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REVIEW

Arsenite transport in plants

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Abstract Arsenic is a metalloid which is toxic to living organisms. Natural occurrence of arsenic and human activities have led to widespread contamination in many areas of the world, exposing a large section of the human population to potential arsenic poisoning. Arsenic intake can occur through consumption of contaminated crops and it is therefore important to understand the mechanisms of transport, metabolism and tolerance that plants display in response to arsenic. Plants are mainly exposed to the inorganic forms of arsenic, arsenate and arsenite. Recently, significant progress has been made in the identification and characterisation of proteins responsible for movement of arsenite into and within plants. Aquaporins of the NIP (nodulin26-like intrinsic protein) subfamily were shown to transport arsenite in planta and in heterologous systems. In this review, we will evaluate the implications of these new findings and assess how this may help in developing safer and more tolerant crops.

Keywords Arsenic · Arsenate · Arsenite · Aquaporin · NIP

Introduction

The occurrence and usage of arsenicals

Arsenic (As) is a metalloid which occurs ubiquitously in the earth's crust. In addition, As is often present in the atmosphere and in surface and ground waters. Natural processes like weathering of rocks and volcanic emissions, and human activities such as combustion of fossil fuels, mining, smelting of ores or the application of arsenical pesticides, herbicides and wood preservatives, are the main sources which contribute to As contamination in the environment [1].

Arsenic exists in either organic or inorganic form but is normally not encountered in its elemental state. Typically, the inorganic fraction contains oxygenated As anions or more complex As salts with, for example, sulphur and iron, of which arsenopyrite (FeAsS) is the most abundant [2, 3]. However, the most prevalent inorganic As species are the pentavalent As^{V} arsenate (occurring as $H_2AsO_4^-$ and $HAsSO_4^{2-}$ in most environments) and the trivalent As^{III}, arsenite (As_2O_3), which dissolves as $As(OH)_3$.

In addition, large numbers of organic As compounds can be found. Living organisms can contain arsenolipids and arsenosugars that may be incorporated into cellular metabolism. Those that persist in the environment tend to be mostly methylated arsenicals such as monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) [4]. In addition, environmental organic arsenicals may derive from pesticides, herbicides and preservatives.

Medicinal properties of arsenic have been exploited by humans for a considerable time: for example, the German pharmacologist Paul Ehrlich introduced the arsenic compound arsphenamine for the treatment of syphilis in 1909 [5, 6]. Arsenic compounds have also been used to treat diseases like trypanosomiasis, amoebic dysentery, sleeping fever and promyelocytic leukaemia [7]. Besides their medicinal use, arsenicals have been employed since the Bronze age when they were often added during smelting to create a harder metal alloy. More recent applications include widespread usage in the production of herbicides,

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pesticides, insecticides, defoliants and wood preservatives [8]. Furthermore, arsenic helps remove impurities during glass making, it is a colorant in fireworks and a doping material in semiconductor manufacturing [9].

Arsenic toxicity

Arsenic is toxic to all living organisms. It has been defined as a group 1 carcinogen and is placed in the highest health hazard category by the international agency for research on cancer [10–12]. Elevated risks of cancer of the lung, skin and prostate result from prolonged exposure to medium and high levels of arsenicals (arsencosis). In addition, low level chronic arsencosis can cause non-cancerous afflictions such as hypo- and hyperpigmentation, keratosis, heart problems and diabetes [13].

At the cellular level, arsenic toxicity depends to a large extent on the nature of the arsenical: As^{III} has a high affinity to bind with sulfhydryl groups found in the amino acid cysteine. Binding of arsenic to these residues disrupts protein structure and protein–protein interactions, thus affecting many key metabolic processes in the cell such as fatty acid metabolism, glucose uptake and glutathione production [14, 15]. In addition, the binding ability of As^{III} to the reducing agent glutathione can lead to glutathione depletion and therefore increased levels of damaging reactive oxygen species (ROS) [16].

Being a phosphate analogue, As^{V} can substitute inorganic phosphate in a plethora of biochemical processes. For example, synthesis of triphosphate nucleotides like ATP can be affected which impacts on energy homeostasis, carbon metabolism and nucleic acid synthesis. This can also negatively affect DNA repair and DNA methylation and thus impact on gene expression [17].

Organic arsenic can take many forms such as methylated species, arsenic betaine, arsenosugars and arsenolipids. Generally, the toxicity of these compounds is lower than that of inorganic arsenic species [18]. However, there may be exceptions to this rule: in the plants *Spartina patens* and radish, DMA^{V} appeared to be more toxic than inorganic arsenic [19, 20], whereas in human hepatocytes, cytotoxicity decreased as: $MMA^{III} > As^{III} > As^{V} > MMA^{V} = DMA^{V}$ [21].

The damaging effects of arsenicals on humans manifest themselves predominantly through contact with arsenic in contaminated drinking water and through the food chain. In combination, these factors account for around 99% of the total human arsenic ingestion [22]. It is estimated that more than 100 million people are exposed to water which contains arsenic above the WHO safety limit of 0.01 ppm [23], and arsenic contamination of ground water is a particularly big problem in countries with naturally elevated arsenic soil contents such as Bangladesh [24], India [25], China, Vietnam, United States of America and Mexico [1, 26].

