

## **The Twin-Arginine Translocation (Tat) Pathway is Essential for Viability in *Caulobacter crescentus***

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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.

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Revealed by Loss of Ectoderm Membrane Rafts in Chicken Embryos**

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