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Toxic Heavy Metal and Metalloid Accumulation in Crop Plants and Foods

Stephan Clemens[1] and Jian Feng Ma[2]

[1]Department of Plant Physiology, University of Bayreuth, 95440 Bayreuth, Germany; email: stephan.clemens@uni-bayreuth.de

[2]Institute of Plant Science and Resources, Okayama University, Kurashiki 710-0046, Japan; email: maj@rib.okayama-u.ac.jp

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Keywords

food safety, arsenic accumulation, cadmium accumulation, natural variation, metal transporters

Abstract

Arsenic, cadmium, lead, and mercury are toxic elements that are almost ubiquitously present at low levels in the environment because of anthropogenic influences. Dietary intake of plant-derived food represents a major fraction of potentially health-threatening human exposure, especially to arsenic and cadmium. In the interest of better food safety, it is important to reduce toxic element accumulation in crops. A molecular understanding of the pathways responsible for this accumulation can enable the development of crop varieties with strongly reduced concentrations of toxic elements in their edible parts. Such understanding is rapidly progressing for arsenic and cadmium but is in its infancy for lead and mercury. Basic discoveries have been made in *Arabidopsis*, rice, and other models, and most advances in crops have been made in rice. Proteins mediating the uptake of arsenic and cadmium have been identified, and the speciation and biotransformations of arsenic are now understood. Factors controlling the efficiency of root-to-shoot translocation and the partitioning of toxic elements through the rice node have also been identified.

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INTRODUCTION

With his *Recherches Chimiques sur la Végétation*, published in 1804, Nicolas-Théodore de Saussure laid the foundation of plant nutrition (41). He demonstrated through careful quantitative analysis that growing plants increased in dry weight by more than their fixed carbon. Since that time, the mineral compositions of plants and how they arise have been fundamental questions of biology. Plants acquire essential and beneficial elements from the soil, but because their selectivity is imperfect, they can also take up nonessential elements when those elements are bioavailable. Some of these inadvertently accumulated elements are highly toxic, the most important ones being arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd) (49). These four elements rank first, second, third, and seventh, respectively, on the US Agency for Toxic Substances and Disease Registry (ATSDR) 2013 Priority List of Hazardous Substances, which includes organic toxicants (7).

Consumption of crops containing trace amounts of these toxic elements contributes to potentially health-threatening human exposure, albeit to varying degrees because of differences between the elements in the efficiency of transfer from the soil to aboveground tissues of crops (100). Nonoccupational Cd exposure of the (nonsmoking) general population is >90% attributable to the ingestion of cereals, vegetables, and other plant-derived food (21, 29). The percentage for smokers is lower, but only because they inhale Cd accumulated in tobacco leaves. Poisoning with As is widespread in many countries, especially in South and Southeast Asia. Even in areas such as Bangladesh, where contamination of drinking water is a major cause of As exposure (42), the consumption of rice contributes substantially to the overall As burden (148). The importance of rice as an As source, which is due to unique features of this species detailed below, also applies to human populations outside of Asia, as has been shown for US women (35).

By contrast, plant-derived food apparently plays only a minor role in Hg exposure, except in areas with Hg contamination of farmland (107). Methylmercury, which is far more toxic and more bioavailable than inorganic Hg, is taken up by humans predominantly via seafood, because methylmercury readily bioaccumulates through aquatic food chains (31, 83). For Pb, the situation is less well understood, because little is known about the mobility of Pb within plants. Keeping Pb

bioavailable for plant uptake poses technical challenges that have severely hampered physiological studies (33). As with Hg, root-to-shoot translocation factors for Pb are generally low (100), but Pb is nonetheless detectable in a large number of cereal and vegetable samples (e.g., 92). However, it is often not clear how much of the Pb content is in fact taken up from the soil solution. Especially in mining-affected areas, there can be substantial aerial deposition on the surfaces of leaves, seeds, or fruits.

Mechanistic insights into the pathways of toxic metal/metalloid accumulation can aid the development of crop plants with strongly reduced toxic element content, thereby limiting human health risks. Consistent with each element's soil-to-plant mobility and importance as a metal/metalloid contaminant in plant-derived food, the molecular understanding of accumulation pathways is far greater for As and Cd than for Pb and Hg. This difference is reflected in our article, which emphasizes the substantial progress made since several excellent reviews discussed the detoxification and accumulation of As and Cd by plants (81, 129, 146, 148). Pb and Hg are only sporadically covered.

Not discussed in this review is the accumulation of selenium (Se). In the 1990s, Se was regarded as one of the most relevant elemental food contaminants other than As, Cd, Pb, and Hg (75); since then, however, it has in fact become a target of biofortification efforts (73). Se is an essential trace element for animals and humans. Plant-derived food represents the main dietary source of Se, and insufficient Se intake is associated with several human diseases (43).

ARSENIC, CADMIUM, AND OTHER TOXIC ELEMENTS IN THE ENVIRONMENT

Most of the bioavailable Cd in the environment is of anthropogenic origin. The main sources are release by metal mining or smelting activities and application of phosphate fertilizer with Cd impurities (125). In the earth's crust, As is more abundant than Cd. Accordingly, As contamination stems from natural processes such as volcanism as well as from human activities. The latter include the use of As-based pesticides, herbicides, and wood preservatives and the burning of coal (150). In Bangladesh and other countries affected by As contamination of groundwater, irrigation further increases soil As levels (79).

Cd is present only in inorganic form and in one oxidation state (+2). Its bioavailability depends largely on soil conditions. It is more available in aerobic soils than in anaerobic soils because of the formation of insoluble CdS in the latter. Several different forms of As can be present in soil solutions: Besides inorganic As in the oxidation states +3 (arsenite) and +5 (arsenate), various pentavalent methylated As species [e.g., monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)] exist. Their relative proportions are highly variable depending on sources of contamination and soil conditions (145); for instance, in aerobic soils, As(V) is predominant, whereas under reducing conditions in flooded rice paddy fields, As(III) is predominant. Microbial activities influence the proportion of methylated As in the soil (145). Most of the As deliberately released into the environment—as pesticides, for instance—is organic As. (For a detailed discussion of As in the environment and the soil factors influencing bioavailability, see 148 and 150.)

Recent experiments have demonstrated that MMA and DMA are produced by bacteria in the rhizosphere and not through methylation of inorganic As in planta. Respective enzymatic activities have been well documented in bacteria, fungi, algae, and mammals. They are catalyzed by diverse *S*-adenosylmethionine-dependent As(III) methyltransferases and play an important role in the biogeochemical cycling of As (150). However, apparently no such activities exist in higher plants. When tomato, red clover, or rice plants were exposed to inorganic As in axenic culture, no methylated As species were detected; only after exposure to methylated As did the respective As species accumulate in roots and shoots (67). This result is consistent with other data demonstrating

a strong decrease in the concentrations of MMA and DMA in hydroponically cultivated rice when bacterial growth was suppressed by chloramphenicol (5). Furthermore, there is to date no evidence for the existence of genes encoding As(III) methyltransferases in genomes of higher plants (142), whereas the presence of methylated As in soil-grown rice plants coincides with the presence of bacterial *As(III) S-adenosylmethionine methyltransferase* (*arsM*) genes in these soils (51). Thus, the presence of methylated As, especially in rice, is due to the microbial synthesis of methylated As species in the rhizosphere and their uptake and translocation by the plant.

