

Project ID: **55278**

Project Title: **Molecular Genetics of Metal Detoxification: Prospects for Phytoremediation**

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Research Objective:

We seek to define the genes involved in heavy metal tolerance and sequestration. Initially, two complementary strategies 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. During the course of the investigation, we added a third strategy to the existing plan. In this third strategy, we sought to isolate the cDNAs that are specifically expressed during exposure to Cd.

For the past year, the success in isolating a large number of genes using the second and third strategy has redirected our emphasis to concentrating on the characterization of these genes. Consequently, some of the work that was initiated with the first strategy has been put on hold.

Research Progress and Implications:

Strategy #1: Mutant analysis (update from previously reports).

Mutant JS563: This work has been published (*J Biol Chem* 274:12350-13257, 1999). Cd sensitivity in this mutant was found to be due to a mutation in a gene encoding a mitochondrial sulfide:quinone oxidoreductase, which we have named HMT2. Its normal function appears to detoxify excess sulfide in the mitochondria resulting from metal-induced sulfide production. Interestingly, homologous enzymes may be widespread in higher organisms. Sulfide oxidizing activities have been described previously in animal mitochondria, and genes of unknown function, but with similarity *hmt2*⁺, are present in the genomes of flies, worms, rats, mice and humans. This suggests a common tolerance mechanism in diverse higher organisms.

Mutant JS282: The analysis of a 7 kb genomic DNA that complements this mutant, which hypoproduces phytochelatins, led to an interesting 0.8 kb mRNA that is specifically induced by Cd, but not by other metals (Cu, Hg, Se, Zn) or agents that cause oxidative stress or heat stress. The expression of this gene is Cd concentration-dependent, with higher levels of mRNA accumulation corresponding to higher levels of Cd exposure. Removal of Cd from the media leads to rapid down-regulation of mRNA abundance. The promoter for this gene contains putative AP-1 binding motifs and the 3' untranslated region contains four AUUUA motifs that are important for mRNA degradation or stability. We assume that the AUUUA motifs are at least partial responsible for the rapid disappearance of the 0.8 kb transcript upon removal of Cd. The genetic signals that provide metal-specific transcript initiation and stability could be used for engineering of metal-responsive remediation genes.

Strategy #2. Genes that confer hypertolerance.

S. pombe cDNAs. A collection of cDNAs derived from *S. pombe* has been shown to confer high level Cd-tolerance when hyperexpressed in *S. pombe*. Upon further screening and sequencing, we have limited the analysis to 19 unique clones. These have been grouped into 6 classes: 1) Cd binding, 2) Signal transduction, 3) Biochemical pathway genes, 4) Stress related genes, 5) Ribosomal genes, and 6) Unknown (novel) genes. A subset of the genes also confers tolerance to oxidative stress agents such as diamide and tbHOOH.

Brassica juncea cDNAs. 9 cDNAs from Indian Mustard, a metal-hyperaccumulating plant, can confer Cd-hypertolerance to *S. pombe*. To date, 5 have been sequenced, which revealed 4 unique genes, but two of the 4 are different members of the same gene family. Interesting, none shows extensive sequence similarity to genes outside of the plant kingdom. These suggest that they could encode functions that may be unique to plants. One cDNA appears to encode a metalloredutase-like protein.

Strategy #3. Genes expressed specifically during Cd-stress.

Subtractive hybridization was used to isolate a collection of ~250 clones that are expressed during Cd stress. 50 of these have been sequenced. Since this screen was based on expression, but not on function, most of the cDNA clones are not full length. Based on the sequence of these partial genes, many of the deduced peptides appear to share similarity with proteins associated with oxidative stress, while a few have metal binding domains and may be involved in Cd binding. Of particular interest is a clone which encodes a product with sequence similarity to ABC transporters, the class of proteins in which a member has been shown to be involved in vacuolar sequestration of Cd.

Planned Activities:

Due to the large number of genes isolated from *S. pombe* and *B. juncea* by the second strategy, most of the planned activities for the coming year will be devoted to the characterization of these genes. Since these cDNAs already exhibit a metal-hypertolerance function that would be useful for bioremediation, our highest priority is to test their activity in a plant. The planned experiments are: 1) For the 19 *S. pombe* cDNAs, we will engineer their expression in Arabidopsis. 2) For 9 *B. juncea* cDNAs, we will complete the DNA sequence for of the remaining 4 clones. We will then engineer the expression of these cDNAs in Arabidopsis. The transgenic plants will be tested for tolerance and accumulation of Cd. For the *B. juncea* clone that shows sequence similarity to a metalloredutase, we will test biochemical activity.

From the third strategy clones, we will narrow our focus to a few promising candidates. Full length cDNAs from these selected candidates will be isolated and expressed in *S. pombe* and Arabidopsis to test for functional expression.

Information Access:

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Optional Proprietary Information

We have not reported on the identities of the cDNAs described in this report. Should their expression in plants enhance tolerance to or accumulation of Cd, then they could have commercial utility for phytoremediation. In that case, we may file for patent rights.