Signaling and Communication in Plants

Markus Geisler *Editor*

Plant ABC Transporters

Springer

Signaling and Communication in Plants

Series Editor

František Baluška
Department of Plant Cell Biology, IZMB, University of Bonn, Bonn, Germany

For further volumes:
http://www.springer.com/series/8094

Markus Geisler
Editor

Plant ABC Transporters

Springer

Editor
Markus Geisler
Plant Biology
University of Fribourg Department of Biology
Fribourg, Switzerland

ISSN 1867-9048 ISSN 1867-9056 (electronic)
ISBN 978-3-319-06510-6 ISBN 978-3-319-06511-3 (eBook)
DOI 10.1007/978-3-319-06511-3
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014946212

© Springer International Publishing Switzerland 2014
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

ABC (ATP-binding cassette) proteins are ubiquitous, membrane-intrinsic transporters that catalyze the primary (ATP-dependent) movement of their substrates over biological membranes. Until know, the special challenge of ABC transporter work lies in the identification of ABC transporter substrates. Therefore, in higher plants, ABC transporters were—in functional analogy to their mammalian orthologs—initially identified as central part of a detoxification process in that they sequester conjugated xenobiotics from the cytoplasm into the central vacuole. However, mainly genetic work in the last decade has provided the discovery of unexpectedly diverse ABC transporter substrates that include beside chlorophyll catabolites and xenobiotic conjugates, heavy metals, lipids, terpenoids, lignols, and organic acids. According to their associated wide biological function, plant ABCs were found beside on vacuolar membranes also on the plasma membrane and on membranes of other organelles, including the plastids, peroxisomes, mitochondria and the endoplasmic reticulum. The discovery that members of the ABCB and ABCG family are involved in the cellular and intercellular movement of phytohormones, such as auxins (IAA and IBA), abscisic acid (ABA), cytokinin, and most probably also strigolactones, has boosted their investigation and provided a new perception of the whole family. Further, recent work on ABC transporters has left the Arabidopsis limits and been extended to dicot and monocot crop plants, which has caused considerable interest also outside the plant community.

This book is therefore devoted to the exciting plethora of plant ABC transporter substrates and highlights their tightly connected biological functions that accordingly reach from cellular detoxification, over development, to symbiosis and defense. It contains a special focus on "phytohormone transporters" and several chapters converging on the trafficking, regulation, and structure–function of ABCB-type auxin transporters. Moreover, it especially emphasizes the role of ABC transporters in plant defense and the symbiosis between plant and microorganisms, such as arbuscular mycorrhiza and rhizobia root nodules. Finally, it encompasses also a set of chapters that center on ABC protein structure and ABC evolution.

I would like to express my deepest gratitude to the ABC transporter community for pushing this exciting field so tremendously forward in the last decade. But also for contributing to this little milestone, making it what it was initially meant to be: a complete, timely, and beautiful overview on *Plant ABC Transporters* for the expert. And a teaser for the beginner: there is much more to explore!

Fribourg, Switzerland Markus Geisler
March 17, 2014

Contents

ABC Transporters and Heavy Metals

Won-Yong Song, Jiyoung Park, Cornelia Eisenach, Masayoshi Maeshima, Youngsook Lee, and Enrico Martinoia

Abstract The first evidence showing that ABC transporters are involved in heavy metal resistance in eukaryotic cells has been obtained from experiments in *Schizosaccharomyces pombe* and *Saccharomyces cerevisae*, where a half-size transporter of the ABCB subclass and an ABCC-type transporter, respectively, have been shown to confer heavy metal tolerance. Biochemical studies have indicated that vacuolar ABC transporters should also play an important role in heavy metal detoxification in plants. But it was only recently that two ABCC-type transporters, AtABCC1 and AtABCC2, have been identified as major apo-phytochelatin and phytochelatin-heavy metal(oid) complex transporters. Several plasma membrane transporters have also been shown to confer heavy metal resistance. However, with the exception of STAR1, an UDP glucose exporter, which—by altering cell wall composition—confers aluminum tolerance, the substrates required to be transported to confer heavy metal resistance by these plasma membrane-localized ABC proteins are still not elucidated. A mitochondrial ABC transporter AtATM3 was shown to be required for plant growth and development.

W.-Y. Song • Y. Lee
POSTECH-UZH Cooperative Laboratory, Department Integrative Bioscience and Biotechnology, Pohang University of Science and Technology, Pohang, South Korea

J. Park
Division of Biological Sciences, University of California, San Diego, La Jolla, CA, USA

C. Eisenach
Institute of Plant Biology, University Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland

M. Maeshima
Laboratory of Cell Dynamics, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

E. Martinoia (✉)
POSTECH-UZH Cooperative Laboratory, Department Integrative Bioscience and Biotechnology, Pohang University of Science and Technology, Pohang, South Korea

Institute of Plant Biology, University Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland
e-mail: enrico.martinoia@botinst.uzh.ch

M. Geisler (ed.), *Plant ABC Transporters*, Signaling and Communication in Plants 22,
DOI 10.1007/978-3-319-06511-3_1, © Springer International Publishing Switzerland 2014

The different studies indicate that this transporter is important for the production of cytosolic iron sulfur complexes and molybdenum cofactors, prosthetic groups required for several enzymes. However, the final proof as to which substrate is transported by AtATM3 is still missing. Several laboratories took advantage of the fact that ABC transporters are involved in heavy metal tolerance to generate transgenic plants suitable for phytoremediation. The results show that overexpression of ABC proteins alone is not sufficient to produce plants that can efficiently decontaminate soils, but they indicate that this class of transporters, when combined with other transporters and enzymes involved in heavy metal transport and detoxification, may prove a good solution to produce plants that can stabilize, and in the long term clean up, soils contaminated with heavy metals.

