LOCATING MANGANESE TRANSPORTERS IN PLANT CELLS

Manny Delhaize, Ben D. Gruber, Rosemary G. White, Diane M. Hebb, Helen Leung, Peter R. Ryan, Alan E. Richardson
CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.
email: rosemary.white@csiro.au

KEY WORDS: manganese transport, GFP labelling, tonoplast, pre-vacuolar compartments

INTRODUCTION: Plant members of the cation diffusion facilitator (CDF) family of proteins can confer tolerance to metals when overexpressed in yeast or plants. Four proteins (AtMTP8 to AtMTP11) that form a distinct group on a phylogenetic tree of the CDF proteins within Arabidopsis thaliana are closely related to the protein encoded by the Stylosanthes hamata gene; ShMTP8 [1]. Both ShMTP8 and AtMTP11 confer Mn tolerance, and cause Mn accumulation in transgenic plants expressing these proteins [1,2]. Here, we investigate the subcellular location of GFP-fusions of ShMTP8 and AtMTP11.

RESULTS: In tobacco (Nicotiana tabacum) and Arabidopsis stably expressing ShMTP8:GFP (GFP fused to the C-terminus of the full ShMTP8 protein), fluorescence was localised to a discrete membrane that mostly tracked the cell wall, except where it invaginated around the nucleus, chloroplasts and other large cytoplasmic organelles [1]. Plants expressing 35S::GFP showed fluorescence within the nucleus and thin layer of cytoplasm. In young Arabidopsis root cap cells, 35S::GFP fluorescence was detected within their relatively large nuclei and cytoplasmic volume, whereas expression of ShMTP8:GFP outlined multiple small vacuoles that eventually coalesced to occupy most of the volume in older cells. In contrast, transient expression of either AtMTP11:GFP (GFP fused to C-terminus of AtMTP11) or GFP:AtMTP11 (GFP fused to N-terminus of AtMTP11) after bombardment into intact leek (Allium ampeloprasum) cells gave numerous motile fluorescent organelles which co-localised with the pre-vacuolar compartment (PVC) marker mRFP:AtVSR2 but which were largely distinct from the Golgi marker STtmd:DsRed [2]. After treatment with 20 μM wortmannin, AtMTP11:GFP-tagged organelles became less motile and many became vacuolated, similar to wortmannin-induced vacuolation of PVCs [3]. Brefeldin A, which disrupts the Golgi apparatus, did not affect these organelles.

DISCUSSION AND CONCLUSIONS: These results suggest that at least two members of the CDF family, ShMTP8 and AtMTP11, confer Mn tolerance by sequestering Mn into internal organelles. The GFP-tagged ShMTP8 and AtMTP11 appear to be located in different but closely related organelles, although it is possible that the GFP-tagging causes some mis-expression of one or both proteins. Confirmation will depend on determining whether the labelled organelles are also sites of Mn accumulation.