POTENTIAL OF PLANT GENETIC ENGINEERING FOR PHYTOREMEDIATION OF TOXIC TRACE ELEMENTS

Nathalie Verbruggen
Laboratoire de Physiologie et Génétique Moléculaire des Plantes, Université Libre de Bruxelles, Campus Plaine (CP 242) Bd du Triomphe, 1050 Brussels, Belgium.

Danika LeDuc
Department of Chemistry & Biochemistry, California State University, East Bay, 25800 Carlos Bee Boulevard, Hayward, CA 94542, USA.

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Summary

Successful phytoremediation of toxic trace elements depend on many factors: plant genotype, the environment and the interactions between the plant genotype and its environment. Potential of plant genetic engineering is here discussed primarily in the framework of improving phytoextraction efficiency. Efforts to optimize remediation of Cd and Hg, two of the most harmful heavy metals and environmental threats, are
emphasized. Potential targets that have been or might be manipulated to improve transport, sequestration or detoxification processes are reviewed. Targets of genetic engineering include genes involved in the uptake of trace elements from the soil to the root, translocation from the root to the shoot, vacuolar sequestration, and chelation. Successes in genetically modifying plants to phytoextract Se or Hg are detailed.

1. General Introduction

The term phytoremediation simply means using plants and their associated microbes to clean up toxic substances in the environment (Terry et al., 2000). The many possible ways in which plants can achieve this include phytoextraction, phytovolatilization, detoxification, and sequestration. Phytoextraction and phytovolatilization are particularly attractive in that their use has the potential of moving the contaminants from the local ecosystem altogether. In phytoextraction, plants take up the contaminant of interest from soil, sediment, or water, and accumulate it. In order to achieve this, the plant may need some mechanism of protecting itself from the toxic effects of the contaminant. As such, phytoextraction often involves phytodetoxification – the conversion of a contaminant from a more toxic to less toxic form – or a means of internal sequestration to a less sensitive part of the plant, such as the vacuole. Phytoextraction is most successful when the plant is able to transport the contaminant from the roots to the shoots. In such a case, harvesting the aboveground biomass succeeds in removing the contaminant from the local ecosystem. Phytovolatilization is relevant to those trace element contaminants that can be metabolized to a volatile form. Well-studied examples are selenium (Se), volatile forms of which include dimethylselenide and dimethylselenide amongst several others, and mercury (Hg), which is volatile in its elemental form (Terry et al., 2000; Rugh et al., 1996). The advantage of phytovolatilization is clear – the plant does the work of synthesizing the volatile form and releases it to the atmosphere where it is diluted and joins the global cycle of the corresponding element.

It is important to remember that these processes occur naturally in plants, with wide variation observed depending on plant species (and ecotype), trace element contaminant, and geologic and environmental conditions. The primary advantages of phytoremediation are its low cost and low management requirements (Bañuelos et al., 2002). Growing plants is much cheaper than the excavating, removing, soil-washing, or capping technologies often used (Cunningham et al., 2000). Management of a phytoremediation plot becomes a matter of farming – fertilization, perhaps, and irrigation when needed, whereas bacteria-based bioremediation (biopiles) requires constant amendments (Cookson, 1995). The disadvantage is the long timescale that would be necessary for natural, phytoremediation processes to remediate a local environment.

Spurred on by the potential benefits of widespread implementation of phytoremediation, researchers have sought plant species that could remove contaminants at especially fast rates. Plants suitable for phytoextraction should possess exceptional qualities such as high capacities for absorption, root/shoot translocation, and detoxification of the metals or non-metals to be extracted and, last but not least, a high biomass and preferably a rapid growth rate.
High levels of tolerance to specific metals are known to occur but the majority of metal tolerant species are excluders. The combination of high tolerance and high accumulation of heavy metals can be found in a rare class of plants called hyperaccumulators. These species (more than 400 have been identified) can accumulate exceptional concentrations of heavy metals in their aerial parts without visible toxicity symptoms. Brooks defined a hyperaccumulator as a plant able to accumulate high concentrations of heavy metals in the shoot. The concentrations accumulated are about 100 times those that occur in non-accumulator plants growing in the same substrates (Brooks, 1998). Levels to define hyperaccumulators are 1% of shoot dry weight (SDW) for zinc (Zn), 0.1 % SDW for nickel (Ni), lead (Pb), copper (Cu), and cobalt (Co); and 0.01% SDW for cadmium (Cd). Hyperaccumulators of non-metal toxic trace elements like Se have also been identified (> 0.1% SDW). However, most hyperaccumulators do not develop sufficient biomass, even in the absence of high concentrations of heavy metals to be useful in phytoextraction and/or are not amenable to agronomic practices (mechanical harvest). However this argument does not apply to all hyperaccumulator species. Some of the 300 Ni hyperaccumulators identified, such as Alyssum murale, seem to develop sufficient biomass and consequently important mineral mass (Li et al. 2003, Chaney et al. 1997, 2005). Stanleya pinnata is a selenium hyperaccumulator that could also be used in phytoremediation as well (Feist and Parker 2001; Parker et al. 2003).