1 Introduction

All living cells require heavy metals such as Fe, Zn, Mn, Cu, Ni, or Mo as cofactors for enzymes and transcription factors. As other organisms, plants have to tightly regulate uptake, allocation, and storage of these essential heavy metals in order to allow for optimal growth. On the one side, a deficiency in these metals causes growth retardation and developmental defects throughout a plant's entire life cycle; on the other side, the uptake of excess heavy metals would lead to toxicity syndromes.

Non-essential, toxic heavy metals are present in natural soils, and volcanic eruptions are often accompanied by the release of toxic heavy metals. During the industrialization period of the late nineteenth and early twentieth century anthropogenic release of heavy metals has increased dramatically. Mining, waste incinerators, pesticides, and fertilizers contaminated our environment. Furthermore, in Bangladesh and India water wells initially built to supply clean water to the population were later found to contain toxic amounts of arsenic, which led to a contamination of rice paddy fields (Zhao et al. 2010; Meharg and Rahman 2003; Williams et al. 2005).

Non-essential heavy metals may hijack transporters required for the uptake of essential heavy metals, because of the two metal species' chemical similarity, and consequently enter and accumulate in the plant cell. It has been shown that the iron/zinc transporter IRT1 is the major entry point for toxic cadmium (Vert et al. 2002), while high-affinity phosphate transporters and members of the MIP family import arsenate(V) and arsenite (III), respectively (Zhao et al. 2010).

A multitude of transporters belonging to different classes are involved in uptake, distribution and sequestration of metal ions. Uptake of heavy metals occurs mainly by cation channels and symporters, such as members of the ZRT/IRT1 protein family, which are responsible for the uptake of iron and zinc or the copper transporter COPT1 (Palmer and Guerinot 2009). P-type ATPases have been

shown to be required for the export of zinc into the xylem and its translocation to the shoot (Hussain et al. 2004; Verret et al. 2004). Alternatively, antiporters may also release heavy metals into the xylem, as has been suggested for the putative iron exporter ferroportin (Morrissey et al. 2009). P-type ATPases have also been demonstrated to play an important role in transporting copper from the cytosol into the chloroplast stroma and from the stroma into the thylakoid lumen (Shikanai et al. 2003; Burkhead et al. 2009). Storage and concomitant detoxification of excess heavy metals generally occurs within the vacuole (Martinoia et al. 2007). The last decade saw the identification of a large number of vacuolar heavy metal transporters that import excess heavy metals but that also export them, if the plant metabolism requires the supply of a specific heavy metal (Martinoia et al. 2007, 2012). In order to further reduce the toxicity of heavy metals, plants produce chelating agents, mainly carboxylates and the glutathione-derived phytochelatins (Grill et al. 1989; Clemens 2006), which are also transported into the vacuole.

In contrast to bacteria, to date no plant ABC transporter has been shown to be involved in essential heavy metal uptake or release at the plasma membrane, while many bacterial ABC transporters were reported for essential metal ion transport (Self et al. 2003; Napolitano et al. 2012). Instead, many plant ABC transporters identified up to now are involved in toxic metal transport and thus protect plants from the harmful effects of toxic heavy metals. It will be worthwhile to investigate why higher organisms have evolved ABC transporters that are mainly exporters, whereas bacteria evolved to possess a higher numbers of nutrient importers.

2 The Complex Functions of HMTs/ATMs

Plants, fungi and some yeast are known to produce glutathione-derived heavy metal chelators, phytochelatins, in response to the presence of cadmium, lead, arsenic, and some other heavy metals (Grill et al. 1989; Cobbett 2000). The first putative vacuolar phytochelatine transporter was identified in *Schizosaccharomyces pombe* and named SpHMT1. SpHMT1 is a half size ABC transporter of the ABCB subclass. The corresponding mutant, *hmt1*, was hypersensitive to cadmium and the authors could link the sensitivity to a strongly reduced amount of high-molecular weight phytochelatin–cadmium (HMWPC-Cd-S-2) complexes (Ortiz et al. 1992). Transport assays with vesicles isolated either from *hmt1 S. pombe* or from *S. pombe* form hmt1 mutant having empty vector SpHMT1 revealed that HMT1 transports apo-phytochelatins as well a phytochelatin–cadmium complexes, but not Cd^{2+}, GSH, GSSG, or glutathione conjugates (Ortiz et al. 1995). Intriguingly, and in contrast to the phytochelatine biosynthesis mutant, the *hmt1* mutant was not sensitive to As or Hg. In order to see, whether cadmium resistance mediated by SpHMT1 required a transport process, they produced a catalytic SpHMT1 mutant not able to bind ATP. This mutated form of SpHMT1 did not confer cadmium resistance anymore even in the presence of phytochelatins.

