Conservation and Variation between *Rhodobacter capsulatus* and *Escherichia coli* Tat Systems*††*

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The Tat system allows the translocation of folded and often cofactor-containing proteins across biological membranes. Here, we show by an interspecies transfer of a complete Tat translocon that Tat systems are largely, but not fully, interchangeable even between different classes of proteobacteria. The Tat apparatus from the α-proteobacterium *Rhodobacter capsulatus* was transferred to a Tat-deficient *Escherichia coli* strain, which is a γ-proteobacterium. Similar to that of *E. coli*, the *R. capsulatus* Tat system consists of three components, rc-TatA, rc-TatB, and rc-TatC. A fourth gene (rc-tatF) is present in the rc-tatABC operon which has no apparent relevance for translocation. The translational starts of rc-tatC and rc-tatF overlap in four nucleotides (ATGA) with the preceding tat genes, pointing to efficient translational coupling of rc-tatB, rc-tatC, and rc-tatF.

We show by a variety of physiological and biochemical assays that the *R. capsulatus* Tat system functionally targets the *E. coli* Tat substrates TorA, AmiA, AmiC, and formate dehydrogenase. Even a Tat substrate from a third organism is accepted, demonstrating that usually Tat systems and Tat substrates from different proteobacteria are compatible with each other. Only one exceptional Tat substrate of *E. coli*, a membrane-anchored dimethyl sulfide (DMSO) reductase, was not targeted by the *R. capsulatus* Tat system, resulting in a DMSO respiration deficiency. Although the general features of Tat substrates and translocons are similar between species, the data indicate that details in the targeting pathways can vary considerably.

The twin-arginine translocation (Tat) system is known to translocate folded proteins across bacterial energy-transducing membranes (26). Three functionally distinct components of Tat systems have been identified, namely, TatA, TatB, and TatC. TatA and TatB resemble each other, and single-amino-acid exchanges can render TatA to a TatB-substituting component (6). Some organisms contain paralogs of individual components, and many organisms contain only one TatAB-like component (13). Substrates of the Tat system are synthesized with an N-terminal signal sequence which contains the eponymous twin-arginine pattern (26). As the individual Tat substrates differ between species, it appears possible that Tat systems preferentially recognize their respective substrates. This view has been supported by studies on the transport of glucose/fructose oxidoreductase (GFOR) from *Zymomonas mobilis* in *Escherichia coli* (7). GFOR was not compatible with the Tat system from *E. coli* but became compatible when the signal sequence was exchanged with that from *E. coli* trimethylamine N-oxide (TMAO) reductase. Also, a *Bacillus subtilis* PhoD signal LacZ fusion was not translocated by the *E. coli* Tat system but became translocated by the PhoD-specific *Bacillus* Tat system (30). On the other hand, the Tat substrates alkaline phosphatase from *Thermus thermophilus* (1), high-potential iron-sulfur protein (HiPIP) from *Allochromatium vinosum* (9), and PlcH from *Pseudomonas aeruginosa* (42) were compatible with the *E. coli* Tat translocon, and *E. coli* TorA was compatible with Tat systems from *Salmonella enterica* serovar Typhimurium and *Agrobacterium tumefaciens* (27).

In this study, we intended to use a heterologous Tat system to address the unclear aspects of substrate specificity. The Tat system from *Rhodobacter capsulatus* was transferred to a Tat-deficient *E. coli* strain, and the functionality of the Tat transport was analyzed with physiological and biochemical assays. The Tat system from *R. capsulatus* fully complemented most established phenotypes of Tat deficiency. The fourth gene product of the *R. capsulatus* tat operon rc-tatF was not required for this complementation. Despite a high degree of compatibility between the tested Tat systems, the typical and well-studied Tat substrate DmsA was not membrane targeted by the heterologous *R. capsulatus* Tat system. These observations point to variations between Tat systems which can lead to exceptional rejections of certain Tat substrates.

