A naturally occurring bacterial Tat signal peptide lacking one of the 'invariant' arginine residues of the consensus targeting motif

Andrew P. Hinsley[a], Nicola R. Stanley[a,b], Tracy Palmer[a,b], Ben C. Berks[a,*]

[a] *Centre for Metalloprotein Spectroscopy and Biology, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK*
[b] *Department of Molecular Microbiology, John Innes Centre, Norwich NR4 7UH, UK*

Received 19 March 2001; revised 20 April 2001; accepted 20 April 2001

First published online 3 May 2001

Edited by Gunnar von Heijne

Abstract Currently described substrates of the bacterial Tat protein transport system are directed for export by signal peptides containing a pair of invariant arginine residues. The signal peptide of the TtrB subunit of *Salmonella enterica* tetrathionate reductase contains a single arginine residue but is nevertheless able to mediate Tat pathway transport. This naturally occurring example of a Tat signal peptide lacking a consensus arginine pair expands the range of sequences that can target a protein to the Tat pathway. The possible implications of this finding for the assembly of electron transfer complexes containing Rieske proteins in plant organelles are discussed.

Key words: Sec-independent; Bacterial protein export; Tat pathway; Twin–arginine signal peptide; Tetrathionate reductase; Rieske protein

1. Introduction

The cytoplasmic membrane of most bacteria contains the two general protein export systems Sec and Tat to which proteins are targeted by means of amino-terminal signal peptides. The Sec system utilises signal peptides that lack consensus sequence motifs but have overall physicochemical similarity, namely a basic amino-terminus and a helical hydrophobic core [1]. Proteins are targeted to the Tat pathway by signal peptides bearing a consensus amino-terminal SRRxFLK motif in which the consecutive arginine residues are invariant [2,3]. Tat signal peptides also require a hydrophobic core, though this is more polar than that of corresponding Sec signal peptides [4]. The Sec system operates by a threading mechanism in which the unstructured precursor protein is extruded across the membrane and only folds on reaching the extracytoplasmic compartment. In contrast the Tat system functions to move folded proteins from one side of the membrane to the other. In many cases this allows the substrate protein to bind a cofactor molecule in the cytoplasm prior to transport [5]. Sec and Tat pathways are both also operative in the endosymbiont-derived thylakoid membrane of plant chloroplasts [6]. Plant mitochondria may likewise possess a Tat system since a homologue of the essential bacterial Tat pathway component TatC is encoded by the mitochondrial genome of higher plants [7].

Studies with bacterial and chloroplast Tat signal peptides have demonstrated that both invariant arginine residues of the consensus motif are critically important for the Tat transport with even conservative substitution of just one of the arginine residues by lysine normally blocking translocation [3,8–12]. Recently, however, transport was observed for single lysine for arginine substituted variants of the *Escherichia coli* protein SufI [3]. Although the rate of transport measured with these variants was much slower than that of the wild-type protein, and probably physiologically unviable, these observations raise the possibility that Tat signal peptides with arginine substitutions might occur naturally. In order to investigate this possibility we undertook a database search for bacterial proteins possessing otherwise plausible Tat signal peptides but with single lysine for arginine substitutions. This search recovered the TtrB subunit of the tetrathionate reductase of *Salmonella enterica* (formerly *Salmonella typhimurium*) strain LT2a.