Pb was one of the first anthropogenic environmental pollutants (97). Pb mining dates back to before 3000 BCE because Pb is a soft, easy-to-process metal with a wide range of uses. It continues to be released anthropogenically by mining, smelting, and battery manufacturing. Enormous quantities are also present in the environment owing to the use of leaded gasoline for several decades (63). Pb is highly persistent in soils and hardly bioavailable under most conditions owing to low solubility at pH values greater than 5 and strong interaction with organic matter.

Hg in soil is largely a function of atmospheric deposition resulting from natural processes such as volcanism as well as human activities. Among the latter, the dominant Hg sources are fossil-fuel burning, artisanal small-scale gold mining, metal manufacturing, and the use of Hg in the chloralkali industry (103). In soil, anoxic conditions and higher temperatures favor the formation of methylmercury by bacteria, e.g., in rice paddy fields.

TOXICITY TO PLANTS AND HUMANS

As, Cd, Pb, and Hg are all highly toxic to both plants and humans in their ionic forms; As and Hg are also toxic in their methylated forms (36, 58). Whereas methylated As, at least in the pentavalent state, is generally considered less toxic than As(III) and As(V), methylated Hg is more toxic than Hg(II) for most organisms (45, 65). The primary targets of toxicity for these elements are not known, and there may not be any such targets; these elements can damage different cellular structures and a variety of tissues and organs. One major cause of toxicity is the strong interaction with sulfhydryl groups; another is the interference with the homeostasis of essential elements (for a comprehensive discussion of Cd as an example, see 74). The thiol reactivity can impair protein function and indirectly causes oxidative stress. Cd is known to interfere with calcium (Ca) metabolism in mammals, causing Itai-itai disease upon severe exposure. Both Cd and Pb can replace zinc (Zn) in proteins. Arsenate disturbs phosphate metabolism because of the chemical similarity of the two anions. Cd and inorganic As are classified as carcinogens.

Low-dose exposure far below the thresholds for acute toxicity can cause disease because of long-term bioaccumulation in the human body. A slow poisoning by chronic Cd and As exposure has been implicated in overall increases in mortality and a range of diseases, including various types of cancer and cardiovascular disease in the case of As (53) and renal damage and osteoporosis in the case of Cd (50). Public health concerns related to Pb have focused on neurotoxicity associated with negative effects on the intellectual abilities of children even at low exposure levels (12). Similarly, neurons are the main targets of methylmercury toxicity (83).

For As and Cd, there is general agreement that consumption of plant food contributes substantially to human exposure. Many populations have, for instance, a Cd intake above the provisional tolerable weekly intake (PTWI) levels determined by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (78). Moreover, the available data have led epidemiologists to question this PTWI (2). The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain called for the PTWI to be lowered from 5.8 to 2.5 μg per kilogram of body weight (29), a level that is near the average intake around the world (21).

The PTWI for Pb—which is exceeded, for instance, in some regions of China, according to recent analyses (147)—was deemed no longer appropriate in light of available epidemiological data (30), and the Joint FAO/WHO Expert Committee on Food Additives even concluded that the current PTWI must be withdrawn because this level of exposure is associated with measurable impairment of neurodevelopment in children (32). Thus, it is clearly in the interest of improving public health to lower dietary exposure to toxic elements.

MECHANISTIC INSIGHTS INTO THE ACCUMULATION OF TOXIC ELEMENTS

Toxic nonessential elements move into and through a plant along the same pathways as essential and beneficial elements. Thus, the molecular elucidation of plant metal homeostasis and mineral nutrition has laid the foundation for dissecting nonessential element accumulation. For example, several of the metal transporters identified in the early phase of discovery—which was driven largely by yeast expression experiments—were shown to be Cd permeable (22, 60, 121). Reverse genetics approaches in the model systems *Arabidopsis thaliana* and rice and analysis of natural variation in ionomic profiles of *A. thaliana* and crop species have in recent years yielded tremendous mechanistic understanding (57, 108). Work on rice has been leading the way because rice is both a model species and globally the most relevant source of human As and Cd intake (126, 148). Additional insight has come from investigation of metal-hyperaccumulating species (61).

The path of elements from the soil into vegetative and reproductive organs of a plant can be structured into distinct processes that vary in relevance depending on, for instance, the plant species, the habitat, the developmental state, and the tissue in question (23, 26, 81). Possibly following active mobilization from the soil or the apoplast, elements are taken up by transporters residing in the plasma membrane of root cells. Translocation to the shoot is dependent on the rate of trapping in different compartments of root cells, the mobility within the root symplast and across barriers such as the endodermis, loading into the xylem, and upward mobility in the xylem. Element accumulation in leaves requires uptake from the xylem and the apoplast, transport to sites of usage and into storage compartments, and the availability of binding molecules. Distribution between leaves and the loading of seeds are a function of phloem loading and mobility, the efficiency of xylem-to-phloem transfer, the rates of various symplast-to-apoplast and apoplast-to-symplast transport processes, and the availability of storage sites. Key questions addressed with respect to essential/beneficial and nonessential elements include (*a*) which transporters, metal-handling proteins, and low-molecular-weight metal chelators are involved in these processes; (*b*) where and when metals are localized in which ligand environments; and (*c*) which factors explain the genotype-dependent fraction of natural variation in element accumulation.

This last question arose from the screening of large cultivar collections and diversity panels. Such studies have in part been motivated by the concept of biofortification, i.e., the goal of fighting nutrient malnutrition (so-called hidden hunger) by developing crop varieties with higher micronutrient densities (10, 131). In model systems and in several crop species, variation in the ionomic profiles of leaves and seeds has been documented, a substantial fraction of which is genotype dependent. Cd and As have often been included in the list of monitored elements because a complementary goal of biofortification must be the reduction of toxic element accumulation. This is particularly important for Cd because Cd concentrations correlate strongly with Zn and iron (Fe) concentrations (55).

Genotypic variation in Cd accumulation by different types of crop plants was documented in the 1980s and 1990s (37, 75). Since then, a considerable number of more extensive studies have expanded this knowledge, e.g., for leafy vegetables (130), wheat (62), and barley (135). Significant

potential for breeding low-Cd and low-As varieties has been shown particularly well in rice (e.g., 4, 19, 46, 94, 95, 122). In the largest survey published to date, more than 1,700 rice accessions were cultivated over two years in flooded and nonflooded conditions (102). Consistent with many previous observations, the watering regime strongly influenced As and Cd grain accumulation in opposite ways. On average, flooded conditions led to a 30-fold increase in As accumulation, and nonflooded conditions led to 10-fold increase in Cd accumulation; these increases resulted from the higher bioavailability of As under reducing conditions [as As(III)] and the higher bioavailability of Cd under oxidizing conditions. The range of concentrations (maximum:minimum ratio) was higher for As and Cd than for most other elements (approximately 150 and 125 for Cd and As, respectively, under nonflooded conditions), and heritability was high (>0.6) for As under both conditions and for Cd under nonflooded conditions.