**MATERIALS AND METHODS**

**Strains and growth conditions.** The *E. coli* strain MC4100 (10) and its tat-deficient derivative DADE (MC4100 ΔtatABCDE) (49) were used for functional analyses as well as for expression of *E. coli* tatABCStrep. The strain BL21-DE3 was used for expression of rc-tatF, and the strain XL1-Blue Mf’ Kan (Stratagene) was used for all cloning steps. *E. coli* was grown at 37°C on LB medium (1% tryptone, 1% NaCl, 0.5% yeast extract) in the presence of the appropriate antibiotics (100 µg/ml ampicillin, 12.5 µg/ml tetracycline). TMAO or dimethyl sulfide (DMSO) respiration was tested by anaerobic growth on M9 medium supplemented with 0.5% glycerol, trace elements (SL12 [28]), and 1.1% (wt/vol) TMAO or 0.7% (vol/vol) DMSO, respectively. *R. capsulatus* strain B10S (22) was grown aerobically on 1.5% Bacto peptone, 1.5% yeast extract, 0.8% glucose, and trace elements. The strain B10S was transferred to a Tat-deficient *R. capsulatus* strain B10S (22) was grown aerobically on 0.15% Bacto peptone, 0.15% yeast extract, 0.8 mM MgCl2, and 0.5 mM CaCl2.

**Plasmids and genetic methods.** The tat operon of *R. capsulatus* B10S was amplified from chromosomal DNA using the primers Rc-tat-HindIII-F (5’-GTT CGA AGC TTC CGT GTG TCA TCG 3’) and Rc-tat-XbaI-R (5’-GCC GCT CTA GAC TGC TCC CCC-3’). The PCR fragment was restricted with HindIII and XbaI and cloned behind the lac promoter into the corresponding sites of the low-copy RK2 orl’-containing plasmid pRK415 (21).

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resulting in pRK-rec-tatABC. An rc-tatABC complementation vector was constructed by amplification of rc-tatABC, using the primers Rc-tatHindIII-F (see above) and Rec-tatABC-XbaI (5′ TCG GGT GTA GAC CAC AAA AAC GCA TCC 3′), restriction of the PCR fragment with HindIII and XbaI, and cloning into the corresponding sites of pRK415, resulting in pRK-rec-tatABC.

For expression of rc-tatF, the gene was amplified from chromosomal DNA using the primers F-NdeI-R (5′ GAC GAC ATA TGA CCG ATC CCC TGA CGC GGA TTG 3′) and F-HindIII-R (5′ CTT CAA AGC TTC AGC CGG TGC CGC GCC AGG 3′). The PCR fragment was restricted with NdeI and HindIII and cloned into the corresponding sites of pET22-b (+) (Novagen), resulting in pEX-rec-tatF-H6. For expression of ec-tatABCSTrep, the complete tat operon of E. coli was amplified from chromosomal DNA, using the primers Pat-XbaI-F (5′ ATT CTA GAT TAA AGA TCA GGT CGG TAT TCC TGC 3′) and tatD-XbaI-R (5′ TAT CTC GAG GGC AAT CCC AAA CAG TGT TTG GAC 3′), restricted with XbaI and Xhol, and cloned into the corresponding sites of pABS-tatABCSTrep (5), resulting in pABS-tatABCSTrep. All constructs were confirmed by sequencing and restriction analyses.

For purification of rc-TatF, E. coli BL21-DE3 was transformed with pEX-rec-tatF-H6 and grown at high aeration at 37°C to an optical density at 600 nm of 1, induced for 2 h with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), harvested. Cells were resuspended in precooled 20 mM Tris HCl, pH 8.0, 400 mM NaCl, and 5 mM imidazole (5 ml buffer/g wet weight). The cell debris was removed by centrifugation (18,000 × g, 30 min, 4°C), and the soluble proteins were loaded on a 2-ml Ni-nitrilotriacetic acid His bind resin (Merck) equilibrated with the above buffer. After being washed with a further 12 ml of buffer, rc-TatF was eluted with 20 mM Tris HCl, pH 8.0, 400 mM NaCl, and 200 mM imidazole, dialyzed against 20 mM Tris HCl, pH 8.8, and 0.5% Tween 20, and loaded on a Superose 6 gel filtration column (flow rate of 0.4 ml/min, equilibrated with 20 mM Tris HCl, pH 8.8, 1 M NaCl, and 0.5% Tween 20, Phenomenex).

