the thylakoidal system is known to require the ΔpH across the membrane but not to require any form of nucleoside triphosphate hydrolysis [17,18]. However, these unusual aspects, combined with the relatively recent discovery of the system in bacteria, have also meant that the structure and mechanism of the system are rather poorly understood. Nevertheless, the first details of the Tat system organization have appeared during the last year or so. First, it has been shown that TatB and TatC form a structural and functional unit within the *E. coli* Tat system, being tightly associated in a 1:1 ratio and remaining active even as a TatB–TatC chimera [16]. Secondly, studies on the thylakoidal system [19,20] indicate that this TatB–TatC complex forms the initial binding site for substrates, with TatA being recruited at a later step in the overall translocation process. Thirdly, mutagenesis of TatC has revealed the presence of critical residues on the *cis* side of the membrane, consistent with a role in substrate binding [21,22].

While the above studies have started to explore the structure and function of the Tat system, many details have yet to be resolved. In particular, the functions of the TatA protein are as yet unknown and it is equally unclear how it carries out a function distinct from TatB despite being homologous in certain regions. Several highly conserved TatA/B characteristics have been pinpointed as potentially important, but their relevance has not been addressed experimentally. The mode by which TatA interacts with the TatBC core is also poorly understood at the present time. In this study we have used site-specific mutagenesis to probe the structure–function relationships in TatA and TatB. The data illustrate that a highly conserved 'hinge' region plays an important function in both TatA and TatB whereas another conserved TatA/TatB feature, an amphipathic helical region, is surprisingly unimportant.

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0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00068-1

2. Materials and methods

2.1. Bacterial strains, plasmids and growth conditions

E. coli strain MC4100 [23] was the parental strain; Δ*tatAE*, Δ*tatB*, Δ*tatC*, Δ*tatABCDE* have been described before [10–12,24], and arabinose-resistant derivatives were used as described [25]. Plasmid pBAD-ABC has been described before [25]. *E. coli* was aerobically grown at 37°C in modified low salt Luria broth (lsLB) [26], or anaerobically in lsLB-GT medium, consisting of lsLB supplemented with glycerol (0.5%), trimethylamine *N*-oxide (TMAO; 0.4%), and ammonium molybdate (1 μM), or in minimal TMAO/glycerol medium [25]. Medium supplements were used at the following final concentrations: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG); arabinose 200 μM.

Site-specific mutagenesis was used to generate a vector that encoded the *tat* operon within pBAD-ABC with point mutations in the *tatA* or *B* genes, using the Quickchange™ mutagenesis system (Stratagene) according to the manufacturer's instructions. The effects of these mutations were analyzed using an assay for TMAO reductase activity as detailed below. For studies on the effects of these mutations on green fluorescent protein (GFP) export, the *tatABC* sequences were removed from pBAD-ABC using *Nhe*I and *Xba*I, and cloned into the pEXT22 vector [27] using the *Xba*I site, generating pEXT-ABC and mutant derivatives which are compatible with pJDT1 encoding TorA-GFP. An identical strategy was used to analyze TatC mutants in a previous study [21] and further details are given in this paper.

2.2. Cell fractionations

Cells were grown aerobically (in lsLB medium) or anaerobically (in lsLB-GT medium) in the presence or absence of IPTG or arabinose (as stated in the text), and periplasm and spheroplasts were prepared by the ethylenediamine tetraacetic acid (EDTA)/lysozyme/cold osmoshock procedure [28]. Spheroplasts were lysed by sonication, and intact cells and cellular debris were removed by centrifugation (5 min at 10 000 × *g*). Membranes were separated from the cytoplasmic fraction by centrifugation (30 min at 250 000 × *g*). Protein concentration was determined using a BCA-linked assay (Pierce). Protein fractions were separated on a 10% non-denaturing polyacrylamide gel and analyzed for TorA activity [8].