Natural variation in Pb accumulation by crops has not been studied as intensively. Variation clearly exists (19, 132), but little information is available on genotype influence. Data from a recent survey of rice (96) suggest, however, that this influence is much smaller for Pb than for As and Cd. Most of the variation is apparently due to environmental factors such as mining impact. Even less is known about Hg and methylmercury; as indicated above, researchers have only recently begun studying accumulation in crops grown in areas with Hg mining and smelting activities (107).

Uptake of Toxic Elements from Soil into Root Cells

Because they have no biological functions, nonessential elements enter cells as hitchhikers through transporters for essential or beneficial elements (20). The classic examples are Cd uptake via the Fe(II) transporter iron-regulated transporter 1 (IRT1) in *A. thaliana* (27) and via ZRT- and IRT-like protein 8 (ZIP8) and divalent metal transporter 1 (DMT1) into mammalian cells (28, 38). These transporters belong to the ZIP and natural resistance–associated macrophage protein (Nramp) families of metal transporters. Various members of these families (OsIRT1, OsIRT2, and OsNramp1) have been implicated in Cd uptake in rice (reviewed in 21, 126). Sasaki et al. (111) recently characterized OsNramp5 as a transporter important for manganese (Mn) acquisition and demonstrated that it represents the major pathway for Cd entry into rice roots (**Figure 1**). A knockout line and several knockdown lines showed much lower Mn and Cd concentrations in roots and shoots after growth in hydroponic culture or in soil. Short-term Mn and Cd uptake activity was strongly reduced. Localization of the OsNramp5 protein in the plasma membrane at the distal side of exodermis and endodermis cells was consistent with a role of OsNramp5 as the major uptake system for Mn and Cd.

Independently, Ishikawa et al. (47) isolated three plants with drastically reduced Cd grain concentrations from a mutagenized population in the background of a popular Japanese rice cultivar, Koshihikari. After growing in Cd-contaminated soil, these plants had concentrations of <0.05 μg per gram of dry weight, as compared with a mean of approximately 1.7 μg per gram of dry weight for their parents. The Cd concentrations in their roots and shoots were also much lower, indicating that root Cd uptake played a role in the reduced concentrations. Positional cloning revealed that all three plants carried mutations in the *OsNramp5* gene.

The finding that a loss-of-function allele can result in strongly reduced Cd accumulation offers exciting prospects for the development of low-Cd rice because these alleles can be easily integrated into breeding programs, especially when present in widely grown cultivars (47). An important question is whether a concomitant negative effect on essential element acquisition could cause yield penalties. Growth of *osnramp5* mutant plants in paddy fields did not reveal any biomass reduction even though the Mn concentrations in their tissues were much lower than those in wild-type plants (47). Lab experiments with a controlled Mn supply, by contrast, revealed strong

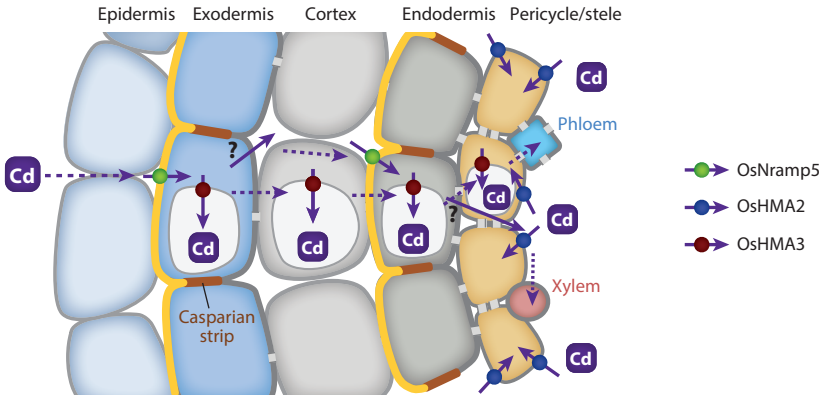

Figure 1

Transporters involved in uptake and root-to-shoot translocation of Cd in rice roots. OsNramp5 localized at the distal side of both the exodermis and endodermis (highlighted in *yellow*) is responsible for the transport of Cd from the apoplast into the root cells. OsHMA3 in the tonoplast sequesters Cd into vacuoles, and OsHMA2 at the plasma membrane of pericycle cells is hypothesized to transport Cd from the apoplast to the symplast to facilitate translocation via the phloem. Dashed arrows indicate possible passive diffusion of Cd.

growth impairment upon Mn limitation (111, 141), suggesting that the *osnramp5* mutant alleles will be useful primarily when field conditions afford ample Mn. Assuming that *Nramp5* orthologs play a similar role for Cd uptake in other cereals, this question will have to be addressed for these species as well.

There is always more than one As species present in plant tissues (76). A survey of a wide range of plant species indicated that As(V) and the major forms of methylated As in soils (MMA and DMA) can be taken up by every plant, with As(V) showing on average the highest efficiency of entry into roots (106). Thus, different As uptake routes need to be distinguished.

It has long been known that As(V) uptake occurs through transporters for the chemically similar macronutrient phosphate (**Figure 2**). For instance, work on *Holcus lanatus* and other plants with naturally selected As hypertolerance showed that this trait is at least partly explained by a suppression of high-affinity phosphate uptake (77). Subsequent studies in *A. thaliana* showed that phosphate transporter mutants are indeed less sensitive to As(V) (e.g., 114; reviewed in 148). In corresponding experiments with rice, Wu et al. (136) analyzed the actual As(V) uptake rates of a phosphate transporter mutant defective in OsPHF1 and lines overexpressing either a phosphate transporter (OsPht1;8) or a positive regulator of phosphate starvation response (OsPHR2) and found that phosphate transporter activity clearly contributes to As(V) uptake and As accumulation. However, when the plants were cultivated in flooded soil, phosphate transporter overexpression had no effect on As accumulation in straw or grains. Under such conditions, As(III) is the dominant species. Furthermore, As(III) is more bioavailable than As(V), which is one reason why the soil-to-plant transfer factor of As in rice is nearly ten times that in other cereals (148). From these results, it follows that most human As intake through food is attributable to rice consumption, whereas many different crop species contribute substantially to Cd intake.