The discovery that *Caenorhabditis elegans* produces phytochelatins, as do plants and some fungi, led to the discovery of a HMT1 homologue, CeHMT1, in this organism and generated the corresponding RNAi lines (Vatamaniuk et al. 2005). As observed for the *S. pombe hmt1* mutant, *Caenorhabditis elegans* was hypersensitive to cadmium when CeHMT1 was absent. Surprisingly, the worms carrying the RNAi construct were even more susceptible to cadmium than those mutated in phytochelatin synthase (PCS). In a subsequent report, Schwartz et al. (2010) showed that in contrast to SpPHMT1, CeHMT1 could confer heavy metal resistance not only to cadmium but also to As and Cu, albeit in a phytochelatin-independent manner. Interestingly, CeHMT1 and PCS are co-expressed in highly endocytic cells, called coelomocytes, suggesting that these cells play a central role in heavy metal detoxification in *C. elegans*. The observations that HMTs conferred cadmium resistance, but were unable to confer tolerance against As and Hg—both of which are known to form strong conjugates with phytochelatins—inspired two laboratories to re-evaluate the role of HMTs. Preveral et al. (2009) observed that SpHMT1 confers heavy metal resistance in *Saccharomyces cerevisiae* as well as in *E. coli*, both of which do not produce phytochelatins. However, SpHMT1 required glutathione to confer cadmium resistance. These results indicate that SpHMT1-dependent heavy metal resistance requires glutathione but not phytochelatins. Furthermore, Sooksa-nguan et al. (2009) showed that a HMT1 homologue from *Drosophila*, *DmHMT1,* which does not produce phytochelatins, was targeted to the vacuolar membrane in *S. pombe* and could rescue cadmium sensitivity in the *sphmt1* mutant. However, no phytochelatin transport activity could be observed in *S. pombe expressing DmHMT1*. In addition, the authors re-addressed the question of phytochelatin transport for SpHMT1 and could observe a slight but consistent decrease in vacuolar PC_2 content, but not for longer chain PCs. Consequently, Sooksa-nguan et al. (2009) suggested that SpHMT1 and PCS do not act in a direct, linear way and that SpHMT1 may contribute in a minor way to phytochelatin transport, while acting mainly in a way that is cadmium specific.

To date, HMT1 homologues that might reside in the vacuolar membrane of plants have not been identified, even though plants do encode for HMT1 homologues. Instead, in *Arabidopsis* (Chen et al. 2007; Rea 2007) as well as in *Chlamydomonas* (Hanikenne et al. 2005), the HMT1 homologues reside in mitochondria. Indeed, Atm1p, the HMT1 homolog in *S. cerevisiae*, was reported to reside in the inner mitochondrial membrane, and its transport activity was predicted to occur from the mitochondrial matrix to both, the intermembrane space as well as the cytosol (Leighton and Schatz 1995). Based on the observation that *atm1p* mutants accumulate iron within mitochondria, Atm1p has been suggested to function as a mitochondrial exporter for iron–sulfur clusters. Deletion of AtATM3/AtABCB25—one out of three Atm1p/HMT1 homologues of Arabidopsis—has a dramatic effect, causing dwarfism and chlorosis (Fig. 2a; Kushnir et al. 2001). The observation that AtATM3/AtABCB25 complements the yeast *atm1p* mutant indicates that both genes exhibit a similar function (Kushnir et al. 2001; Chen et al. 2007). In a later, detailed work, Bernard et al. (2009) provided further evidence that out of the three ATM *Arabidopsis* homologues, only ATM3 was

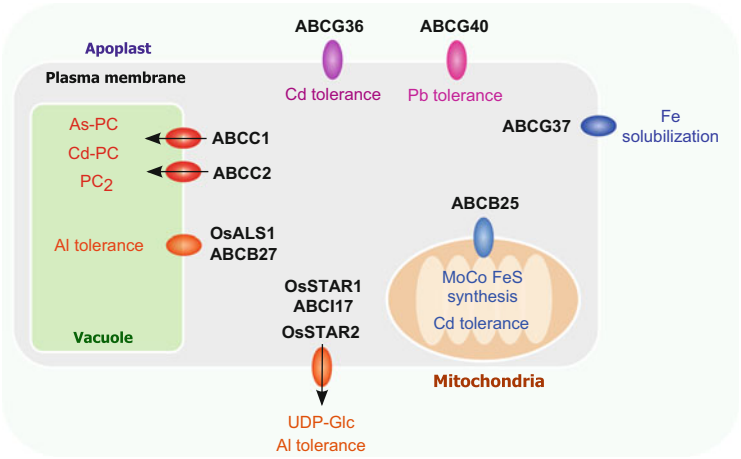

Fig. 1 Plant ABC transporters involved in heavy metal tolerance ABC transporters that have been reported to transport heavy metal(loid)s (marked with *arrows*) or involved heavy metal tolerance. Indicated transport proteins are from *Arabidopsis thaliana* unless specified otherwise

important for plant growth. Using a set of *atatm3* mutants, the group aimed to obtain information as to which substrate might be transported by AtATM3. Investigating enzymes containing Fe–S and molybdenum cofactors (MoCo) as prosthetic groups, they concluded that AtAMT3 may transport either at least two distinct compounds required for FeS and MoCo assembly in the cytosol, or a single compound required for the production of both cofactors. The central role of ATM3 in the production of MoCo was confirmed in a subsequent study, which showed that nitrate reductase and sulfite reductase activity, both of which require MoCo, were reduced to approximately 50 % from wild-type plants, while those which depend also on FeS were virtually undetectable (Fig. 1, Teschner et al. 2010). *Atatm3* mutants accumulate the first intermediate of MoCo synthesis, pyranopterin, which is produced in mitochondria. Nevertheless, the authors did not provide transport data and therefore the substrate(s) of ATM3 are still to be identified.

