Structural analysis of chloroplast tail-anchored membrane protein recognition by ArsA1

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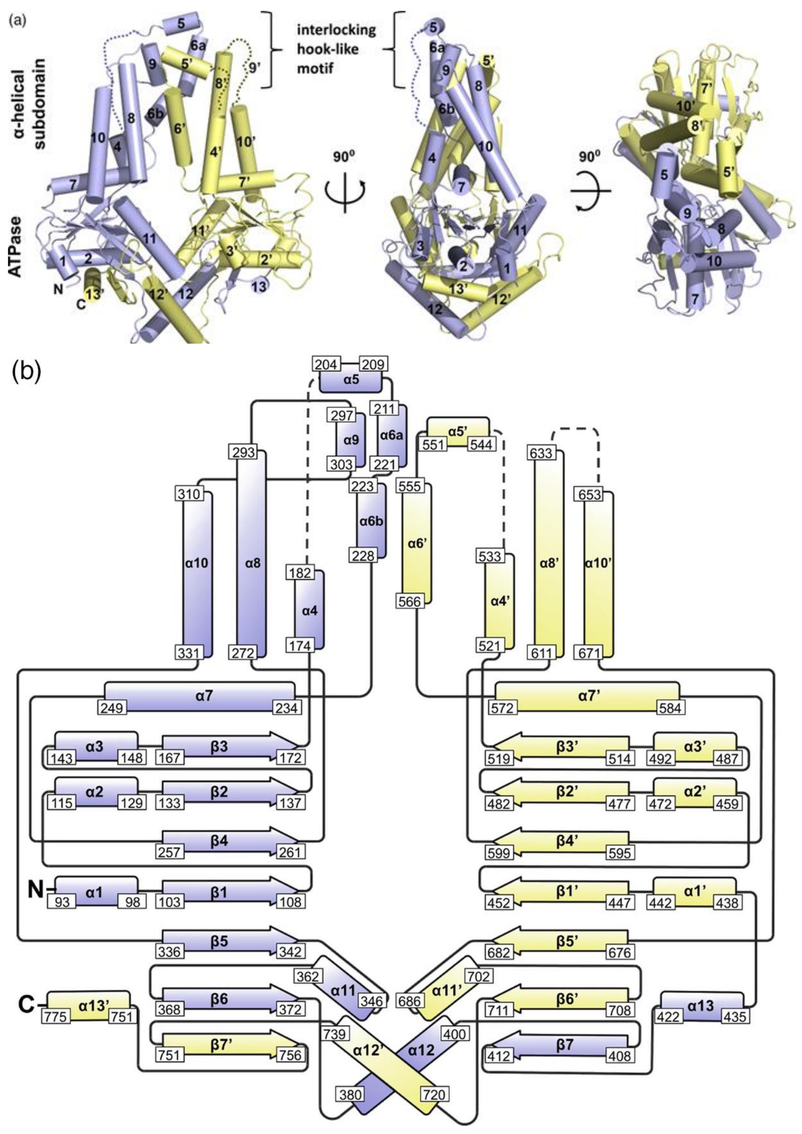
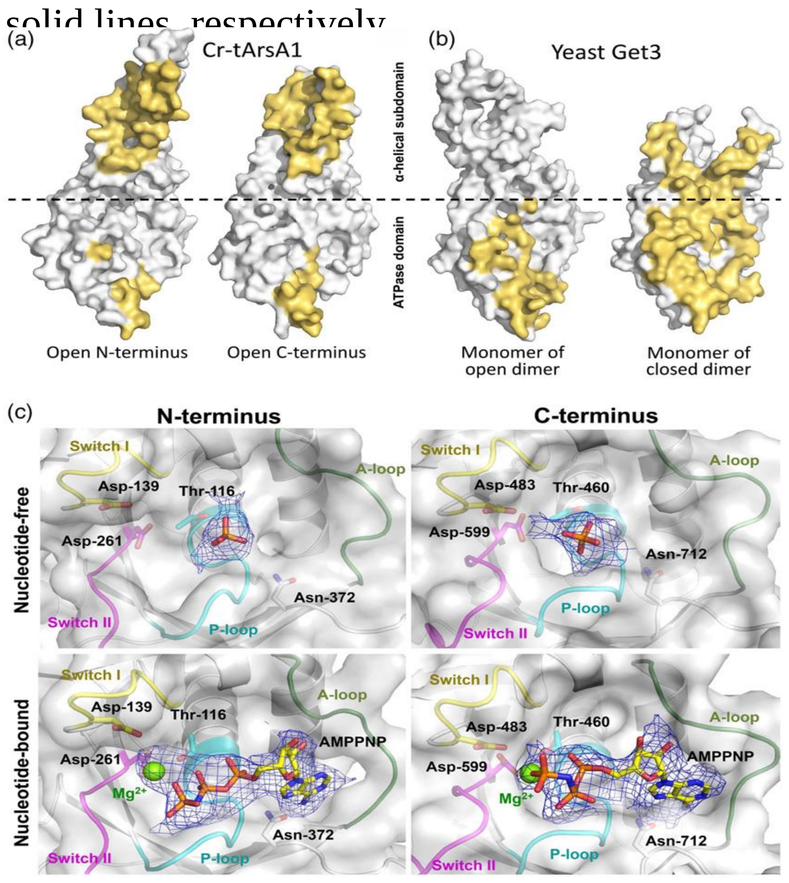
Abstract

