4-2009

Genetic Engineering to Enhance Mercury Phytoremediation

Oscar N. Ruiz

Henry Daniell
University of Pennsylvania

Recommended Citation

Ruiz, O. N., & Daniell, H. (2009). Genetic Engineering to Enhance Mercury Phytoremediation. *Current Opinion in Biotechnology, 20* (2), 213-219. http://dx.doi.org/10.1016/j.copbio.2009.02.010

Genetic Engineering to Enhance Mercury Phytoremediation

Abstract

Most phytoremediation studies utilize *merA* or *merB* genes to modify plants via the nuclear or chloroplast genome, expressing organomercurial lyase and/or mercuric ion reductase in the cytoplasm, endoplasmic reticulum or within plastids. Several plant species including Arabidopsis, tobacco, poplar, rice, Eastern cottonwood, peanut, salt marsh grass and Chlorella have been transformed with these genes. Transgenic plants grew exceedingly well in soil contaminated with organic (~400 μM PMA) or inorganic mercury (~500 μM HgCl2), accumulating Hg in roots surpassing the concentration in soil (~2000 μg/g). However, none of these plants were tested in the field to demonstrate real potential of this approach. Availability of metal transporters, translocators, chelators and the ability to express membrane proteins could further enhance mercury phytoremediation capabilities.

Disciplines

Dentistry

Published in final edited form as:
Curr Opin Biotechnol. 2009 April ; 20(2): 213–219. doi:10.1016/j.copbio.2009.02.010.

GENETIC ENGINEERING TO ENHANCE MERCURY PHYTOREMEDIATION

Oscar N. Ruiz[1] and **Henry Daniell**[2,*]

1 *Department of Natural Sciences, Inter American University of Puerto Rico, 500 Dr. John Will Harris Road, Engineering Building, Room G-336, Bayamon, P.R. 00957-6257, USA*

2 *Department. of Molecular Biology and Microbiology, University of Central Florida, College of Medicine, Biomolecular Science Building, Room 336, Orlando, FL 32816-2364, USA*

Summary

Most phytoremediation studies utilize *merA* or *merB* genes to modify plants via the nuclear or chloroplast genome, expressing organomercurial lyase and/or mercuric ion reductase in the cytoplasm, endoplasmic reticulum or within plastids. Several plant species including Arabidopsis, tobacco, poplar, rice, Eastern cottonwood, peanut, salt marsh grass and Chlorella have been transformed with these genes. Transgenic plants grew exceedingly well in soil contaminated with organic (~400 μM PMA) or inorganic mercury (~500 μM $HgCl_2$), accumulating Hg in roots surpassing the concentration in soil (~2000 μg/g). However, none of these plants were tested in the field to demonstrate real potential of this approach. Availability of metal transporters, translocators, chelators and the ability to express membrane proteins could further enhance mercury phytoremediation capabilities.

Phytoremediation is the use of plants to clean up contaminated environments. It is a cost-effective, environmentally friendly approach with great advantages for large-scale clean up of contaminated sites [1,2,3]. Current remediation methods for heavy metal contamination are environmentally invasive, expensive and inefficient, especially for large scale clean up [4]. However, plants have limited capabilities for treating high levels of heavy metal contamination.

Mercury (Hg), one of the most toxic pollutants threatening our health and ecosystems, has been introduced from natural and anthropogenic sources. Recent estimates calculate the annual global emissions between 4,800–8,300 tons per year [5]. In the United States alone coal burning power plants emit about 48 tons of mercury annually [6]; the combined estimates for Asia and Africa surpass the 1,500 tons [7*]. The cost of remediation of each pound of mercury from the environment using current technologies is in the range of tens of thousands of dollars. Therefore, finding alternate remediation approaches is an urgent need.

Mercury is usually released in the metal or ionic form, accumulating in sediments where it becomes methylated by anaerobic sulfate-reducing bacteria to produce methyl mercury, a highly toxic organomercurial compound. The toxicity of mercury greatly depends on the form

*Corresponding author: Henry Daniell, Ph.D. Pegasus Professor & University Board of Trustee Chair, University of Central Florida, College of Medicine, 4000 Central Florida Blvd, Dept. of Molecular Biology & Microbiology, Biomolecular Science Building, Room 336, Orlando, FL 32816-2364, Phone: 407-823-0952, Fax: 407-823-0956, E-mail: E-mail: daniell@mail.ucf.edu.

present in the environment. Inorganic mercury forms are usually less harmful than organic forms, in part because they bind strongly to soil components that reduce their availability and absorption. On the other hand, organomercurials are highly toxic because of their hydrophobicity, which facilitates their movement across cell membranes and accumulation in membrane bound organelles, inhibiting essential oxidative and photosynthetic pathways. The toxic effects of methyl mercury are further magnified at higher trophic levels due to increased accumulation in tissues [8,9]. Organomercurials are potent neurotoxins with over 90% absorption into the blood stream from the intestinal track, while mercury salts and elemental mercury show reduced absorptions of less than 10% and 0.1%, respectively [10]. In plants, ionic mercury tends to affect the plasma membrane where it damages membrane transporters such as aquaporins, leading to nutrient and water disruption [11]. Organomercurials rapidly localize to plastids where they accumulate and disrupt important metabolic functions including electron transport, oxygen evolution [12], photophosphorylation, Hill reaction, chlorophyll content and chlorophyll fluorescence [13,14].

