The twin-arginine translocation (Tat) protein export pathway

Tracy Palmer[1] *and Ben C. Berks*[2]

Abstract | The twin-arginine translocation (Tat) protein export system is present in the cytoplasmic membranes of most bacteria and archaea and has the highly unusual property of transporting fully folded proteins. The system must therefore provide a transmembrane pathway that is large enough to allow the passage of structured macromolecular substrates of different sizes but that maintains the impermeability of the membrane to ions. In the Gram-negative bacterium *Escherichia coli*, this complex task can be achieved by using only three small membrane proteins: TatA, TatB and TatC. In this Review, we summarize recent advances in our understanding of how this remarkable machine operates.

Signal peptides
Short, cleavable peptides that are usually found at the amino termini of proteins and direct their transport.

Bacterial and archaeal proteins that are located outside the cytoplasm are normally synthesized with amino-terminal signal peptides that target them to either the Sec or the Tat (twin-arginine translocation) protein export pathway. The key functional difference between these two export systems is that the Sec apparatus translocates polypeptides in an unstructured state, whereas the Tat pathway transports proteins that have already folded. The Sec pathway is universally conserved, essential and normally the main route of protein export. The Tat pathway, by contrast, is found in some, but not all, bacteria and archaea and has been identified as essential (at least, under standard laboratory conditions) in only a few organisms, including the human pathogen *Mycobacterium tuberculosis*[1], some haloarchaea[2,3], the symbiotic nitrogen-fixing bacterium *Sinorhizobium meliloti*[4] and the predatory bacterium *Bdellovibrio bacteriovorus*[5]. The Tat system is evolutionarily conserved in plant thylakoids and certain mitochondria (see BOX 1 for details). The Tat pathway normally transports fewer substrates than the Sec system[6], but nevertheless has key roles in many cellular processes, including respiratory and photosynthetic energy metabolism, iron and phosphate acquisition[7–11], cell division[12], cell motility[13,14], quorum sensing[15], organophosphate metabolism[16], resistance to heavy metals[7] and antimicrobial peptides[17], and symbiotic nitrogen fixation[18,19]. Importantly, in animal and plant pathogens the Tat pathway has been found to be required for virulence in almost all examples tested (reviewed in REF. 20).

Proteins are targeted to the Tat machinery by signal peptides that contain a conserved twin-arginine motif (BOX 2). Transport is carried out by integral membrane proteins from the TatA and TatC families. In some organisms, single TatA and TatC components are sufficient to mediate transport through the Tat pathway. However, in other cases, Tat-mediated transport involves a further member of the TatA family termed TatB. In these organisms, both TatA and TatB are essential for transport throught the Tat pathway, showing that these two homologous proteins have non-identical functions[21,22]. However, trace Tat-mediated transport can be detected in the absence of TatB with sensitive genetic reporters[23,24]. TatAB pairs have apparently arisen from a common ancestor multiple times in evolution, suggesting that there are limited structural differences between TatA and TatB[25,26]. Indeed, single amino acid substitutions are enough to convert *E. coli* TatA into a protein that can sustain both TatA and TatB function[24]. Moreover, TatA from *Bacillus subtilis*, an organism that does not possess TatB, is able to substitute for either TatA or TatB in *E. coli*[27]. In light of these observations, we refer to the TatA of TatAC systems as dual-function TatA, to indicate that it fulfils the roles of both TatA and TatB in the TatABC systems. TABLE 1 summarizes the distribution of TatABC and TatAC systems in representative organisms. In general, TatAC systems are found in Gram-positive bacteria with low-GC-content genomes (including *B. subtilis*) and in archaea, whereas TatABC systems are present in Gram-negative bacteria (including *E. coli*,), in Gram-positive bacteria with high-GC-content genomes (such as members of the phylum Actinobacteria) and in plant chloroplasts. Many organisms have multiple copies of Tat components. These extra Tat components can be functional duplications of TatA (for example, *E. coli* TatE[21]), additional Tat

[1]Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.
[2]Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK.
e-mails: t.palmer@dundee.ac.uk; ben.berks@bioch.ox.ac.uk
doi:10.1038/nrmicro2814
Published online 11 June 2012

© 2012 Macmillan Publishers Limited. All rights reserved

Box 1 | Tat-mediated translocation in eukaryotic systems

The twin-arginine translocation (Tat) pathway is not unique to bacteria and archaea, but can also be found in some eukaryotes. The full Tat pathway found in the cyanobacterial ancestors of eukaryote chloroplasts has been conserved in the thylakoid membrane of plant and algal chloroplasts[125]. The thylakoid Tat system is required for the assembly of photosystem II and of the cytochrome b_6f complex, so a functional Tat system is essential for photosynthesis[51]. Substrates of the thylakoid Tat pathway (formerly termed the ΔpH-dependent pathway) are encoded in the nucleus, so are initially transported into the chloroplast stroma before they are imported by the Tat apparatus into the thylakoid lumen. A homologue of the TatC component from the bacterial and archaeal Tat pathway is encoded in the mitochondrial genomes of plants and many protists[25]. It has been speculated that the mitochondrial TatC protein is involved in assembly of the Rieske iron–sulphur protein subunit of the cytochrome bc_1 complex[126] because this protein is a Tat substrate in bacteria[49]. The Tat pathway is absent from fungal mitochondria and among animal mitochondria is found only in the aspiculate homoscleromorph sponges[127]. A protein that might substitute for the Tat pathway in transporting the yeast Rieske protein has recently been identified[128].

pathways that are expressed under specific environmental conditions (for example, phosphate starvation-induced TatAdCd in *B. subtilis*[28]) or TatC homologues of unknown function (for example, *Archaeoglobus fulgidus* TatC2).

In this Review, we outline our current understanding of substrate selection and the mechanism of transport by the Tat pathway.

Rationale for use of the Tat pathway

Why does a bacterium need to transport certain proteins in a folded state using the Tat apparatus when most proteins can be transported in an unfolded conformation by the Sec pathway? There is no single answer to this question, and for many Tat substrates it is not clear why they are targeted to this system. However, three main reasons for use of the Tat pathway have been definitively established experimentally: the need for the insertion of complex cofactors, the avoidance of metal ions that compete for insertion into the active site of the proteins, and transport as a hetero-oligomeric complex (FIG. 1). Other possible general rationales for the use of the Tat pathway are a requirement for cytoplasmic factors for protein folding or maturation, and difficulties in keeping the protein unfolded before post-translational transport. It has been proposed that the reason why halophilic archaea route most of their secretion through the Tat system is either that they have problems folding proteins in the high extracellular salt concentrations of their environments in the absence of chaperones[29] or, conversely, that the high internal salt concentrations promote cytoplasmic folding and aggregation of unfolded proteins[30,31].

Many exported proteins containing non-covalently bound cofactors use the Tat pathway because the protein has to fold around the cofactor. Cofactor insertion before transport sidesteps the requirement for additional mechanisms to export the cofactor and to catalyse its insertion into the protein in the periplasm[32]. It should be stressed that only proteins containing certain types of cofactor use the Tat pathway[33], and these cofactors can be broadly classified as metal–sulphur clusters or nucleotide-based cofactors (FIG. 1a). The Cu_Z copper–sulphur cluster[34] found in nitrous oxide reductases is associated with the Tat pathway but is an interesting anomaly, as there is now good evidence that this metal cluster is inserted in the periplasm[35]. Proteins containing cofactor types that are not associated with the Tat pathway (for example, haem) are instead transported by the Sec pathway and receive their cofactors in the periplasm.

As a general rule, Tat-associated cofactors are found in both periplasmic and cytoplasmic proteins, whereas Sec-dependent cofactors are found only in periplasmic proteins. This suggests that the Tat pathway is used when cytoplasmic mechanisms of cofactor assembly and insertion are already available[32]. A few periplasmic proteins are known to bind both Tat-associated cofactors and Sec-associated cofactors, and in these cases the protein is transported by the Sec pathway. Examples are single-polypeptide *c*-type haem- and flavin-binding flavocytochromes *c*[33] and the *c*-type haem- and Cu_Z copper–sulphur cluster-containing nitrous oxide reductase of *Wolinella succinogenes*[36].

Metal ions compete for binding sites in proteins, so cells need mechanisms to ensure that only the required metal ion is inserted. Analysis of the biogenesis of MncA, a periplasmic Mn^{2+}-binding protein in *Synechocystis* sp. PCC 6803, shows that Tat targeting can be used to control this process[37]. Compared with other transition metal divalent cations, the Mn^{2+} ion has a considerably weaker affinity for ligands, but by using the Tat system, MncA can fold and bind its Mn^{2+} cofactor in the cytoplasm, where competing metal ions are kept at low levels (FIG. 1b). The same bacterium possesses a close homologue of MncA that binds Cu^{2+} rather than Mn^{2+}. This homologue is targeted to the Sec pathway rather than the Tat pathway, as expected if it is metal ion selection, and not the protein fold, that determines which export pathway is used.

Is control of metal ion incorporation likely to be a common feature of Tat-targeted metalloproteins? For enzymes that bind metal ions at a substrate-accessible active site, we think that this is unlikely, because the ion-binding site would be exposed to other metal ions in the periplasm, and these could displace the bound ion if it were less strongly binding than the periplasmic ions. Conversely, the mechanism may be important for buried electron transfer centres, which are inaccessible to competing ions when the protein is folded but vulnerable during folding. For example, proteins with iron–sulphur clusters may be Tat targeted because cytoplasmic insertion protects the cluster from incorporating competing metal ions, such as Co^{2+} or Cu^{2+}, during assembly[37,38].

The Tat pathway translocates a number of hetero-oligomeric complexes in which only one of the subunits possesses a Tat-targeting signal peptide and the other subunits are carried in a 'piggy-back' manner. For such complexes, the partner subunits can recognize each other and form a complex only when they are folded. A good example is provided by the protein complex SoxYZ, which has no cofactor and is involved in thiosulphate oxidation (FIG. 1c). Only SoxY has a

Halophilic
Living in a high-ionic-strength (salty) environment.

Cu_Z copper–sulphur cluster
The catalytic [4Cu–2S] cluster that is found at the active site of nitrous oxide reductase.

Metalloproteins
Protein that bind one or more metal ions either directly or as part of a more complex cofactor.

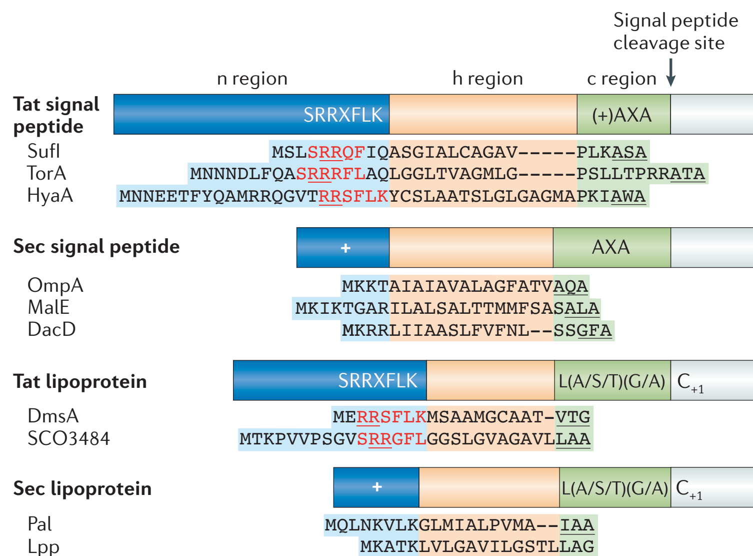
© 2012 Macmillan Publishers Limited. All rights reserved

Box 2 | Tat signal peptides

Proteins that are targeted to the Sec and twin-arginine translocation (Tat) machineries have amino-terminal signal peptides that have a similar overall architecture and are normally cleaved by an externally facing signal peptidase[46,61,117]. Signal peptides have a tripartite structure with a basic n region at the N terminus, a hydrophobic h region in the middle and a polar c region at the carboxyl terminus (see the figure for examples of the Tat signal peptides of various *Escherichia coli* proteins, dimethyl sulphoxide reductase (DmsA) of *Shewanella oneidensis* and SCO3483 of *Streptomyces coelicolor*, and the Sec signal peptides of various *E. coli* proteins and lipoproteins). The c region generally contains a signal peptidase recognition site for either a type I or a type II signal peptidase. Type II signal peptidase, also known as lipoprotein signal peptidase, has a consensus recognition sequence that is typically L_{-3}(A/S/T)$_{-2}$(G/A)$_{-1}$ along with an absolutely conserved cysteine at the +1 position of the mature protein; this cysteine is lipidated prior to cleavage. Despite the Sec- and Tat-targeting signal peptides being superficially similar, there are key differences that are crucial for targeting to the correct export pathway and, in some cases, for avoiding mistargeting to the incorrect machinery. Tat signal peptides contain a highly conserved twin-arginine motif, defined as SRRXFLK, in which the consecutive arginines are almost always invariant; the other motif residues occur with a frequency of >50%, and the amino acid at the X position is usually, but not always, polar[32,41,129]. Numerous studies have shown that the twin arginines have a crucial role in Tat signal peptide function, whereas mutation of the other conserved residues generally results in a less notable or even undetectable protein transport defect (reviewed recently in REF. 40). However, the presence of a twin-arginine motif in a signal peptide does not in itself prevent interaction with the Sec pathway — indeed, some Sec-targeting sequences also have twin arginines in their n regions (as shown in D-alanyl-D-alanine carboxypeptidase (DacD), see the figure) — and it is other features of the Tat signal peptide and/or the passenger domain of Tat substrates that confer Sec incompatibility.