Entry of As(III) into rice roots is mediated by the nodulin 26–like intrinsic protein (NIP) aquaglyceroporin Lsi1 (OsNIP2;1) (72) (**Figures 2** and **3**). Uptake is passive and driven by a concentration gradient. Lsi1 is not the only member of the NIP family that transports As(III). The first evidence came from expression of several plant NIPs in yeast cells (9). The physiological function of Lsi1 is the uptake of silicic acid (69). Rice accumulates silicon (Si) up to very high

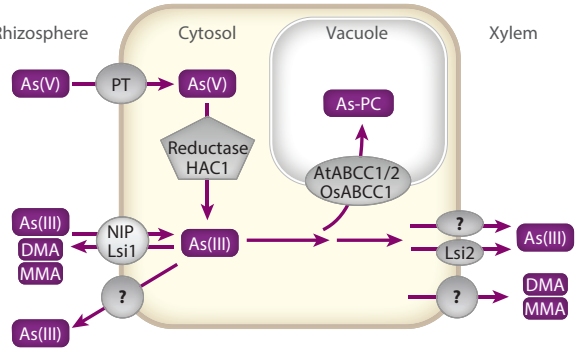

Figure 2

Cellular processes involved in As uptake and assimilation in plant roots. As(V) is taken up through phosphate transporters (PTs) and then reduced to As(III) by As(V) reductases such as HAC1 in *Arabidopsis thaliana*. Under reducing conditions (e.g., in rice paddy fields), As(III) is the dominant form in the soil. In rice, it is taken up by Lsi1 (a Si channel); in other species (e.g., *A. thaliana*), it is taken up by other nodulin 26–like intrinsic proteins (NIPs). Rice Lsi1 is also permeable to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). One fraction of As(III) is effluxed to the rhizosphere by aquaglyceroporins (such as Lsi1 in rice) and unidentified transporters. Another fraction of As(III) is chelated by phytochelatins (PCs). ABC transporters such as AtABCC1 and -2 in *A. thaliana* and OsABCC1 in rice sequester As-PC complexes in the vacuole. In rice, the efflux transporter Lsi2 transports the remaining As(III) to the xylem; in *A. thaliana*, As(III) efflux is not understood. No transporters involved in xylem loading of methylated As species have been identified.

levels and thereby gains protection against biotic and abiotic stresses (70). The strong activity of this pathway in rice relative to those in other cereals is a second reason why rice is the dominant source of As in food (148).

More recently, Li et al. (64) found that Lsi1 is also involved in the uptake of methylated As. This study showed that MMA and DMA uptake rates are significantly higher for roots of wild-type plants than for roots of *lsi1* mutant plants, and that Lsi1 expression in *Xenopus laevis* oocytes confers MMA and DMA uptake activity. Finally, because aquaglyceroporins mediate facilitated diffusion and not active transport, Lsi1 can mediate efflux of As(III) as well (**Figure 2**), a process that has been observed in several plant species (146). Experiments with As(V)-exposed rice plants revealed a significant, albeit minor, reduction in As(III) efflux in *lsi1* plants compared with wild-type plants (144), implying the existence of additional efflux transporters (e.g., other NIPs). The efflux activity of Lsi1 could be one reason why the loss of Lsi1 function has only a small effect on the long-term accumulation of As in the straw and grains of field-grown rice (72).

In contrast to the tremendous recent progress in dissecting the entry of Cd and As into plant cells, there is no molecular understanding of uptake pathways for Pb ions, inorganic Hg, or methylmercury.

X-ray absorption spectroscopy (XAS): a technique that provides information on local atomic geometry and the chemical state of absorbing atoms

HPLC-ICP-MS/ESI-MS: a speciation analysis technique that combines high-performance liquid chromatography with inductively coupled plasma mass spectrometry (for elemental analysis) and electrospray ionization mass spectrometry (for identification of organic ligands)

Arsenic Biotransformations In Planta

Advances in speciation techniques such as X-ray absorption spectroscopy (XAS) and HPLC-ICP-MS/ESI-MS (which combines high-performance liquid chromatography with inductively coupled plasma mass spectrometry and electrospray ionization mass spectrometry) (149) have enabled researchers to analyze the different chemical forms of As both in the soil and in plant organs. One important finding was that many plant species rapidly reduce As(V) to As(III) inside cells (e.g., 101, 105) (**Figure 2**). Even the observed conversion of As(V) to As(III) in plant growth medium

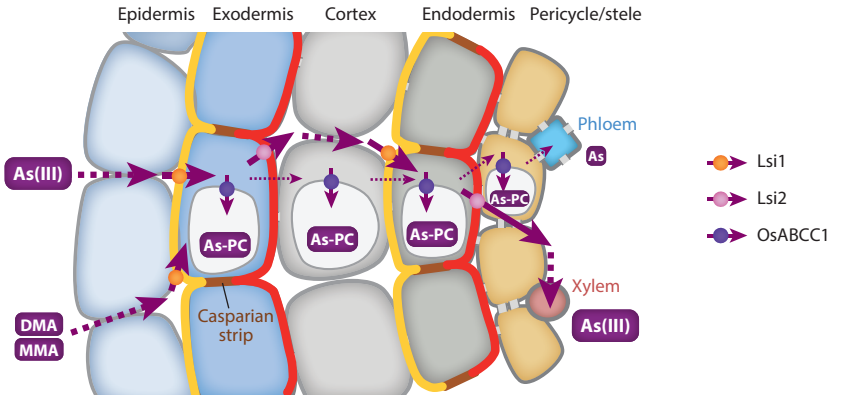

Figure 3

Lateral transport of As in rice roots. As(III) is transported toward the stele by two Si transporters, Lsi1 and Lsi2, localized at the distal (highlighted in *yellow*) and proximal (highlighted in *red*) sides of the exodermis and endodermis. OsABCC1 in the tonoplast sequesters As-phytochelatin (As-PC) in the vacuoles and thereby restricts the availability of As(III) for movement across the root. Dashed arrows indicate possible passive diffusion of As; the arrow thickness reflects the relative contribution to overall transport. Additional abbreviations: DMA, dimethylarsinic acid; MMA, monomethylarsonic acid.

is the result not of microbial activity but of intracellular reduction of As(V) and subsequent efflux of As(III) (137).

Molecularly, this reduction had been enigmatic for a long time. The responsible As(V) reductases were only recently identified in *A. thaliana* after an analysis of knockout mutants could not confirm the existence of an earlier reported reductase, arsenate reductase 2 (ACR2) (66). In a study of natural diversity in As(V) tolerance, Sánchez-Bermejo et al. (109) detected a major quantitative trait locus (QTL) by analyzing a recombinant inbred line population derived from two parents representing the more As(V)-tolerant and more As(V)-sensitive accessions. They fine-mapped this QTL [designated *arsenate tolerance QTL 1* (*ATQ1*)] to a region containing a candidate gene that encodes a protein that functions as an As(V) reductase when expressed in *Escherichia coli*.

QTL: quantitative trait locus

In an independent and parallel study, Chao et al. (16) investigated natural variation in leaf As accumulation in *A. thaliana*. Genome-wide association mapping showed that the same gene, *High Arsenic Content 1* (*HAC1*), explains a large proportion of the variation in leaf As. This result demonstrated that the newly identified As(V) reductase strongly influences not only tolerance but also aboveground As accumulation in plants grown on soil containing trace amounts of As at a realistic level. HAC activity in outer root cell layers enables efflux of As(III) and facilitates trapping of As(III) complexed with thiols in root cells, as detailed below (**Figure 2**). Putative *AtHAC1* orthologs are present in both dicot and monocot genomes (16). The physiological importance in paddy fields will have to be tested because As is predominantly present as As(III).

