MOLECULAR CLONING AND CHARACTERIZATION OF A PLASMA MEMBRANE ABC-TYPE TRANSPORTER IN NICOTIANA PLUMBAGINIFOLIA

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The ATP-binding cassette (ABC) superfamily of membrane transporters is widely spread throughout all species from the archaea to humans (Higgins, 1992). Members of this family are involved in active transport of numerous chemically and structurally unrelated compounds and have been shown to play important roles in cell detoxification (Rea *et al.*, 1998). Recent studies have identified several ABC genes in plants. Most of them encode enzymes of the tonoplast (Rea, 1999).

We report here on the identification of *Np*ABC1, a transporter of the plasma membrane, isolated from *Nicotiana plumbaginifolia*. Our approach consisted in searching for chemicals that, when added to cultures cells, would induce the synthesis of a transporter in the plasma membrane. We have developed a sensitive system allowing us to label *in vivo* proteins with ^{35}S and compare protein profiles derived from cells growing in the absence or presence of the tested chemicals. Introduction of sclareolide, a terpene compound, to the medium caused the appearance of a 160 kDa band. Partial amino acid sequence information was obtained from this protein and homology to the ABC transporter superfamily was found. On the basis of this sequence, we designed a set of degenerated primers and, by successive RACE-PCR, we obtained a complete cDNA sequence yielding an open reading frame of 4311 bp corresponding to a protein of 161,848 Da. Comparison of the deduced amino acid sequence with protein data bases confirmed that this clone is a member of the ABC family.

A 96-amino acid sequence from the *Np*ABC1 C-terminal region was produced in *E coli* and used for rabbit immunisation. Antibodies used for western blot analysis showed induction of the protein after sclareolide addition. By *in situ* subcellular localisation, *Np*ABC1 was found in the plasma membrane. We observed that sclareol, a diterpene from the leaf surface of *Nicotiana* species, also induced *Np*ABC1. It has been shown that this compound is toxic for some fungi (Bailey *et al.*, 1975). This could suggest that *Np*ABC1 might be involved in plant pathogen interaction.

References

Bailey, J.A., Carter, G.A., Burden, R.S., Wain, R.L. (1975) *Nature* **255,** 328-329.
Higgins, C.F. (1992) *Ann. Rev. of Cell Biol.* **8,** 67-113.
Rea, P.A., Li, Z.S., Lu, Y.P., Drozdowicz, Y.M., Martinoia, E. (1998) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **49,** 725-760.
Rea, P.A., (1999) *J. Exp. Bot.* **50,** 895-913.