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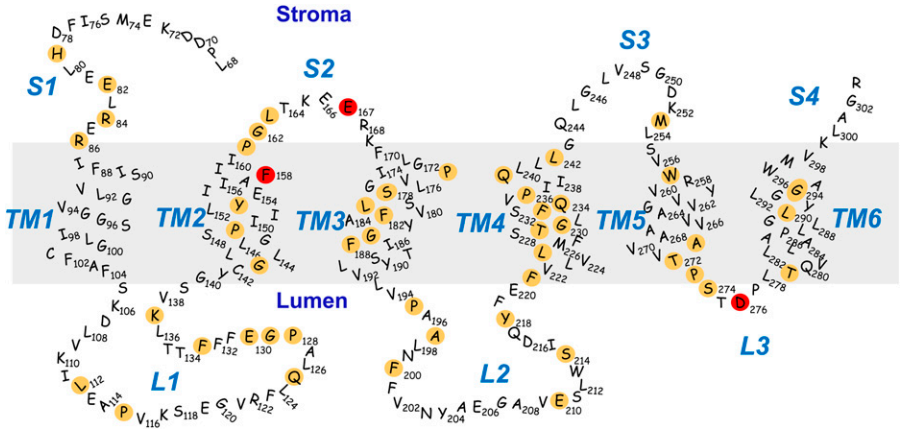
IN BRIEF

Special Delivery: In Vitro Functional Examination of the Twin-Arginine Transport Complex Core Component cpTatC

Moving proteins across a membrane can be tricky. Much as the postman has to deliver the mail without letting the dog out, proteins must be translocated while preventing movement of ions across the membrane. Some proteins will fit through the "mail slot" of Sec-mediated protein transport, where proteins are unfolded to pass through a membrane channel. However, other proteins are transported fully folded; for this, prokaryotes and chloroplasts use the twin-arginine transport (Tat) system (reviewed in Palmer and Berks, 2012). The Sec pathway requires an opening that can accommodate the width of the polypeptide chain, roughly 12 Å; by contrast, the *Escherichia coli* Tat pathway can accommodate up to 70 Å. Why do some proteins use (and perhaps require) Tat-mediated special delivery? This may depend on the nature of the package; for example, proteins that are very tightly folded, have cofactors or metal ions, or are preformed as heterodimers would not tolerate unfolding for transport.

Although much research has examined the "why" and "how" of Tat transport, many questions remain, particularly about the interaction with the substrate protein and formation of the translocase complex. In plants, the chloroplast (cp) cpTat pathway transports folded proteins, including essential photosystem I and II components, into the chloroplast thylakoid (reviewed in Celedon and Cline, 2013). **Ma and Cline (pages 999–1015)** examine the function of cpTatC, the cpTat subunit that binds the twin-Arg-containing signal peptide of the substrate protein and triggers assembly of the translocase. As a first step, the authors establish an in vitro model system to examine cpTatC function in isolated pea (*Pisum sativum*) chloroplasts. To do this, they show that imported cpTatC protein incorporates in the thylakoid membrane, functions in transport, and is affected by known mutations. Using this system to examine the function of mutant forms of cpTatC, they find that Ala-scanning mutations in the S1 and S2 segments (see figure) affect signal peptide recognition, but mutations in L1 and L2 affect Tat complex assembly. Binding assays and Cys-Cys cross-linking confirmed that S1 and S2 interact with the twin-Arg domain of the substrate signal peptide. The authors also show that L1 and L2 regions are important for the cpTatC–cpTatC interaction and for the interaction of cpTatC with other cpTat components.



Mutagenesis of cpTatC. The cpTatC protein spans the thylakoid membrane (grey). Amino acids 1 to 67 are not shown. Conserved residues are indicated in tan, and residues corresponding to *E. coli* TatC essential residues are indicated in red. (*Reprinted from Ma and Cline [2013], Figure 4A.*)

In addition to examining individual components, this in vitro system can also be used to study the mechanism of the translocation step. The authors showed that substrate is efficiently transported while still cross-linked to the S1 or S2 domains of imported cpTatC. Thus, this system can be used to test the effects of mutant cpTatC proteins on translocation. This will enable examination of the mechanism of protein transport by the cpTat system to help solve outstanding questions, such as the means by which Tat transport harnesses the energy of the proton gradient.

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