Plants cannot successfully detoxify or interconvert mercury to more benign forms. Genetic engineering can integrate genes from other organisms to enhance phytoremediation capabilities in plants. This article reviews our current knowledge of transgenic plant systems for Hg phytoremediation, provides insight on mechanistic aspects and directions for further studies.

MerAB Engineered via the Nuclear Genome

A well characterized phytoremediation system is the use of the bacterial *merA* (mercuric ion reductase) and *merB* (organomercurial lyase) genes to genetically engineer plants for the remediation of Hg [15–26]. Organomercurial lyase facilitates protonolysis of organic-Hg to Hg^{2+} while mercuric ion reductase reduces Hg^{2+} to Hg^0, which is volatilized from plants. Initial attempts to use the native bacterial *merA* gene in nuclear transgenic plants failed to express the mercuric ion reductase [24*,25]. When the coding sequence was modified to plant preferred codons, transgenic *Arabidopsis thaliana*, yellow poplar, peanut, and Eastern cottonwood plants were resistant to levels up to 25 –100 μM $HgCl_2$ [24*–26]. Kim et al, [22*] demonstrated that root-specific expression of the *merA* gene in *Arabidopsis thaliana* provided resistance up to 80 μM $HgCl_2$ (Table 1). Heaton et al, [27] showed that the root of *merA* transgenic plants grew directly into Hg “hotspots” while the wild type grew away from the source. These results indicated that root protection was important for phytoremediation and that root is the main organ affected by Hg. One drawback of this finding was the implication that Hg was being detoxified to Hg^0 in the root where it may become volatilized, though Hg^0 could also be transported through the xylem to the leaves where it is transpired.

Heaton et al, [20] developed *merA* transgenic rice (*Oryza sativa*) resistant to 250 μM $HgCl_2$; the wild type was resistant up to 150 μM $HgCl_2$. This was the first report of a transgenic monocot for phytoremediation of Hg. Also, salt marsh cordgrass (*Spartina alterniflora*) modified for Hg phytoremediation was resistant up to 500 μM $HgCl_2$ [19*]. It should be pointed out that both these monocots showed natural tolerance to Hg and further studies are needed to understand the mechanism of such resistance. Recently, Huang et al, [7*] demonstrated that transgenic *Chlorella* expressing the *merA* gene withstood up to 40 μM $HgCl_2$. Microalgae have certain advantages over land plants for phytoremediation, including larger surface area in contact with the contaminant in water, and a natural capacity to bind Hg [28].

Unfortunately, mercuric ion reductase did not protect against the more toxic and environmentally relevant organic-Hg. Both the *merA* and *merB* genes are needed to protect cells from organic-Hg. When a codon optimized *merB* was used to transform the *Arabidopsis thaliana* nuclear genome, it provided resistance up to 1 μM phenylmercuric acetate (PMA) [17]; although this is a very low level of resistance, this concentration was lethal to

untransformed plants. The organic-Hg concentration needed to inhibit growth in untransformed plants was 100-fold less than the level required for $HgCl_2$, confirming higher toxicity. Transgenic Eastern cottonwood trees expressing *merB* failed to confer resistance to PMA [18].

In 2000, Bizily et al, [16**] reported the first transgenic plant expressing both the *merA* and *merB* genes; *Arabidopsis thaliana* transgenic lines showed resistance up to 5 μM PMA and 10 μM CH_3Hg, a 5-fold increase in resistance when compared to transgenic plants expressing only the *merB* genes. Transgenic Eastern cottonwood trees expressing both genes were tolerant up to 10 μM of PMA [23]. Bizily et al, [15] showed that transgenic plants in which organomercurial lyase was localized to the endoplasmic reticulum (ER) and to the cell wall (CW) conferred as much resistance (up to 5 μM PMA) as transgenic plants expressing both genes in the cytoplasm. Organomercurial lyase localized to the ER showed 41-times as much activity as organomercurial lyase in the cytoplasm, suggesting that organic-Hg may be localized to membrane bound organelles (Table 1).

MerAB Engineered via the Chloroplast Genome

In order to provide higher levels of resistance (> 5–10 μM PMA), the plastids may need protection from toxic mercury. There are different types of plastids present in plant organs and tissues. Chloroplasts present in green tissues are metabolically very active and are involved in photosynthesis, sulfur/nitrogen metabolism and synthesis of starch, amino acids, fatty acids, pigments, vitamins, etc. Chloroplasts are the primary source of world's food and oxygen, sustaining life on this planet. Other types of plastids include colorful chromoplasts present in flowers, fruits and vegetables. Amyloplasts are present in tubers like potato or in endosperm tissues in seeds, primarily performing the function of storage. Non-green proplastids are present in roots and developing tissues. In spite of variation in their appearance and functions, all plastids contain identical genome structure and sequence.

