Targeting of proteins to the twin-arginine transport pathway

Tracy Palmer\textsuperscript{1†} and Phillip J. Stansfeld\textsuperscript{2}

\textsuperscript{1}Biosciences Institute, Molecular and Cellular Microbiology Theme, Centre for Bacterial Cell Biology, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4AX

\textsuperscript{2}School of Life Sciences & Department of Chemistry, University of Warwick, Gibbet Hill Campus, Coventry, CV4 7AL, United Kingdom

tray.palmer@newcastle.ac.uk and phillip.stansfeld@warwick.ac.uk

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\textsuperscript{†}for correspondence tray.palmer@newcastle.ac.uk

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Abstract
The twin-arginine protein transport (Tat pathway) is found in prokaryotes and plant organelles and transports folded proteins across membranes. Targeting of substrates to the Tat system is mediated by the presence of an N-terminal signal sequence containing a highly conserved twin-arginine motif. The Tat machinery comprises membrane proteins from the TatA and TatC families. Assembly of the Tat translocon is dynamic and is triggered by the interaction of a Tat substrate with the Tat receptor complex. This review will summarise recent advances in our understanding of Tat transport, focusing in particular on the roles played by Tat signal peptides in protein targeting and translocation.

Introduction
The transport of proteins across lipid membranes is an essential biological process. In prokaryotes, the general secretory (Sec) and twin arginine transport (Tat) pathways operate in parallel to transport proteins across the cytoplasmic membrane. The Sec system transports unfolded proteins through a narrow channel, in a process that can either be co-translational or post-translational (Fig 1A; reviewed in Tsirigotaki et al., 2017, Collinson et al., 2015). Following translocation, proteins fold in the extracellular compartment, usually aided by periplasmic chaperones (Stull et al., 2018). The twin arginine transport (Tat) pathway, by contrast, exports proteins that are folded in the cytosol, and is therefore strictly post-translational (Fig 1A; Cline, 2015, Berks, 2015, Hamsanathan and Musser, 2018). Both of these pathways are also able to integrate hydrophobic segments of transmembrane proteins into the bilayer.

The Sec pathway is ubiquitous and essential as the majority of both extracytoplasmic proteins and polytopic membrane proteins require this system for their localisation. The Tat pathway transports a smaller number of substrates and, as a consequence, is not essential for survival under most growth conditions (reviewed in Palmer and Berks, 2012). Indeed, the Tat pathway is absent from some classes of bacteria and archaea. Nevertheless, the Tat system plays an important role in prokaryote physiology as it transports a subset of proteins that must be exported in a folded state. These proteins are primarily those that non-covalently bind prosthetic groups in the cytoplasm, for example iron sulphur clusters or the molybdopterin cofactor (Weiner et al., 1998, Sargent et al., 1998). One of the most important Tat substrates is the Rieske iron-sulphur protein (Fig 2A) of the cytochrome bc$_1$ and bc$_6$f respiratory complexes which is essential for bacterial photosynthesis and for many types of respiratory metabolism (Meloni et al., 2003, Bachmann et al., 2006, De Buck et al., 2007, Aldridge et al., 2008, Keller et al., 2012, Hinsley et al., 2001). The Tat machinery is conserved in the chloroplasts and mitochondria of plants, but
with the exception of homoscleromorph sponges it has been lost from the mitochondria of animals (Settles et al., 1997, Carrie et al., 2016, Pett and Lavrov, 2013, Petru et al., 2018).

**Tat signal peptides**

Proteins are targeted to the Sec and Tat pathways by N-terminal signal peptides that are superficially very similar. They each share a tripartite structure with a positively charged n-region, a hydrophobic h-region, at least 12 amino acids in length with a propensity for helix formation, and a polar c-region containing a cleavage site for signal peptidase (Dalbey and Wickner, 1985, Yahr and Wickner, 2001, Lüke et al., 2009; Fig 1B). One of the key differences between the two targeting signals is that the n-regions of Tat signal peptides almost always have a pair of consecutive arginines, for which the Tat system is named, whereas the Sec signal peptide n-regions are simply positively charged with no specific sequence constraints (Berks, 1996). Numerous studies have highlighted the twin arginines in Tat signal peptides as essential for efficient Tat transport, with even conservative substitution with lysine being poorly tolerated (e.g. Stanley et al., 2000, DeLisa et al., 2002). By contrast, there appears to be no mechanistic difference between lysine and arginine in Sec signal peptides (Sasaki et al., 1990).