The enzyme tetrathionate reductase permits the use of tetrathionate as a respiratory electron acceptor when oxygen is unavailable [13]. The tetrathionate reductase of *S. enterica* is a three-subunit membrane-bound enzyme encoded by the *ttrBCA* operon [14]. It couples the two electron oxidation of membrane quinols to the reduction of tetrathionate ($^{-}O_3S$-S-S-SO_3^{-}) to thiosulphate ($^{-}$S-SO_3^{-}). Sequence analysis suggests that TtrC is an integral membrane protein and probably carries the quinol oxidising site. TtrA and TtrB are predicted to be peripheral membrane subunits of the electron transfer complex with TtrA containing the molybdenum *bis*(molybdopterin guanine dinucleotide) active site cofactor. Electron flow from TtrC to the molybdopterin cofactor is probably by way of the four and one [4Fe–4S] clusters located within, respectively, the TtrB and TtrA subunits. TtrA and TtrB are synthesised with apparent signal peptides suggesting that they are located at the periplasmic side of the cytoplasmic membrane. On the basis of the types of cofactor that they bind both TtrA and TtrB would be predicted to be exported via the Tat rather than the Sec pathway [2]. Indeed the TtrA signal peptide has the features typical of a Tat-targeting signal [5]. However, while the TtrB signal peptide has sequence similarity to the predicted Tat signal peptides of homologous periplasmic iron–sulfur proteins a lysine residue replaces the first of the otherwise invariant arginine residues of the Tat consensus motif (Fig. 1a). The sequence of the TtrB signal peptide is invariant amongst the *Salmonella* strains and species for which relevant genome data are currently available. Thus the presence of the lysine–arginine pair is not a strain-specific mutation.

Given the precedent of slow transport of single lysine for

*Corresponding author. Fax: (44)-1603-592250.
E-mail: b.berks@uea.ac.uk

PII: S0014-5793(01)02428-0

arginine substituted SufI variants [4] we considered here the possibility that TtrB is a naturally occurring example of a bacterial Tat signal peptide lacking one of the 'invariant' twin–arginine residues.

2. Materials and methods

Previously described strains and plasmids used in this study were: *E. coli* strains MC4100 (F^- *ΔlacU169 ara139 rpsL150 relA1 ptsF rbs flbB5301* [15], BILKO (MC4100 Δ*tatC*) [7], K38 (*HfrC phoA4 pit-10 tonA22 ompF 627 relA1 spoT1* λ^+) [16] and NRS-1 (K38 Δ*tatC*::Ω Spc^R) [3]; *S. enterica* LT2a (wild-type, B.N. Ames); plasmids pAH26 (*ttrRSBCA*$^+$) [14] and pGP1-2 [17]. Plasmid pAH56 for the in vivo synthesis of the TtrB signal peptide–SufI fusion was constructed as follows. A 409-bp PCR fragment covering the region from 303 bp upstream of the start codon to the end of the signal peptide coding region of *ttrB* was amplified using the primers 5′-GCTCTAGAC-AACGGTTGCCAGTGGCTA-3′ and 5′-CGCATATGAAATTTC-GCTT CAGCCAGCGG-3′ with *S. enterica* LT2a chromosomal DNA as the template. The product was digested with *Xba*I and *Nde*I and cloned into pNR17 [18] which contains the *sufI* mature protein coding region. The resultant construct was digested with *Bam*HI and *Xba*I and the TtrB–SufI fusion was cloned into pT7.5, [17] to give plasmid pAH56. Site-specific mutations in pAH56 were constructed by the QuikChangeTM system (Stratagene). All constructs were verified by sequencing. Anaerobic culture in tetrathionate-containing media and tetrathionate reductase activity measurements were carried out as described in [14]. Pulse-chase experiments and preparation of periplasmic fractions by osmotic shock were performed as described [3].

3. Results

The *S. enterica ttrBCA* operon encodes the three tetrathionate reductase structural proteins. Transcription from the *ttrB* promoter is completely dependent on a two-component regulator encoded by the divergently transcribed *ttrRS* operon and on the presence of the anoxia-responsive transcriptional regulator FNR [14]. *E. coli* is able to functionally express the *S.*

a Bacterial `16Fe ferredoxins'

```
S. enterica   TtrB   MWTGVNMDSSKRQFLQQLGVLTAGASLVPLAEA
A. fulgidus   AF0499          MSRRKFLLLTGAAAAGAILTPQISA
E. coli       NrfC          MTWSRRQFLTGVGVLAAVSGTAGRVVA
H. influenzae NrfC        MTVCSRRNFVSGMGAVILMTGTSLPAFA
E. coli       F239         MSFTRRKFVLGMGTVIFFTGSASSLLA
D. vulgaris   HmcB           MDRRRFLTLLGSAGLTATVATAGTAKA
E. coli       HybA           MNRRNFIKAASCGALLTGALPSVSHAA
Tat consensus                 SRR FLK
```