The idea of protecting essential metabolic reactions occurring within plastids, has been our working hypothesis for expressing *merA* and *merB* genes within plant chloroplasts [29**]. Chloroplast has been shown to be the main target for Hg poisoning [12,30,31]. Transgene containment by the maternal inheritance of the plastid genome [32] or cytoplasmic male sterility [33], is another major advantage of the chloroplast transformation system. Chloroplast genetic engineering is especially advantageous for the development of transgenic plants when multiple genes are required for effective phytoremediation [34]. By using chloroplast transformation, multigenetic pathways can be developed in a single transformation event without the need for backcrosses of independent lines or re-transformation. Because plastids have retained the genetic machinery from bacterial ancestor, their genomes can transcribe and translate operons [34]. Chloroplast genetic engineering also circumvents common drawbacks of nuclear transformation, including gene silencing and position effect. Site-specific transgene integration by homologous recombination [35] eliminates the position effect. High expression levels, up to 47% of total leaf protein in healthy plants [36**] or accumulation of transcripts 150 fold higher than nuclear transgenic plants [37] did not result in post-transcriptional or post-translational gene silencing. Such high expression levels are achieved because there are up to 10,000 copies of transgenes in each transformed plant cell. Also, the addition of transcriptional and translational enhancer elements is needed to optimize expression of transgenes. However, there is no need for codon optimization of bacterial genes for their expression in plastids [29**].

In spite of these advantages, there are a few limitations of the chloroplast transformation technology. Unlike transformation of the nuclear genome of different crop species using the same transformation vectors, species specific vectors are necessary for chloroplast

transformation. Therefore, it is important to have the chloroplast genome sequence, especially the intergenic spacer regions and endogenous regulatory sequences (promoter, 5′UTR, 3′UTR) to create species specific chloroplast vectors. Until 2004, complete genome sequences were available for only six crop species. However, plastid genome sequences are becoming available at a rapid pace, facilitating the application of chloroplast genetic engineering to more species than ever before [38–42]. The second challenge is achieving homoplasmy (integration of transgenes into each chloroplast genome and elimination of untransformed chloroplast genome). This has been easily accomplished in plant species that are regenerated via organogenesis, after several rounds of selection. Chloroplast genomes of more than ten crop species have been transformed via organogenesis, including cauliflower, lettuce, cabbage, petunia, poplar, tobacco, potato, tomato, etc [43]. However, achieving homoplasmy in crops regenerated via somatic embryogenesis is challenging although carrot, cotton, and soybean chloroplast genomes have been transformed already, with desired agronomic traits [43]. The versatility of the chloroplast system has led to its application in developing transgenic plants with enhanced agronomic traits [43]. Therefore, chloroplast genetic engineering is an ideal approach for mercury phytoremediation [29**].

In these studies, the native bacterial *merA* and *merB* genes were integrated into the tobacco chloroplast genome [29**,44**]. The transgenic plants were resistant to very high concentrations of PMA, up to 400 μM. This is the only report to date in which transgenic plants not only survived such high concentrations of PMA but also showed better growth than control untreated plants [29**]. Transgenic plants grew exceedingly well in soil contaminated with 300 μM PMA or $HgCl_2$, accumulating Hg in the root to levels surpassing the concentration in soil, up to 2000 μg/g [44**]. There are several reasons for successful phytoremediation via the chloroplast genome. Mercury, especially in the organic form, is targeted to the chloroplast. Mercuric ion reductase functions better in chloroplasts because of the abundance of NADPH. Protection of the root in the transgenic plant was possible because root proplastids are active and express the *mer* genes under the control of a constitutive promoter. Kumar et al, [45] showed that root proplastids expressed up to 70% of foreign protein as leaf chloroplasts. Interestingly, transgenic plants were able to accumulate 100-fold and 4-fold more Hg in the shoot in the presence of PMA or $HgCl_2$ than untransformed plants. This shows that organic-Hg is transported more efficiently than inorganic forms. Finally, the transgenic plants were shown to volatilize Hg^0 efficiently, independent of the form of Hg in the soil, confirming that both mercuric ion reductase and organomercurial lyase were active in transgenic chloroplasts [44**].

Other Mechanisms for Phytoremediation of Mercury

The mer operon has three known membrane transporter genes involved in the process of translocating Hg^{2+} into the cell: *merC*, *merP*, and *merT* [46,47]. Recently, Sasaki et al, [48**] showed that expression of the *merC* gene in *Arabidopsis thaliana* enhanced hypersensitivity to otherwise sub-lethal concentrations of $HgCl_2$. Therefore, plants can be genetically modified with bacterial metal transporters to enhance heavy metal uptake. In a subsequent report, *Arabidopsis thaliana* transformed with the *merP* gene showed resistance to 10 μM $HgCl_2$ instead of becoming hyper-sensitive [49**]. Because *merP* requires *merT* for Hg^{2+} translocation, it is possible that *merP* was trapping Hg at the cell membrane, thereby protecting the cytoplasm. To take advantage of these transport mechanisms, transgenic plants should be modified with the *merA* gene as well; this should provide resistance against higher levels of transported Hg. Alternatively, the *merC* can be coupled to a chelator gene like polyphosphate kinase (*ppk*) or metallothionein (*mt*), to develop transgenic plants that could accumulate Hg. Recently, it has been shown that by using chloroplast genetic engineering, membrane proteins can be targeted to the chloroplast inner envelope membrane and

accumulated at very high levels [50**]. This opens the possibility of transforming the chloroplast genome with *mer* transporters to enhance Hg accumulation or phytoremediation.

