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Perspectives for genetic engineering for the phytoremediation of arsenic-contaminated environments: from imagination to reality?

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Abstract

Phytoremediation to clean up arsenic-contaminated environments has been widely hailed as environmentally friendly and cost effective, and genetic engineering is believed to improve the efficiency and versatility of phytoremediation. Successful genetic engineering requires the thorough understanding of the mechanisms involved in arsenic tolerance and accumulation by natural plant species. Key mechanisms include arsenate reduction, arsenic sequestration in vacuoles of root or shoot, arsenic loading to the xylem, and volatilization through the leaves. Key advances include the identification of arsenic (As) translocation from root to shoot in the As hyperaccumulator, *Pteris vittata*, and the characterization of related key genes from hyperaccumulator and nonaccumulators. In this paper we have proposed three pathways for genetic engineering: arsenic sequestration in the root, hyperaccumulation of arsenic in aboveground tissues, and phytovolatilization.

Introduction

Arsenic is introduced into the environment through both geological and anthropogenic processes and is considered as a global contaminant. Arsenic is among the top carcinogens, and arsenic elevation in soil (and thus food) and drinking water has been reported to affect millions of people around the globe [1,2]. Long-term exposure to arsenic is associated with a variety of diseases, including cancer and diabetes [3–5]. Worldwide, substantial effort has been directed to the removal of arsenic from water and soil through chemical and physical remediation processes, but these are expensive and therefore have limited applicability in many areas where arsenic contamination and poverty coexist, as a result, the consumption of arsenic-tainted water and food remains the major route of arsenic exposure in humans. Alternative and environmental-friendly technologies are thus urgently needed to combat global arsenic contamination.

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Among various technologies available so far or under development currently, phytoremediation is considered to be the most environmentally friendly and cost effective. The phytoremediation of arsenic-contaminated environments includes the following types: firstly, phytostabilization, which refers to the use of plants (or vegetation) to minimize arsenic dispersion from soil to water or air; secondly, phytoextraction (or phytofiltration), which refers to the use of plants to remove arsenic from soil or water; thirdly, phytovolatilization is a newly conceived and specialized form of phytoremediation and involves the production of volatile arsenic compounds and their emission from plants. However, a common feature of most types of phytoremediation is arsenic tolerance and accumulation, and both tolerance and accumulation involve arsenic compartmentation and translocation. This paper will thus review some recent progress in genetic engineering related to arsenic compartmentation and translocation in plants, and will discuss some of the key issues of how to make phytoremediation work in reality.

Brief overview of plant uptake and metabolism of arsenic

Plant uptake and metabolism of arsenic has recently been reviewed by Tripathi *et al.* [6] and Zhao *et al.* [7••], so here we will only provide a brief overview. Arsenic in the environment mainly exists in two inorganic oxidation states, arsenate (As(V)) and arsenite (As(III)). As(V) and As(III) enter plant cells via phosphate transporters and aquaglyceroporins, respectively, as reviewed in [8]. Once taken up, As(V) is reduced to As(III), catalyzed largely by arsenate reductases, members of the superfamily of protein tyrosine phosphatase (PTPase) [9]. As(III) can then be complexed with glutathione (GSH) or phytochelatins (PCs), Raab *et al.* [10] identified up to 14 different species of arsenic complexes in sunflower plants. As(III) or complexed As(III) is then transported across the tonoplast and sequestered in the vacuole. Most data support the idea that arsenic is translocated from the roots to the tissues above ground, mostly in the form of As(III) [11,12••]. As(III) can be methylated to form monomethylarsenate (MMAs(V)), dimethylarsenate (DMAs(V)), and trimethylarsine oxide (TMAO(V)) *in planta* [7••,13].

