The Entire N-Terminal Half of TatC is Involved in Twin-Arginine Precursor Binding†

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ABSTRACT: Translocation of twin-arginine precursor proteins across the cytoplasmic membrane of *Escherichia coli* requires the three membrane proteins TatA, TatB, and TatC. TatC and TatB were shown to be involved in precursor binding. We have analyzed in vitro a number of single alanine substitutions in *tatC* that were previously shown to compromise in vivo the function of the Tat translocase. All *tatC* mutants that were defective in precursor translocation into cytoplasmic membrane vesicles concomitantly interfered with precursor binding not only to TatC but also to TatB. Hence structural changes of TatC that affect precursor targeting simultaneously abolish engagement of the twin-arginine signal sequence with TatB and block the formation of a functional Tat translocase. Since these phenotypes were observed for *tatC* mutations spread over the first half of TatC, this entire part of the molecule must globally be involved in precursor binding.

Prokaryotic organisms export proteins across the cytoplasmic membrane via one of two major protein translocation machineries, named Sec and Tat translocases. The Tat (twin-arginine translocation) pathway (*1*) is restricted to folded substrates (*2*) that carry the consensus motif S-R-R-x-F-L-K in their signal sequences. A homologous Tat machinery has been conserved in the thylakoid membrane of plant chloroplasts. In *E. coli*, a minimal set of three membrane proteins, TatA, TatB, and TatC are required to form a functional Tat translocase. Whereas TatC is multispanning (cf. Figure 1A), TatA and TatB each have a single transmembrane domain with their N-termini in the periplasm. A flexible association of TatA with membrane lipids ranging from topology inversion (*3*) up to soluble intermediates (*4*) are being discussed. *E. coli* cells express a fourth Tat component, TatE, which is functionally equivalent to TatA (*5*) and probably arose from a cryptic gene duplication of *tatA* (*6*). Although TatA and TatB of *E. coli* perform distinct functions, they are likely derivatives of a common ancestor, as *tatA* mutants were isolated that can compensate for a complete loss of TatB (*7*).

The major function thus far elucidated for TatB and TatC is the specific recognition of a Tat substrate via its RR-motif (*8*, *9*), with TatC being the primary recognition site (*8*). TatA is believed to constitute the major part of the protein-conducting channel. As a result of its propensity to assemble into homo-oligomeric complexes (*10*) of different sizes (*11*, *12*), TatA would be most suitable to provide variable pores for a formfitting passage of folded Tat substrates. Although no mechanistic details are known, the transmembrane H^+-motive force is the likely source of energy for moving Tat substrates entirely across the membrane (*13*). It does not, however, seem to influence initial translocation steps up to cleavage of the signal peptide (*14*). Unexpectedly, at this stage the Tat translocase is still able to reject a client protein (*15*).

A current model predicts that after binding of an RR-signal sequence to TatBC, TatA starts to oligomerize (*16*) and to be recruited to the TatBC−precursor complex in a H^+-motive force-dependent manner (*17*). A major issue is the mechanism of quality control by which the Tat translocase senses the folding state of targeted proteins (*18*). For some RR-