The chain formation phenotype was assessed by differential contrast microscopy as described previously (5). Complementation of the sodium dodecyl sulfate (SDS) sensitivity of the Tat mutant strain DADE was quantified as described by Lte et al. (19). Cell fractionation, SDS-polyacrylamide gel electrophoresis, and Western blotting were carried out as described previously (9). TMAO reductase was detected in native gels as described elsewhere (41). HiPip detection was carried out as described previously (9). Formate dehydrogenase (FDH) activity was quantified by 2,6-dichlorophenolindophenol reduction, with phenazine methosulfate as the mediator, as described elsewhere (12).

**Prediction of Tat substrates.** The genome sequence and the amino acid sequences of all predicted proteins from R. capsulatus strain SB1003 was obtained via ERGO Light (Integrated Genomics). The sequences were analyzed by TATFIND 1.2 as described previously (13, 31). As a slight modification, the Tat substrate was not allowed to be translationally initiated at position (Xm) of the RR pattern (Xm−1)(R)m (Xm−1)(X)m (Xm−1)(X)m (Xm−1), nor to have a P at position (Xm−1), or a Q at position (Xm), or an I/L/C at position (Xm). With these additional restrictions, obvious false positives in R. capsulatus were eliminated. Four further likely Tat substrates, such as the well-characterized Tat substrate NosZ (18), could be identified by nonautomated proteome analysis. The reason for the nondetection of these Tat substrates by TATFIND 1.2 was the restriction to only a subset of amino acids at position (Xm−1). TatP (3), a second Tat substrate identification program, recognized all but four of the listed putative Tat substrates, which were different from the four not recognized by TATFIND 1.2. At least one of the four unrecognized substrates, the cytchrome bc1 complex Rieske subunit, has been clearly established as a Tat substrate in other organisms (25).

**RESULTS**

It is proposed that Tat translocons are adapted to their natural set of substrates (7). Only single natural Tat substrates have been tested for their compatibility with Tat translocons from a different organism (1, 7, 9, 27, 42). To test in vivo the adaptation of a Tat translocon to its substrates, we decided to exchange the Tat translocon of Escherichia coli with that of Rhodobacter capsulatus. With this strategy, all E. coli Tat substrates are left in their native environment, where they fulfill their natural role, thus allowing physiological assessments of Tat substrate functionality with certain substrates. E. coli is best suited for such an experiment, as various physiological assays for Tat functionality are established for this organism (5, 19, 43). Moreover, a mutant strain of this γ-proteobacterium is available which is devoid of all known Tat components (49). The Tat system of the α-proteobacterium R. capsulatus was chosen, as it differs significantly from that of E. coli with respect to its predicted set of Tat substrates, whereas it is similar to that of E. coli with respect to translocon composition.

**The set of Tat substrates in R. capsulatus.** The genome of R. capsulatus has been sequenced by Integrated Genomics and made publicly available via the ERGO Light homepage (http://www.ergo-light.com/ERGO/). Using the programs TATFIND and TatP (3, 31), we could identify genes for 29 likely Tat substrates in the genome of R. capsulatus (see Table S1 in the supplemental material). Among these are six predicted to be cofactor-containing redox enzymes, eight periplasmic binding proteins, two hydrolases, five enzymes involved in biogenes pathways (such as N2O reductase maturation, periplasmic disulfide formation, and glucan biosynthesis), and eight hypothetical proteins without functional assignments. One known Tat substrate from R. capsulatus, a DMSO reductase with high similarity to E. coli TMAO reductase (40), was not detected in the available genome data of the sequenced strain. The high number of transporter-associated binding proteins is unexpected and in marked contrast to that in E. coli, which has only one predicted Tat substrate among its binding proteins. Only 6 out of the 29 predicted R. capsulatus Tat substrates have homologs among the Tat substrates of E. coli. Thus, the E. coli system differs considerably in its substrate range from that of R. capsulatus.

