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Review

The Tat system of Gram-positive bacteria ☆

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ABSTRACT

The twin-arginine protein translocation (Tat) system has a unique ability to translocate folded and co-factor-containing proteins across lipid bilayers. The Tat pathway is present in bacteria, archaea and in the thylakoid membranes of chloroplasts and, depending on the organism and environmental conditions, it can be deemed important for cell survival, virulence or bioproduction. This review provides an overview of the current understanding of the Tat system with specific focus on Gram-positive bacteria. The 'universal minimal Tat system' is composed of a TatA and a TatC protein. However, this pathway is more commonly composed of two TatA-like proteins and one TatC protein. Often the TatA-like proteins have diverged to have two different functions and, in this case, the second TatA-like protein is usually referred to as TatB. The correct folding and/or incorporation of co-factors are requirements for translocation, and the known quality control mechanisms are examined in this review. A number of examples of crosstalk between the Tat system and other protein transport systems, such as the Sec–YidC translocon and signal peptidases or sheddases are also discussed. Further, an overview of specific Gram-positive bacterial Tat systems found in monoderm and diderm species is detailed. Altogether, this review highlights the unique features of Gram-positive bacterial Tat systems and pinpoints key questions that remain to be addressed in future research. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

1. Introduction to the Tat system

The twin-arginine translocation (Tat) pathway is found in the membrane of bacteria, archaea and in the thylakoids of chloroplasts. It is unique in that before proteins are moved from the cytoplasm into or over the membrane, they are correctly folded and, if appropriate, co-factors are attached [1–7]. The cargo proteins are globular and the pathway must allow for variable-sized cargo and, therefore, a range of potential pore sizes [8]. A second distinctive identifier of the Tat system is the eponymous motif containing twin-arginine residues in the signal peptide of proteins destined for transport via this pathway.

The Tat system is most studied in the Gram-negative bacterium *Escherichia coli*. However, in recent years, the understanding of the Tat system in Gram-positive bacteria, such as *Bacillus subtilis* and corynebacteria has expanded extensively. Combined, these studies have highlighted a number of strong similarities and some interesting differences between the bacterial Tat systems. Components of the *B. subtilis* Tat system are able to functionally replace all components of the Tat system in *E. coli* [9,10]; they all have similar signal peptide requirements [11–13] and there are numerous examples of interspecies crossover with regard to substrates [10,14–18]. Differences seem to come in at the quality control and chaperone level. These similarities suggest that, at a fundamental level, the Tat system follows similar mechanisms in both Gram-positive and Gram-negative bacteria. This review focuses specifically on the Tat system in Gram-positive bacteria, although it will refer to investigations performed in *E. coli*.

1.1. The core Tat system

Cargo proteins are destined for the Tat system by virtue of specific N-terminal signal peptides. The signal peptide of the Tat system is similar to that of the Sec system, the main bacterial protein secretion pathway, but there are several distinctions between the two. Signal peptides are generally composed of three defined regions; a N-terminal domain, a hydrophobic region and a polar C-terminal domain containing the signal peptidase recognition site [19,20]. On average, the hydrophobic region of Tat signal peptides is larger and less hydrophobic than that of Sec signal peptides [21]. The canonical N-terminal twin-arginine motif is S/T-RRxFLK (with x referring to a polar amino acid) and it is central to Tat-dependent translocation [22,23]. There are a few instances where Tat-dependently translocated proteins do not have signal peptides. However, in these cases the cargo protein is associated with a second Tat signal peptide-containing protein and a piggyback or hitchhiker mechanism allows for its translocation [24,25].

The current consensus model for the Tat system involves a docking complex and a pore complex. In essence this requires two types of proteins: a large integral membrane protein named TatC and a small membrane protein named TatA. Multiple TatA-like proteins are present in most organisms, and are known as TatA, TatB or TatE [22,26] (Fig. 1). Translocation is initiated once a cargo protein with the correct signal

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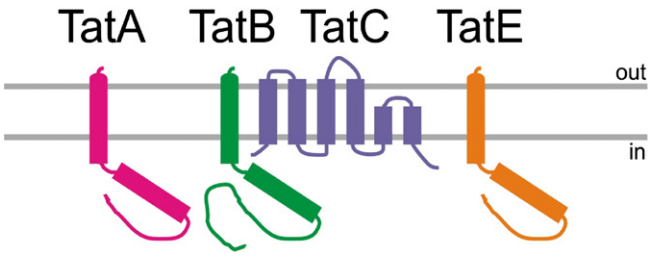

Fig. 1. Basic Tat system components. The universally confirmed components of the Tat system are illustrated. The TatA-like proteins TatA, TatB and TatE are shown in pink, green and orange respectively, while the large transmembrane protein TatC is shown in purple.