For other metals, hyperaccumulating plants have not yet been identified or seem to be very rare. With respect to Cd, for instance, only three low biomass, slow growing Brassicaceae species (Thlaspi caerulescens, Thlaspi praecox and Arabidopsis halleri) have been described as hyperaccumulators. Moreover, their development of leaves in rosettes at the ground level impedes mechanical harvest. Nevertheless, some authors believed in the potential of the polymetallic hyperaccumulator, Thlaspi caerulescens, for direct use in phytoremediation (Chaney et al. 2005) because of its exceptional capacity of heavy metal (Zn, Cd, and Ni) accumulation.

To create suitable plants for phytoremediation, one possibility is the selection of high biomass plants like poplar, Brassica juncea, or tobacco with the highest tolerance and accumulation capacities, provided that the species displays sufficient genetic variation for those characteristics. Another avenue of recent research is the association of metal tolerant micro-organisms like bacteria or fungi to enhance tolerance. In this alternative, the genetic engineering of plant-associated bacteria seems promising (Barac et al. 2004; Taghavi et al. 2005; van der Lelie et al. 2005). Improvement of hyperaccumulator biomass is also envisaged by the genetic manipulation of plant hormone levels (Eapen and D’Souza 2005). However, no concrete example has been published up to now.

Finally, genetic engineering of plants can substantially improve phytoremediation efficiency of plants. This review will focus on the genetic engineering of plants to improve the efficiency of phytoextraction of heavy metals and Se (which is a non-metal) from contaminated soils. Of major concern with respect to health and environment concerns are the metalloids, arsenic and selenium, and the metals, cadmium and mercury. Genetic manipulation has aimed to improve accumulation, tolerance and detoxification capacities of high biomass and rapid growing plants in order to optimise the phytoextraction process.
2. General Consideration in Choosing Target Genes for Phytoremediation

Heavy metal tolerance is not a monogenic trait. Some researchers have proposed that there is no correlation between metal accumulation and tolerance and that these characters are independent, which means that several genes may have to be transferred to optimize phytoremediation performance (Baker and Walker 1990; Bert et al. 2003; Macnair et al. 1999). The choice of genes to be transferred is difficult. Many genes are involved in metal uptake, translocation, and sequestration. The genes to be transferred depend on the heavy metal to be extracted from the soil. At least three categories of genes are generally considered: transporters, proteins involved in metal chelation, and metabolic enzymes involved in detoxification. Transgenic plants with improved metal uptake and/or sequestration have been developed for cadmium, zinc, lead, mercury, arsenic and selenium.

The genes that have been transferred, thus far, are mainly cloned from micro-organisms for which the tolerance or detoxification mechanisms were known or from the non-tolerant, non-accumulator model plant species, Arabidopsis thaliana but usually not from hyperaccumulators. A lack of understanding of the mechanisms involved and responsible genes has impeded rapid progress. A common strategy to identify genes necessary to tolerate heavy metals or non-metals is to study plants that are naturally adapted to those toxic trace elements. Hyperaccumulators constitute an exceptional biological material and gene reservoir to understand adaptation to extreme metallic environments. Recently transcriptomic studies on hyperaccumulators have provided novel insights into the molecular mechanisms underlying metal tolerance and accumulation as well as access to the identification of a large array of genes which are constitutively (in the absence of excess of metallic ions) overexpressed and are thought to be involved in the hyperaccumulation trait (Weber et al. 2004; Weber et al. 2006; Becher et al. 2004; Talke et al. 2006; Craciun et al. 2006; van de Mortel et al. 2006, Filatov et al. 2006). Classic genetic studies and recent QTL analysis of hyperaccumulators support the existence of only few genes that are responsible for tolerance or accumulation (Assunção et al. 2006; Deniau et al. 2006, Courbot et al. 2007, Willems et al. 2007). Only one gene co-localizing with a QTL for Cd tolerance and Zn tolerance has been published (Courbot et al. 2007, Willems et al. 2007). Not understood is the origin of constitutive gene overexpression in hyperaccumulators. Interestingly, constitutive expression of existing genes seems to be a common process in the adaptation of plants to extreme environments (Taji et al. 2004).