The hydrophobicity of Tat signal peptide h regions is usually less than that of Sec-targeting sequences[130], and this is a major distinguishing feature for these two peptides. Increasing the hydrophobicity of the Tat signal peptide h region reroutes passenger proteins to the Sec pathway (for example, see REF. 130). In addition, the c regions of some Tat signal peptides contain basic residues, which are seldom found in the c region of Sec signal peptides. Although the presence of a basic amino acid in the c region is not a requirement for recognition by the Tat machinery, it has been shown to prevent engagement of the signal peptide with the Sec machinery[130–132]. Several bioinformatics programs can be used to predict Tat signal peptides[6,30,133,134].

One of the most unusual aspects of the Tat pathway is that it is also capable of transporting proteins that lack any sort of targeting sequence[21]. This is achieved because such proteins form complexes with partner subunits that have N-terminal twin-arginine signal peptides, a phenomenon termed hitch-hiking[135]. A good example of this is the large subunit of exported hydrogenase enzymes. These lack signal peptides but are transported to the periplasmic side of the membrane because they bind tightly to their small-subunit partner proteins (for example, hydrogenase 1 small subunit (HyaA); see the figure), which carry Tat signal peptides[135,136].



In the figure, residues that match the Tat consensus are shown in red, with the twin arginines underlined. A '+' indicates a basic region. Signal peptidase recognition sequences in the c regions are underlined. Lpp, major outer-membrane lipoprotein; MalE, maltose-binding periplasmic protein; OmpA, outer-membrane protein A; Pal, peptidoglycan-associated lipoprotein; TorA, trimethyl-amine-*N*-oxide reductase.

Tat-targeting signal peptide, so SoxZ must be exported in complex with SoxY. Certain bacterial genomes encode close homologues of SoxYZ in which the two polypeptides are fused to form a single polypeptide. Remarkably, these fused SoxYZ proteins always possess a Sec-targeting rather than a Tat-targeting signal peptide. As the two-subunit SoxYZ complexes and the fused SoxYZ should have similar structures[39], it is unlikely that the individual SoxY and SoxZ subunits are inherently incompatible with the Sec apparatus. Thus, use of the Tat pathway by the two-subunit complex can be linked directly to the requirement to co-translocate two polypeptides chains.

Tat substrates

The number of proteins that are exported using the Tat system varies widely in different organisms (TABLE 1). Gram-positive Firmicutes members such as *B. subtilis* and *Staphylococcus aureus* have few predicted Tat substrates[6]. By contrast, enteric bacteria have about

© 2012 Macmillan Publishers Limited. All rights reserved

Table 1 | **Occurence of Tat components and substrates in selected organisms**

Organism	Tat components present	Genetic organization of Tat components	Predicted substrate number	Refs
Gram-negative bacteria				
Escherichia coli	2 x TatA (TatA, TatE); 1 x TatB; 1 x TatC	*tatABC*; *tatE*	27	40
Salmonella enterica	2 x TatA (TatA, TatE); 1 x TatB; 1 x TatC	*tatABC*; *tatE*	29	40
*Bdellovibrio bacteriovorus**	2 x TatA (TatA1, TatA2); 1 x TatB; 1 x TatC	*tatBC*; *tatA1*; *tatA2*	21	5
*Sinorhizobium meliloti**	1 x TatA; 1 x TatB; 1 x TatC	tatABC	94	6,18
Myxococcus xanthus	1 x TatA; 1 x TatB; 1 x TatC	*tatBC*; *tatA*	43	145
Campylobacter jejuni	1 x TatA; 1 x TatB; 1 x TatC	*tatBC*; *tatA*	15	146
Rickettsia prowazekii	1 x TatA; 1 x TatC	*tatA*; *tatC*	1	6
Gram-positive bacteria				
Bacillus subtilis	3 x TatA (TatAd, TatAy, TatAc); 2x TatC (TatCd, TatCy)	*tatAdCd*; *tatAyCy*; *tatAc*	5-7	6
Staphylococcus aureus	1 x TatA; 1 x TatC	tatAC	1	147
Streptomyces coelicolor	2 x TatA (TatA1, TatA2); 1 x TatB; 1 x TatC	*tatA1C*; *tatB*; *tatA2*	145–189	6,42,133
Streptomyces scabies	1 x TatA; 1 x TatB; 1 x TatC	*tatAC*; *tatB*	126	41
*Mycobacterium tuberculosis**	1 x TatA; 1 x TatB; 1 x TatC	*tatAC*;. *tatB*	31	6
Archaea				
*Haloferax volcanii**	2 x TatA (TatAo, TatAt); 2 x TatC (TatCo, TatCt)‡	*tatCo tatCt* (divergently transcribed); *tatAo*; *tatAt*	68	6
*Halobacterium salinarum**	1 x TatA; 2 x TatC (TatC1, TatC2)‡	*tatC1 tatC2* (divergently transcribed); *tatA*	60	2
Archaeoglobus fulgidus	2 x TatA (TatA1, TatA2); 2 x TatC (TatC1, TatC2)	*tatA1*; *tatA2*; *tatC1*; *tatC2*	9	6
Sulfolobus solfataricus	3 x TatA (TatA1, TatA2, TatA3); 2 x TatC (TatC1, TatC2)	*tatA1A2C1*; *tatA3*; *tatC2*	5	6

Tat, twin-arginine translocation. *The Tat machinery is essential in these organisms[1–5]. ‡One of the two TatC proteins encoded by these organisms is much longer and contains two distinct TatC domains, probably arising as the result of a gene duplication event.

20–30 substrates (reviewed in REF. 40). The biggest users of the Tat pathway, in terms of substrate numbers, are species of the Gram-positive genus *Streptomyces*. In *Streptomyces coelicolor* and *Streptomyces scabies*, there are many more than 100 predicted or experimentally verified substrates (up to one-sixth of the total secretome)[41,42]. Some halophilic archaea seem to use the Tat pathway for 50% or more of their protein traffic. For example, more than 40% of the predicted secreted proteins of *Haloferax volcanii* are candidate Tat substrates, as are more than 55% of the secreted proteins of *Natrialba magadii*[6,30,43].

The Tat pathway is unexpectedly versatile with regard to the types of proteins that it can export. It has recently been shown that lipoproteins can be substrates of the Tat pathway[43–47]. Lipoproteins differ from other exported proteins, having type II signal peptides that are cleaved by lipoprotein signal peptidase adjacent to a lipidated cysteine residue (BOX 2). The Tat pathway can also assemble some membrane-bound proteins. These may be N-terminally anchored proteins in which the uncleaved twin-arginine signal sequence acts as a transmembrane signal anchor — the Rieske iron–sulphur protein, for example[48–52]. They can also be anchored by a single transmembrane carboxyl helix — for example, the small subunits of bacterial respiratory formate dehydrogenases and uptake hydrogenases[53,54]. Such tail-anchored proteins are difficult for the cell to assemble because Tat substrates are exported post-translationally and so aggregation of the hydrophobic transmembrane helix must be prevented in the cytoplasm.

Signal anchor
A non-cleaved signal sequence.

Tat structure and translocation cycle

The key mechanistic feature of the Tat system is that it can transport folded proteins across an ionically tight membrane. This task is so difficult that only one other protein transport system has been found to exhibit this capability with a structurally diverse set of substrate proteins (namely, the peroxisomal import pathway[55]). Other protein transport systems that are found in sealed cellular membranes translocate only unstructured substrate proteins. To understand why transport of folded proteins is so challenging, we need to recognize that any transport apparatus in a sealed membrane must maintain the permeability barrier to small molecules and ions both during the transport process and when not in use. In the case of an unfolded substrate, the transporter needs to provide a pathway that is the same width as a polypeptide chain (that is, about 12 Å in diameter). In the Sec translocase, this pathway is provided by an aqueous channel passing through the middle of a single transporter protein and can be opened and closed by simple movements of the helices[56]. By contrast, the *E. coli* Tat system is able to transport substrates of up to 70 Å in diameter[57]. It is difficult to envisage how a transmembrane pathway of this size could be opened

