Copper (Cu) homeostasis is a key process in plants because Cu is an essential micronutrient whose lack provokes some deficiency symptoms, and its excess causes toxicity. Several proteins are involved in Cu homeostasis in chloroplasts, including the heavy metal ATPase8 (HMA8). HMA8 belongs to a subgroup of 1B P-type ATPase monovalent cation transporters, and it is responsible for Cu⁺ transport across the thylakoid membranes. In soybean, there are two different genes subjected to alternative splicing leading to the formation of several transcripts that could also lead to different proteins. First, we tried to overexpress this membrane protein in bacteria. Second, we expressed several HMA8 splicing forms in yeast, and made complementation analysis with the Ccc2 yeast strain mutant that lacks the Golgi-localized Cu-transporting P-type ATPase. And third, we are using several Arabidopsis (A.) thaliana mutants to undertake the functional characterization of these proteins.

INTRODUCTION

Copper (Cu) homeostasis is a key process in plants because Cu is an essential micronutrient whose lack provokes some deficiency symptoms, and its excess causes toxicity. Several proteins are involved in Cu homeostasis in chloroplasts, including the heavy metal ATPase8 (HMA8). HMA8 belongs to a subgroup of 1B P-type ATPase monovalent cation transporters, and it is responsible for Cu⁺ transport across the thylakoid membranes. In soybean, there are two different genes subjected to alternative splicing leading to the formation of several transcripts that could also lead to different proteins. First, we tried to overexpress this membrane protein in bacteria. Second, we expressed several HMA8 splicing forms in yeast, and made complementation analysis with the Ccc2 yeast strain mutant that lacks the Golgi-localized Cu-transporting P-type ATPase. And third, we are using several Arabidopsis (A.) thaliana mutants to undertake the functional characterization of these proteins.

EXPERIMENTAL DESIGN

OBJECTIVES

1. Overexpression in E. coli.
2. Expression in S. cerevisiae.
3. Overexpression in A. thaliana.

RESULTS

1. Overexpression in E. coli
   - Immunoblot analysis of HMA8 in E. coli.

2. Expression in S. cerevisiae
   - Immunoblot analysis of HMA8 in yeast.

3. Yeast complementation
   - Functional analysis of HMA8 in yeast.

4. Overexpression in A. thaliana
   - A. thaliana mutants used in the characterization of the HMA8 proteins. A) paa2-1 mutant (adapted from Abdel-Ghany et al., 2005). B) paa1-1 mutant (adapted from Shikanai et al., 2003).

CONCLUSIONS

1. The overexpression of HMA8 was not observed in E. coli.
2. Two of the spliced forms were expressed in S. cerevisiae.
3. The HMA8 proteins did not complement the yeast Golgi-localized Cu-transporting ATPase Ccc2 function.
4. At present, we are working on the overexpression of HMA8 proteins in A. thaliana mutants to carry out their functional characterization.

REFERENCES


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