Cell PRESS

CURT1,CAAD-containing aaRSs, thylakoid curvature and gene translation

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CURT1 proteins induce membrane curvature to grana margins in *Arabidopsis* (*Arabidopsis thaliana*) thylakoids. A domain sharing sequence and structural features with CURT1 is found in some cyanobacterial aminoacyl-tRNA synthetases (aaRSs) that show an unusual localization to the thylakoid membranes. Evolutionary scenarios and functional implications are discussed in this article.

In cyanobacteria and chloroplasts, thylakoid membranes act as a structural support for protein supercomplexes involved in light harvesting, photosynthetic electron transport, and production of ATP by photophosphorylation. Functional compartmentalization by asymmetric distribution of protein complexes in membrane subdomains has been proposed to be advantageous for the efficiency of photosynthetic processes (see [1] and references therein). Chloroplast thylakoids show a complex and intricate architecture. They are composed of stacks made of flattened discs, called grana, and non-stacked membrane domains that protrude into the stroma and connect the grana, named stroma lamellae. A recent report by Armbruster *et al.* presents a thorough characterization of a family of proteins that have a role at inducing curvature to the edges of grana sacs. The authors named these proteins as CURT1, followed by letters A, B, C or D for individual members of the *Arabidopsis* family [2]. CURT1B has first been described a decade ago as a phosphoprotein of the thylakoid membranes of *Arabidopsis* and was named TMP14 (thylakoid membrane phosphoprotein of 14 kD) [3]. Homologs of TMP14 were found in cyanobacteria and plants (Figure 1A) with evidence for their association with photosystem I [4,5]. Thus, a new name, PSI-P, was proposed. Although the association with PSI is in conflict with the results presented by Armbuster *et al.* and merits further investigation, a shared feature to all the cited studies is the thylakoidal localization of CURT1/TMP14/PSI-P proteins, pointing to a function related to these membranes.

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Keywords: membrane curvature; thylakoids; aminoacyl-tRNA synthetases; protein sorting.

1360-1385/$ – see front matter
 http://dx.doi.org/10.1016/j.tplants.2013.12.004

Secondary structure of CURT1 is predicted to contain two central transmembrane helices flanked by one amphipathic helix at the N terminus and a fourth helix at the C terminus (Figure 1A, B). CURT1 proteins insert into the thylakoid membrane with their N and C termini facing the stroma, and they localize predominantly at the edges of grana discs, where the membrane shows the most pronounced curvature. The absence of individual CURT1 proteins induces a wider shape of the thylakoid grana, with few stacked discs, whereas double, triple and quadruple *curt1* mutants are completely devoid of grana. Conversely, in plants overexpressing CURT1A grana are more abundant and exhibit a slimmer shape of a reduced diameter. *In vitro*, CURT1A oligomerizes and provokes membrane curvature, inducing liposomes to adopt a tubular shape [2]. All these pieces of evidence indicate that CURT1 proteins function at inducing membrane curvature to the edges of grana discs, which is consistent with the presence of a predicted amphipathic helix, a structural feature that in other proteins induces membranes to bend [6].

CURT1 proteins are nuclear-encoded although their genes are of cyanobacterial origin, with homologs only found in cyanobacteria, algae, and plants. The absence of homologs among early diverging cyanobacterial lineages such as *Gloeobacter violaceus* and *Synechococcus* PCC 7336, suggests that the CURT1 ancestor originated during cyanobacteria diversification.

Notwithstanding extensive similarities and analogies in function and protein composition between thylakoids from chloroplasts and cyanobacteria, the architecture of these two types of thylakoids differs widely. In cyanobacteria, thylakoid membranes do not form grana, and despite their morphology being diverse in different species, in general they have the appearance of a folded sac. These sacs form concentric shells at the periphery of the cytoplasm (which would be topologically equivalent to the chloroplast stroma). Most cyanobacteria possess phycobilisomes, a thylakoid membrane-associated extrinsic protein complex that imposes a physical restriction against membrane stacking [7]. Partial complementation by *Arabidopsis* CURT1A of a *Synechocystis* mutant, defective in the homologous *synCurt1* gene, points to an evolutionary conservation of function [2]. However, the failure of CURT1A to fully complement the *Synechocystis* mutant and the different architecture of plant and cyanobacterial thylakoids suggest some degree of functional divergence, which may result from sequence and/or structural features acquired or lost during evolution. For instance, not all cyanobacterial CURT1-like proteins are predicted to

