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# Mechanisms to cope with arsenic or cadmium excess in plants

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The metalloid arsenic and the heavy metal cadmium have no demonstrated biological function in plants. Both elements are highly toxic and of major concern with respect to their accumulation in soils, in the food-chain or in drinking water. Arsenate is taken up by phosphate transporters and rapidly reduced to arsenite, As(III). In reducing environments, As(III) is taken up by aquaporin nodulin 26-like intrinsic proteins. Cd<sup>2+</sup> enters the root via essential metal uptake systems. As(III) and Cd<sup>2+</sup> share some similarity between their toxicology and sequestration machineries. Recent progress in understanding the mechanisms of As and Cd uptake and detoxification is presented, including the elucidation of why rice takes up so much arsenic from soil and of mechanisms of As and Cd hypertolerance.

## Addresses

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**Current Opinion in Plant Biology** 2009, **12**:1–9

This review comes from a themed issue on  
Physiology and metabolism  
Edited by David Salt and Lorraine Williams

1369-5266/\$ – see front matter  
Published by Elsevier Ltd.

DOI 10.1016/j.pbi.2009.05.001

## Introduction

The metalloid arsenic and the heavy metal cadmium are often considered to be biologically nonessential. However, under Zn limitation many diatoms use Cd instead of Zn in carbonic anhydrase [1]. As(III) can be used as the sole electron donor for anoxygenic photosynthesis in bacteria from hot spring biofilms [2\*]. Arsenic may also be beneficial in methionine metabolism and gene silencing in animals [3]. Essential or beneficial functions for Cd or As have not been reported in higher plants, except for some Cd-hyperaccumulating populations of *Thlaspi caerulescens*, which require Cd for optimum growth [4,5]. However, the possibility cannot be excluded that the growth promoting effects of Cd in these populations may be an indirect effect of interference with the

plant-internal availability of ‘real’ nutritional elements. The essential character of elements is thought to be a consequence of their chemical characteristics and availability [6]. Arsenic and cadmium are found naturally at low concentrations in the earth’s crust and may not have been recruited during evolution because of their lower abundance compared to phosphorus and zinc, respectively, which are the neighboring elements in the respective columns of the periodic table. The similarity between these pairs makes Cd and As potentially toxic for the cell because they tend to substitute for Zn and P, respectively, in cellular metabolism. However, unlike P, which is always present as phosphate in cells, cellular As can be present as arsenate, As(V), which is a phosphate chemical analog, but also as arsenite, As(III), which behaves as a sulphur-seeking heavy metal ion, rather like Cd<sup>2+</sup>.

Since cellular As(V) is usually rapidly reduced to As(III) in cells, either enzymatically or nonenzymatically, there is a degree of similarity between the toxicologies of, and the sequestration machineries for Cd and As.

Arsenic and cadmium are both potential threats for human health and the environment, through their accumulation in the soil, in the food-chain and locally in drinking water [7,8\*]. Human activities (metallic industries, contaminated fertilizers, herbicides or insecticides, irrigation with As-contaminated groundwater, and use of contaminated sewage sludge) are largely responsible for the accumulation of above-background levels of As and Cd in soils.

Despite the toxicity of As and Cd, high tolerance levels to these elements have evolved in a number of plant species, mainly through mechanisms of exclusion. Some plant species, belonging to the class of hyperaccumulators, can accumulate exceptional concentrations of As (above 0.1%) and Cd (above 0.01%) in their shoot dry weight without toxicity symptoms. Arsenic hyperaccumulation seems to be confined to the Pteridaceae family of ferns. Cd hyperaccumulation is present only in some populations of *T. caerulescens*, *T. praecox*, and *Arabidopsis halleri*, all belonging to the Brassicaceae family, and *Sedum alfredii* (Crassulaceae).

Interest in As and Cd tolerance and accumulation capacities of plants is driven by potential applications in phytoremediation and food security (which is reviewed by Zhao *et al.* in this issue).