3. Results

3.1. Conserved residues in TatA and TatB

TatA and TatB are homologous proteins that carry out very different functions in the *E. coli* Tat system. Each contains a single transmembrane (TM) helix (N-terminus on the periplasmic side of the membrane), and this span is followed by a predicted amphipathic α-helical region on the cytoplasmic face [1,2]. TatA is a relatively small protein (89 residues) with only a short cytoplasmic domain following the amphipathic region, whereas TatB is larger (171 residues) with more extended amphipathic and cytoplasmic domains. Fig. 1 shows an alignment of various bacterial TatA and TatB proteins, in which TM spans and amphipathic regions are indicated and conserved residues or regions boxed or indicated by asterisks. The sequences exhibit surprisingly little sequence homology and only a few residues are invariant throughout bacteria (shown in bold). These include the FG sequence just after the TM span in TatA and the GP sequence at a similar position in TatB. It should, however, be noted that these motifs are not absolutely diagnostic of TatA/TatB identity since in plants, the Tha4 and Hcf106 proteins (believed to be homologous to TatA/TatB, respectively) each contain both motifs (i.e. the sequence FGP).

The residues in *E. coli* TatA and TatB targeted for mutagenesis are shown underlined in Fig. 1. We focused particular attention on the FG sequence in TatA and the GP sequence in TatB since the highly conserved turn-inducing Gly/Pro residues may be indicative of a 'hinge' function for this region. We also substituted the Glu8 residue in TatB (by both Ala and Gln) since this residue is totally conserved throughout TatB proteins and apparently located in the TM span. The presence of an acidic residue in such a position raises the possibility of a role in proton conductance, and the thylakoidal Tat system is indeed driven by a proton-motive force [17,18]. These are in fact the only invariant residues among TatA/TatB sequences but others are highly conserved and several were substituted (by alanine) at positions indicated in Fig. 1. Mutations were made within the *tatABC* operon cloned into the arabinose-inducible pBAD24 plasmid, as used previously for studies on TatC function [21]. The TatC protein encoded by this plasmid (pBAD-ABC) contains a C-terminal Strep II tag to aid identification of TatC and purification of the complex by Streptactin affinity chromatography. The mutated operons were expressed in a Δ*tatABCDE* strain lacking all known Tat components. All of the mutants were analyzed to assess the levels of TatABC expressed, and the TatB and TatC levels were found to be similar in all cases (data not shown). The levels of TatA varied to a slightly greater extent for unknown reasons.

3.2. Important residues in TatA and TatB

We first analyzed the export of an authentic Tat substrate, TMAO reductase (TorA), in cells expressing TatA/B proteins that contained mutations in the invariant residues. These tests used a native gel electrophoresis system which was specifically stained for TMAO reductase activity; the assay is only semi-quantitative at best but drastic effects on export are readily apparent. In wild-type cells expressing pBAD-ABC, TorA is located primarily in the periplasm (P), as expected, whereas the activity is exclusively in the cytoplasm (C) of Δ*tatABCDE* cells. The data obtained with TatA/B mutants show that TorA is exported in cells expressing TatA/F20A, TatA/G21A, TatB/G21A and TatB/P22G. However, a more slowly migrating form of TorA (denoted TorA*) is observed in each case, indicating an inhibition of export. In the same experiment we analyzed TatB mutants containing Ala or Gln in place of the invariant Glu8 residue. Surprisingly, both mutants export TorA with wild-type efficiency suggesting that this residue does not play a key role in the export process.

TorA export assays were also conducted with the other TatA/B mutants generated in this study, but it was found that more precise information could be obtained using a TorA-GFP export assay that is described below. However, the effects of two further mutations are shown in Fig. 2 because they proved to drastically affect the Tat export process. These are TatA/G33A and TatB/P26A; Fig. 2 shows that these mutations severely affect export in that considerable TorA activity is found in the cytoplasm.

3.3. Export of TorA-FGP in cells expressing mutated TatA/B subunits

Strains expressing the mutant TatA/B proteins were also tested for their ability to export a chimeric protein comprising the TorA signal peptide linked to green fluorescent protein (GFP). This chimera is exported exclusively by the Tat pathway [26] and was used to assess the effects of TatC mutations in an earlier study [21]. The assay is far more quantitative than the TorA assay and for these experiments we used a plasmid, pJDT1 [26] encoding TorA-GFP in the arabinose-