Here we will focus on genes encoding for transporters or involved in metal chelation whose overexpression already conferred higher tolerance or accumulation to heavy metals and some non-metals or are thought to be good candidate genes to improve those traits in high biomass plants. Many proof of concept studies have been published with the model plant Arabidopsis thaliana, awaiting validation in plant species more amenable for phytoremediation. Up to now, the constitutive 35S CaMV promoter was extensively used. However to fine-tune toxic elements accumulation, targeted expression of genes encoding transporters or enzymes involved in chelation will be necessary in order to avoid futile cycles. For example, the constitutive overexpression (in all tissues of the plant), of a plasma membrane pump or transporter involved in the efflux of a toxic trace element may result in futile cycling inside the plant without
substantial accumulation in harvestable parts. Novel powerful tools to control the timing, the location and the level of transgene expression are expected from the characterization of native promoters of hyperaccumulators.

3. Genetic Engineering of Metal Transporters

Transport mechanisms of metal or metalloid ions are extremely complex and diverse. Metal uptake, metal partitioning to plant organs and cell types, and metal delivery in sub-cellular localizations including metal storage in vacuoles require metal transporters. For simplicity, the processes to be considered are either localized at the soil – root passage, root to shoot translocation, or at the cellular level, the storage to the vacuole from the cytoplasm. Depending on the limiting steps for the envisaged metals, one, two, or all three processes may need to be enhanced. Metal transporters that we will consider here in light of their relevance to phytoremediation are involved in:

1. Uptake of metal from the soil to the root
2. Translocation from the root to the shoot
3. Detoxification by storage in the vacuoles

3.1. Uptake of Metal from the Soil to the Root

Several genes encoding transporters at the plasma membrane of root epidermal cells have been identified. There is to our knowledge no published example of genetic manipulation within the framework of phytoremediation to enhance that transport system in order to increase absorption of toxic metals or metalloids from the soil solution to the roots.

Toxic trace elements that we consider in this review (e.g. Cd, Hg, As, and Se) enter plant cells via uptake systems for essential ions. Some of the transporters of these essential ions have been identified, for example, by virtue of their induction upon mineral deficiency conditions, like Zn or Fe deficiency. For example, several ZIP (zinc-regulated transporter, iron-regulated transporter protein) transporters are induced upon deficiency and are involved in the cellular uptake of Zn across plasma membrane. Interestingly, several ZIP members are overexpressed in A. halleri and T. caerulescens (ZIP 3, 4, 6, 9, and 10 and IRT3) and are thought to play a role in the enhanced root metal uptake of those Zn hyperaccumulators (Kramer et al 2007). IRT1, one of the first discovered members of the large ZIP family, is involved in the uptake of Fe$^{2+}$ from the soil. IRT1 is induced upon Fe deficiency and also is responsible for the uptake of heavy metals such as zinc, manganese, cobalt, and cadmium. Plants overexpressing IRT1, engineered to improve Fe nutrition, also accumulated more cadmium (Connolly et al. 2002).

Unless specific modifications of the transporters would increase their affinity towards the pollutants, overproduction of uptake systems for essential ions might deplete the soil in those nutrients. Nevertheless, there are several examples of enhanced absorption of metals as a result of increased capacity to sequester them in the vacuoles (see point 3 of this section).
3.2. Translocation from the Root to the Shoot

Translocation from the root to the shoot is a major step for phytoextraction, as above-ground biomass will be harvested. Efficient translocation of metal ions to the shoot requires radial symplastic passage, an active loading into the xylem, and the availability of suitable ligands in the xylem (Clemens, 2006).

The study of Zn and Cd hyperaccumulators has allowed the identification of P-ATPase as an important player for the passage from the cytosol of root cells into the vascular tissues.

-P-ATPase-HMA

The P1B-type ATPases, also known as the Heavy Metal transporting ATPases (HMAs), play an important role in transporting transition metal ions against their electrochemical gradient using the energy provided by ATP hydrolysis. They have been identified in all living organisms from archaea to humans, including yeast and plants. The HMAs cluster into two classes: those transporting monovalent cations (Cu/Ag group) and those transporting divalent cations (Zn/Co/Cd/Pb group: Axelsen and Palmgren 2001). Although all classes of P1B-type ATPases have been detected in prokaryotes (Rensing et al. 1999), in non-plant eukaryotes only Cu/Ag P1B-type ATPases have been identified to date. A. thaliana and Oryza sativa contain eight HMA genes (Baxter et al. 2003), suggesting that these enzymes play important roles in the transport of metals in plants. In A. thaliana, analysis of the complete genome sequence predicts the distribution of these eight HMAs in two classes: HMA1-4 for the transport of Zn/Co/Cd/Pb, and HMA5-8 for Cu/Ag.