One public concern regarding the use of the merAB system is the release of Hg^0 into the atmosphere. Therefore, an alternative approach would be the sequestration of ionic mercury inside the cell by binding it to a chelating molecule. The bacterial *ppk* gene coding for polyphosphate kinase synthesized polyphosphates, which are negatively charged; the long phosphate polymers reduced cytotoxicity of heavy metals by chelation [51]. Evidence for this was first provided when nuclear transgenic tobacco plants expressing the *ppk* gene showed enhanced tolerance and accumulation of Hg^{2+} when grown in 10 μM $HgCl_2$ [52,53**]. Although the levels of resistance were low, the possibility of using metal-scavenging molecules for phytoremediation was demonstrated.

Future Prospects for Mercury Phytoremediation

It is clear that plants can be genetically modified to enhance their tolerance, uptake, translocation, accumulation and volatilization capabilities for Hg phytoremediation. Several general conclusions can be drawn from the discussed reports. Transgenic plants grow exceedingly well in soil contaminated with PMA or $HgCl_2$, accumulating Hg in the root to levels surpassing the concentration in soil, up to 2000 μg/g or volatilizing mercury supplied in organic or inorganic form. However, none of these plants were tested in the field to demonstrate real potential of this approach. Availability of metal transporters, translocators, and chelators could further enhance phytoremediation capabilities. Our recent ability to express membrane proteins opens the possibility of transforming the chloroplast genome with *mer* transporters to enhance Hg accumulation or phytoremediation. Alternatively, the *merC* can be coupled to a chelator gene like polyphosphate kinase (*ppk*) or metallothionein (*mt*), to develop transgenic plants that could accumulate Hg.

Plants tend to accumulate most mercury in roots, and translocation to shoot tends to be a major limitation, dependent upon concentration and cellular integrity [20,27,44**]. It seems that for translocation to occur, saturation of the roots to levels only withstood by transgenic plants is required. A recent report of a hundred fold increase in Hg translocation to the shoot [44**] is quite encouraging. However, in order to utilize the large biomass above ground, the translocation problem should be addressed further. Other interesting observations include promotion of growth in *mer* transgenic lines compared to untreated controls, and increased chlorophyll content when grown in the presence of mercury [25,29**,54].

Conclusions

Toxicity of methyl mercury is magnified due to its high accumulation in tissues; over 90% of methyl mercury is absorbed into the blood stream from the gastrointestinal tract when compared to 0.1% of elemental mercury [10]. Methyl mercury accumulated in fish is therefore 990-fold more neuro-toxic than elemental mercury in thermometers. US EPA cautions pregnant women against frequent consumption of fish. Therefore, it is very important to develop technologies to clean up our environment. The potential of transgenic plants for Hg phytoremediation has been demonstrated, but important questions need to be answered to explore the full potential of this technology. A better understanding of Hg-forms transported and accumulated, and their saturation and steady-state levels in different tissues would help develop phytoremediation systems that are cost-effective. New mercury phytoremediation technologies would rely on different gene combinations to enhance uptake, translocation, chelation or detoxification and release of Hg^0 into the atmosphere (Figure 1). Ultimately, the scientific community should make an effort to address the most important questions that deter the application of phytoremediation and produce plants with the best capabilities, including

plants with large biomass, rapid growth rate, adaptation to wider climatic conditions, and expression of multiple genes in different cellular compartments. The use of edible plant species should be avoided in phytoremediation applications, while plant species that out-cross with wild relatives or cultivated plants may be used only when containment measures are applied including harvesting transgenic plants prior to the plant reproductive age, using a male sterility or maternal inheritance system, or planting away from wild relatives to minimize outcross.

Acknowledgments

Chloroplast genetic engineering studies reviewed in this article were supported in part by grants from United States Department of Agriculture 58-3611-7-610 and National Institutes of Health R01 GM 63879 to Henry Daniell. Oscar N Ruiz is supported in part by grants from National Science Foundation CBET-0755649 and Puerto Rico Space Grant Consortium NASA-IDEAS ER 2006-08. Authors thank Gricel Ruiz for help with Figure 1.

References Cited

1. Doucleff M, Terry N. Pumping out the arsenic. Nat Biotechnol 2002;20:1094–1095. [PubMed: 12410252]
2. Kramer U. Phytoremediation: novel approaches to cleaning up polluted soils. Curr Opin Biotechnol 2005;16:133–41. [PubMed: 15831377]
3. Salt DE, Smith RD, Raskin I. Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 1998;49:643–668. [PubMed: 15012249]
4. Kärenlampi S, Schat H, Vangronsveld J, Verkleij JAC, Lelie D, Mergeay M, Tervahauta AI. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soil. Environ Pollut 2000;107:225–231. [PubMed: 15092999]
5. EPA. Mercury Human Exposure. US Environmental Protection Agency. 2008. Retrieved February 8, 2009 from www.epa.gov/mercury/exposure.htm
6. EPA. Clean Air Mercury Rule. U.S. Environmetal Protection Agency; Washington, DC: 2005.
*7. Huang CC, Chen MW, Hsieh JL, Lin WH, Chen PC, Chien LF. Expression of mercuric reductase from Bacillus megaterium MB1 in eukaryotic microalga Chlorella sp. DT: an approach for mercury phytoremediation. Appl Microbiol Biotechnol 2006;72:197–205. [PubMed: 16547702]This is the first report of a genetically modified green alga for mercury remediation with resistance up to 40 μM $HgCl_2$. Phytoremediation of mercury from polluted water is a serious concern
8. Harada M. Minamata Disease Research Group Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 1995;25:1–24. [PubMed: 7734058]
9. Patra M, Sharma A. Mercury Toxicity in Plants. The Botanical Review 2000;66:379–422.
10. Goldman LR, Shannon MW. The committee on environmental health technical report. Mercury in the environment: implication for pediatricians. Pediatrics 2001;108:197–205. [PubMed: 11433078]
11. Zhang WH, Tyerman SD. Inhibition of water channels by $HgCl_2$ in intact wheat root cells. Plant Physiol 1999;120:849–858. [PubMed: 10398721]
12. Bernier M, Carpentier R. The action of mercury on the binding of extrinsic polypeptides associated with water oxidizing complex of photosystem II. FEBS Letters 1995;360:251–254. [PubMed: 7883042]
13. Kupper H, Kupper F, Spiller M. Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. J Exp Bot 1996;47:259–266.
14. Sinha S, Gupta M, Chandra P. Bioaccumulation and biochemical effects of mercury in the plant Bacopa monnieri (L). Environ Toxicol Water Quality 1996;11:105–112.
15. Bizily SP, Kim T, Kandasamy MK, Meagher RB. Subcellular targeting of methylmercury lyase enhances its specific activity for organic mercury detoxification in plants. Plant Physiol 2003;131:463–471. [PubMed: 12586871]
**16. Bizily S, Rugh CC, Meagher RB. Phytoremediation of hazardous organomercurials by genetically engineered plants. Nat Biotechnol 2000;18:213–217. [PubMed: 10657131]This is the first report of nuclear genetic engineering for phytoremediation using *merA* and *merB* genes in plants. *A.*