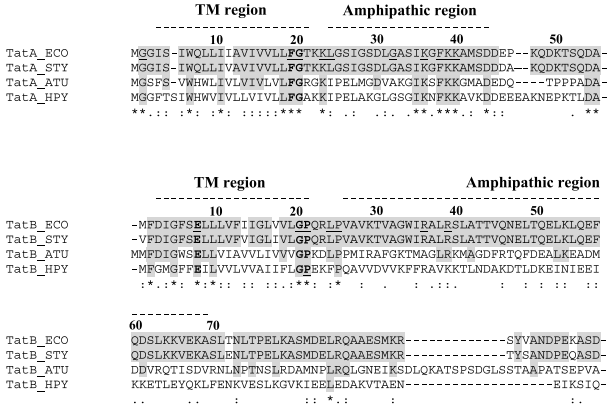

Fig. 1. Primary structures of bacterial TatA/B proteins. The figure shows an alignment of the amino-terminal regions of TatA/TatB proteins from *E. coli* (ECO), *Salmonella typhimurium* (STY), *Helicobacter pylori* (HPY) and *Agrobacterium tumefaciens* (ATU). The single transmembrane spanning regions and predicted amphipathic regions are indicated and conserved residues are boxed and denoted by asterisks. The C-terminus of TatB is located in the cytoplasm since it interacts with the N-terminus of TatC [16] which is known to be cytoplasmically located [30]. TatA is predicted to adopt the same topology because it is homologous to TatB. Residues that are absolutely conserved throughout bacteria are shown in bold; those mutated in this study are underlined.

inducible pBAD24 vector, with the mutant *tatABC* operons cloned into the compatible, IPTG-inducible pEXT22 vector. The wild-type *tatABC* operon in pEXT22 (plasmid pEXT-ABC) supports efficient export of TorA-GFP in Δ*tatABCDE* cells [21].

Fig. 3A shows assays for the export of TorA-GFP by the strains expressing mutant Tat proteins, where cells were fractionated and the samples immunoblotted using antibodies to GFP. In the control assay, GFP is found predominantly as mature size protein in the periplasmic fraction (lanes P) in cells expressing both TorA-GFP and pEXT-ABC, as found previously [21]. No export is observed when pJDT1 is expressed in the Δ*tatABCDE* strain, and the protein instead accumulates as the precursor form in the cytoplasm and membrane fractions (pJDT1/Δtat panel). The upper panels show the data obtained with mutant TatA proteins. The G2A mutant exhibits a slight export defect, with some of the GFP found in the cytoplasm and membrane fractions, and substitution of the conserved F20 and G21 residues leads to a slightly more severe export defect, with more of the majority of the GFP now in the cytoplasm and membrane. A mild export defect is also observed with the K24A mutant, whereas the L25A and F39A mutants support nearly wild-type export efficiencies. Finally, the export of GFP is almost totally blocked in the G33A mutant, indicating that this residue is essential for efficient export of TorA-GFP.

The effects of the TatB mutations are shown in the lower panel of Fig. 3A. Substitution of the invariant G21 residue by Ala hardly affects export efficiency at all while the P22G and P22L mutants exhibit only a moderate export defect. The result with the P22L is surprising since the same mutation in the chromosomal *tatB* gene was found to block export in *E. coli* [9]. The likely explanation is that the mutation does not completely block TatB function and moderate levels of export activity are sustained by the increased levels of TatB present in our system due to the higher copy number of the plasmid-borne *tatB* gene. Fig. 3B shows a comparison of TatA and TatB levels in the Δ*tatABCDE* strain (lanes tat-), wild-type *E. coli* (wt) and the strain expressing the P22L mutant after induction with IPTG. The data clearly show a significant increase in Tat subunit levels (typically 10–20-fold over wild-type levels after quantitation of these blots; not shown). Of the remaining mutants, L25A and L63A export TorA-GFP with high efficiency whereas export is almost totally blocked in the P26A mutant, which is clearly the most drastically affected of the TatB mutants in terms of export activity.

We conclude from these data that the highly conserved 'hinge' regions between the transmembrane spans and predicted amphipathic regions in TatA and TatB are indeed required for efficient export but they are by no means essential. Moreover, substitutions in this region do not have severe effects on the export of GFP. Much more important, for both TorA and GFP export, are the G33 residue in TatA and the P26 residue in TatB.