Arsenic contamination through food consumption results mainly from crop irrigation with arsenic contaminated water. For several reasons, the problem is particularly acute where rice is concerned [27]. The flooded conditions in which paddy rice is cultivated leads to mobilisation of As^{III} which would otherwise be sequestered in the soil [28]. Among the cereals, rice accumulates relatively high proportions of arsenic in its edible parts, with amounts of grain arsenic that range from 0.08 to 0.20 mg/kg on non-contaminated soils [29] and values as high as 2.0 mg/kg in contaminated areas [30]. Thus, with rice being the major calorific food stuff for billions of people and with its main production in southeast Asian countries that have high levels of arsenic in their aquifers, it forms a major focus of research to mitigate human arsenic contamination. Exposure to arsenic can also result from diets that are rich in (shell)fish. In these organisms, arsenic is mainly present as arsenobetaine and arsenocholine which are relatively non-toxic.

Arsenic resistance and transport in non-plant organisms

General mechanisms of arsenic detoxification

In most organisms, detoxification of arsenicals relies on multiple strategies, but some general mechanisms can be identified. Pentavalent arsenic compounds (e.g. arsenate) are often rapidly reduced to As^{III} which has a high affinity for –SH groups and thus can be bound to sulfhydryl-rich chelators such as glutathione and phytochelatins. Conjugation removes the reactivity and allows relatively safe intra- and intercellular transport of the complexed arsenic, for example into tissues and cellular compartments with low sensitivity. In both prokaryotes and mammals, generic methylation of mainly trivalent arsenic occurs, which can increase mobility and volatility of arsenicals. This allows secretion of methylated arsenic through the skin and urine in mammals and direct release into the atmosphere by bacteria.

Arsenic transport and resistance in prokaryotes

In prokaryotes (Fig. 1a), arsenate and arsenite enter through phosphate transporters and aquaglyceroporins, respectively. *Escherichia coli* contains two phosphate transporters, Pit and Pst, that participate in As^{V} transport [31]. The Pit system provides the main arsenate uptake pathway [32, 33]. Two main pathways for As^{III} uptake were also identified in prokaryotes: the *E. coli* glycerol facilitator, GlpF, was the first bacterial member of the

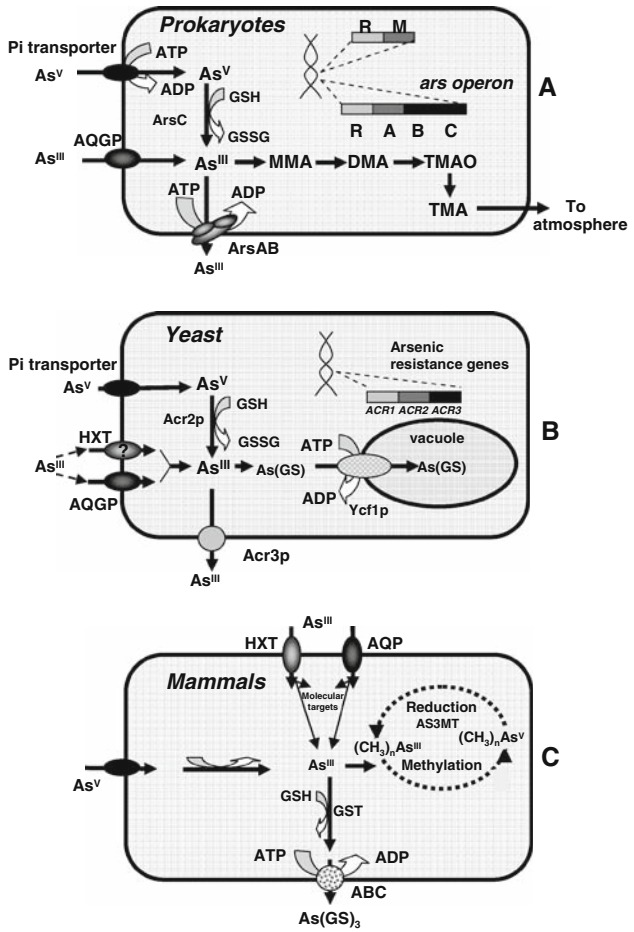

Fig. 1 Arsenic metabolism in prokaryotes and eukaryotes. **a** The main uptake of arsenate (As^V) into bacterial cells occurs via PhoS, PstC, and PstB phosphate (P_i) transporters. Arsenite (As^{III}) enters the bacterial cells via the GlpF aquaglyceroporin (AQGP). As^V is reduced to As^{III} by the bacterial ArsC arsenate reductase using glutathione (GSH) as reductant. ArsB, an As^{III}:H^+-antiporter, or ArsAB, an As^{III} ATPase, extrude As^{III} into the external environment. In addition, As^{III} can be released into the environment in volatile form after subsequent methylation steps carried out by As^{III}-*S*-adenosylmethionine methyltransferase. *MMA* monomethylarsonic acid, *DMA* dimethylarsinic acid, *TMAO* trimethylarsine oxide, *TMA* trimethylarsine. **b** In yeast, uptake of arsenate is facilitated by Pho87 type phosphate transporters, and arsenite is taken up mainly via the AQGP Fps1p but may also enter cells through hexose permeases (HXTs). The reduction of arsenate to arsenite in yeast cells is provided by the Acr2p arsenate reductase via glutathione oxidation (GSH to GS). Removal of cellular As^{III} can occur through conjugation to glutathione ($As(GS)_3$) which is sequestered into vacuoles by the ABC transporter Ycf1p, or through extrusion of As^{III} via the plasma membrane carrier Acr3. **c** As in yeast, uptake of As^{III} in mammalian cells can occur via aquaporins such as AQP7 and AQP9 and via HXTs. Specific proteins responsible for arsenate uptake and arsenate reduction in mammals have yet to be identified. The main efflux mechanisms for As^{III} in mammals appear to be ABC transporters from the MRP and MDR subfamilies. Methylation of As^{III} by arsenic methyltransferases such as AS3MT increases mobility of arsenicals in the body and facilitates removal through skin and urine