The close relatedness of *A. halleri* and *T. caerulescens* to *Arabidopsis thaliana* has allowed the use of high-throughput technologies, in particular *Arabidopsis*

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DNA chips. A large array of genes are constitutively highly expressed in Cd hyperaccumulators compared to a nonhyperaccumulating related species, as reviewed in [9]. Gene duplication and modification of *cis*-regulatory elements are demonstrated mechanisms of enhanced expression [10<sup>••</sup>]. The importance of *trans*-regulatory elements or modified epigenetic regulation has not or been little studied. The molecular study of these Cd hyperaccumulators has further unraveled the role of genes involved in metal homeostasis and detoxification previously identified in *A. thaliana*. The major metal(-loid) detoxification mechanisms in plants are: transport to the major storage organs or tissues, chelation, sub-cellular compartmentalization, or efflux from the plant body. This review will present recent progress in the understanding of the latter mechanisms in hyperaccumulating and nonhyperaccumulating plants, which is illustrated in Figure 1.

### Uptake of As and Cd from the soil into the root

Depending on the soil redox status, soil As can be present as arsenite, As(III), or arsenate, As(V), whereas Cd is always in the divalent form. Bioavailability depends on pH, soil structure, soil organic matter, and chemical speciation, but Cd is usually more bioavailable than As.

As(V) is easily incorporated into plant cells through the high-affinity Pi transport system. Naturally selected As(V) hypertolerance in plants, apart from As hyperaccumulators, generally relies on decreased As(V) uptake, because of suppression of the high-affinity Pi uptake system (reviewed in [11]). As shown in *Holcus lanatus*, As(V) hypertolerance is not associated with any hypertolerance to As(III), because of the different uptake mechanisms of As(V) and As(III) [12]. As(III) is mainly taken up through members of the NIP (nodulin 26-like intrinsic protein) subfamily of aquaporins [13<sup>•</sup>,14]. As(III) uptake is of particular importance for flooded rice with its roots growing in reducing environments. Arsenite has a molecular size and structure similar to that of silicic acid and the silicon transporter OsNIP2;1/Lsi1 was recently demonstrated to be responsible for most of the As(III) influx into rice roots [15<sup>••</sup>], explaining why rice, which has a high silicon requirement, takes up so much arsenic from soil. As(III) efflux from the roots into the rhizosphere has been demonstrated in a number of plant species. In nonhyperaccumulators very considerable fractions (50–85%) of the arsenic taken up may be removed from the plant body via root As(III) efflux; however, in the As hyperaccumulator, *P. vittata*, hardly any As(III) efflux was observed (Figure 1) (reviewed in [8<sup>•</sup>]).

The uptake of Cd from the soil seems to occur mainly via Ca<sup>2+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>, and Zn<sup>2+</sup> transporters [6] (Figure 1). The best-studied nonspecific transporter is the ZIP IRT1, which is the major transporter respon-

sible for high-affinity iron uptake from the soil. While the specificity of AtIRT1 could be increased by mutagenesis, no mutated forms of AtIRT1 transporting Fe and devoid of Cd transport activity have yet been identified [16]. However, in *T. caerulescens* there is no evidence that TcIRT1 can transport Cd above Fe [17<sup>•</sup>], which would be particularly useful in Fe biofortification strategies.