Radial Transport and Xylem Loading

Toxic elements taken up at the epidermis or exodermis must be radially transported across the cortex, endodermis, and pericycle cells for subsequent translocation from the roots to the shoots. Transporters are required for this radial transport, at least in some plant species. Especially in rice, the roots are characterized by two Casparian strips at both the exodermis and endodermis. Furthermore, mature roots have a highly developed aerenchyma in which almost all of the cortex

cells between the exodermis and endodermis are destroyed. Therefore, movement from the soil solution to the stele requires both influx and efflux transporters in both the exodermis and endodermis of the root. However, most transporters required for such transfer of toxic elements have not been identified. An exception is Lsi2, which effluxes As(III) toward the stele in rice (**Figure 3**). Lsi2 is structurally unrelated to Lsi1. It belongs to a family of anion transporters and effluxes silicic acid from the cytosol at the proximal side of exodermis and endodermis cells (71). The *lsi2* mutant accumulates much less As in straw and grains owing to an approximately 90% reduction in As(III) concentrations in the xylem sap (72).

The rate of root-to-shoot translocation is a key factor in food contamination for two reasons. First, as indicated above, the long-distance transport from roots as the site of uptake to edible leaves and seeds strongly varies between the relevant toxic elements. Pb and Hg are effectively trapped in the roots, with the notable exception of methylmercury, which appears to be highly mobile in rice (82). The root trapping of Pb can be explained at least in part by strong interaction with the cell wall (11). Translocation rates of As are also low in most crop species investigated to date. The crucial exception here is rice. A direct comparison with wheat and barley, for instance, showed that translocation in rice is 68–78% and 250–440% higher, respectively, depending on the form of inorganic As applied (117), and comparisons with other crop plants, such as tomato, have yielded qualitatively similar results (67). Thus, higher As mobility is one reason for the massively higher soil-to-shoot As transfer factors in commercially grown rice relative to wheat and barley (133). Among the relevant toxic elements, Cd generally shows the highest translocation rates. Because this applies to many crop plants, the human Cd intake via food cannot be attributed almost entirely to a single species, as is the case with As intake and rice.

Second, a large fraction of the observed intraspecific variation in toxic element accumulation by edible aboveground plant organs is attributable to variation in root-to-shoot translocation rates. This is exemplified by a study of genetically diverse rice accessions: Uraguchi et al. (128) found strong correlations between Cd concentrations in the xylem sap and those in shoots and grains, but only minor differences in root uptake of Cd across different accessions. Using a positron-emitting tracer imaging system (PETIS) to image Cd in real time, Ishikawa et al. (48) demonstrated that high-Cd rice cultivars transfer Cd from roots to shoots more efficiently than low-Cd rice cultivars. Wu et al. (135) also found a good correlation between shoot Cd accumulation and root-to-shoot translocation in a barley core collection. Results from smaller-scale and less-detailed studies with other crops—e.g., wheat (40)—are consistent with these findings.

Positron-emitting tracer imaging system (PETIS): an imaging technique that uses positron-emitting tracers such as ^{107}Cd to noninvasively monitor an element

Heavy metal–transporting ATPases (HMAs): plant P_{1B}-type ATPases that function as integral membrane proteins coupling ATP hydrolysis to metal cation transport

The movement of elements in the root toward the xylem can be restricted by sequestration in root cell vacuoles (126) (**Figures 1** and **3**). This was clearly demonstrated by the identification of the P_{1B}-type ATPase *Oryza sativa* heavy metal–transporting ATPase 3 (OsHMA3) as a major determinant of Cd root-to-shoot translocation in rice (86, 124) (**Figure 1**). In a mapping population derived from a cross between an *indica* variety with high grain Cd (Anjana Dhan) and the *japonica* cultivar Nipponbare, which has comparatively low grain Cd, Ueno et al. (124) isolated the gene underlying a major QTL for this trait. Anjana Dhan carries a loss-of-function allele of *OsHMA3* and is thus defective in Cd transport across the tonoplast of root cells, where the transporter is localized. Ueno et al. (123) corroborated this finding in another high-Cd cultivar, Jarjan. Independently, Miyadate et al. (86) identified a lack of *OsHMA3* function as the major cause of strong Cd accumulation in the cultivar Cho-Ko-Koku.

Further support for the key role of OsHMA3 in restricting Cd root-to-shoot translocation comes from overexpression effects. A study of transgenic lines showed greatly increased Cd accumulation in roots and strongly reduced Cd accumulation in shoots (110). Takahashi et al. (118) reported similar but much less pronounced effects in rice plants overexpressing *OsNramp1*. The actual function of this plasma membrane–localized Cd transporter is not understood.

It is highly likely that *HMA3* orthologs and *HMA3* polymorphisms play similar roles in many other plants, including dicot crops. A genome-wide association study of *A. thaliana* showed that natural variation in shoot Cd accumulation could be explained largely by the existence of divergent *AtHMA3* haplotypes that differ in functionality, with inactive gene variants causing Cd accumulation in shoots (17).

Genome-wide association study: an approach in which common polymorphisms are analyzed in many individuals to search for associations of polymorphisms with a particular trait

Phytochelatins (PCs): cysteine-rich peptides with the general structure (γ-Glu-Cys)$_n$-Gly (where $n = 2$–5) that are essential for metal tolerance in plants

In a simplified model, the movement of metal cations (including Cd^{2+}) across a root can be described as a function of uptake into the cytosol (which is energetically favorable because of the negative plasma membrane potential) and efflux into the vacuole or the xylem as an apoplastic space. This efflux needs to be energized because it occurs against the plasma membrane potential or the inside positive potential of the vacuole (98). HMAs are responsible for myriad metal efflux activities in all kingdoms of life (6). In rice roots, OsHMA2 is responsible for the root-to-shoot translocation of Cd. A reduction in OsHMA2 activity resulting from transposon insertion or RNA interference (RNAi) decreases the translocation of Cd to the shoot (112, 119, 140). OsHMA2 is a transporter of Zn in rice and is localized in the plasma membrane of root pericycle cells at the vegetative growth stage. In contrast to other HMA members, HMA2 has been hypothesized to function as an influx transporter (140) (**Figure 1**). Knocking out this transporter significantly decreased the Zn concentration in the root tips and shoot elongating zone, suggesting that OsHMA2 transports Zn and Cd from the apoplast to the symplast to facilitate translocation via the phloem.

The barley ortholog HvHMA2 shows Zn and Cd transport activity in yeast and is able to rescue the defect in root-to-shoot translocation of Zn in the *A. thaliana hma2 hma4* double mutant (84). Generally, there is again strong evidence that the proposed importance of HMA2 for root-to-shoot translocation extends beyond monocots. In both *A. thaliana* (134) and the metal hyperaccumulator *Arabidopsis halleri* (39), HMA2 and/or its close homolog HMA4 account for much of the xylem loading of Cd.