**The Tat translocon components in R. capsulatus.** The Tat translocon components from R. capsulatus resemble those of E. coli. We identified by BLAST analyses one operon which encodes orthologs of TatA, TatB, and TatC (in the following termed rc-TatA, rc-TatB, and rc-TatC, respectively). The corresponding genes are organized together with a fourth gene, rc-tatF (Fig. 1). This situation resembles that of E. coli, which has a tatABC operon connected with a fourth gene, tatD, which
is a nuclease with no relevance for translocation (49). E. coli contains two TatA paralogs, TatA and TatE, of which TatE is encoded by a monocistronic gene (38). rc-TatA, rc-TatB, and rc-TatC show significant sequence identity to their orthologs in E. coli. As expected from phylogenetic studies (51), TatA/E and TatB orthologs from both species resemble each other, with 20 to 37% overall identity and certain conserved boxes with over 40% identity. rc-TatA has the highest similarity to TatA (57% overall identity, 50% in residues 6 to 43). rc-TatB has the highest similarity to TatB (27% overall identity, 69% in residues 1 to 26, and 42% in residues 122 to 145). The TatC proteins from both organisms show similar overall sequence identities (27%), and there are three conserved boxes with an identity higher than 40% (61% in residues 8 to 25, 43% in residues 89 to 143, and 45% in residues 186 to 238). The fourth gene product of the R. capsulatus tatABCF operon, rc-TatF, has no detectable sequence homology to any gene product of E. coli. The rc-tatC stop codon overlaps with the start codon of rc-tatF (ATG ACC), strongly suggesting a translational coupling of the two genes. An efficient translational coupling by this four-nucleotide overlap is very likely, as the genes for rc-TatB and rc-TatC show the same overlap (ATG ACC), and it is known that TatB and TatC form stable complexes (26). In the only other sequenced Rhodobacter species, R. sphaeroides, the two orthologs are in the same position and share the same four nucleotides, suggesting that the translational coupling of the tatF and tatC orthologs is conserved within the genus.

Sequence analyses revealed that the rc-tatABCF operon allows the formation of a RNA hairpin structure downstream of rc-tatC. This is similar to the tatABCD operon from E. coli (49), in which a hairpin is found in an untranslated region between tatC and tatD (Fig. 1). In the case of R. capsulatus, there is no untranslated region between rc-tatC and rc-tatF, and the hairpin is predicted to be encoded 54 bases downstream of rc-tatC within rc-tatF (CGG CCC CCG AGTTT CGG GGG CGG). As it is known that such hairpins can block 3’ to 5’ exoribonuclease activities (23), this hairpin is likely to stabilize the rc-tatABC portion of the transcript. Therefore, although rc-tatF is translationally coupled to rc-tatC, it is likely that the gene product TatF is less abundant than TatABC.

In summary, the Tat translocon components from R. capsulatus resemble those of E. coli. However, the fourth component encoded by the rc-tat operon, rc-TatF, is unrelated to components from E. coli.

The R. capsulatus Tat translocase is functional in E. coli. To test the functionality of the Tat translocon from R. capsulatus, the complete rc-tat operon was transferred on a low-copy vector to an E. coli mutant strain (DADE) which is devoid of all known endogenous Tat components (49). In a first approach, Tat system functionality was assessed by the so-called chain formation phenotype which is observed when the two Tat substrates AmiA and AmiC are not translocated. These two proteins are murine amidases which are important for the hydrolysis of the cell division septum and thus for the separation of cells after cell division (17). When AmiA and AmiC are not translocated into the periplasm of Tat-deficient bacteria, cell chains can be readily observed by microscopy (4, 19).