In mammals and yeast, tail-anchored (TA) membrane proteins destined for the post-translational pathway are safely delivered to the endoplasmic reticulum (ER) membrane by a well-known targeting factor, TRC40/Get3. In contrast, the underlying mechanism for translocation of TA proteins in plants remains obscure. we present crystal structures of algal ArsA1 (the Get3 homolog) in a distinct nucleotide-free open state and bound to adenylyl-imidodiphosphate. This approximately 80-kDa protein possesses a monomeric architecture, with two ATPase domains in a single polypeptide chain. It is capable of binding chloroplast (TOC34 and TOC159) and mitochondrial (TOM7) TA proteins based on features of its transmembrane domain as well as the regions immediately before and after the transmembrane domain. Our data provide insights into the molecular basis of the highly specific selectivity of interactions of algal ArsA1 with the correct sets of TA substrates before membrane targeting in plant cells.

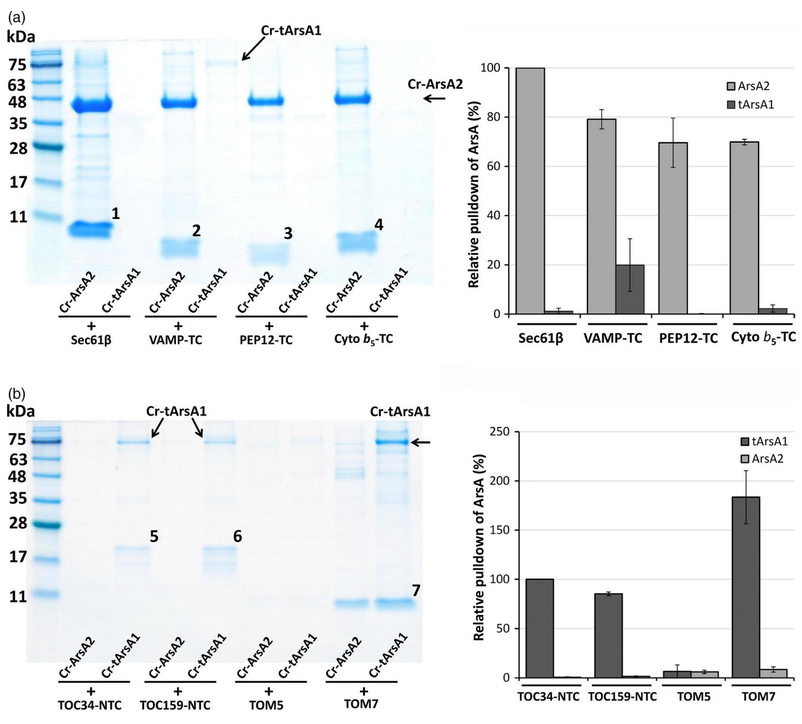
The structure of Cr-ArsA1



Overall structure of Cr-tArsA1.(a) Three views of the crystal structure of nucleotide-free Cr-tArsA1 in the 'open' state. The N- and C-terminal domains are colored in light blue and yellow, respectively. Three disordered regions in the α-helical subdomain, α4–α5, α4′–α5′ and α8′–α10′, are depicted by dotted lines. The flexible structure between α8′ and α10′ is represented as a disordered α-helix (α9′) based on alignments of primary and tertiary structures.
(b) Topology of the secondary structure of Cr-tArsA1, colored as in (a). α-helices, β-strands, disordered regions and loops are shown as cylinders, arrows, dotted lines and solid lines, respectively.