b Bacterial Rieske proteins

```
P. denitrificans  FbcF MSHADEHAGDHGATRRDFLYYATAGAGTVAAGAAAWTLV...
B. subtilis       QcrA       MGGKHDISRRQFLNYTLTGVGGFMAASMLMPMV...
Synecocystis 6083 PetC MTQISGSPDVPDLGRRQFMNLLTFGTITGVAAGALYPAV...
```

c Plant chloroplast Rieske proteins

```
Pea    PetC   ...QATSIPADRVPDMSKRKTLNLLLLGALSLPTAGMLVPYG..
Potato PetC   ...QATSIPADNVPDMQKRETLNLLLLGALSLPTGYMLLPYA..
```

d Plant mitochondrial Rieske proteins

```
Maize  Rieske ...DEYNHERYPPGDPSKRAFAYFVLSGGRFIYASLLRLLVL...
Potato Rieske ...DDSNHERYPPGDPSKRAFAYFVLTGGRFVYASSVRLLIL...
```

e Animal and yeast Rieske proteins

```
Human          Rieske ...VLDSTKSSRESSEARKGFSYLVTGVTTVGVAYAAKNAVT...
S. cerevisiae  Rieske ...NFDDVLKENNDADKGRSYAYFMVGAMGLLSSAGAKSTVE...
```

Fig. 1. (a) The TtrB signal peptide is compared with those of other homologous, predicted periplasmic, proteins that bind four [4Fe–4S] clusters. The remainder of the figure shows signal-anchor peptides of selected Rieske proteins from (b) bacterial cytochrome bc_1 and cytochrome b_6f complexes, (c) cytochrome b_6f complexes of higher plant chloroplasts, (d) cytochrome bc_1 complexes of higher plant mitochondria and (e) cytochrome bc_1 complexes from other mitochondrial sources. In each case residues matching the consensus *E. coli* SRRxFLK motif are shown in bold. The Rieske proteins extend beyond the sequences shown where indicated (...).