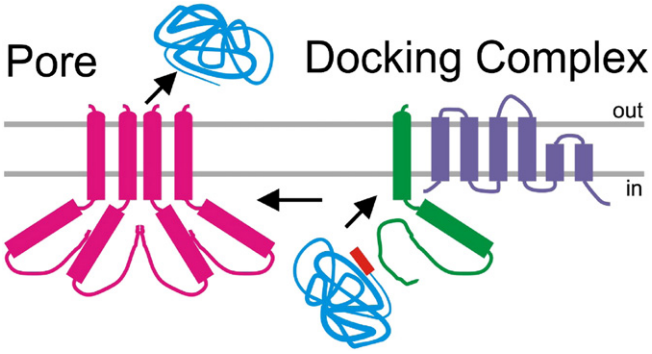

Fig. 2. Consensus model for protein translocation via the Tat system. A simple model for protein translocation via the Tat system is depicted as follows: The docking complex is composed of TatC and a TatA-like protein (often TatB). This docking complex forms the primary cargo-Tat interaction point. The Tat-dependent cargo protein in its 'translocation-approved' form interacts with the docking complex, where after pore-forming TatA-like proteins are recruited. The pore adapts to the globular nature of the cargo, which is then translocated across the membrane and released from the Tat machinery.

peptide interacts with a docking complex composed of TatC and TatA-like proteins [27,28]. The specific interaction between the cargo protein and the docking complex has been implicated in substrate proofreading [22,29–32], and the TatC component of the docking complex inserts the cargo proteins into the membrane [33]. The cargo-docking complex then recruits the pore-forming TatA-like proteins and the proton-motive force is used as an energy source for translocation [29,34,35] (Fig. 2). Tat complexes of different sizes have been observed and are described in Table 1.

Phylogenetic studies of Tat systems in all kingdoms of life have defined the composition of the principal Tat system as one TatC and two TatA-like components [36,37]. The replication of TatA or TatA-like proteins is probably a result of direct gene duplication as the gene for the second TatA-like component is often found elsewhere on the genome. In some cases these duplicated TatA-like proteins have diverged substantially from the 'original' TatA protein [37]. This divergence is observed in a number of bacterial species and thylakoids where the second TatA-like proteins are termed TatB or Hcf106a, respectively. Though structurally similar, these diverged TatA-like proteins have specialized to a point where TatB explicitly associates with TatC in the docking complex and TatA assembles into the pore complex [38,39].

The TatA–TatB divergence is not universal nor is the core system always defined as TatA, TatB and TatC, as demonstrated in staphylococci and *B. subtilis*. In staphylococci only one TatA protein is present [40,41], and in *B. subtilis* the Tat system is composed of two parallel TatA–TatC pathways each with a single unique TatA [42]. Similarly, in *Corynebacterium glutamicum*, which contains three TatA-like proteins (TatA, TatB, TatE), the essential components for translocation are TatA and TatC only [43]. Hence, the optimum Tat system seems to be composed of three components (TatA, TatB and TatC), while the essential core could be defined as a binary TatA–TatC system.

1.2. The TatC protein

TatC is a protein with six membrane-spanning domains and highly conserved cytoplasmic loops [37,44,45]. As mentioned above, TatC interacts with TatA-like proteins (often TatB) to form the docking complex and its role is to identify and verify cargo [46,47]. A high degree of protein conservation between species is often a good indication of the importance of regions and residues within a protein. This is indeed the case in the first cytoplasmic loop of TatC, which directly interacts with cargo proteins [45,48–52]. Furthermore, both cytoplasmic loops are directly associated with TatA [53]. The transmembrane spanning domains of TatC interact directly with other TatC molecules [45,54] as well as with the cargo [33,51]. Residues within the first two extracytoplasmic loops of TatC are not highly conserved between species. However, the secondary structure of these extracytoplasmic loops must be vital, as random mutagenesis studies have shown that these regions were specifically sensitive to substitutions [48,55]. Further, mutations in the C-terminal cytoplasmic region of *B. subtilis* TatCy block substrate translocation, implying this region is also important [52]. Hence, structural and functional studies investigating TatC have shown a number of regions that directly interact with other

Table 1
Tat complexes of *B. subtilis* and *E. coli* and their sizes.

E. coli			
TatA (11 kDa)	50–500 kDa [73,158,159]	TatAC	Aggregates and no clear complex [158]
TatB (18 kDa)	100–880 kDa [158] OR 100 kDa [59]	TatBC	440 kDa and 580 kDa [158,159] OR 430 kDa [59] OR 370 kDa [73]
TatE (7 kDa)	50–110 kDa [57]	TatAB	Aggregates with hints of a ladder [158] OR pools at the bottom of the gel [73]
TatC (30 kDa)	250 kDa [158] OR 220 kDa [59]	TatABC	440 kDa and 580 kDa [158] OR 430 kDa [59] OR 370 kDa [73]
B. subtilis			
TatAy (5 kDa)	Membrane: 200 kDa* [16] Cytoplasm: 5 MDa* [16]	TatAdCd	230 kDa [10]
TatAd (7 kDa)	Membrane: 270 kDa* [10,74] OR 160 kDa* [10] OR 200 kDa* (his-tagged) Cytoplasm: 2 MDa* [10] Laddering described but size not discussed by Walther et al. [67]	TatAy–TatCy	200 kDa* [16]
TatAc (6 kDa)	100 kDa [74,126]	TatAcCd	230 kDa [126]
TatCd (27 kDa)	66 kDa and 100 kDa [126]	TatAcCy	200 kDa [126]
TatCy (28 kDa)	66 kDa [126]		

The predicted protein sizes according to gene products are indicated in brackets next to each protein. Complex sizes were mostly determined by blue-native gel electrophoresis, except for a few cases where gel filtration chromatography was used (indicated by *).

components of the Tat system, and these regions have specific roles with regard to efficient Tat-dependent translocation.