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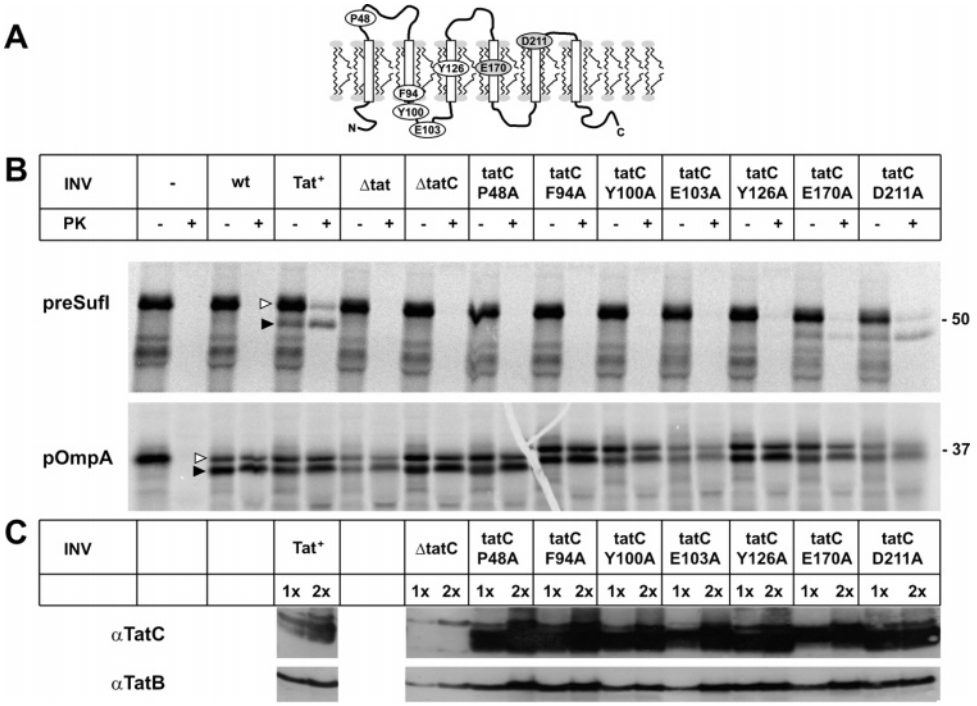

FIGURE 1: Influence of various single alanine substitutions in TatC on the translocation of preSufI into inside-out, inner membrane vesicles of *E. coli*. (A) topography model of TatC according to ref 23 and location of the alanine substitutions investigated. (B) The precursors of SufI (preSufI) and OmpA (pOmpA) were synthesized by a cell-free *E. coli* transcription/translation system in the absence or presence of the indicated inside-out, inner membrane vesicles (INV). INV had been obtained from wild-type cells (wt), a *tatABCDE* deletion strain (Δtat), the *tat* deletion strain transformed with plasmid pQE-TatABC (Tat^+), and the *tat* deletion strain transformed with derivatives of pQE-TatABC, in which the indicated mutations in tatC had been introduced. ΔtatC-INV (ΔtatC) were obtained from a transformant, in which pQE-TatABC encoded an early truncate of TatC. Amounts of INV added were adjusted to the same protein content as determined by the absorbance of the vesicle suspensions at 280 nm. Radiolabeled translation products were separated by SDS-PAGE and visualized by phosphorimaging. For translation products of OmpA, half of each reaction was digested with proteinase K (PK) as described (*8*) prior to precipitation with 5% (final) trichloroacetic acid, whereas in the case of SufI two-thirds were treated in the same way. The non-digested parts of each reaction were directly precipitated with trichloroacetic acid. Marked are the precursors of SufI and OmpA (open arrowheads) and their signal sequence-less forms (closed arrowheads). Note that under these experimental conditions, translocation into INV as indicated by the vesicle-dependent appearance of PK-resistant species usually leads to some uncleaved precursor. (C) Immunoblot of INV prepared from the indicated strains. The blot was decorated using antisera against TatC and TatB (αTatC, αTatB). Totals of 1.0 μL and 0.5 μL (2× and 1×, respectively) of each vesicle suspension normalized to an absorbance of 25 at 280 nm were applied to SDS-PAGE.

precursors that undergo a complex maturation process, dedicated cytosolic chaperones seem to fulfill a proof-reading function by preventing premature membrane-targeting (*19*, *20*).

The actual recognition site for the arginine pair at TatC has so far remained elusive. Several point mutations in *tatC* were described (*21*−*23*) that affect RR-dependent translocation in *E. coli* cells to varying degrees. The P48A and F94A mutations lead to a complete block in translocation activity (*21*, *22*), whereas the Y100A, Y126A, and E170A mutations lead to a partial block (*23*). The E103A and D211A TatC mutants were described as completely inactive in one study (*23*) but active in another (*22*). Here we have analyzed those *tatC* mutants with respect to their individual influence on precursor targeting and subsequent translocation steps.