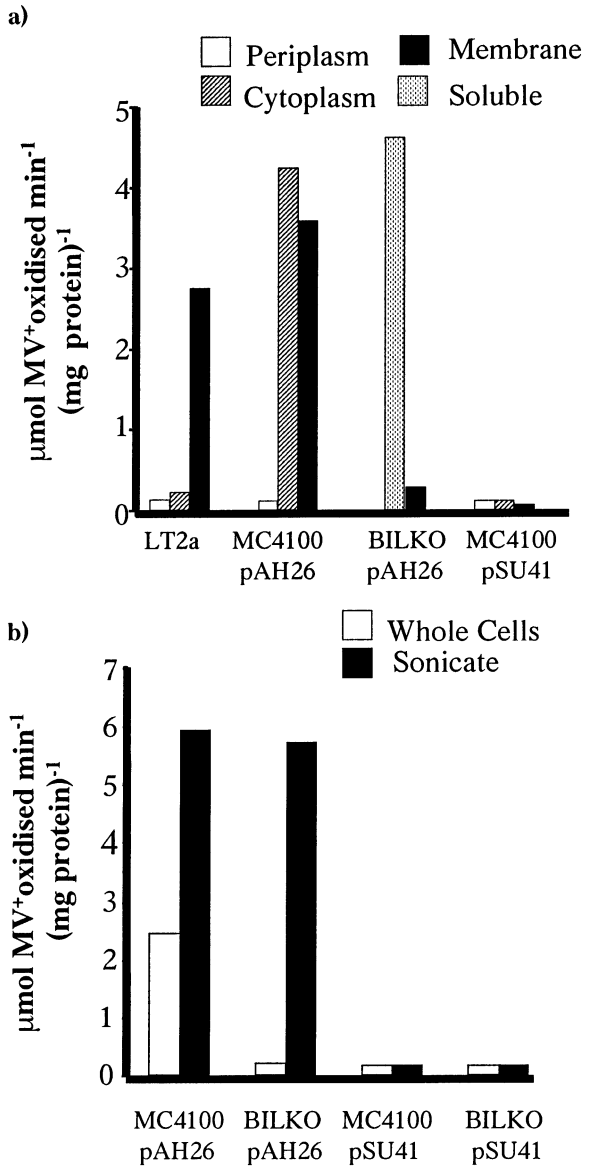

Fig. 2. Subcellular localisation of tetrathionate reductase activity. Tetrathionate reductase activities were measured for whole cells or subcellular fractions of *E. coli* strains MC4100 and BILKO (MC4100 Δ*tatC*) transformed with either plasmid pSU41 or pAH26 (pSU41 with a *S. enterica ttrRSBCA* insert). Tetrathionate reductase activities in subcellular fractions of wild-type *S. enterica* strain LT2a were also determined in (a). Cells were cultured on LB tetrathionate medium and tetrathionate reductase activities were measured using methyl viologen radical as the electron donor. (a) Periplasmic fractions were prepared by osmotic shock. The resulting sphaeroplasts were lysed by passage through a French pressure cell and then cytoplasmic and membrane fractions prepared by differential ultracentrifugation. Preparation of distinct periplasmic and cytoplasmic fractions was not possible for BILKO (pAH26). Data are therefore shown for the entire water-soluble fraction. The protein concentration used in the specific activity calculations is that of the cells from which the subcellular fractions were prepared. This allows direct comparison with the experiments shown in (b). (b) Tetrathionate reductase activities were determined before and after disruption of the cells by sonication.

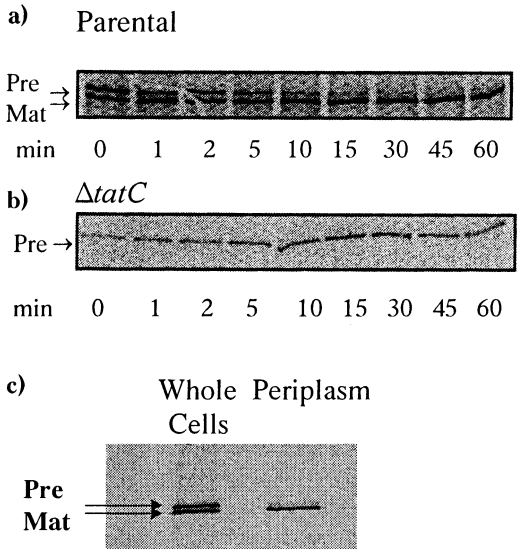

Fig. 3. The signal peptide of *S. enterica* TtrB directs Tat-dependent protein transport in *E. coli*. sTtrB–SufI was expressed in *E. coli* strains K38[pGP1-2] (a,c) or the Δ*tatC* derivative NRS-1[pGP1-2] (b), pulse-labelled for 5 min by addition of [^{35}S]methionine and then chased from time zero with unlabelled methionine. Precursor synthesis and processing were assessed by SDS–PAGE of whole cells followed by autoradiography. In (c) labelled protein was analysed at a chase time of 10 min either in whole cells or in the periplasmic fraction produced by osmotic shock.

enterica tetrathionate reductase when transformed with plasmid pAH26, which carries the *ttrSRBCA* gene cluster, and then cultured under anaerobic conditions in the presence of tetrathionate [14].