Although duplications of TatA-like proteins are common, the Tat system in *B. subtilis* is distinctive in that there are two copies of *tatC* on the genome, *tatCd* and *TatCy* [37,56]. TatCd and TatCy each form complexes with TatAd or TatAy respectively, which operate in parallel as detailed in the following sections.

1.3. TatA and TatA-like proteins

Sequence comparisons of TatA and TatA-like proteins have shown that variations have arisen due to gene duplications followed by further sequence divergence [37]. In some cases the sequence divergence has resulted in proteins with specialized functions, which is particularly evident in *E. coli* and *Streptomyces coelicolor* where there are three *tatA*-like genes on the genomes. TatA and TatE are interchangeable and either of them can form part of the pore during translocation [57,58], while TatB functionality has specialized to form part of the docking complex [2,32,3359]. *E. coli* TatA and TatB only share 20% sequence similarity [60]. However, small mutations in TatA allow for complementation of a TatB mutant strain [61,62] and a chimeric TatA–TatB protein is able to complement for the absence of both TatA and TatB [63]. Further, *B. subtilis* TatAd is a bifunctional protein that is able to compensate for the absence of TatA and TatB in *E. coli* [10]. This underscores the view that, although TatA and TatB have evolved their own specific functions, they are structurally similar and with minor tweaking can replace one another.

TatA and TatA-like proteins are composed of a short N-terminal domain which sticks outward from the membrane, a single transmembrane domain, a short flexible hinge region and an amphipathic helix that is thought to lie flat against the membrane [64–66]. The amphipathic helix region leads onto a second flexible end region containing a large number of densely charged residues [67]. These densely charged residues have been suggested to form charged zippers that allow for the self-assembly of the TatA pore-complex. Most structural regions are involved in TatA functioning, and the hinge and amphipathic helix are particularly important [9,10,60,63,68–72]. Only the C-terminal region was shown to be dispensable for TatB or TatA activity in *E. coli* [63].

Blue native gel electrophoresis of *E. coli* TatA has revealed a laddering of TatA complexes (Table 1) and electron microscopy studies have subsequently confirmed that *E. coli* TatA forms complexes with variations in size [8,73]. This has led to the theory that by changing the number of TatA components the pore size can adapt to the size of the substrate to be translocated. Though highly attractive, this theory is hard to reconcile with regard to the apparent lack of major size variations in complexes of *E. coli* TatE [58] or *B. subtilis* TatAd [74]. Notably, the blue native laddering effect is greatly diminished for *E. coli* TatE and *B. subtilis* TatAd, and it remained so far undetected for *B. subtilis* TatAc [57,67,74]. Nevertheless, *E. coli* TatE and *B. subtilis* TatAd and TatAc are able to compensate for TatA deficiency in *E. coli* [10,57,75]. This raises the question how pore complexes composed of only *E. coli* TatE or *B. subtilis* TatA proteins accommodate cargo proteins of very different sizes while maintaining membrane integrity.

2. Quality control and pre-processing of cargo proteins

The twin-arginine residues in the signal peptide region define the Tat system and its substrates. However, the signal peptide is not the only requirement for Tat-dependent translocation. Also, the correct folding and co-factor incorporation of cargo proteins is vital in Tat systems. If the cargo protein is not correctly folded or if co-factors are inserted incorrectly, translocation is terminated and the cargo degraded [76–78]. For example, it has been proposed for certain Tat-dependently transported proteins, such as YkuE of *B. subtilis* and the cyanobacterial MncA protein, that divalent metal ions lower down the Irving William series (e.g. Mn^{2+} and Fe^{2+}) are particularly important co-factors [79,80]. These co-factors need to be inserted prior to translocation to ensure their stability and proper function [79]. Strong evidence exists that the Tat machinery, and in particular the docking complex, is directly involved with Tat substrate quality control [2,3,33,81]. Further, if essential for survival, suppressor mutations can arise in the docking complex (TatC and TatB) to ensure translocation, as shown with the selectable reporter TEM-1 β-lactamase in several folded states [3].

The folding requirement of cargo proteins for translocation via Tat has been very clearly illustrated with proteins that need disulfide bonds for their stability and/or activity. This includes single-chain Fv antibody fragments, heterodimeric F(AB) antibody fragments, human tissue plasminogen activator and *E. coli* TorA–PhoA fusion proteins [1,2,4,82,83]. However, disulfide bond formation is not the only folding requirement for export of these proteins via Tat. When using the TorA–PhoA fusion protein as a model, translocation was hindered substantially when the protein was truncated while the region with disulfide bonds was not altered [4].