### Toxicity and plant responses

#### Primary targets of As and Cd toxicity

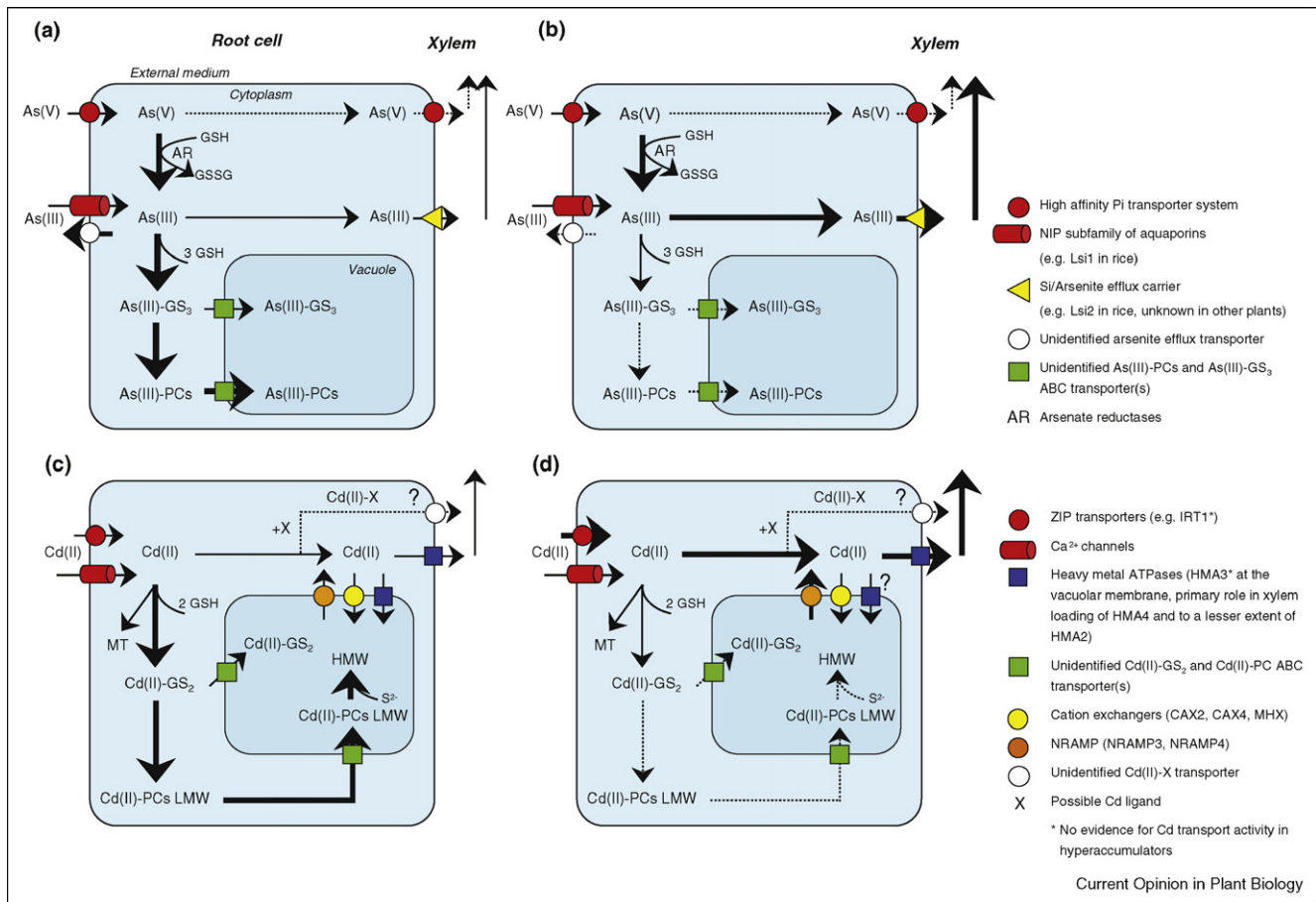
The primary targets of Cd and As toxicity are unknown. As(V) is potentially toxic because it can substitute for phosphate in phosphorylation reactions, including ATP synthesis [8<sup>•</sup>]. An important source of Cd toxicity is its chemical similarity with essential elements, in particular Zn, but also Ca and Fe, deregulating the homeostasis of the latter elements or causing their displacement from proteins.

However, in plant cells As(V) is rapidly reduced to As(III), catalyzed by ACR2 arsenate reductases [12,18]. Toxicity of As(III) like that of Cd is probably primarily due to high sulphhydryl reactivity. Arsenic, either supplied as As(V) or As(III), causes oxidative stress, like Cd, and can deplete reduced glutathione, an important cellular antioxidant, through the formation of As(III)–glutathione or Cd–glutathione complexes [As(III)–GS<sub>3</sub> or Cd(II)–GS<sub>2</sub>] and As(III)-induced or Cd-induced phytochelatin (PC) synthesis.

As in animals, the mitochondrial electron transfer chain of plant cells is thought to be one of the major targets of Cd toxicity, and is the site of the most rapid Cd-induced ROS production [19]. The photosynthetic electron transfer chain is another reported target of Cd<sup>2+</sup> toxicity (reviewed in [20]). Increased ROS production induces lipid peroxidation. It was recently shown that vitamin E (alpha-tocopherol, the main antioxidant in membranes) is crucial in the tolerance of *A. thaliana* to oxidative stress induced by Cd [21]. Cd also inhibits water transport, causing proline accumulation as a consequence of water stress [22].