Genetic engineering for arsenic sequestration in vacuole

Complexation of As(III) with PCs or GSH is an efficient way to detoxify arsenic, probably because the complexes are pumped and sequestered in the vacuole catalyzed by the homologs of multidrug resistance proteins (MRPs), members of the ABC superfamily [14,15]. Targeting increased accumulation or synthesis of PCs and/or GSH may be one way to develop arsenic phytoremediation. Increased expression of phytochelatin synthase (PCS), the rate-limiting step in PC biosynthesis, has been attempted to increase plant tolerance to and accumulation of arsenic. Gasic and Korban [16] found that the overexpression of PCS in Indian mustard increased its tolerance to arsenic but did not enhance arsenic accumulation significantly. The lack of response in accumulation could be due to the fact that PC synthesis is also limited by the production of GSH. A more recent study by Guo *et al.* [17] showed that overexpressing AtPCS1 and GSH1, which encodes γ-glutamylcysteine synthetase (γ-ECS), the rate-limiting step in GSH biosynthesis, individually in *Arabidopsis thaliana* increased arsenic tolerance and accumulation. Although these studies indicated the feasibility of overexpressing PCS and/or γ-ECS for increasing arsenic accumulation and

concomitantly tolerance, there are no direct data on the site of arsenic storage in these transgenic lines; thus it remains unclear whether the complexed As(III) is primarily vacuolar or remains in the cytoplasm. It is possible that transport of complexed As(III) or even free As(III) across the tonoplast membrane is potentially the rate-limiting step in overall arsenic tolerance and accumulation. Yet, to date, there are no reports of genetic engineering of tonoplast transport.

Even if arsenic is sequestered in the vacuole, its overall effect on tolerance will depend on the spatial expression of the relevant genes *in planta*. If these genes are overexpressed in root, this could result in reduced arsenic translocation from root to shoot, which is of practical relevance for phytostabilization. On the contrary, if these genes were specifically overexpressed in above ground tissues, then the genetically modified plant might be useful for phytoextraction. Of course, the ultimate efficiency of spatial distribution may depend on the machinery of arsenic translocation from root to shoot, as discussed below.

Genetic engineering for arsenate reduction

It is clear that both arsenic sequestration and translocation are closely linked to the forms of arsenic that exist within plant tissues. Trivalent arsenite is most easily trapped in the root through vacuole sequestration of thiol conjugates, but, under aerobic conditions, much of the arsenic coming into the cells is as pentavalent arsenate. Therefore the reduction of arsenate to arsenite is a key step in arsenic metabolism. To express the genes for arsenate reductases, genetically engineering plants have been applied for phytoremediation. Dhankher *et al.* [18] found that overexpressing the gene for the *Escherichia coli* arsenate reductase gene, *arsC*, in *A. thaliana* under the control of a light-responsive transcription factor (so that it would be expressed only in above ground tissues) led to hypersensitivity to arsenic. Although this seemed at first a counter-intuitive result, the authors considered the possibility that the product, arsenite, was more toxic than the substrate, arsenate, without sufficient thiols to form the $As(GS)_3$ conjugate. For that reason the gene for the *E. coli* γ-ECS, which would increase the rate of GSH biosynthesis, was coexpressed with *arsC*, resulting in higher tolerance to and accumulation of arsenic. This is consistent with tolerance being related to the sequestration of As(III)–thiol conjugates in the vacuole.

Although phytoremediation might be facilitated by the expression of heterologous arsenate reductases in above ground tissues, it is now clear that plants have their own such enzymes that are expressed constitutively [19•,20]. One reason for the slow translocation of arsenic from root to shoot could be due to endogenous arsenate reductase activity in the root, so that the arsenate entering root cells is partly reduced to arsenite, conjugated with thiols, and sequestered in the root vacuole. Therefore, if expression of the native arsenate reductase gene(s) could be reduced in the root, more arsenate might be available for movement to aboveground tissues. In support of this possibility, Dhankher *et al.* [21] reported that silencing the arsenate reductase gene AtACR2 in the root of *A. thalina* resulted in the hyperaccumulation of arsenic in the shoot, although the authors did not present direct evidence on the speciation of arsenic in different plant tissues and in the xylem.