AtATM3 and a homologue from *Chlamydomonas*, CrCds1, have been shown to also play a role in cadmium tolerance (Hanikenne et al. 2005; Kim et al. 2006). In the case of *Chlamydomonas* the authors searched for mutants sensitive to cadmium. One of these mutants encoded for a HMT1 homologue. The mutant accumulated even more phytochelatins than the corresponding wild type and was also hypersensitive to iron. In order to identify *Arabidopsis* ABC transporters involved in heavy metal transport and/or resistance, Bovet et al. (2005) used a microarray specific for Arabidopsis ABC transporters. Among other genes, they observed that AtATM3/AtABCB25, was strongly upregulated in the roots of cadmium-treated plants. Overexpression of AtABCB25 enhanced Cd resistance, while a T-DNA insertion in this gene led to increased sensitivity (Kim et al. 2006). However, since *atatm3* mutants are already affected in their growth, the increased sensitivity might also reflect the decreased fitness of the *atatm3* mutant. Interestingly, when compared to

wild-type *atabcb25* mutants produce more glutathione in the presence of cadmium. This observation indicates that this mutant suffers higher oxidative stress and may be the link between the Fe–S, MoCo production and the cadmium phenotype.

3 Vacuolar ABCCs Are Involved in Heavy Metal Detoxification

The first ABC transporter of the ABCC family involved in heavy metal resistance was identified in *S. cerevisiae,* a fungus not producing phytochelatins. In the absence of Yeast Cadmium Factor 1 (YCF1), yeast is sensitive to cadmium (Szczypka et al. 1994) as well as to arsenic (As) and antimony (Sb) (Ghosh et al. 1999). It could be shown that YCF1 requires glutathione to confer heavy metal resistance and that YCF1-mediated detoxification of cellular heavy metals/metalloids occurs by transporting the glutathione-heavy metal complexes GS_2–Cd and GS_3–As, respectively (Li et al. 1997; Ghosh et al. 1999). A similar mode of action has been postulated for the human MRP1/HsABCC1, which partially complements the yeast mutant *ycf1* (Tommasini et al. 1996). It has also been suggested that detoxification of toxic heavy metals in plants may be partially mediated by glutathione heavy metal complexes mechanism, however, so far the importance of such a mechanism in plants is still elusive.

Plants are known to produce glutathione-derived heavy metal chelators, so-called phytochelatins that are produced in response to increased levels of cadmium, lead, arsenic, and some other heavy metals (Grill et al. 1989; Clemens 2006). Heavy metal–phytochelatin complexes are more stable compared to those including glutathione, and therefore they are more efficient in detoxifying potentially toxic heavy metals. Using vacuoles isolated from oat roots, Salt and Rauser (1995) provided evidence that apo-phytochelatin as well as Cd–phytochelatin is taken up by vacuoles in a strictly MgATP-dependent manner. While uptake of cadmium could be inhibited by both vanadate and NH_4^+, the latter of which abolishes pH gradients, phytochelatin transport was inhibited by vanadate only. These results indicated that cadmium uptake into the vacuole can be mediated by ABC transporters and H^+ gradient-dependent transporters, while phytochelatins are likely to be transported exclusively by an ABC transporter. Most ABC transporters found in the tonoplast belong to the ABCC clade. Expression studies showed that among the ABCC clade of *Arabidopsis*, it is mainly ABCC3, which is highly induced by cadmium (Bovet et al. 2005). Cadmium-dependent transcript increase was also observed for AtABCC6, 8, 10, and 12 (Bovet et al. 2005; Gaillard et al. 2008). Studies using T-DNA insertion lines for AtMRP3/AtABCC3 as well as AtAMRP6/AtABCC6 presented evidence that, indeed, these two ABC transporters are likely to be involved in cadmium resistance. AtABCC3 was able to partially complement the cadmium-sensitive phenotype of *ycf1* yeast (Tommasini et al. 1998), indicating that it could transport glutathione–cadmium complexes.

However, only a very slightly higher sensitivity was observed for the *atabcc3* mutant in the presence of cadmium (Klein and Martinoia, unpublished). While *AtABCC6* could not be expressed in yeast (Gaillard et al. 2008), the authors presented evidence that, at an early developmental stage, leaves of *atabcc6* knock-out mutants exhibited impaired growth in the presence of cadmium. No effect could be observed at the root (Gaillard et al. 2008). Nevertheless, the mechanism through which these two ABC transporters might be involved in heavy metal resistance remains elusive. Wang and Wu (2006) presented further evidence that in plants ABCCs are involved in heavy metal tolerance. These authors created insertional mutants in *Chlamydomonas reinhardtii* and screened them for cadmium sensitivity. Amongst them, the authors identified a mutant that carried a deletion of an ABCC-type transporter gene. This ABC transporter, *CrMRP2*, was strongly upregulated by cadmium and conferred cadmium tolerance when heterologously expressed in *ycf1* yeast, indicating that CrMRP2 acts as a glutathione–Cd transporter, although it cannot be excluded that phytochelatins may also be transported. Since this *Chlamydomonas* mutant is still producing similar amounts of phytochelatins as the wild type, CrMRP2 it is more likely to be a glutathione-transporter.