Finally As, either supplied as As(V) or As(III), and Cd are both mutagenic [23]. Cd inactivates DNA mismatch repair in yeast and human cells [24] and the same mechanisms may act in plants. A putative chromatin remodeling factor, named OXS3 was recently identified in a screen for Cd tolerance of a *Brassica juncea* cDNA library in *Schizosaccharomyces pombe* [25]. An *oxs3* mutant was hypersensitive to Cd and overexpression improved Cd tolerance. The authors postulate that OXS3 might protect DNA or alter its transcriptional selectivity. Interestingly OXS3 overexpression also enhanced tolerance to As, Cu, and Zn or oxidizing chemicals like diamide.

Figure 1



Mechanisms to cope with As or Cd excess in roots. A schematic representation of main processes involved in As (a) and (b) and Cd (c) and (d) uptake, possible metabolism, vacuolar sequestration, and translocation in roots of nonhyperaccumulators (a and c) and hyperaccumulators (b and d). Inside the cell only the vacuole is shown. Line thickness relates to flux rate. Question marks refer to poorly characterized processes. For simplicity, neither root tissue specificity nor efficiency of radial symplastic route, nor long-distance transport through phloem is represented. Panels a and b are adapted from [8]. As can enter root cells as As(V) or As(III). As(V) (the main form of As taken up by the hyperaccumulator ferns) is taken up by the high-affinity Pi transport system and is rapidly reduced in As(III) by arsenate reductases (AR). As(III), which is the major As form in reducing environments, is taken up by nodulin 26-like protein (NIP) subfamily of aquaporins. In nonhyperaccumulators, a large fraction of As(III) is effluxed from the roots to the rhizosphere by an unidentified transporter. This efflux is negligible in hyperaccumulators. As(III) can form complexes with glutathione, and As(III)-GS<sub>3</sub> can be transported into the vacuole by an unidentified ABC transporter, or act as substrate for phytochelatin (PC) synthase. Nonhyperaccumulators mainly rely on PC complexation and sequestration of As(III)-PCs complexes in the root vacuoles for As detoxification and tolerance. As loaded into the xylem is mainly As(III). In rice, As(III) is transported to the xylem by the Si/arsenite effluxer Lsi2 (with no similarity to the silicon influx transporter Lsi1). In hyperaccumulators, there is little PC complexation of As(III) and the majority of arsenite is loaded into the xylem by an efflux carrier, which has not been identified to date. Cd<sup>2+</sup> ions are taken up by Fe<sup>2+</sup> and Zn<sup>2+</sup> ZIP transporters and possibly by Ca<sup>2+</sup> transporters/channels. In nonhyperaccumulators like *Arabidopsis*, the ZIP transporter IRT1 seems to be a main entry for Cd. As described for As, the main detoxification pathway of Cd in roots of nonhyperaccumulators relies on PC complexation and vacuolar transport of Cd-PCs complexes of low molecular weight (LMW). In the vacuole, high-molecular-weight complexes (HMW) may be formed that contain sulphides (S<sup>2-</sup>). The stability of those complexes and the fate of PCs are not well understood. Cd(II) can also be transported to the vacuole by the activity of different transporters (cation exchangers, HMA3) or as Cd-GS<sub>2</sub> complexes by an unidentified ABC transporter. Part of the vacuolar Cd(II) pool can be effluxed back into the cytosol by NRAMP activity. Metallothioneins (MT) are also potential Cd ligand in the cytosol. A considerable fraction of Cd loaded into the xylem seems to be in the ionic form. HMA4 and to a lesser extent HMA2 are involved in Cd xylem loading. Cd may also be loaded as bound to an unidentified (X) ligand. In Cd hyperaccumulators, a higher influx of Cd into the roots is thought to be mediated by ZIP transporters. In *Thlaspi caerulescens* there is no conclusive evidence that IRT1 homologs have a Cd transport activity. Vacuolar storage of Cd in roots is limited, either as Cd(II) or Cd(II)-GS/Cd(II)-PC complexes. There is no evidence that HMA3 has Cd transport activity in hyperaccumulators. In addition NRAMP3 and 4 genes are highly expressed, suggesting a higher vacuolar efflux. The majority of Cd(II) is loaded into the xylem by the activity of HMA4.