Genetic engineering for increased translocation from root to shoot

One of the key properties of arsenic hyperaccumulators such as *Pteris vittata* is a highly efficient system of arsenic translocation from root to shoot [11,22], while most non-hyperaccumulators usually have a low mobility rate compared to *P. vittata*. Arsenic mobility from root to shoot varies considerably among different plant species, suggesting that it is under genetic control. A key step in arsenic translocation from root to shoot is arsenic loading to the xylem, a process that is not well understood. Recently, Ma *et al.* [23,24] identified a gene encoding an efflux protein, Lsi2, which is responsible for loading arsenite into the xylem, as arsenite is the dominant arsenic species in the xylem. An Lsi2 mutation resulted in a nearly 50% reduction in arsenic accumulation in the shoot. Lsi2 is a homolog of the *E. coli* ArsB, which is an As(III)/H^+ exchanger that confers bacterial arsenite resistance [25]. The plant efflux protein apparently transports both metalloids As(III) and Si(IV). It will be interesting to examine whether overexpression of genes such as Lsi2 will enhance arsenic translocation from root to shoot.

Genetic engineering for volatilization

Many organisms, including bacteria, fungi, and animals, methylate arsenic. Methylated arsenic species have been detected in several plant species, including rice grain [26,27], and recent data suggest that this is the result of endogenous methylation by the plants themselves [13]. The final product of the methylation pathway is the gas trimethylarsine (TMAs(III)), which can be volatilized from the plant. Recently, Qin *et al.* [28••] cloned a gene encoding an As(III)-*S*-adenosylmethionine methyltransferase (*arsM*) from the soil bacterium *Rhodopseudomonas palustris*. Expression of the *arsM* gene in an arsenic-sensitive strain of *E. coli* that had been genetically engineered to remove all arsenic detoxification genes resulted in the biosynthesis of several methylated forms of arsenic, including volatile TMAs(III) and concomitant arsenic tolerance. These results indicate that the expression of the single methyltransferase gene is sufficient to produce both volatilization of and tolerance to arsenic. More recently Rosen and coworkers have identified the gene for an ArsM homolog in a primitive plant, the eukaryotic alga *Cyanidioschyzon merolae* [29••]. Cells expressing CmArsM similarly methylate As(III), as does the purified enzyme. Whether similar processes are also present in higher plants remains unclear, but, in a rice microarray study, a putative gene annotated as a methyltransferase was upregulated by exposure to arsenate in the growth solution [30•]. These results point to the possibility of engineering arsenic volatilization for the phytoremediation of arsenic-contaminated water and soil and also to improve the safety of the food supply by reducing the arsenic content of the rice grain.

Conclusions

Successful phytoremediation depends largely on our understanding of the bioavailability of arsenic in soil, and of plant tolerance to and accumulation of arsenic. We have discussed the complexity of plant tolerance and accumulation of arsenic; it is clear that arsenic accumulation in plants is modulated by a network of functional genes, their expression patterns, and temporal/spatial coordination. Here we summarize the key mechanisms of

arsenic tolerance and accumulation and point out key pathways that can be manipulated for phytoremediation (Figure 1). To date, there have been a few studies that indicate the feasibility of manipulating one or more genes for the phytoremediation of arsenic-contaminated environments. However, successful phytoremediation may require a more complex system-wide approach that combines many of these single methodologies. Finally, whether transgenic plants designed for the remediation of arsenic-contaminated soil or water can work under field conditions is a major question. Practical applications of phytoremediation will require the analysis of the following topics:

- Further elucidation of the molecular mechanisms of arsenic tolerance and accumulation, particularly where and how arsenic is translocated and stored in plants.
- Genetic modification of fast-growing and high-biomass crop plants to accumulate substantial arsenic in above ground tissues for phytoextraction and/or volatilization, or to sequester arsenic in roots for phytotstabilization.
- Integration (molecular design) of multiple pathways of arsenic uptake and metabolism for the optimization of phytoremediation using genetically modified ‘smart’ plants.