aquaporin family to be identified and this protein is ubiquitously distributed across prokaryotes. Although the physiological function of GlpF is in glycerol uptake, it was shown to be also permeable to arsenite: A *ΔglpF* deletion strain of *E. coli* showed an 80% decrease in arsenite uptake compared to wild type [34].

Prokaryotic arsenic resistance is controlled by the *arsRBC* operon. *ars*R is a small metallo-regulatory protein that functions as an As-sensing repressor protein. The presence of As removes arsR from its binding site to initiate expression of the operon. *arsC* encodes the glutathione dependent reductase which reduces As^V to As^{III} and arsB extrudes As^{III} from the cell, using H^+ antiport as a driving force [30]. A second family of arsenate reductases, which is also widely distributed in bacteria, is typified by the *arsC* gene product of *Staphylococcus aureus* but relies on thioredoxin as the source of reducing potential rather than glutathione.

In some bacteria, the *arsRBC* operon acquired extra genes and evolved into *arsRDABC* [35]. arsA is an ATPase which can bind to arsB converting the As^{III} efflux carrier to an ATP-driven extraction pump which presumably has a much larger capacity than arsB alone. arsD is a weak As^{III} responsive repressor of the operon with a function similar to arsR [36].

In some bacteria, additional detoxification mechanisms have been identified: the *Sinorhizobium meliloti ars* operon includes an aquaglyceroporin (*aqpS*) instead of *arsB* [37]. aqpS may provide another pathway for As^{III} extrusion from the cell. In *Rhodopseudomonas palustris*, the *arsM* is part of the *ars* operon and encodes an As^{III}-*S*-adenosyl-methionine-methyltransferase regulated by the *arsR* repressor. This enzyme mediates the sequential methylation of As^{III} to volatile trimethylarsine oxide which is released into the atmosphere [38].

Arsenic transport and resistance in yeast

The eukaryotic model system *Saccharomyces cerevisiae* has been extensively studied in relation to arsenic tolerance and detoxification. In yeast, As^V enters cells through high affinity phosphate transporters such as Pho84 (Fig. 1b), whereas As^{III} influx occurs through the aquaglyceroporin Fps1 [30, 39]. In addition, it has been proposed that glucose permeases are involved in yeast arsenic uptake [40]. Expression of yeast hexose carriers restored As sensitivity in the yeast mutant Δ*fps1* which is As^{III} tolerant [41].

Arsenic tolerance in yeast is provided by the gene cluster *ACR1, ACR2* and *ACR3. ACR1* encodes a putative transcription factor that regulates *ACR2* and *ACR3* transcription, possibly by directly sensing cellular As levels. *ACR2*

encodes an arsenate reductase and *ACR3* has been shown to encode a plasma membrane expressed As^{III}-efflux transporter. The gene cluster therefore provides a mechanism for sensing, reduction and efflux of As. Nevertheless, a second pathway for detoxification is present in yeast in the form of vacuoles: cytosolic As^{III} complexed with glutathione can be sequestered into this compartment through an ABC-type transporter, YCF1, that also transports conjugates of other harmful compounds [42].

Arsenic transport and resistance in mammals

Ingested As^{V} is taken up in the gut via carriers that normally process phosphate such as the Na^{+}: P_i cotransporter present in the intestinal membranes (Fig. 1c). The main mechanisms for As^{III} uptake in mammals are aquaglyceroporins (AQPs) and the hexose permeases (HXTs). Expression of the rat *AQP9* in yeast increased uptake of both As^{III} and MMA^{III} [43, 44]. Expression of *AQP7* and *AQP9* from either rat or human in *Xenopus* oocytes also increased As^{III} uptake [45].

Recent work has shown that Glut1, a mammalian glucose permease, can facilitate uptake of As^{III} and MMA^{III} when expressed in yeast and *Xenopus* oocytes. Interestingly, competition between glucose and MMA^{III} did not take place, suggesting that translocation of each substrate occurs independently in this protein [41].

Membrane proteins are also key players in arsenic removal from mammalian cells. Members of two subfamilies of ABC transporters (MRP and MRD) were shown to be involved in arsenic efflux from cells. Exposure of rat derived cell lines to various arsenicals led to elevated expression of *MRP1*, *MRP2* and *MDR1* [46, 47]. In contrast, mice carrying loss of function mutations in these transporters are considerably more sensitive to arsenic toxicity and accumulated more arsenicals in their tissues [48]. Close association of GSTP1, a glutathione transferase, and MRPs and MDRs in membrane vesicles suggests, that complexation of As^{III} compounds by GSH provides the substrate for the ABC transporters which mediate extrusion of arsenicals from the cell [49].