**Sensing and signaling of As and Cd excess**

Plant responses to nonessential metal(loid)s are thought to be triggered by the damage occurring as a consequence of excessive exposure.

Arsenic stress signaling, contrary to Cd, has scarcely been studied in plants thus far. Signaling of As(V) or Cd stress may occur through their impact on homeostasis of essential elements. In *A. thaliana*, As(V) represses the activation

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of genes specifically involved in Pi uptake, while inducing others transcriptionally regulated by As(V), suggesting that converse signaling pathways are involved in plant responses to As(V) and low Pi availability [26<sup>\*</sup>]. Cd<sup>2+</sup> exposure seems to rapidly induce apparent Zn deficiency, maybe through binding to a Zn sensor protein [27,28].

As-induced or Cd-induced increase in ROS production may act as a cellular signal triggering the stress response. Stress-responsive MAP kinases seem to be involved in transcriptional responses to Cd as they are activated possibly by ROS under Cd<sup>2+</sup> excess, but more slowly than upon Cu<sup>2+</sup> treatment [29]. It is thought that MAPK activation by excess copper and cadmium is mediated by ROS. ROS production by Cd is delayed supporting the concept that oxidative injury by Cd is a secondary effect. Another putative player in Cd-induced oxidative stress signaling is AtOS1, a member of the Abc1 family localized in the chloroplasts. AtOS1 does not transport Cd but seems to be crucial for Cd tolerance, possibly through a putative kinase activity [30]. Similar to other abiotic stresses, different plant hormones and growth regulators may participate in the plant response to metal(loid) stress. Exposure to Cd can induce an increase in jasmonate, ABA, ethylene, and salicylic acid (reviewed in [20]). However, Weber *et al.* [27] tested growth of SA-deficient *nahG* and ethylene *etr1-1* and *ein2-1* mutants in the presence of Cd<sup>2+</sup> and none of them was significantly compromised in Cd<sup>2+</sup> tolerance, which argues against a role of SA or ethylene in mediating the protective response to Cd<sup>2+</sup>.

Exposure to As [31] or Cd [27,32,33] has been reported to induce many different transcription factors. Some of them are constitutively upregulated in *A. halleri* or *T. caerulescens*. However, there are no clues regarding their precise functions as yet.

##### Detoxification

A major strategy to detoxify nonessential trace metal(loid)s is the synthesis of specific low-molecular-weight chelators to avoid binding to physiologically important proteins and to facilitate their transport into the vacuoles. The favored ligands of As(III) and Cd<sup>2+</sup> are thiols, present in glutathione and PC.

The tripeptide glutathione (Glu-Cys-Gly), GSH, is synthesized by gamma-glutamylcysteine synthetase ( $\gamma$ -ECS) and glutathione synthetase (GS). GSH can bind to several metals and metalloids and is also a key metabolite in cellular redox balance, which is usually one of the targets of metal(loid) toxicity. Increasing GSH synthesis is considered a means of increasing metal(loid) binding capacity as well as a way to increase cellular defense against oxidative stress. Since glutathione is the precursor of PC, overexpression of  $\gamma$ -ECS or GS usually also leads to higher rates of PC accumulation under metal exposure.

However, increasing GSH (and PC) synthesis alone seems to be insufficient to achieve more than marginal enhancements of Cd and As tolerance or accumulation (reviewed in [34]). Glutathione conjugates can be transported into the vacuoles. In *Saccharomyces cerevisiae*, which does not rely on PC detoxification, the vacuolar Cd-GS<sub>2</sub> transporter YCF1 is a crucial factor for Cd and As tolerance. Overexpression of ScYCF1 confers higher capacity for lead and cadmium accumulation in *Arabidopsis thaliana* by enhanced transport of Cd-GS<sub>2</sub> into the vacuoles [35], although As accumulation has not yet been tested. To date a clear homolog of YCF1 in plants has not been identified. However, there is clear evidence for the involvement of an ABC transporter of As(III)-GS<sub>3</sub> and Cd-GS<sub>2</sub> into vacuoles of plant cells [12,34]. ABC transporters for Cd have been identified in *A. thaliana*, ATM3 in the mitochondria [36] and PDR8 in the plasma membrane of root hairs and epidermis [37]. However, ATM3 seems to be primarily involved in Fe homeostasis and PDR8 in pathogen defense.