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• of special interest

•• of outstanding interest

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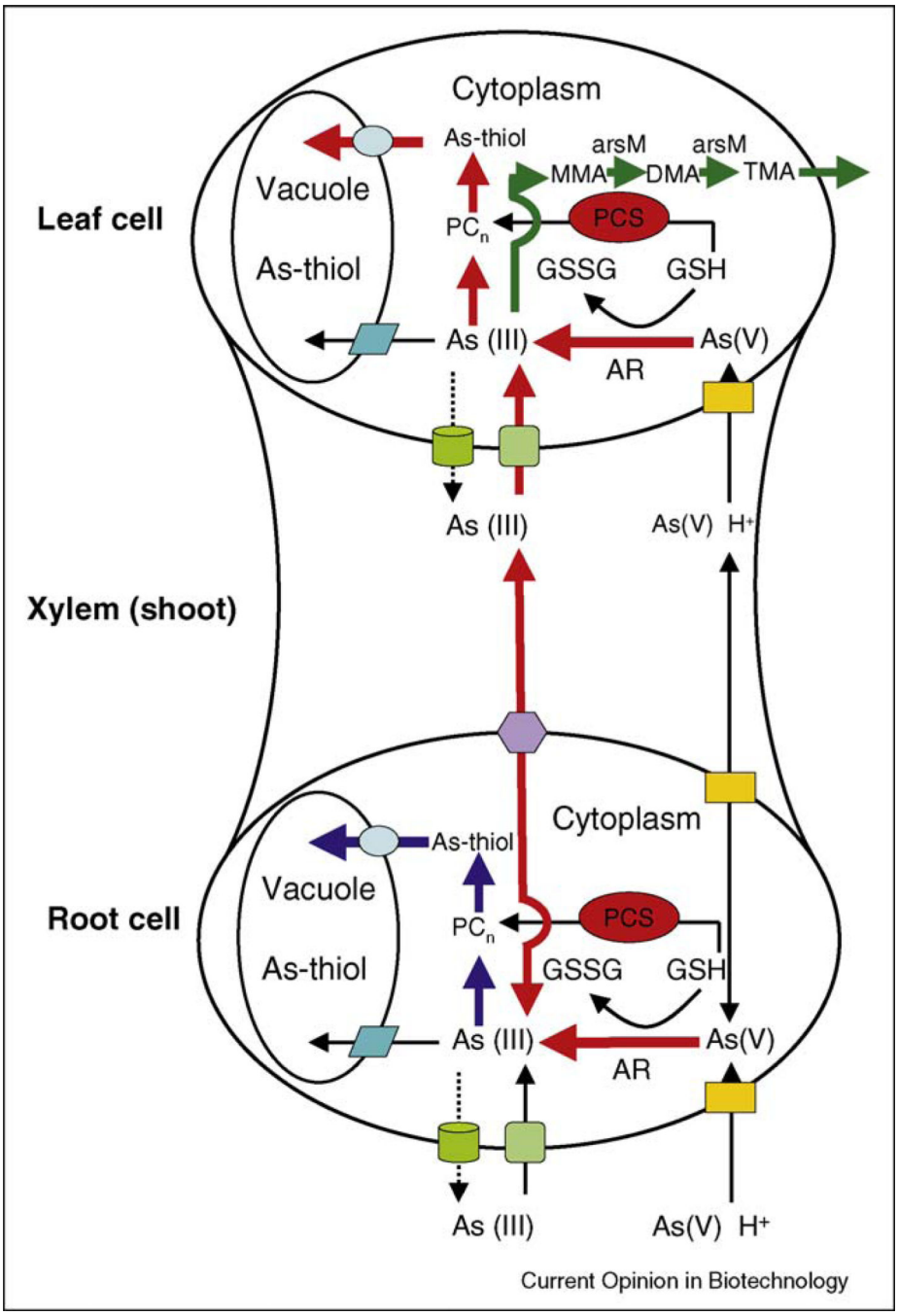

Figure 1.

Schematic diagram of arsenic uptake and metabolism in plants and possible genetic manipulations that can be designed for efficient phytoremediation. The pathway in *red* is for phytoextraction, in *dark blue* for phytostabilization, and in *dark green* for phytovolatilization. AR, arsenate reductase; PCS, phytochelatin synthase; arsM, As(III)-*S*-adenosylmethionine methyltransferase. Phosphate/arsenate transporter; plasma membrane aquaporin channel; unidentified arsenite efflux transporter; As-thiol

transporter; tonoplast aquaporin channel for As(III) transporter to vacuole; arsenite efflux carrier Lsi2.