The Tat-deficient strain DADE carrying only the empty vector formed the expected cell chains (Fig. 2A). The R. capsulatus Tat system as well as the E. coli Tat system completely cured this morphological defect, resulting in single cells with wild-type shape (Fig. 2B and C). As the absence of AmiA or AmiC alone already results in short cell chains, the full complementation indicates that both Tat substrates are localized properly (17). Therefore, the heterologous R. capsulatus Tat system translocates AmiA and AmiC in E. coli.

FIG. 2. Complementation of cell chain formation and SDS sensitivity phenotypes of tat-deficient E. coli by recombinant Tat systems from R. capsulatus and E. coli. Phase-contrast micrographs of (A) E. coli DADÉ (MC4100 ΔtatABCDE)/pRK415 (empty vector control) (B) E. coli DADE (MC4100 ΔtatABCDE)/pRK-ec-tatABC (positive control), and (C) E. coli DADE (MC4100 ΔtatABCDE)/pRK-rec-tatABC (complementation with R. capsulatus Tat system). (D) SDS sensitivity as expressed in percent survival after 3 h of growth in LB medium containing the indicated SDS concentrations (19). Diamonds, E. coli DADE/pRK415 (empty vector control); squares, E. coli DADE/pRK-rec-tatABC (complementation with E. coli Tat system, positive control); filled circles, E. coli DADE/pRK-rec-tatABC (complementation with R. capsulatus Tat system). Error bars indicate the standard deviations as derived from triplicate assays.
The transport block of AmiA and AmiC also contributes to a cell envelope defect which renders the cells sensitive towards detergents such as SDS, and an SDS sensitivity assay has been established which quantifies the tolerance of the growing bacteria towards this detergent (19). We thus tested the SDS sensitivity of the heterologous translocon-containing strain (Fig. 2D). In agreement with the morphological observations, the complementation of this phenotype was complete, indicating that the R. capsulatus Tat translocon can translocate AmiA and AmiC.

Another physiological assay for Tat system functionality is growth by TMAO respiration. The TMAO reductase TorA, which is the terminal reductase of this anaerobic respiration, is a bis(molybdopterin guanine dinucleotide) molybdenum cofactor-containing Tat substrate which requires Tat system functionality for its translocation into the periplasm (36). E. coli thus does not grow on TMAO media with glycerol as a non-fermentable carbon source in the absence of a functional Tat system. We therefore tested the functionality of the R. capsulatus Tat system by complementation of the TMAO respiratory growth of the Tat-deficient E. coli strain DADE. TMAO growth of the strain containing the empty vector was tested as a negative control, and the complementation by the E. coli Tat system was tested as a positive control. As shown in Fig. 3A, the R. capsulatus Tat system fully complemented the TMAO growth deficiency of the complete tat knockout strain. As expected, the growth deficiency was also complemented by the tatABC genes from E. coli. The vector-only negative control strain did not grow, indicating that the growth conditions were selective.

TorA export was also monitored by detection of TorA in the periplasm of cells shifted to anaerobic growth in the presence of TMAO. TorA activity can be detected in native gels soaked with dithionite-reduced methyl viologen. When the dark-blue gel is transferred into a TMAO-containing solution, it is locally destained at the TorA bands, due to TMAO-dependent oxidation of reduced methyl viologen. Subcellular fractions from various strains were thus tested for the presence of TorA activity (Fig. 3B). TorA was equally active in the corresponding subcellular fractions from strains containing either the E. coli or the R. capsulatus Tat system but was absent from the periplasm and the membrane fraction in the strain which lacked a Tat system (Fig. 3B). The absence of TorA activity from these fractions indicated that the Tat mutant strain did not translocate TorA. In contrast, strains containing the R. capsulatus or the E. coli Tat systems translocated this enzyme equally well. Significant activity was detected in the cytoplasm and most likely corresponded to a precursor or TorA, as has been reported earlier (36). Interestingly, TorA activity was also detected in the membrane fraction, and here, it depended on the presence of a functional Tat system. This indicates that at least a significant portion of TorA associates with the membrane after translocation, probably by the specific interaction with the membrane-bound pentaheme c-type cytochrome TorC, which has been clearly demonstrated (16). As TorA is the inducible TMAO reductase of E. coli, we also checked for the inducibility of the activity in our assays. Bacteria were grown anaerobically on LB-0.5% glycerol-0.4% nitrate to an optical density at 600 nm of about 0.5, and TorA was induced by the addition of 1.1% TMAO. After 1 h of continued growth, periplasm was prepared and analyzed by the described activity staining (Fig. 3B, right). The data show that the detected activity was indeed inducible by TMAO. Together, these results confirm that periplasmic and membrane-associated TMAO reductase activity by TorA depends on a functional Tat system and show that the R. capsulatus Tat system functions well with TorA.