Arsenic and plants

Arsenic toxicity and tolerance

Arsenic is readily taken up by plant roots, in most cases as arsenate (As^{V}), the dominant form of arsenic in aerobic environments [4]. Specific transporters have been identified that are believed to mediate a large part of the observed As^{V} influx and these include the *A. thaliana* Pht1;1 and 1;4 high and medium affinity phosphate uptake systems [50]. In anaerobic soils, arsenic mostly exists as inorganic As^{III} due to abundant reducing activity of microbial organisms. Thus, in most aquatic plants, uptake of As^{III} is at the root of arsenic toxicity. Organic forms of arsenic occur in some soils, but usually in a small proportion, and consist mostly of methylated arsenic, such as MMA^{V} and DMA^{V}. Uptake of these compounds by plants is lower than that for inorganic As species [19, 51] (Fig. 2).

Plants vary greatly in arsenic tolerance, from sensitive species that include all major crops, to tolerant plants such as some ecotypes of the grass *Holcus lanatus*, to extremophiles such as the hyperaccumulator *Pteris vittata* (Chinese break fern) which can accumulate 2% of its dry weight as arsenic [52]. In spite of this ecophysiological diversity, many common responses to arsenic exposure have been observed. For example, most intracellular As^{V} is reduced to As^{III} by the action of specific arsenate reductases. Such proteins have been identified in many plant species including *Arabidopsis* [53], *H. lanatus* [54],

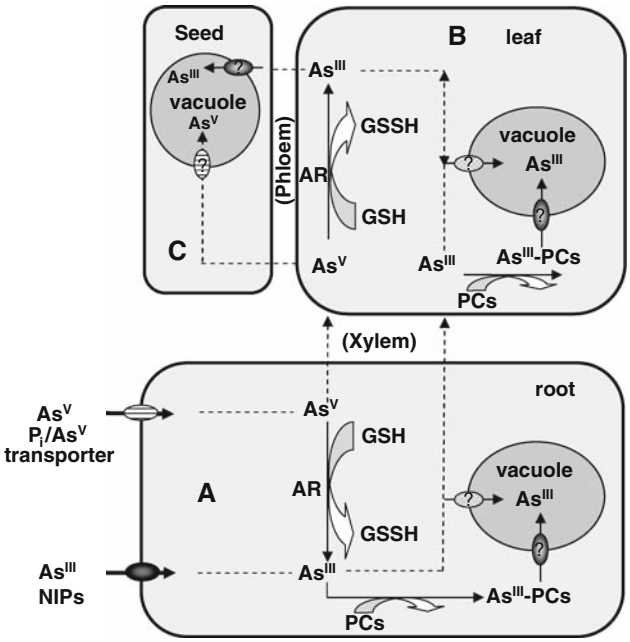

Fig. 2 Mechanisms of arsenic uptake in plants **a** Plants take up As^{V} through phosphate transporters (Pi) such as those belonging to the PHT1 family. As^{III} influx occurs through aquaglyceroporins of the NIP (nodulin like intrinsic protein) subfamily. As^{V} is reduced to As^{III} by arsenate reductase (AR) using glutathione (GSH) as a reductant and As^{III} can form complexes with thiol groups from glutathione and phytochelatins (PCs) to lower its cytotoxicity. The complexed As^{III} and inorganic As^{III} are believed to be mostly sequestered into the central vacuole via as yet unknown transporters. Inorganic As^{V} and As^{III} are the major arsenicals found in the xylem sap of plants. **b** Most plant species act as 'excluders' i.e. a very small proportion of arsenic is translocated to shoot tissue where similar reduction and sequestration mechanisms are present. **c** Via the phloem, some of the total arsenic content ends up in the vacuoles and other tissues of edible parts such as seeds

P. vittata [55] and rice [56]. Many plant arsenate reductases are bifunctional and also show tyrosine phosphatase activity. They are homologous to the human cell-cycle dual-specificity phosphatases, CDC25s, and often contain the conserved motif HCX_5R. Expression of plant arsenate reductases in *E. coli* and yeast mutants that lack endogenous reductases restored As^V resistance, and several have been found to be induced by plant exposure to As^V [53, 55, 56]. However, the physiological relevance of these enzymes is not always clear since in some species disruption of their activity had little impact on arsenic tolerance [57], and alternative mechanisms of As^V reduction, such as non-enzymatic reduction, may be present in plants.

Arsenic toxicity is largely manifested in the cytoplasm and a common mechanism of detoxifying cytoplasmic metals and metalloids is the complexation via sulphur bonds [30, 36]. Although variation between species is likely, it is believed that a substantial proportion of As^{III} may be chelated in this way to minimise cytoplasmic exposure. In plants, this is typically carried out by glutathione and glutathione-based phytochelatins (PCs) which have a general structure $(\lambda\text{-Glu-Cys})_n$-Gly. The –SH functional group of the cysteine in glutathione and PCs has a high affinity for As^{III}. Many studies point to the essential role of PCs in both constitutive and adaptive plant tolerance to arsenic [58]. For example, an *Arabidopsis* mutant that lacked the capacity to synthesise PCs was found to become hypersensitive to arsenate [59].

Interestingly, however, the proportion of complexed arsenic in hyperaccumulators such as *P. vittata* [60, 61] and *P. cretica* [58] is very small, suggesting that such species rely on tolerance mechanisms that do not involve complexation of arsenic.