thaliana was engineered to provide resistance to low concentrations of phenylmercuric acetate, up to 5–10 μM

17. Bizily S, Rugh CC, Summers AO, Meagher RB. Phytoremediation of methylmercury pollution: *merB* expression in *Arabidopsis thaliana* plants confer resistance to organomercurial. Proc Natl Acad Sci U S A 1999;96:6808–6813. [PubMed: 10359794]
18. Che D, Meagher RB, Heaton AC, Lima A, Rugh CL, Merkle SA. Expression of mercuric ion reductase in Eastern cottonwood (Populus deltoides) confers mercuric ion reduction and resistance. Plant Biotechnol J 2003;1:311–319. [PubMed: 17163907]
*19. Czakó M, Feng X, He Y, Liang D, Márton L. Transgenic *Spartina alterniflora* for phytoremediation. Environ Geochem Health 2006;28:103–110. [PubMed: 16528587]This is the first report of a genetically engineered wetland grass for phytoremediation that is naturally resistant to mercury. Resistance was demonstrated up to 500 μM $HgCl_2$. Phytoremediation of mercury from polluted water is a serious concern
20. Heaton AC, Rugh CC, Kim T, Meagher RB. Toward detoxifying mercury-polluted aquatic sediments with rice genetically engineered for mercury resistance. Environ Toxicol Chem 2003;22:2940–2947. [PubMed: 14713034]
21. He YK, Sun JG, Feng XZ, Czakó M, Márton L. Differential mercury volatilization by tobacco organs expressing a modified bacterial merA gene. Cell Res 2001;11:231–236. [PubMed: 11642409]
*22. Kim T, Balish RS, Heaton AC, McKinney EC, Dhankher OP, Meagher RB. Engineering **a root-specific, repressor-operator gene complex. Plant Biotechnol J 2005;3:571–582. [PubMed: 17147628]This report demonstrated that plant root is the main organ affected by Hg^{2+} and that root-specific expression of the *merA* gene can confer protection up to 80 μM $HgCl_2$
23. Lyyra S, Meagher RB, Kim T, Heaton A, Montello P, Balish RS, Merkle SA. Coupling two mercury resistance genes in Eastern cottonwood enhances the processing of organomercury. Plant Biotechnol J 2007;5:254–62. [PubMed: 17309680]
*24. Rugh CL, Senecoff JF, Meagher RB, Merkle SA. Development of transgenic yellow poplar for mercury phytoremediation. Nat Biotechnol 1998;16:925–928. [PubMed: 9788347]This is the first report of utilization of a forest tree for phytoremediation, transforming poplar with the *merA* gene, conferring resistance up to 50 μM $HgCl_2$
25. Rugh CC, Summers AO, Meagher RB. Mercuric ion reductase and resistance in transgenic Arabidopsis thaliana expressing modified bacterial merA gene. Proc Natl Acad Sci U S A 1996;93:3182–3187. [PubMed: 8622910]
26. Yang H, Nairn J, Ozias-Akins P. Transformation of peanut using a modified bacterial mercuric ion reductase gene driven by an actin promoter from Arabidopsis thaliana. J Plant Physiol 2003;160:945–52. [PubMed: 12964870]
27. Heaton ACP, Rugh CL, Wang NJ, Meagher RB. Physiological Responses of Transgenic *merA*-TOBACCO (*Nicotiana tabacum*) to Foliar and Root Mercury Exposure. Water Air Soil Pollut 2005;161:137–155.
28. Schmitt D, Müller A, Csögör Z, Frimmel FH, Posten C. The adsorption kinetics of metal ions onto different microalgae and siliceous earth. Water Res 2001;35:779–85. [PubMed: 11228977]
**29. Ruiz ON, Hussein HS, Terry N, Daniell H. Phytoremediation of organomercurials via the chloroplast genetic engineering. Plant Physiol 2003;132:1344–1352. [PubMed: 12857816]This is the first report of chloroplast genetic engineering for phytoremediation. The native bacterial *merA* and *merB* genes were integrated into the tobacco chloroplast genome and provided resistance to very high concentrations of phenylmercuric acetate, up to 400 μM
30. Sabat SC. Copper ion inhibition of electron transport activity in sodium chloride washed Photosystem II particle is partially prevented by calcium ion. Z Naturforsch 1996;51:179–184.
31. Sinha S, Gupta M, Chandra P. Bioaccumulation and biochemical effects of mercury in the plant Bacopa monnieri. Environ Toxicol Water Quality 1996;11:105–112.
32. Daniell H. Transgene containment by maternal inheritance: effective or elusive? Proc Natl Acad Sci U S A 2007;104:6879–6880. [PubMed: 17440039]
33. Ruiz ON, Daniell H. Cytoplasmic male sterility via the chloroplast genome. Plant Physiol 2005;138:1232–1246. [PubMed: 16009998]