In addition to influx and efflux activities, the formation of complexes with ligands governs the mobility of metals and metalloids. The dominant ligands for toxic elements in plants are phytochelatins (PCs) (20, 81, 129). A defect in PC synthesis is tantamount to a loss of cytosolic buffering and causes strong hypersensitivity to Cd, As (25), and Pb (33) as well as slight Hg hypersensitivity (15, 25). PC synthesis is activated by exposure to any of these toxic elements, and the formation of metal/metalloid-PC complexes suppresses interaction with sensitive targets. A large fraction of the toxic elements inside plant cells is bound in these complexes, as was recently demonstrated for, e.g., rice roots exposed to low Cd doses (91).

As discussed above, most inorganic As in plants is present as As(III). The majority of this As(III) is complexed with thiol ligands (i.e., either glutathione or PC) throughout the plant (101). Raab et al. (105) identified several different As species in an analysis of sunflower plants exposed to either As(V) or As(III), finding complexes of As(III) with glutathione and (γ-Glu-Cys)$_2$-Gly [= GS-As(III)-PC2] and with (γ-Glu-Cys)$_3$-Gly [= As(III)-PC3] in all analyzed tissues. It is generally assumed that this speciation of As as As(III) complexes with thiols principally applies to all plants, with the exception of As-hyperaccumulating ferns (148). In a recent study, Kopittke et al. (59) used the fluorescence X-ray absorption near-edge structure (XANES) technique to analyze As speciation across wheat and rice roots with high spatial resolution. They detected As(V) in As(V)-exposed roots only in the rhizodermal cell layer. Farther inside the root, all As was present as As(III)-thiol complexes, indicating that As(V) contributes little if anything to As translocation in cereals (**Figure 2**). Consistent with this speciation, Batista et al. (8) found a large number of different PC variants and As(III)-PC complexes in rice roots exposed to As. Their comparative analysis of rice cultivars with contrasting As accumulation behavior revealed negative correlations between the concentrations of PCs and the root-to-grain and shoot-to-grain transfer factors, suggesting that natural variation in rice As accumulation could be due in part to differences in PC synthesis rate.

In a wide range of plants, the most efficiently translocated As species are in fact methylated As species (106). Much of the As(III) is retained in root cells because of the transport of As(III)-PC complexes into vacuoles (**Figures 2** and **3**). Elemental imaging of rice roots by high-resolution secondary ion mass spectrometry (SIMS) revealed strong colocalization of As and sulfur in the vacuoles of endodermal and pericycle cells, which is consistent with such transport (89). After a long search, Song et al. (115) identified the ABC transporters responsible for the vacuolar sequestration of metal/metalloid-PC complexes. *A. thaliana* ABCC1 and -2 account for nearly all of the vacuolar uptake of As-PCs and Cd-PCs (99, 115). They also confer Hg tolerance, suggesting Hg-PC transport activity (99). Rice apparently has only one gene with high similarity to *AtABCC1* and -2, designated *OsABCC1* (116). *OsABCC1* is expressed throughout the plant, and the encoded transporter resides in the tonoplast. In root cells, loss of OsABCC1 function abolishes the transport of thiols into the vacuoles, which in wild-type plants occurs upon exposure to As (**Figure 3**). All of these observations are consistent with OsABCC1 being the main transporter for the vacuolar sequestration of As-PC complexes in rice. In contrast to *A. thaliana abcc1 abcc2* double-mutant plants, the rice *osabcc1* mutant is hypersensitive only to As and not to Cd, supporting the idea that in rice roots, vacuolar sequestration of Cd is dependent mainly on OsHMA3 (116) (**Figure 1**).

As(III) is the dominant As species in the xylem of most plants analyzed to date (129, 146). The xylem sap of rice plants contains mostly As(III) even when plants are exposed to external As(V) (117). Obviously As(III) is not quantitatively trapped in root cells; a fraction is available for efflux and eventual loading into the xylem.

The passage of methylated As toward the xylem is not understood at a molecular level (**Figure 2**). The much higher root-to-shoot translocation rate relative to inorganic As [a survey of 46 plant species showed median transfer factors of 0.09 for As(V) and 0.81 for DMA (106)] suggests much less trapping of methylated As in root cells. MMA, but not DMA, has been detected in thiol complexes (105). How the efflux of methylated As into the apoplast occurs is unknown. Unlike Lsi1, which transports both undissociated $As(OH)_3$ [the dominant form of As(III) at pH < 8] and methylated As, Lsi2 apparently transports only $As(OH)_3$ (64).

Long-Distance Transport of Toxic Elements

Transfer of toxic elements between organs requires long-distance transport via the xylem and phloem. Mechanistic knowledge of toxic elements is essentially confined to As and Cd. Important questions concern the processes that take place after the xylem loading discussed above, including speciation in the xylem and phloem, xylem unloading, phloem loading and unloading, and intervascular transfer (e.g., partitioning between vascular bundles or xylem-to-phloem transfer in rice nodes). Intervascular transfer is of major relevance for the accumulation of toxic elements in food and is discussed separately below.

Knowledge of the actual metal/metalloid speciation in the xylem and phloem is still limited because of many analytical challenges related to sampling techniques and purity (3). The transport forms of As and Cd in the xylem are generally assumed to be As(III) and ionic Cd. In As(V)-exposed plants in which As(V) is not fully reduced during root passage, As(V) can additionally be present in the xylem (129)—as demonstrated, e.g., for wheat and barley (117). Methylated forms of As can be detected when plants are exposed to them (64).

Phloem is particularly difficult to sample. A classic model to obtain phloem sap is *Ricinus communis*. Analyses have found As(III) and a small fraction of As(V) but no As-thiol complexes, even though glutathione and PC2 were present (143). By contrast, there is evidence that Cd is complexed by PCs in the phloem in other plants, including *Brassica napus* (80) and rice (54). The apparent difference in the role of thiol complexation could be explained by the higher stability

of Cd-PC complexes at alkaline pH compared with As-PC complexes, which are more stable at acidic pH (52, 113). Alternatively, phloem sap in rice might differ from that in *R. communis* (e.g., it could have a slightly lower pH); there is now strong evidence that As(III) phloem mobility is restricted in rice by the sequestration of As-thiol complexes in the vacuoles of phloem companion cells. Synchrotron X-ray fluorescence and high-resolution SIMS elemental mapping in the nodes, internodes, and leaf sheaths of rice plants found As in the vacuoles of companion cells colocalized with sulfur (87). In feeding experiments with excised rice panicles, treatment with buthionine sulfoximine (an inhibitor of glutathione and therefore PC biosynthesis) resulted in higher mobility of As from node I toward the flag leaf and the grain (18).

PC influence on the mobility of toxic elements in the phloem is likely to be a general phenomenon in crops. PC synthesis is ubiquitous in plants (24), and it is noteworthy that, according to translatome data (available so far only for *A. thaliana*), the main PC synthase AtPCS1 is highly expressed in phloem companion cells (55, 90). Several other potential binding partners, such as nicotianamine and phytosiderophores, exist in the phloem sap (for a comprehensive overview, see 3). However, the strong preference of the relevant toxic elements for sulfur ligands makes it very likely that thiols, and possibly proteins, are dominant.