The paired arginines of the Tat signal peptide are part of a larger motif (S-R-R-x-F-L-K; Fig 1B). The amino acids at the other motif positions are only semi-conserved and none of them are essential for Tat transport (Stanley et al., 2000). After the twin arginines, the consensus phenylalanine has the highest frequency (for example it is found in two thirds of *Escherichia coli* Tat signals; Palmer et al., 2010). Mutational analysis has indicated that amino acid hydrophobicity at this position is an important factor and substitutions that reduce hydrophobicity decrease transport efficiency (Stanley et al., 2000).

A second critical difference between Sec and Tat signal peptides is the degree of h-region hydrophobicity. Highly hydrophobic Sec signal sequences interact with the signal recognition particle (SRP) when they emerge from the ribosome exit tunnel to engage in co-translational translocation. By contrast, moderately hydrophobic Sec signals escape SRP and mediate post-translational translocation through interaction with SecA and other chaperones (Tsirigotaki et al., 2017). Tat signal peptide h-regions are generally less hydrophobic than Sec signals and contain significantly more glycines and fewer leucines (Cristóbal et al., 1999). However, there is overlap in hydrophobicity scores for naturally-occurring Sec and Tat signal peptides, and more than half of *Escherichia coli* Tat signals are able to mediate some degree of productive engagement with the Sec pathway if they are fused to a Sec-compatible reporter protein (Tullman-Ercek et al., 2007).
Hamsanathan et al. (2017) recently noted that Tat signal peptides contain one or more helix-
destabilising residues (for example glycine, proline or serine) located between 12 and 17 residues
distal to the twin arginine motif (Hamsanathan et al., 2017). Evidence discussed below suggests
that these residues are required to allow the signal peptide to undergo conformational changes
during interaction with the Tat machinery.
Finally, Tat signal peptides frequently contain at least one basic amino acid in their c-regions.
This is not required for targeting to the Tat system and can be readily substituted for a neutral or
even negatively-charged amino acid without affecting the rate of Tat transport (Stanley et al.,
2000). Instead it has been shown that the positive charge acts as a Sec-avoidance motif,
reducing functional engagement of the signal peptide with the Sec pathway (Bogsch et al., 1997,
Cristóbal et al., 1999, Blaudeck et al., 2001) consistent with earlier studies that found that c-
region basic residues interfere with the function of Sec signal peptides (Li et al., 1988, Geller et
al., 1993).

Functional overlap between Tat and Sec targeting sequences
In prokaryotes and plastids, the Tat pathway always co-exists with Sec. It is imperative that Sec
and Tat substrate proteins are sorted to the correct transport pathway. The Sec system cannot
tolerate folded proteins, which can lead to lethal jamming of the machinery (Cosma et al., 1995,
van Stelten et al., 2009), whereas the Tat system is unable to transport most unfolded proteins
(e.g. Santini et al., 1998, Halbig et al., 1999, DeLisa et al., 2003). Correct targeting is particularly
relevant in the case of an unusual class of cytoplasmic polytopic membrane proteins that rely on
the activity of both Sec and Tat for their correct assembly. This class is exemplified by the Rieske
iron-sulphur protein from Actinobacteria. Most Rieske proteins have a single transmembrane
domain at their N-terminus, which in bacteria and plant thylakoids is an uncleaved Tat signal
sequence (Molik et al., 2001, Bachmann et al., 2006, De Buck et al., 2007). However,
Actinobacterial Rieske proteins, usually have three or occasionally five transmembrane helices
preceding the iron sulphur cluster-containing domain (Fig 2A; Niebisch and Bott, 2001, Keller et
al., 2012, Hopkins et al., 2014, Tooke et al., 2017). These unusually long Rieske proteins are
initially handled like most other polytopic membrane proteins, with the highly hydrophobic first
transmembrane helix engaging SRP for co-translational membrane insertion by the Sec pathway.
However, the final transmembrane helix resembles a typical Tat signal sequence, in accord with a
requirement for extracellular iron sulphur proteins to acquire their cofactors in the cytoplasm
(Berks, 1996, Berks et al., 2000, Keller et al., 2012). Detailed mechanistic analysis has indicated
that it is a combination of low relative hydrophobicity coupled with the presence of numerous
positive charges at the C-terminal side of the helix that render the Sec system unable to fully
translocate the C-terminus of this domain across the membrane. As a result, the Sec apparatus releases the transmembrane domain which most probably forms a re-entrant loop in the membrane (Tooke et al., 2017; Fig 2B). Following cofactor insertion into the Rieske domain, the membrane-tethered Tat signal sequence is recognised by the Tat system to complete localisation and assembly of the protein (Fig 2B).