PC are a family of peptides with the general structure ( $\gamma$ -Glu-Cys)*n*-Gly where *n* = 2–11. They are synthesized from GSH, catalyzed by PC synthase (PCS). PCS is constitutively expressed, but requires post-translational activation by metal(loid)s, of which As and Cd belong to the most effective activators. The formation of As-GS<sub>3</sub> or Cd-GS<sub>2</sub> thiolates, which act as high-affinity substrates for the enzyme, seems to be sufficient for its activation [38]. PC are found in all plants, some fungi and animals. PC synthesis seems to be the main factor for basal Cd and As tolerance [6,39], but not in hypertolerant plants or hyperaccumulators [40] (Figure 1). In *A. thaliana* PC deficiency leads to Cd and As hypersensitivity [41]. In roots of nonhypertolerant plants the major part of Cd is chelated by PC. This chelation event is followed by the transport of PC-Cd complexes into the vacuole, which is catalyzed by the ABC transporter HMT1 in *S. pombe*. The same scenario is proposed for arsenite [8<sup>\*</sup>]. However, overexpression of HMT1 in *S. pombe* or in *S. cerevisiae* did not increase tolerance to As(III or V), but only to Cd, and the phenotype was independent of PC synthesis but dependent on GSH synthesis, demonstrating that glutathione-Cd conjugates are also substrates of HMT1, contrary to what was previously believed [42<sup>\*</sup>]. In addition, HMT1-deficient *S. pombe* cells are specifically hypersensitive to Cd<sup>2+</sup>, not to Hg(II) and As(III), whereas PCS-deficient cells are hypersensitive to all these metals. Moreover Cd hypersensitivity in *hmt1* mutant cells is complemented by the HMT1 ortholog from *Drosophila*, an organism without PCS, and SpHMT1 is either incapable or not essential for PC-Cd or apoPC transport across the tonoplast [43]. While PC-Cd or PC-As complexes have been observed in plant vacuoles, the responsible transporter has not been identified yet but is most likely an ABC-type one, as appeared from biochemical characterization [44].

PCS overexpression has variable effects on Cd tolerance, ranging from sensitization to enhanced tolerance, depending on the donor and receiver species [45–47]. For unknown reasons, *PCS1* overexpression in *A. thaliana* enhanced tolerance to As but decreased tolerance to Cd [45]. In several studies, PCS overexpression led to the accumulation of  $\gamma$ -Glu-Cys, but to a strong depletion of GSH, which might explain the sensitization to Cd. Apparently, PCS exerts insufficient control over the rate of GSH synthesis. This might explain why Pomponi *et al.* [46] found substantially enhanced Cd tolerance and accumulation only after exogenous GSH supply in PCS-overexpressing tobacco. Likewise, increasing both glutathione and PC synthesis in *A. thaliana* improved the tolerance and accumulation of both cadmium and arsenic (III and V) [48].

It is noteworthy that the first step in thiol-based arsenate detoxification is the reduction of As(V) to As(III), since only As(III) can bind to thiols and activate PCS. Plants possess arsenate reductases showing homology with the yeast arsenate reductase, *Acr2*, which seem to be essential for basic As(V) tolerance [12,49]. Recent studies on the *acr2* mutant showed that phenotypes are strongly dependent on plant P-status (M Arnetoli, H Schat, unpublished). Moreover, as demonstrated in As hypertolerant populations of *H. lanatus*, PC synthesis under As(V) exposure may be limited by the arsenate reductase capacity, rather than by PC synthetic capacity itself [12]. This might explain why only combined expression of  $\gamma$ -ECS and arsenate reductase (*ArsC*) substantially increased As(V) tolerance in *A. thaliana* [50]. Homologous overexpression of *AtACR2* alone, on the other hand, produced a dual phenotype: enhanced tolerance at slightly toxic As(V) exposure levels, but hypersensitivity at more toxic levels, the latter possibly owing to the saturation of the PC-based As(III) sequestration capacity [12].