HMA4 was the first plant P1B-type ATPase of the divalent transport group to be cloned and characterized in A. thaliana (Mills et al. 2003). A role for HMA4 in Zn homeostasis, Cd detoxification, and in the translocation of these metals from the root to the shoot has been demonstrated in A. thaliana (Hussain et al. 2004; Mills et al. 2003, 2005; Verret et al. 2004, 2005). In the Cd/Zn hyperaccumulators, A. halleri and T. caerulescens, HMA4 is more highly expressed in both roots and shoots compared with Cd/Zn-sensitive close relatives (Bernard et al. 2004, Papoyan et al. 2004, Talke et al. 2006, Courbot et al. 2007). The elevated expression of HMA4 in two different Zn/Cd hyperaccumulator species that evolved independently strongly supports the idea that HMA4 plays an important role in tolerance to both metals. In A. halleri at least, this hypothesis is reinforced by the QTL analysis of Cd and Zn tolerance performed, indicating the co-localization of major QTLs of Zn and Cd tolerance with the HMA4 gene (Willems et al. 2007; Courbot et al. 2007). HMA4 is currently the only gene for which there is genetic evidence for a role in both Zn and Cd tolerance. HMA4 would be involved in the detoxification of roots by translocating Cd and Zn to the shoot. As observed in AtHMA4-overexpressing lines (Verret et al. 2004), which translocate more Cd and Zn to the shoot and are also more tolerant to Cd and Zn excess, the increased expression of HMA4 in A. halleri or T. caerulescens might be related to the increased Cd and Zn tolerance and hyperaccumulation phenotype of these species (Bernard et al. 2004, Courbot et al. 2007, Popayan et al. 2004, Talke et al. 2006 and Willems et al. 2007).
The study of hyperaccumulators has also revealed the overexpression of metal ligands or metal-ligand complexes transporters, which are thought to play a role in metal long-distance transport.

**MATE**

MATE is a large family of multi-drug and toxin extrusion (or efflux) membrane proteins. Some members of the family were shown to function as drug/cation antiporters that remove toxic compounds and secondary metabolites from the cytosol by exporting them out of the cell or sequestering them to the vacuole (Delhaize et al. 2007). FRD3 is a member of the MATE subfamily, which is thought to efflux citrate in the root vasculature. Citrate is necessary for the transport of Fe and most likely also that of Zn (Durrett et al. 2007). Interestingly FRD3 is constitutively overexpressed in *A. halleri* compared to its close relative *A. thaliana* and may play a role in Zn translocation (Talke et al. 2006). Few MATE members have been characterized in plants up to now. MATE members other than FRD3 might be involved in metal transport.

**OPT:**

OPT is a superfamily of oligopeptide transporters including the yellow-stripe 1-like (YSL) subfamily. The AtYSL gene family was identified in Arabidopsis following cloning and characterization of maize YS1. YSL is a plasma membrane-localized transporter protein that is necessary for acquisition of Fe by uptake of Fe(III)-phytosiderophores (Curie et al., 2001). Dicotyledons do not synthesize phytosiderophores, but the structurally related non-protein amino acid nicotianamine (NA). Some YSL transporters are involved in the loading and unloading of nicotianamine-metal chelates. There is evidence for a role of YSL transporters in the Zn and Ni hyperaccumulation of *Thlaspi caerulescens*, especially for TcYSL3 and TcYSL7 expressed around the root vascular tissues (Gendre et al. 2007). YSL3 was shown to transport Ni-NA chelates.

Other OPT members may be involved in the transport of metal chelates, such as AtOPT6, which is able to transport glutathione derivatives and Cd-glutathione complexes (Cagnac et al. 2004). However no demonstration of a clear role relevant to phytoextraction has been published up to now.

**3.3. Vacuolar Transporters**

For metals that cannot be volatilised, vacuolar storage in the leaves is thought to be the main pathway of detoxification, and this process is usually limiting in non-tolerant species.

**CDF-MTP:**

The family of Cation Diffusion Facilitators also named in plants Metal Transporter Proteins contains members involved in the transport of Zn$^{2+}$, Fe$^{2+}$, Cd$^{2+}$, Co$^{2+}$, and Mn$^{2+}$ not only from cytoplasm to organelles or apoplasm, but also from the cytoplasm to the endoplasmic reticulum (Peiter et al. 2007). ZAT (Zinc transporter of *Arabidopsis thaliana*) recently renamed AtMTP1 encodes a Zn transporter suggested to be involved
in vacuolar sequestration in *Arabidopsis thaliana*. Overproduction of the Zn transporter ZAT in *A. thaliana* resulted in higher Zn tolerance and a 2-fold higher Zn accumulation in roots (Van der Zaal et al. 1999). ShMTP is involved in the vacuolar storage of Mn in the Mn tolerant *Stylosanthes hamata*, which is a tropical legume. *ShMTP* conferred higher tolerance and accumulation of Mn when overexpressed in *Arabidopsis thaliana* (Delhaize et al. 2003).

