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Project Title: **Molecular Dissection of the Cellular Mechanisms Involved in Nickel Hyperaccumulation in Plants**

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MOLECULAR DISSECTION OF THE CELLULAR MECHANISMS INVOLVED IN NICKEL HYPERACCUMULATION

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PROJECT SUMMARY

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 determines 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.

- We will clone key genes involved in histidine biosynthesis.
- We will characterize their transcriptional and post transcriptional regulation by histidine, Ni.
- We will determine if any of these genes are essential and sufficient for Ni hyperaccumulation by their expression in the non-hyperaccumulator *Arabidopsis thaliana*.

CLONING THE GENES INVOLVED IN HISTIDINE BIOSYNTHESIS IN *T. goesingense*

In our last progress report (1997 – 1998) we outlined how we had successfully cloned and characterized *THG1*, *THD1* and *THB1*, encoding for the enzymes ATP phosphoribosyltransferase (ATP PRT), imidazoleglycerol phosphate dehydratase (IGPD), and histidinol dehydrogenase (HD), respectively. We have now used these cloned genes to investigate the role of histidine in Ni hyperaccumulation in *T. goesingense.*

REGULATION OF HISITIDINE BIOSYNTHESIS BY NICKEL IN *T. goesingense*

In *E. coli* ATP PRT is an important control point for histidine biosynthesis, being regulated at the level of transcription, translation and allosteric activation/inhibition. The highly regulated nature of ATP PRT in *E. coli* suggests that it might also play a key regulatory role in plants, making it a likely target for regulation of histidine biosynthesis by Ni. However, Northern analysis of the *THG1* RNA message clearly demonstrated that Ni does not induce or suppress transcription of the *THG1* mRNA in either the roots or shoots of *T. goesingense* (Fig 1). Because it is possible that ATP PRT is not the key regulated step in histidine biosynthesis in plants we also analyzed expression of two other genes in the histidine biosynthetic pathway, *THB1* and *THD1*, which encode the enzymes IGPD and HDH respectively. The IGPD enzyme catalyzes the conversion of imidazolglycerol phosphate to imidazoleacetol phosphate, the first step after the branch point, which feeds into the purine recycling pathway. This enzyme also catalyzes the conversion of L-histidinol phosphate to L-histidinol. Because of its key position in the histidine biosynthetic pathway this enzyme may also be regulated. Histidinol dehydrogenase (HDH) catalyzes the oxidation of L-histidinol to L-hisitidine, the final step in histidine biosynthesis. Because this catalytic step uses NAD^+ as an oxidant it is possible that it is also regulated.

Northern analysis of the mRNA levels for both *THB1* and *THD1* clearly showed that expression of these mRNAs is not induced or repressed by Ni treatment in either the roots or the shoots of *T. goesingense* (Fig 1). Because *THG1*, *THB1* and *THD1* mRNA expression levels are not changed by Ni treatment, it is

unlikely that control of the histidine biosynthetic pathway, at the transcriptional level, by Ni is involved in Ni hyperaccumulation in *T. goesingense*.

To determine if Ni modifies histidine biosynthesis at the post-translational level in *T. goesingense* we also analyzed the concentration of free histidine in root, xylem sap and shoot tissue. It is clear from this data (Table 1) that histidine concentrations remain basically unchanged after Ni exposure in both the xylem sap and the shoots. Interestingly, we did observe a large decrease in histidine concentrations in the root tissue after Ni exposure, however this was not reflected in the expression levels of *THG1*, *THB1* or *THD1* in the roots. At present we have no explanation for this loss of histidine, however it may reflect either increased catabolism or efflux of histidine in roots. However, recent analysis of *T. goesingense* root exudate showed no increases in rates of histidine exudation from roots after exposure to Ni (Salt et al., 1999a).