It has been shown previously that a Δ*tatC* mutation blocks Tat-dependent protein transport in *E. coli* [7]. An *E. coli* strain bearing both pAH26 and the Δ*tatC* mutation failed to metabolise tetrathionate when cultured anaerobically on a complex carbon source (data not shown) suggesting that tetrathionate respiration is Tat-dependent. When assayed with the non-physiological electron donor methyl viologen tetrathionate reductase activity in wild-type *S. enterica* localises predominantly to the membrane (Fig. 2a). In an *E. coli* strain bearing plasmid pAH26 tetrathionate reductase activity is distributed more or less evenly between the membrane and cytoplasmic fractions suggesting that *E. coli* is able to correctly assemble some, but not all, of the Ttr proteins produced by the multicopy plasmid (Fig. 2a). However, in the presence of a Δ*tatC* mutation tetrathionate reductase activity is no longer found in the membranes of the *E. coli* strain but is completely water-soluble (Fig. 2a). It is not certain that this tetrathionate reductase activity is located in cytoplasm since, as we have observed for some other *tat* mutant/plasmid combinations, the Δ*tatC* pAH26 strain lyses when sphaeroplast preparation is attempted precluding production of separate cytoplasmic and periplasmic fractions. However, the accessibility experiment shown in Fig. 2b would support a cytoplasmic location for the tetrathionate reductase activity in the Δ*tatC* mutant. In contrast to the parental strain, tetrathionate reductase activity measured with the membrane impermeant substrates tetrathionate and methyl viologen is only detected in the Δ*tatC* mutant when the cells are disrupted. This implies that tetrathionate reductase catalytic subunit is exposed to the periplasmic environment in the parental strain but not in the Δ*tatC* mutant. In summary, these experiments demonstrate that the Tat system is required for the correct subcellular localisation of tetrathionate reductase. Nevertheless, these observations are insufficient to show that the Tat pathway recognises the putative TtrB signal peptide since the TtrA subunit also has a predicted Tat signal peptide.

To directly test the function of the TtrB signal peptide we constructed plasmid pAH56 that directs expression of a hybrid protein, hereafter termed sTtrB–SufI, in which the TtrB signal peptide is fused to the amino-terminus of the mature region of the *E. coli* protein SufI. SufI is a water-soluble, monomeric and cofactorless native *E. coli* Tat substrate [3,18]. The *ttrB′–sufI′* gene fusion on plasmid pAH56 is under the control of a phage T7 ϕ10 promoter. Upon provision of T7 RNA polymerase, and in the presence of rifampicin to

```
SufI                  MSLSRRQFIQASGIALCAGAVPLKASA
SufI-R5K              MSLSKRQFIQASGIALCAGAVPLKASA
TtrB            MWTGVNMDSSKRQFLQQLGVLTAGASLVPLAEA
TtrB-R12K       MWTGVNMDSSKKQFLQQLGVLTAGASLVPLAEA
TtrB-K11R       MWTGVNMDSSRRQFLQQLGVLTAGASLVPLAEA
TtrB-K11R-R12K MWTGVNMDSSRKQFLQQLGVLTAGASLVPLAEA
```