**CAX:**

CAX is the acronym of CAtion eXchanger. It is a large family of membrane proteins, which was recently subdivided in “true” CAX and CCH (calcium cation exchanger). Some of the CAX members of *A. thaliana* have been characterized, and all seem to be involved in metal vacuolar sequestration up to now. *In vitro* studies have shown a Cd²⁺/H⁺ antiport activity in tonoplast-enriched vesicles from oat roots with a *Kₘ* of 5.5 µM (Salt and Wagner 1993). In his review, Clemens questioned the availability of Cd²⁺ ions in the micromolar range (Clemens, 2006). Still, there is experimental evidence that AtCAX2 and AtCAX4 can transport Cd²⁺ in the vacuoles. Under the activity of the constitutive 35S CaMV promoter, expression of *AtCAX2* and *AtCAX4* resulted in higher accumulation of Cd in the root vacuoles (Korenkov et al. 2007). It would be interesting to test overexpression of CAX vacuolar transporters using a leaf specific promoter to see whether it can also increase Cd accumulation in the leaves.

**ABC:**

The superfamily of ABC (ATP-Binding Cassette) transporters is involved in many physiological processes. Some of the ABC transporters are involved in vacuolar sequestration of various metals or xenobiotics. In two subfamilies, MRP and PRD, members are involved in the transport of chelated heavy metals or in the organic acid necessary for the transport of heavy metals.

Overexpression of the YCF1 MRP member of *Saccharomyces cerevisiae* conferred higher capacity of lead and cadmium accumulation in *Arabidopsis thaliana* by enhanced transport of Cd-GS2 in the vacuoles (Song et al. 2003).

Hmt1 is the transporter (ABC half-size family) of PC-Cd complexes in the vacuoles of *S. pombe* (Ortiz et al. 1995). Enhancing this process may be relevant in the framework of optimizing phytoremediation efficiency. However, there is no true homolog of Hmt1 identified in any plant up to now, and overexpression of *Hmt1* in plants has not been reported thus far. The closest homolog in plant is the mitochondrial ATM3, also involved in Cd tolerance (Hanikenne et al. 2005; Kim et al. 2006)
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LeDuc D.L., AbdelSamie M., Montes-Bayon M., Wu C.P., Reisinger S.J., Terry N. (2006) Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phyto remediation traits in Indian mustard. Environ. Poll. 144: 70-76. [The authors constructed and characterized transgenic Brassica juncea overexpressing both ATP sulfurylase and selenocysteine methyltransferase to combine increased selenate uptake with increased selenocysteine detoxification.]

Lee J., Donghwan S., Song W. Y., Hwang N. Lee Y. (2004) Arabidopsis metallothioneins 2a and 3 enhance resistance to cadmium when expressed in Vicia faba guard cells. Plant Molecular Biology 54: 805–815. [The experiments presented here constitute a strong support for the in vitro capacity of 2 Arabidopsis thaliana metallothioneins to chelate Cd$^{++}$. Two Arabidopsis thaliana metallothioneins were expressed in guard cells of Vicia faba after biolistic bombardment and transformed cells were analyzed after Cd$^{++}$-treatment.]


Li Y.M., Dankher O.P., Carreira L., Lee D., Schroeder J.I., Balish R.S., Meagher R.B. (2004) Overexpression of phytochelatin synthase in Arabidopsis leads to enhanced arsenic tolerance and cadmium hypersensitivity. Plant Cell Physiology 45: 1787-1797. [The paper presents the analysis of Arabidopsis thaliana plants overexpressing phytochelatin synthase 1, which are more sensitive to cadmium stress while being more resistant to arsenic.]

Logan H.M., Cathala N., Grignon C., Davidian J.C. (1996) Cloning of a cDNA encoded by a member of the Arabidopsis thaliana ATP sulfurylase multigene family: expression studies in yeast and in relation to plant sulfur nutrition. J. Biol. Chem. 271: 12227-12233. [This paper reports the cloning of an ATP sulfurylase cDNA sequence in Arabidopsis thaliana that is highly similar to a sequence in Brassica napus with increased expression upon sulfur starvation.]

Lund P.A. and Brown N.L. (1987) Role of the merT and merP gene products of transposon Tn501 in the
induction and expression of resistance to mercuric ions. *Gene* 52: 207-214. [This paper describes the roles of merT and merP gene products in mercury binding and transport.]

Macnair M.R., Bert V., Huitson S.B., Saumitou-Laprade P., Petit D. (1999) Zinc tolerance and hyperaccumulation are genetically independent characters. *Proc Biol Sci* 266: 2175-2179. [This paper supports the independence of Zn tolerance and hyperaccumulation characteristics in Arabidopsis halleri. Results also support the existence of a single major gene for Zn tolerance.]