In most plants, only a fraction of the arsenic is translocated to shoot tissue, a widespread tolerance mechanism that is also observed for other toxic ions. However, in hyperaccumulators, the opposite is the case: the majority of potentially harmful metals/metalloids is deposited in the shoot. This is exemplified in *P. vittata* where around 80% of the arsenic is deposited in the shoot. In contrast, only around 5–10% of total arsenic ends up in leaves of non-accumulating species such as the fern *P. tremula* [62], *Arabidopsis* [63] and rice [64]. The form in which arsenic is translocated from root to shoot appears to be mainly as inorganic As^{III}, accounting for 60–100% of the total arsenic [57, 64, 65].

Whether in root or shoot tissue, normally most arsenic will ultimately end up in the vacuolar compartment. In yeast, the vacuolar ABC transporter YCF1 (yeast Cd factor 1) is involved in vacuolar sequestration of metals and metalloids as glutathione-*S*-conjugates which include As^{III}-$(GS)_3$ [42]. However, there are to date no reports on the actual mechanism(s) of As transport across the plant vacuolar membrane (tonoplast). Evidence from a limited number of studies on cadmium suggests that Cd–PC complexes can be transported across the tonoplast of oats as Cd–PC_3 [66], and in a tonoplast enriched vesicle preparation from *H. lanatus*, ATP dependent accumulation of As^{III}-GS_3 was recorded [54]. These findings point to the possible contribution of ABC transporters in these processes, but the sheer size of plant ABC transporter gene families, with typically over 100 isoforms, greatly hampers the identification of specific proteins.

The role of aquaporins in As^{III} uptake

It is clear from multiple studies that plant arsenic toxicity mainly derives from exposure to inorganic arsenate (As^V) and arsenite (As^{III}). Since As^V is rapidly reduced, intracellular arsenic is predominantly in the form of inorganic or complexed As^{III}. The molecular identity of the proteins that participate in uptake, efflux, compartmentation and long-distance transport of As^{III} is largely unknown, but great progress has been achieved recently in identifying a number of them, particularly those associated with As^{III} uptake.

The uptake of As^{III} rather than As^V is especially pertinent for plants that grow in reducing environments such as paddy-grown rice and aquatic species. In analogy to prokaryotes, yeast and mammalian organisms, it was hypothesised that plants too take up As^{III} through aquaporins [67]. However, with large aquaporin gene families ($n = 30$–40) typically present in plant genomes, pinpointing specific isoforms was not straightforward. Plant aquaporins cluster in four subfamilies called PIPs (plasma membrane intrinsic proteins), TIPs (tonoplast intrinsic proteins), SIPs (small basic intrinsic proteins) and NIPs (nodulin26-like intrinsic proteins). The tertiary structure of aquaporin subunits is composed of a two times three transmembrane domain (TMD) structure (Fig. 3a) which both contain the canonical NPA motif. The two domains fold onto each other with the NPA motifs forming part of the central restrictive pore, and is often referred to as the 'hourglass model' [68]. Four subunits form a functional aquaporin, each with a central pore.

Aquaporin selectivity is mainly derived from the Ar/R (aromatic-arginine) pore region which is located a few angstroms from the canonical NPA-NPA motif (Fig. 3b). It consists of two residues from TMD2 and TMD5 and two residues from loop E. The composition of the Ar/R domain defines the pore size, pore hydrophobicity and hydrogen bonding between pore and substrate, and hence greatly impacts on selectivity and function of aquaporins (Table 1). Archetypal aquaporins contain a narrow pore with a width of around 0.3 nm, just wider than a water