EXPERIMENTAL PROCEDURES

Plasmids. For the synthesis of wild type pOmpA, preSufI, and TorA-23K, plasmids pDMB (*24*), pKSMSufI-RR (*8*), and pMW18 (*25*) were used, respectively. The construction of plasmids pSufF8-RR and pSufL16-RR encoding the respective TAG codon mutants of *sufI* have been described (*8*). Stop codons were introduced into the TorA signal sequence-encoding DNA using plasmid pMW18 as template and the following two pairs of oligonucleotides: T23K-F14F (5′-CAC GTC GGC GTT AGC TGG CAC AAC-3′, with the stop codon underlined) and T23K-F14R (5′-GTT GTG CCA GCT AAC GCC GAC GTG-3′) and T23K-L31F (5′-GGG GCC GTC ATA GTT AAC GCC GCG-3′) and T23K-L31R (5′-CGC GGC GTT AAC TAT GAC GGC CCC-3′), resulting in plasmids pBluSK-T23K-F14 and pBluSK-T23K-L31, respectively.

Using the primers TaNcoFor (5′-CCG TCC ATG GGT GGT ATC AGT ATT TG-3′ with an *Nco*I-cleavage site underlined) and TcQ60Rev (5′-GGC CCA GAT CTA TTA TTC TTC AGT TTT TTC G-3′ with a *Bgl*II- cleavage site underlined) the *tatABC* operon was amplified by PCR from the wild type template p8737 (*26*), and from templates encoding the *tatC* mutations P48A (*22*), F94A (*27*), Y100A, Y126A, E170A (all constructed according to ref 27), and E103A and D211A (constructed in p8737). PCR products were cut with *Nco*I/*Bgl*II and ligated into pQE60 (Qiagen) linearized with the same enzymes. The resulting plasmids pQE60-TatABC encoding wild type and mutant TatCs were transformed into strain DADE (MC4100, Δ*tatABCD*, Δ*tatE* (*28*)) that beforehand had been transformed with pREP4 (Qiagen). All constructs were verified by sequencing.

In Vitro Synthesis. Synthesis of stop codon mutants of preSufI and TorA-23K by coupled transcription/translation and binding of the resulting translation products to post-

translationally added membrane vesicles were performed as described (*8*). To assay transport of preSufI and pOmpA, membrane vesicles were added 10 min after starting the synthesis reaction and incubated for 30 min (*26*). Together with the vesicles, ATP, DTT, creatine phosphate, and creatine phosphokinase were added again at their initial concentrations. At the time of addition of INV,[1] reactions were also made 0.5 M in K-acetate which has been found to support RR-dependent translocation into *E. coli* INV.[2] Reactions were stopped as further detailed in the legend to Figure 1.

Preparation of Membrane Vesicles. INV were prepared from *E. coli* wild type strain MC4100 (F^-, Δ*lacU169*, *araD139*, *rpsL150*, *relA1*, *ptsF*, *rbsR*, *flbB5301*) (*29*) and from transformants of the *tat* deletion strain DADE (MC4100, Δ*tat*ABCD*tat*E) (*28*) according to the method described in refs 8 and 26 except that the expression from plasmid-borne *tatABC* genes was induced by 2 mM isopropyl thio-β-D-galactopyranoside.

Miscellaneous. Cross-linking following UV irradiation and immunoprecipitation with antibodies against TatB and TatC (*26*) and FkpA were carried out as described (*8*) except that proteins were denatured in sodium dodecyl sulfate (SDS) at 37 °C rather than at 95 °C when TatC was to be immunoprecipitated.

