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Final Report
DE-FG07-97ER20292

Title: Characterization of a new family of metal transporters

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Summary:

Metal ions are critical nutrients, yet overaccumulation of these same metals can also be toxic. To maintain appropriate intracellular levels, cells require specific metal uptake systems that are subject to precise homeostatic regulation. The long-range goal of our research is to define the molecular mechanism(s) and regulation of metal ion uptake in eukaryotic cells. Integrating genetic, molecular biological and biochemical approaches, we have examined these processes in the yeast *Saccharomyces cerevisiae* and the plant *Arabidopsis thaliana*. Both are proven model systems for studying fundamental cellular processes. Our work has focused on the ZIP family of metal transporters which we identified; this family has representatives in bacteria, fungi, plants and animals. IRT1, one of the founding members of the ZIP family, is an essential cation transporter that is expressed in the epidermal cells of iron deficient plant roots and is responsible for uptake of iron from the soil. We now know that there are 15 ZIP genes in the Arabidopsis genome which can be divided into four different classes, based on their intron/exon arrangements and the similarities among their encoded gene products. The ZIP family members display different substrate specificities for metals and different tissue distributions in Arabidopsis. Moreover, the family members respond differentially to metal deficiencies. For example, *IRT1*, *ZIP6* and *ZIP9* mRNA are expressed mainly in the roots of iron deficient plants whereas *ZIP4* responds to both iron and zinc deficiency. Work in both yeast and Arabidopsis has addressed substrate specificity as well as how these transporters are regulated in response to metal availability.

Our project was broken down into four specific aims. Significant progress was made on all four aims. I have listed the publications which have resulted under the relevant specific aim.

1 Determine the role of ZIP transporters in Arabidopsis metal ion uptake and metabolism

Using functional expression studies in yeast and genetic, cytological, and biochemical analyses of *Arabidopsis*, the role of ZIP transporters in plants will be determined.

Korshunova, Y.O., D. Eide, W. Gregg Clark, M.L. Guerinot and H.B. Prakasi. 1999. The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. **Plant Mol. Biol.** 40: 37-40

Fox, T. and M.L. Guerinot. 1998. Molecular biology of cation transport in plants. *Annu. Rev. Plant Physiol.* **Plant Mol. Biol.** 49: 669-96

Guerinot, M.L. and D. Eide. 1999. Zeroing in on zinc uptake in yeast and plants. **Curr. Opin. Plant Biol.** 2: 244-249.

Guerinot, M.L. 2000. The ZIP family of metal transporters. **Biochim. Biophys. Acta.** 1465: 190-198.

Vért, G., N. Grotz, F. Dédaldéchamp, F. Gaymard, M.L. Guerinot, J-F. Briat and C. Curie. 2002. IRT1: an Arabidopsis transporter essential for iron uptake for the soil. **Plant Cell** In Press.

2) Identify residues that determine the substrate specificity and affinity of ZIP transporters

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Residues that are critical for substrate affinity and specificity will be identified using random, site-directed, and domain-swapping mutagenesis procedures. Our ultimate goal will be to create transporters with increased specificity for particular heavy metals.

Rogers, E.R., D.J. Eide and M.L. Guerinot. 2000. Altered Selectivity in an Arabidopsis metal transporter. **Proc. Natl. Acad. Sci. U.S.A.** 97: 12356-12360.

3) *Characterize the feedback inactivation mechanism of ZIP transporter regulation*

We will dissect the endocytic pathway taken by ZIP proteins, examine the role of intracellular versus extracellular metal in the signaling process, and identify the *cis*-acting sites responsible for ZIP feedback inactivation. *Trans*-acting factors will also be identified.

Gitan, R., Luo, H., Rodgers, J., Broderius, M., and Eide, D. Zinc-induced inactivation of the yeast ZRT1 zinc transporter occurs through endocytosis and vacuolar degradation. **J. Biol. Chem.** 273:28617-28624 (1998).

Gitan, R.S. and Eide, D. Zinc-regulated ubiquitin conjugation signals endocytosis of the yeast ZRT1 zinc transporter. **Biochem. J.** 346:329-336 (2000).

