

Environmental Management Science Program

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Characterization of a New Family of Metal Transport Proteins

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Research Objective

Soils at many DOE sites are contaminated with metals and radionuclides. Such soils obviously pose a risk to human and animal health. Unlike organic wastes which can be metabolized, metals are immutable and cannot be degraded into harmless constituents. Phytoremediation, the use of plants to remove toxic materials from soil and water, may prove to be an environmentally friendly and cost effective solution for cleaning up metal-contaminated sites. The success of phytoremediation will rely on the availability of plants that absorb, translocate, and tolerate the contaminating metals. However, before we can engineer such plants, we need more basic information on how plants acquire metals. An important long term goal of our research program is to understand how metals such as zinc, cadmium and copper are transported across membranes. Our research is focused on a new family of metal transporters which we have identified through combined studies in the yeast *Saccharomyces cerevisiae* and in the model plant *Arabidopsis thaliana*. We have identified a family of 19 presumptive metal transport genes in a variety of organisms including yeast, trypanosomes, plants, nematodes, and humans. This family, which we have designated the “ZIP” genes, provides a rich source of material with which to undertake studies on metal transport in eukaryotes. The project has three main objectives:

Objective 1: Determine the sub-cellular location of the ZIP proteins in *Arabidopsis*.

Objective 2: Carry out a structure/function analysis of the proteins encoded by the ZIP gene family to identify regions of the protein responsible for substrate specificity and affinity.

Objective 3: Engineer plants to overexpress and underexpress members of the ZIP gene family and analyze these transgenic plants for alterations in metal accumulation. We now know that manipulation of transporter levels will also require an understanding of post-transcriptional control of ZIP gene expression.

Research Progress and Implications

We are currently in year one of a three-year project. We are concentrating on Arabidopsis ZIP family members that variously transport iron, zinc and manganese when expressed in yeast. In plants, three of the family members, ZIP1, ZIP3 and ZIP4, are up-regulated by zinc deficiency and one, IRT1, is up-regulated by iron deficiency. We have constructed ten site-directed mutations in IRT1 by changing conserved histidine, aspartic acid and glutamic acid residues to alanine residues. While most of the mutations abolish both iron and zinc uptake activity, three mutations, E103A, H197A and E305A, have little or no zinc uptake activity but retain wild type levels of iron uptake activity. In addition, three chimeric proteins between IRT1 and either ZIP1 or ZIP3 have been constructed. However, all of the constructs assayed to date have little or no iron or zinc activity. Transgenic plants engineered to either overexpress or underexpress a particular transporter gene are allowing us to test the relative importance of each transporter in maintaining ion balance. This year, we have focused on analyzing lines carrying IRT1 sense constructs. In wild-type Arabidopsis plants, IRT1 mRNA is only expressed in iron-deficient roots, whereas transgenic plants carrying a CaMV 35S::IRT1 construct show expression of IRT1 mRNA in roots and shoots under both iron-deficient and iron-sufficient conditions. However, IRT1 protein is only detected in the roots of iron-deficient transgenic plants. Metal analysis

of transgenic plants showed that several lines accumulate iron, zinc and manganese as compared to wildtype; these lines have higher levels of IRT1 protein than wildtype under permissive (iron-deficient) conditions, indicating a correlation between IRT1 protein level and metal accumulation. In addition, lines that have elevated levels of IRT1 protein show an enhanced sensitivity to cadmium under iron-deficient conditions and yeast expressing IRT1 also show an enhanced sensitivity to cadmium.

Planned Activities

During the next year, we will begin analysis of additional transgenic plant constructs, including *IRT1* antisense plants, and sense and antisense constructs for *ZIP1*, *ZIP2* and *ZIP3*. We will continue our structure-function studies, focusing on the random mutagenesis approach. We will also continue to prepare family member specific antibodies for use in localization studies. At the present time, we have antibodies directed against IRT1 and against ZIP3.

Other Access To Information

Grotz, N., T. Fox, E. Connolly, W. Park, M.L. Guerinot and D. Eide. 1998. Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. *Proc. Natl. Acad. Sci. U.S.A.* 95: 7220-7224.
Fox, T. and M.L. Guerinot. 1998. Molecular biology of cation transport in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49: 669-96.