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Review

Transport and proofreading of proteins by the twin-arginine translocation (Tat) system in bacteria☆

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ABSTRACT

The twin-arginine translocation (Tat) system operates in plant thylakoid membranes and the plasma membranes of most free-living bacteria. In bacteria, it is responsible for the export of a number of proteins to the periplasm, outer membrane or growth medium, selecting substrates by virtue of cleavable N-terminal signal peptides that contain a key twin-arginine motif together with other determinants. Its most notable attribute is its ability to transport large folded proteins (even oligomeric proteins) across the tightly sealed plasma membrane. In Gram-negative bacteria, TatABC subunits appear to carry out all of the essential translocation functions in the form of two distinct complexes at steady state: a TatABC substrate-binding complex and separate TatA complex. Several studies favour a model in which these complexes transiently coalesce to generate the full translocase. Most Gram-positive organisms possess an even simpler "minimalist" Tat system which lacks a TatB component and contains, instead, a bifunctional TatA component. These Tat systems may involve the operation of a TatAC complex together with a separate TatA complex, although a radically different model for TatAC-type systems has also been proposed. While bacterial Tat systems appear to require the presence of only a few proteins for the actual translocation event, there is increasing evidence for the operation of ancillary components that carry out sophisticated "proofreading" activities. These activities ensure that redox proteins are only exported after full assembly of the cofactor, thereby avoiding the futile export of apo-forms. This article is part of a Special Issue entitled Protein translocation across or insertion into membranes.

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1. Introduction

The twin-arginine translocation protein transport system, or Tat system, has attracted a great deal of interest in recent years. Most protein translocases transport their substrate proteins in an unfolded

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conformation, and there are good reasons for doing it this way; the translocation channel can be kept to a minimal diameter, and a variety of substrates can be transported using the same basic channel. In a sense, this broad type of mechanism offers a "one size fits all" advantage, although there is now good evidence that most protein translocases are rather flexible and able to adjust to the type of substrate being translocated. Few protein translocases are able to transport fully folded proteins and the Tat system appears to be unique in its ability to transport relatively massive folded proteins–even oligomeric proteins–across energy-transducing membranes. In this review we discuss recent studies on the structure and mechanism of this remarkable system. While much of the previous work has been carried out on the *Escherichia coli* Tat system, we have sought to highlight the properties of the Tat system from Gram-positive organisms in order to consider bacterial (and plant) Tat systems in a wider sense.

2. The Tat system subunits

The subunits of the Tat transport machinery in thylakoids and Gram-negative bacteria all share a high degree of sequence homology, and this has helped to identify the presence of Tat proteins in a wide range of bacteria [1–3]. The essential members of the Tat translocation apparatus in *E. coli* are the TatABC subunits, and their counterparts in plants are Tha4, Hcf106 and cpTatC respectively [1–6]. However, most Gram-positive bacteria contain only TatA and TatC-type proteins; these thus comprise a minimal Tat translocation system [7,8] that will be discussed in more detail below. Tat subunits are resident in the inner membrane of Gram-negative bacteria and their homologues in plants are located in the thylakoid membrane, where they mediate the transport of proteins into the lumen.

In *E. coli*, TatA and B are small proteins of 9.6 and 18.4 kDa, respectively, and each contains a single transmembrane span. The two subunits are homologous, and each is predicted to contain an N-terminal transmembrane (TM) helix followed by a short hinge region and an amphipathic helix ending in an unstructured and charged C-terminus [1,2,4]. Studies using circular dichroism (CD) and oriented CD (OCD) have largely confirmed the presence of these secondary structures, and there is now good evidence that the N-terminal TM α-helix does traverse the membrane with the amphipathic helix lying along the surface of the membrane [9,10]. Further studies using solid state NMR have shown the TM helix to be 14–16 residues long with a 17° tilt compared to normal in the membrane [11]. However, it should be pointed out that while most studies have proposed an N-out topology for TatA, this is not universally accepted and a study using thiol-reactive reagents concluded that the N terminus is in the cytoplasm rather than the previously assumed periplasm [12]. TatA and TatB share 25% sequence identity and the two subunits have a similar secondary structure; TatB is, however, significantly longer as it has an extended C-terminal domain and a key point is that the two subunits cannot substitute for each other, even when over-expressed [13].