© 2012 Macmillan Publishers Limited. All rights reserved

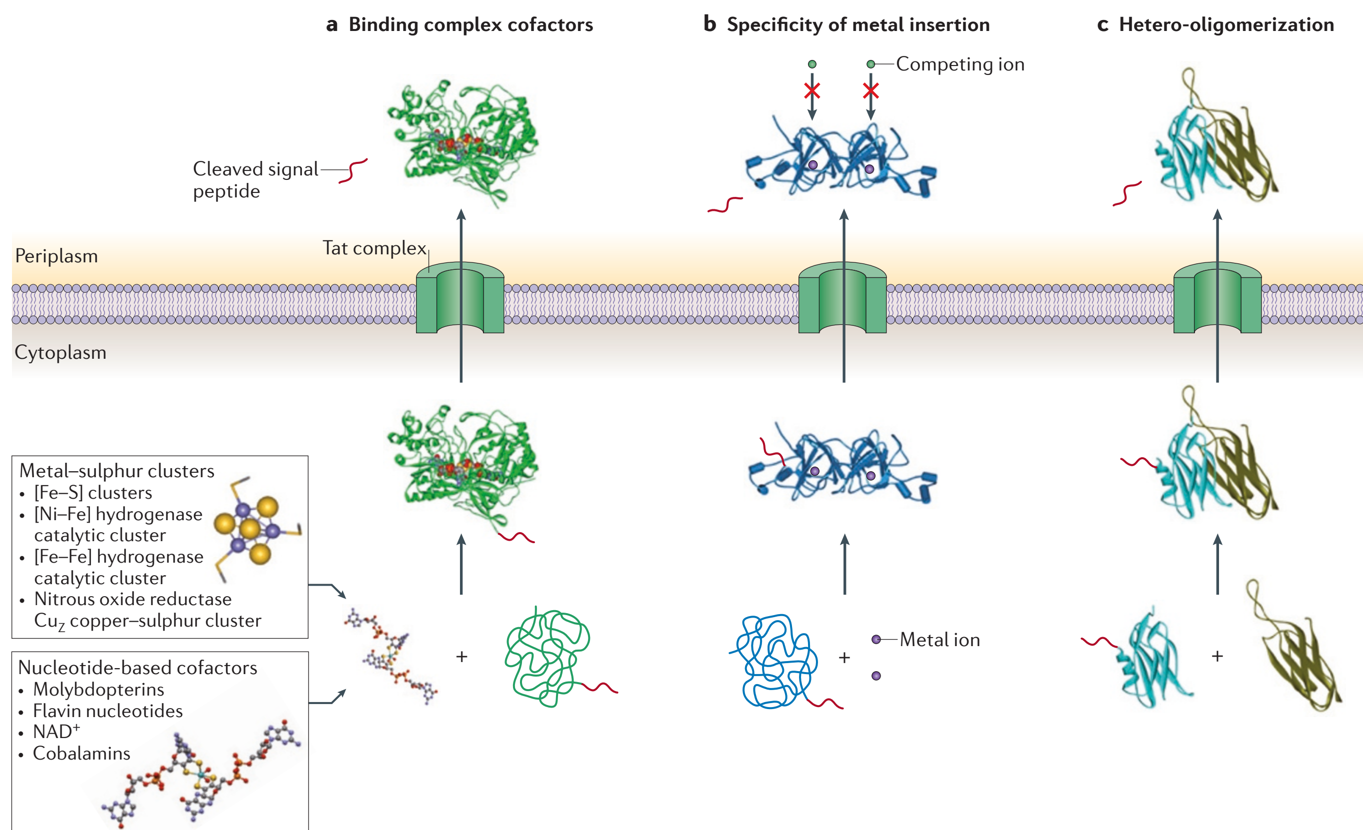


Figure 1 | **Physiological rationales for transporting proteins in a folded state using the Tat system.** Three reasons that proteins use the twin-arginine translocation (Tat) pathway have been definitively identified. The fate of the signal peptide following cleavage is uncertain, so this peptide is arbitrarily shown as being released into the periplasmic compartment. **a** | Tat targeting allows proteins to acquire their cofactor before transport across the cytoplasmic membrane. The example shown is insertion of a molybdopterin cofactor into trimethylamine-*N*-oxide reductase. Only certain cofactors are associated with Tat-mediated transport. These can be broadly divided into metal–sulphur clusters and cofactors containing a nucleotide moiety. **b** | Metal ions compete for binding sites in proteins. Use of the Tat system allows a protein to obtain metal ions under controlled conditions in the cytoplasm. The example shown is the Mn^{2+}-binding periplasmic protein MncA from the cyanobacterium *Synechocystis* sp. PCC 6803; MncA picks up its Mn^{2+} cofactors in the protected cytoplasmic environment to avoid competing ions that are present in the periplasm[37]. **c** | The Tat pathway allows hetero-oligomeric complexes to form in the cytoplasm and then be transported by a signal peptide in just one of the constituent subunits. The example shown here is the SoxYZ complex, which is involved in thiosulphate oxidation[39].

and closed by conformational changes in a transporter protein. An additional complication is that the Tat system must transport proteins with a range of diameters (between 20 and 70 Å in *E. coli* and as small as a single protein strand in engineered systems[57–59]). A pathway that is large enough to accommodate the larger substrates would be too big to seal tightly around smaller substrates. Unfolded substrates, by contrast, have an approximately constant cross-sectional area, allowing a single-size channel to fit tightly around any substrate. The Tat mechanism is therefore likely to be distinct from those of other protein transporters. Indeed, the Tat components have no detectable similarity to other proteins.

Tat is an active transport system that is energized by the transmembrane proton motive force (PMF)[60,61] (or possibly by the sodium motive force in some organisms[62]). It has been estimated that each protein translocated by the Tat system requires the removal of around 10^5 protons from the proton gradient[63], the equivalent to the energy that is stored in approximately 10^4 molecules of ATP. This is of the same order of magnitude as estimates of the energetic cost of Sec transport[64] but is a huge cost in comparison with small-molecule transport. It is possible that the bulk of this ion movement results from imperfect sealing of the membrane during transport rather than mechanical work involved in protein transport.

Our current understanding of the Tat translocation cycle is based on experiments that were carried out using either inner-membrane vesicles of *E. coli* or isolated pea thylakoids. It is assumed that the mechanism of Tat transport is conserved between these two systems. However, it remains possible that the pathways differ in some details, particularly as the split of TatA and TatB functions from a common ancestor with both functions seems to have occurred independently in the two lineages[26]. We also note that plant TatC has an N-terminal

© 2012 Macmillan Publishers Limited. All rights reserved

stromal domain of unknown function that is not found in *E. coli* TatC[65,66]. A consensus model for Tat-mediated transport, as derived from these studies, is summarized in FIG. 2.

E. coli and plant chloroplasts both use three-component TatABC systems. In these systems, TatB and TatC form an integral membrane complex containing an estimated 6–8 copies of each protein[67,68]. The TatB and TatC components probably form distinct substructures in the complex[69–71], with the TatC component constructed from TatC dimer units[33,72,73] and TatB forming tetrameric units[69]. TatB contains one, and TatC six, transmembrane helices[74–76]. Low-resolution structures of the *E. coli* TatBC complex have been obtained by electron microscopy[68] and are shown in FIG. 3C.

The transport cycle is initiated by substrates binding to the TatBC complex via their signal peptides[77,78]. Some substrate proteins can interact with the phospholipid surface of the membrane and may find the TatBC complex most efficiently by two-dimensional diffusion on the membrane surface[79,80]. Some reports have also indicated that proofreading chaperones could be involved in substrate targeting to the TatBC complex[81,82] (BOX 3).

When a substrate protein binds to the TatBC complex, the signal peptide twin-arginine motif is positioned close to TatC, whereas the hydrophobic h region of the signal peptide and the folded passenger domain are in the vicinity of TatB[83–85]. It is likely that TatC forms a specific binding site for the twin-arginine motif, as it is this motif that mediates the selectivity of binding to the TatBC complex[77]. Indeed, *tatC* mutants have been isolated that switch the specificity to a twin-lysine-containing motif[86]. Crosslinking data suggest that the signal peptide-binding site of TatC is centred around the cytoplasmic N-terminal tail and the first cytoplasmic loop of the protein[87]. For TatB, it is less clear whether proximity to the substrate reflects binding interactions. A model has been proposed whereby multiple TatB molecules envelop the substrate molecule[85]. Such an interaction would be expected to strengthen binding of the substrate to TatBC, but the limited data available suggest that the passenger domain has no effect on these binding interactions[78].

Physiological plant substrates show only weak binding to TatBC in de-energized thylakoids, so many interaction studies have relied on the use of more strongly interacting engineered substrates[78]. For some of these substrates, binding is moderate in strength, predominantly electrostatic in nature and leaves the signal peptide accessible at the face of the membrane. For others, binding is close to irreversible, with the signal peptide becoming deeply buried in the TatBC complex. Importantly, in the presence of a PMF, all types of substrate seem to be (reversibly) bound in the deep-insertion mode, suggesting that under physiological conditions the PMF drives the conversion of an initial weak TatBC–signal peptide complex into a more strongly bound arrangement.

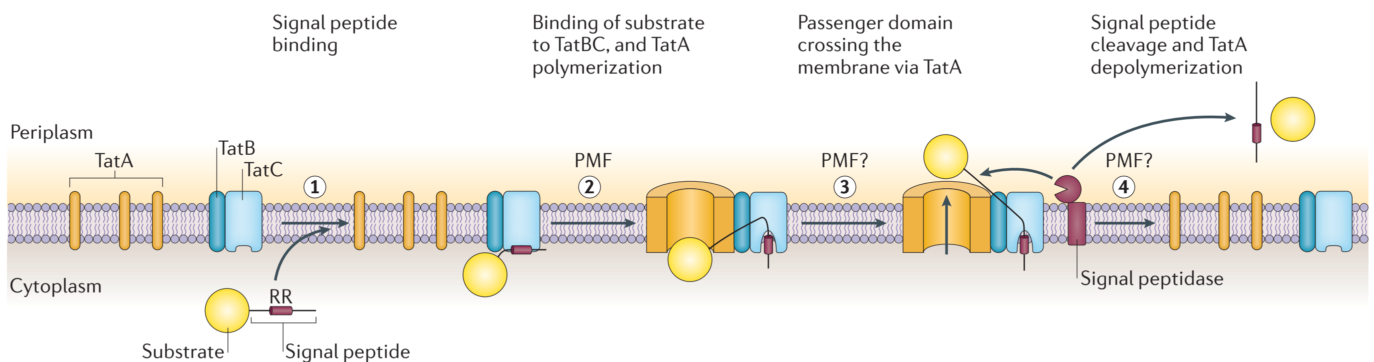


Figure 2 | **Model for the Tat translocation cycle in *E. coli* and plant chloroplasts.** The experimental evidence underlying this model for the twin-arginine translocation (Tat) pathway is outlined in the main text. At the start of the cycle, TatB and TatC associate as a complex, whereas TatA is present as dispersed protomers. The TatBC complex contains multiple copies of each protein, but only single copies are depicted, for clarity. The number of subunits in a TatA protomer is uncertain. Step 1: to initiate substrate translocation, the TatBC complex binds the signal peptide of a substrate protein in an energy-independent step; the twin-arginine (RR) consensus motif in the signal peptide is specifically recognized by a site in TatC . The remainder of the signal peptide and the substrate passenger domain are close to TatB. Step 2: in the plant thylakoid Tat system, the proton-motive force (PMF) causes the substrate signal peptide to become more tightly bound to the TatBC complex and less accessible from the cytoplasm. However, the amino-terminus of the signal peptide remains at the cytoplasmic side of the membrane. Some evidence suggests a potentially analogous, but PMF-independent, tightening of TatBC–substrate interactions in the *Escherichia coli* Tat system. TatA protomers are recruited to the TatBC complex and polymerized in a step that depends on the PMF. The resulting TatABC complex is the active translocation site. At this stage, the signal peptide is in contact with all three Tat components[83]. Step 3: the passenger domain of the substrate protein crosses the membrane via the polymerized TatA component, and the signal peptide remains bound to the TatBC complex. It is not known whether this step requires energization by the PMF. Step 4: when the passenger domain has reached the far side of the membrane, the signal peptide is normally proteolytically removed by a signal peptidase at the periplasmic face of the membrane, and TatA dissociates from TatBC and depolymerizes back to free protomers. The fate of the signal peptide following transport is uncertain, so this peptide is arbitarily shown as being released into the periplasmic compartment.