In the *E. coli* Tat system, certain cargo proteins have been associated with their own specialized chaperones and in some instances the signal peptide region of the cargo protein has been shown to directly interact with them. These chaperones allow for the incorporation of specific cofactors and the correct folding of the cargo proteins [78,84]. Such specific chaperone–substrate relationships include the hydrogenase-2 (NiFe) chaperoned by HybE [78] and TorA chaperoned by TorD [78]. A chaperone function has also been proposed for DmsD in chaperoning the DmsA subunit of DMSO reductase [84], but a subsequent study showed that DmsD is completely dispensable for DmsA export [85].

In the case of Gram-positive bacteria no clear Tat-specific chaperones have as yet been identified. Nevertheless, yeast two-hybrid (Y2H) studies performed with the *B. subtilis* Tat proteins against a library of proteins have suggested a number of Tat-interacting partners with potential chaperone or proofreading activity. For example, this study showed that WprA, an extracytoplasmic cell wall-bound protease, is important for export of the Tat-dependent substrate EfeB [86]. Further, the soluble chemoreceptor HemAT and the large transmembrane pentose transporter CsbC were shown to affect the amount of TatAd observed in the cell and, thus, to be important for export of the Tat-dependent substrate PhoD [86]. Quantitative proteomic studies in *B. subtilis tat* mutant strains have also highlighted a number of proteins potentially associated with quality control. One of these, the iron–sulfur scaffold protein SufS, was found in decreased amounts in the cytoplasm of a *tat* mutant strain (total-*tat*) devoid of all Tat components [6]. Furthermore, a chaperone associated with heat shock and iron–sulfur cluster assembly, DnaJ, was observed in higher amounts in the total-*tat* mutant strain [6,87,88]. Although these proteomic approaches have as yet not confirmed any Gram-positive Tat-associated chaperones, they have put forward potential protein–protein partners and suggest a larger more complicated Tat-associated protein network than has previously been considered.

There has been some discussion in the field as to whether or not TatA molecules interact with the substrate in the cytoplasm before the cargo is inserted into the membrane. This would be an enticing theory, where TatA or TatA-like proteins interact with the cargo protein prior to the docking complex interactions. These discussions all revolve around identification and co-localization of TatA in the cytoplasm and contrasting results have been documented. Studies in *E. coli* with YFP-labeled TatA showed that TatA localized only at the membrane [89], while TatA labeled with ^{35}S-methionine showed both membrane and cytoplasmic localization [90]. Analyses in *B. subtilis* with green fluorescent protein (GFP) labeled TatAc, TatAy and TatAd showed these proteins localized at the poles and membrane [91]. However, other studies indicate that TatAd may associate with its substrate, pre-PhoD, in the cytoplasm and the membrane [53,92,93]. When expression is induced in *E. coli*, TatAy [16] and TatAd [10] have also been observed in the cytoplasm as well as the membrane. Similarly, in *Streptomyces lividans*, TatA was shown to interact with TatB or its cargo in the

cytoplasm [94,95]. Further studies in *S. lividans* showed that TatA or TatB complexes interact with the signal peptide region of Tat-dependent substrates in the cytoplasm [96]. This led to theories where TatA-like proteins might have chaperone activities that lead/recruit substrates to the membrane translocation pore. An interesting additive is that, when the whole Tat system is hyper-expressed in *E. coli*, TatA forms tubes in the cytoplasm and the presence of these tubes is dependent on TatC [97]. Under non-hyper-expressed conditions TatC also stabilizes and helps retain TatAd in the membrane [53]. Cytoplasmic TatA may therefore have a broader role in the Tat translocation system. However, this may not reflect real physiological Tat-associated interactions, since all these studies have been performed using induced Tat proteins with subsequent cell disruption and subcellular fractionation. This harsh treatment of the cells may well lead to a (partial) dissociation of TatA from the membrane, which cannot be readily distinguished from the possible presence of soluble TatA that is in the process of being inserted into the membrane.

Altogether, there are multiple proteins and quality control checkpoints associated with correct translocation of Tat substrates. These include protein interactions between substrate specific chaperones, the signal peptide region of the substrate and the docking complex, to ensure correct folding of substrates and co-factor insertion. Many ongoing research efforts are aimed at the molecular dissection of these checkpoints, and this is particularly necessary for an improved understanding of mechanisms determining Tat pathway function in Gram-positive bacteria.

3. Crosstalk between pathways

Cellular systems are complex and proteins can often have overlapping functions. The core Tat system has its select components, however proteins associated with it allow for crosstalk with other cellular systems. These systems can include other secretion systems, translation systems as well as post-translocational interactions.