Based on the assumption that the vacuolar phytochelatin transporter should be a member of the ABCC family, Song et al. (2010) performed a large screen of T-DNA insertion mutants in all *Arabidopsis* ABCCs using arsenic as toxic heavy metalloid known to be detoxified by phytochelatins. The rationale behind this assumption was that only ABCCs had so far been demonstrated to unequivocally reside on the vacuolar membrane. Furthermore, two ABCC members had already been suggested to be involved in heavy metal detoxification (Tommasini et al. 1998; Gaillard et al. 2008). In the presence of arsenic-containing herbicide DSMA, *AtABCC1* and *AtABCC2* T-DNA insertion lines showed a slightly impaired root growth. However, the corresponding double mutant exhibited a very drastic phenotype and was extremely sensitive to arsenate and disodium methanearsonate (DSMA) when grown on both, agar and soil (Figs. 1 and 2b). A first indication that these transporters could indeed mediate the transfer of phytochelatins into the vacuole was gained from the observation that the arsenic sensitivity of *ycf1* yeast could be restored only by coexpressing a phytochelatine synthase and either AtABCC1 or AtABCC2. Transport experiments with vesicles isolated from yeast expressing either AtABCC1 or AtABCC2 revealed that both proteins transported apoPC and with an even higher capacity As-PC_2. The concentration-dependent uptake of As-PC_2 did not exhibit classical saturation kinetics but followed a sigmoid curve, indicating that As-PC_2 transport is regulated allosterically. This characteristic may be important to maintain a low cytosolic PCs pool under non-stress conditions, allowing for apoPCs to accumulate in the cytosol where they interact with heavy metals before being transported into the vacuole. Transport experiments performed with vacuoles isolated from *atabcc1 atabcc2* double knock-out *Arabidopsis* plants showed that these vacuoles exhibit a residual As(III)-PC_2 transport activity of only 10–15 %, indicating that these two ABC transporters are the major PC transporters in *Arabidopsis*. Overexpression of the transporters alone did not result in plants with an increased As tolerance, but the additional

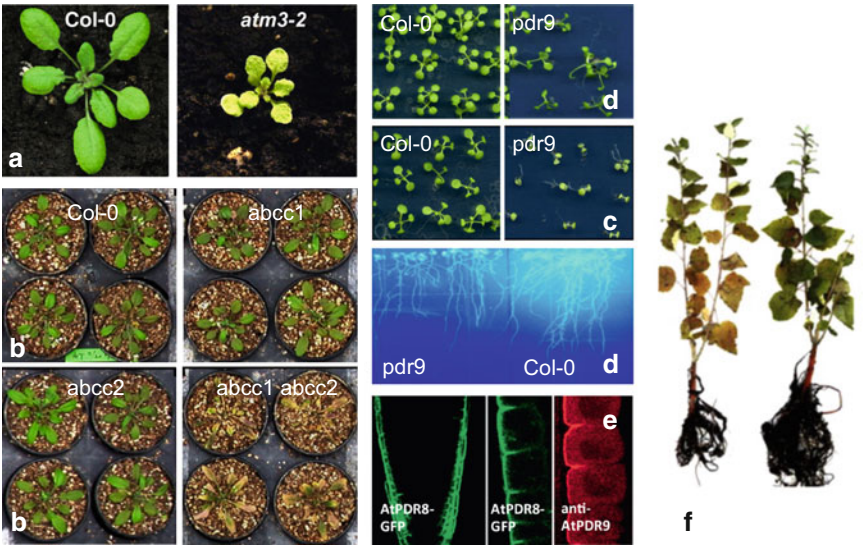

Fig. 2 Phenotypes and localization of ABC transporters involved in heavy metal allocation or detoxification. The *Arabidopsis* mutant of the mitochondrial ATM3 protein is chlorotic and exhibits strong growth defects (Teschner et al. 2010) (**a**). Phenotypes of wild-type *Arabidopsis* plants, the *atabcc1*, *atabcc2*, and *atabcc1xatabcc2* double mutants grown on arsenic-containing soil. Only the double mutant shows a high sensitivity against arsenic (Song et al. 2010) (**b**). *atabcg37*/ *pdr9* mutant plants are strongly impaired in growth and development when iron is a limiting factor in the medium (**c**, *lower panel*), while their growth is only slightly retarded under sufficient iron conditions (**c**, *upper panel*) (Fourcroy et al. 2013). This is due to a reduced iron mobilization as a consequence of impaired excretion of coumarylic compounds (**d**). AtABCG36/ PDR8 and AtABCG37/ PDR9 are localized on the lateral side of the rhizodermis, allowing for an efficient excretion of compounds to the soil (**e**) (Modified from Bätz and Martinoia 2014). Transgenic poplar trees transformed with the yeast YCF1 ABC transporter are less chlorotic than the corresponding wild-type trees and form a much larger and denser root system (Shim et al. 2013)

coexpression of phytochelatin synthase was necessary to attain this desired As-tolerant phenotype.

