

Environmental Management Science Program

Project ID Number 55278

Molecular Genetics of Metal Detoxification: Prospects for Phytoremediation

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June 1, 1998

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Research Objective

We seek to define the genes involved in heavy metal tolerance and sequestration. Two complementary approaches were taken: 1) clone and characterize the genes that complement cadmium hypersensitive mutants of fission yeast, specifically those responsible for production of metal-binding complexes, and 2) isolate genes that can confer cadmium hypertolerance to wild type strains of fission yeast.

Research Progress and Implications

This work summarizes present status of a 3 year project beginning September 1, 1996.

Mutant analysis: Mutant JS282 hypoproduces phytochelatin: A 7 kb genomic DNA fragment complements the mutant. However, the genomic clone on a multicopy vector causes hypersensitivity to cadmium or selenium in wild type strains. Two cDNAs have been isolated and sequenced. A 0.75 kb cDNA is probably not full length, but shows 70% sequence similarity to a *S. cerevisiae* gene suspected to encode a membrane protein. Expression of this cDNA does not confer hypersensitivity to Cd or Se in wild type strains. A second cDNA of 1.8 kb was isolated, but it lacks sequence similarity to proteins in the data base. This 1.8 kb cDNA could confer Cd and Se hypersensitivity to wild type cells.

Mutant JS246 also hypoproduces phytochelatin: A genomic clone that restores Cd tolerance has been isolated. Interestingly, wild type strains harboring the genomic clone on a multicopy plasmid are hypertolerant to cadmium. Three cDNAs corresponding to this genomic clone have been sequenced. cDNA#1 is about 1.3 kb and shows sequence similarity with a bacterial oxidative stress protein; cDNA#2 is about 1 kb and shows similarity to a *S. cerevisiae* gene of unknown function; cDNA#3 is only 0.4 kb and does not show similarity to genes in the data base.

Mutant JS237 fails to accumulate the sulfide-containing phytochelatin-cadmium complex. This mutant was found to have a mRNA splicing defect in a gene homologous to the human Wiskott-Aldrich Syndrome protein (WASP). The human disease symptom is associated with defects in cytoskeletal organization. Likewise, mutant JS237 exhibits aberrant arrangement of polymerized actin. The fission yeast WASP has been found to co-localize with cortical actin and to interact with actin associated proteins. The genomic clone complements the defect associated with the polymerized actin organization, but did not fully restore Cd tolerance. Hence, we suspected that another gene is encoded by the genomic fragment. This led to the identification of an upstream mRNA that overlaps the WASP coding region. This mRNA encodes a P-type ATPase. Most members of this family are associated with cation transport, however, this clone shows strongest sequence similarity to aminophospholipid translocases. We tested for but failed to observe aminophospholipid translocase activity. Thus, the possibility of it being a cation transporter remains open. Constructs have been made to express this protein for production of polyclonal antibodies.

S. pombe cDNAs that confer hypertolerance to cadmium: A cDNA expression library was made in a fission yeast vector such that selection for clones that confer high level tolerance can be made. Some 40 clones were isolated and sequenced. These cDNAs reveal a variety of genes, including one that encodes a Class I (animal-type) metallothionein protein. Hence, fission yeast also has a metallothionein which is not used in response to metal stress, but when overproduced through artificial means, can nonetheless confer tolerance. Other genes encode enzymes in glutathione biosynthesis and proteins that are involved in calcium binding or oxidative stress. Some proteins show no sequence similarity to known proteins.

Plant metallothioneins that confer cadmium hypertolerance to fission yeast: A cDNA library was made from the mRNA of *Brassica juncea* (Indian Mustard), a metal-hyperaccumulating plant. When introduced into fission yeast, cadmium hypertolerant clones were isolated and found to encode Class II metallothionein genes. 3 cDNAs were found which encoded distinct members of the Class II metallothionein (MT) family. A smaller cDNA (369 bp) encoding a Class II type 1 MT (45 amino acids) and two larger cDNAs (492 and 535 bp) encoding a Class II type 2 MTs (80 amino acids each) were sequenced.

Metal binding peptides in *Brassica juncea*: Studies were initiated to define the distinct roles of phytochelatin and metallothioneins in metal hyperaccumulation in *B. juncea*. *B. juncea* seedlings were treated with Cd and/or Cu and were harvested for analysis of the accumulation of phytochelatin peptides and metallothionein mRNA. Treatment of *B. juncea* seedlings with 12.5, 50 and 200 mM Cd resulted in increasing levels of phytochelatin (PCs) predominantly from n=2 to 4 (n is the number of Glu-Cys units). Cu treatment caused only an increase of PC accumulation at 12.5 and 50 mM Cu. At the 200 mM Cu treatment, an inhibition of PC(n=2) and PC(n=4) occurred with a concomitant appearance of an as yet unidentified compound. Based upon the detection method using DTNB, it can only be concluded that this compound contains a reduced sulfhydryl group.

Planned Activities

Molecular genetic characterization of the mutants is progressing from the stages of gene isolation and characterization to the stages of determining protein localization and function. Localization of the protein products will involve generating antibodies against the proteins. Functional analysis, especially of the cDNAs that confer hypertolerance, will involve gene disruption studies to reveal the loss-of-function phenotype. For physiological characterization of metal binding complexes in *B. juncea*, mRNA probes from the metallothionein genes will determine the relationship between their expression and the appearance of phytochelatin peptides. In addition, amino acid composition will be determined for the undefined metal-binding species observed during the HPLC analysis of phytochelatin.

Other Access To Information

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