RESULTS

Mutations in TatC that Abolish RR-Dependent Translocation into Inside-Out Inner Membrane Vesicles of E. coli. Figure 1A illustrates the predicted topography of TatC and the locations of the seven single alanine substitutions analyzed in this study. To obtain translocation of an RR-precursor protein into inside-out inner membrane vesicles (INV), vesicles have to be prepared from *E. coli* strains that overproduce Tat proteins (*26*, *30*). This is demonstrated for in vitro synthesized preSufI in Figure 1B. Signal sequence cleavage (black arrowhead) and proteinase K (PK) protection of preSufI and SufI, both reflecting translocation into the vesicle lumen, were observed only with INV that had been prepared form an *E. coli* strain expressing the *tatABC* genes from a multicopy plasmid under *lac* induction (Tat^+). INV from cells containing the chromosomal copies of *tatABC* under their own promoter (wt) were as inactive in translocation of SufI as vesicles prepared from strain DADE carrying chromosomal deletions of *tatABCD* and *tatE* (Δtat) or a *tatC* deletion mutant (ΔtatC).

The *tatABC* operons of wild type cells and the seven *tatC* mutants were therefore amplified by PCR and placed under *lac* promoter control in pQE60. The resulting plasmids were transformed into the *tat* deletion strain DADE along with plasmid pREP4 coding for the *lac* repressor. IPTG-induced overexpression of the individual plasmid-encoded *tat* operons should then allow the isolation of translocation-proficient INV.

Membrane vesicles prepared from the *P48A*, *F94A*, *Y100A*, *E103A*, and *Y126A tatC* mutants did not give rise to signal sequence cleavage, suggesting that the preSufI is not transported, and this is confirmed by the absence of resistance to PK (Figure 1B). This was the case although the pQE60-expressed *tatC* mutant proteins in general were recovered from INV at even higher levels than overexpressed wild-type TatC (Figure 1C). Since none of these five mutations interfered with the expression of TatB (Figure 1C) or with the Sec-dependent translocation of pOmpA (Figure 1B), they directly and specifically inactivated the Tat translocase. In contrast, a significant level of translocation was observed with INV prepared in the same manner from the *E170A* and *D211A tatC* mutants. A similar lack of phenotype of those two mutations had also been reported previously (*22*).

Translocation-Deficient tatC *Mutations Do Not Allow Association of PreSufI with TatC and TatB* . Using site-specific cross-linking we have previously shown (*8*) that upon membrane targeting, TatC serves as the primary recognition site for the RR consensus motif of the SufI signal sequence, which only in the presence of TatC also interacts with TatB. We therefore tested whether any of the translocation defects associated with five of the above *tatC* mutations resulted from an impaired ability of preSufI to bind to TatC and/or TatB. To experimentally address these questions we expressed in vitro two stop codon mutants of preSufI by including in the transcription/translation reaction a suppressor tRNA which had been chemically loaded with Tmd-Phe (*L*-4′-(3-[trifluoromethyl]-3*H*-diazirin-3-yl) phenylalanine). Tmd-Phe is a photoactivatable derivative of phenylalanine. Following in vitro synthesis, UV irradiation is used to convert the diazirine group of Tmd-Phe into a highly reactive carbene which forms covalent bonds to molecules coming as close as about 2 Å.

As shown in Figure 2A, the two TAG stop codons were engineered into the coding sequence of the preSufI signal sequence replacing either a phenylalanine two residues downstream of the twin-arginine pair (F8) or a leucine residue within the hydrophobic core of the signal sequence (L16). Both stop codon mutants of preSufI were efficiently suppressed since in vitro synthesis gave rise to the full-length 52 kDa translation products of preSufI (Figure 2B,C, preSufI-L16 and preSufI-F8, white arrowheads). Suppression of stop codons was paralleled by the incorporation of the cross-linker because UV irradiation yielded discrete radioactively labeled products that were larger than preSufI (Figure 2B,C, compare lanes 1 and 2). A prominent 80 kDa adduct was obtained in the absence of membrane vesicles (lane 2, arrows). Immunoprecipitation revealed that this cross-linking partner was the periplasmic protein FkpA (Figure 2D, lanes 2 and 3) obviously contaminating the cytosolic extract used for cell-free translation. When preSufI was, however, incubated with Tat^+-INV under conditions that allow binding but no translocation, UV-activated Tmd-Phe at position L16 predominantly cross-linked to a smaller protein (Figure 2B, lane 4, black star) which cross-reacted with antibodies against TatB (Figure 2D, lanes 4 and 5). In contrast, Tmd-Phe at position F8 cross-linked to TatC (panels C, lane 4, and D, lanes 8 and 9, white stars). These results confirm the previously established contact sites between preSufI and TatBC (*8*).

