The twin-arginine translocation (Tat) pathway is essential for viability in *Caulobacter crescentus*

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Abstract

Unlike the general secretory (Sec) pathway, which exports unfolded proteins out of the cytoplasm, the twin-arginine transport (Tat) pathway translocates folded proteins. Substrates of the Tat pathway contain cleavable N-terminal signal peptides with a highly conserved double arginine motif, hence the name of the pathway. In bacteria, the Tat system often exports proteins that bind cofactors and serve critical functions in metabolism, cell envelope biogenesis, and virulence. Analysis of sequenced bacterial genomes indicated that a relatively large fraction of exported proteins in *Caulobacter crescentus* are secreted via the Tat pathway, compared to other species. Therefore, we investigated the role of the Tat system in *Caulobacter* physiology. We found that all three components of the Tat system--TatA, TatB, and TatC--are essential for viability: depletion of any of the three components causes *Caulobacter* cells to bloat and stop dividing. In addition, we used a plasmid loss assay to demonstrate that colony formation does not occur without any of the *tat* genes. For example, a strain was generated in which the only copy of *tatA* was carried on a plasmid along with the *Escherichia coli lacZ* gene. If the plasmid could be lost, then colonies would appear white on plates containing 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal). Instead, we found that all colonies turned distinctively blue, as the *lacZ* gene product converted X-gal into a blue precipitate, indicating that the plasmid containing the sole copy of the *tatA* must be maintained. To our knowledge, this is the first demonstration that the Tat pathway is essential for viability. Because the Tat pathway is well-conserved, we are currently determining if components of the *E. coli* Tat system can replace their respective homologs in *Caulobacter*. Such analysis will provide insight into how components of the Tat system function as a unit. A better understanding of the mechanisms of the Tat pathway may lead to advances in antimicrobial research and in the production of desired protein products for biotechnology.

Background

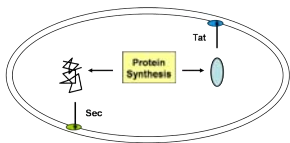

Figure 1. Sec vs. Tat pathway

General secretory (Sec) pathway exports unfolded proteins, while the twin- arginine translocation (Tat) pathway exports folded proteins.

Export of mRFP1 and GFP/YFP

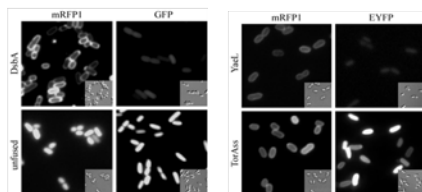

Figure 2. Export of mRFP1 and GFP/YFP via Tat and Sec pathways

- GFP/YFP does not fold properly or fluoresce when exported via the Sec pathway in *E. coli*
- Tat system allows GFP to fold in the cytoplasm prior to export
- mRFP1 folds properly regardless of export pathway

Model of Tat-dependent transport

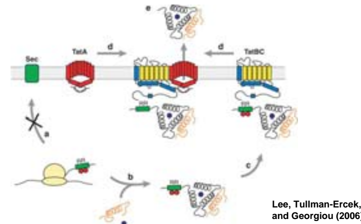

Figure 3. Model of Tat transport

(a) Signal peptide and/or binding of chaperone help avoid other export pathways
(b) Cofactor and/or other subunits added for proper folding
(c) Binding to TatB and TatC
(d) Transfer to TatA pore complex for translocation using proton motive force
(e) Cleavage of signal peptide and release of substrate

Role of Tat pathway in virulence

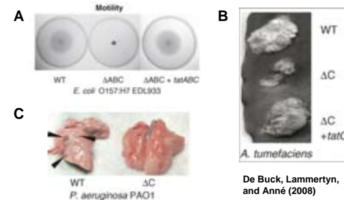

Figure 4. Multiple phenotypes of *tat* mutant pathogens

(**A**) Motility assay plates of *E. coli* demonstrate that a *tatABC* mutant (ΔABC) lacks motility, while a complemented mutant (ΔABC + *tatABC*) is motile.
(**B**) Production of tumors by *A. tumefaciens* (WT), *tatC* mutant (ΔC), and complemented *tatC* mutant vary on susceptible plant tissue.
(**C**) Pulmonary abscesses were detected on rat lungs that were infected with *P. aeruginosa* (WT) but were not observed on lungs infected with *tatC* mutant bacteria (ΔC).