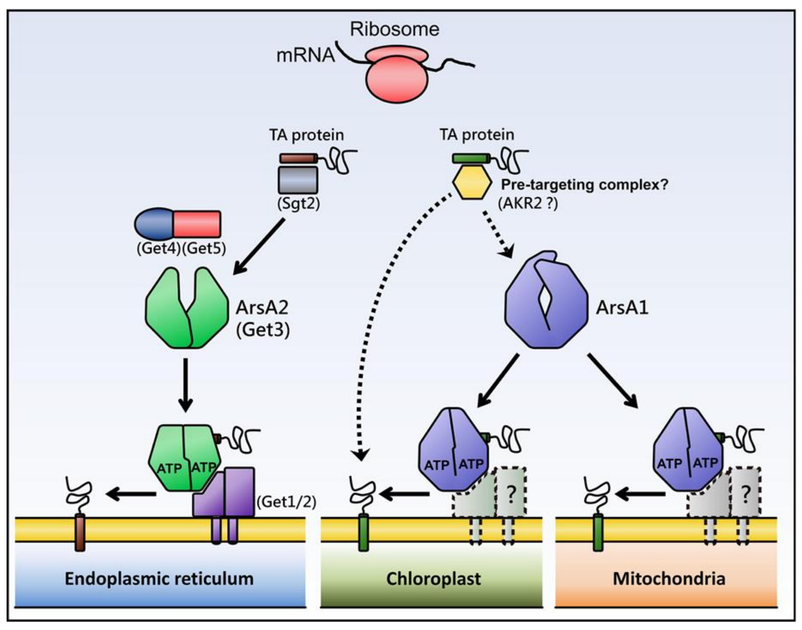


Results

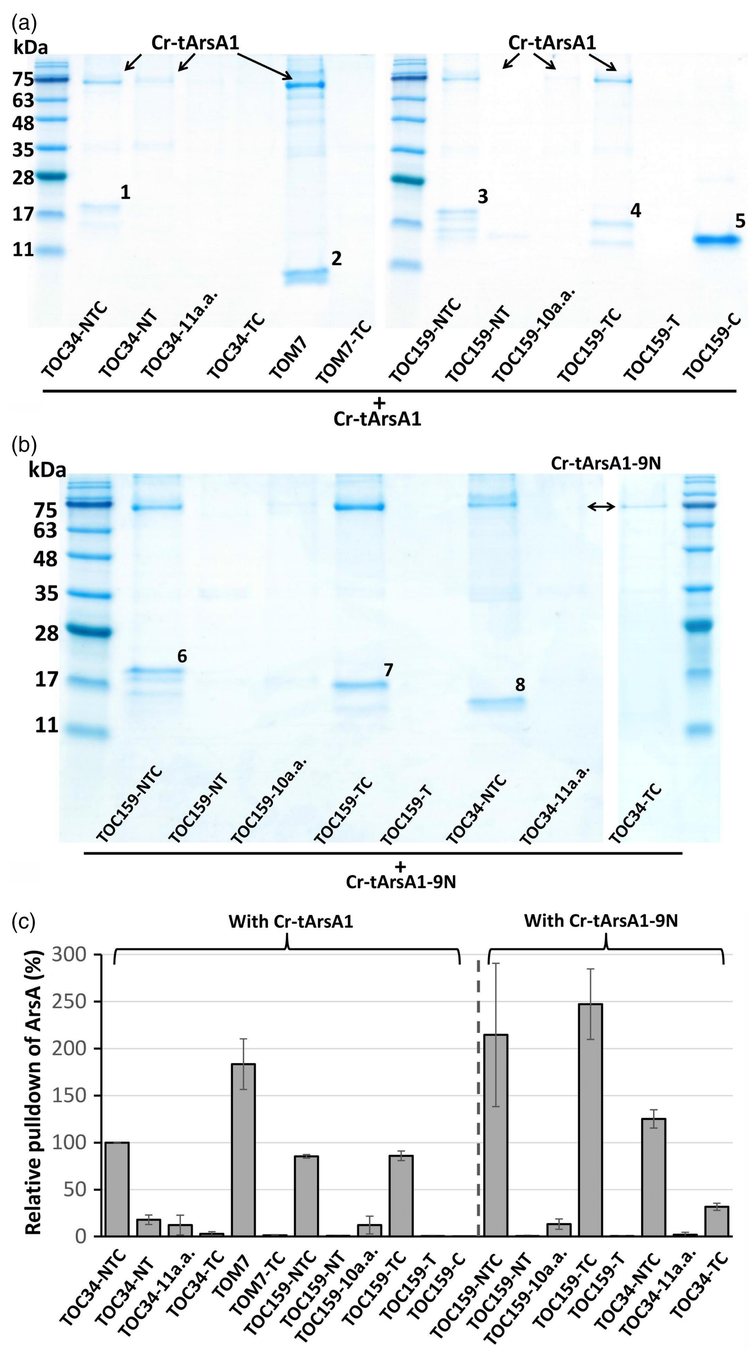


Characterization of Cr-ArsA protein pull-down assays for Cr-tail-anchored TA protein substrate interactions. (a) Cr-ArsA proteins were each purified with C-terminal 6× His-tag fused TA substrates by recombinant co-expression. Arrows and numbers indicate Cr-tArsA1/ VAMP-TC and Cr-ArsA2/Sec61β (1), Cr-ArsA2/ VAMP-TC (2), Cr-ArsA2/PEP12 -TC (3), and Cr-ArsA2/Cyto b_5-TC (4). (b) Cr-tArsA1/TOC34-NTC (5), Cr-tArsA1/ TOC159-NTC (6) and Cr-tArsA1 /TOM7 (7), respectively.

Conclusions



Model for the role of ArsA orthologs in post-translational membrane protein targeting in the alga *Chlamydomonas reinhardtii*.



Tail-anchored (TA) protein substrates are recognized by Cr-tArsA1 based on features of the transmembrane domain (TMD) and its upstream N-terminal sequence (NTS) and downstream C-terminal sequence (CTS) regions. The NTS plus TMD plus CTS (NTC) region of these chloroplast/mitochondria -localized TA proteins constitutes the minimal recognition motif for Cr-tArsA1 binding. (a) Cr-tArsA1 was purified with C-terminal 6× His-tag-fused TA substrates by recombinant co-expression. Arrows and numbers indicate Cr-tArsA1/TOC34-NTC (1), Cr-tArsA1/TOM7 (2), Cr-tArsA1/ TOC159-NTC (3), Cr-tArsA1/TOC159-TC (4) and TOC159-C (5).(b) Arrows and numbers indicate Cr-tArsA1-9N/ TOC159-NTC (6), Cr-tArsA1-9N/ TOC159-TC (7) and Cr-tArsA1-9N/ TOC34-NTC (8).(c) Bar graphs represent quantitative analysis of the pull-down assays and each value is relative to Cr-tArsA1/ TOC34-NTC. Cr-tArsA1 binding to TA substrates (TOC34-NT, TOM7, TOC159 -10a.a. and TOC159-TC) were also confirmed via mass spectrometry

References

Tai-Wen Lin , Chi-Chih Chen , Shu-Mei Wu , Yu-Ching Chang , Yi-Chuan Li, Yu-Wang Su , Chwan-Deng Hsiao , Hsin-Yang Chang (2019) Structural analysis of chloroplast tail-anchored membrane protein recognition by ArsA1 . *The Plant Journal*. https://doi.org/10.1111/tpj.14316