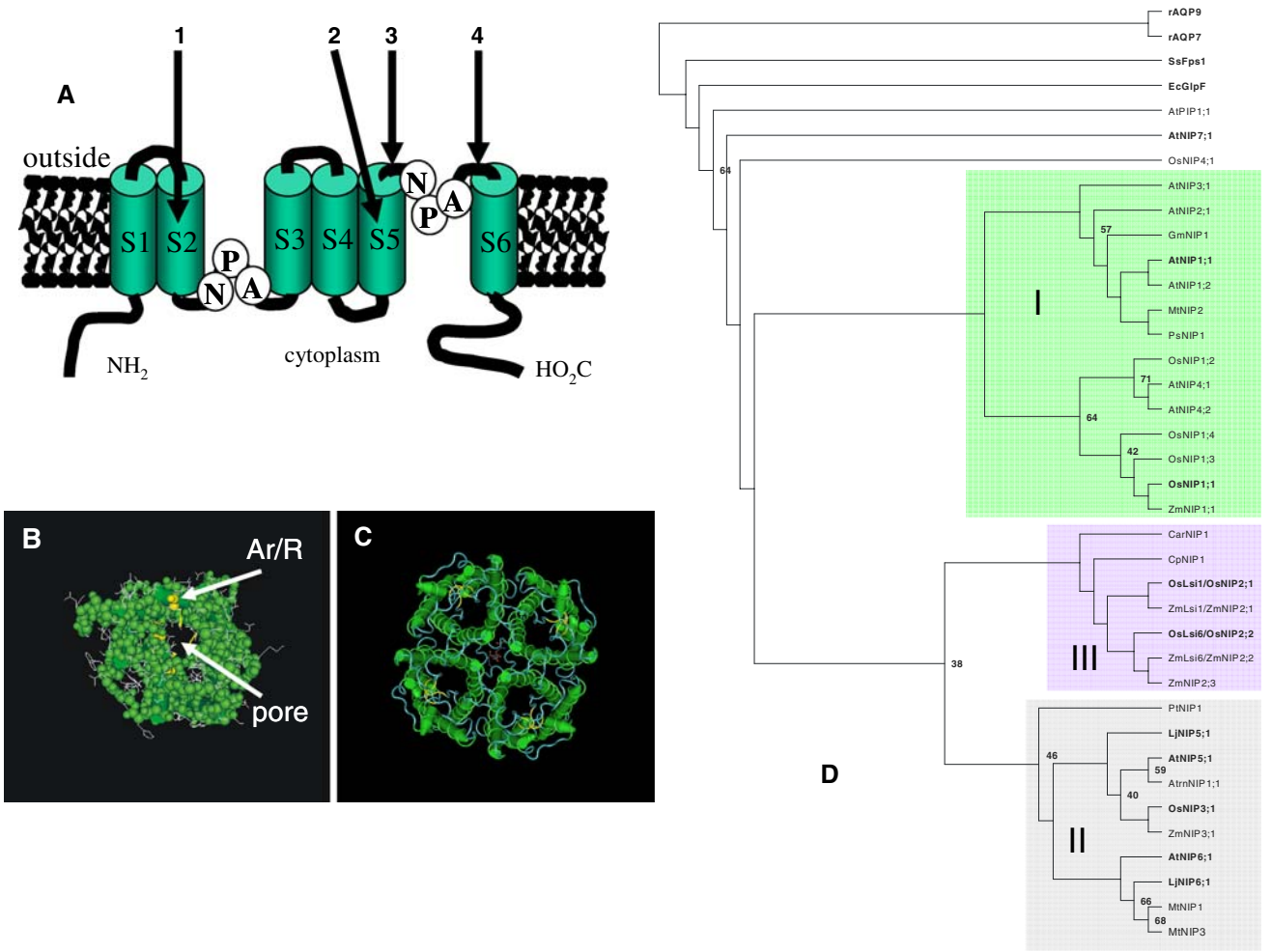

Fig. 3 The involvement of NIPs in plant arsenite transport. **a** Generalised secondary structure of an aquaporin monomer with six transmembrane domains (S1–S6) and two canonical NPA motifs between S2 and S3 and S5 and S6. The *arrows* 1–4 point to the approximate positions of residues that make up the Ar/R (aromatic/arginine) region that is essential for channel selectivity. **b** Each monomer forms a pore (*arrow*) with the Ar/R residues (*in yellow*) forming the narrowest part of the channel pore. **c** Four monomers form a functional aquaporin. Views in (**b**) and (**c**) are perpendicular to the membrane from the outside. **d** Phylogenetic tree of plant NIPs. At *Arabidopsis thaliana*, Os *Oryza sativa*, Zm *Zea mays*, Mt *Medicago truncatula*, Cp *Cucubrita pepo*, Nal *Nicotiana alata*, Lj *Lotus japonicus*, Pt *Pinus taeda,* Atrn *Atriplex nummularia*, Car *Cicer arietinum*, Gm *Glycine max*, Ps *Pisum sativum*, Ec *Escherichia coli*, Ss *Saccharomyces cerevisiae*. For comparison, AtPIP1;1, a non-NIP plasma membrane intrinsic protein, and As^{III} conducting aquaglyceroporins from mammals (AQP7 and AQP9), yeast (Fps1) and bacteria (GlpF) are included. The *color fields* represent different subgroups of the NIP proteins and isoforms that have been shown to be able to conduct As^{III} are shown in *bold*. The alignment of amino acid sequences was performed using ClustalX 2.0.9

molecule (~0.28 nm). This tight fit, and an energetic barrier near the channel NPA motifs that shapes the pore water-wire, ensure high water selectivity, a very low proton conductance and no or very little permeability for any other substrates. However, in several of the plant aquaporin subfamilies such as the NIPs, the Ar/R composition creates a larger pore size, thus allowing additional substrates to permeate such as neutral metalloids, undissociated acids and small solutes like glycerol. This class of aquaporin is therefore often referred to as aquaglyceroporins. On the basis of the Ar/R domain composition, the plant NIP subfamily can be further divided into groups I, II and III (Fig. 3d), with group I containing the archetypal Nodulin26 and NIPs with permeability to water, glycerol and lactic acid. NIPs in groups II and III have a predicted larger pore size than those of the NIP I group, with permeability for larger solutes such as antimonite, urea, formamide, silicic acid and boric acid, but very low water permeability. In contrast to group I members, group II and III isoforms are sometimes referred to as metalloid transporters and also include the outlier AtNIP7;1 [63, 68–70]. Several isoforms in this group have been shown to fulfil physiological functions in planta: AtNIP5;1 was shown to catalyse B uptake, presumably as the neutral boric acid $B(OH)_3$, in

Table 1 Aquaporin Ar/R selectivity filter sequences. The essential amino acid residues in the 2nd and 5th transmembrane domain (TMD) and in the 'E' loop are shown for the archetypal aquaporin AtMIP1 and for group I, II and III NIP (nodulin26-like intrinsic proteins) aquaglyceroporins

