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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 iron 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 24 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.

## Research Progress and Implications

We are currently in year two of a three-year project. IRT1, a member of the ZIP family of transporters, is expressed in the roots of iron-deficient plants and can rescue an iron uptake mutant of yeast. It is not, however, exclusively an iron transporter, as it can also transport zinc, manganese and cadmium when expressed in yeast (Korshunova et al., 1999). In order to determine which residues of IRT1 are involved in substrate recognition and transport, single amino acid changes were introduced into IRT1, replacing various charged residues that were likely to be metal ligands. Two mutations had a significant effect on the functional characteristics of the transporter. One mutant, E103A, transports iron, manganese and cadmium but not zinc and another, D100A, transports zinc and cadmium but no longer transports iron or manganese. Both of these residues, E103 and D100, are predicted to be within an extracellular loop, suggesting that this loop is involved in metal recognition. Such information may allow us to construct transgenic plants in aid of phytoremediation that can specifically accumulate one cation while excluding others.

Transgenic plants expressing IRT1 from the CaMV 35S promoter only accumulate IRT1 protein in the roots of iron-deficient transgenic plants. We believe that IRT1 protein is rapidly turned over unless plants are truly iron-deficient, based on our observations on the behavior of ZRT1 in yeast. ZRT1 is subject to post-transcriptional regulation such that its activity is reduced in the presence of high substrate concentrations. Our recent studies have defined the mechanism of this regulation and demonstrated that down-regulation of the transporters is mediated by zinc-induced endocytosis and vacuolar degradation of the protein (Gitan et al., 1998). We have further characterized the signal transduction pathway that controls ZRT1 endocytosis in response to zinc. Our results establish that ubiquitination is an essential part of the pathway that leads to the endocytosis of ZRT1 in response to zinc. This conclusion is based on the following observations. First, we have established that ZRT1

is mono- and di-ubiquitinated in response to zinc treatment. Moreover, we have shown that this ubiquitination occurs prior to endocytosis; the ubiquitinated protein accumulates in zinc treated mutant cell lines that are blocked for endocytosis. Given that transporter activity does not greatly decrease in these mutants, it appears that ubiquitin addition does not directly interfere with their zinc uptake function. Second mutations that impair components of the ubiquitin conjugation pathway also impair ZRT1 ubiquitination and endocytosis. Specifically, mutations in the functionally overlapping UBC4 and UBC5 E2 ubiquitin conjugating enzymes and the RSP5/NPI1 E3 ubiquitin protein ligase are defective in these processes. These components are known to provide substrate specificity to the ubiquitination process and, consistent with this, other E2 and E3 mutants have no effect on zinc-induced ZRT1 endocytosis. Third, a mutation within ZRT1 that alters a potential ubiquitination site eliminates both ubiquitination and endocytosis. Ubiquitin is conjugated to lysine residues in target proteins and lysine 195 of ZRT1 is the apparent site of ZRT1 modification. These data, combined with the studies of others showing that ubiquitin serves as a tag for endocytosis of other plasma membrane proteins, demonstrate the important role of this modification in signaling endocytosis.

We have also begun studies on the human members of the ZIP family. hZIP2 expression has been observed in uterine epithelial cells (where it is induced by contact inhibition), infant brain, and prostate (based on the EST dbase). hZIP1 appears to be much more widely expressed. hZIP2 is 22% identical and 53% similar to IRT1. We have expressed hZIP2 cells in K562 erythroleukemia cells from the CMV promoter and find that it significantly increases (3-fold) zinc uptake activity. Immunofluorescence experiments using a functional epitope tagged gene demonstrate plasma membrane localization.

## Planned Activities

During the next year, we will begin analysis of additional transgenic plant constructs, including *IRT1* plants carrying mutations predicted to eliminate feedback inactivation, and sense and antisense constructs for *ZIP1*, *ZIP2* and *ZIP3*. We will also continue our structure-function studies. In yeast, we will determine if intracellular or extracellular zinc is the signal for endocytosis of ZRT1 and we will identify the region of ZRT1 that serves as the zinc sensor. We will also begin a screen for EMS-generated mutants of the CaMV35S:IRT1 line that have altered sensitivity to cadmium.

## Information Access

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