Mills R.F., Krijger G.C., Baccarini P.J., Hall J.L.,Williams L.E. (2003) Functional expression of AtHMA4, a P-1B-type ATPase of the Zn/Co/Cd/Pb subclass. *Plant J* 35: 164–176. [This is the first report about a P-1B-type ATPase of the Zn/Co/Cd/Pb subclass in plants.]


Misra T.K., Brown N.L., Haberstroh L., Schmidt A., Goddette D., Silver S. (1985) Mercuric reductase structural genes from plasmid R100 and transposon Tn501: functional domains of the enzyme. *Gene* 34: 253-262. [The coding regions following merC from R100 and Tn501 are compared to show high sequence similarity, and amino acids in the mercuric reductase active site and substrate binding positions are shown to be highly conserved.]

Moffat A.S. (1995) Plants proving their worth in toxic metal cleanup. *Science* 269: 302-303. [This Science news article discusses early work in phytoremediation and potential concerns of its field use including transgene spread and how risk-benefit analysis must be done to ensure that harm to the ecosystem is reduced in the accumulation and volatilization of toxins via plants.]

Moore B. (1960) A new screen test and selective medium for the rapid detection of epidemic strains of...
Staphylococcus aureus. Lancet ii: 453-458. [This paper describes the discovery of mercury-resistant bacteria.]

Murillo M. and Leustek T. (1995) Adenosine-5'-triphosphate-sulfurylase from Arabidopsis thaliana and Escherichia coli are functionally equivalent but structurally and kinetically divergent: nucleotide sequence of two adenosine-5'-triphosphate-sulfurylase cDNAs from Arabidopsis thaliana and analysis of a recombinant enzyme. Arch. Biochem. Biophys. 323:195-204. [This manuscript reports on the cloning of the second and third discovered ATP sulfurylase cDNAs in Arabidopsis thaliana and describes specific activities of their enzyme products.]


Noctor G., Strohm M., Jouanin L., Kunert K.J., Foyer C.H., Rennenberg H. (1996) Synthesis of glutathione in leaves of transgenic poplar overexpressing γ-glutamylcysteine synthetase. Plant Physiology 112: 1071-1078. [This paper is an example of how glutathione content can be increased in plants through the overexpression of the limited γ-glutamylcysteine synthetase and also describes how cysteine is the limiting reactant in the production of glutathione.]

Nussbaum S., Schmutz D., Brunold C. (1988) Regulation of assimilatory sulfate reduction by cadmium in Zea mays L. Plant Physiology 88: 1407-1410. [Here it is shown that ATP sulfurylase and adenosine 5'-phosphosulfate sulfotransferase activities were upregulated in plants by cadmium challenge, but activities decreased once a certain threshold cadmium concentration was surpassed.]
by the overproduction of His in response to Ni.]

Phartiyal P., Kim W.-S., Cahoon R.E., Jez J.M., Krishnan H.B. (2006) Soybean ATP sulfurylase, a homodimeric enzyme involved in sulfur assimilation, is abundantly expressed in roots and induced by cold treatment. *Arch. Biochem. Biophys.* 450: 20-29. [This paper reports the first cloning of an ATP sulfurylase cDNA from soybean whose expression was most abundant in roots and induced by cold treatment.]

Pianelli K., Mari S., Marquez L., Lebrun M., Czernic P. (2005) Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana*. *Transgenic Res.* 14: 739-748. [This manuscript explores the potential of genetic engineering of nicotianamine in phytoremediation. Arabidopsis plants overexpressing a nicotianamine synthase cDNA from Thlaspi caerulescens and grown on nickel-contaminated soil are analyzed.]


Pickering I.J., Prince R.C., George M.J., Smith R.D., George G.N., Salt D.E. (2000) Reduction and coordination of arsenic in Indian mustard. *Plant Physiology* 122: 1171-1177. [Using, among other techniques, X-ray absorption spectroscopy, the authors determine that arsenate is reduced to arsenite, which is then chelated by thiolates.]


Presser T.S. and Ohlendorf H.M. (1987) Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA. *Environ. Manage.* 11: 805-821. [This paper describes the effects of the Kesterson Reservoir disaster in which selenium-containing drainage water was emptied into a wildlife refuge from 1978 to 1982, resulting in bird deaths and deformities first observed in the 1983 nesting season.]

Pomponi M., Censi V., Di Girolamo V., De Paolis A., di Toppi L.S., Aromolo R., Costantino P., Cardarelli M. (2006) Overexpression of *Arabidopsis* phytochelatin synthase in tobacco plants enhances Cd\(^{++}\) tolerance and accumulation but not translocation to the shoot. *Planta* 223: 1432-2048. [This paper support the importance of phytochelatins for Cd\(^{++}\) tolerance but not for Cd\(^{++}\) translocation from the root to the shoot.]