This biochemical data strongly supports the molecular evidence that free histidine concentrations in *T. goesingense* are not regulated by Ni exposure. It is possible, however, that the constitutive concentration of free histidine observed in *T. goesingense* is sufficient to fulfill its theorized role in Ni hyperaccumulation. To test this hypothesis we compared the histidine concentration in *T. goesingense* and the non-accumulator *T. arvense*. This comparison revealed that the non-accumulator *T. arvense* contained equal concentrations of histidine in both roots, shoots and xylem sap as that found in *T. goesingense* during Ni exposure. The histidine concentration in the xylem sap of *T. goesingense* after Ni exposure is also similar to that measured in other non-accumulators. This strongly supports the case that there are no significant differences in histidine metabolism between the accumulator and non-accumulator species of *Thlaspi*.

If free histidine is involved in the hyperaccumulation of Ni, as has been suggested occurs in *Alyssum* species, we would predict that histidine binds Ni within the plant. To directly address this hypothesis we used X-ray absorption spectroscopy (XAS) to determine the *in planta* coordination environment of the Ni in both the hyperaccumulator and non-accumulator *Thlaspi* species (Table 2). From this data it is clear that histidine or a histidine-like molecule is involved in coordinating Ni in both the roots and shoots of *T. goesingense* and *T. arvense*. However, the concentration of the Ni-histidine complex in the shoots of the non-accumulator *T. arvense* appears to be approximately 5-10 fold higher than in *T. goesingense*, and equal in the roots. Again, supporting the conclusion that free histidine does not play a key role in Ni hyperaccumulation in *T. goesingense.*

FUTURE DIRECTIONS

We are now in the process of overexpressing *THG1*, *THB1* and *THD1* in *A. thaliana*, to determine if any of these genes are essential and sufficient for Ni hyperaccumulation.

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Table 1. Free histidine content of *Thlaspi goesingense* tissues exposed to 50 µM Ni for 7 days

	SHOOT	ROOT	XYLEM SAP
	nmol g^{-1} fresh biomass		*µmol L^{-1}*
T. goesingense			
Control	136 ± 37 (4)	742 ± 188 (4)	7.4 ± 3 (4)
Nickel treated	107 ± 62 (5)	68 ± 30 (7)	18.2 ± 9 (6)
T. arvense			
Control	73 ± 16 (4)	43 ± 9 (3)	57 ± 31 (8)
Nickel treated	n.a.	n.a.	n.a.

Histidine was measured by HPLC as the phenylthiocarbamyl amino acid derivative with methionine sulfoxide as the internal standard. n.a.: not available.

Table 2. Nickel coordination by histidine ligands in *T. goesingense* and *T. arvense* measured by X-ray absorption spectroscopy (XAS)

	T. goesingense		*T. arvense*	
Time (d)	Shoot	Root	Shoot	Root
	mmol Kg^{-1} Dry Biomass			
1	0.2	2.5	0.4	2.9
3	0.1	2.5	1.0	3.9
5	0.4	2.6	1.2	4.3
7	0.2	4.1	1.3	6.1

Plants were exposed to 10 µM $Ni(NO_3)_2$ in the hydroponic solution. By fitting X-ray absorption spectra of aqueous Ni^{2+} and Ni^{2+} coordinated with histidine (6.66 mM $Ni(NO_3)_2$, 80 mM histidine, 30% glycerol, pH 7.0), citrate (6.66 mM $Ni(NO_3)_2$, 70 mM citrate, 30% glycerol, pH 8), glutamine (1 mM $Ni(NO_3)_2$, 4 mM glutamine, 30% glycerol, pH 7.3), and isolated *T. goesingense* shoot cell wall material, we were able to determine the contribution of histidine as a ligand of Ni^{2+}.

