Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Protein transport by the bacterial Tat pathway ☆

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ARTICLE INFO

Article history:
Received 6 December 2013
Received in revised form 10 February 2014
Accepted 15 February 2014
Available online xxxx

Keywords:
Tat
Twin arginine
Protein secretion
Signal peptide
Secretory pathway

ABSTRACT

The twin-arginine translocation (Tat) system accomplishes the remarkable feat of translocating large – even dimeric – proteins across tightly sealed energy-transducing membranes. All of the available evidence indicates that it is unique in terms of both structure and mechanism; however its very nature has hindered efforts to probe the core translocation events. At the heart of the problem is the fact that two large sub-complexes are believed to coalesce to form the active translocon, and 'capturing' this translocation event has been too difficult. Nevertheless, studies on the individual components have come a long way in recent years, and structural studies have reached the point where educated guesses can be made concerning the most interesting aspects of Tat. In this article we review these studies and the emerging ideas in this field. This article is part of a Special Issue entitled: Protein Trafficking & Secretion.

1. Introduction

The twin arginine translocase (Tat) is a protein transport pathway that exists in Archaea, bacteria and plant chloroplasts. In bacteria, it exports proteins across the plasma membrane and is important for many processes including energy metabolism, formation of the cell envelope, biofilm formation, heavy metal resistance, nitrogen-fixing symbiosis, bacterial pathogenesis and others [1,2]. What makes this protein transport system unusual compared to other transport systems (such as the general secretory, or Sec pathway) is its ability to transport fully folded proteins across membranes. This remarkable feat has no requirement for ATP as an energy source, and relies solely on the proton motive force (PMF) [3–5].

The mechanism of translocation remains poorly understood, in part due to a lack of high resolution structural information on this complex and its individual components. That said, a number of recent biophysical and structural studies have provided a more detailed picture of the action and composition of this translocase, particularly with respect to the early events prior to the actual translocation event. This review discusses the key information from each of these studies. Much of this article will focus on the *Escherichia coli* (*E. coli*) Tat system, but relevant data on the Gram-positive homologs from *Bacillus subtilis* (*B. subtilis*) and the chloroplast Tat system are also mentioned. A more detailed analysis of Gram-positive Tat systems is given elsewhere in this volume by Goosens et al. [6].

2. The Tat system's substrates

The extent to which different organisms utilise the Tat pathway varies significantly. Gram-positive bacteria such as *Staphylococcus aureus* or *B. subtilis* have few predicted substrates [7–9], whereas enteric bacteria typically possess around 20–30 substrates [10]. Whilst the rationale for using this translocase remains unclear for some Tat substrates, three key underlying factors have been identified. The first is a requirement for the enzymatic insertion of complex cofactors in the cytoplasm prior to transport, thereby bypassing the requirement for extra mechanisms to firstly, separately export the cofactor and then subsequently catalyse its insertion in the periplasm [1]. The second motive is avoidance of metal ions that compete for insertion into the active site, and lastly, the transport of hetero-oligomeric complexes that optimally assemble in the cytoplasm [11,12]. The latter is achieved through proteins forming complexes with other proteins that possess an N-terminal Tat signal peptide [13].

Navigation to the Tat translocase is dictated by the presence of an N-terminal signal peptide that possesses an overall tripartite architecture of: a polar amino terminal (N) domain, hydrophobic core (H) region and a polar carboxyl (C) domain (Fig. 1). Despite the Sec- and Tat-type signal peptides having the same basic structure and a similar terminal Ala-X-Ala motif, studies on Tat signals revealed a highly conserved SRRxFLK motif [14,15] located at the junction of the N- and H-domains. The twin-arginine motif gives this translocase its name. Both arginines are critical in chloroplast Tat signals [16], but less so in bacteria, where mutation of a single arginine in bacteria only affects the rate of translocation, whereas mutation of both completely abolishes transport [17–19]. Within the SRRxFLK motif, three determinants are important: the twin-arginine pair, the hydrophilic −1 residue

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http://dx.doi.org/10.1016/j.bbamcr.2014.02.013
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Fig. 1. The Tat signal peptide. A polar amino domain (N-region), hydrophobic core (H-region), and polar carboxyl domain (C-region) comprise the tripartite structure of a Tat signal peptide, which is located at the N-terminus of the substrate protein. On average they are less hydrophobic than Sec-specific signals, as well as being longer (on average 38 to 24 amino acids, respectively). Tat signal peptides are distinguished by their conserved twin-arginine motif in the N-region. The C-terminal region houses an A-x-A motif, which is a consensus cleavage site for removal of the signal peptide by signal peptidase.

and the hydrophobic +2 residue (+/− relative to the twin-arginine pair). However, while Tat and Sec signal peptides share a similar overall architecture, it is not the twin arginine pair alone that prevents mistargeting to the Sec pathway. Tat signal peptides are less hydrophobic than those used in Sec-targeting [20], and the C-region of certain Tat signal peptides houses basic residues, which are seldom found in the same region of Sec signal peptides. The latter is believed to hinder engagement with Sec machinery [21–23].