Rüegsegger A., Schmutz D., Brunold C. (1990) Regulation of glutathione synthesis by cadmium in *Pisum sativum* L. *Plant Physiology* 93: 1579-1589. [In this work, the authors conclude that glutathione is a precursor of phytochelatins and that cadmium increases the activity of enzymes in the sulfur assimilation pathway to make more cysteine, the limiting reagent in glutathione synthesis.]


Schat H., Llugany M., Vooijs R., Hartley-Whitaker J., Bleeker P.M. (2002) The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and nonhyperaccumulator metallophytes. *Journal of Experimental Botany* 53: 2381-2392. [This article demonstrates that tolerance of heavy metal hyperaccumulators does not depend on phytochelatins, however, as tolerance is dependent on PC-based sequestration.]

Schmöger M.E.V., Oven M., Grill E. (2000) Detoxification of arsenic by phytochelatins in plants. *Plant Physiology* 122: 793-801. [This paper reviews the role of phytochelatins in arsenic detoxification including knowledge of how phytochelatin production is induced by arsenic and evidence for the existence of arsenic-phytochelatin complexes isolated from plants.]


Schottel J.L. (1978) The mercuric and organomercurial detoxifying enzymes from a plasmid-bearing strain of *Escherichia coli*. *J. Biol. Chem.* 253: 4341 – 4349. [This paper reports the early purification and characterization of a mercuric reductase and organomercurial hydrolase, including information on their cofactors, *K_a*’s, molecular weight, isoelectric point, and optimal pH.]

Setya A., Murillo M., Leustek T. (1996) Sulfate reduction in higher plants: molecular evidence for a novel 5'-adenylylsulfate reductase. *Proc. Natl. Acad. Sci. USA* 93: 13383-13388. [This manuscript describes the cloning of three Arabidopsis thaliana cDNA’s that are shown to encode APS reductases suggesting that plants may not use PAPS reductases.]

Shaw W.H. and Anderson J.W. (1972) Purification, properties, and substrate specificity of adenosine triphosphate sulphurylase from spinach leaf tissue. *Biochem. J.*,127: 237-247. [This early work describes the purification and characterization of an ATP sulphurylase from spinach, including determining co-
factors, substrates (such as selenate), and $K_m$’s.]


Song W.Y., Sohn E.J., Martinoia E., Lee Y.J., Yang Y.Y., Jasinski M, Forestier C., Hwang I., Lee Y. (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. *Nature Biotechnology* 21:914 – 919. [This article shows that the overexpression of the Saccharomyces cerevisiae ABC transporter, YCF1, which transports heavy metal-glutathione complexes to the vacuole, can increase both the accumulation and the tolerance to Pb (II) and Cd (II) in plants. These results are discussed in the perspective of phytoremediation.]


Strohm M., Jouanin L., Kunert K.J., Pruvost C., Polle A., Foyer C.H., Rennenberg H. (1995) Regulation of glutathione synthesis in leaves of transgenic poplar (*Populus tremula x P. alba*) overexpressing glutathione synthetase. *Plant J.* 7: 141-145. [By studying transgenic poplar, the authors determined that glutathione synthesis is controlled by the availability of both cysteine and γ-glutamylcysteine and is related to the expression of γ-glutamylcysteine synthetase and glutathione synthetase.]


Talke I., Hanikenne M., Krämer U. (2006) Zinc dependent global transcriptional control, transcriptional de-regulation and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. *Plant Physiol*, 142: 148–167. [In this article, the use of Arabidopsis thaliana gene chips to identify genes overexpressed in the closely related hyperaccumulator species Arabidopsis halleri is presented. This study shows that under normal growth conditions, roots of the Zn hyperaccumulator behave as Zn deficient, when considering expression of several genes involved in Zn transpiration. It also further supports the role of HMA4 in Zn translocation.]

differences between sulfur and selenium assimilation in plants, and how this knowledge could be applied to phytoremediation.]

Terry N., Banuelos G. (2000) Phytoremediation of contaminated soil and water. Lewis Publishers. [This book covers work in phytoremediation from bench to field demonstration projects with analysis of the potential for this field of study and future applications.]


van de Mortel J., Villanueva L.A., Schat H., Kwekkeboom J., Coughlan S., Moerland P.D., Ver Loren van Themaat E., Koornneef M., Aarts M.G.M. (2006) Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of Arabidopsis thaliana and the related metal hyperaccumulator Thlaspi caerulescens. Plant Physiol. 142: 1127–1147. [This is a global transcriptional analysis of Thlaspi caerulescens using gene chips of Arabidopsis thaliana. It highlights the up-regulation of genes involved in metal homeostasis but also genes involved in lignin biosynthesis and genes of unknown function. The article also discusses the higher deposition of lignin in root endoderm in the metal hyperaccumulator.]