Distribution of Toxic Elements

Long-distance transport via the phloem represents the main pathway for toxic element accumulation in seeds and grains consumed by humans. This has been demonstrated for Cd (120) and As(III) (14) in rice [methylated As can also reach the rice grain via the xylem (14)]. In addition, the phloem can principally mediate mobilization from leaves and reallocation from shoots to roots (81, 126), even though feeding experiments with flag leaves have demonstrated—at least for rice—that, in contrast to organic As, inorganic As is immobile (13).

How As, Cd, and other elements are loaded into the phloem is not clear. They are presumably taken up into phloem companion cells, but the responsible transporters have not been identified (55). An exception is rice, in which the available evidence suggests that phloem loading by intervascular transfer in node I is a major pathway for the movement of toxic elements toward the grain.

In grasses, nodes are critically important for distributing mineral nutrients and toxic elements (139). Nodes, especially node I, exert control over the distribution of elements toward leaves or grains. This is essential because transpiration as a driving force for the movement of minerals would strongly favor accumulation in leaves with a high transpiration rate. Nodes have a complex but well-organized vascular system (139). Rice nodes contain two major vascular bundles: enlarged vascular bundles (EVBs) and diffuse vascular bundles (DVBs). EVBs come from the lower nodes and are connected to the leaf, whereas DVBs surrounding the EVBs start at the node and are connected to the upper nodes or panicles. Therefore, transport of toxic elements to grains requires an intervascular transfer from the EVBs to the DVBs at the nodes, which presumably requires many transporters.

Both Cd and As are concentrated more in the nodes than in the internodes (34, 87). When Fujimaki et al. (34) used ^{107}Cd PETIS to perform a noninvasive analysis of Cd movement through a rice plant, they found a strong accumulation in the nodes caused by a slowing of xylem flow. Tracing of ^{109}Cd in a mature rice plant produced evidence for downward movement of Cd from a node, suggesting intervascular bundle transfer (56). Synchrotron micro-X-ray fluorescence spectrometry analysis of a rice node detected Cd mostly in the xylem and the parenchyma cells bridging the bundles (138).

Oryza sativa low-affinity cation transporter 1 (OsLCT1) and OsHMA2 are involved in intervascular transfer. *OsLCT1* transcript accumulation is strongly upregulated during the reproductive

stage in rice (127). The transcript was detected in cells surrounding the EVBs and DVBs of node I, as well as in the phloem parenchyma cells of EVBs. RNAi-mediated knockdown of *OsLCT1* resulted in up to a 50% reduction of grain Cd after the cultivation of plants in soil with average Cd contamination. Analysis of xylem and phloem sap showed that the Cd concentration is reduced only in the phloem sap, strongly suggesting that OsLCT1 plays a role in xylem-to-phloem transfer. OsLCT1 resides in the plasma membrane, and in yeast cells it apparently mediates Cd efflux. The transport orientation in planta remains to be determined.

OsHMA2 is expressed not only in roots but also in the upper nodes during the reproductive stage (140). OsHMA2 was detected in the plasma membrane of phloem companion cells in EVBs and DVBs. Two transposon insertion mutants contained less Zn and Cd specifically in the tissues above node I (the rachis, flag leaf, and grain), suggesting that OsHMA2 plays a role in the preferential delivery of Zn (and inadvertently of Cd) to these tissues. These results led to the hypothesis that, in contrast to other HMA proteins, OsHMA2 seems to function as an influx transporter of both Zn and Cd.

In the nodes of rice plants, Moore et al. (87) detected As in the phloem of the different types of vascular bundles, mostly in the companion cells. The proposed filtering role of the node restricting As movement to the grain was discussed above with respect to As(III)-PC trapping. Song et al. (116) recently demonstrated that OsABCC1 is essential for this process. OsABCC1 is localized to the tonoplast of phloem companion cells of DVBs in the nodes. Short-term feeding experiments with rice plants cut during the milky stage below the second node revealed a strong increase in grain As of *osabcc1* mutants, consistent with a defect in As phloem retention caused by a lack of vacuolar sequestration of As(III)-PC complexes (116) (**Figure 4**).

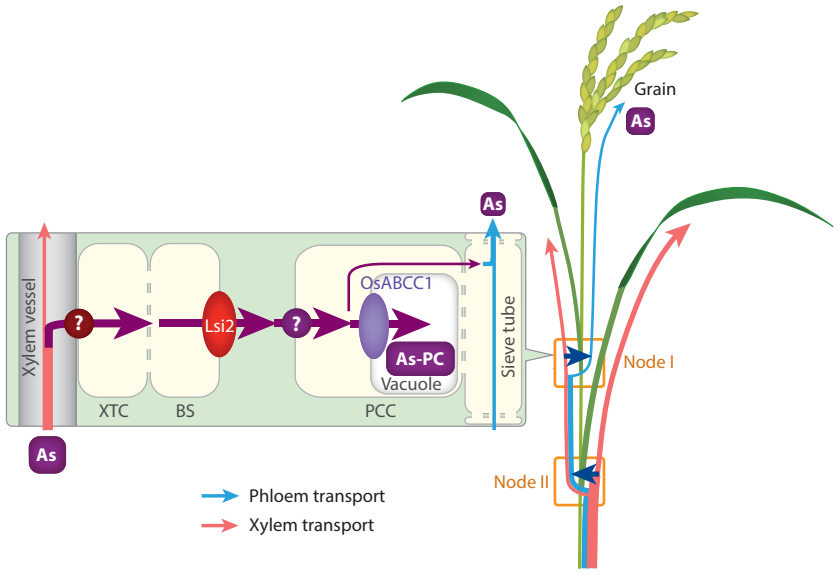

Figure 4

Transporters controlling As distribution in nodes of rice. The Si transporter Lsi2 is involved in the intervascular transfer of As (indicated by *dark blue arrows*). OsABCC1 localized at the tonoplast of phloem companion cells (PCCs) plays an important role in restricting As movement to the grain by sequestering As-phytochelatin (As-PC) complexes in vacuoles. The thickness of the arrows in the PCC reflects partitioning of As between the pathways. Question marks indicate unidentified transporters. Additional abbreviations: BS, bundle sheath; XTC, xylem transfer cell.

Partitioning of As between grains and flag leaves via DVBs and EVBs, respectively, is apparently also influenced by Lsi2, which in the node is localized in parenchyma cells that bridge the two types of vascular bundles (**Figure 4**). In feeding experiments that compared wild-type and *lsi2* mutant plants with excised panicles, the *lsi2* mutants accumulated less As in the grain and more in the flag leaf than the wild type (18).

Overall, many transporters involved in the distribution of toxic elements remain unidentified. Especially in dicots, the molecular mechanisms underlying the distribution are also unknown.

Seed Loading and Storage of Toxic Elements

The transfer of micronutrients into the different parts of a seed (the seed coat, embryo, and endosperm) requires symplastic phloem unloading, efflux from the symplast into the apoplast, and uptake into the symplast. The number and nature of apoplastic barriers vary across species (98), and molecular knowledge of the transport processes is extremely limited. This applies equally to toxic elements (55).