Crosstalk between the Sec and Tat secretion systems has been shown in *Streptomyces*. Here, overproduction of the Tat system had a direct effect on the amount of Tat-dependently and Sec-dependently secreted substrates, as there was an increase in Tat-dependent secretion and a decrease in Sec-dependent secretion [98]. Furthermore, the deletion of *tatB* increased Sec-dependent secretion in *S. lividans* [99]. Collaboration between translocation systems has been documented in *E. coli* expressing an unusual Rieske iron–sulfur protein from *S. coelicolor*. A portion of this normally Tat-dependent cargo protein was shown to use a Sec–YidC-dependent membrane insertion mechanism while the remaining portion was translocated Tat-dependently [7].

Further convergence between the Tat and Sec systems relates to signal peptide processing. Signal peptide processing is necessary for the release of Sec-translocated proteins from the membrane [19,100]. Signal peptide processing of Tat-dependent cargo precursors in *E. coli* [101] and in *B. subtilis* [6,56] has been observed. The Rieske-iron protein QcrA has widespread Tat-dependency [6,102–104]. Intriguingly, the QcrA protein of *B. subtilis* is receptive for signal peptidase processing, resulting in the release of a smaller form into the growth medium [6]. This processing and subsequent release of the extracytoplasmic QcrA domain is hard to reconcile with the function of QcrA in electron transfer, which suggests a potential sheddase function of signal peptidases [100]. The view that the *B. subtilis* signal peptidase can act as sheddases is supported by the previous observation that certain polytopic membrane proteins can also be cleft by these enzymes [105,106].

Another example of Sec and Tat pathway crosstalk is associated with the cell wall-bound and extracellular quality control protease WprA, which is known to degrade Sec-dependently translocated proteins at the membrane–cell wall interface [107,108]. The afore-mentioned Y2H studies have shown direct protein–protein interactions between TatAy–TatCy and WprA [86]. Accordingly, a *wprA* mutation resulted in a decreased amount of the TatAy–TatCy-dependently translocated EfeB and YkuE proteins, suggesting a functional WprA–TatAy–TatCy–EfeB/YkuE interaction [86]. Further, studies in *B. subtilis* have shown that multiple extracytoplasmic proteases are involved in degrading EfeB [109]. Unexpectedly, in strains lacking these extracellular proteases the need for WprA in EfeB biogenesis was suppressed suggesting that WprA is somehow needed to protect EfeB against the activity of these other proteases [109].

4. Specific Gram-positive bacterial Tat systems

Gram-positive bacteria were originally defined by the results of the Gram stain. The properties of Gram-positive bacteria are such that their thick peptidoglycan layer retains the Gram stain and with it the purple–blue color of the stain. Although quite rudimental the Gram stain does successfully describe and delineate fundamental features of the bacterial envelope. Nonetheless, it is important to mention that within the Gram-positive phylogenetic tree there is a subdivision of bacteria that, though testing positive in the traditional Gram staining, contain a different cellular envelope structure. Instead of a single membrane and a cell wall, the envelope of these bacteria includes a peptidoglycan-mycolic acid wall structure and notably an extra membrane (Fig. 3). It is therefore more accurate to define them as diderms [23,110–112]. Bacteria that have this extra membrane include mycobacteria, corynebacteria, rhodococci and nocardiae. Hence, for the purpose of this review the bacterial groups detailed below are defined as monoderm and diderm Gram-positive bacteria.

4.1. Monoderm Gram-positive bacterial Tat systems

4.1.1. B. subtilis

B. subtilis is the predominant model organism for Gram-positive bacteria. It has a Generally Regarded As Safe (GRAS) status, is highly amenable to genetic engineering, and is able to produce large titres of secreted proteins [19,113,114]. Within the genus *Bacillus* there are other industrially relevant workhorses, such as *Bacillus licheniformis* and *Bacillus amyloliquefaciens*, but also pathogenic species such as *Bacillus anthracis* and *Bacillus cereus*. This genus is therefore of interest for fundamental scientific research, medical microbiological aspects, and applications in bio-industrial settings [114].

As mentioned before in this review, the *B. subtilis* Tat machinery is composed only of TatA and TatC proteins. There are three TatA components in *B. subtilis*: TatAc, TatAd and TatAy. Together with TatC, two of these TatA components combine to form two parallel TatA–TatC pathways, namely TatAy–TatCy and TatAd–TatCd. The TatAy–TatCy and TatAd–TatCd pairs are expressed operonically [115–117],

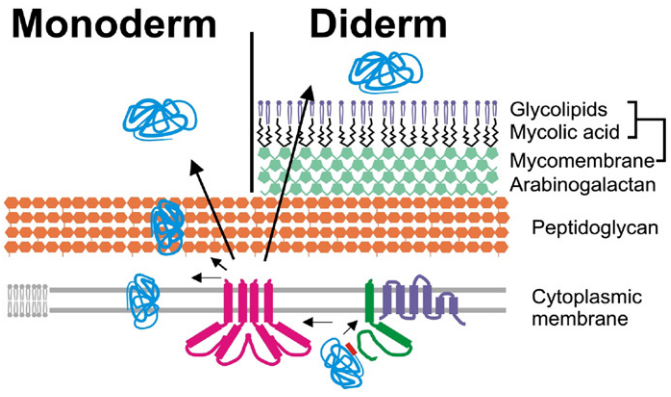

Fig. 3. Gram-positive bacterial cell envelope structure. Schematic representation of the envelope structure of diderm and monoderm Gram-positive bacteria. Note that proteins can be Tat-dependently targeted to the membrane (e.g. the Rieske protein QcrA of *B. subtilis*), the cell wall (e.g. the metallo-phosphoesterase YkuE of *B. subtilis*), or the growth medium (e.g. the Dyp-type peroxidase EfeB [YwbN] of *B. subtilis*).