34. Quesada T, Ruiz ON, Daniell H. Characterization of heterologous multigene operons in transgenic chloroplasts: transcription, processing and translation. Plant Physiology 2005;138:1–17. [PubMed: 15888672]

35. Daniell H, Ruiz ON, Dhingra A. Chloroplast genetic engineering to improve agronomic traits. Methods Mol Biol 2004;286:111–137. [PubMed: 15310917]

**36. DeCosa, B.; Moar, W.; Lee, SB.; Miller, M.; Daniell, H. Nature Biotechnol. Vol. 19. 2001. Hyper-expression of the Bt Cry2Aa2 operon in chloroplasts leads to formation of insecticidal crystals; p. 71-74.This is the first report of multigene engineering via the chloroplast genome and highest level of foreign gene expression in healthy transgenic plants and opened the door for studies on phytoremediation

37. Dhingra A, Portis AR Jr, Daniell H. Enhanced translation of a chloroplast-expressed RbcS gene restores small subunit levels and photosynthesis in nuclear RbcS antisense plants. Proc Natl Acad Sci U S A 2004;101:6315–6320. [PubMed: 15067115]

38. Daniell H, Wurdack KJ, Kanagaraj A, Lee SB, Saski C, Jansen RK. The complete nucleotide sequence of the cassava (Manihot esculenta) chloroplast genome and the evolution of atpF in Malpighiales: RNA editing and multiple losses of a group II intron. Theor Appl Genet 2008;116:723–737. [PubMed: 18214421]

39. Jansen RK, Cai Z, Raubeson LA, Daniell H, Depamphilis CW, Leebens-Mack J, Müller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, Chumley TW, Lee SB, Peery R, McNeal JR, Kuehl JV, Boore JL. Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc Natl Acad Sci U S A 2007;104:19369–19374. [PubMed: 18048330]

40. Jansen RK, Wojciechowski MF, Sanniyasi E, Lee SB, Daniell H. Complete plastid genome sequence of the chickpea (Cicer arietinum) and the phylogenetic distribution of rps12 and clpP intron losses among legumes (Leguminosae). Mol Phylogenet Evol 2008;48:1204–17. [PubMed: 18638561]

41. Samson N, Bausher MG, Lee SB, Jansen RK, Daniell H. The complete nucleotide sequence of the coffee (Coffea arabica L.) chloroplast genome: organization and implications for biotechnology and phylogenetic relationships amongst angiosperms. Plant Biotechnol J 2007;5:339–353. [PubMed: 17309688]

42. Saski C, Lee SB, Fjellheim S, Guda C, Jansen RK, Luo H, Tomkins J, Rognli OA, Daniell H, Clarke JL. Complete chloroplast genome sequences of Hordeum vulgare, Sorghum bicolor and Agrostis stolonifera, and comparative analyses with other grass genomes. Theor Appl Genet 2007;115:571–590. [PubMed: 17534593]

43. Verma D, Daniell H. Chloroplast vector systems for biotechnology applications. Plant Physiol 2007;145:1129–1143. [PubMed: 18056863]

**44. Hussein H, Ruiz ON, Terry N, Daniell H. Phytoremediation of mercury and organomercurials in chloroplast transgenic plants: Enhanced root uptake, translocation to shoots and volatilization. Environ Sci Technol 2007;41:8439–8446. [PubMed: 18200876]This report demonstrated that chloroplast transgenic plants expressing *merA* and *merB* can accumulate Hg in roots to levels surpassing the concentration in soil, up to 2000μg/g without any detrimental effect and could accumulate 100-fold more Hg in leaves than untransformed plants

45. Kumar S, Dhingra A, Daniell H. Plastid-expressed betaine aldehyde dehydrogenase gene in carrot cultured cells, roots, and leaves confers enhanced salt tolerance. Plant Physiol 2004;136:2843–2854. [PubMed: 15347789]

46. Morby AP, Hobman JL, Brown NL. The role of cysteine residues in the transport of mercuric ions by the Tn501 MerT and MerP mercury-resistance proteins. Mol Microbiol 1995;17:25–35. [PubMed: 7476206]

47. Wilson JR, Leang C, Morby AP, Hobman JL, Brown NL. MerF is a mercury transport protein: different structures but a common mechanism for mercuric ion transporters? FEBS Lett 2000;472:78–82. [PubMed: 10781809]