The best-studied Tat system is that of *E. coli*, and there is clear evidence that the TatABC subunits carry out the key functions in protein translocation. However, this organism also contains a TatE subunit, encoded by a monocistronic gene that is outside the main *tatABC* operon. This subunit is thought to result from a cryptic gene duplication of *tatA* and the proteins share up to 50% amino acid sequence homology. Expression of the *tatE* gene partially complements *tatA* null mutants, but the TatE subunit is present at much lower levels than TatA and appears to be largely redundant [2,13].

TatC is the largest subunit of the Tat machinery (28.9 kDa in *E. coli*) and has been predicted to contain six TM domains from its primary sequence [14]. CD and OCD performed on TatCd (from *Bacillus subtilis*) showed a high helical content (~50%), with the helices apparently exhibiting significant tilt in the bilayer. However, it could not be determined whether this was due to the six helices lying at an angle in the membrane or, for example, because two of the helices lie on the surface of the membrane [15]. The latter case would signify the presence of only four TM helices (also suggested in a previous study [16]). Other work, using introduced cysteine residues along the length of TatC and membrane-permeant and non-permeant thiol reactive reagents, pointed to the presence of six TM helices [17], and this appears to be the most likely scenario at present.

None of the Tat subunits shares real sequence homology with other proteins in the database. The Tat system is thus unrelated to other known transport systems and this point, together with the apparently unique reaction mechanism (below) makes the Tat system both interesting and challenging. The basic structures of Tat subunits are shown in Fig. 1.

3. Structures of Tat complexes

3.1. Tat sub-complexes in Gram-negative bacteria

Many studies have focused on the structures and compositions of Tat complexes, and the results have been used to help build models for the Tat mechanism. In Gram-negative bacteria, two separate complexes are found at steady state: a TatABC complex and homo-oligomeric TatA complex [18,19]. This is a key point that has been instrumental in building the mechanistic models described below. The TatABC complex has a mass of about 370 kDa according to Blue-native electrophoresis studies [20,21] and within this complex, TatB and TatC form a functional unit, with the two subunits present in a 1:1 ratio [18]. The TatBC subunits are furthermore functional when translationally fused together [18]. Each subunit contacts other cognate TatB/C subunits in a larger complex, but there is also evidence that TatB and TatC form autonomous units within the TatABC complex [22]. Some TatA co-purifies with the TatBC subunits, and several Gram-negative bacteria are believed to contain a TatABC complex. TatB and TatC play important roles in substrate binding (see below) but the role of TatA in the complex is unclear, although the complex appears to be less stable in its absence [23,24]. The corresponding complex in plants (Hcf106-TatC) does not contain the TatA homologue (Tha4) suggesting that the TatA subunits in the bacterial TatABC complex may play a structural role, or perhaps a role in interaction

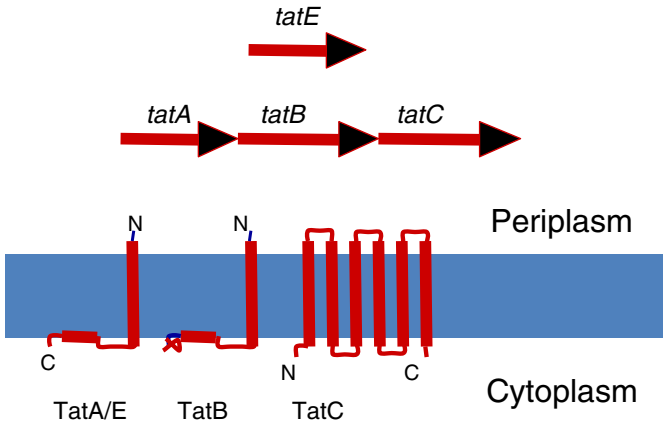

Fig. 1. Overall structure of subunits in the *E. coli* Tat system. Gram-negative bacteria usually contain 3-component Tat systems with TatABC subunits. In *E. coli*, these proteins are encoded by the *tatABC* operon as shown, but this organism also contains a TatA paralogue, TatE, which is encoded elsewhere in the genome. Most Gram-positive bacteria lack *tatB* genes and the TatA subunit is bifunctional. The diagram shows the proposed overall structure for the subunits of the TatABC system; the TatA and TatB proteins contain a single TM span, a very short N-terminal section in the periplasmic space and an amphipathic helix lying along the cytoplasmic face of the plasma membrane. TatC is believed to contain 6 TM spans with the N- and C-termini in the cytoplasm.