Complementation of the *cad1-3* PC-deficient mutant with root-specific and shoot-specific expression of *PCS1*, grafting experiments in *A. thaliana*, and analysis of xylem and phloem saps in *Brassica napus* support a role for PC in long-distance bidirectional transport between root and shoot via the phloem [34,51,52]. There is also good evidence that in nonhyperaccumulators, like *B. juncea*, Cd is transported in the xylem (in which PC concentrations are negligible), as the hydrated cation [53]. As(V) accounts for a variable but considerable fraction of the total xylem As of nonhyperaccumulators although As(III) is always more abundant than As(V) [54,8\*].

However, efficient root to shoot transport in hyperaccumulators of As or Cd occurs mainly via the xylem and does therefore not depend on PC synthesis. There is even a negative correlation between translocation efficiency and

the degree of As(III) or Cd–thiol coordination in roots [55,56\*\*]. It seems that Cd and As hyperaccumulators transport the metals upward via the xylem, presumably mainly as free  $Cd^{2+}$  [57] or inorganic arsenite,  $H_3AsO_3$  [58].

Metallothioneins (MT) are small cysteine-rich proteins. MT–metal complexes seem to reside in the cytosolic compartment. A clear role for Cd binding has been demonstrated for MT in animals. Their exact roles in plants are poorly known but a large body of evidence associates MT primarily with copper homeostasis [39,59]. Nevertheless the ectopic expression of MT can increase tolerance to Cd [60] and *mt1a mt2b A. thaliana* mutants showed a decrease in Cd tolerance, although only in a PC-deficient *pcs1* mutant background [61].

While most Cd is chelated before its transport to the vacuole,  $Cd^{2+}$  can be directly transported into the vacuoles by  $Cd^{2+}$ /proton antiporters like CAX2 and CAX4 and possibly also by MHX [62,63]. The importance of this activity is not clear, but the overexpression of *AtCAX2* or *AtCAX4* in tobacco enhanced Cd and Zn transport into root tonoplast vesicles and enhanced Cd accumulation in roots of plants exposed to Cd [62]. The  $P_{1B}$ -ATPase HMA3 seems to be also involved in the vacuolar storage of Cd in nonhyperaccumulators, as recently demonstrated in *A. thaliana* [64]. *AtHMA3* has a high expression level in guard cells, hydathodes, vascular tissues, and the root apex [64]. In *A. halleri* shoots and in *T. caerulescens* roots, *HMA3* is constitutively highly expressed (reviewed in [9]). However, heterologous expression of *AhHMA3* in yeast supports transport activity for Zn but not for Cd [65].

In the leaves of *T. caerulescens* where Cd is mainly sequestered as vacuolar malate complexes [57], the identity of  $Cd^{2+}$  vacuolar transporters is still unknown. Likewise putative vacuolar As(III) transport in the shoot of As hyperaccumulators needs further investigation.

In contrast, NRAMP3 and NRAMP4 are responsible for  $Cd^{2+}$  efflux from the vacuole [66]. Their overexpression increased Cd sensitivity in *Arabidopsis*, as did loss-of-function mutations. The latter phenotype seems to be due to the impairment of Fe homeostasis, as NRAMP3 and NRAMP4 are responsible for the release of vacuolar  $Fe^{2+}$ . NRAMP3 and NRAMP4 are overexpressed both in roots and shoots of *T. caerulescens* where their roles are still unclear [67].

Efflux from the roots represents another potential strategy for metal detoxification. This process consists of uptake of As(V) and intracellular reduction to As(III), followed by the efflux of As(III) [68]. Plant As(III) effluxers have been identified in yeasts and bacteria but not in plants.

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In contrast to Cd, As can be methylated in plants, which is another potential detoxification mechanism, because methylated As species are less toxic than inorganic ones. As(V) exposure has been shown to upregulate several methyltransferases [31]. Methylated As species are much less abundant in plants than in animals, suggesting that methylation is not the primary strategy for As detoxification in plants. However, methylation may be important in specific organs, for example, seeds.