More insight has been gained into the distribution of elements in seeds. Knowledge about element localization is relevant for food safety because it provides information about accumulation in the parts that are actually consumed (e.g., the endosperm) and because colocalization with other elements provides hints about the pathways involved in loading of the seed. Among the toxic elements, the available data are restricted largely to As. In spite of tremendous advances in imaging methods, it remains difficult to detect Cd by synchrotron-based techniques in a concentration range realistic for cultivated crops (149).

Speciation analysis of As in rice grains found inorganic and methylated species. The ratios varied substantially for different parts of the grain, indicating differences in mobility and/or loading pathways for inorganic and organic As within the grain. A XANES-based study showed that a large fraction of the inorganic As is present in As-thiol complexes, suggesting that PCs also play a role in depositing As in grains (68). A higher-resolution nanoscale secondary ion mass spectrometry (nanoSIMS) study detected As associated with the protein fraction, consistent with thiol complexation, whereas phosphorus (P) localization was distinctly different, in line with the dominant role of As(III) mobility in the plant (88). Apart from rice grains, very little information is available on storage forms of toxic elements in consumed parts of crop plants.

Nanoscale secondary ion mass spectrometry (nanoSIMS): a technique in which secondary ions are ejected by an ion beam from the surface of a sample and analyzed with a mass spectrometer to generate a chemical map

Targeting induced local lesions in genomes (TILLING): a technique that isolates allelic variants in genes of interest from mutant populations or diversity panels

TOWARD SAFER CROP PLANTS WITH REDUCED TOXIC ELEMENT ACCUMULATION

Several agronomic mitigation strategies beyond the scope of this review have been proposed and explored to reduce toxic element accumulation in crops. These strategies include liming and the application of organic material to reduce the bioavailability of Cd and Pb in the soil, Si fertilization to saturate the Si transport pathway that mediates As(III) accumulation, and careful management of irrigation regimes to control Cd and As bioavailability (147). Over the past few years, approaches based on the molecular knowledge described above have rapidly gained momentum as arguably the most promising way to achieve higher food safety.

Comparatively easy to implement is the use of loss-of-function alleles of metal/metalloid transporters in breeding programs. Such alleles can come from mutagenized populations, preferably in the genetic background of commonly planted elite cultivars. Isolation of alleles can be achieved by unbiased mutant screening, as demonstrated impressively for OsNramp5 (47), or by specifically targeting a gene—for example, in targeting induced local lesions in genomes (TILLING) populations, as is currently being explored for OsLCT1 (S. Uraguchi, N. Tanaka, T.

Ushijima, H. Utsushi, H. Hasegawa, et al., unpublished research). Other potentially interesting low-accumulation alleles can be expected to emerge from the molecular dissection of natural variation in toxic element accumulation (see below).

As discussed above for OsNramp5, an obvious caveat associated with loss-of-function alleles is potential interference with essential mineral homeostasis; this is also exemplified by OsHMA2. Loss of function resulted not only in a reduction of grain Cd but also in a reduction of grain Zn (140). Thus, it would be preferable to alter the specificity of transporters involved in toxic element movement (55). Respective variants could either be searched for in existing populations or introduced into crops by genome editing techniques. A few examples exist that demonstrate the feasibility of this approach. Pottier et al. (104) isolated versions of AtNramp4 with modified Cd and Zn transport but wild-type Fe transport activity, which is very promising given the importance of Nramps for Cd accumulation. For ZnT, a mammalian Zn transporter of the cation diffusion facilitator (CDF) family [the metal tolerance protein (MTP) family in plants], Hoch et al. (44) identified amino acid residues critical for discrimination against Cd. However, not every transporter will be amenable to selectivity engineering. For aquaglyceroporins, for instance, it appears to be rather difficult to find variants that discriminate against As(III) (85).

Altering the expression levels of transporters through transgenic approaches can be successful even when the selectivity is not changed. Overexpression of *OsHMA3* in rice cultivars with naturally low expression achieved strong reduction in grain Cd without affecting Zn and Fe nutrition, and there was no yield penalty even when the plants were grown in Cd-contaminated soil (124). The latter result could be attributed to compensatory upregulation of four genes related to Zn transport in the overexpressed lines (110).

OUTLOOK

Tremendous progress has been made in recent years in discerning the pathways of As and Cd accumulation and in identifying some of the molecular players involved, especially through exciting work with rice. Nonetheless, we are far from a comprehensive understanding. This is clear from the genetic analysis of variation in Cd accumulation. Several QTLs have been detected in different mapping populations, mainly in rice (e.g., 1, 46, 122) and also recently in barley (135). With the exception of *OsHMA3* (124), the underlying genes have not been identified. Similarly, studies have found multiple loci associated with grain As in rice but no colocalization with genes known to be involved in As accumulation (93). Thus, many factors influencing toxic element accumulation have yet to be identified and understood in different crops.

SUMMARY POINTS

1. Human As intake via plant-derived food is attributable almost entirely to rice consumption, whereas many different crop species contribute substantially to human Cd exposure.
2. Rice is the dominant source of dietary As intake because it predominantly takes up the more readily bioavailable As(III) and transports As(III) through the Lsi1- and Lsi2-dependent Si uptake pathway, which is particularly active in rice.
3. Exploration of natural variation in *Arabidopsis thaliana* As tolerance and accumulation has enabled the identification of plant arsenate reductases and demonstrated their role in restricting As root-to-shoot translocation.

4. Tremendous advances in elemental imaging and speciation analysis have provided a high-resolution map of As biotransformations, mobility, and accumulation in crop plants.
5. Phytochelatins (PCs) are important ligands for As(III) in plants. They restrict the movement of As in both root cells and phloem companion cells. OsABCC1 accounts for most of the transport of As-PC complexes into the vacuole.
6. OsNramp5 and OsHMA3 have been identified as the transporters representing the major Cd uptake route and the main pathway for Cd retention in the roots of rice plants.
7. OsLCT1 and OsHMA2 mediate the intervascular transfer of Cd in rice nodes toward the grain.
8. The recent identification and functional characterization of toxic element pathways in rice provide excellent opportunities for the development of varieties with strongly reduced As and Cd concentrations.

FUTURE ISSUES

1. A detailed understanding of toxic element accumulation pathways requires knowledge of the function of metal transporters and ligands with cellular resolution, as exemplified by the intricate interactions of cells to direct the flow of metals and metalloids through the nodes of rice plants.
2. The regulation of metal transport processes in response to a fluctuating nutrient supply and the consequences of such regulation for toxic element accumulation need to be elucidated.
3. The transporters and ligands mediating the passage of toxic elements across apoplastic barriers into seeds are unknown.
4. The exciting progress in rice highlights the lack of molecular tools to develop low-Cd varieties of other important crops and sources of human Cd intake (e.g., wheat). For instance, the transporters involved in the transfer of toxic elements from soil to edible parts need to be identified.
5. The mechanisms of toxic element uptake into leaves and tubers of crops remain to be identified.
6. The contribution of Pb accumulation in crops to the threat of slow Pb poisoning needs to be clarified.
7. Accumulation pathways for Pb, Hg, and methylmercury in crops will need to be investigated with respect to natural variation and molecular mechanisms.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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