© 2012 Macmillan Publishers Limited. All rights reserved

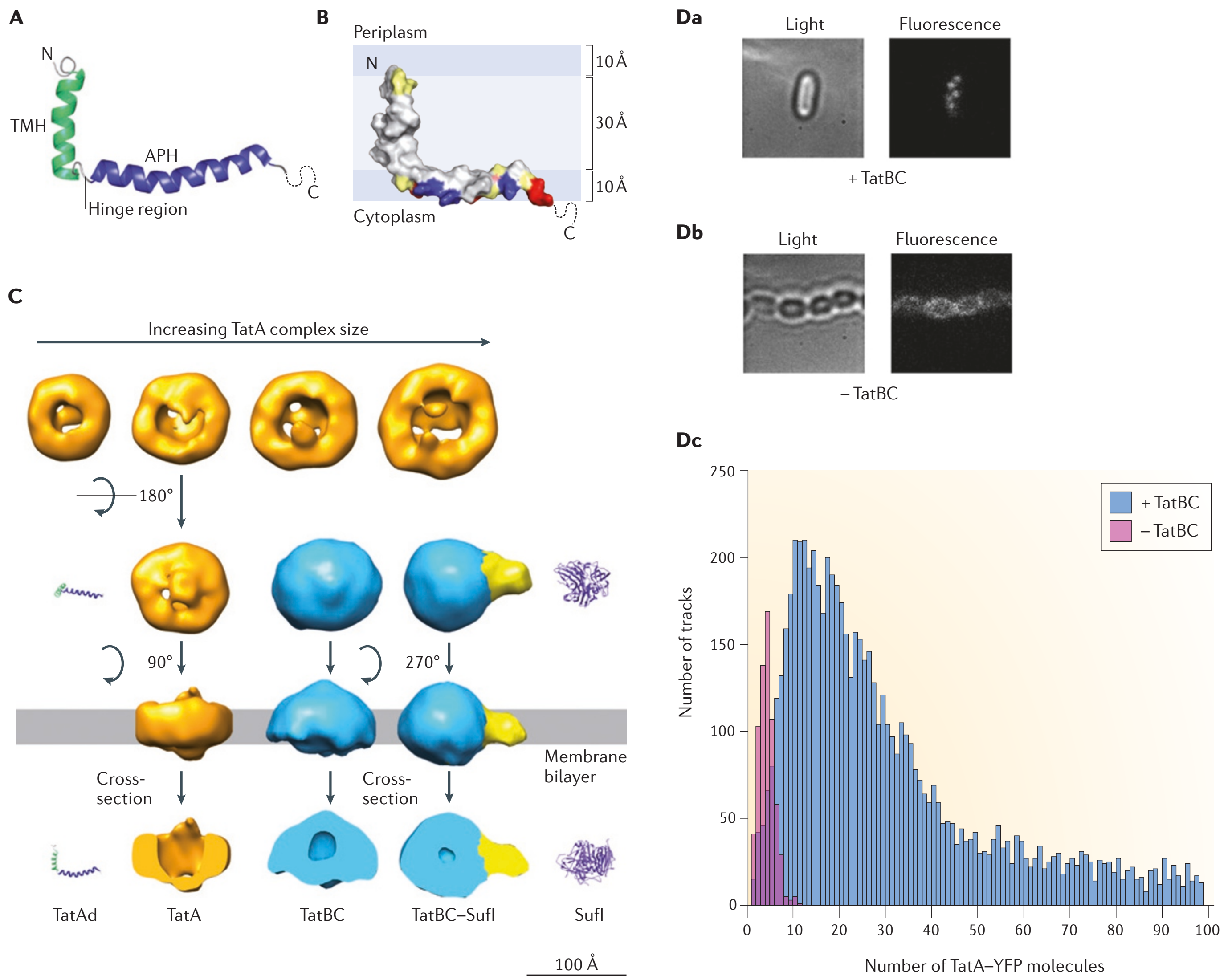


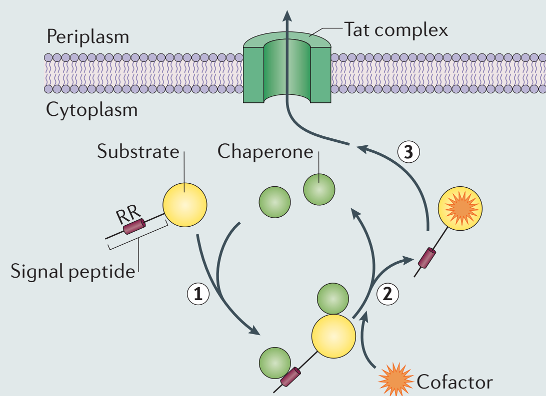
Figure 3 | **Structural and organizational features of Tat transporter components. A** | Structure of a monomer of the *Bacillus subtilis* twin-arginine translocation (Tat) pathway protein TatAd in micelles of the detergent dodecyl-phosphocholine (DPC)[97]. The structure was determined by solution NMR methods and is depicted in a ribbon backbone representation. The transmembrane helix (TMH), amphipathic helix (APH), hinge region and protein amino and carboxyl termini are indicated. The 22 amino acid C-terminal tail of the protein is unstructured and represented as a dotted line. **B** | Possible orientation of the *B. subtilis* TatAd monomer (excluding the unstructured C-terminal tail and shown in space-filling representation) in the cell membrane. Polar amino acids are red (acidic), blue (basic) or yellow (neutral). The bilayer is represented to scale: polar head groups are 10 Å wide each side of the membrane and lipid tails are 30 Å wide along the membrane normal. The position of TatAd is suggested on the basis of TatAd surface polarity. Note that a solid-state NMR study of the same protein in bicelles containing short-chain phospholipids suggests that the TMH is tilted 13° from the membrane normal and that the APH is inclined 26° out of the membrane plane[104]. **C** | Low-resolution structures of *Escherichia coli* Tat complexes in detergent solution, as determined by single-particle negative-stain electron microscopy[68,112]. Surface representations of TatA complexes, the TatBC complex, and TatBC in complex with one copy of the substrate molecule SufI (also known as FtsP) are shown to scale. The upper panel shows four size classes of TatA complexes. The third panel shows the inferred position of the complexes in the membrane bilayer. For comparative purposes, high-resolution structures of the *B. subtilis* TatAd monomer (coloured as in part **a**) and *E. coli* SufI are shown in ribbon backbone representations. The orientations shown are proposed to correspond to the orientation of these molecules in the adjacent electron microscopy structures. **D** | Stoichiometry of the TatA complex, as determined by direct visualization of TatA–YFP (yellow fluorescent protein) fusions expressed at native levels in living *E. coli* cells[93] (movies are available in REF. 93). The TatA–YFP fusion was expressed in the presence (part **Da**) or absence (part **Db**) of TatBC. Note that the cells in part **Db** show a chaining phenotype that is due to the mislocalization of Tat-dependent periplasmic amidases[144]. The graph (part **Dc**) shows the TatA–YFP complex stoichiometry distribution for cells expressing or missing TatBC. Parts **A** and **B** are courtesy of J. Schnell (University of Oxford, UK); part **C** is courtesy of H. Saibil (Birkbeck College London, UK); part **Da** is reproduced, with permission, from REF. 93 © (2008) US National Academy of Sciences; part **Db** is courtesy of N. Greene and M. Leake (University of Oxford, UK); and part **Dc** is plotted from data in REF. 93.

© 2012 Macmillan Publishers Limited. All rights reserved

Box 3 | **Tat proofreading and folding quality control**

Some cofactor-containing twin-arginine translocation (Tat) substrates are subject to chaperone-mediated 'proofreading' to ensure that they are correctly assembled (and any co-exported partner proteins are bound) before they interact with the Tat system. In this process, a signal peptide is bound tightly by a dedicated chaperone (see the figure; step 1), and a second chaperone binds elsewhere on the preprotein. The cofactor is then loaded (see the figure; step 2) before the chaperones are released and the protein can interact with the Tat machinery (see the figure; step 3). For example, TorD is a proofreading chaperone that is dedicated to the maturation of the Tat substrate trimethylamine-*N*-oxide reductase (TorA), which contains a molybdopterin guanine dinucleotide cofactor. TorD mediates efficient cofactor insertion by binding to sequences in the mature region of TorA[137]. TorD also binds to the TorA signal peptide[136,138,139]. It has been proposed that signal peptide binding prevents TorA from interacting with the export machinery until cofactor insertion has taken place[140]. Chaperone-mediated proofreading has probably evolved independently on more than one occasion. For example, periplasmic nitrate reductases that are homologous to TorA have signal peptide-binding chaperones that are structurally unrelated to the TorD family[141].

It has been proposed that, in addition to carrying out chaperone-mediated proofreading, the Tat pathway has an intrinsic capacity to differentiate between folded and unfolded substrate proteins by a process termed folding quality control. This hypothesis arose from studies in which alkaline phosphatase (PhoA) was targeted to the *Escherichia coli* Tat pathway[142]. The active conformation of PhoA depends on disulphide bond formation, and Tat-mediated transport was found to occur only in strains that had been manipulated to allow disulphide bond formation in the cytoplasm. This implies that the Tat pathway permits export of only fully folded proteins. *In vitro* experiments showed that discrimination of the PhoA folding state occurs after targeting to the translocase[90]. However, more recent studies have shown that the Tat pathway is able to transport natively unstructured proteins provided that these proteins are neither too long (exceeding ~100 amino acids) nor too hydrophobic[58,59]. Indeed, some truncated forms of PhoA can be transported in the absence of disulphide bond formation[143]. Unstructured proteins that exceed the size limit abort transport and are released into the membrane[58]. These observations would argue against the Tat machinery having an intrinsic quality control feature but suggest that proteins which are too large or expose too many hydrophobic stretches are incompatible with the translocation mechanism[58,59,114]. One interesting possibility is that the stalling of Tat substrates with exposed hydrophobic residues is related to the mechanism of membrane integration for Tat substrates that have carboxy-terminal hydrophobic anchor sequences.



Solution NMR
Structural analysis of a molecule in an aqueous solution by NMR spectroscopy.

Amphipathic helix
A protein helix that has one polar and one nonpolar face.

E. coli TatBC–substrate complexes have been isolated, and their structures have been characterized at low resolution by electron microscopy[68]. These structures suggest that the folded passenger domain of the substrate is located at the periphery of TatBC and that TatBC undergoes a conformational change on substrate binding (FIG. 3C). Although TatBC complexes contain multiple TatC subunits and thus multiple potential signal peptide-binding sites, only one or two substrate molecules are bound to each complex. This suggests that not all TatC subunits in the TatBC complex have the same binding properties and that there is therefore functional asymmetry within the complex[68]. A mixture of tight- and loose-binding sites could explain why translocase-bound substrate molecules are readily exchangeable in experiments with *E. coli* membranes[80,88], although some substrate molecules are bound sufficiently tightly to survive purification[68]. It is possible that these two types of binding site parallel the two binding modes seen in plants. However, none of the interactions observed in *E. coli* requires the PMF.

Formation of a TatBC–substrate complex triggers the association of TatA with TatBC[89]. This association requires the PMF and lasts only until the substrate has crossed the membrane[58,83,89]. During the transport process, the substrate is probably in simultaneous contact with all three Tat components, suggesting that a TatABC complex mediates transport[84,89]. However, it has also been proposed that the substrate is transferred from TatBC to TatA[83,90].

Crosslinking studies in plant thylakoids suggest that TatA undergoes polymerization on recruitment by the TatBC–substrate complex[91,92]. This is supported by direct imaging of yellow fluorescent protein (YFP)-labelled TatA in *E. coli* cells[93], which showed that TatA forms only low-order oligomers (mean ~4 subunits) in the absence of TatBC but larger complexes (with a mean of 25 subunits) when TatBC is present (FIG. 3D). A recent crosslinking study suggests that monomeric TatA is associated with the *E. coli* TatBC complex before a substrate is bound[94]. This is supported by the observation of TatA-dependent differences in substrate–TatBC interactions[88] and by the fact that small amounts of TatA are co-purified with TatBC in the absence of substrate[67,95]. TatBC-associated TatA monomers may provide the nucleation site for TatA polymerization[94]. At this juncture, it is worth noting a recent study which suggests that a PMF-dependent, substrate-independent priming step occurs in *E. coli*[88]. If this step can be equated to TatA polymerization, then we can infer that a substrate is not necessary to trigger TatA assembly in *E. coli*, in contrast to assembly in plant thylakoids. The overall transport time for Tat substrates seems to be of the order of 1 or a few minutes[88,89,96], and it is likely that the time required for diffusion and capture of TatA during polymerization is responsible for much of this time[88].

Structural studies have begun to provide a framework for understanding the function and behaviour of TatA. The structure of a detergent-solubilized monomer of the dual-function TatAd from *B. subtilis* has recently been determined by solution NMR[97] (FIG. 3A). The structure confirms earlier predictions[98,99] that TatA has a single N-terminal transmembrane helix (TMH) followed by a basic amphipathic helix (APH) and then a highly charged, natively unstructured C-terminal tail. The C-terminal tail is not essential for TatA function[100,101]. TatA is bent around an invariant glycine residue at the TMH–APH junction, resulting in these helices being positioned at right angles to each other. This L-shaped arrangement would allow the APH to lie parallel to the membrane surface, in contact with the phospholipid headgroups (FIG. 3B), in agreement with biophysical data[99,102,103]; however, solid-state NMR analysis of the dual-function TatAd

© 2012 Macmillan Publishers Limited. All rights reserved

of *B. subtilis* in bicelles suggests that the whole molecule is slightly tilted so that the end of the APH projects out of the membrane[104]. The N terminus of TatA, including the TMH, is scarcely long enough to span the hydrophobic interior of the cytoplasmic membrane (FIG. 3B), suggesting that TatA either does not fully cross the bilayer or thins the membrane in its vicinity. TatA is oriented in the membrane such that the APH and C-terminal tail are located at the cytoplasmic side of the membrane[69,105–107].

TatB is likely to have a similar overall structure to TatA, although TatB proteins are usually substantially longer than their TatA counterparts owing to an insertion between the APH and the C-terminal tail[100,108]. TatB-specific functions are associated with this insertion, with the N terminus of the protein and with the TMH[24,109]. By contrast, it is the APH that is crucial for TatA-specific functions[26,69,101,110,111] (one study has reported the opposite conclusions[100], but the data could have been affected by large differences in the expression levels of the domain-swapped proteins used).

When *E. coli* TatA is solubilized from the cytoplasmic membrane with mild detergents, the protein is typically found in large oligomers of variable size[112–114]. Single-particle electron microscopy shows that these oligomers have a ring-shaped morphology, with additional density partly blocking one end of the central cavity[68,112] (FIG. 3C). Particles of similar morphology have also been observed for the TatA homologue TatE[115]. TatA oligomerization in detergent solution has been shown to depend on specific interactions between the TMHs[99,116], suggesting that the walls of the rings are formed by the TMHs. However, comparison of the *B. subtilis* TatAd monomer structure with the electron microscopy map of *E. coli* TatA shows that there is a poor correspondence between the length of the *B. subtilis* TMH and the height of the *E. coli* ring (FIG. 3C, lower panel), although this is of uncertain significance at the low resolution of the *E. coli* maps. As TatA is a small protein with a single TMH, tens of TatA molecules would be needed to build the ring structures that are observed by electron microscopy.