Aquaporin	TMD2	TMD5	E loop	E loop	Relative conductance			Other known substrates	Ref
					As	Sb	H_2O		
MIPs									
AtPIP/OsPIP	F	H	T	R	–		+++		[73]
NIP-I									
AtNIP1;1	W	V	A	R	–/++	–	+++	Glycerol	[68, 72]
AtNIP1;2	W	V	A	R	++				[72]
OsNIP1;1	W	V	A	R	++/–				[64, 71]
NIP-II									
AtNIP5;1	A	I	G	R	++	+		B, urea	[69, 71]
AtNIP6;1	A	I	A	R	++	+	–	B, urea, NH_3	[70, 71]
AtNIP7;1	A	V	G	R	+	+++	–		[63, 71]
OsNIP3;1	A	A	G	R	+		B		[64]
OsNIP3;2	A	A	A	R	+++	++			[71]
NIP-III									
OsNIP2;1	G	S	G	R	+++	+++		Si	[64, 71]
OsNIP2;2	G	S	G	R	+	+		Si	[64, 71]

Relative conductance to various substrates is indicated as: – none or very low, + low to medium, ++ medium, +++ high, *n.d.* not determined

boron limited conditions [69]. AtNIP6;1 also transports B but functions in root to shoot movement of this mineral [70]. OsNIP2;1 (Lsi1) is a major pathway for uptake of Si (as $Si(OH)_4$), a beneficial nutrient that is required in large amounts by rice [71]. Several other group II and III NIPs are also Si permeable but their physiological function remains unclear [64].

To assess the possibility that NIPs form arsenic transporters, several groups recently studied the physiological and transport characteristics of these proteins. Homozygous T-DNA lines for the three group II *A. thaliana* NIPs (NIP5;1, 6;1 and 7;1) showed that loss of function in NIP7;1 led to plant tolerance to As^{III} but not As^{V}, whereas arsenic tolerance in *Atnip5;1* and *Atnip6;1* mutants was not significantly affected [63]. Total arsenic content in *Atnip7;1* was reduced and heterologous expression of this protein increased As^{III} sensitivity in the *Δfps1* yeast strain that lacks the arsenite uptake aquaglyceroporin FSP1. Transport assays showed that expression of AtNIP7;1 also increased short-term As^{III} uptake in yeast.

In rice, the group III NIP2;1 was previously shown to provide a silicon uptake pathway and is therefore also referred to as Lsi1 [71]. Loss of function in NIP2;1 not only affects silicon accumulation but resulted in greatly reduced arsenite influx into roots compared with the wild-type rice. Heterologous expression of OsNIP2;1 in *Xenopus laevis* oocytes showed that OsNIP2;1 has a high As^{III} conductivity but did not transport As^{V}. Loss of function in another group II NIP (OsNIP2;2) did not significantly change plant arsenic levels, in spite of As^{III} transport capacity in oocytes, presumably because of a very low in planta expression level of this isoform.

Further work, based primarily on heterologous expression of NIPs in yeast, confirmed the As^{III} transport capacity of AtNIP7;1 and OsNIP2;1, but in addition showed that all group II NIPs are capable of As^{III} transport [72]. Indeed, several of the NIPs derived from *A. thaliana, Lotus japonicus* and rice were capable of mediating bidirectional As^{III} transport which could have implications for their in planta role.

Although suggested by some authors, the idea that only 'metalloid' NIPs have significant As^{III} permeability, and therefore a role in As^{III} uptake and distribution in plants, has been shown to be too narrow. A forward genetics screen with *A. thaliana* identified the group I NIP1;1 as a determinant of As^{III} tolerance [73]. Expression of AtNIP1;1 in oocytes showed As^{III} transport in these cells as did its close homologue AtNIP1;2, another member of the group I NIPs. Work with rice provided similar insights for group I NIPs from this species, with OsNIP1;1 and OsNIP3;1 both capable of mediating As^{III} uptake in oocytes, albeit significantly less so for OsNIP3;1 [64]. In total, these recent findings suggest that all NIP aquaporins may have some permeability to As^{III} but that specific isoforms are more important than others where plant arsenic tolerance is concerned, depending on expression levels and tissue localisation.

As^{III} efflux through aquaporins and other systems

Arsenic tolerance in prokaryotes and unicellular eukaryotes relies heavily on As^{III} efflux and the relevant membrane transporters have been well characterised [34]. The question whether As^{III} efflux is an important component of plant arsenic tolerance in plants has yet to be answered definitively, but several studies have shown that plants release As^{V} and As^{III} into the external medium [57]. As^{III} efflux occurs soon after plants are exposed to arsenate, but the mechanistic details of this process are unclear [74].

