Environmental Management Science Program

Project ID Number 54898

Molecular Dissection of the Cellular Mechanisms Involved in Nickel Hyperaccumulation in Plants

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June 1, 1998

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Research Objective

Phytoremediation, the use of plants for environmental cleanup of pollutants, including toxic metals, holds the potential to allow the economic restoration of heavy metal and radionuclide contaminated sites. A number of terrestrial plants are known to naturally accumulate high levels of metals in their shoots (1-2 % dry weight), and these plants have been termed metal-hyperaccumulators. Clearly, the genetic traits that determine metal-hyperaccumulation offers the potential for the development of practical phytoremediation processes. Our long-term objective is to rationally design and generate plants ideally suited for phytoremediation using this unique genetic material.

Initially, our strategy will focus on isolating and characterizing the key genetic information needed for expression of the metal-hyperaccumulation phenotype. Recently, histidine has been shown to play a major role in Ni hyperaccumulation. Based on this information we propose to investigate, at the molecular level, the role of histidine biosynthesis in Ni hyperaccumulation in *Thlaspi goesingense*, a Ni hyperaccumulator species

Research Progress and Implications

We have been successful at cloning three key genes involved in histidine biosynthesis in plants, THG1, THD1 and THB1, encoding for ATP phosphoribosyltransferase (ATP PRT), imidazoleglycerol phosphate dehydratase (IGPD), and histidinol dehydrogenase (HD) (Fig. 1), respectively. The genes were cloned by functional complementation of *Escherichia coli* mutants lacking the gene of interest. Complementation of the *E. coli* mutant CGSC 4289 - which lacks a functional ATP PRT gene - by the

Figure 1. Amino acid alignments of histidine biosynthetic genes cloned from the Ni hyperaccumulator *T. goesingense* with the same genes from other species.

T. goesingense THG1 gene is shown in Figure 2 as an example. This type of functional complementation provides strong evidence for the identify of the cloned genes.

We have isolated mRNA from *T. goesingense* exposed to Ni and we are now in the process of using THG1, THB1 and THD1 as probes to study the transcriptional regulation of these genes by nickel, using Northern analysis. All three *T. goesingense* genes (THG1, THB1, THD1) have also been engineered into an *E. coli* expression vectors for expression and purification of the recombinant proteins. We have recently been successful at expressing THG1 in *E. coli* and we are now in the process of purifying the recombinant His-tagged ATP PRT (Fig. 3). From the SDS-PAGE gel, the plant ATP PRT has a molecular weight of approximately 48 kd, which compares well with that of other ATP PRT's from different sources. Once purified, the activity of ATP PRT will be studied for allosteric regulation by nickel and hisitdine. The ATP PRT enzyme from *E. coli* is strongly inhibited by its product, phosphoribosyl ATP, and our preliminary studies suggest this is also true for our plant enzyme.

Figure 2. Functional complementation of a HisG⁻ mutant *E. coli* (CGSC 4289), unable to grow in the absence of histidine, with the *Thlaspi* THG1 gene. Minimal medium lacking histidine; A, HisG⁻ transformed with pTrip-THD1; B, HisG⁻ transformed with pTrip-THG1; C, Wild-type TOP10F⁺ with p Trip-THG1. Similar results have also been with THD1 and THB1.

To efficiently assay the *T. goesingense* ATP PRT activity, it will be necessary to remove the product of the reaction by adding the product degrading next enzyme in the sequence, phosphoribosyl ATP pyrophosphatase. This enzyme is not available commercially, therefore, we are in the process of cloning it by PCR from *E. coli* (Fig. 4). Plasmid DNA containing the *E. coli* histidine operon has been obtained from Dr. D.J. Savic and Dr. C.B. Bruni. Using primers specific to the His I gene, encoding phosphoribosyl ATP pyrophosphatase, we have successfully amplified a 680 bp DNA fragment which should correspond to the His I gene (Fig. 4). We will clone this fragment and check its identity by functional complementation of a His I⁻ *E. coli* mutant. Once the identify of the fragment as His I (encoding phosphoribosyl ATP pyrophosphatase) is confirmed, we will engineer it into an *E. coli* expression vector, purify the recombinant protein for use in the ATP PRT enzyme activity assay.

Figure 3. SDS-PAGE gel of total protein from *E. coli* transformed with the pET-30 expression vector containing the THG1 gene. Cells were induced at 22°C with 0.4 mM IPTG for 3 h, resulting in expression of the THG1 gene and accumulation of the gene product (see arrow).

Figure 4. PCR products from amplification of *E. coli* His I gene. A. DNA ladder; B, PCR reaction containing expected 680 bp fragment (see arrow)

Planned Activities

If Northern analysis of mRNA shows significant regulation of THG1, THD1 and THB1 by Ni, we will raise antibodies against the recombinant proteins and measure the level of the proteins in plants after exposure to Ni. We will also perform *in-situ* hybridizations using the THG1, THD1 and THB1 genes as probes to determine the tissue localization of Ni regulation of these genes. We will also make transgenic *Arabidopsis thaliana* over expressing THG1, THD1 and THB1 to determine if increased production of histidine is sufficient to allow increased accumulation of Ni by plants.