As expected, the cross-links between preSufI and TatBC were not obtained with INV lacking TatABCD, (Figure 2BC, lane 5, Δtat). In the presence of these INV, preSufI instead remained cross-linked to FkpA, while INV of cells expressing TatABC only at wild-type level gave rise to a mixed phenotype with cross-links to both TatBC and FkpA (lane

[1] Abbreviations: INV, inside-out, inner membrane vesicles; Tmd-Phe, *L*-4′-(3-[trifluoromethyl]-3*H*-diazirin-3-yl) phenylalanine.

[2] Lüke, I., Moser, M., and Müller, M. Unpublished observation.

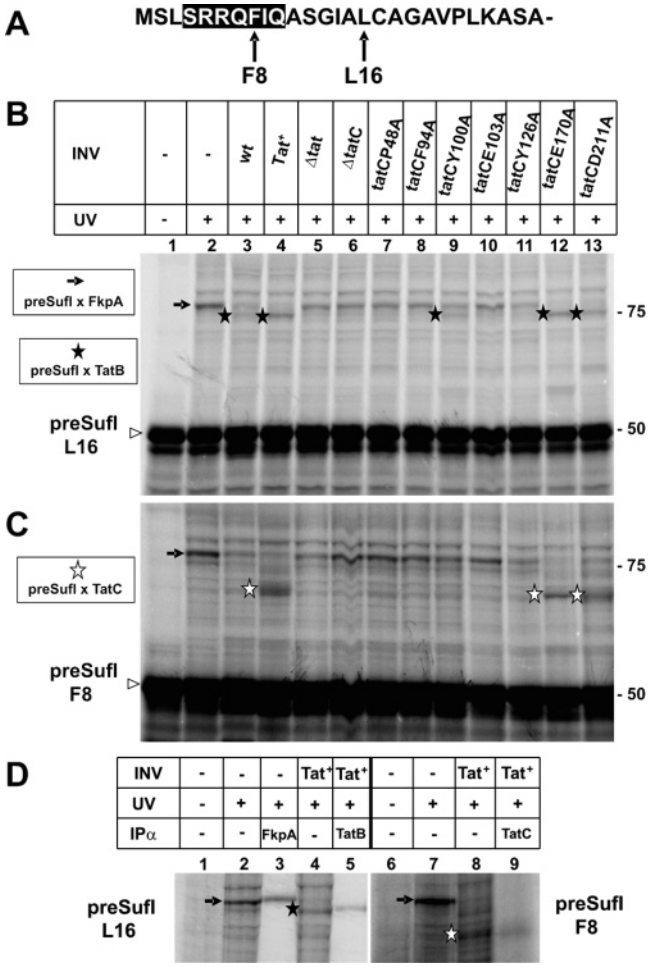

FIGURE 2: Influence of various single alanine substitutions in TatC on the targeting of preSufI to TatC and TatB. (A) Signal sequence of preSufI with the consensus motif highlighted by a black box. The positions are indicated where stop codons were introduced into the *sufI* DNA. Capital letters in front of the numerals indicate the amino acids which were replaced by Tmd-Phe. Numbering starts with the N-terminal methionine of the signal sequence of preSufI. (B) preSufI carrying Tmd-Phe at position L16 was synthesized in vitro. INV obtained from the indicated strains (for details see the legend to Figure 1) were added post-translationally. Where indicated, samples were irradiated (UV). Following precipitation with 5% trichloroacetic acid, proteins were resolved by SDS-PAGE. The position of preSufI on SDS-PAGE after phosphorimaging is indicated (preSufIL16, open arrowhead). Marked are cross-links of preSufI with FkpA (arrow) and TatB (black star). (C) As in panel B except that experiments were performed with preSufI carrying Tmd-Phe at position F8 (preSufIF8, open arrowhead). This area of the signal sequence rather cross-links to TatC (white star) than to TatB. (D) As in panels B and C. Immunoprecipitation was performed on fourfold (IP αFkpA and αTatB) and eightfold (IP αTatC) scaled-up reactions.