Subsequent experiments revealed that AtABCC1 and AtABCC2 also play a predominant role in conferring Cd and Hg(II) tolerance (Park et al. 2012). The *atabcc1 atabcc2* double knock-out displayed similar degrees of sensitivity to Hg (II) and arsenic. The sensitivity response in the presence of cadmium was strong but less pronounced compared to As and Hg, which was probably due to the fact that at pH 7 phytochelatin–cadmium complexes are very stable, while those with As(III) or Hg(II) are less stable. In summary, phytochelatin–cadmium complexes are stable in the cytosol, conferring a significant, although not complete level of tolerance to Cd without the ABCC transporters, whereas the phytochelatin–Hg and –As complexes absolutely require the ABCC transporters to be stably detoxified. The role of ABCC1 and ABCC2 in Cd sequestration is supported by the observation that in

cadmium-treated plants a Cd-sensing fluorescent signal marked the vacuole of wild-type plants, but marked the cytosol in *atabcc1 atabcc2* cells. The *atabcc1* single knock-out mutant was more sensitive than the wild type to Cd(II) and Hg(II), however by contrast, the *atabcc2* knockout mutant did not exhibit any dramatic difference in its sensitivity from the wild type. These results suggest that both AtABCC1 and AtABCC2 contribute to Cd(II) and Hg(II) tolerance, and that AtABCC1 can confer a significant level of tolerance to these divalent heavy metals in the absence of AtABCC2. Most of the work on ABCCs, phytochelatins, and heavy metals has been done with *Arabidopsis*. A recent work performed with barley vacuoles (Song et al. 2014) indicates that although the general mechanism for PCs-As transport is conserved in *Arabidopsis* and barley, the transport characteristics for PCs–heavy metal complexes may be different between plant species, and that PC-essential heavy metal complexes can be formed and transported into vacuole by ABC transporters. Together with the previous report suggesting a role of phytochelatin in Zn homeostasis (Tennstedt et al. 2009), this raises the question how plant cells can maintain essential heavy metal homeostasis when they take up non-essential, toxic heavy metals.

Interestingly, it has been shown in rice that a P-type ATPase, HMA3, is the major vacuolar transporter conferring cadmium resistance (Ueno et al. 2010). Its *Arabidopsis* homologue, AtHMA3, acts mainly as a zinc transporter, and the cadmium phenotype observed for the corresponding mutant plants is relatively weak (Morel et al. 2009). However, OsHMA3 has a far higher specificity for cadmium than AtHMA3 and the corresponding mutant is highly sensitive to cadmium. It will therefore be of interest to investigate the role of phytochelatin-transporters in rice and to investigate their role in cadmium tolerance. A tonoplast-localized ABC transporter in rice is involved in aluminum tolerance (Huang et al. 2012). OsALS1, a close homologue to the animal TAP peptide transporters is a member of ABCB subfamily and expressed mainly in roots (Fig. 1). A loss-of-function mutant of *osals1* exhibited sensitivity specifically to Al but not to other heavy metals. Although no Al transport activity of OsALS1 could be detected visual examination of Al using morin staining suggested that Al levels increase in the cytoplasm of *osals1* mutant roots. An *Arabidopsis* homolog, AtALS1, is also localized to the tonoplast, but the mutant phenotype of AtALS1 is limited to narrow ranges of Al concentrations (Larsen et al. 2007).

4 Plasma Membrane-Localized ABC Transporters Involved in Heavy Metals Resistance and Uptake

Transport processes mediating detoxification of Na^+ occur at both the vacuolar membrane as well as at the plasma membrane. Internal and external excretion processes are responsible for keeping Na^+ at nontoxic concentrations. Na^+/H^+ antiporters are responsible for the accumulation of Na^+ within the vacuole, where

it acts as "cheap" osmoticum. Excretion back into the apoplast and finally into the soil ensures the maintenance of nontoxic, intracellular Na^+ levels (Zhu 2002). Although, as described above, ABC transporters are involved in vacuolar deposition of heavy metals, to date no direct export of heavy metals from root to soil has been documented to our knowledge. Nevertheless, two plasma membrane-localized ABCGs and one bacterial-type ABC protein play an important role in heavy metal tolerance.