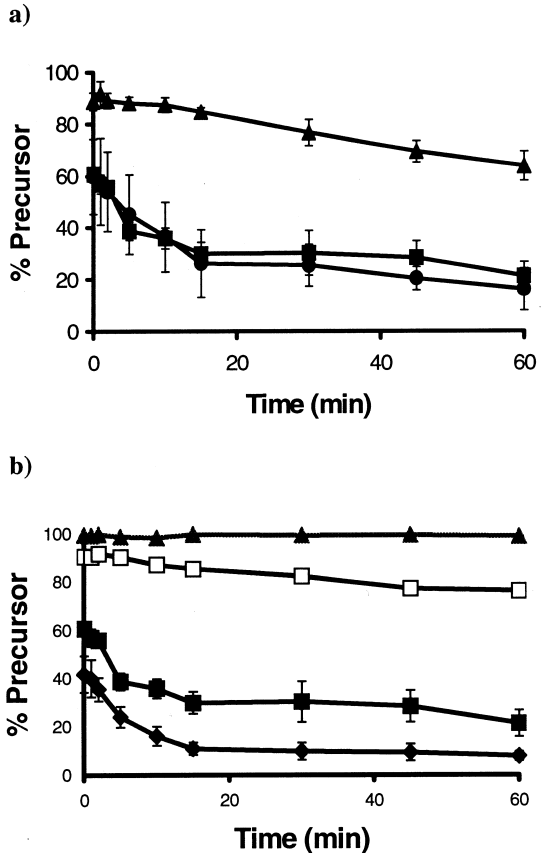

Fig. 4. Analysis of the effects of site-specific amino acid substitutions in the TtrB signal peptide. Pulse-chase experiments were performed as described for Fig. 3a. The mean percentage of total SufI protein remaining in precursor form in whole cells at each of the indicated time points is plotted ($n = 3$). The bars represent the standard error of the mean. (a) The export of wild-type pre-SufI (■) is compared with that of SufI–R5K (▲) and sTtrB–SufI (●). (b) The export of sTtrB–SufI (■) is compared with that of precursors with alterations in the TtrB signal peptide: TtrB–R12K–SufI (▲), TtrB–K11R–SufI (♦) and TtrB–K11R,R12K–SufI (□). The sequences of the engineered TtrB signal peptides are given at the top of the figure.

block transcription from *E. coli* promoters, this arrangement allows specific in vivo radiolabelling of the hybrid protein [3].

Pulse-chase experiments were carried out to determine the transport behaviour of the sTtrB–SufI hybrid precursor protein. *E. coli* cells were co-transformed with plasmid pAH56 bearing the *ttrB′–sufI′* gene fusion and plasmid pGP1-2 encoding T7 RNA polymerase. After induction of the T7 RNA polymerase gene encoded on the helper plasmid, the cells were pulse-labelled with [^{35}S]methionine, followed by the addition of excess unlabelled methionine. The sTtrB–SufI radiolabelled during the pulse period was converted over time to a smaller mature form (Fig. 3a). Neither form is present when a plasmid lacking the *ttrB′–sufI′* insert is used (data not shown), indicating that these proteins correspond to the precursor and a processed form of sTtrB–SufI. Subcellular fractionation experiments demonstrate that the mature form represents protein that has been exported to the periplasm (Fig. 3c). These experiments show that the TtrB signal peptide is capable of directing transport of a passenger protein from cytoplasm to periplasm. When the pulse-chase experiment was repeated in a Δ*tatC* background the precursor protein remains unprocessed for at least 60 min indicating that the observed transport of sTtrB–SufI is entirely Tat-dependent (Fig. 3b). We conclude that the TtrB signal peptide is capable of functional interaction with the Tat transport pathway.

The rate of export of sTtrB–SufI is comparable to that of wild-type SufI assayed under identical conditions (Fig. 4a). sTtrB–SufI is transported more rapidly than a SufI variant (SufI-R5K; [3]) that like TtrB substitutes a lysine for the first consensus arginine position in the Tat consensus motif (Fig. 4a).

Since the TtrB signal peptide mediates reasonably efficient Tat-dependent transport we wondered whether the consensus arginine positions in the TtrB signal peptide might be less important in signal peptide function than in other Tat signal peptides. To investigate this question we analysed the export behaviour of site-directed variants of sTtrB–SufI (depicted at the top of Fig. 4) and compared this with the previously reported transport behaviour of analogous substitutions in SufI precursors assayed under identical conditions [3]. sTtrB–SufI variants are given shorthand designations made up of the substituted amino acid type and position followed by the replacement amino acid. Thus sTtrB–R12K–SufI carries a lysine substitution of the arginine at position 12 in the TtrB signal peptide. Fractionation experiments were used to verify that the mature protein detected in the pulse-chase experiments was located in the periplasm (data not shown). No processing was observed for any of the variant precursor proteins in a Δ*tatC* background confirming that the transport observed occurred by means of the Tat pathway (data not shown).