The finding that the Tat signal peptides of such dual-targeted proteins initially engage with the Sec apparatus has significance for the targeting mechanism of soluble Tat substrates. Analysis of the known and predicted Tat substrates in E. coli and Salmonella shows that the overwhelming majority have the basic c-region or mature domain N-terminus required to avoid Sec transport. The inference is that such signal peptides often initially engage with the Sec pathway and abort at a late stage when the C-terminal positive charges are recognised and are inserted in the membrane as re-entrant loops. This would mean that the Tat system frequently recognises membrane-associated signal peptides (Musser and Theg, 2000, Ma and Cline, 2000, Shanmugham et al., 2006, Bageshwar et al., 2009).

Despite the overwhelming conservation of the twin arginines in Tat signal peptides, recent studies demonstrate that they are not mechanistically essential for operation of the Tat pathway. Specifically, inactivating substitutions in either the paired arginines or their binding site in the Tat translocon can be overcome by increasing the hydrophobicity of the signal peptide h-region (Ulfig et al., 2017, Huang and Palmer, 2017). Some of these hydrophobic suppressors are able to direct significant levels of export, approaching 30% of wild type transport activity, and it was noted that even signal peptides with a marked increase in hydrophobicity (approaching those that target the SRP pathway) could productively engage with Tat (Ulfig et al., 2017, Huang and Palmer, 2017).

Collectively these results indicate that the functional requirements for Tat signal peptides are remarkably similar to Sec, i.e. one or more positive charge in the signal peptide n-region coupled with a relatively hydrophobic h-region. Indeed, it has been shown that two canonical Sec signal peptides, OmpA and DsbA (Fig 1B) are able to interact with the Tat machinery and mediate Tat-dependent transport of a reporter protein. A conservative estimate predicts that almost half of E. coli Sec signals have features that would permit engagement with the Tat pathway (Huang and Palmer, 2017). However, in vivo, it is unlikely that such substrates would ever reach the Tat machinery because their signal peptides would interact with either SRP or SecA and be channelled into the Sec pathway. This places extraordinary constraints on Tat signal sequences which must evolve to escape recognition by these targeting factors. Indeed, it is likely that the twin arginine motif, and its cognate recognition site on the translocon, arose to increase the affinity of the Tat system for the weakly hydrophobic signal peptides.
By the same token, although signal peptides with paired arginines are compatible with the Sec pathway, only 0.02% of *E. coli* Sec signals contain this feature, whereas paired lysines are much more common (Huang and Palmer, 2017). This implies that there may also be evolutionary constraints acting on Sec targeting sequences. Interestingly, while some Tat substrate proteins are clearly incompatible with the Sec pathway because they must be folded in the cytoplasm, some protein families are compatible with either export route. A good example of this is the cell wall amidase family which in *E. coli* has the three members; AmiA, AmiB and AmiC. While AmiA and AmiC are Tat substrates, AmiB (which has 40% sequence identity to AmiC), is a Sec substrate (Ize *et al.*, 2003, Bernhardt and de Boer, 2003). Similarly, many solute binding proteins that would normally be expected to utilise the Sec pathway are Tat substrates in *Streptomyces* bacteria. (Widdick *et al.*, 2006, Joshi *et al.*, 2010). Intriguingly, the Tat machinery is localised to the tips of growing hyphae in *Streptomyces coelicolor*, so it is plausible that the Tat-dependent export of these proteins may reflect a requirement for them to be secreted at the region of active growth (Willemse *et al.*, 2012).