The best characterized among them are the bacterial-type ABC proteins OsSTAR1 and OsSTAR2 that play an important role in Aluminum (Al) tolerance in rice. Al is a major toxic metal that limits crop production in acidic soil (Fig. 1). A well-known mechanism to cope with aluminum toxicity is the excretion of citrate by MATE transporters (Magalhaes et al. 2007) and malate secretion by ALMTs into the rhizosphere (Delhaize et al. 2004; Meyer et al. 2010). These organic acids chelate Al, preventing the entry of the toxic Al^{3+} into the root (Ryan et al. 2011). OsSTAR1 and OsSTAR2 were identified in a screen to discover new genes involved in Al tolerance (Huang et al. 2009). As is often observed in bacteria, the cytosolic and membrane domains of an ABC transporter are encoded by two different genes. OsSTAR1 corresponds to the nucleotide-binding domain of the transporter and OsSTAR2 to its membrane domain. The combined ABC transporter complex was shown to locate mainly to membrane vesicles of root cells. Coexpression of OsSTAR1 and OsSTAR2 in oocytes of *Xenopus laevis* revealed that both proteins form a functional ABC transporter able to transport UDP-glucose. The discovery that UDP-glucose is involved in aluminum tolerance was surprising. The authors suggested that UDP-glucose may be used to alter the composition of the cell wall, thus avoiding migration of Al into proximity of the plasma membrane. The same group reported that a close homologue, AtSTAR1 exists in *Arabidopsis* (Huang et al. 2010). The knockout mutant of AtSTAR1 (ABCI17) was also sensitive to aluminum. The observation that OsSTAR1 could rescue the aluminum sensitive phenotype of the *Arabidopsis* mutant indicates that both genes code for proteins exhibiting a similar function.

In the transcriptome-based screen described above, Bovet et al. (2005) discovered that AtPDR8/AtABCG36/PEN3 is highly upregulated by cadmium. Further studies showed that this is also true for lead. Like all full-size ABCG transporters described so far, AtPDR8/AtABCG36 is localized at the plasma membrane (Kobae et al. 2006; Stein et al. 2006). *Arabidopsis* plants overexpressing PDR8 were more resistant to cadmium and lead, while RNAi and T-DNA mutant plants were more sensitive to it (Fig. 1, Kim et al. 2007). At the same time, plants overexpressing AtPDR8 contained less cadmium, while RNAi/T-DNA mutants contained more. Flux experiments using Arabidopsis mesophyll protoplasts indicated that Cd^{2+} export was more pronounced in AtPDR8 overexpressing plants than in the corresponding mutants. This result indicates that AtPDR8 is able to export Cd, however, whether this export involves Cd as Cd^{2+} or a Cd complex is yet unknown. Even an indirect activation of a Cd efflux transporter cannot be excluded. Interestingly, AtPDR8/AtABCG36/PEN3 transporter has been shown to play an important role in plant pathogen defense (Kobae et al. 2006; Stein et al. 2006). It will be

therefore interesting to see, whether the substrate(s) conferring heavy metal resistance and the ones involved in the plant–pathogen reaction are the same, or whether AtABCG36, as other ABC transporters, transports structurally unrelated compounds (Kang et al. 2011) and exerts its dual function in this way.

In a screen aimed to identify whether ABC transporters could be involved in lead detoxification, Lee et al. (2005) (Fig. 1) observed that the transcript levels of AtPDR12/AtABCG40 increased when Pb was present in the medium. As for AtPDR8, plants overexpressing AtPDR12 were more resistant to Pb^{2+} and accumulated less of the heavy metal, while knockout mutants of this transporter were more sensitive and accumulated more Pb^{2+}. The observation that inhibition of glutathione biosynthesis had a much stronger effect than the absence of AtABCG40 indicates that there is an additional glutathione-dependent detoxification pathway that plays a more important role than that mediated by AtABCG40. Later it was found that ABCG40 specifically transports abscisic acid (ABA) (Kang et al. 2010). Since Pb did not compete with ABA for transport via ABCG40 (Kang and Lee, unpublished result), the role of the transporter in Pb tolerance might be indirect via uptake of the stress hormone ABA. However, it cannot be excluded that ABCG40 might also transport Pb directly, since some ABC transporters can transport diverse substrates of different structures (Kang et al. 2011).

Iron deficiency is often paralleled by the upregulation of genes of the phenylpropanoid pathway and release of phenolics from the root to the soil (Schmid et al. 2014). Recently it has been shown that *Arabidopsis* plants that cannot excrete phenolics are more sensitive to iron starvation under conditions of low iron availability. Coexpression studies showed that AtABCG37 was strongly upregulated by iron deficiency (Fourcroy et al. 2013; Rodríguez-Celma et al. 2013) and it was therefore questioned whether this transporter exports phenolics that may complex and solubilize Fe. Indeed AtABCG37 mutants were impaired in their growth under iron limiting conditions (Figs. 1 and 2c). In a careful study Fourcroy et al. (2013) could show that AtABCG37 was required for the secretion of coumarin compounds and hence to supply iron to plants under conditions of sparse iron availability (Fig. 2d). Their role in root excretion is underlined by the observation that AtABCG37, as ABCG36 exhibits a polar localization on the distal part of the rhizodermis (Fig. 2e). However, ABCG37 was also reported to transport auxinic compounds, including the auxin precursor, IBA (Růžička et al. 2010; see chapter "IBA Transport by PDR Proteins").

5 ABC Transporters and Their Potential in Heavy Metal Phytoremediation

Heavy metal-contaminated soils pose a problem for food security in many parts of the world. On the one side, there is a natural presence or deposition of heavy metals in certain regions. On the other side, industrialization and mining have

contaminated large regions worldwide. In reality, the main contaminants are cadmium and arsenic. For over 20 years, scientists have tried to develop plants that can efficiently purify soils (Clemens et al. 2002; Verbruggen et al. 2009; Singh et al. 2011). Plants that can accumulate large amounts of heavy metals, so-called hyper-accumulators, have been known for a long time. However, these plants produce only very small biomass and are therefore not suited to purify soils (Krämer 2010). Hence, the goal of scientists is to either find plants that produce high biomass and that at the same time accumulate considerable amounts of heavy metals, or produce transgenic plants that display these features. Many approaches have been taken to either produce higher amounts of glutathione or phytochelatins in plants, or to express transporters that allocate more heavy metals either to the shoot or to the vacuole.