We also analyzed the Tat-dependent targeting of formate dehydrogenase by means of its activity. E. coli contains three formate dehydrogenases. Two of them, FDH-N and FDH-O, are present under anaerobic conditions during growth with nitrate as the final electron acceptor (39). The periplasmic subunits of these two formate dehydrogenases are Tat substrates which are translocated as heterodimers of nearly 150 kDa and thus are so far the largest Tat substrates known (37). These homologous enzymes are attached to a membrane anchor subunit after translocation. R. capsulatus does not encode a formate dehydrogenase Tat substrate in its sequenced genome. We thus measured formate dehydrogenase activity in membrane and soluble fractions after anaerobic growth on nitrate. The specific FDH activity was 173 ± 18 U/mg (mean ± standard deviation) in the wild-type strain MC4100 carrying
the empty vector, 11 ± 4 U/mg in the Tat-deficient strain DADE carrying the empty vector, 172 ± 4 U/mg in the strain DADE with a complementing E. coli Tat system, and 62 ± 10 U/mg in the strain DADE with a complementing R. capsulatus Tat system. Targeting of formate dehydrogenase to the membrane fraction thus clearly depended on a functional Tat system. While the targeting deficiency of a Tat-deficient strain was fully compensated by expression of the tatABC genes in trans from pRK-tatABC, the targeting efficiency was near 35\% of the wild-type level when the E. coli Tat system was exchanged with that of R. capsulatus. Therefore, the R. capsulatus Tat system can translocate formate dehydrogenases.

To test whether the observed function of the heterologous R. capsulatus Tat translocon was independent of rc-TatF, we tested TMAO respiration with a shortened operon lacking rc-tatF. The absence of rc-TatF had no influence on the functional complementation of TMAO respiration (data not shown). Similarly, TatF was not relevant for AmiA and AmiC transport, as monitored by SDS sensitivity and complementation of chain formation (data not shown). TatF is therefore not essential for the translocation activity of the Tat translocon of R. capsulatus in E. coli.

The location of the rc-tatF gene behind rc-tatC in R. capsulatus is analogous to the position of tatD behind the tatC gene in E. coli. Although the two gene products, TatD and rc-TatF, do not exhibit any sequence similarity, it was possible that they could resemble each other functionally. As TatD is a DNase, one thus could imagine that rc-TatF exhibits DNase activity, too. To test this, rc-TatF was tagged with a C-terminal His\_tag and purified from E. coli BL21 DE3 by affinity chromatography, followed by a gel filtration step. The thus highly enriched His\_tagged rc-TatF migrated in SDS-polyacrylamide gel electrophoresis as a single band at 30 kDa, which is in agreement with the calculated molecular mass of 32 kDa (data not shown). rc-TatF was stable at pH 8.5 in buffers containing 0.5% Tween 20. Purified rc-TatF had no nuclease activity (data not shown). DNase activity, which was present after His tag affinity chromatography, was not due to rc-TatF, as the DNase eluted prior to rc-TatF in the gel filtration chromatography purification step. Unless the tag affects activity, this observation indicates that rc-TatF is not only structurally but also functionally unrelated to TatD of E. coli.