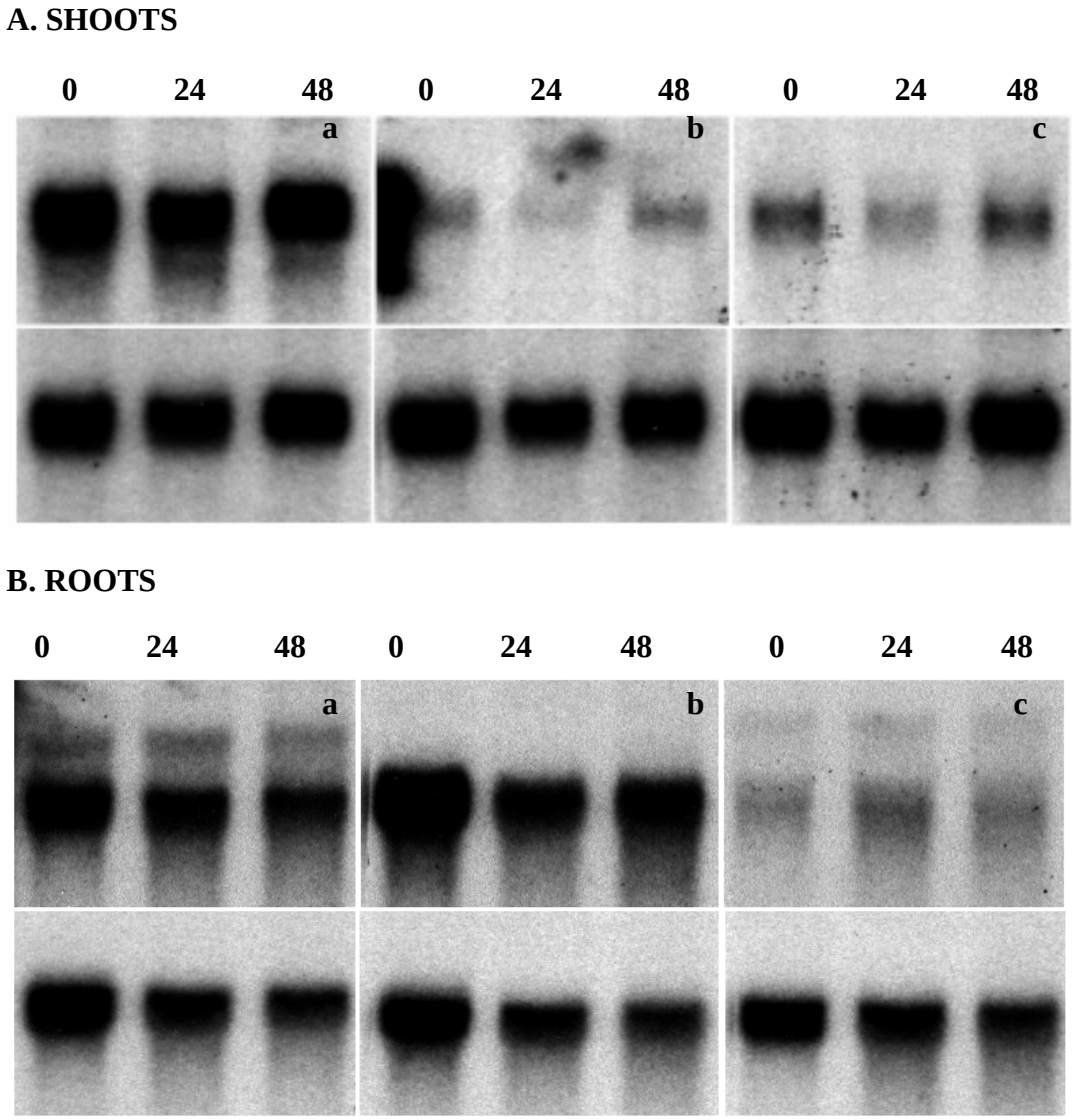

Figure 1. Northern blot analysis of the expression of *THG1*, *THD1*, and *THB1*. Total RNA was isolated from *T. goesingense* root and shoots tissue and probed with ^{32}P labeled *THG1*(**a**), *THB1*(**b**) and *THD1*(**c**) cDNA's probes (upper panel). Total RNA was also probed with an *A. thaliana* ^{32}P labeled actin cDNA fragment as a loading control (lower panel).