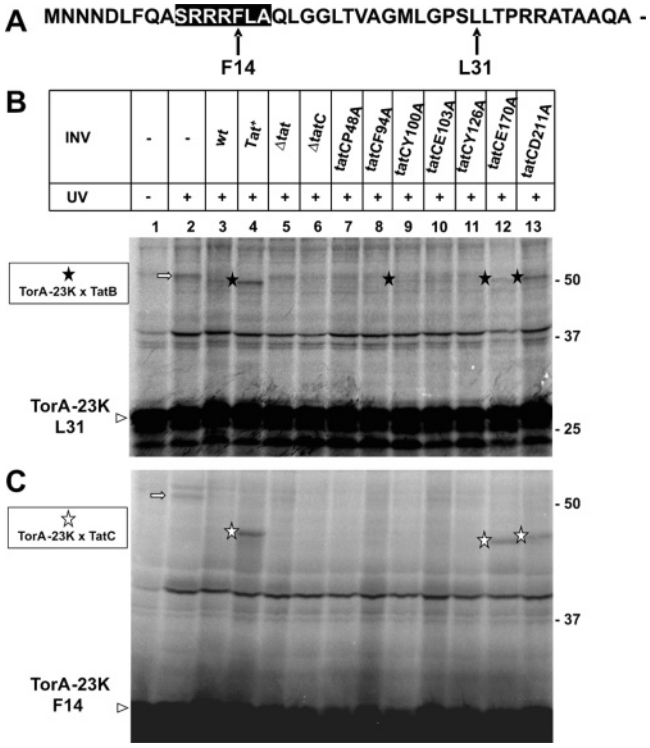

FIGURE 3: Influence of various single alanine substitutions in TatC on the targeting of TorA-23K to TatC and TatB. (A) Signal sequence of TorA. For further details see legend to Figure 2. (B and C) Cross-links of the TorA signal sequence to TatB and TatC (black and white stars) and an unidentified soluble protein that has not been further characterized (white arrow). Note that SDS-PAGE was performed using 12% (B) and 9% (C) polyacrylamide gels.

3). Thus cross-linking of preSufI to FkpA conveniently served as an indication for impaired membrane targeting.

The data obtained with the 7 *tatC* mutants are shown in lanes 7−13 of Figures 2B and 2C. Similar to a *tatC* deletion mutant (lane 6, ΔtatC), most of the TatC mutant vesicles did not release cross-linking to FkpA, with the notable exceptions of the *tatCE170A* and *tatCD211A* mutations. INV of those two mutants, which also supported Tat-dependent transport as shown in Figure 1, showed the same cross-linking phenotype as Tat^+-INV (compare lane 4 with lanes 12,13); that is, preSufI cross-links to FkpA were almost totally replaced by those to TatB and TatC (black and white stars). A similar dual cross-linking to FkpA and TatB as seen with INV harboring limiting amounts of wild-type TatABC (lane 3) was also observed with the translocation-deficient INV prepared from the Y100A mutant of TatC (lane 9). Thus for most of the *tatC* point mutant vesicles tested, a lack of translocation activity was paralleled by an impaired or abolished association of preSufI with both TatB and TatC. The combined data provide strong evidence that the 5 TatC mutations P48A, F94A, Y100A, E103A, and Y126A interfere with the binding of preSufI substrate to the TatBC receptor site, causing a block in translocation activity.

The Targeting Defect of Distinct tatC *Mutations Is Verified for a Different RR-Signal Sequence.* Thus far the specific events during targeting of an RR-precursor protein to the Tat translocase of *E. coli* had been analyzed by site-specific cross-linking using the signal sequence of preSufI only. To corroborate our findings we performed experiments with a second RR-substrate. We employed the fusion protein TorA-23K consisting of the signal sequence of *E. coli* trimethylamine *N*-oxide reductase (TorA) and the 23 kDa subunit of the photosystem II oxygen-evolving complex from spinach chloroplasts (*31*). The 23kDa protein with its authentic signal sequence is a substrate of the thylakoidal Tat pathway. We introduced stop codons into the consensus motif and the hydrophobic core of the TorA signal sequence (Figure 3A, F14 and L31, respectively) at positions that were analogous to those of preSufI.