**Tat signal peptides trigger assembly of the active Tat translocon**

The Tat machinery comprises membrane proteins from the TatA and TatC families. TatA proteins are monotopic, with an amphipathic C-terminal domain located at the cytoplasmic side of the membrane (Koch *et al.*, 2012, Aldridge *et al.*, 2012; Fig 3A). In most Gram-negative bacteria and some Gram-positive bacteria (for example *Streptomyces*) two functionally distinct TatA family proteins, TatA and TatB, are present (Chanal *et al.*, 1998, Sargent *et al.*, 1999, De Keersmaeker *et al.*, 2005, Hicks *et al.*, 2006). By contrast TatB proteins are not found in the Tat systems of archaea, Gram-positive bacteria of the firmicutes phylum, and some obligate intracellular Gram-negative bacteria, (Jongbloed *et al.*, 2004, Dilks *et al.*, 2005, Nunez *et al.*, 2012). NMR structures of the helical regions of TatA and TatB have been determined (Fig 3A), with both proteins having a relatively short N-terminal transmembrane helix that is only marginally long enough to span the bilayer (Hu *et al.*, 2010, Rodriguez *et al.*, 2013, Zhang *et al.*, 2014).

TatC family proteins are polytopic, with six transmembrane helices. X-ray structures of *Aquifex aeolicus* TatC show that the six helices form a glove-shape with the arrangement stabilised by a structured periplasmic cap (Rollauer *et al.*, 2012, Ramasamy *et al.*, 2013; Fig 3A). Notably, the final two transmembrane helices of TatC (TM 5 and 6) are of similar length to the N-terminal transmembrane helices of TatA and TatB and molecular dynamics simulations indicate that the bilayer will be thinned in their vicinity (Rollauer *et al.*, 2012, Ramasamy *et al.*, 2013).

Most mechanistic studies on the bacterial Tat pathway have used *Escherichia coli* as a model, with plant thylakoids also providing an excellent system for mechanistic analysis of the related
eukaryotic Tat pathway. Current evidence points to a model whereby the active Tat translocon assembles 'on demand' upon interaction with a substrate protein, and that it disassembles once translocation is complete (Mori and Cline, 2002, Alami et al., 2003, Alcock et al., 2013, Rose et al., 2013; Fig 4).

In the resting state the Tat receptor complex comprises multiple (probably three or four) copies of TatA, TatB and TatC in a 1:1:1 ratio (Bolhuis et al., 2001, Alcock et al., 2016, Habersetzer et al., 2017). Crosslinking studies alongside sequence co-evolution analysis and molecular simulations have generated a robust model for the receptor complex (Blümmel et al., 2015, Alcock et al., 2016, Habersetzer et al., 2017; Fig 3B). In the resting state the transmembrane domain of TatB makes extensive contacts along the length of transmembrane helix 5 of TatC and the top of helix 6. Contacts between the opposite face of the TatB transmembrane helix and the first transmembrane helix of an adjacent TatC facilitate oligomerisation of the complex (Blümmel et al., 2015, Alcock et al., 2016). While a complex of TatB and TatC is stable to purification and retains the ability to interact with Tat signal peptides (Bolhuis et al., 2001, de Leeuw et al., 2002, Tarry et al., 2009), in vivo TatA is also associated with the complex in the resting state (Zoufaly et al., 2012, Aldridge et al., 2014, Alcock et al., 2016, Habersetzer et al., 2017). The TatA binding site was localised through cysteine crosslinking and molecular modelling to transmembrane helix 6 of TatC, adjacent to the TatB binding site which lies primarily on helix 5 (Fig 3B; Habersetzer et al., 2017).

