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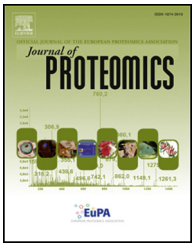

Proteomic analysis of chloroplast biogenesis (*clb*) mutants uncovers novel proteins potentially involved in the development of *Arabidopsis thaliana* chloroplasts☆

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ABSTRACT

Plant cells outstand for their ability to generate biomass from inorganic sources, this phenomenon takes place within the chloroplasts. The enzymatic machinery and developmental processes of chloroplasts have been subject of research for several decades, and this has resulted in the identification of a plethora of proteins that are essential for their development and function. Mutant lines for the genes that code for those proteins, often display pigment-accumulation defects (e.g., albino phenotypes). Here, we present a comparative proteomic analysis of four chloroplast-biogenesis affected mutants (*cla1-1*, *clb2*, *clb5*, *clb19*) aiming to identify novel proteins involved in the regulation of chloroplast development in *Arabidopsis thaliana*. We performed 2D-PAGE separation of the protein samples. These samples were then analyzed by computational processing of gel images in order to select protein spots with abundance shifts of at least twofold, statistically significant according to Student's *t*-test ($P < 0.01$). These spots were subjected to MALDI-TOF mass-spectrometry for protein identification. This process resulted in the discovery of three novel proteins potentially involved in the development of *A. thaliana* chloroplasts, as their associated mutant lines segregate pigment-deficient plants with abnormal chloroplasts, and altered mRNA accumulation of chloroplast-development marker genes.

Biological significance

This report highlights the potential of using a comparative proteomic strategy for the study of biological processes. Particularly, we compared the proteomes of wild-type seedlings and four mutant lines of *A. thaliana* affected in chloroplast biogenesis. From this proteomic analysis it was possible to detect common mechanisms in the mutants to respond to stress

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and cope with heterotrophy. Notably, it was possible to identify three novel proteins potentially involved in the development or functioning of chloroplasts, also it was demonstrated that plants annotated to carry T-DNA insertions in the cognate genes display pigment-deficient phenotypes, aberrant and underdeveloped chloroplasts, as well as altered mRNA accumulation of chloroplast biogenesis marker genes.

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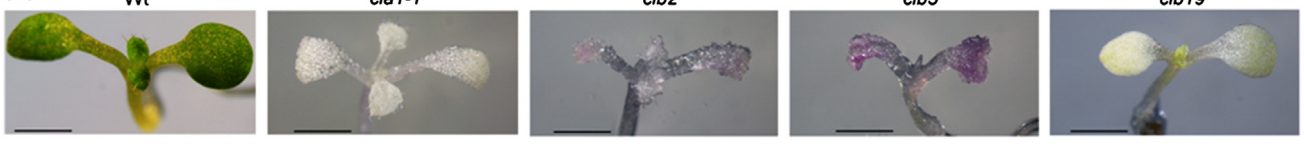
1. Introduction

Among eukaryotes, plant cells are notable for their ability to generate biomass from CO_2 and sunlight via the process known as photosynthesis. This process takes place in the membranes and compartments of a remarkable organelle, the chloroplast. Given their ability to perform photosynthesis, chloroplasts represent the main source of food (sugars) for plant cells and are the keystone for the entire food chain sustaining animal life. The chloroplasts are not only responsible for the production of carbon resources, they are also the biosynthesis site of many other important metabolites, such as amino acids, lipids, hormones, vitamins, and isoprenoids [1–3]. These functions make chloroplasts an essential organelle for the development and survival of plants. Thus, abnormal development of chloroplasts often results in lethality [4–7].

Thus far, several mutant lines harboring defects in chloroplast development have been isolated, and the mutated genes have been found to encode for several different components of the plastid machinery for protein import, isoprenoid biosynthesis, RNA processing, protein maturation, plastid gene expression, thylakoid biogenesis, chloroplast to nucleus signaling, and other important processes [8–19]. Examples include the chloroplast-biogenesis (*clb*) mutants, which were originally isolated based on the phenotypes of impaired pigment accumulation, rendering plants albino, yellow, or pale green [19]. In vitro cultured *clb* mutants contain underdeveloped chloroplasts, showing reduced organelle diameter, low accumulation of thylakoid membranes, low levels of photosynthetic pigments, and deficiencies in the expression of several nuclear and chloroplast encoded genes. Several *clb* mutants have been fully characterized, and the genes affected in such mutants are now known. For instance, *cla1-1*, *clb4*, and *clb6* mutant plants are affected in the expression of deoxy-xylulose-5-phosphate synthase (DXS1), hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS), and hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR), respectively; these three enzymes each catalyze a different step in the chloroplastic pathway for isoprenoid biosynthesis (Fig. 1A, B) [9,10,19]. Other *clb* mutants, such as *clb19*, affect the expression of a PPR domain-containing protein known to be involved in the editing of the *ClpP* and *rpoA* plastid mRNAs (Fig. 1A, B), which code for the catalytic subunit of the main protease complex in plastids and the α-subunit of the plastid-encoded RNA polymerase, respectively [19]. Another characterized *clb* mutant is *clb2*, whose affected locus was very recently assigned in our laboratory using next-generation sequencing methods, to a homogentisate prenyltransferase gene, which is known to be involved in the biosynthesis of plastoquinone 9 (Fig. 1A, B; unpublished data) [19,20]. Finally, during the time of production of this research, the *clb5* mutant line was found to be affected in the expression of ZDS (ζ-carotene desaturase) enzyme, which is responsible of the biosynthesis of the essential carotenoid lycopene [21]. Despite all the information that has been obtained through the characterization of *clb* mutants, there are still some *clb* lines (*clb1 and clb3*) whose characterization is still under investigation.

Since these chloroplasts are essential for plant development, an understanding of the precise mechanisms underlying their development has been the subject of intense

A



B

Locus	Mutant	Description	Phenotype	Reference
At4g15560	*cla1-1*	1-deoxy-D-xylulosa 5-phosphate synthase; chloroplastic pathway for isoprenoid synthesis.	Albino	Mandel M *et al*, 1996.
At1g05750	*clb19*	Pentatricopeptide repeat protein; editing of clpP and rpoA plastid transcripts.	Yellow	Chateigner-Boutin A *et al*, 2009.
At3g11945	*clb2*	Homogentisate prenyl transferase; plastoquinone-9 biosynthesis.	Albino	Gutiérrez-Nava M *et al*, 2004. Tian L *et al*, 2007.
NA	*clb5*	Unknown.	Albino	Gutiérrez-Nava M *et al*, 2004.

Fig. 1 – Seedlings used in this analysis. A) Wild-type (Wt; 8 DAG) and mutant (16 DAG) seedling phenotypes. Plants were germinated and grown in vitro and collected after the emergence of the first pair of true leaves. B) Loci affected by the *clb* mutations under analysis; a short description of the affected proteins is included. Scale bar represents 10 μm.

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