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Heavy Metal Pumps in Plants

Jeffrey F. Harper
Scripps Research Institute
Division of Plant Biology
BCC283
10550 North Torrey Pines Road
La Jolla, California 92037
Phone: 619-784-2862
E-mail: harper@scripps.edu

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Research Objective

The purpose of the proposed DOE research is to determine the function of AMA1, a novel heavy metal pump identified in a model plant system, Arabidopsis. Heavy metal pumps belong to a superfamily of P-type ATPases which include the plasma membrane Na/K-ATPase in animals and the plasma membrane H^+-ATPase in plants and fungi. Heavy metal pumps have been implicated in heavy metal resistance (e.g., cadmium) and regulation of essential micronutrients (e.g., copper). Although several heavy metal pumps have now been identified in plants, their isoform specific functions have not been investigated. Our results suggest that AMA1 is a molydenum uptake pump. We are exploring the possibility to engineer the ion specificity of these pumps to take up other heavy metals from the soil.

Research Progress and Implications

This report summarizes work after 2 years of a 3 year project. As of June 1998, we have identified and cloned a gene encoding a heavy metal pump from Arabidopsis, called AMA1 (Arabidopsis Heavy Metal ATPase-isoform 1). Sequence analysis indicates that AMA1 encodes a P-type ATPase with a predicted molecular weight of 102 kDa. Eight putative transmembrane domains are predicted with a "CPC" motif, characteristic of heavy metal P-type ATPases, located in the middle of transmembrane 4. AMA1p is distinquished from other heavy metal pumps by a unique 26kD C-terminal domain with multiple metal binding motifs rich in histidine and cystein, with cysteins commonly found as CC pairs.

AMA1p is localized to the plasma membrane, as indicated by aqueous two phase partioning and immunodetection of the pump using two different polyclonal antibodies raised against the N- and C-terminal domains. Western blot analysis of root and shoot tissues indicates that AMA1p is primarily expressed in roots and flowers.

To genetically investigate the biological function of AMA1, we identified a plant line containing a T-DNA disrupted allel, ama1-1. The ama1-1 knock-out was identified by screening a collection of Agrobacteria-transformed Arabidopsis using PCR reactions. A T-DNA was found inserted in the N-terminal coding region, at a site immediately before the first transmembrane domain. This research identifies the first mutation of a heavy metal pump in plants.

In comparison to a wild-type parent, homozygous ama1-1 knock out plants were healthy and showed normal development under standard growth conditions. However, metal analysis indicated that ama1-1 plants had a reduced accumulation of one metal, molybdenum. Heavy metal concentrations were measured using Ion Conductive Plasma-Optical Emission Spectrometry (ICP-OES). In 4 week old plants grown on 0.5x Murashige and Skoog media, molybdenum accumulation was reduced to 25% (2.3 ppm) in knock-out plants, while normal metal concentrations were measured for calcium, cobalt, copper, iron, magnesium, manganese, and zinc.

Our results are consistent with the hypothesis that AMA1p functions as a molybdenum uptake pump in plant roots. Although molybdenum is an essential metal for enzymes involved in nitrogen and sulfur metabolism, little is known about how this metal is transported within cells. This research provides the first evidence for a P-type ATPase involved in molybdenum transport.

A continued effort is recommended to engineer P-type ATPases to transport heavy metals for bioremidiation purposes.

Planned Activities

A long term goal is to engineer new ion specificities into P-type ATPases. This would allow the design of new pumps to transport other essential and toxic heavy metals, such as manganese, cadmium, chromium, copper or gold. As a first step we need to better understand the structural basis for ion selectivity. To produce enzymes for biochemical and structural analysis we have been developing protocols for functional expression of P-type ATPases in yeast. Our short term goal is to evaluate the functional activity of AMA1 when expressed in yeast.

Our research on P-type ATPases extends to other pumps which translocate calcium, sodium, protons, and manganese. A comparative study of these different pumps should help us understand how a common enzymatic mechanism has evolved to specifically transport so many different ions.

Specific objectives for the next year:

1. Confirm that the ama1-1 knock out plant has a disruption of a heavy metal uptake system. The approach is to measure uptake rates of molybdenum and other heavy metals. Metals will be detected using radioactive labels or with ICP-OES.
2. Determine if AMA1p alters heavy metal homeostasis when expressed in yeast. The approach is to express AMA1p in wild type yeast and a mutant harboring a disruption of both endogenous heavy metal pumps. Metals will be detected using ICP-OES.
3. Consider strategies to use yeast to establish a genetic selection system to identify mutant pumps with altered ion specificities.