Tat transport is initiated by the interaction of a Tat signal peptide with the receptor, with the twin arginine motif recognised by a conserved surface patch on the cytoplasmic face of TatC (Alami et al., 2003, Rollauer et al., 2012). Following initial binding, the signal peptide subsequently transitions to bind more deeply within the receptor complex (Alami et al., 2003, Gérard and Cline, 2007, Blümmel et al., 2015, Hamsanathan et al., 2017; Fig 4). This promotes a reorganisation of the receptor complex in which TatB is displaced from its resting state binding site on TatC, allowing this site to be occupied by TatA (Habersetzer et al., 2017, Alcock et al., 2016). In this activated state of the receptor complex, we expect that TatB now occupies the helix 6 binding site. Crosslinking experiments also suggest that TatC molecules adopt a tail-to-tail orientation following activation, homodimerising through transmembrane helices 5 and 6 (Cléon et al., 2015, Huang et al., 2017, Habersetzer et al., 2017). The precise order of these events is unclear. However, based on current evidence, it is likely that is the receptor re-organisation is triggered by interaction of the signal peptide h-region with TatB, consistent with the extensive contacts TatB makes with this region of the signal peptide (Alami et al., 2003, Panahandeh et al., 2008, Gérard and Cline, 2006). This mechanistic model is supported by genetic suppressor analysis, where a group of suppressor substitutions were identified in the transmembrane helix of TatB that restored
Tat transport activity to signal peptides with inactivating substitutions of the twin arginine motif and to TatC variants that had inactivating substitutions in the twin arginine recognition site. Biochemical analysis of these suppressors revealed signal peptide-independent structural reorganisation of the receptor complex (Huang et al., 2017) and for the strongest suppressor, TatB F13Y, constitutive TatB vacation of the TM5 site and occupancy of the TM6 site (Tooke and Palmer, unpublished).

The structure of the signal peptide-activated form of the receptor complex is not known. However, it has been shown that covalently attaching the twin arginine motif to its binding site on TatC does not inhibit translocation of a substrate across the membrane, indicating that the twin arginine residues remain at the cytoplasmic face of the membrane (Gérard and Cline, 2006). Extensive crosslinks have been detected throughout the signal peptide h-region with TatB (Alami et al., 2003, Panahandeh et al., 2008, Gérard and Cline, 2006), and a site-specific crosslink observed between a cysteine residue in the C-terminal end of the h-region and a cysteine in TatC TM5 (Aldridge et al., 2014). Applying these constraints to modelling the TatC-signal peptide complex places the signal peptide bound at the cytoplasmic face of one TatC protomer with the h-region interacting with TatB bound to TM5 of the adjacent TatC protomer (Aldridge et al., 2014, Alcock et al., 2016; Fig 5). The identification of TatC variants that have dominant negative activity (i.e. that inhibit Tat transport activity in the presence of a wild type copy of TatC) strongly supports the inference that hetero-oligomerisation of the receptor complex is a functional requirement (Cléon et al., 2015).

A recent study has confirmed that Tat signal peptides bind to the receptor complex in a hairpin conformation. Fluorescence quenching experiments place the C-terminal end of the signal peptide h-region at the tip of the hairpin, directly preceding the helix-destabilising residue (Fig 1B; Hamsanathan et al., 2017). The second arm of the hairpin would then be formed from the signal peptide c-region and potentially residues at the N-terminus of the mature domain (Fig 5). Indeed, crosslinking of both of these regions to TatB have been detected (Hamsanathan et al., 2017, Gérard and Cline, 2006). A role for the early mature domain of the substrate protein in receptor binding is supported by the isolation of suppressor substitutions in this region that can compensate for inactivating twin-arginine substitutions (Ulfig and Freudl, 2018). Collectively these results point to a model where the signal peptide may make contact with two separate TatB molecules; the c-region and early mature domain contacting TatB bound to TM5 of the same TatC protomer and the h-region with TatB bound at TM5 of a neighbouring TatC (Fig 5). In principle, contacts of the signal peptide with one or other of these TatB molecules (or potentially both) could drive productive reorganisation of the receptor.
This signal peptide-induced structural rearrangement primes the receptor for the recruitment of further TatA molecules, in a process that is dependent on the protonmotive force (Mori and Cline, 2002, Alami et al., 2003, Dabney-Smith et al., 2006, Dabney-Smith and Cline, 2009, Alcock et al., 2013, Rose et al., 2013, Aldridge et al., 2014; Fig 2B). The mechanism of TatA oligomer assembly is not understood. However, it has been speculated that the concave face of TatC may form a platform to support multimerisation (Rollauer et al., 2012). Intriguingly, activation of the receptor complex results in TatA occupancy at the TatC TM5 binding site which lies at the edge of this face and could potentially act as a nucleation point for TatA polymerisation (Alcock et al., 2016, Habersetzer et al., 2017). Some support for this mechanistic model comes from the work of Aldridge et al. who observed recruitment of Tha4 (the thylakoid orthologue of TatA) to a site at the concave face of TatC under protein transport conditions (Aldridge et al., 2014). At present it is not clear whether TatA forms an oligomer of fixed size, or a series of size-variable assemblies (Oates et al., 2005, Richter and Brüser, 2005, Gohlke et al., 2005, Leake et al., 2008, Beck et al., 2013, Dabney-Smith et al., 2006, Dabney-Smith and Cline, 2009). Further studies are required to understand the formation and arrangement of the TatA oligomer.

