

PCP1 AND ATOSA1: PLASTIDIAL PROTEINS INVOLVED IN OXIDATIVE STRESS RESPONSE AND METAL HOMEOSTASIS IN *ARABIDOPSIS* CHLOROPLASTS

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The Activity-of-bcl-complex protein family was firstly characterized in *Saccharomyces cerevisiae*, in which the protein acts as a chaperone-like protein essential for the proper conformation and the efficient functioning of the mitochondrial *bcl* complex. In *Arabidopsis thaliana* 17 genes cluster as Activity-of-bcl-complex proteins, even though knowledge about their putative functions is still limited.

This work is focusing on the characterization of two proteins in *A. thaliana* belonging to this family, in order to understand their involvement in plant development or stress responses. The first, PCP1 (putative chloroplast protein 1) is encoded by an homolog of a gene of *Brassica juncea* modulated upon cadmium treatment. The second, AtOSA1 is an oxidative stress-related protein involved in plant response to stress generated by Cd²⁺, hydrogen peroxide and excessive light. These proteins share 45% aminoacidic identity, and both hold the domain characteristic of the family and two transmembrane spans at the C-terminus. Under standard growth conditions, single mutants and the crossed-double mutant did not show morphological or developmental abnormalities, with the exception of the pale-green phenotype showed by *atosal* and double mutants. This was supported by pigment analysis that revealed a reduced total chlorophyll content and an increased *chl a/b* ratio in these mutants. Since AtOSA1 is involved in plant response to oxidative stress, we tested the hydrogen peroxide effect on mutants. *pcp1* and *pcp1/atosal* plants showed a reduced root length, suggesting that root growth in mutants is more sensitive to hydrogen peroxide than in WT.

The analysis of cellular localisation of FLAG-tagged AtOSA1 and PCP1 proteins revealed that both are localised in chloroplasts. To address whether photosynthesis might be affected in mutant plants, analysis of the photosynthetic machinery was performed by Western-Blot and electron transport in thylakoid membrane was investigated. No particular differences in protein composition in PSI and PSII were observed, but protein content of the *b6f* complex is severely reduced in mutants. Analysis of chlorophyll fluorescence showed that mutant and WT plants have similar maximum quantum yield, effective PSII quantum yield and excitation pressure (1-qP value). An increase in light intensity caused a NPQ induction in all genotypes, particularly marked in the *atosal* mutant.

Due to the difference in chlorophyll content, mutant plants could be impaired in transport of metal ions that are required for photosynthetic apparatus building. We are currently measuring the content of metals in chloroplasts, comparing WT to mutants, with particular concern to the major metals involved in the chloroplast physiology and oxidative stress-counteracting mechanisms.

Plastidial co-localization and high sequence similarity suggest that AtOSA1 and PCP1 may have evolved functional redundancy. Their overlapping function is observed in root length,

analysing plant response to oxidative stress. Nevertheless, the two single mutants do not display similar pigment composition and are characterised by a different NPQ induction. The latter results suggest that either the two proteins do not share a complete functional redundancy or that their different expression level partially complement the lack of gene function of the single mutants.