Whether the TatA oligomers seen in detergent solution are related to the substrate-induced TatA oligomers observed in membranes is an open question. TatA oligomer formation in the membrane requires a substrate (at least, in thylakoids), the TatBC complex and the PMF, but large TatA complexes form in detergent solution in the absence of any of these factors[112], suggesting that detergent extraction induces TatA polymerization and that the TatA complexes which are seen in detergent solution are non-physiological, at least regarding their route of assembly. Nevertheless, it seems unlikely that a protein which has evolved to polymerize in a membrane would oligomerize in a distinctly different way in detergent solution. The diffusion of YFP-labelled TatA complexes in cells is best modelled when TatA is given a ring morphology, as seen for detergent-solubilized TatA, rather than a close-packed structure[93]. Moreover, TatA–YFP complexes have diverse oligomeric states (FIG. 3D), as seen in detergent solution[93]. It has been argued that detergent extraction drives the TatA equilibrium towards the highly oligomerized state by removing phospholipids that stabilize the dispersed state in membranes[93].

Other unresolved issues include the oligomeric state of TatA before substrate-induced polymerization and whether this protomer acts as the building block from which the polymerized TatA complexes are assembled. Some crosslinking and single-molecule fluorescence data suggest that the TatA protomer in the membrane is a homotetramer and that this unit is used to build the substrate-induced complexes[92,93]. A tetramer unit would also be consistent with a literal interpretation of the minimal size differences between TatA oligomers in detergent solution, as measured by blue native PAGE[112,113]. Crosslinking data have been interpreted to suggest that, whereas TatA tetramers are held together by interactions between the TMHs, substrate-induced polymerization involves interactions between the C-terminal tails[92]. By contrast, solid-state NMR diffusion measurements of a TatA fragment that was reconstituted in a bicelle environment suggest that the TatA protomer unit is a monomer[104]. Likewise, it is difficult to reconcile spin-labelling measurements from detergent-solubilized TatA complexes with a structural model in which the repeating unit is larger than a single TatA subunit[116].

Following assembly of a TatABC–substrate complex, the substrate is translocated across the membrane to the periplasm, where the signal peptide is normally removed by a signal peptidase[47,61,117]. The TatABC complex then disassembles[58,89]. The N terminus of the signal peptide most probably remains at the cytoplasmic side of the membrane during the transport process, as a structured domain fused to the signal peptide N terminus is not transported[106] and the topology of the uncleaved Tat signal peptide of bacterial Rieske iron–sulphur proteins is such that the N terminus is cytoplasmic and the C terminus is periplasmic[52]. By contrast, the C terminus of the signal peptide must reach the periplasmic face of the membrane to contact the active site of the signal peptidase[118]. Thus, during the transport cycle, the signal peptide has its C-terminal end flipped to the far side of the membrane. The fate of the signal peptide after transport is unclear. The transmembrane location of the uncleaved Tat signal peptides on bacterial Rieske iron–sulphur proteins suggest that the signal peptide can be released laterally from the transporter into the membrane bilayer. However, outer-membrane targeting of *Dickeya dadantii* pectate lyase (PnlH) is mediated by an uncleaved Tat signal peptide following Tat transport, providing clear evidence that signal peptides can also be released at the periplasmic side of the bilayer[119]. Although it has been shown that Tat signal peptides can be cleaved by the intramembrane protease RseP in *E. coli*[120], these experiments are not definitive because they were carried out with fusion proteins that were not capable of releasing the signal peptide to the periplasmic side of the membrane.

Mechanistic conundrums

Having outlined our current understanding of the Tat transport cycle, we now consider to what extent it is possible to answer three key questions regarding the

© 2012 Macmillan Publishers Limited. All rights reserved

mechanism of transport: how can a folded protein be transported across a membrane without compromising the membrane permeability barrier, how can this pathway be sealed when no substrate is present and how is the PMF transduced to drive protein transport?

When trying to answer these questions, one important factor to bear in mind is that we do not know with complete confidence which particular component (or components) of the Tat system is responsible for these functions. Here, we take the widely held assumption that TatA is responsible for the physical transport of the substrate across the membrane, as shown in FIG. 2. This inference is based on the observation that translocation takes place only when TatA has been recruited to TatBC, and is supported by the discovery of a transport-arrested substrate that has interactions exclusively with TatA[90]. The TatBC complex shows no evidence of an internal cavity that is large enough to form a transmembrane route for protein transport, even after substrate binding (FIG. 3C, lower panel), and the passenger domain of the substrate is outside of the TatBC complex, where TatA should also be bound.

In thylakoids, the transport-active TatA polymer assembles in response to a substrate. This suggests that the Tat system solves the problem of sealing the membrane when not in use by building and maintaining a translocation site only when substrate is present.

The cavities that are seen in the detergent-solubilized *E. coli* TatA complexes (FIG. 3C, upper and lower panels) are large enough to accommodate the known Tat substrates, indicating that substrate could be transported through the centre of the complex[112]. In the electron microscopy structures, the cavity contains stain and so must be filled with water. This suggests that transport would be through an aqueous channel. In this scenario, the channel sides would need to be lined by the APHs because the TMHs are predominantly nonpolar (FIG. 3B). It has been proposed that dynamic variation in the oligomeric state of TatA allows the channel to pack tightly around substrates of different sizes, thus ensuring a tight seal during transport (the bespoke channel model[93,112]), with TatA protomers being recruited to the channel until it becomes large enough to let the substrate through. This model is consistent with the wide range of sizes observed for TatA oligomers both in detergents and in membranes[68,93,112,113] and with evidence of rapid subunit exchange between TatA oligomers in detergent solution[116]. The model is challenged by crosslinking experiments suggesting that signal peptides (without a passenger domain) and complete substrate proteins induce TatA oligomers of the same size[91].

Alternative models predict that concentration of the membrane-active APHs by polymerization weakens or remodels the membrane so that application of force to the substrate is sufficient to move it through the membrane bilayer. This transient route[58] could be within a TatA ring, such that displaced lipids exit through transient openings between TMHs, or it could be through a less structured membrane patch. In one model, multiple APHs fold into the membrane in response to force on the substrate, and the polar sides of the APHs wrap around the polar face of the substrate, shielding it from the bilayer interior[91]. In this model, the membrane seal is retained because the substrate is transported as soon as sufficient APHs have been recruited to allow the substrate to move across the membrane. A second model suggests that the multiple APHs induce membrane disorder, which presumably allows the reorientation of phospholipid head groups around the substrate as it is driven through the membrane[72]. A third model posits that a ring of APHs inserted into the cytoplasmic leaflet of the membrane bilayer facilitates inward bending of the membrane towards the centre of the ring owing to expansion of the surface area of the cytoplasmic leaflet[26]. All these schemes require finely balanced APH–phospholipid interactions, an assumption that is in agreement with both the high sensitivity of the APH to amino acid substitutions[26,110] and the strong influence of the membrane phospholipid composition on transport[121,122] and on APH–membrane interactions[99,103]. The first and third models require the APH to dip at least transiently into the membrane bilayer from an initial interfacial position; this is supported by experiments with topology reporter fusions, which have been interpreted as showing that the APH 'flips' across the membrane during transport[123]. However, a recent study using a membrane-impermeable labelling reagent found no evidence for periplasmic exposure of TatA during transport[107]. The structure of the *B. subtilis* TatAd monomer shows that the APH and the TMH are held rigidly at right angles to each other owing to side chain packing interactions around the TMH–APH joint (FIG. 3A), and this would seem to preclude APH movement relative to the TMH[97]. Furthermore, it seems unlikely that the single glycine at this junction would provide sufficient conformational flexibility to allow the APH to bend much further towards the TMH, even if the packing interactions were disrupted. Consequently, any movement of the APH into the membrane might be expected to also lead to a reorientation of the TMH, which would strain the interactions between the TMHs in the TatA oligomer. Thus, it is conceivable that the proposed APH insertion triggers the TatA depolymerization that is associated with the end of transport. The inability of the APH to bend down towards the TMH would seem to preclude the aqueous-channel model for Tat transport, as this model requires the APH to be arranged parallel to the TMH in order to provide a polar lining to the channel.

All of these models emphasize that the mechanism of Tat translocation is likely to be different from that exhibited by a permanent channel structure.

Although it is known that the PMF is necessary for TatA to associate with the substrate-activated TatBC complex, the molecular basis for this requirement has not been established. Furthermore, it has been inferred that the PMF is involved in at least two distinct steps in the transport cycle[63,96]. One consequence of transporting a folded passenger domain is that the C-terminal end of the signal peptide reaches the periplasmic side of the membrane only when the passenger domain has been transported. In other words, completion of signal peptide flipping is linked to completion of passenger

© 2012 Macmillan Publishers Limited. All rights reserved

protein translocation. This linkage could be achieved by different scenarios. TatBC could use the energy of the PMF to actively flip the C-terminal end of the signal peptide across the membrane, forcing the folded passenger domain to follow passively through TatA. Alternatively, the PMF might be used to drive active transport of the passenger domain by TatA, causing the TatBC-bound peptide to reorient. Finally, it is possible that transport of the passenger domain occurs via Brownian motion but that the substrate becomes trapped when it reaches the far side of the membrane (rectified diffusion). In this case, the role of the PMF would be to power the formation of a transport-permissive holotranslocase (as observed), which would disassemble after transport to prevent reversal of transport and/or ensure that the transport cycle moves in the forward direction. Active flipping of the signal peptide is supported by the observation that the PMF drives isolated signal peptides into the interior of the plant TatBC complex[78]. It is also consistent with the observation that the signal peptide is flipped and cleaved even for substrates that subsequently fail to fully transport, as this shows that the N-terminal region of the protein (that is, the signal peptide) is transported first[58,124]. The Tat system is able to transport natively unstructured passenger domains that are substantially longer than the width of the membrane bilayer[58,59]. Because transport continues after the N terminus of the passenger domain has reached the far side of the membrane (and is therefore a drawn-out process), this observation has been interpreted as showing that the Tat system exerts a sustained (perhaps a peristaltic?) force on the substrate[58] rather than using a single (and quick) power stroke, as might be envisaged for active signal peptide flipping. However, there is an upper limit to the length of unstructured polypeptide that can be translocated[58,59], a finding that is more consistent with a single rather than a sustained transport event[59]. It is equally possible that unstructured polypeptides which are longer than the bilayer are transported through the translocase by Brownian motion following anchoring of their N termini at the far side of the membrane by signal peptide flipping.

Conclusions

Rapid progress has been made in characterizing the Tat system. Nevertheless, we are still a long way from elucidating the Tat mechanism at the molecular level and cannot explain how Tat is able to transport folded proteins across a membrane. Ongoing developments in three areas will be crucial to determining the mechanism of Tat-meditated transport. First, high-resolution structures of the Tat components and their complexes are a necessity. These may reveal the broad operating principles of Tat transport and will be essential for underpinning mechanistic studies and our understanding of their results. Structure determination will be extremely challenging because Tat components are integral membrane proteins and form complexes of inferred high conformational and compositional flexibility. We anticipate that a wide range of structural methods in combination with modelling are likely to be necessary. The NMR structure of the *B. subtilis* dual-function TatAd protein[97] (FIG. 3A,B) is a first exciting step on this road. Second, quantitative kinetic analysis is necessary to understand any catalytic mechanism, but it is still almost completely unavailable for the Tat system. Quantitative real-time methods for monitoring the steps of transport will need to be developed, along the lines of the pioneering study of Whitaker *et al.*[88]. Third, because the transport cycle of the Tat system involves dynamic changes in complex composition that are perturbed by extracting the Tat components from the membrane with detergents, we need to develop methods that allow detailed molecular analysis of the Tat system in the native membrane environment. The recent application of single-molecule fluorescence imaging to determine the composition of the TatA complex in cells[93] (FIG. 3D) represents one possible way to do this.