and each pathway works independently and has its own substrate specificities [6,56,117]. Studies have shown that TatAd–TatCd is expressed only under conditions of low phosphate, which corresponds to the expression of its one known substrate PhoD [56,116–118]. In contrast, TatAy–TatCy is expressed consistently over many tested conditions and has a broader range of native substrates including EfeB (YwbN), QcrA, YkuE and potentially a few more [6,19,56,80]. The three known TatAy–TatCy-dependent cargo proteins have known co-factors (Fig. 4), and in the case of QcrA it not only has a Rieske iron–sulfur 2Fe–2S cofactor, but also a known disulfide bond essential for correct folding [119–123].

The TatAd–TatCd pathway shows more flexibility with regard to substrate specificity, as when overexpressed, TatAd–TatCd is able to translocate the normally TatAy–TatCy-dependent substrate EfeB [124]. Reversed, however, TatAy–TatCy is not able to translocate the TatAd–TatCd dependent PhoD [124]. Inter-species inter-pathway variation is observed when the *B. subtilis* pathways are expressed in *E. coli*, or when cargo proteins with *E. coli* signal peptides are expressed in *B. subtilis*. Both *B. subtilis* pathways are able to translocate GFP with Tat-dependent *E. coli* signal peptides when the pathways are expressed in *E. coli*. However, when the same cargo proteins were expressed in *B. subtilis*, translocation was not exclusively Tat-dependent [10,16,125]. This suggests that, although the *B. subtilis* Tat pathway is technically able to translocate these cargo proteins, other mechanisms in *B. subtilis* itself may prevent the Tat-dependent translocation of these heterologous GFP fusion proteins [10,16,125]. This underscores the view that the Tat-associated quality control and proofreading mechanisms in *E. coli* differ from those of *B. subtilis*.

The role of the third *B. subtilis* TatA component, TatAc, has until recently remained ambiguous. It is consistently expressed [116], but *tatAc* mutant strains have as yet not shown any phenotypes [11,19,56], nor could TatAc compensate for the absence of TatAy or TatAd in translocation of EfeB or PhoD, respectively [124]. However, when expressed in *E. coli*, complexes of TatAc–TatCd or TatAc–TatCy were fully functional and translocated the *E. coli* cargo proteins TorA, AmiC and AmiA [126]. Furthermore, when expressed in *E. coli*, TatAc was also able to complement for the absence of *E. coli* TatA and TatB [74]. Protein–protein interaction studies with Y2H techniques have shown that TatAc may not only interact directly with itself, with TatAd and TatAy, but also with the 'haem-based aerotactic transducer' HemAT [86]. Importantly, HemAT was also shown to be important for secretion of PhoD under low phosphate conditions. Therefore, the observed Y2H interaction with HemAT is biologically relevant [86].

In *B. subtilis* various phenotypes associated with Tat-deficiencies have been observed. These phenotypes may be due to the absence of specific cargo proteins. However they could also suggest relationships associated with quality control, compensatory mechanisms or even crosstalk between systems. Therefore, these phenotypes may imply direct or indirect responses. Quantitative proteomic studies showed that a number of biofilm-associated proteins were found in decreased amounts in *tatAy–tatCy* deficient strains. This led to the observation that a deficiency in *tatAy–tatCy* results in a delayed biofilm formation phenotype at the liquid–air interface [6]. However a direct link between this delayed biofilm phenotype and the Tat system is not yet clear and probably relates to an indirect response. Phenotypes directly related to the absence of the dependent cargo proteins have also been described. In particular, EfeB has a ferrous iron scavenging function and forms part of the membrane associated EfeUOB iron transporter [127]. Consistent with its role in iron scavenging, the absence of active EfeB in *tatAy–tatCy* deficient strains results in a lowered growth rate under limited iron availability [128]. Interestingly this EfeB-iron-associated phenotype is amplified in LB media without salt (i.e. NaCl), where active EfeB becomes essential for normal growth [128]. EfeB is part of the *efeUOB* operon, however, it is also independently controlled by cell envelope stress factors [116,127]. The specificity of EfeB to scavenge ferrous iron has implications under microaerobic conditions, where ferrous iron is more abundant than ferric iron, giving strains producing EfeB a growth advantage. Notably, the acquisition of ferrous iron involves the H_2O_2-dependent oxidation of ferrous iron to ferric iron by EfeB, thereby effectively eliminating potentially detrimental reactive oxygen species at membrane–cell wall interface [127].