Evidence suggests that unhinging of the signal peptide hairpin may be a critical step in substrate translocation, and deliberate locking of the hairpin by internal crosslinking inhibits transport (Hamsanathan et al., 2017). How transport of the passenger domain is achieved, however, is still open to debate. Mechanisms for substrate transport are discussed in recent reviews (Cline, 2015, Berks, 2015, Hamsanathan and Musser, 2018) and will not be described in detail here. However, according to current models, the assembled TatA oligomer forms the substrate translocation pathway either through formation of a (size-variable) channel or by promoting localized membrane weakening and transient bilayer disruption (Gohlke et al., 2005, Leake et al., 2008, Brüser and Sanders, 2003, Rodriguez et al., 2013). Following passage of substrate across the membrane, the signal peptide is cleaved (Fig 4), the TatA oligomeric pore dissociates as the assembled translocation system rearranges to the resting state. Currently almost nothing is known about the mechanism of Tat translocon disassembly, and whether it is an obligate step for each round of substrate transport.

**Future perspectives**

Significant progress towards understanding the mechanism of protein transport by the Tat pathway has been catalysed by the determination of high-resolution structures for TatC and the helical regions of TatA and TatB (Rollauer et al., 2012, Ramasamy et al., 2013, Rodriguez et al., 2013, Zhang et al., 2014). However, currently we still lack a molecular level understanding of protein translocation, which ideally requires structural resolution of protein complexes and
transport intermediates. The highly dynamic nature of the Tat system makes this particularly challenging, but may be facilitated through isolation of mutations that lock the translocon in intermediate states. The identification of a substitution that promotes constitutive translocon assembly (e.g. *E. coli* TatBF13Y; Huang *et al.*, 2017) could offer insight into the nature of the assembled translocon, and has the potential to address questions including whether the assembled TatA oligomer is of fixed size, and how TatA molecules are scaffolded. Finally, it is unclear how translocon disassembly is initiated, and whether this process is related to the mechanism by which the Tat system fails to transport some unfolded proteins (Richter and Brüser, 2005, Panahandeh *et al.*, 2008).
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Figure legends

Figure 1. Targeting to the Sec and Tat pathways. A. The Sec pathway transports unfolded proteins. During co-translational targeting to Sec, the signal sequence is recognised at the translating ribosome by ribosome-bound signal recognition particle (SRP) and the nascent chain is guided via the SRP receptor to the Sec translocon where the energy of protein synthesis is harnessed to drive protein transport. In the post-translational pathway, the substrate is maintained in an unfolded conformation and guided to the Sec translocon by the ATPase, SecA. ATP hydrolysis by SecA provides the driving force for Sec-dependent post-translational protein export (Tsirigotaki et al., 2017, Collinson et al., 2015, Rapoport et al., 2017, Lycklama et al., 2012). The Tat pathway transports folded proteins without the requirement for targeting factors. B. Signal peptides that target to Sec and Tat pathways share a similar tripartite organisation with a positively-charged n-region, hydrophobic h-region and polar c-region containing a signal peptidase cleavage site (AxA). Tat signal peptides have an almost invariant pair of arginines that are embedded within an SRRxFLK motif (Berks, 1996). A helix destabilising residue (#), often a glycine, serine or proline towards the C-terminal end of the h-region, provides flexibility at this region of the signal peptide (Hamsanathan et al., 2017). A basic residue (+) is frequently found in the Tat signal peptide c-region and serves as a Sec avoidance motif (Bogsch et al., 1997). The arrow indicates the position of signal peptide cleavage. Amino acid sequences of two E. coli Sec signal peptides, OmpA (post-translational Sec targeting; Fekkes et al., 1998) and DsbA (co-translational targeting; Schierle et al., 2003) – basic residues in the n-region and the signal peptidase cleavage site in the c-region are underlined and shown in bold. Two well-studied E. coli Tat signal peptides, SufI and TorA, are also shown. Residues that match the twin arginine consensus are in red, the Sec avoidance signal in bold typeface and the signal peptidase cleavage site in underline.

