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

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Research Progress

Soils with high levels of heavy metals such as Cd, Cr and Pb are detrimental to human and animal health. Many human disorders have been attributed to environmental contamination by heavy metals. Removal of heavy metals from highly contaminated soils is therefore a very costly but necessary process that is currently being pursued. Excavation and re-burial of top layer soils is the current, relatively inefficient, approach for decontamination of specific sites. Recent research indicates that uptake of heavy metals into plants via the root system may provide a cost effective approach for decontamination of heavy metal-laden soils. Several mechanisms have been identified which allow detoxification of heavy metals once they have reached the inside of plant cells, including binding to chelating substrates and transporting heavy metals into the plant cell vacuoles which function as storage organelles. However, the molecular biological mechanisms by which heavy metals are transported across the plasma membrane into root cells remain completely unknown. It has been suggested that passive uptake by ion channels, by heavy metal transporters, and by conjugated-metal transporters together could allow concentration of heavy metals by approximately 105-fold inside plant cells with respect to soil concentrations. However, no information has been obtained regarding the plant cDNAs that encode plasma membrane transporters which are able to catalyze the uptake of heavy metals.

By pursuing a genetic approach, we have cloned a cDNA from wheat roots, named PMT1, that shows a novel primary structure and contains several hydrophobic putative membrane-spanning domains. Expression of the PMT1 cDNA enhances the sensitivity of growth to Cd2+. Furthermore, PMT-expressing yeast cells show enhanced uptake of Cd, Pb and Ca2+. Isolation of the wheat root PMT1 cDNA will allow us to address questions regarding mechanisms of heavy metal uptake across the plasma membrane of plant cells and may contribute to testing future bioengineering of heavy metal removal by plants. We propose studies which will test the following hypotheses:

1. PMT1 functions as a plasma membrane heavy metal and Ca2+ uptake transporter isolated from higher plant roots.
2. Overexpression of the PMT1 cDNA in plants can be used to enhance plasma membrane heavy metal uptake into plant tissues.

To test these hypotheses we suggest experiments with the following specific aims:

I. Characterize the selectivity of PMT1 for uptake of toxic heavy metals such as Cd, Pb, Cr, Cu and nutrients such as Ca2+ and Mg2+. Competition among nutrient metals and heavy metals for uptake will further be studied.
II. Determine whether PMT1 itself represents a metal transporting membrane protein. The plasma membrane localization of the PMT1 protein will be analyzed and structural mutations in PMT1 which enhance heavy metal uptake will be isolated using a genetic selection system in yeast.
III. Low-stringency hybridizations will be used to determine whether PMT1 is a member of a larger gene family and RNA expression levels of PMT1s will be analyzed. PMT1 hybridization to mRNAs isolated from known heavy metal hyper accumulating plants will be pursued.

IV. PMT1 and PMT1 mutants that enhance heavy metal uptake will be overexpressed in transgenic plants to determine whether PMT1 expression can enhance heavy metal uptake. Transgenic PMT1 overexpressing plants will be quantitatively analyzed for growth phenotypes, and heavy metal and nutrient accumulation.