Apart from the EfeB-associated response, studies have suggested that the salinity of the environment in which *B. subtilis* finds itself has direct implications with regard to the Tat-dependency of other substrates. In LB medium with high (6%) NaCl concentrations some ectopically expressed Tat-dependent cargo proteins showed independence to the Tat system [125,128]. Conversely, in LB without NaCl, *B. subtilis* strains expressing the *B. cereus* or *Staphylococcus aureus* TatA–TatC translocases showed increased Tat-dependent secretion of ectopically expressed EfeB-myc [69]. Although intriguing, exactly why and how environmental salinity changes the dependency of cargo proteins on Tat is presently not clear. However, some data suggest that TatA–TatC translocases of Gram-positive bacteria are intrinsically sensitive to salt, which would be indicative of mechanistically relevant electrostatic interactions within the Tat translocon [67,69].

4.1.2. Streptomyces

Streptomycetes are mycelial bacteria normally found in the soil. However, due to their ability to secrete large amounts of proteins they have become workhorses in various industrial applications, particularly

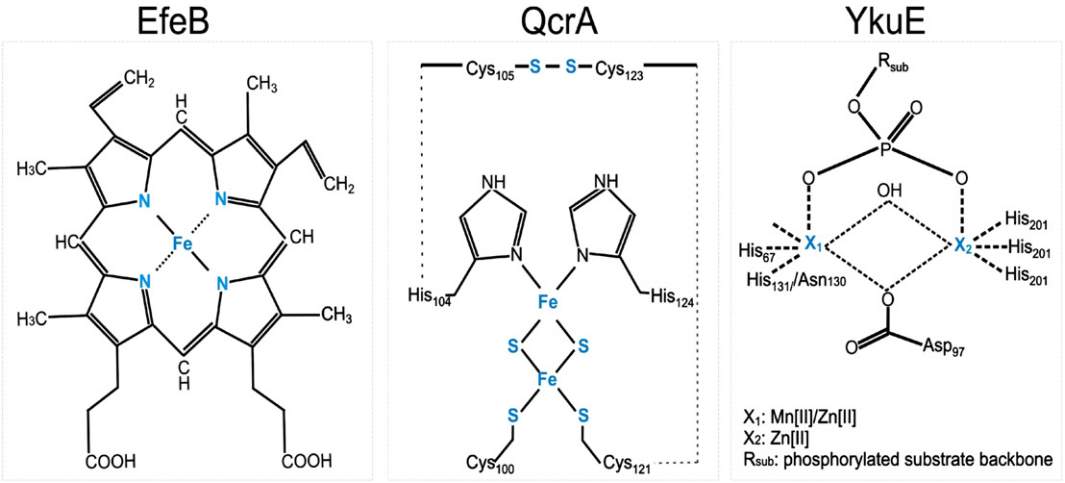

Fig. 4. The co-factors of known *B. subtilis* Tat-dependent cargo. The three TatAy–TatCy dependent cargo proteins each have known co-factors: the Dyp-type peroxidase EfeB (YwbN) has a heme group [127], the Rieske protein QcrA has a Rieske-iron–sulfur co-factor and contains a disulfide bond [119–121], and the metallo-phosphoesterase YkuE has a metal co-factor [80].

regarding the production of antibiotics [129]. Studies on the Tat systems of *S. coelicolor*, *Streptomyces scabies* and *S. lividans* have shown that, unlike all other bacterial systems, the *Streptomyces* Tat system is a major secretion pathway in terms of the numbers of cargo proteins [130–132].

The essential Tat system in streptomycetes is composed of one TatC and two TatA-like proteins with specialized TatA and TatB functions [94,133]. Studies have indicated that both TatA and TatB associate with Tat-dependent pre-proteins in the cytoplasm [94,95]. This has led to theories of TatA-like cytoplasmic chaperone activity, as discussed in the previous section.

The *Streptomyces* Tat system can be engineered in various ways. By simply inducing expression of *tatABC*, the secretion of the Tat-dependent substrate xylanase C was increased [98]. Also, the induced production of the phage shock protein PspA improves Tat-dependent protein secretion in both *S. lividans* and *E. coli* [134,135]. Although the exact role and mechanism of how PspA assists the Tat system is not clear, PspA binds phospholipids [136] and direct interactions between PspA and the N-terminal domain of *E. coli* TatA have been shown [137]. In both *E. coli* and *Streptomyces*, PspA is induced and shown to be important under conditions of extracytoplasmic stress [138,139]. It has been suggested that PspA suppresses proton leakage [136]. Therefore its role may be associated with membrane integrity and helping the cell to maintain the proton-motive force during protein translocation via Tat.