1. Saint-Joanis, B. *et al.* Inactivation of Rv2525c, a substrate of the twin arginine translocation (Tat) system of *Mycobacterium tuberculosis*, increases β-lactam susceptibility and virulence. *J. Bacteriol.* **188**, 6669–6679 (2006).
2. Dilks, K., Gimenez, M. I. & Pohlschroder, M. Genetic and biochemical analysis of the twin-arginine translocation pathway in halophilic archaea. *J. Bacteriol.* **187**, 8104–8113 (2005).
3. Thomas, J. R. & Bolhuis, A. The *tatC* gene cluster is essential for viability in halophilic archaea. *FEMS Microbiol. Lett.* **256**, 44–49 (2006).
4. Pickering, B. S. & Oresnik, I. J. The twin arginine transport system appears to be essential for viability in *Sinorhizobium meliloti*. *J. Bacteriol.* **192**, 5173–5180 (2010).
5. Chang, C. Y. *et al.* The *Bdellovibrio bacteriovorus* twin-arginine transport system has roles in predatory and prey-independent growth. *Microbiology* **157**, 3079–3093 (2011).
6. Dilks, K., Rose, R. W., Hartmann, E. & Pohlschroder, M. Prokaryotic utilization of the twin-arginine translocation pathway: a genomic survey. *J. Bacteriol.* **185**, 1478–1483 (2003).
7. Ize, B. *et al.* Novel phenotypes of *Escherichia coli tat* mutants revealed by global gene expression and phenotypic analysis. *J. Biol. Chem.* **279**, 47543–47554 (2004).
8. Letoffe, S., Heuck, G., Delepelaire, P., Lange, N. & Wandersman, C. Bacteria capture iron from heme by keeping tetrapyrrol skeleton intact. *Proc. Natl Acad. Sci. USA* **106**, 11719–11724 (2009).
9. Mickael, C. S. *et al. Salmonella enterica* serovar *Enteritidis tatB* and *tatC* mutants are impaired in Caco-2 cell invasion *in vitro* and show reduced systemic spread in chickens. *Infect. Immun.* **78**, 3493–3505 (2010).
10. Jongbloed, J. D. *et al.* TatC is a specificity determinant for protein secretion via the twin-arginine translocation pathway. *J. Biol. Chem.* **275**, 41350–41357 (2000).
11. Monds, R. D., Newell, P. D., Schwartzman, J. A. & O'Toole, G. A. Conservation of the Pho regulon in *Pseudomonas fluorescens* Pf0-1. *Appl. Environ. Microbiol.* **72**, 1910–1924 (2006).
12. Stanley, N. R., Findlay, K., Berks, B. C. & Palmer, T. *Escherichia coli* strains blocked in Tat-dependent protein export exhibit pleiotropic defects in the cell envelope. *J. Bacteriol.* **183**, 139–144 (2001).
13. Ochsner, U. A., Snyder, A., Vasil, A. I. & Vasil, M. L. Effects of the twin-arginine translocase on secretion of virulence factors, stress response, and pathogenesis. *Proc. Natl Acad. Sci. USA* **99**, 8312–8317 (2002).
14. Ding, Z. & Christie, P. J. *Agrobacterium tumefaciens* twin-arginine-dependent translocation is important for virulence, flagellation, and chemotaxis but not type IV secretion. *J. Bacteriol.* **185**, 760–771 (2003).
15. Stevenson, L. G. *et al.* Rhomboid protease AarA mediates quorum-sensing in *Providencia stuartii* by activating TatA of the twin-arginine translocase. *Proc. Natl Acad. Sci. USA* **104**, 1003–1008 (2007).
16. Gorla, P., Pandey, J. P., Parthasarathy, S., Merrick, M. & Siddavattam, D. Organophosphate hydrolase in *Brevundimonas diminuta* is targeted to the periplasmic face of the inner membrane by the twin arginine translocation pathway. *J. Bacteriol.* **191**, 6292–6299 (2009).
17. Weatherspoon-Griffin, N. *et al.* The CpxR/CpxA two-component system up-regulates two Tat-dependent peptidoglycan amidases to confer bacterial resistance to antimicrobial peptide. *J. Biol. Chem.* **286**, 5529–5539 (2011).
18. Meloni, S. *et al.* The twin-arginine translocation (Tat) system is essential for *Rhizobium*–legume symbiosis. *Mol. Microbiol.* **48**, 1195–1207 (2003).
19. Krehenbrink, M. & Downie, J. A. Identification of protein secretion systems and novel secreted proteins in *Rhizobium leguminosarum* bv. *viciae*. *BMC Genomics* **9**, 55 (2008).
20. De Buck, E., Lammertyn, E. & Anne, J. The importance of the twin-arginine translocation pathway for bacterial virulence. *Trends Microbiol.* **16**, 442–453 (2008).
21. Sargent, F. *et al.* Overlapping functions of components of a bacterial Sec-independent protein export pathway. *EMBO J.* **17**, 3640–3650 (1998).
This study identifies TatA and TatE as functionally equivalent components of a novel pathway for the transport of proteins with twin-arginine signal peptides in *E. coli*.

© 2012 Macmillan Publishers Limited. All rights reserved

22. Sargent, F., Stanley, N. R., Berks, B. C. & Palmer, T. Sec-independent protein translocation in *Escherichia coli*. A distinct and pivotal role for the TatB protein. *J. Biol. Chem.* **274**, 36073–36082 (1999).
23. Ize, B. *et al. In vivo* dissection of the Tat translocation pathway in *Escherichia coli*. *J. Mol. Biol.* **317**, 327–335 (2002).
24. Blaudeck, N., Kreutzenbeck, P., Muller, M., Sprenger, G. A. & Freudl, R. Isolation and characterization of bifunctional *Escherichia coli* TatA mutant proteins that allow efficient Tat-dependent protein translocation in the absence of TatB. *J. Biol. Chem.* **280**, 3426–3432 (2005).
This article describes the isolation of mutant *E. coli* TatA proteins that allow the Tat system to export native substrates in the absence of TatB. This indicates that TatB is not mechanistically essential for operation of the Tat pathway.
25. Yen, M. R., Tseng, Y. H., Nguyen, E. H., Wu, L. F. & Saier, M. H. Jr. Sequence and phylogenetic analyses of the twin-arginine targeting (Tat) protein export system. *Arch. Microbiol.* **177**, 441–450 (2002).
26. Greene, N. P. *et al.* Cysteine scanning mutagenesis and disulfide mapping studies of the TatA component of the bacterial twin arginine translocase. *J. Biol. Chem.* **282**, 23937–23945 (2007).
27. Barnett, J. P., Eijlander, R. T., Kuipers, O. P. & Robinson, C. A minimal Tat system from a Gram-positive organism: a bifunctional TatA subunit participates in discrete TatAC and TatA complexes. *J. Biol. Chem.* **283**, 2534–2542 (2008).
28. Jongbloed, J. D. *et al.* Two minimal Tat translocases in *Bacillus*. *Mol. Microbiol.* **54**, 1319–1325 (2004).
This work shows that TatB proteins are not components of the Tat machinery in some Gram-positive bacteria.
29. Pohlschroder, M., Dilks, K., Hand, N. J. & Wesley Rose, R. Translocation of proteins across archaeal cytoplasmic membranes. *FEMS Microbiol. Rev.* **28**, 3–24 (2004).
30. Rose, R. W., Bruser, T., Kissinger, J. C. & Pohlschroder, M. Adaptation of protein secretion to extremely high-salt conditions by extensive use of the twin-arginine translocation pathway. *Mol. Microbiol.* **45**, 943–950 (2002).
31. Bolhuis, A. Protein transport in the halophilic archaeon *Halobacterium* sp. NRC-1: a major role for the twin-arginine translocation pathway? *Microbiology* **148**, 3335–3346 (2002).
32. Berks, B. C. A common export pathway for proteins binding complex redox cofactors? *Mol. Microbiol.* **22**, 393–404 (1996).
This review proposes the existence of a bacterial protein transport system that translocates folded, cofactor-containing proteins using signal peptides that contain a conserved twin-arginine motif.
33. Berks, B. C., Palmer, T. & Sargent, F. The Tat protein translocation pathway and its role in microbial physiology. *Adv. Microb. Physiol.* **47**, 187–254 (2003).
34. Pomowski, A., Zumft, W. G., Kroneck, P. M. & Einsle, O. N_2O binding at a [4Cu:2S] copper–sulphur cluster in nitrous oxide reductase. *Nature* **477**, 234–237 (2011).
35. Zumft, W. G. Biogenesis of the bacterial respiratory Cu_A, Cu-S enzyme nitrous oxide reductase. *J. Mol. Microbiol. Biotechnol.* **10**, 154–166 (2005).
36. Simon, J., Einsle, O., Kroneck, P. M. & Zumft, W. G. The unprecedented *nos* gene cluster of *Wolinella succinogenes* encodes a novel respiratory electron transfer pathway to cytochrome *c* nitrous oxide reductase. *FEBS Lett.* **569**, 7–12 (2004).
37. Tottey, S. *et al.* Protein-folding location can regulate manganese-binding versus copper- or zinc-binding. *Nature* **455**, 1138–1142 (2008).
This article demonstrates that export by the Tat pathway allows a metalloprotein to acquire Mn^{2+} in the cytoplasm, where the abundance of competing ions is much lower than in the periplasm.
38. Ranquet, C., Ollagnier-de-Choudens, S., Loiseau, L., Barras, F. & Fontecave, M. Cobalt stress in *Escherichia coli*. The effect on the iron-sulfur proteins. *J. Biol. Chem.* **282**, 30442–30451 (2007). 39. Sauve, V., Bruno, S., Berks, B. C. & Hemmings, A. M. The SoxYZ complex carries sulfur cycle intermediates on a peptide swinging arm. *J. Biol. Chem.* **282**, 23194–23204 (2007).
40. Palmer, T., Sargent, F. & Berks, B. C. The Tat protein export pathway. In Escherichia coli *and* Salmonella*: Cellular and Molecular Biology*. (eds Böck, A. *et al.*) chapter 4.3.2. *EcoSal* [online] http://www.ecosal.org/ (American Society for Microbiology Press, 2010).