Both stop codon mutants of TorA-23K were suppressed giving rise to full-size translation products of about 27 kDa. When UV-irradiated in the absence of membrane vesicles,

both TorA-23K constructs yielded adducts with an increase in size of about 30 kDa (Figure 3B,C, lane 2, white arrows). Upon longer exposure, these adducts were resolved into several species, two weaker ones of which coimmunoprecipitated with antisera directed toward FkpA and TorD, a TorA-specific chaperone, respectively;[3] the identity of the major soluble binding factor has not yet been determined. These 30 kDa cross-links disappeared in the presence of translocation-proficient INV (Tat^+) in favor of smaller adducts that were identified by immunoprecipitation (data not shown) as TatB in the case of TorA-23K carrying Tmd-Phe at position L31 and as TatC for the cross-linker placed at F14 (panels B and C, lane 4, black and white stars, respectively). Thus the same contact sites between the signal sequence of TorA-23K and TatC/TatB were obtained upon membrane targeting as previously observed for preSufI.

Cross-linking of the TorA-23K constructs to TatB and TatC was also seen for the two functionally active *tatC* substitutions *E170A* and *D211A* (lanes 12 and 13). In contrast, none of the other *tatC* point mutants (lanes 7−11) yielded substantial TatB/C cross-links, with the exception of Y100A which again gave a weak cross-link to TatB. At least in the case of TorA-23K with Tmd-Phe at position L31 (panel B) the impaired cross-linking to TatB seemed to be paralleled by a sustained interaction with the undefined 30 kDa soluble proteins (white arrow). However, the latter cross-links were in general less strong than the FkpA−preSufI interactions (compare Figures 2 and 3).

DISCUSSION

We show here that the five single alanine substitutions P48A, F94A, Y100A, E103A, and Y126A of TatC, that interfere with RR-dependent translocation in vivo (*22*, *23*), also abolish translocation of the Tat substrate preSufI into isolated membrane vesicles of *E. coli*. This defect was specific as Sec-dependent translocation of pOmpA into *tatC* INV was not impaired. All translocation-defective *tatC* mutations impaired membrane-targeting of two RR-substrates, preSufI and TorA-23K, both to TatC and to TatB suggesting that they prevented a functional interaction of these RR-precursors with the Tat translocase.

Previous studies suggested that in the Tat pathway both of *E. coli* and of chloroplasts, TatC represents the primary binding site for RR-precursors. Thus RR-signal sequences interact with TatC even if TatB is missing (*8*). In contrast, contacts of the signal sequence with TatB strictly depend on the presence of TatC and can be quenched by its overproduction (*8*). Cross-linking analyses revealed that the RR-consensus motif of signal sequences preferentially binds to TatC, whereas TatB also associates with the hydrophobic core downstream of the RR-pair (*8*, *32*). The latter contacts do not seem to strictly require an intact RR-motif (*8*) for which reason they might occur subsequently to the first recognition of the RR-consensus by TatC. Binding of the signal sequence to TatC, however, appears to persist during the whole translocation process (*32*).

The requirement for TatC in mediating contact with TatB is underlined by our findings that basically all defective *tatC* mutations, with the possible exception of Y100A (see below), impair precursor binding to TatC in the same manner as to TatB. On the other hand, among the tested single alanine substitutions in TatC, there was none that would have allowed association of the signal sequence with TatC but blocked that with TatB. This would have been expected if recognition of precursor and mediation of contact with TatB resided in two independent domains of the TatC molecule.