### Long-distance transport

While nontolerant plants and tolerant excluders mainly accumulate Cd and As in roots, the capacity to translocate these elements to the shoot is, however, a factor in tolerance. More efficient root to shoot translocation has evolved in hyperaccumulators, in which it seems to be a crucial mechanism of hypertolerance.

The mechanism of As translocation is not well understood yet, but in most plants As is present both as As(V) and As(III) in the xylem, suggesting that both forms can be loaded into the xylem, As(V) presumably by Pi transporters and As(III) by aquaporins. Root to shoot transport in rice is largely mediated by the silicon transporter, *Lsi2* [15\*\*]. In the As hyperaccumulator *P. vittata*, xylem As is almost exclusively present as As(III), and As(III) efflux from roots into the rhizosphere is negligible, compared to non-As-hyperaccumulators (reviewed in [8\*]) (Figure 1). It is unknown to what extent root to shoot As translocation is essential for As hypertolerance in this species.

The first identified transporter involved in Cd translocation from the root to the shoot is the P<sub>1B</sub>-type ATPase *AtHMA4* [69–71]. Recently a role for *AtHMA2* in Cd translocation was also demonstrated in *Arabidopsis*. However, loss of *HMA2* activity only influenced Cd transport in a *hma4* mutant background, resulting in the almost complete loss of root to shoot translocation [56\*\*]. *Hma4* and *hma2hma4* mutants showed increased sensitivity to Cd, but to a much lesser extent than a *pcs* mutant [56\*\*]. In the Cd/Zn hyperaccumulators *A. halleri* and *T. caerulescens*, *HMA4* is more highly expressed in both roots and shoots compared to Cd/Zn-sensitive close relatives [9] (Figure 1). 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 and/or accumulation to both metals. In *A. halleri* at least, this hypothesis was reinforced by the colocalization of major QTLs of Zn and Cd tolerance with the *HMA4* gene [72,73]. Recently, Hanikenne *et al.* [10\*\*] demonstrated the importance of *HMA4* in the hyperaccumulation of Zn and hypertolerance to Cd using RNAi-mediated silencing. *A. halleri* plants (from a Zn hyperaccumulator, Cd hypertolerant accession) with lower expression of *HMA4* were shown to translocate less Zn from the root to the shoot and to be more sensitive to Cd and Zn treatments (Cd translocation

was not measured). Interestingly, when expressed in *A. thaliana* under the activity of its native promoter, *AhHMA4* increased sensitivity to Zn and Cd, because of strongly increased metal accumulation in the shoot, which lacks efficient detoxification mechanisms [10\*\*].

### Other adaptive responses

Transcriptomic studies suggest that oxidative stress and protein denaturation are important components of Cd toxicity ([28,34] and references therein). Protective responses include enhanced antioxidative defense and higher synthesis of protein chaperones (like HSP and sHSP), ability to remove oxidized proteins, changes in cell wall composition, as well as in lignin deposition [33]. Responses to Cd also include the induction of genes involved in sulphur and glutathione metabolism ([33] and references therein).

### Concluding remarks

Much progress has been recently made into the mechanisms of uptake, distribution, and detoxification of As and Cd. Major highlights include the elucidation of efficient As accumulation by rice, of the major determinants of root to shoot Cd translocation, allowing strategies to be designed to enhance food security. Hyperaccumulators of As and Cd, which constitute fascinating materials for studying mechanisms of adaptation to extreme environments, are also exceptional gene reservoirs for phytoremediation applications. This year the first demonstration in a hyperaccumulator species of a major gene for Zn hyperaccumulation and Cd detoxification has been published. This study opens the way for other gene functional studies by RNAi. Further attention should be paid to the tissue-specific gene expression network required for the spatial distribution of metal(loid)s, in particular in hyperaccumulators, and the regulation of protein levels and activity.

### Acknowledgements

NV and CH thank the Belgian Science Policy (project IAP IV/33) and the Fonds National de la Recherche Scientifique (FRS-FNRS) for financial support. The authors thank A Smith for critical reading of this manuscript, S Clemens for his feedback on Figure 1, and the COST839 network for providing excellent opportunities of discussion.

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