CHARACTERIZATION OF KNOCK OUT MUTANTS IN THE PPI GENE FAMILY: A T-DNA INSERTION CAUSING ABERRANT SPLICING 'AT DISTANCE'

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PPI1 (PROTON PUMP INTERACTOR 1) is a protein identified in a two hybrid screen whose N-terminus binds to the *Arabidopsis thaliana* plasma membrane proton pump (PM H^+-ATPase). When added to purified membranes of Arabidopsis, the entire PPI1 protein, or fragments thereof, expressed as fusion protein in *E. coli*, are able to stimulate the activity of the proton pump (Morandini et al., 2002 Plant J. 31:487-97).

To study the function of the PPI1 protein we make use of Arabidopsis lines bearing a T-DNA inserted in the gene. Two lines were characterized in detail for *Ppi1*: an insertion in intron I (named N93) and one in exon VII (F09) at aa 525.

RT-PCR analysis on leaves and cell culture tissue confirmed that the transcript is aberrant in both insertion lines. In N93 homozygous plants, a low level of transcript is detectable, but the distal 5' UTR, normally present in the mRNA, is missing. In the case of F09 insertion, the message runs into the T-DNA region and presents premature terminations codons. Surprisingly a new, larger RNA molecule accumulates in this KO line because the splicing of the V intron is extensively prevented. The presence of the intron in the new RNA molecule was confirmed by PCR and by direct sequencing. The presence of premature termination codons causes similar phenomena in animal cells (Wang et al., 2002 Mol Cell 10:951-7).

Western analysis performed on extracts from homozygous knock-out plants and from culture cells using an anti-PPI1 serum, confirms that the protein is absent in both insertion lines. In contrast, the protein as well the mRNA are present in the early stages of growth of wt seedlings and in leaves.

In an alternative approach to study the pattern of *Ppi1* expression, we also produced reporter constructs with the GUS gene under the control of *Ppi1* promoter. Since the gene presents a large intron before the beginning of the coding region (a so-called 'leader intron'), we decided to test three different constructs. The first contains both the promoter and the leader intron; the second one is lacking the leader intron but still contains with the *Ppi1* 5'UTR; the last one is missing the intron and the 5'UTR derives from the TMV.

The *Ppi1* KO lines do not show evident phenotype when grown in pots. Different growth conditions are being tested for identifying phenotypic differences in respect to wt. Two further insertion lines in Ppi3 and one in Ppi4 are being characterized at the molecular level.