Conservative substitution of the single consensus arginine residue in the TtrB signal peptide with lysine prevents transport of the variant precursor (sTtrB–R12K–SufI; Fig. 4b). Thus, as found for SufI, Tat transport has a minimum requirement of one consensus arginine residue even in a signal peptide that is evolutionarily adapted to function with just a single arginine residue. Introduction of a consensus twin–arginine pair into the TtrB signal peptide (sTtrB–K11R–SufI) enhances the rate with which the precursor protein is exported (Fig. 4b). This suggests that optimisation of the TtrB signal peptide for transport with a lysine–arginine pair does not fully replace the functionality of the standard arginine–arginine pair. Finally, if the order of the lysine–arginine pair in the TtrB signal peptide is reversed (sTtrB–K11R,R12K–SufI) then the rate of export is significantly slowed, but not blocked (Fig. 4b). Thus the TtrB signal peptide is optimised to function without the first consensus arginine. This situation contrasts with that observed for Lys–Arg and Arg–Lys SufI variants where the two engineered precursor proteins were found to behave in a similar manner [3].

4. Discussion

The experiments described here demonstrate that the *S. enterica* TtrB signal peptide directs Tat-dependent transport even though one of the otherwise invariant arginine residues of the Tat consensus motif is conservatively substituted by a lysine. We found that the rate of transport mediated by the TtrB signal peptide could be enhanced by introduction of the consensus arginine pair suggesting that the TtrB signal peptide has not been optimised for rapid transport. The reason that the TtrB protein employs (or alternatively tolerates) a sub-optimal targeting signal is not clear. Experiments in which mutations have been introduced at the twin–arginine consensus residues of TtrB (this work), SufI, and another *E. coli* protein YacK [3], suggest that it is unlikely that a Tat signal peptide can function in *E. coli* without either two arginine residues, or one arginine residue and one lysine residue. Intriguingly however, it has been reported that the thylakoid membrane protein Pftf is processed by the Tat pathway even when the twin–arginine-containing signal peptide has been deleted [19] indicating that currently uncharacterised Tat-targeting signals exist.

The TtrB signal peptide is the first identified naturally occurring Tat signal sequence that functions with a single consensus arginine residue. We have been unable to identify further candidate variant Tat signal peptides in the bacterial protein databases using as our search criteria either lysine plus arginine consensus motifs or the binding of Tat-associated cofactor types to the mature domain. Intriguingly, however, an important class of proteins found in plant organelles may utilise Tat-targeting signals with a Lys–Arg pair. These are the Rieske iron–sulfur protein subunits of the chloroplast cytochrome b_6f complex and the mitochondrial cytochrome bc_1 complex. The Rieske subunits consist of a membrane-extrinsic globular domain containing a [2Fe–2S] cluster and an amino-terminal signal-anchor sequence that mediates membrane attachment. In bacterial cytochrome b_6f and bc_1 complexes the iron–sulfur cluster-binding domain of the Rieske subunit is located at the periplasmic face of the membrane and the proteins have apparent, uncleaved, Tat signal sequences (Fig. 1b; [2]). In plant thylakoids the iron–sulfur cluster domain of the Rieske protein is located at the lumenal side of the thylakoid membrane but the iron–sulfur cluster is probably inserted in the stroma [20]. Although no currently identified substrates of the thylakoid Tat system contain cofactors the pathway transports tightly folded substrates and, by analogy to Rieske targeting in the ancestral cyanobacterium, is the obvious candidate to transport the Rieske protein [8,21,22]. The effects of ionophores on Rieske protein transport in whole chloroplasts would be consistent with this hypothesis [23]. Nevertheless, the signal-anchor sequences of chloroplast

Rieske proteins have a lysine–arginine pair rather than the classical Tat twin–arginine motif (Fig. 1c). The demonstration in the current study that a Tat-targeted bacterial iron–sulfur protein functions with a lysine–arginine pair indicates that such a substitution should no longer be regarded as incompatible with Tat transport. Given the importance of the b_6f complex to chloroplast function and the possible involvement of a variant Tat-targeting motif in the process an experimental study of the route of Rieske protein targeting in plant thylakoids would be of high interest.

