

Jeff Harper, The Scripps Research Inst., Division of Plant Biology.
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Heavy Metal Pumps in Plants

Original Abstract

Plants have been proposed as a bioremediation tool to help remove toxic heavy metals from contaminated land and water. However, little is known about how plants take up heavy metals from the soil and transport them to different parts of the plant. An important long term goal is to understand how heavy metals, such as copper and cadmium, are transported across the plasma membrane of plant cells. The proposed research is focused on a putative heavy metal uptake pump, AXA2p [Arabidopsis X (unknown heavy metal) ATPase, isoform 2 protein], identified in a model plant, Arabidopsis. AXA belongs to a super-family of ion-translocating P-type ATPases and is the first heavy metal pump cloned from plants. AXA2 is most similar to a subfamily of pumps recently identified in bacteria, yeast and humans which appear to pump heavy metals such as copper and cadmium. Three specific aims are proposed: 1) Determine the ion specificity of the AXA2 pump, 2) Determine how pumping activity is regulated, and 3) Determine if an increased uptake of specific heavy metals can be achieved by engineering a transgenic plant with a hyper-active pump. The hypothesis being tested is that AXA2 encodes a high affinity uptake pump for copper, with lower affinity for metals such as cadmium, zinc and nickel. Fundamental research on heavy metal transporters may eventually permit transgenic plants to be engineered with specific heavy metal uptake systems useful for bioremediation.

Progress Report

The long term goal of the proposed research is to understand how heavy metals, such as copper and cadmium, are taken up from the soil and translocated throughout the plant. The focus is on a putative heavy metal pump, AXA2p [Arabidopsis X (unknown heavy metal) ATPase, isoform 2 protein], identified in a model plant, Arabidopsis. AXA2 belongs to a large family of ion-translocating P-type ATPases. AXA2p is the first heavy metal pump cloned from plants and is most similar to a subfamily of heavy metal pumps recently identified in bacteria, yeast and humans.

Specific Aims

1) Determine which ions are translocated by the AXA2 pump. The approach is to over-express AXA2 in yeast, and use purified membrane vesicles to evaluate ion stimulated ATPase activity. The metals to be tested include copper, cadmium, cesium, cobalt, chromium, lead, manganese, molybdenum, nickel and zinc.

Progress:

We have successfully expressed AXA2p in yeast and shown that it accumulates in a yeast endomembrane system. However, at present we have been unable to detect any metal stimulated ATPase activity, using Cu(I), Cu (II), Zn, Co, Cd, Fe, Mn, or Mo. In parallel studies (partially supported by this grant), we have successfully demonstrated Mn²⁺ and Ca²⁺-pumping activities for two other P-type ATPases identified in plants.

Continuation Plan:

This biochemical approach will be continued in two directions: 1) We will test whether any of the above metals stimulates a phospho-intermediate in AXA2p. This would indicate that a specific metal was recognized by the pump. Although this assay is similar to the ATPase assays already employed, it may avoid the complication that even the correct heavy metal will inhibit the ATPase activity of the pump when used at an “inappropriately high” concentration. 2) We will exhaustively analyze all possible assays for the specific metals showing changes in uptake properties in an AXA2 “knock-out” plant (see below).

2) Determine whether a deletion of the C-terminal or N-terminal domain will generate a hyper-active pump. The approach is to express deletion mutants in yeast, and to evaluate their metal stimulated ATPase activity in purified membrane vesicles. The hypothesis being tested is that an autoinhibitor is located in the N- or C-terminal domain.

Progress:

We have engineered and successfully expressed C- and N-terminal truncations in yeast. However, as with the full-length enzyme, we have been unable to detect an ATPase activity. Nevertheless, our preliminary results indicate that a C-terminal truncation mutant does appear to provide a growth phenotype in yeast.

Continuation Plan:

The exciting result that yeast growth properties can be altered by a mutant AXA2 will be pursued to obtain functional evidence to test the hypothesis that the pump translocates copper, and is regulated by a C-terminal autoinhibitor.

3) Determine if heavy metal uptake can be increased in a transgenic plant by expressing a hyper-active heavy metal pump. The approach is to transform plants with an AXA2 mutant in which the putative autoinhibitory domain has been disrupted.

Progress:

The most significant progress has been the identification of the first “knock-out” of a heavy metal pump in plants. We are now characterizing a homozygous plant line harboring a T-DNA insertion in the N-terminal end of the AXA2 coding sequence (i.e. “knock-out”). In addition, we have obtained 4 plant lines which are over-expressing AXA2p, as determined by immunodetection on Western blots.

Continuation Plan:

‘The knock out and over-expression plants should allow us to evaluate the role of this pump in heavy metal ion accumulation. Plants are currently being grown for ion measurements using an ICP-coupled Mass Spectrometry. If any of the plants show changes in the accumulation of a specific heavy metal, we will refocus our biochemical investigations to exhaustively study that metal. At present, our working hypothesis is still that AXA2 is an uptake pump for copper or molybdenum.

Summary

We are making significant progress towards our original three specific aims. In addition, the identification of an AXA2 knock-out plant line provides an unprecedented opportunity to study the role of heavy metal pumps in the uptake and accumulation of metals in plants.

Dr. Jeff Harper
The Scripps-Research Institute
Dept. of Cell Biology/Plant Division BCC 283
10550 North Torrey Pines Road
La Jolla, CA 92037

Tel.: 619-784-2862
Fax.: 619-784-9840