**48. Sasaki Y, Hayakawa T, Inoue C, Miyazaki A, Silver S, Kusano T. Generation of mercury-hyperaccumulating plants through transgenic expression of the bacterial mercury membrane transport protein MerC. Transgenic Res 2006;15:615–625. [PubMed: 16830224]This is the first report of expression of a mercury transporter gene in plants. The bacterial MerC protein was shown to be associated with the cell membrane and render plants hypersensitive to $HgCl_2$

**49. Hsieh JL, Chen CY, Chiu MH, Chein MF, Chang JS, Endo G, Huang CC. Expressing a bacterial mercuric ion binding protein in plant for phytoremediation of heavy metals. J Hazard Mater 2009;161:920–925. [PubMed: 18538925]Expression of the bacterial *merP* gene provided resistance up to 10 μM $HgCl_2$ possibly by a chelation mechanism. The MerP protein was shown to be associated with the cell membrane

**50. Singh, ND.; Li, M.; Lee, SB.; Schnell, D.; Daniell, H. Plant Cell. 2008 Dec 5. Arabidopsis Tic40 expression **in tobacco chloroplasts results in massive proliferation of the inner envelope membrane and upregulation of associated proteins. Epub ahead of print. This is the first report of the expression of a membrane protein via the chloroplast genome. Over-expression of the membrane protein resulted in massive proliferation of the inner envelope membrane (up to 19 layers in electron micrographs) and this opens the possibility of engineering mercury transporters in plant cells

51. Pan-Hou H, Kiyono M, Omura H, Omura T, Endo G. Polyphosphate produced in recombinant Escherichia coli confers mercury resistance. FEMS Microbiol Lett 2002;207:159–164. [PubMed: 11958934]

52. Nagata T, Ishikawa C, Kiyono M, Pan-Hou H. Accumulation of mercury in transgenic tobacco expressing bacterial polyphosphate. Biol Pharm Bull 2006;29:2350–2353. [PubMed: 17142961]

**53. Nagata T, Kiyono M, Pan-Hou H. Engineering expression of bacterial polyphosphate kinase in tobacco for mercury remediation. Appl Microbiol Biotechnol 2006;72:777–782. [PubMed: 16514513]This is the first report of mercury phytoremediation by genetically engineering the nuclear genome of tobacco plants with a chelator gene. Expression of the *ppk* gene increased polyphosphate abundance and tobacco tolerance to $HgCl_2$ up to 10 μM

54. Heaton ACP, Rugh CL, Meagher RB. Phytoremediation of mercury-and- Methylmercury-polluted soils using genetically engineered plants. J Soil Contamination 1998;7:497–509.