The Rieske proteins of plant mitochondria also have Tat-like signal sequences containing a lysine–arginine rather than twin–arginine pair (Fig. 1d) raising the possibility that the Rieske protein is the currently unidentified substrate of the plant mitochondrial Tat system. In contrast the signal sequences of mitochondrial Rieske proteins from animals and yeast, which do not have Tat homologues encoded in their genomes, do not contain sequences with reasonable similarity to Tat consensus sequences (Fig. 1e).

In conclusion, our demonstration that TtrB has a functional Tat-targeting signal relaxes the sequence constraints expected of naturally occurring Tat signal peptides both in bacteria and plants.

Acknowledgements: This work was supported by Grant 83/P11074 to B.C.B. from the Biotechnology and Biological Research Council of the United Kingdom and by the Norwich Research Park. T.P. is a Royal Society Research Fellow.

References

[1] Pugsley, A.P. (1993) Microbiol. Rev. 57, 50–108.
[2] Berks, B.C. (1996) Mol. Microbiol. 22, 393–404.
[3] Stanley, N.R., Palmer, T. and Berks, B.C. (2000) J. Biol. Chem. 275, 11591–11596.
[4] Cristóbal, S., de Gier, J.-W., Nielsen, H. and von Heijne, G. (1999) EMBO J. 18, 2982–2990.
[5] Berks, B.C., Sargent, F. and Palmer, T. (2000) Mol. Microbiol. 35, 260–274.
[6] Keegstra, K. and Cline, K. (1999) Plant Cell 11, 557–570.
[7] Bogsch, E., Sargent, F., Stanley, N.R., Berks, B.C., Robinson, C. and Palmer, T. (1998) J. Biol. Chem. 273, 18003–18006.
[8] Chaddock, A.M., Mant, A., Karnauchov, I., Brink, S., Herrmann, R.G., Klösgen, R.B. and Robinson, C. (1995) EMBO J. 14, 2715–2722.
[9] Dreusch, A., Bürgisser, D.M., Heizmann, C.W. and Zumft, W.G. (1997) Biochim. Biophys. Acta 1319, 311–318.
[10] Henry, R., Carrigan, M., McCaffrey, M., Ma, X. and Cline, K. (1997) J. Cell Biol. 136, 823–832.
[11] Gross, R., Simon, J. and Kröger, A. (1999) Arch. Microbiol. 172, 227–232.
[12] Halbig, D., Wiegert, T., Blaudeck, N., Freudl, R. and Sprenger, G.A. (1999) Eur. J. Biochem. 263, 543–551.
[13] Barrett, E.L. and Clark, M.A. (1987) Microbiol. Rev. 51, 192–205.
[14] Hensel, M., Hinsley, A.P., Nikolaus, T., Sawers, G. and Berks, B.C. (1999) Mol. Microbiol. 32, 275–288.
[15] Casadaban, M.J. and Cohen, S.N. (1979) Proc. Natl. Acad. Sci. USA 76, 4530–4533.
[16] Lyons, L.B. and Zinder, N.D. (1972) Virology 49, 45–60.
[17] Tabor, S. and Richardson, C.C. (1985) Proc. Natl. Acad. Sci. USA 82, 1074–1078.
[18] Stanley, N.R. (2000) PhD thesis, University of East Anglia, Norwich.
[19] Summer, E.J., Mori, H., Settles, A.M. and Cline, K. (2000) J. Biol. Chem. 275, 23483–23490.
[20] Nuccio, M.L., Russell, B.L., Nolte, K.D., Rathinasabapathi, B., Gage, D.A. and Hanson, A.D. (1998) Plant J. 16, 487–496.
[21] Clark, S.A. and Theg, S.M. (1997) Mol. Biol. Cell 8, 923–934.
[22] Hynds, P.J., Robinson, D. and Robinson, C. (1998) J. Biol. Chem. 273, 34868–34874.
[23] Madueño, F., Bradshaw, S.A. and Gray, J.C. (1994) J. Biol. Chem. 269, 17458–17463.