41. Joshi, M. V. *et al.* The twin arginine protein transport pathway exports multiple virulence proteins in the plant pathogen *Streptomyces scabies*. *Mol. Microbiol.* **77**, 252–271 (2010).
42. Widdick, D. A. *et al.* The twin-arginine translocation pathway is a major route of protein export in *Streptomyces coelicolor*. *Proc. Natl Acad. Sci. USA* **103**, 17927–17932 (2006).
43. Storf, S. *et al.* Mutational and bioinformatic analysis of haloarchaeal lipobox-containing proteins. *Archaea* **2010**, 410975 (2010).
44. Gralnick, J. A., Vali, H., Lies, D. P. & Newman, D. K. Extracellular respiration of dimethyl sulfoxide by *Shewanella oneidensis* strain MR-1. *Proc. Natl Acad. Sci. USA* **103**, 4669–4674 (2006).
45. Valente, F. M. *et al.* The [NiFeSe] hydrogenase from *Desulfovibrio vulgaris* Hildenborough is a bacterial lipoprotein lacking a typical lipoprotein signal peptide. *FEBS Lett.* **581**, 3341–3344 (2007).
46. Thompson, B. J. *et al.* Investigating lipoprotein biogenesis and function in the model Gram-positive bacterium *Streptomyces coelicolor*. *Mol. Microbiol.* **77**, 943–957 (2010).
47. Widdick, D. A. *et al.* Dissecting the complete lipoprotein biogenesis pathway in *Streptomyces scabies*. *Mol. Microbiol.* **80**, 1395–1412 (2011).
48. Aldridge, C., Spence, E., Kirkilionis, M. A., Frigerio, L. & Robinson, C. Tat-dependent targeting of Rieske iron-sulphur proteins to both the plasma and thylakoid membranes in the cyanobacterium *Synechocystis* PCC6803. *Mol. Microbiol.* **70**, 140–150 (2008).
49. Bachmann, J., Bauer, B., Zwicker, K., Ludwig, B. & Anderka, O. The Rieske protein from *Paracoccus denitrificans* is inserted into the cytoplasmic membrane by the twin-arginine translocase. *FEBS J.* **273**, 4817–4830 (2006).
50. De Buck, E. *et al.* The twin-arginine translocation pathway is necessary for correct membrane insertion of the Rieske Fe/S protein in *Legionella pneumophila*. *FEBS Lett.* **581**, 259–264 (2007).
51. Molik, S., Karnauchov, I., Weidlich, C., Herrmann, R. G. & Klosgen, R. B. The Rieske Fe/S protein of the cytochrome b_6/f complex in chloroplasts: missing link in the evolution of protein transport pathways in chloroplasts? *J. Biol. Chem.* **276**, 42761–42766 (2001).
52. Berks, B. C., Palmer, T. & Sargent, F. Protein targeting by the bacterial twin-arginine translocation (Tat) pathway. *Curr. Opin. Microbiol.* **8**, 174–181 (2005).
53. Jormakka, M., Tornroth, S., Byrne, B. & Iwata, S. Molecular basis of proton motive force generation: structure of formate dehydrogenase-N. *Science* **295**, 1863–1868 (2002).
54. Hatzixanthis, K., Palmer, T. & Sargent, F. A subset of bacterial inner membrane proteins integrated by the twin-arginine translocase. *Mol. Microbiol.* **49**, 1377–1390 (2003).
This study shows that the bacterial Tat pathway assembles substrate proteins containing TMHs.
55. Schliebs, W., Girzalsky, W. & Erdmann, R. Peroxisomal protein import and ERAD: variations on a common theme. *Nature Rev. Mol. Cell Biol.* **11**, 885–890 (2010).
56. Park, E. & Rapoport, T. A. Mechanisms of Sec61/SecY-mediated protein translocation across membranes. *Annu. Rev. Biophys.* **41**, 21–40 (2012).
57. Berks, B. C., Sargent, F. & Palmer, T. The Tat protein export pathway. *Mol. Microbiol.* **35**, 260–274 (2000).
58. Cline, K. & McCaffery, M. Evidence for a dynamic and transient pathway through the TAT protein transport machinery. *EMBO J.* **26**, 3039–3049 (2007).
59. Richter, S., Lindenstrauss, U., Lucke, C., Bayliss, R. & Bruser, T. Functional Tat transport of unstructured, small, hydrophilic proteins. *J. Biol. Chem.* **282**, 33257–33264 (2007).
References 58 and 58 demonstrate that some small, unfolded proteins can be transported through the Tat system in plants and bacteria.
60. Mould, R. M. & Robinson, C. A proton gradient is required for the transport of two lumenal oxygen-evolving proteins across the thylakoid membrane. *J. Biol. Chem.* **266**, 12189–12193 (1991).
61. Yahr, T. L. & Wickner, W. T. Functional reconstitution of bacterial Tat translocation *in vitro*. *EMBO J.* **20**, 2472–2479 (2001).
This report describes the development of an *in vitro* system for the analysis of the *E. coli* Tat transport pathway and demonstrates that ATP is not required for Tat-mediated transport.
62. Kwan, D. C., Thomas, J. R. & Bolhuis, A. Bioenergetic requirements of a Tat-dependent substrate in the halophilic archaeon *Haloarcula hispanica*. *FEBS J.* **275**, 6159–6167 (2008).
63. Alder, N. N. & Theg, S. M. Energetics of protein transport across biological membranes. a study of the thylakoid ΔpH-dependent/cpTat pathway. *Cell* **112**, 231–242 (2003).
This is the most detailed study on the energetics associated with protein transport by the Tat pathway.
64. Driessen, A. J. Bacterial protein translocation: kinetic and thermodynamic role of ATP and the protonmotive force. *Trends Biochem. Sci.* **17**, 219–223 (1992).
65. Mori, H., Summer, E. J. & Cline, K. Chloroplast TatC plays a direct role in thylakoid ΔpH-dependent protein transport. *FEBS Lett.* **501**, 65–68 (2001).
66. Martin, J. R., Harwood, J. H., McCaffery, M., Fernandez, D. E. & Cline, K. Localization and integration of thylakoid protein translocase subunit cpTatC. *Plant J.* **58**, 831–842 (2009).
67. Bolhuis, A., Mathers, J. E., Thomas, J. D., Barrett, C. M. & Robinson, C. TatB and TatC form a functional and structural unit of the twin-arginine translocase from *Escherichia coli*. *J. Biol. Chem.* **276**, 20213–20219 (2001).
This is the first article to report the purification of the TatBC complex and to show that TatB and TatC are present at equal stoichiometry in the complex.
68. Tarry, M. J. *et al.* Structural analysis of substrate binding by the TatBC component of the twin-arginine protein transport system. *Proc. Natl Acad. Sci. USA* **106**, 13284–13289 (2009).
This investigation yields the first low-resolution structures of purified TatBC with bound substrates.
69. Lee, P. A. *et al.* Cysteine-scanning mutagenesis and disulfide mapping studies of the conserved domain of the twin-arginine translocase TatB component. *J. Biol. Chem.* **281**, 34072–34085 (2006).
70. Behrendt, J., Lindenstrauss, U. & Bruser, T. The TatBC complex formation suppresses a modular TatB-multimerization in *Escherichia coli*. *FEBS Lett.* **581**, 4085–4090 (2007).
71. Orriss, G. L. *et al.* TatBC, TatB, and TatC form structurally autonomous units within the twin arginine protein transport system of *Escherichia coli*. *FEBS Lett.* **581**, 4091–4097 (2007).
72. Bruser, T. & Sanders, C. An alternative model of the twin arginine translocation system. *Microbiol. Res.* **158**, 7–17 (2003).
73. Maldonado, B., Buchanan, G., Muller, M., Berks, B. C. & Palmer, T. Genetic evidence for a TatC dimer at the core of the *Escherichia coli* twin arginine (Tat) protein translocase. *J. Mol. Microbiol. Biotechnol.* **20**, 168–175 (2011).
74. Ki, J. J. *et al.* A periplasmic fluorescent reporter protein and its application in high-throughput membrane protein topology analysis. *J. Mol. Biol.* **341**, 901–909 (2004).
75. Behrendt, J., Standar, K., Lindenstrauss, U. & Bruser, T. Topological studies on the twin-arginine translocase component TatC. *FEMS Microbiol. Lett.* **234**, 303–308 (2004).
76. Punginelli, C. *et al.* Cysteine scanning mutagenesis and topological mapping of the *Escherichia coli* twin-arginine translocase TatC Component. *J. Bacteriol.* **189**, 5482–5494 (2007).
77. Cline, K. & Mori, H. Thylakoid ΔpH-dependent precursor proteins bind to a cpTatC–Hcf106 complex before Tha4-dependent transport. *J. Cell Biol.* **154**, 719–729 (2001).
This is the first study to show that the TatBC complex binds substrate proteins.
78. Gerard, F. & Cline, K. The thylakoid proton gradient promotes an advanced stage of signal peptide binding deep within the Tat pathway receptor complex. *J. Biol. Chem.* **282**, 5263–5272 (2007).
79. Shanmugham, A., Wong Fong Sang, H. W., Bollen, Y. J. & Lill, H. Membrane binding of twin arginine preproteins as an early step in translocation. *Biochemistry* **45**, 2243–2249 (2006).
80. Bageshwar, U. K., Whitaker, N., Liang, F. C. & Musser, S. M. Interconvertibility of lipid- and translocon-bound forms of the bacterial Tat precursor pre-SufI. *Mol. Microbiol.* **74**, 209–226 (2009).
81. Papish, A. L., Ladner, C. L. & Turner, R. J. The twin-arginine leader-binding protein, DmsD, interacts with the TatB and TatC subunits of the *Escherichia coli* twin-arginine translocase. *J. Biol. Chem.* **278**, 32501–32506 (2003).