3.4. Evidence that the amphipathic regions in TatA and TatB do not play critical roles in export

The TM regions of TatA/B are closely followed by positively charged regions that are strongly predicted to form amphipathic α-helices as indicated in Fig. 1. A recent study has shown these regions to be functionally important since the TatA and TatB proteins are active after truncation by up to 40 or 70 residues from the C-terminus, respectively, but further truncation abolished export competence [29]. Alignments of TatA/B proteins show that, although the precise positions of the basic residues in this region are not always absolutely conserved, the predicted amphipathic character is highly conserved (data not shown). The invariant FG or GP motifs (in TatA/B, respectively) lie between these regions.

Fig. 4A shows the locations of basic residues in the relevant

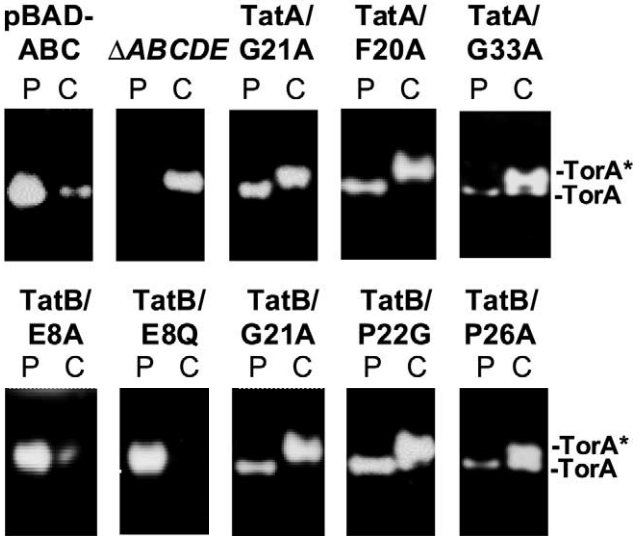

Fig. 2. Export of TMAO reductase (TorA) in cells expressing TatA/B mutants. *tatA* and *tatB* mutants in the pBAD-ABC vector were expressed in the Δ*tatABCDE* strain as detailed in Section 2. After induction with 0.2 M arabinose, cells were fractionated to generate samples of cytoplasm (C) and periplasm (P). Control samples were also analyzed: MC4100 wild-type cells expressing the pBAD24 vector or the Δ*tatABCDE* strain as indicated. These samples were electrophoresed on a native polyacrylamide gel followed by staining for TMAO reductase activity. TorA, mature TMAO reductase; TorA*, more slowly migrating form that accumulates in the cytoplasm.

