DECIPHERING THE MOLECULAR MECHANISMS INVOLVED IN INTRACELLULAR PROTEIN TRAFFICKING IN PLANT CELLS

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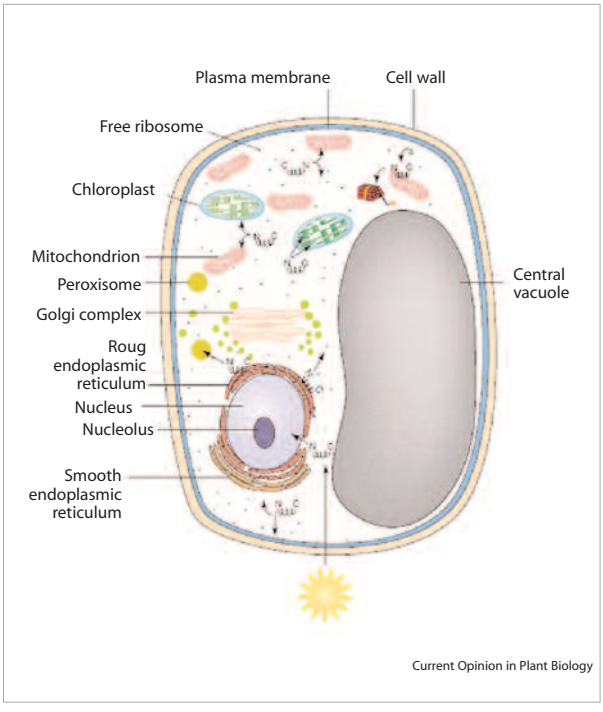
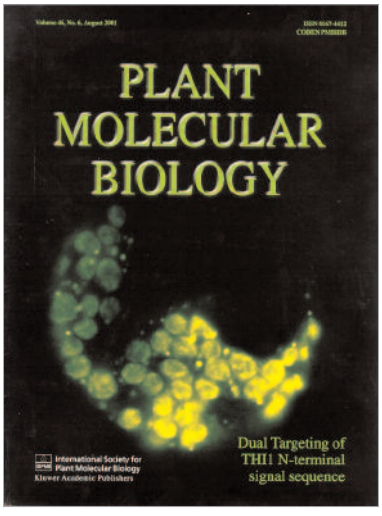


One of our main objectives is to understand the molecular mechanisms responsible for the protein localization inside plant cells, especially the proteins directed to mitochondria and chloroplasts. In this proposal, it is intended to advance in the characterization of the regulatory mechanisms involved in the intracellular localization of different proteins, also integrating an evolutionary approach. Four sub-projects are presented. The first one aims to identify proteins interacting with THI1 protein of *Arabidopsis thaliana* on thiamin biosynthesis, by using the yeast double-hybrid technique. Moreover, it is intended to identify proteins responsible for the control of the double targeting of THI1, since it was shown by our group that this protein is directed simultaneously to mitochondria and chloroplasts by a post-transcriptional mechanism. The second sub-project aims to study the thiamin biosynthesis in plants. Preliminary studies have suggested that biosynthesis of thiamin in plants can involve more than one subcellular compartment, opposing previous works that suggests the occurrence of the pathway only in the plastids. In addition to in-silico analysis, experiments with yeast functional complementation of plant genes involved on thiamin biosynthesis will be carried out. Furthermore, the determination of the subcellular localization of all proteins involved in the process will be provided by gene fusions with the green fluorescent protein (GFP). The third subproject aims to understand the gene regulation mechanism, know as riboswitches. The presence of structures in mRNA capable to interact directly with molecules intervening with its own translation has driven the attention of several groups worldwide. In the case of the *Thic* genes (involved in thiamin biosynthesis in *Arabidopsis thaliana)*, results have shown that its mRNA presents an aptamer-like structure that allows its binding to either thiamin pirophosfate (TPP) or thiamin, regulating its expression. It is intended to verify experimentally if the occurrence of this structure intervenes with the expression of the *Thic* gene, as well as, to the subcellular localization of the *Thic* protein. Moreover, a study will be carried in silico with all genes whose products are involved in thiamin biosynthesis, in order to identify the occurrence of aptamer-like structures and, in positive case, to verify its function experimentally. The fourth subproject involves the characterization of the mechanism responsible for the localization of one thylakoid membrane metaloprotesease, the FtsH-p1. Members of this family have been described as belonging to the Tat system (twin-arginine translocation system) for insertion in the thylakoid membrane. In a previous work of our group, it was shown that a member of this family does not present a classical RR motif responsible for its insertion in the membrane. Therefore, it is intended to characterize the mechanism responsible for the localization of protein, which suggests a distinct mechanism of those already described in the literature.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our group has shown that a protein named THI1, from *Arabidopsis thaliana,* is encoded by a single gene and is subsequently translocated to mitochondria and chloroplasts by an alternative translation initiation mechanism based on two in-frame AUG start codons. When translation initiates in the first AUG, the protein is translocated to plastids, whereas translocation from the second AUG, the protein is delivered into mitochondria. Previous examples of dual-targeting to mitochondria and chloroplasts involved ambiguous targeting sequences recognized by both sets of import machinery.



MAIN PUBLICATIONS

Ribeiro DT, Farias LP, Almeida JD, Kashiwabara PM, et al. 2005. Functional characterization of the *Thi*1 gene promoter region from *Arabidopsis thaliana*. *J. Exp. Bot.* **56**: 1797-1804.

Macasev D, Whelan J, Newbigin E, et al. 2004. Tom22′, an 8 kDa *trans*-site receptor in plants and protozoans, a conserved feature of the TOM complex that appeared early in the evolution of eukaryotes. *Mol. Biol. Evol.* **21**: 1557-1564.

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