© 2012 Macmillan Publishers Limited. All rights reserved

82. Kostecki, J. S., Li, H., Turner, R. J. & DeLisa, M. P. Visualizing interactions along the *Escherichia coli* twin-arginine translocation pathway using protein fragment complementation. *PLoS ONE* **5**, e9225 (2010).
83. Alami, M. *et al.* Differential interactions between a twin-arginine signal peptide and its translocase in *Escherichia coli. Mol. Cell* **12**, 937–946 (2003).
This work uses site-specific crosslinking to show that TatC interacts with the signal peptide twin-arginine motif, whereas TatB contacts the entire signal sequence.
84. Gerard, F. & Cline, K. Efficient twin arginine translocation (Tat) pathway transport of a precursor protein covalently anchored to its initial cpTatC binding site. *J. Biol. Chem.* **281**, 6130–6135 (2006).
85. Maurer, C., Panahandeh, S., Jungkamp, A. C., Moser, M. & Muller, M. TatB functions as an oligomeric binding site for folded Tat precursor proteins. *Mol. Biol. Cell* **21**, 4151–4161 (2010).
86. Strauch, E. M. & Georgiou, G. *Escherichia coli tatC* mutations that suppress defective twin-arginine transporter signal peptides. *J. Mol. Biol.* **374**, 283–291 (2007).
87. Zoufaly, S. *et al.* Mapping the precursor-binding site on the TatC subunit of the twin-arginine-specific protein translocase by site-specific photo cross-linking. *J. Biol. Chem.* **287**, 13430–13441 (2012).
88. Whitaker, N., Bageshwar, U. K. & Musser, S. M. Kinetics of precursor interactions with the bacterial Tat translocase detected by real-time FRET. *J. Biol. Chem.* **287**, 11252–11260 (2012).
89. Mori, H. & Cline, K. A twin arginine signal peptide and the pH gradient trigger reversible assembly of the thylakoid ΔpH/Tat translocase. *J. Cell Biol.* **157**, 205–210 (2002).
This article demonstrates that thylakoid TatA interacts with the thylakoid TatBC complex only during substrate transport.
90. Panahandeh, S., Maurer, C., Moser, M., DeLisa, M. P. & Muller, M. Following the path of a twin-arginine precursor along the TatABC translocase of *Escherichia coli. J. Biol. Chem.* **283**, 33267–33275 (2008).
91. Dabney-Smith, C., Mori, H. & Cline, K. Oligomers of Tha4 organize at the thylakoid Tat translocase during protein transport. *J. Biol. Chem.* **281**, 5476–5483 (2006).
92. Dabney-Smith, C. & Cline, K. Clustering of C-terminal stromal domains of Tha4 homo-oligomers during translocation by the Tat protein transport system. *Mol. Biol. Cell* **20**, 2060–2069 (2009).
References 91 and 92 use chemical crosslinking to show that TatA polymerization is induced in thylakoids by the substrate-bound TatBC complex and by the PMF.
93. Leake, M. C. *et al.* Variable stoichiometry of the TatA component of the twin-arginine protein transport system observed by *in vivo* single-molecule imaging. *Proc. Natl Acad. Sci. USA* **105**, 15376–15381 (2008).
This study uses *in vivo* imaging of fluorophore-tagged TatA to show that it forms large assemblies of variable stoichiometry in a TatBC-dependent manner.
94. Frobel, J., Rose, P. & Muller, M. Early contacts between substrate proteins and TatA translocase component in twin-arginine translocation. *J. Biol. Chem.* **286**, 43679–43689 (2011).
95. de Leeuw, E. *et al.* Oligomeric properties and signal peptide binding by *Escherichia coli* Tat protein transport complexes. *J. Mol. Biol.* **322**, 1135–1146 (2002).
96. Bageshwar, U. K. & Musser, S. M. Two electrical potential-dependent steps are required for transport by the *Escherichia coli* Tat machinery. *J. Cell Biol.* **179**, 87–99 (2007).
This article demonstrates that the electrical potential component of the PMF is required for two distinct steps in Tat-mediated transport.
97. Hu, Y., Zhao, E., Li, H., Xia, B. & Jin, C. Solution NMR structure of the TatA component of the twin-arginine protein transport system from Gram-positive bacterium *Bacillus subtilis. J. Am. Chem. Soc.* **132**, 15942–15944 (2010).
This paper reports the solution NMR structure of the TMH and APH domains of a TatA protein.
98. Settles, A. M. *et al.* Sec-independent protein translocation by the maize Hcf106 protein. *Science* **278**, 1467–1470 (1997).
This article describes the identification of a protein component that is essential for protein transport by the plant Tat pathway, and the recognition that similar components are encoded in the genomes of bacteria.
99. Porcelli, I. *et al.* Characterization and membrane assembly of the TatA component of the *Escherichia coli* twin-arginine protein transport system. *Biochemistry* **41**, 13690–13697 (2002).
100. Lee, P. A., Buchanan, G., Stanley, N. R., Berks, B. C. & Palmer, T. Truncation analysis of TatA and TatB defines the minimal functional units required for protein translocation. *J. Bacteriol.* **184**, 5871–5879 (2002).
101. Dabney-Smith, C., Mori, H. & Cline, K. Requirement of a Tha4-conserved transmembrane glutamate in thylakoid Tat translocase assembly revealed by biochemical complementation. *J. Biol. Chem.* **278**, 43027–43033 (2003).
102. Lange, C., Muller, S. D., Walther, T. H., Burck, J. & Ulrich, A. S. Structure analysis of the protein translocating channel TatA in membranes using a multi-construct approach. *Biochim. Biophys. Acta* **1768**, 2627–2634 (2007).
103. Chan, C. S., Haney, E. F., Vogel, H. J. & Turner, R. J. Towards understanding the Tat translocation mechanism through structural and biophysical studies of the amphipathic region of TatA from *Escherichia coli. Biochim. Biophys. Acta* **1808**, 2289–2296 (2011).
104. Walther, T. H., Grage, S. L., Roth, N. & Ulrich, A. S. Membrane alignment of the pore-forming component $TatA_d$ of the twin-arginine translocase from *Bacillus subtilis* resolved by solid-state NMR spectroscopy. *J. Am. Chem. Soc.* **132**, 15945–15956 (2010).
105. Mori, H., Summer, E. J., Ma, X. & Cline, K. Component specificity for the thylakoidal Sec and Delta pH-dependent protein transport pathways. *J. Cell Biol.* **146**, 45–56 (1999).
106. Fincher, V., McCaffery, M. & Cline, K. Evidence for a loop mechanism of protein transport by the thylakoid Delta pH pathway. *FEBS Lett.* **423**, 66–70 (1998).
107. Koch, S., Fritsch, M. J., Buchanan, G. & Palmer, T. *Escherichia coli* TatA and TatB proteins have an N-out, C-in topology in intact cells. *J Biol Chem* **287**, 14420–14431 (2012).
This work demonstrates that the APH of TatA is not exposed at the periplasmic side of the membrane during Tat-mediated transport.
108. Maldonado, B. *et al.* Characterisation of the membrane-extrinsic domain of the TatB component of the twin arginine protein translocase. *FEBS Lett.* **585**, 478–484 (2011).
109. Barnett, J. P., Lawrence, J., Mendel, S. & Robinson, C. Expression of the bifunctional *Bacillus subtilis* TatAd protein in *Escherichia coli* reveals distinct TatA/B-family and TatB-specific domains. *Arch. Microbiol.* **193**, 583–594 (2011).
110. Hicks, M. G., Lee, P. A., Georgiou, G., Berks, B. C. & Palmer, T. Positive selection for loss-of-function *tat* mutations identifies critical residues required for TatA activity. *J. Bacteriol.* **187**, 2920–2925 (2005).
111. Frobel, J., Rose, R. & Muller, M. Twin-arginine translocation: early contacts between substrate proteins and TatA. *J. Biol. Chem.* **286**, 43679–43689 (2011).
112. Gohlke, U. *et al.* The TatA component of the twin-arginine protein transport system forms channel complexes of variable diameter. *Proc. Natl Acad. Sci. USA* **102**, 10482–10486 (2005).
This article reports low-resolution structures of purified TatA complexes in detergent solution.
113. Oates, J. *et al.* The *Escherichia coli* twin-arginine translocation apparatus incorporates a distinct form of TatABC complex, spectrum of modular TatA complexes and minor TatAB complex. *J. Mol. Biol.* **346**, 295–305 (2005).
114. Richter, S. & Bruser, T. Targeting of unfolded PhoA to the TAT translocon of *Escherichia coli. J. Biol. Chem.* **280**, 42723–42730 (2005).
115. Baglieri, J., Beck, D., Vasisht, N., Smith, C. & Robinson, C. Structure of the TatA paralog, TatE, suggests a structurally homogeneous form of Tat protein translocase that transports folded proteins of differing diameter. *J. Biol. Chem.* **287**, 7335–7344 (2011).
116. White, G. F. *et al.* Subunit organization in the TatA complex of the twin arginine protein translocase: a site-directed EPR spin labeling study. *J. Biol. Chem.* **285**, 2294–2301 (2010).
117. Luke, I., Handford, J. I., Palmer, T. & Sargent, F. Proteolytic processing of *Escherichia coli* twin-arginine signal peptides by LepB. *Arch. Microbiol.* **191**, 919–925 (2009).
118. Paetzel, M., Dalbey, R. E. & Strynadka, N. C. Crystal structure of a bacterial signal peptidase in complex with a β-lactam inhibitor. *Nature* **396**, 186–190 (1998).
119. Ferrandez, Y. & Condemine, G. Novel mechanism of outer membrane targeting of proteins in Gram-negative bacteria. *Mol. Microbiol.* **69**, 1349–1357 (2008).
120. Saito, A. *et al.* Post-liberation cleavage of signal peptides is catalyzed by the site-2 protease (S2P) in bacteria. *Proc. Natl Acad. Sci. USA* **108**, 13740–13745 (2011).
121. Mikhaleva, N. I., Santini, C. L., Giordano, G., Nesmeyanova, M. A. & Wu, L. F. Requirement for phospholipids of the translocation of the trimethylamine *N*-oxide reductase through the Tat pathway in *Escherichia coli. FEBS Lett.* **463**, 331–335 (1999).
122. Sikdar, R. & Doerrler, W. T. Inefficient Tat-dependent export of periplasmic amidases in an *Escherichia coli* strain with mutations in two DedA family genes. *J. Bacteriol.* **192**, 807–818 (2010).
123. Gouffi, K., Gerard, F., Santini, C. L. & Wu, L. F. Dual topology of the *Escherichia coli* TatA protein. *J. Biol. Chem.* **279**, 11608–11615 (2004).
124. Di Cola, A. & Robinson, C. Large-scale translocation reversal within the thylakoid Tat system *in vivo. J. Cell Biol.* **171**, 281–289 (2005).
125. Cline, K. & Theg, S. M. in *Molecular Machines Involved in Protein Transport across Cellular Membranes* (eds Dalbey, R.E., Koehler, C.M. & Tamanoi, F.) 463–492 (Elsevier, 2007).
126. Hinsley, A. P., Stanley, N. R., Palmer, T. & Berks, B. C. A naturally occurring bacterial Tat signal peptide lacking one of the 'invariant' arginine residues of the consensus targeting motif. *FEBS Lett.* **497**, 45–49 (2001).
127. Gazave, E. *et al.* Molecular phylogeny restores the supra-generic subdivision of homoscleromorph sponges (Porifera, Homoscleromorpha). *PLoS ONE* **5**, e14290 (2010).
128. Wagener, N., Ackermann, M., Funes, S. & Neupert, W. A. Pathway of protein translocation in mitochondria mediated by the AAA-ATPase Bcs1. *Mol. Cell* **44**, 191–202 (2011).
129. Stanley, N. R., Palmer, T. & Berks, B. C. The twin arginine consensus motif of Tat signal peptides is involved in Sec-independent protein targeting in *Escherichia coli. J. Biol. Chem.* **275**, 11591–11596 (2000).
130. Cristobal, S., de Gier, J. W., Nielsen, H. & von Heijne, G. Competition between Sec- and TAT-dependent protein translocation in *Escherichia coli. EMBO J.* **18**, 2982–2990 (1999).
This study shows that the hydrophobicity of the signal peptide h region is an important factor in selective targeting to the Tat, rather than the Sec, pathway.
131. Blaudeck, N., Kreutzenbeck, P., Freudl, R. & Sprenger, G. A. Genetic analysis of pathway specificity during posttranslational protein translocation across the *Escherichia coli* plasma membrane. *J. Bacteriol.* **185**, 2811–2819 (2003).
132. Bogsch, E., Brink, S. & Robinson, C. Pathway specificity for a ΔpH-dependent precursor thylakoid lumen protein is governed by a 'Sec-avoidance' motif in the transfer peptide and a 'Sec-incompatible' mature protein. *EMBO J.* **16**, 3851–3859 (1997).
133. Bendtsen, J. D., Nielsen, H., Widdick, D., Palmer, T. & Brunak, S. Prediction of twin-arginine signal peptides. *BMC Bioinformatics* **6**, 167 (2005).
134. Bagos, P. G., Nikolaou, E. P., Liakopoulos, T. D. & Tsirigos, K. D. Combined prediction of Tat and Sec signal peptides with hidden Markov models. *Bioinformatics* **26**, 2811–2817 (2010).
135. Rodrigue, A., Chanal, A., Beck, K., Muller, M. & Wu, L. F. Co-translocation of a periplasmic enzyme complex by a hitchhiker mechanism through the bacterial tat pathway. *J. Biol. Chem.* **274**, 13223–13228 (1999).
136. Jack, R. L. *et al.* Coordinating assembly and export of complex bacterial proteins. *EMBO J.* **23**, 3962–3972 (2004).
This article describes chaperone-mediated proofreading of cofactor-containing Tat substrates in *E. coli* and demonstrates that proofreading chaperones are specific for their cognate Tat signal peptides.
137. Pommier, J., Mejean, V., Giordano, G. & Iobbi-Nivol, C. TorD, a cytoplasmic chaperone that interacts with the unfolded trimethylamine *N*-oxide reductase enzyme (TorA) in *Escherichia coli. J. Biol. Chem.* **273**, 16615–16620 (1998).

© 2012 Macmillan Publishers Limited. All rights reserved

138. Li, S. Y., Chang, B. Y. & Lin, S. C. Coexpression of TorD enhances the transport of GFP via the TAT pathway. *J. Biotechnol.* **122**, 412–421 (2006).
139. Hatzixanthis, K. *et al.* Signal peptide-chaperone interactions on the twin-arginine protein transport pathway. *Proc. Natl Acad. Sci. USA* **102**, 8460–8465 (2005).
140. Sargent, F. Constructing the wonders of the bacterial world: biosynthesis of complex enzymes. *Microbiology* **153**, 633–651 (2007).
141. Maillard, J. *et al.* Structural diversity in twin-arginine signal peptide-binding proteins. *Proc. Natl Acad. Sci. USA* **104**, 15641–15646 (2007).
142. DeLisa, M. P., Tullman, D. & Georgiou, G. Folding quality control in the export of proteins by the bacterial twin-arginine translocation pathway. *Proc. Natl Acad. Sci. USA* **100**, 6115–6120 (2003).
This study demonstrates that a non-natively folded protein cannot be transported by the Tat pathway and forms the basis for the proposal that the Tat pathway mediates folding quality control.
143. Maurer, C., Panahandeh, S., Moser, M. & Muller, M. Impairment of twin-arginine-dependent export by seemingly small alterations of substrate conformation. *FEBS Lett.* **583**, 2849–2853 (2009).
144. Ize, B., Stanley, N. R., Buchanan, G. & Palmer, T. Role of the *Escherichia coli* Tat pathway in outer membrane integrity. *Mol. Microbiol.* **48**, 1183–1193 (2003).
145. Kimura, Y., Saiga, H., Hamanaka, H. & Matoba, H. *Myxococcus xanthus* twin-arginine translocation system is important for growth and development. *Arch. Microbiol.* **184**, 387–396 (2006).
146. Hitchcock, A. *et al.* Roles of the twin-arginine translocase and associated chaperones in the biogenesis of the electron transport chains of the human pathogen *Campylobacter jejuni. Microbiology* **156**, 2994–3010 (2010).
147. Biswas, L. *et al.* Role of the twin-arginine translocation pathway in *Staphylococcus. J. Bacteriol.* **191**, 5921–5929 (2009).

Acknowledgements:
The authors thank members of their laboratories, past and present, their collaborators and their colleagues in the Tat transport field for many insightful discussions on the Tat system, and apologize for being unable to cite many relevant papers owing to space constraints. Work in the authors' laboratories has been supported by the Biotechnology and Biological Sciences Research Council UK, the Medical Research Council UK, the Wellcome Trust, the Royal Society UK, the Leverhulme Trust, the European Molecular Biology Organization, the E. P. Abraham Cephalosporin Trust, the University of Oxford and the University of Dundee.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Tracy Palmer's homepage:
http://www.lifesci.dundee.ac.uk/groups/tracy_palmer/
Ben C. Berks's homepage:
http://www.bioch.ox.ac.uk/aspsite/index.asp?pageid=566
ALL LINKS ARE ACTIVE IN THE ONLINE PDF

© 2012 Macmillan Publishers Limited. All rights reserved