We found the signal sequence of preSufI when synthesized under our in vitro conditions cross-linked to the periplasmic protein FkpA. These cross-links predominated both in the absence of INV and in the presence of INV devoid of a functional Tat translocase. Thus the occurrence of FkpA-SufI adducts is the equivalent of an abrogated targeting to the Tat translocase. FkpA-SufI cross-links likely are an in vitro artifact, because it is difficult to conceive how, in vivo, the signal sequence of SufI should interact with the periplasmically localized FkpA prior to targeting to TatBC. FkpA may, however, fortuitously mimic the function of some as yet unidentified cytosolic chaperone which conceivably is required to protect the signal sequence of preSufI following release from the ribosome. A general function as chaperone has in fact been described for FkpA, which interacts with early folding intermediates, prevents aggregation of proteins, and reactivates inactive protein independently of its peptidyl-prolyl *cis*,*trans*-isomerase activity (*33*, *34*). Similar prominent cross-links with soluble proteins of about 30 kDa size were also obtained for the TorA signal sequence but were only partly due to interactions with FkpA and the dedicated chaperone of TorA, TorD (*20*, *35*).

Our finding that in the presence of INV, which lack TatABC, both RR-signal sequences cross-linked to a soluble protein to the same extent as in the complete absence of membranes does not support the idea of membrane targeting sites outside of TatBC. Such an "unspecific" membrane targeting of RR-signal sequences that precedes the first contact with the Tat translocase has been proposed (*36*−*38*). Our results do not, however, rule out the possibility that the signal sequence of an RR-precursor binds to unspecific docking sites at the membrane if soluble chaperones become limiting.

Using the persistence of precursor-FkpA adducts as an indication for a compromised membrane-targeting due to a defective Tat translocase, we observed at least in the case of the Y100A *tatC* mutation an intermediate phenotype with cross-links to both FkpA and TatB (cf. Figures 2B and 3B, lanes 9). The same mutation probably also allowed some interaction with TatC, but this is more difficult to assess in Figures 2C and 3C, as cross-links with TatC in general were less well resolved from the background noise. In any case, the conformational change of TatC brought about by the Y100A mutation is probably less severe leading to a leaky targeting phenotype. This would be compatible with the initial phenotypic characterization of this mutant as affecting an important yet non-essential residue in TatC (*23*). Simultaneous cross-linking of preSufI to FkpA and TatB was not only typical for the TatCY100A mutation but also obtained when INV contained limiting levels of wild-type TatABC. Therefore the failure to translocate preSufI into TatCY100A INV, despite its binding to the Tat translocase, is probably not so much due to a dissociation of the targeting and translocation functions by this TatC mutation but may only

[3] Panahandeh, S., Holzapfel, E., Moser, M., and Müller, M. Unpublished data.

reflect a drop in translocation efficiency below detection level.

We did, however, obtain translocation with INV prepared from the *E170A* and *D211A tatC* mutants which is in contrast to their original characterization as translocation-defective in vivo (*23*). Further studies on the various mutants described here concluded that only P48A and F94A are consistently and completely translocation-defective in vivo (*21*−*23*). The E103A and D211A TatC mutants were reported not to support detectable levels of RR-dependent translocation when expressed at low levels (comparable to wild-type levels), but expression on a multi-copy plasmid led to efficient translocation of Tat substrates. In this study we have shown that E170A and D211A do support translocation into INV, confirming that these mutations do not block translocation. In turn, these data rule out the possibility that the data obtained with the other *tatC* mutants (P48A, F94A, Y100A, E103A, Y126A) simply result from overexpression of TatC because the TatC mutants were all overexpressed to similar levels.

The key question of where in TatC the actual binding site for the twin-arginine consensus motif resides remains open. The finding that amino acid substitutions dispersed over the N-terminal half of the TatC molecule all affect RR-precursor binding renders a linear RR-recognition motif in TatC less likely and favors the idea of an epitope of TatC that is involved in the decoding of the RR-motif. It has been shown that the TatC P48A mutation leads to a disintegration of the TatABC complex (*39*), and this would explain the complete block in both translocation and precursor binding. The F94A mutation, on the other hand, appears to be structurally intact, suggesting that this mutation does affect the substrate binding site. The other TatC mutants have not been characterized in structural terms

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