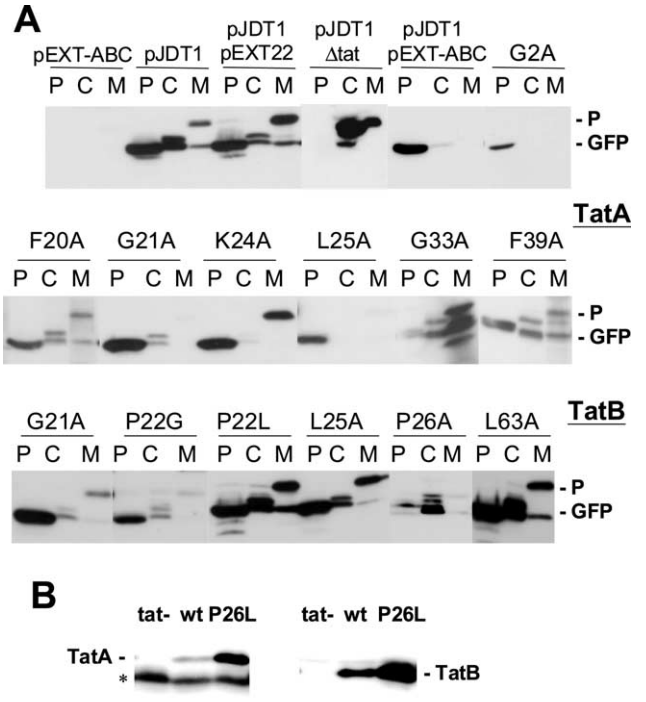

Fig. 3. Assays for the export of TorA-GFP by cells expressing mutant forms of TatA/B. *E. coli tatABC* operons encoding mutant forms of TatA/B were cloned into the pEXT22 vector (generating pEXT-ABC) and expressed in the *E. coli* Δ*tatABCDE* strain, together with a TorA-GFP fusion protein (plasmid pJDT1) as detailed in Section 2. A: Export assays. The control assays in the upper panel show, from left to right, a negative control for the GFP antibody in wild-type MC4100 cells expressing pEXT-ABC but not pJDT1 (pEXT-ABC panel), and the export of TorA-GFP in MC4100 cells (pJDT1 panel), in MC4100 in the presence of the pEXT22 vector, in the Δ*tatABCDE* strain and in MC4100 in the presence of pEXT-ABC. Additional panels indicate the identities of the TatA/B mutants analyzed. Cells were fractionated into periplasm, cytoplasm and membrane fractions (P, C, M) and sodium dodecyl sulfate (SDS)–polyacrylamide gels were immunoblotted using GFP antibodies. The mobilities of the TorA-GFP precursor protein (pGFP) and mature GFP are indicated. B: Immunoblot of samples of Δ*tatABCDE* cells ('tat-'), wild-type MC4100 cells ('wt') and cells expressing the TatB P22L mutation described in A, using antisera against TatA and TatB. The mobilities of the TatA/B bands are indicated; a polypeptide reacting non-specifically with the TatA antiserum is denoted by *.

regions of TatA and TatB, and these were substituted in groups by hydrophilic but uncharged Gln or Asn residues. TatA mutants lacking three or four Lys residues (as shown) are denoted TatA/−3K and TatA/−4K, respectively. Similarly, TatB mutants lacking two Arg or three Lys are denoted TatB/−2R and TatB/−3K, respectively. These Tat mutants were analyzed using the TMAO reductase (TorA) native gel assay and the data are shown in Fig. 4B. In the control sample, TorA activity is present in the periplasm of wild-type cells expressing pBAD-ABC, but in the cytoplasm of the Δ*tatABCDE* strain, as expected since this strain lacks all known Tat components. The remaining panels show that efficient export is also apparent in all of the mutants. Low levels of cytoplasmic TorA are apparent in the TatA/−4K and TatB/−3K mutants, indicating that these sets of mutations do have a slight adverse effect, but the data otherwise indicate that the amphipathic nature of this region can be removed over long stretches without affecting export in a significant manner. As these regions represent possible areas of interaction between TatA and TatB, we next tested the combined effects of multiple TatA plus TatB mutations; the lower section of Fig. 4B shows that even a combination of [TatA/−4K+TatB/−3K], or [TatA/−3K+TatB/−2R] fails to severely affect export of TorA. Export is slightly inhibited with the TatB/−3K mutant since the cytoplasmic form of TorA is more apparent, but the overall conclusion is that these sets of basic residues do not play vital roles in the export process. TorA-GFP is similarly exported with almost wild-type efficiency in all of these mutants (data not shown).

4. Discussion

The Tat system catalyzes a unique form of protein translocation process but the actual translocation mechanism is presently obscure. In this study we have focused attention on the TatA/B proteins in order to probe the roles of these subunits, by site-specific mutagenesis of conserved residues in the *E. coli* proteins. The TatA/B proteins lack significant homology to any other proteins in the database except each other, and the primary sequences thus yield few clues regarding their functions. It is also unusual that, although TatA and TatB are homologous proteins, they carry out completely differing roles and cannot substitute for each other [12]. They are also positioned very differently in the TatABC complex, since