The R. capsulatus Tat translocon does not target E. coli DmsA to the membranes. Like TMAO respiration, respiratory growth on DMSO is also dependent on the functionality of the Tat translocon. DmsA, the catalytic subunit of the membrane-anchored DMSO reductase, is Tat dependently targeted as a Tat translocon. DmsA, the catalytic subunit of the membrane-anchored DMSO reductase, is Tat dependently targeted as a Tat translocon. DmsA to the membranes was completely abolished. Therefore, DmsA targeting to the membranes was completely abolished. Therefore, DmsA and DmsB are not functionally targeted to the membrane by the R. capsulatus Tat system, which causes a proteolytic degradation of DMSO reductase and consequently leads to a DMSO respiration deficiency. These results indicate that the tested heterologous Tat system of R. capsulatus, although func-
from pBW-hip with TorA, FDH, AmiA, or AmiC, is not functional with HiPIP from or the recombinant in the E. coli rc-tatABC indicated. Positions of precursor (pre) and mature (mat) forms of HiPIP are specific antibodies. P, periplasm; M, membrane; C, cytoplasm. The rhamnose for 2 hours. HiPIP was detected by Western blotting using specific antibodies to study the recognition of Tat determinants or the ab-

Translocation of HiPIP from Allochromatium vinosum by the Tat translocase of E. coli demonstrates low species specificity of the Tat systems. The functionality of our heterologous Tat system was also assessed with the heterolo-

gous Tat system. The substrates are translocated and functional, thus allowing physiological processes which can be monitored by various assay systems. Therefore, this study underlines that Tat systems, such as immunogold-labeling techniques and proteolytic accessibility assays, which suggested that DmsAB is only membrane targeted by the Tat system but not translocated (32). Despite this controversy, it is interesting that DMSO reductase was not targeted by the Tat system of R. capsulatus (Fig. 3). The exact topology of DmsABC has been extensively studied. Two opinions exist, one placing DmsA on the periplasmic face of the cytoplasmic membrane and the other on the cytoplasmic face. It has been shown that DmsA and DmsB are translocated together across the membrane in a Tat-dependent manner when the membrane anchor subunit DmsC is absent and that the membrane-impermeable substrate TMAO can be specifically reduced by the DmsABC system (44). This contrasts a variety of experiments, such as immunogold-labeling techniques and proteolytic accessibility assays, which suggested that DmsAB is only membrane targeted by the Tat system but not translocated (32). Despite this controversy, it is interesting that DMSO reductase was not targeted by the Tat system of R. capsulatus. The data could be explained by the requirement of a specific interaction of a DmsAB translocon targeting factor with the corresponding Tat system, such as has been suggested for this enzyme (29). Generally speaking, the biogenesis of DmsABC is likely to require additional features of the Tat translocon which are absent in the Tat system of R. capsulatus.

This study shows that Tat signal sequences from very different E. coli Tat substrates are accepted by the R. capsulatus Tat system. The substrates are translocated and functional, thus allowing physiological processes which can be monitored by various assay systems. Therefore, this study underlines that Tat translocons from α- and γ-proteobacteria share basic signal sequence recognition characteristics. Some variations may occur with respect to preferred sequence patterns, and some Tat substrates, such as DmsA in this study, may not be targeted correctly. A complete abolishment of the targeting process may which varies between species. Only little has been done to understand the proposed species specificity of Tat substrate signal sequences. We used a “whole-translocon exchange” experiment to study the acceptance of various Tat substrates by a heterologous translocon. This approach preserves the natural expression and folding conditions of Tat substrates. If some Tat substrates are selectively not translocated by a heterolo-
gous translocon, this is evidence of some selectivity of the translocation machinery.