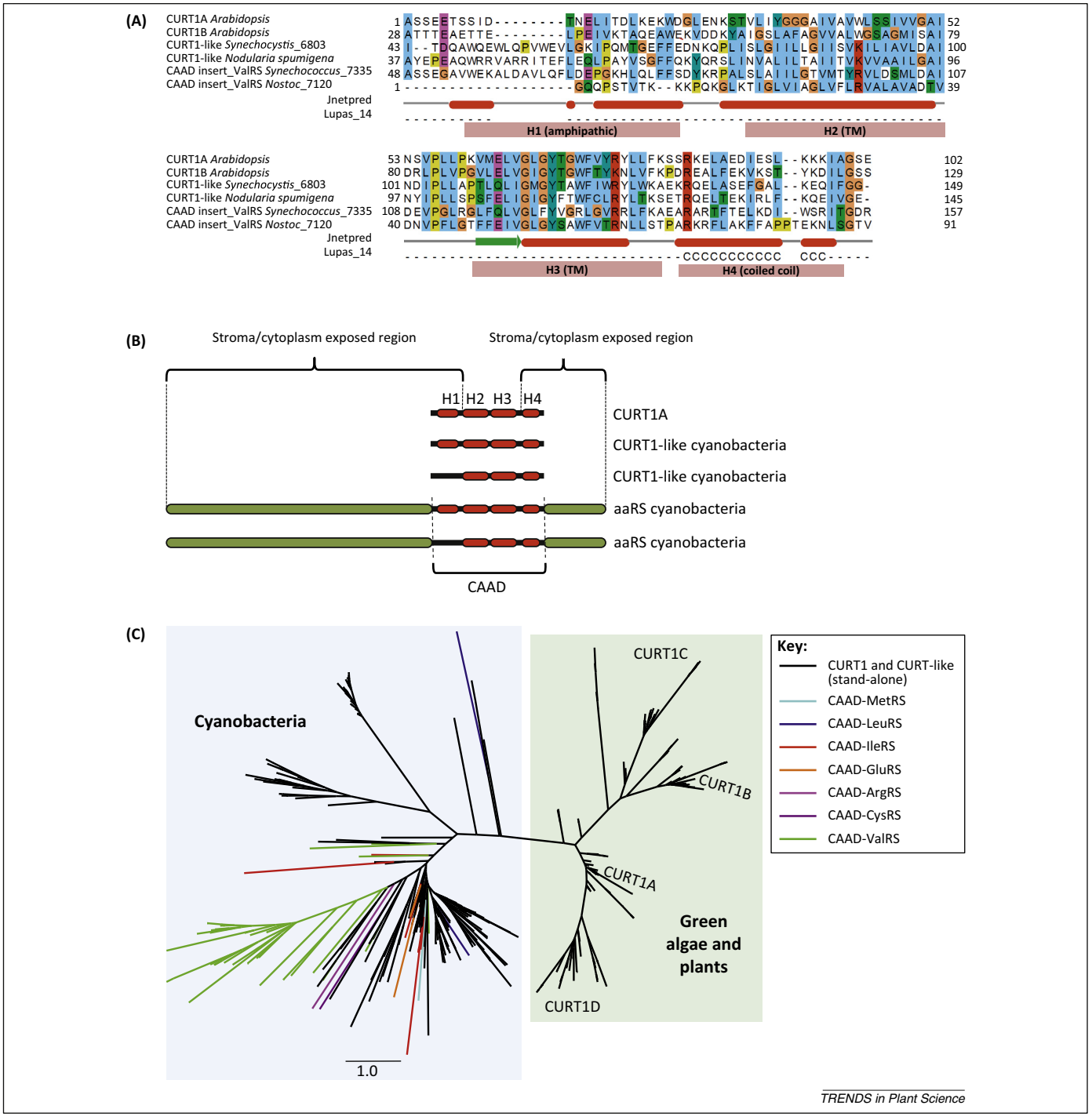

Figure 1. Sequence and structural features of CURT1/CAAD. **(A)** Sequence alignment of CURT1-A and CURT1-B from *Arabidopsis,* CURT1-like proteins form two cyanobacteria and CAAD (CAAD insert_ValRS) from two cyanobacterial species. Secondary structures predicted by JnetPred program are marked by red (∝-helices) and green (ß -sheet). Pink boxes delimit H1-H4 helices according to [2]. Sequences predicted by Lupas_14 to form a coiled coil are indicated by 'C'. Transmembrane helices are labeled as 'TM'. **(B)** Architecture of CURT1, CURT1-like and CAAD-containing proteins. H1-4 helices are indicated in red. Other domains are in green color. **(C)** Phylogenetic relationship of CURT1, cyanobacterial CURT1-like proteins and CAAD from different aaRSs. Branch colors identify CAAD inserts belonging to different aaRS.

possess an amphipathic helix, which is probably essential for inducing membrane curvature. Also, as expected from the absence of thylakoid phosphorylation in cyanobacteria, all CURT1-like proteins lack the phosphorylation site spotted by Hanson and Vener [3]. Complementation assays of *Arabidopsis curt1* mutants with the *synCurt1* gene could help to clarify whether functional divergence is due to sequence variations.

In addition to the stand-alone CURT1-like genes that exist in almost all cyanobacterial genomes, similar sequences were found as a domain fused to or inserted into aminoacyl-tRNA synthetases (aaRSs), a group of enzymes with a major role in gene translation (Figure 1A, B). They catalyze the synthesis of loaded tRNAs, which are substrates for subsequent protein synthesis at the ribosome. AaRSs are generally specific for one