The recent work on the role of plant aquaglyceroporins in As^{III} uptake revealed some evidence that the same class of protein may also contribute to the efflux of As^{III} from cells. Expression of group II and III NIPs from *A. thaliana*, rice and *L. japonicus* in the yeast strain *Δacr3* that lacks its As^{III} efflux carrier led to significantly enhanced yeast tolerance to As^{V} [63, 72]. In both studies, these data were interpreted as evidence for NIP channels forming a shunt pathway for the efflux of arsenite which was produced by the reduction of arsenate inside the yeast cells. There are precedents for aquaglyceroporins acting in cellular As^{III} extrusion, notably in the earlier mentioned *S. meliloti* which expresses *aqpS* in its bacteroid membrane to release As^{III}. Theoretically, similar mechanisms could operate and be beneficial in plants, especially for species exposed to As^{V}. In the case of As^{III} exposed plants like rice, such a mechanism could easily create futile cycling of As^{III} and appears of less advantage. A definitive answer regarding the physiological relevance of aquaglyceroporin mediated As^{III} release has yet to be reached, but data from our laboratories show decreased As^{III} release in As^{V} loaded *nip6;1* and *nip7;1 Arabidopsis* loss of function mutants (F Maathuis et al., unpublished results) and also a significant reduction in As^{III} release in rice *nip2;1* mutants (F Zhao et al., unpublished results).

As^{III} extrusion from the cytosol through aquaporin-type transporters is by definition a passive mechanism dependent on the concentration gradient and may not be very efficient in some conditions. Many organisms show the presence of energy coupled systems to remove As^{III} from the cytoplasm [75] such as the H^+ coupled bacterial ArsB, the ATP dependent arsAB or the yeast ACR3, which is also believed to be H^+ coupled [76]. Whether plants carry out active As^{III} extrusion is not clear; in tomato roots preloaded with As^{V}, As^{III} efflux was found to be sensitive to the protonophore CCCP (carbonylcyanide chlorophenyl hydrazone) suggesting an active mechanism may be involved [74]. However, CCCP-induced depolarisation would also affect passive fluxes. An *Δacr3* yeast complementation approach using a cDNA library from *P. vittata* revealed the presence of an ACR3 like transporter in this species [77]. PvACR3 reduced arsenic accumulation in the complemented yeast, providing strong evidence for its As^{III} efflux capacity. *PvACR3* expression in *P. vittata* is rapidly increased after arsenate exposure, but whether its main function in planta is the actual As^{III} efflux to the apoplast, intracellular compartmentation or long-distance redistribution remains to be revealed. Interestingly, homology analyses show that similar proteins are distributed amongst genomes of lower plants such as mosses and ferns but not in angiosperms.

The similarity in size and pKa of As^{III} and nutritional minerals such as boric acid and silicic acid means that endogenous mechanisms for the latter substrates may also form potential As^{III} efflux systems. Both boric acid and silicic acid are moved through the xylem and can be sequestered in vacuoles, two processes that involve efflux steps. Loss of function in the silicon efflux system Lsi2 impacts greatly on plant arsenic distribution, with a large reduction in both xylem and grain As^{III} concentration [64]. In addition, the xylem As^{III} levels are reduced by silicon indicating that Lsi2 is responsible for a significant proportion of As^{III} loading into the vascular tissue. In combination with the data obtained from the *lsi1* loss of function mutant, the results suggest that in rice, arsenic uptake and distribution closely parallels that of silicon, a nutrient required in large amounts by rice. This may severely limit the scope to engineer rice with improved arsenic tolerance.

Efflux from the cytosol into the large lytic vacuole is a further generic detoxification pathway that has been shown to function in plants with regards to toxic minerals such as Na^+ and heavy metals like Pb^{2+} and Cd^{2+}. The latter are likely to be chelated to compounds such as glutathione and PCs before vacuolar deposition. The form in which vacuolar arsenic is stored appears to be species dependent: for the hyperaccumulator *P. vittata*, it was concluded that vacuolar arsenic consisted almost exclusively of inorganic As^{III} [78], whereas in non-accumulators such as *H. lanatus*, a large proportion was shown to be complexed [58]. The proteins that mediate vacuolar sequestration of arsenic are not known, but in tonoplast-enriched microsomes from *H. lanatus* roots, glutathione complexed As^{III} was efficiently transported into the vesicles after addition of MgATP, and the authors suggested that this phenomenon could be mediated by ABC type transporters [54]. Investigations into altered arsenic tolerance in loss of function mutants for several vacuolar ABC transporters have so far been unsuccessful (F. Maathuis et al., unpublished data).

Conclusions and outlook

Chronic arsenic poisoning is a major threat to large sections of the global population, and food consumption is one

of the biggest contributors to human arsenic exposure. Ideally, this problem should be solved at the source, i.e. through soil remediation and usage of non-contaminated irrigation water. However, this is unlikely to be achieved in the near future, and different strategies may be required to limit arsenic entry to the food chain. Approaches to reduce arsenic uptake in crops, especially in the edible parts, would provide a viable alternative. The latter has far more chance of success if details about uptake, efflux and translocation of the relevant arsenicals are known, and the recent reports on the role(s) of NIP aquaporins in plant arsenic tolerance have further defined this paradigm. These findings can potentially be exploited via overexpression or loss of function mutations in NIPs that affect As^{III} and As^{V} tolerance in plants [72]. In crops like rice, NIP loss of function could limit As^{III} uptake, whereas crops in aerobic soils might benefit from augmented efflux through NIP overexpression. However, such a strategy might interfere with beneficial NIP functions such as boron and silicon nutrition. Altering the composition of NIP Ar/R regions via site-directed mutagenesis could circumvent these negative side effects by altering As^{III} permeability whilst leaving that for beneficial substrates unchanged. NIPs and other As^{III} transporters such as Lsi2 [64] also affect root:shoot arsenic partitioning and can therefore be useful to minimise translocation of arsenicals to edible parts such as grains and fruits.

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