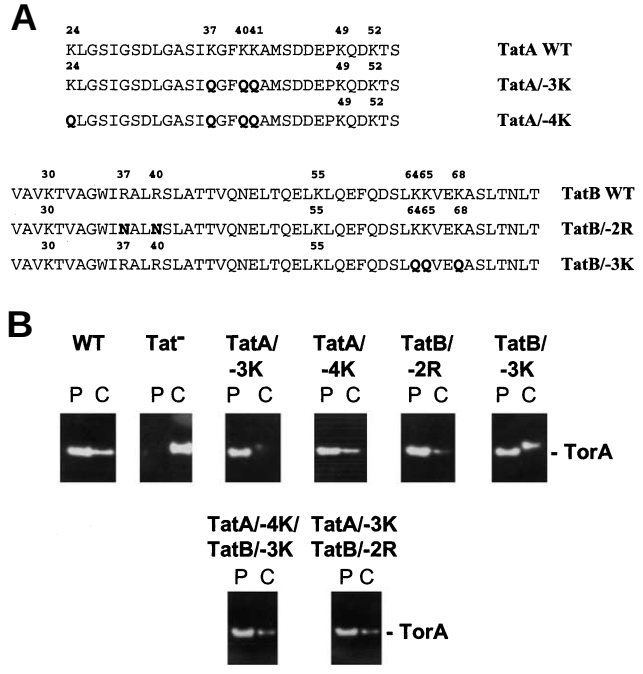

Fig. 4. Multiple mutations in the TatA/B amphipathic regions do not block export. A: Predicted TatA/B amphipathic regions that were targeted for mutagenesis in this study, and the primary sequences of the mutant forms, where the substitutions are shown in bold. Residue numbers are indicated for basic amino acids; further details are given in the text. B: Δ*tatABCDE* cells expressing pBAD-ABC in which the TatA/B amphipathic regions contained multiple substitutions as detailed in A. The upper panel shows fractionations and TMAO reductase (TorA) native gels using periplasmic and cytoplasmic fractions (P, C) from TatA or TatB mutants. The lower panel shows the data obtained from cells expressing pBAD-ABC derivatives in which both the TatA and TatB sequences were mutated.

TatB is a close partner of TatC, present in strictly stoichiometric amounts, whereas TatA is present in great molar excess over TatBC and is much more loosely bound [16].

Very few residues in TatA/B are highly conserved even among eubacteria, and the identities and positions of these invariant residues have therefore prompted speculation regarding their function. The absolutely conserved P22 residue in TatB suggests a possible turn-inducing function, and the presence of invariant Gly residues at essentially identical positions in both the TatA and TatB proteins (residue 21 in *E. coli*) raises other interesting possibilities regarding flexibility between domains. Our data indicate that these residues are indeed important for the functioning of the TatA/B proteins because substitution does adversely affect the Tat-dependent export of TorA. However, these mutants still export TorA with reasonable efficiency, and this means that their presence is not vital. Equally surprising is that substitution of the invariant FG sequence in TatA and GP in TatB does not inhibit the export of a heterologous protein, GFP, to an enormous extent and this raises the intriguing possibility that these apparent hinge regions may assume greater importance for the export of larger or more complex proteins (TorA is three times larger than GFP).

In one sense our data appear to differ from those of Weiner et al. [9], who found that the TatB P22L mutation in the *E. coli* D43 mutant was completely export-deficient. However, this may reflect the differing abundance of the Tat system in these situations, since the D43 mutant contained a chromosomal mutation in the *tatB* gene whereas mutated TatB is encoded by a multicopy plasmid in our studies. While our data show the P22 residue to be important, additional turn-inducing residues in this region appear to be much more significant because substitution of the P26 residue in TatB or the G33 residue in TatA leads to a major export defect in each case, with both TorA and TorA-GFP.

Another highly conserved feature of TatA/B proteins is the presence of a cytoplasmic domain that is strongly predicted to form an amphipathic helix, which would presumably interact with the cytoplasmic face of the plasma membrane. In this study we have mutated many of the positive charges in this region and we find that all of these mutants remain highly efficient in Tat-dependent export. Although not all of the basic residues were targeted in this study, up to seven basic residues were removed in some of the studies involving combinations of TatA and TatB mutants. Our conclusion is that this feature does not execute a central function in the translocation process. It is, however, of interest that the two most important residues identified in this study, G33A in TatA and P26 in TatB, are probably located in these amphipathic regions which points to a critical role for these domains.