The results of this study demonstrate that the Tat translocon of R. capsulatus does not require species-specific auxiliary factors. The heterologous system is compatible with the majority of Tat substrates, demonstrating a high degree of flexibility with regard to accepted Tat substrates. Only one Tat substrate, DmsA, was neither targeted to the membrane fraction nor supportive of DMSO growth. This contrasts the translocation of TorA, AmiA, AmiC, and formate dehydrogenases. Moreover, the efficient translocation of the heterologous Tat sub-

Substrate selectivity may be caused by (i) species-specific vari-

tions in the recognition of Tat determinants or (ii) the ab-

Discussion

In this work, the exchange of a complete Tat translocon was used to study the possibility of translocon substrate selectivity. Substrate selectivity may be caused by (i) species-specific variations in the recognition of Tat determinants or (ii) the absence of translocon-specific targeting factors in the hetero-

FIG. 5. Analysis of translocation of the heterologous Tat substrate HiPIP from A. vinosum by the heterologous Tat system from R. capsulatus in the E. coli host. The detection of HiPIP in subcellular fractions of E. coli containing either the recombinant E. coli Tat system or the recombinant R. capsulatus Tat system is shown. Expression was from pBW-hip in either DADE/prK-rec-tatABC (E. coli Tat system) or DADE/prK-rec-tatABC (R. capsulatus Tat system), induced with 0.4% rhamnose for 2 hours. HiPIP was detected by Western blotting using specific antibodies. P, periplasm; M, membrane; C, cytoplasm. The positions of precursor (pre) and mature (mat) forms of HiPIP are indicated.

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reflect exceptional targeting requirements of rare substrates, such as the membrane targeting of DmsA. Another example from the literature is GFOR from *Zymomonas mobilis* (7).

**Prospects of whole-translocon exchange approaches.** Heterologous systems for physiological Tat system functionality assays are powerful tools, as they can readily provide strong positive evidence. However, our results indicate that if a negative outcome of one physiological assay is observed, it does not necessarily mean that the Tat system is not working. Other physiological assays may turn out to be positive, which proves functionality. In fact, these negative exceptions are the interesting results, as they provoke explanatory studies which lead to new insights. Thereby, one may find additional translocon-specific factors or characteristics which are required for the targeting of a certain substrate, thus abolishing translocation by a heterologous system. One may also consider that different routes could exist for the targeting to the translocon and that heterologous systems may differ in that respect. Certainly, the translocon exchange approach is rapid for the evaluation of the functionality of a proposed Tat translocon of some organism.

**TatF: a novel component associated with Tat and Sec systems?** An interesting aspect of this study is rc-TatF, the fourth gene product of the rc-tatABC operon. rc-TatF is not required for translocation, but there is some evidence which argues for a functionality in association with protein translocation machineries. rc-TatF is the first analyzed member of a new family of proteins found in α-, β-, and δ-proteobacteria. Within the α-proteobacteria, the closest homolog is that of *R. sphaeroides*. It is intriguing that the start codons of the tatF genes from *R. capsulatus* as well as those from *R. sphaeroides* overlap with the stop codon of the corresponding tatC genes, indicating a strict coupling of translation. In other TatF-containing α-proteobacteria (e.g., Agrobacterium, Bradyrhizobium, Brucella, Mesorhizobium, Rhodopseudomonas, and Sinorhizobium species), the Rhizobiales, tatF is localized directly adjacent to the genes encoding YajC and SecDF. The TatF homologs of β- and δ-proteobacteria do not show this proximity to protein translocation-related genes. The strong conservation of yajC and secDF proximity to tatF in Rhizobiales suggests that the TatF homologs may also function somehow in protein translocation in these organisms, albeit in the Sec system and not in the Tat system. TatF also neighbors the YajC homolog in Caulobacter crescentus, a member of the Caulobacterales and another α-proteobacterium. The YajC and SecDF components form a complex which improves the SecA-dependent translocation at the SecYEG translocon (14). One might speculate that the association of TatF orthologs with Sec or Tat protein translocation systems in many organisms may not be coincidental. TatF homologs generally show motifs of ATPases associated with various cellular functions (AAA family). They all belong to an uncharacterized protein family (Pfam entry DUF815 at Sanger). As many AAA family proteins show ATP-dependent chaperone functions, TatF could play a role in the translocation of certain Sec or Tat substrates, depending on the organism, which could explain the differential association of the corresponding genes with the two systems.

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