Figure 2. The Sec and Tat pathways co-operate for the biogenesis of some bacterial Rieske proteins. A. The topological arrangements of selected bacterial Rieske proteins are shown. The vast majority of bacterial Rieske proteins have a single N-terminal transmembrane
helix that is inserted in the membrane by the Tat pathway (Bachmann et al., 2006, De Buck et al., 2007, Goosens et al., 2014). Most Actinobacterial Rieske proteins have three transmembrane helices, but some have five (Keller et al., 2012, Tooke et al., 2017). In each case the final transmembrane helix is integrated by Tat. B. Biogenesis pathway for polytopic Rieske proteins through Sec, to enable initial transmembrane helix insertion (adapted from (Keller et al., 2012, Tooke et al., 2017)) and subsequently, C. through Tat to allow insertion of the final transmembrane helix and transport of the soluble domain.

Figure 3. Structures of the Tat components and a model for the Tat receptor complex. A. Structures of TatA (blue), TatB (orange) and TatC (green) (Rodriguez et al., 2013, Zhang et al., 2014, Rollauer et al., 2012, Ramasamy et al., 2013). TatC has six transmembrane helices with the N- and C-terminus located at the cytoplasmic side of the membrane. Transmembrane helices are numbered. B. A model for the resting state of the TatABC receptor, showing the interactions of the transmembrane helices, with the constituent subunits coloured as in part A. C. Location of the TatA and TatB transmembrane helix binding sites on TatC in the resting state receptor. The TatC stoichiometry of the Tat complex is not established but modelled here for a TatC trimer (adapted from Habersetzer et al., 2017).

Figure 4. A Model for the Tat transport pathway. Step1. A folded Tat substrate docks at the Tat receptor complex, the twin arginines in the signal peptide n-region binding to the cytoplasmic surface of TatC. Step 2. The signal peptide transitions to bind more deeply into the receptor, inserting in a hairpin conformation. The deep insertion of the signal peptide displaces TatB from its resting state binding site on TatC to occupy the TatA binding site at TMH6. A TatA molecule is now recruited to the binding site vacated by TatB. Step 3. The positioning of TatA at the TM5 binding site allows the further recruitment and nucleation of TatA molecules to form a large oligomer. Step 4. The signal peptide hairpin unhinges and the substrate passes across the membrane facilitated by the TatA oligomer. Step 5. The signal peptide is cleaved and the mature domain is released at the periplasmic side of the membrane. Following substrate translocation, the TatA oligomer dissociates and the Tat receptor returns to the resting state.

Figure 5. Hypothetical Structural Model for the signal peptide bound receptor complex. A. Model of the TatABC (blue, orange and green) resting-state complex, with bound SufI substrate (red). Views are from the membrane (left) and the cytoplasm (right). B. The signal peptide hairpin of SufI (blue/yellow/green) binds to the concave face of TatC, such that it can interact with both copies of TatB, either side of a TatC monomer (left). The binding of signal
peptide induces an exchange between TatA and TatB subunits (middle), with the TatA subunits ultimately oligomerising (not shown) to permit the translocation of the SufI mature domain, aided by the unhinging of the signal peptide hairpin (right).
References


Cosma, C. L., Danese, P. N., Carlson, J. H., Silhavy, T. J. and Snyder, W. B. (1995) Mutational activation of the Cpx signal transduction pathway of *Escherichia coli* suppresses the...


binding of SecB and the signal sequence to SecA. Molecular Microbiology, 29, 1179-1190.


Proceedings of the National Academy of Sciences of the United States of America, 85, 7685-7689.


Niebisch, A. and Bott, M. (2001) Molecular analysis of the cytochrome $b_{c_{1}}-aa_{3}$ branch of the Corynebacterium glutamicum respiratory chain containing an unusual diheme cytochrome $c_{1}$. Archives of Microbiology, 175, 282-294.


Sasaki, S., Matsuyama, S. and Mizushima, S. (1990) In vitro kinetic analysis of the role of the positive charge at the amino-terminal region of signal peptides in translocation of


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