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Molecular Characterization of a Novel Heavy Metal Uptake Transporter from Higher Plants & its Potential for Use in Phytoremediation

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Research Objective

Soils and waters contaminated with high levels of heavy metals such as Cadmium, Lead and Copper are detrimental to human and environmental health. Many human disorders have been attributed to environmental contamination by heavy metals. Removal of heavy metals from highly contaminated sites is therefore a very costly but necessary process that is currently being pursued. Recent research in several laboratories indicates that uptake of heavy metals into plants via the root system may provide a cost-effective approach for decontamination of certain heavy metal-laden soils and waters. Several mechanisms have been identified, which allow detoxification in the cytosol and vacuoles of plants. However, the molecular biological mechanisms by which heavy metals are transported from soils across the plasma membrane into roots have remained largely unknown. In recent research, our laboratory has cloned a cation uptake transporter cDNA from plants. Yeast cells expressing this cDNA show enhanced uptake of calcium and cadmium. The proposed research is testing the transport of toxic and nutrient metals by the encoded protein.

Research Progress and Implications

Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. [Daniel P. Schachtman[1], Raman Kumar[1], Julian I. Schroeder[2] and Ellen L. Marsh[1]. [1]Department of Botany, University of Adelaide, Adelaide, SA 5005 Australia; and [2]Department of Biology and Center for Molecular Genetics, University of California at San Diego, La Jolla, CA 92093-0116]

An initial report, describing the cloning of LCT1 was published by Schachtman et al., 1997, Proc. Natl. Acad. Sci. USA 94:11079-11084. The transport of cations across membranes in higher plants plays an essential role in many physiological processes including mineral nutrition, cell expansion, and the transduction of environmental signals. In higher plants, the coordinated expression of transport mechanisms is essential for specialized cellular processes and for adaptation to variable environmental conditions. To understand the molecular basis of cation transport in plant roots, a Triticum aestivum cDNA library was used to complement a yeast mutant deficient in potassium (K+) uptake. Two genes were cloned that complemented the mutant: HKT1 and a novel cDNA described in (Schachtman et al., 1997) encoding a cation transporter, LCT1 (low-affinity cation transporter). Analysis of the secondary structure of LCT1 suggests that the protein contains 8-10 putative trans-membrane helices and a hydrophilic amino terminus containing sequences enriched in Pro, Ser, Thr, and Glu (PEST). The transporter activity was assayed using radioactive isotopes in yeast cells expressing the cDNA. LCT1 mediated low-affinity uptake of the cations Rb^+ and Na^+, and possibly allowed Ca^{2+} but not Zn^{2+} uptake. LCT1 is expressed in low abundance in wheat roots and leaves. The precise functional role of this cation transporter is not known, although the competitive inhibition of cation uptake by Ca^{2+} has parallels to whole plant and molecular studies that have shown the important role of Ca^{2+} in reducing Na^+ uptake and ameliorating Na^+ toxicity. The structure of this higher plant ion transport protein is unqiue and contains PEST sequences. This study was published by: Schachtman et al., 1997, Proc. Natl. Acad. Sci. USA 94:11079-11084.

The plant transporter LCT1 mediates the uptake of calcium and cadmium in yeast. [Stephan Clemens[1], Danuta M. Antosiewicz[1], John M. Ward[2], Daniel P. Schachtman[3] and Julian I. Schroeder[1].

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Non-essential metal ions such as cadmium are most likely taken up by plants via nutrient transporters. To identify possible pathways for Cd^{2+} uptake we tested putative plant cation transporters for Cd^{2+} uptake activity by expressing cDNAs in S. cerevisiae and found that expression of one clone, LCT1 (Schachtman et al., 1997, Proc. Natl. Acad. Sci. USA 94:11079-11084), renders the growth of yeast more cadmium-sensitive. Ion flux assays showed that Cd^{2+} sensitivity is correlated with an increase in Cd^{2+} uptake. LCT1-dependent Cd^{2+} uptake is saturable, lies in the high-affinity range (apparent KM for Cd^{2+} ~33 μM) and is sensitive to block by La^{3+} and Ca^{2+}. Growth assays demonstrated a sensitivity of LCT1-expressing yeast cells to extracellular millimolar Ca^{2+} concentrations. LCT1-dependent increase in Ca^{2+} uptake correlated with the observed phenotype. Furthermore, LCT1 complements the disruption of a non-LCT1-homologous yeast gene encoding a membrane Ca^{2+} influx system We conclude that LCT1 mediates the uptake of Ca^{2+} and Cd^{2+} across the plasma membrane of yeast cells.

Schachtman, D.P., R. Kumar, J.I. Schroeder and E.L. Marsh (1997) Molecular and functional characterization of a novel low-affinity cation transporter (LCT1) in higher plants. Proc. Natl. Acad. Sci. USA 94:11079-11084.

Planned Activities

Because uptake of heavy metals into plant cells is toxic and detoxification mechanisms are essential, we are pursuing research to characterize genes that enhance Cd^{2+} resistance.

Other Access To Information

Clemens, S., D.M. Antosiewicz, J.M. Ward, D.P. Schachtman and J.I. Schroeder (Submitted) The plant transporter LCT1 mediates the uptake of calcium and cadmium in yeast.