4.1.3. Staphylococcus

The staphylococcal Tat system is composed of a single TatA and TatC. The Tat machinery is not present in all staphylococcal species. However, *tatA* and *tatC* have been identified on the genomes of *S. aureus*, *Staphylococcus carnosus*, and *Staphylococcus haemolyticus* [40]. The staphylococcal Tat system is functional in *S. aureus* and *S. carnosus* and is able to translocate a number of non-heterologous proteins (LipA, Spa and GFP) [40,140]. Not many staphylococcal Tat substrates have been identified, and two-dimensional gel electrophoresis of the exoproteome of *tat* mutated staphylococci showed that it did not differ from that of the wild-type strains [40,41]. The sole known staphylococcal Tat-dependent substrate is the iron-dependent peroxidase FepB [40]. Genomic association initially identified this as a potential Tat-dependent cargo protein, as the *febABC* operon is found downstream of the *tatA–tatC* genes [40]. The products of the *febABC* operon are highly reminiscent of the EfeUOB iron uptake system in *B. subtilis*. Further FepB and *B. subtilis* EfeB have 40% sequence similarity, both have peroxidase activity and share the same ferrous iron scavenging function [40,127].

4.2. Diderm Gram-positive bacterial Tat systems

Diderm Gram-positive bacteria have an extra outer-membrane, and cargo proteins are translocated via the Tat system into the inter-membrane space between the cytoplasmic membrane and the outer mycomembrane. It is not clear exactly how the cargo proteins of this Tat system are subsequently secreted into the extracellular milieu and whether secondary secretory mechanisms, similar to those in Gram-negative bacteria, are involved in this process. For example, porins form major integral proteins in the mycomembrane of *C. glutamicum* and are thought to contribute to the cell envelope permeability of these diderm Gram-positive bacteria [111,141,142].

4.2.1. Corynebacterium

Corynebacteria are aerobic or facultative anaerobic non-motile bacteria that have functional Tat systems. This genus includes pathogens such as *Corynebacterium diphtheriae* and *Corynebacterium jeikeium*. On the other hand, non-pathogenic species such as *C. glutamicum* have been widely used in industry [143]. The Tat system in *Corynebacterium* is generally composed of two TatA-like proteins and a TatC. In some instances, such as *C. glutamicum* and *Corynebacterium efficiens*, the gene for a third TatA-like protein, TatE, is also present [144–146]. Although there are three TatA-like proteins in *C. glutamicum*, the essential components for translocation are TatA and TatC only. Notably, the expression of all TatA-like proteins does increase maximum protein secretion [43].

The Tat system in *C. glutamicum* is particularly efficient in translocating heterologous proteins. Industrially relevant co-factor containing proteins, such as carbohydrate oxidases, are secreted in their active form by the Tat system in *C. glutamicum* [17], and enzymes such as isomalthohydrolase, glutaminase and transglutaminase are secreted successfully via Tat to titres of >100 mg/l [14,43]. Further, when comparing the translocation of GFP in three different bacterial industrial workhorses – *C. glutamicum*, *B. subtilis* and *S. carnosus* – only *C. glutamicum* secreted properly folded fluorescent GFP [140].

The overall production of industrially relevant enzymes has been improved by the overproduction of Tat components, in particular TatC [15]. Also the efficiency of secretion can be manipulated by replacing the signal peptide of the cargo proteins, as significant improvements in translocation were observed when the native *C. glutamicum* signal peptide was replaced with a *B. subtilis* signal peptide or an *E. coli* TorA signal peptide [43,140]. Hence using the *C. glutamicum* Tat system in industrial settings for the biotechnological production of relevant enzymes and products is becoming more and more feasible and a promising strategy.

4.2.2. Mycobacterium

The genus *Mycobacterium* includes a number of notorious pathogenic species such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*. *M. tuberculosis* is of particular relevance as it has a functional Tat system, and in 2011 alone, 8.7 million new cases of tuberculosis and 1.4 million deaths were reported [147]. In mycobacteria the Tat system is composed of TatA, TatB and TatC [148]. It follows the similar duplication trend observed in other three-component Tat systems, where the core system is composed of a *tatC–tatA*-like operon with the gene of the third component, *tatB*, found elsewhere on the genome [37,149]. The Tat system in *M. tuberculosis* is unique, especially within the Gram-positive bacteria, in that it is one of the few examples where the Tat system is essential for survival of the organism [26,149,150].

A total of 18 *M. tuberculosis* proteins with functional Tat signal peptides have been confirmed using a β-lactamase reporter system in the related *Mycobacterium smegmatis* [151,152]. Of these 18 known substrates, a number are known to contribute directly to virulence and drug resistance, such as the β-lactamase BlaC and the phospholipase C enzymes PlcA and PlcB [151,153]. The Tat system is absent from humans and combining this with the knowledge that the system is essential in *M. tuberculosis*, has made the Tat system an alluring drug target for future treatment of tuberculosis patients [154].

5. Conclusion

Since first being described in thylakoids and then bacteria nearly 20 years ago [20,38,155–157], our growing understanding of the Tat system has not only enriched fundamental biology, but also proven to be a potential target in pathogenic bacteria and it has demonstrated promise in certain industrial applications. Yet, despite this growing knowledge, several crucial questions remain. Despite a number of elegant theories, there is no hard data regarding the manner in which cargo proteins cross the membrane and, importantly, how the Tat system maintains membrane integrity while translocating large, globular proteins. Of specific interest in future investigations is the interplay between the Tat machinery and other systems, such as chaperones or the phage shock protein PspA, in twin-arginine translocation.

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