References

[1] Robinson, C. and Bolhuis, A. (2001) Nat. Rev. Mol. Cell. Biol. 2, 350–355.
[2] Dalbey, R.E. and Kuhn, A. (2000) Annu. Rev. Cell Dev. Biol. 16, 51–87.
[3] Chaddock, A.M., Mant, A., Karnauchov, I., Brink, S., Herrmann, R.G., Klösgen, R.B. and Robinson, C. (1995) EMBO J. 14, 2715–2722.
[4] Stanley, N.R., Palmer, T. and Berks, B.C. (2000) J. Biol. Chem. 275, 11591–11596.
[5] Clark, S.A. and Theg, S.M. (1997) Mol. Biol. Cell 8, 923–934.
[6] Hynds, P.J., Robinson, D. and Robinson, C. (1998) J. Biol. Chem. 273, 34868–34874.
[7] Berks, B.C. (1996) Mol. Microbiol. 22, 393–404.
[8] Santini, C.-L., Ize, B., Chanal, A., Müller, M., Giordano, G. and Wu, L.-F. (1998) EMBO J. 17, 101–112.
[9] Weiner, J.H., Bilous, P.T., Shaw, G.M., Lubitz, S.P., Frost, L., Thomas, G.H., Cole, J.A. and Turner, R.J. (1998) Cell 93, 93–101.
[10] Sargent, F., Bogsch, E.G., Stanley, N.R., Wexler, M., Robinson, C., Berks, B.C. and Palmer, T. (1998) EMBO J. 17, 3640–3650.
[11] Bogsch, E.G., Sargent, F., Stanley, N.R., Berks, B.C., Robinson, C. and Palmer, T. (1998) J. Biol. Chem. 273, 18003–18006.
[12] Sargent, F., Stanley, N.R., Berks, B.C. and Palmer, T. (1999) J. Biol. Chem. 274, 36073–36082.
[13] Voelker, R. and Barkan, A. (1995) EMBO J. 14, 3905–3914.
[14] Settles, M.A., Yonetani, A., Baron, A., Bush, D.R., Cline, K. and Martienssen, R. (1997) Science 278, 1467–1470.
[15] Walker, M.B., Roy, L.M., Coleman, E., Voelker, R. and Barkan, A. (1999) J. Cell Biol. 147, 267–276.
[16] Bolhuis, A., Mathers, J.E., Thomas, J.D., Barrett, C. and Robinson, C. (2001) J. Biol. Chem. 276, 20213–20219.
[17] Mould, R.M. and Robinson, C. (1991) J. Biol. Chem. 266, 12189–12193.
[18] Cline, K., Ettinger, W.F. and Theg, S.M. (1992) J. Biol. Chem. 267, 2688–2696.
[19] Cline, K. and Mori, H. (2001) J. Cell Biol. 154, 719–729.
[20] Mori, H. and Cline, K. (2002) J. Cell Biol. 157, 205–210.
[21] Allen, S.C.H., Barrett, C.M.L., Ray, N. and Robinson, C. (2002) J. Biol. Chem. 277, 10362–10366.
[22] Buchanan, G., Leeuw, E., Stanley, N.R., Wexler, M., Berks, B.C., Sargent, F. and Palmer, T. (2002) Mol. Microbiol. 43, 1457–1470.
[23] Casadaban, M.J. and Cohen, S.N. (1979) Proc. Natl. Acad. Sci. USA 76, 4530–4533.
[24] Wexler, M., Sargent, F., Jack, R.L., Stanley, N.R., Bogsch, E.G., Robinson, C., Berks, B.C. and Palmer, T. (2000) J. Biol. Chem. 275, 16717–16722.
[25] Bolhuis, A., Bogsch, E.G. and Robinson, C. (2000) FEBS Lett. 472, 88–92.
[26] Thomas, J.D., Daniel, R.A., Errington, J. and Robinson, C. (2001) Mol. Microbiol. 39, 47–52.
[27] Dykxhoorn, D.M., St. Pierre, R. and Linn, T. (1996) Gene 177, 133–136.
[28] Randall, L.L. and Hardy, S.L.S. (1986) Cell 46, 921–928.
[29] Lee, P.A., Buchanan, G., Stanley, N.R., Berks, B.C. and Palmer, T. (2002) J. Bacteriol. 184, 5871–5879.
[30] Gouffi, K., Santini, C.L. and Wu, L.F. (2002) FEBS Lett. 525, 65–70.