4) *Engineer plants to overexpress members of the ZIP gene family and analyze these constructs for alterations in metal accumulation.* Analysis of transgenic plants already constructed indicates that manipulation of transporter levels will require an understanding of feedback inactivation (Specific aim #3). Knowledge acquired will be used to design a second generation of transgenic plants with altered metal uptake abilities.

Guerinot, M.L. and D.E. Salt. 2001. Fortified foods and phytoremediation: two sides of the same coin. **Plant Physiol.** 125: 164-167

Connolly, E.L., J. Fett and M.L. Guerinot. 2002. Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. **Plant Cell** In Press.

In addition to the publications listed above, the work supported by DOE was presented at a number of meetings and a number of universities.

Presentations by David Eide:

University of Kansas Medical Center, Department of Biochemistry and Molecular Biology, "Galvanizing science: the molecular biology of zinc homeostasis," 03/27/99

10th International Conference on Trace Elements in Man and Animals, Evian, France, "The mechanism and regulation of zinc transport in yeast," 05/07/99

Second International Meeting on Copper Homeostasis and its Disorders: Molecular and Cellular Aspects, Ravello, Italy, "Regulation of zinc homeostasis and transport in yeast," 09/21/99

Texas A & M University, Department of Biochemistry, "Galvanizing science: the molecular biology of zinc." 11/17/99

NIH Workshop on Advances in Membrane Transport: Lessons from Model Organisms, Bethesda, MD, "Metal ion transporters in yeast," 12/06/99

University of Michigan, Department of Biological Chemistry, "Molecular biology of cellular zinc homeostasis," 5/16/00

Oregon Health Sciences University, "Molecular mechanisms of cellular zinc homeostasis," 1/03/01

Society for Experimental Biology, Metals and Cells Symposium, University of Kent, Canterbury UK. "Cellular zinc sensors and metal ion homeostasis in yeast," 4/2/01-4/6/01

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FASEB Summer Research Conference on Molecular Mechanisms of Regulation by Dietary Constituents, Saxtons River, VT, 8/23/01

Presentations by Mary Lou Guerinot

- 1997 Metals and Genes Symposium, Society for Experimental Biology, Canterbury, England.
- 1997 Plant Physiology Program, University of Kentucky, Lexington, KY.
- 1997 Biology Department, Northeastern University, Boston, MA.
- 1997 8th International Symposium on Iron Nutrition and Interactions in Plants, Stuttgart, Germany.
- 1997 Plant Biology '97 "Heavy Metal Binding & Transport" Mini-symposium. Vancouver, BC.
- 1997 Biology Department, Yale University, New Haven, CT.
- 1997 Banbury Center Conference on "Genetic Approaches to Analyzing Root Physiology." Cold Spring Harbor, NY.
- 1998 New England Biotechnology Symposium, University of Connecticut, Storrs, CT.
- 1998 Third Annual International Conference on Phytoremediation, Houston, TX
- 1998 Plant Biology '98 "Ion Pumps and Ion Transporters" Mini-symposium, Madison, WI
- 1998 DOE Environmental Management Science Program Workshop, Chicago, IL
- 1999 Plant Biology Program, University of Massachusetts at Amherst, Amherst, MA.
- 1999 Plant Biology, Purdue University, West Lafayette, IN
- 1999 BioIron '99. Sorrento, Italy
- 1999 Laboratoire de Biologie Vegetale et Microbiologie, Universite de Nice-Sophie Antipolis, Nice, France
- 1999 Laboratoire de Biochimie et Physiologie Vegetales, ENSA-INRA, Montpellier, France
- 1999 Frontiers of Plant Biology, Canberra, Australia
- 1999 10th International Conference on Arabidopsis Research, Melbourne, Australia
- 1999 Plant Biology '99. "Biochemical Genetics" symposium. Baltimore, MD
- 1999 Plant Biology Seminar Series, University of Missouri, Columbia
- 1999 Plants, Nutrition and Human Health. 9th Gaitlinburg Symposium. Knoxville, TN.
- 2000 10th International Symposium on Iron Nutrition and Interaction in Plants, Houston, TX
- 2001 Metals and Cells, Society for Experimental Biology, Canterbury, UK
- 2001 Plenary Speaker, Northeast Regional Plant Physiology meeting, Worcester Polytechnic Institute, Worcester, MA.
- 2001 Dept. of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS
- 2001 12th International Plant Membrane Transport Meeting, Madison, WI