3. Tat Translocase components and complexes

3.1. The Tat subunits

Three integral membrane proteins form the minimal set of components for the assembly of the Tat translocase in *E. coli*: TatA, TatB and TatC. These proteins are expressed from the *tatABC* operon and reside in the cytoplasmic membrane arranged as a Tat(A)BC substrate binding complex and a separate TatA complex (Fig. 2). TatA is an 89 amino acid protein (9.6 kDa) that consists of a short periplasmic N-terminal region, a transmembrane helix that is linked via a hinge region to a cytosolically exposed amphipathic helix (APH), and a highly unstructured, cytoplasmically-exposed C-terminal region [24–26]. This arrangement is supported by spectroscopy studies, which indicate that the APH lies along the surface of the membrane [27,28]. Additionally, solid-state NMR has shown the TMH to cross the cytoplasmic membrane at a 17° tilt [29]. An N-out topology is the favoured orientation of TatA in the cytoplasmic membrane and is supported by recent NMR data of the TatA component of a Gram-positive homolog, TatAd [30,31]. Some studies have predicted that TatA may also have a dual topology, on the basis of data that suggest the N-terminal region of TatA can also be accessed from the cytoplasm [32]. Moreover, there is evidence of soluble TatA in bacteria and chloroplasts [33–39]; however the functional relevance of this soluble TatA pool remains controversial.

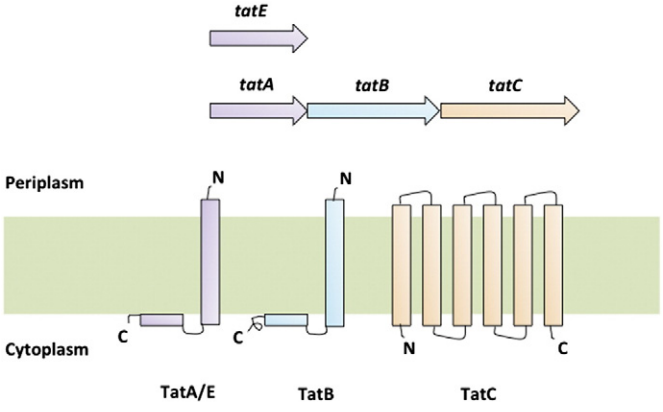

Fig. 2. Component organisation of the *E. coli* Tat system. In Gram-negative bacteria the Tat translocase system is usually made up of three integral membrane proteins, encoded by the *tatABC* operon. Both TatA and TatB are single-span transmembrane proteins that possess: a short periplasmic N-terminal region; single-span transmembrane helix; hinge region; amphipathic helix lying along the cytoplasm-membrane interface and a highly charged, unstructured C-terminus. In contrast, TatC is a polytopic protein that is predicted to contain 6 transmembrane spans, with both the N- and C- termini in the cytoplasm. In *E. coli* a TatA paralog exists, TatE, which is encoded elsewhere in the genome.

TatB consists of 171 amino acids with a molecular mass of 18.5 kDa. Despite sharing a 20% sequence similarity with TatA [40] and a very similar predicted secondary structure (Fig. 2), TatB and TatA carry out functionally distinct roles within the Tat translocase [41].

TatC consists of 258 amino acids with a molecular mass of 28.9 kDa. As predicted by its secondary structure, this protein traverses the membrane 6 times, possessing an N-in C-in topology [42]. The *tatABC* gene products form two distinct membrane complexes at steady state: a TatBC-containing substrate binding complex and a separate TatA complex. It is in this former 370 kDa Tat(A)BC complex where most of TatB and -C are found at a 1:1 stoichiometric ratio [43]. TatA (found at ~25 and 50 fold higher concentrations than TatB and TatC, respectively [44]), is present as highly heterogeneous complexes ranging from 100–500 kDa [43,45,46] and is not required for TatBC complex assembly [47].

The composition of the Tat system differs significantly in most Gram-positive bacteria; all except Streptomycetes contain only *tatAC* genes [48,49]. In those examples studied to date, the TatA protein is bifunctional [50]. The best characterised Tat system in this type of bacteria is found in *B. subtilis* — a non-pathogenic soil bacterium, which contains two discrete Tat systems that operate in parallel, yet possess different substrate specificities [48]. The first of the two is TatAdCd, whose only substrate identified at present is the phosphodiesterase, PhoD [49]. The second translocase is TatAyCy, which exports YwbN, an iron-dependent DyP-peroxidase [49]. There is a third *tatA* gene encoding the TatAc protein, which like TatAd, was recently shown to form small homogeneous complexes and restore export of TorA in a Δ*AE* mutant [51].

Reminiscent of the situation in *E. coli*, the TatAyCy system is composed of two types of membrane protein complexes: TatAyCy and TatAy that have been reported to form ~200 kDa complexes (as judged by gel filtration chromatography) [37,52]. Likewise, TatAdCd exists as a ~230 kDa complex, alongside a separate and discrete TatAd complex of ~160 kDa[50,53]. The TatAd and TatAy proteins are bifunctional fulfilling the role of the TatB protein that would otherwise be present in Gram-negative bacteria [50,54].

In addition to these bacterial TatAC-containing complexes being smaller than their *E. coli* counterparts (TatABC is ~370 kDa on BN gels [43,45,55]), the lack of a *tatB* gene and TatA heterogeneity appear to be conserved features of Gram-positive bacteria. This is an important point because the remarkable heterogeneity of *E. coli* TatA complexes has been considered to be a key element of current translocation models (see below)[53].

Finally, *E. coli* also possesses a TatA paralogue, TatE. This 67 amino acid protein possesses 57% sequence identity to TatA [25] and is thought to have arisen from a gene duplication of *tatA* [56]. Whilst it can fulfil TatA activity if overexpressed [41], there is no evidence for a specific role for this protein, and indeed many Gram-negative bacteria lack a *tatE* gene [46,57].

Please cite this article as: R. Patel, et al., Protein transport by the bacterial Tat pathway, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbamcr.2014.02.013