Van der Zaal B.J., Neuteboom L.W., Pinas J.E., Chardonnens A.N., Schat H., Verkleij J.A.C., Hooykaas P.J.J. (1999) Overexpression of a novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant Physiology 119: 1047–1055. [This is a first report about the genetic manipulation of a member of the Arabidopsis CDF transporters, AtZAT (recently renamed MTP1), to increase Zn tolerance and accumulation.]

Van Huysen T., Terry N., Pilon-Smits E.A.H. (2004) Exploring the selenium phytoremediation potential of transgenic Brassica juncea overexpressing ATP sulfurylase or cystathionine-γ-synthase. Int. J. Phytorem. 6: 111-118. [This study anticipated the later field trial of the APS transgenic plants by evaluating their ability to accumulate selenium on selenium-rich soil in greenhouse studies.]

Van Mantgem P.J., Wu L., Bañuelos G.S. (1996) Bioextraction of selenium by forage and selected field legume species in selenium-laden soils under minimal field management conditions. Ecotoxicol. Environ. Saf. 34: 228-238. [This study demonstrates how the amount of selenium removed by soil depends heavily on the plant species, i.e. the concentration that can be tolerated and the biomass achieved, and on the availability (chemical form) of selenium present in the soil.]


twelve metals. *J. Environ. Qual.* 33:54-60. [This comprehensive study evaluated the ability of the APS Indian mustard to tolerate and accumulate 12 forms of metals in hydroponics experiments.]


Wilber C.G. (1980) Toxicology of selenium: a review. *Clin. Toxicol.* 17: 171-230. [This review explains one of the prevailing theories regarding selenium’s toxicity, i.e. that the misincorporation of selenoamino acids for sulfur amino acids (cysteine and methionine) is responsible.]


Wilson L.G. and Bandurski R.S. (1958) Enzymatic reactions involving sulfate, sulfite, selenate and molybdate. *J. Biol. Chem.* 233: 975-981. [Relevant to the current discussion, this paper shows that ATP sulfurylase activity can use sulfate, selenate, and molybdate as substrates.]


Zayed A.M., Lytle C.M., and Terry N. (1998) Accumulation and volatilization of different chemical species of selenium by plants. *Planta* 206: 284-292. [Using X-ray and atomic absorption spectroscopy, the authors demonstrate that the form of selenium used to challenge plants determines the form accumulated and the rate of selenium volatilisation, suggesting the presence of rate-limiting steps in the selenate assimilation pathway.]

Zenk M.H. (1996) Heavy metal detoxification in higher plants: a review. *Gene* 179: 21-30. [This paper constitutes a review of the important role played by phytochelatins in metal detoxification in plants, including the different chemical forms of phytochelatins, a description of phytochelatin synthase and characterization of the cadmium-sensitive cad1 Arabidopsis thaliana mutant.]


Zhu Y.L., Pilon-Smits E.A.H., Tarun A.S., Weber S.U., Jouanin L., Terry N. (1999) Cadmium tolerance and accumulation in Indian Mustard is enhanced by overexpressing $\gamma$-glutamylcysteine synthetase. *Plant Physiology* 121:1169–1177. [The article shows that increasing glutathione biosynthesis by the overexpression of $\gamma$EC, has a small, but significant, impact on cadmium tolerance and accumulation.]

little but significant impact on cadmium tolerance and accumulation.

Biographical Sketches

Dr Nathalie Verbruggen did her Ph.D. on proline accumulation in plants after salt stress at the Ghent University (Belgium) with Professor Marc van Montagu. Following this, she conducted research as group leader on molecular responses of plants to osmotic stress. In 1999, she became a full-time professor in the University of Brussels and started a new laboratory to study mechanisms underlying metal homeostasis and detoxification in plants. Projects include cadmium hyperaccumulation, copper hypertolerance and magnesium deficiency. N. Verbruggen is also active in explaining the science behind plant genetic engineering and GMO’s.

Dr. Danika LeDuc has been an Assistant Professor of Chemistry & Biochemistry at California State University, East Bay since 2006. She conducts research at the interface of biochemistry and analytical and environmental chemistry, in which she has co-authored over 20 peer reviewed articles and book chapters. Her published work ranges from analysis of selenium and arsenic metabolites to the first field trial using genetically engineered plants for phytoremediation. Her current projects include work on elucidating the molecular mechanism of metal hyperaccumulation in selenium, nickel, and lead accumulating plants using synchrotron X-ray absorption techniques and proteome and transcriptome analyses.