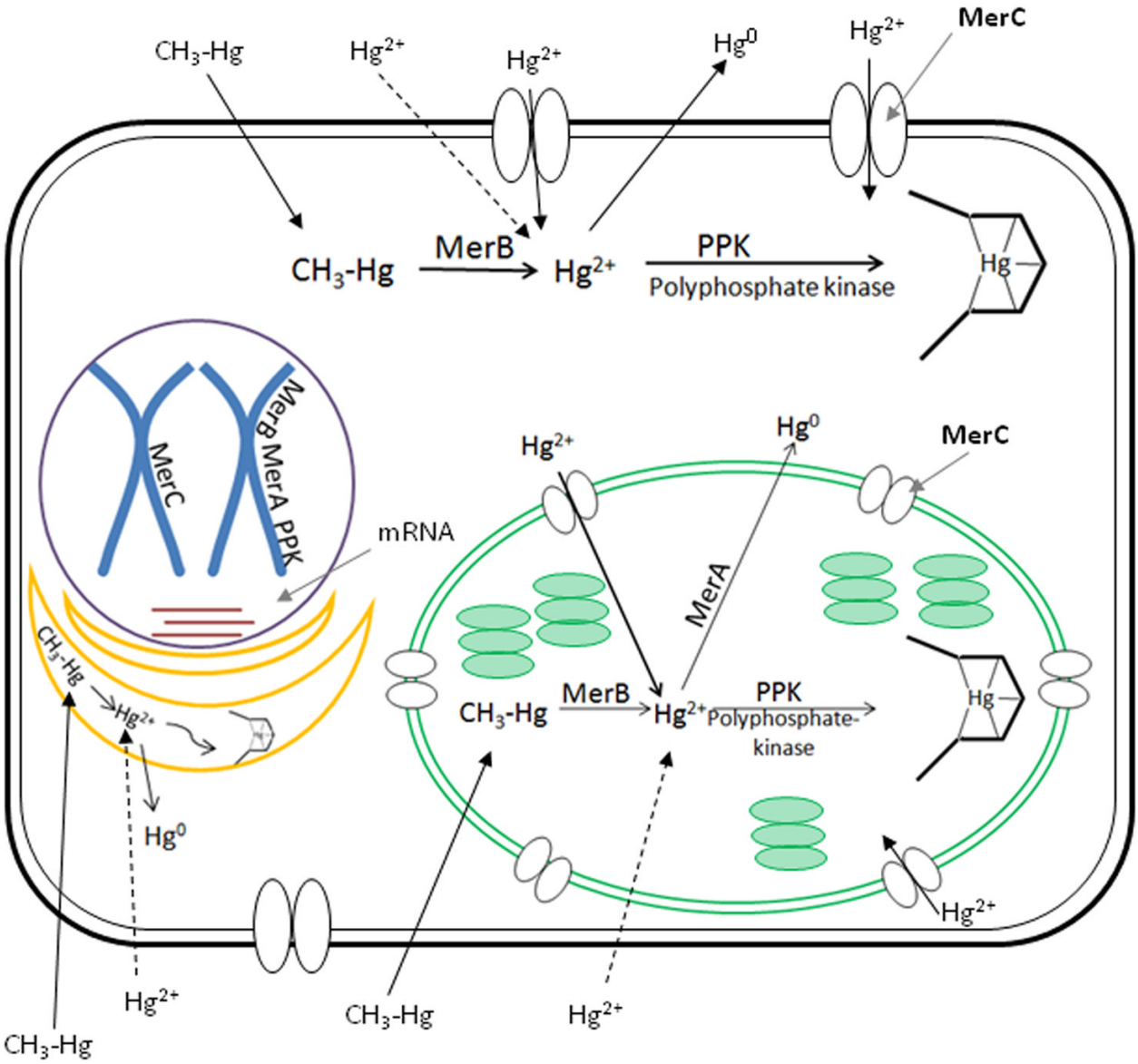

Figure 1. Schematic representation of a genetically modified plant cell for optimal mercury phytoremediation

Mercury phytoremediation technologies should utilize multiple genes to enhance uptake, translocation and detoxification in different cellular compartments, including plastids and endoplasmic reticulum. Both chelation and Hg^0 volatilization are promising approaches for phytoremediation. Solid dark arrows indicate rapid movement across membranes or via specialized Hg transporters (*merC*) while dashed arrows indicate slow movement mediated by cellular ion transporters.

Table 1

Advances in transgenic systems for phytoremediation of mercury

Species	Gene	Expression Compartment	Level of Resistance	Mode of Resistance	Special Future	Year	Ref.
A. thaliana	*merA*	Cytoplasm	100 μM $HgCl_2$	Hg(II) to Hg(0)	First report of *merA* in plants	1996	25
Yellow Poplar (*L. tulipifera*)	*merA*	Cytoplasm	50 μM $HgCl_2$	Hg(II) to Hg(0)	*merA* transformed into a tree species	1998	24*
A. thaliana	*merB*	Cytoplasm	2 μM PMA	PMA to Hg(II)	First report of *merB* in plants	1999	17
A. thaliana	*merA/B*	Cytoplasm	5–10 μM organic- Hg	PMA to Hg(II) to Hg(0)	First report of *merA and merB* in plants	2000	16**
Tobacco (*N. tabacum*)	*merA*	Cytoplasm	50 μM $HgCl_2$	Hg(II) to Hg(0)	*merA* transformed into tobacco	2001	21
Tobacco	*merA/B*	Plastid	400 μM PMA	PMA to Hg(II) to Hg(0)	First chloroplast phytoremediation system	2003	29**
Tobacco	*merB*	Endoplasmic reticulum (ER)	5 μM PMA	PMA to Hg(II)	MerB targeted to the ER	2003	15
Rice (*O. sativa*)	*merA*	Cytoplasm	250 μM $HgCl_2$	Hg(II) to Hg(0)	First monocot for Hg phytoremediation	2003	20
Eastern cottonwood (*P. deltoides*)	*merA*	Cytoplasm	25 μM $HgCl_2$	Hg(II) to Hg(0)	*merA* transformed into a forest tree	2003	18
Peanut (*A. hypogaea*)	*merA*	Cytoplasm	100 μM $HgCl_2$	Hg(II) to Hg(0)	*merA* transformed into peanut	2003	26
A. thaliana	*merA*	Cytoplasm of root cells	80 μM $HgCl_2$	Hg(II) to Hg(0)	Root-specific expression of *merA*	2005	22*
Salt marsh cordgrass (*S. alterniflora*)	*merA*	Cytoplasm	500 μM $HgCl_2$	Hg(II) to Hg(0)	First wetland grass for Hg phytoremediation	2006	19*
Chlorella	*merA*	Cytoplasm	40 μM $HgCl_2$	Hg(II) to Hg(0)	First transgenic alga for Hg bioremediation	2006	7*
A. thaliana	*merC*	Cell membrane	10 μM $HgCl_2$	Hypersensitivity to Hg(II)	First use of Hg membrane transporter	2006	48**
Tobacco	*ppk*	Cytoplasm	10 μM $HgCl_2$	Hg chelation	First use of a Hg-scavenging agent	2006	53**
Eastern cottonwood	*merA/B*	Cytoplasm	10 μM PMA	PMA to Hg(II) to Hg(0)	*merA* and *merB* transformed into a forest tree	2007	23

Species	Gene	Expression Compartment	Level of Resistance	Mode of Resistance	Special Future	Year	Ref.
Tobacco	*merA/B*	Plastid	300 μM PMA/$HgCl_2$	PMA to Hg(II) to Hg(0)	100-fold increase in Hg translocation and accumulation	2007	**44****
A. thaliana	*merP*	Cell membrane	10 μM $HgCl_2$	Possibly Hg(II) chelation	Localization to the cell membrane	2009	**49****

PMA: phenylmercuric acetate; Organic-Hg: PMA and methyl mercury; MerA/B: expression of *merA* and *merB* genes; References in bold: relevant;

* special interest;

** outstanding interest

Curr Opin Biotechnol. Author manuscript; available in PMC 2010 April 1.