Tat pathway is vital in *C. crescentus*

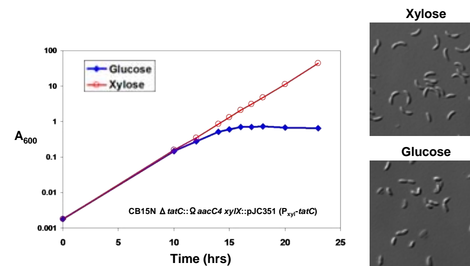

Figure 5. Depletion of TatC using xylose-dependent promoter

A CB15N Δ*tatC* mutant containing a plasmid with P_{xyl}-*tatC* is able to grow normally in the presence of xylose. When the mutant is grown in media with glucose but no xylose, cells stop growing and often bloat and lyse. Similar results were obtained when TatA or TatB were depleted in *C. crescentus.*

Visual assay to determine essentiality

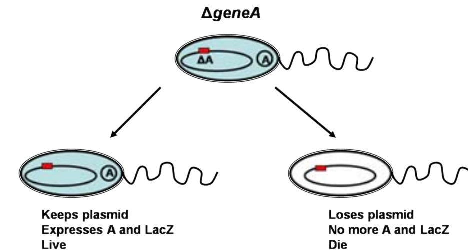

Figure 6. Schematic of visual assay to determine essentiality of *tat* genes

- Plasmid carries only copy of gene (such as *tatA*) in the cell and *E. coli lacZ.*
- Inability to lose plasmid means gene is essential.
- Presence of plasmid detected with X-gal, which turns blue in the presence of beta-galactoside (product of *lacZ* gene).

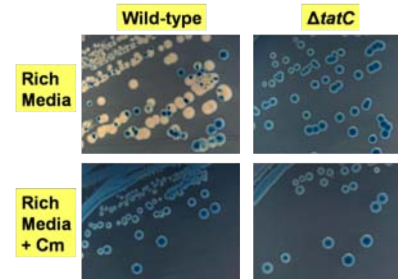

Figure 7. Plasmid loss assay using beta-galactosidase and X-gal to determine if *tatC* is essential in *Caulobacter*

Sectoring assay reveals that a plasmid that carries both *C. crescentus tatC* and *E. coli lacZ* and confers chloramphenicol resistance persists in a Δ*tatC* mutant but is quickly lost by a wild-type strain. In the presence of chloramphenicol, both strains maintain the plasmid. Therefore, TatC is required for *Caulobacter* viability. Similar results were obtained for *tatA* and *tatB.*

Complementation by *E. coli tatABC*

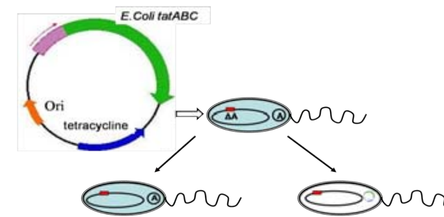

Figure 8. Plasmid loss assay used to determine if *E. coli tatABC* can complement *tat* deletions in *C. crescentus*

Plasmid carrying *E. coli tatABC* was mated into *C. crescentus* Δ*tatA* strain to determine if the *E. coli* genes can substitute for their homologs. If so, then we should be able to observe white colonies due to loss of the plasmid carrying *C. crescentus tatA.* Similar experiments were also performed using Δ*tatB* and Δ*tatC* strains. The results were inconclusive, due to poor growth of strains.

Complementation by individual *E. coli tat* genes

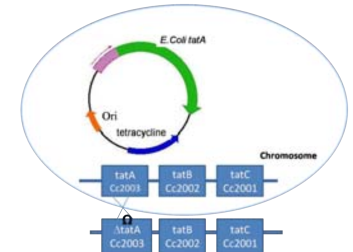

Figure 9. Transduction to determine if the *tat* genes of *E.coli* can complement their respective homologs in *C.crescentus*

- The *E. coli tatA*, *B*, and *C* genes were individually cloned into plasmids under a vanillate-dependent promoter.
- These alleles were mobilized into *C. crescentus*, and an effective level of expression was determined.
- Individual marked deletions were transduced into *C. crescentus* strains carrying the *E. coli* alleles.

C. crescentus deletion mutants	Complementation with *E. coli tatA*	Complementation with *E. coli tatB*	Complementation with *E. coli tatC*
Δ*tatA*	+		
Δ*tatB*		+	
Δ*tatC*			−

Table 1. Results of complementation analysis

Transduction of deletion alleles yielded colonies for strains with *E. coli tatA* or *tatB*; therefore, *tatA* and *tatB* genes are compatible between *E. coli* and *C. crescentus.* However, the strains grow slowly, and cells exhibit aberrant morphology. *E. coli*'s *tatC* appears unable to complement its *C. crescentus* homolog. Further work (not shown) suggests that the entire *E. coli tatABC* complex can complement the *C. crescentus tatC* deletion. Transduction of the Δ*tatC* allele into stains carrying *E. coli tatABC* yielded colonies.

Conclusion

- ***tatABC* is critical to *C. crescentus* viability.**
- **Complementation analysis with individual genes revealed that *E. coli*'s *tatA* and *tatB* can substitute for their respective homologs in *C. crescentus.***
- ***E. coli*'s *tatABC* is capable of restoring the viability of *C. crescentus* Δ*tatC* mutants.**
- **Components of the Tat system may be interchangeable between *E. coli* and *C. crescentus*, indicating functional conservation.**

Future directions

Complementation of *Caulobacter tat* deletions by *E. coli* homologs produced strains with varying growth rates. Strains with more robust growth will be selected and analyzed to elucidate the genetic changes that improve compatibility. Such analysis will lead to better understanding of the relationship between structure and function for components of the Tat pathway.

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