Since some ABC transporters have been shown to play an important role in heavy metal resistance: and accumulation, several approaches were employed to take advantage of their properties: Song et al. (2003) introduced the *Saccaromyces YCF1* into *Arabidopsis*. The authors could show that, as in yeast, YCF1 is targeted to the vacuolar membrane in *Arabidopsis*. Plants expressing YCF1 were more tolerant to cadmium and lead and accumulated significantly more of these heavy metals. Transport studies with isolated vacuoles revealed that, indeed, uptake of GS_2-Cd was strongly increased in the transgenic lines, demonstrating that this transport activity could be exploited to create more heavy metal accumulating plants.

In a subsequent work, Bhuiyan et al. (2011a) introduced *YCF1* in the partially cadmium-resistant crop plant *Brassica juncea*. As in *Arabidopsis* they could observe that the transgenic plants were slightly more tolerant to cadmium and lead and also accumulated up to twofold more cadmium compared to the control plants. *YCF1* has also been introduced into poplar, and transgenic poplar plants have been tested on soil contaminated with several heavy metals under greenhouse and field conditions (Shim et al. 2013). Poplar plants expressing YCF1 were significantly more tolerant to the contaminated soil compared to control plants, produced less necrotic spots, and accumulated up to three times more cadmium under greenhouse conditions. In the field, transgenic plants were still less chlorotic and produced a higher biomass, although the increase in cadmium content was less pronounced compared to greenhouse conditions (Fig. 2f). The YCF1-expressing poplar trees are unlikely to be useful for removing heavy metals from the contaminated soil rapidly and efficiently, due to the low bio-concentration values. Nevertheless, these plants may prove valuable in long-term stabilization of polluted soils such as can be found around mining sites. Transgenic poplar trees could establish larger and more ramified root systems, which can be expected to bind and fortify the soil, thus slowing down erosion and spreading of pollutants. Moreover, the transgenic trees tolerated and accumulated multiple heavy metals and metalloids, such as As, Zn, and Pb and can therefore better survive heterogeneous contaminations as can be found on closed-mine sites than the corresponding control plants. Besides phytostabilization, the trees might actually be able to perform phytoextraction of the heavy metals from the polluted sites if allowed to grow during their whole life

span of more than 30 years. Taking trees has also the advantage that they do not require much maintenance.

As mentioned above, parallel overexpression of AtABCC1/AtABCC2 and PCS results in plants with an increased resistance to arsenic (Song et al. 2010). However, also here the effect is marginal. As already discussed above, overexpression of AtATM3 in *Arabidopsis* led to plants exhibiting a higher cadmium tolerance (Kim et al. 2007). Similar results were obtained when AtATM3 was overexpressed in *Brassica juncea* (Bhuiyan et al. 2011b). Growth of the transgenic plants was less impaired in the presence of cadmium and lead, and a transgenic plant accumulated approximately two times more cadmium. This result is surprising, since it contrasts results from ATM3-overexpressing Arabidopsis plants; they contained less cadmium, while T-DNA mutants or RNAi lines contained more when compared to control plants. However, AtATM3-overexpressing *Brassica* displayed an upregulation of genes involved in glutathione synthesis as well as of genes of some heavy metal transporters. Therefore, the authors hypothesized that the higher cadmium contents in AMT3-overexpressing *Brassica* were due to a modulation of the glutathione-synthesis and heavy metal transporter genes. In fact, to date, we have no knowledge about the exact transport mechanism and substrate specificity of AtATM3 and the use of this ABC transporter in phytoremediation approaches may probably be premature.

In conclusion, overexpression of YCF1 and AtATM3 increases cadmium and lead tolerance and accumulation. AtABCC1/2 has been shown to increase Cd tolerance and accumulation (Song et al. 2010; Park et al. 2012). However, the degree of accumulation is far too small for these plants to be suitable for fast and efficient phytoextraction. For efficient phytoremediation, different strategies have to be combined, where mobilization of heavy metals from the soil, uptake, allocation to the areal part and finally chelating and deposition into the vacuole can be efficiently achieved.

Acknowledgments The work performed in the authors laboratories was supported by The Global Research Laboratory Program of the Ministry of Science, Korea, the Swiss National Foundation and the Ministry of Education, Sports, Culture, Science and Technology of Japan.

References

Bätz U, Martinoia E (2014) Root exudates: the hidden part of plant defense. Trends Plant Sci 19:90–98

Bernard DG, Cheng Y, Zhao Y, Balk J (2009) An allelic mutant series of ATM3 reveals its key role in the biogenesis of cytosolic iron-sulfur proteins in Arabidopsis. Plant Physiol 151:590–602

Bhuiyan MSU, Min SR, Jeong WJ, Sultana S, Choi KS, Lee Y, Liu JR (2011a) Overexpression of AtATM3 in Brassica juncea confers enhanced heavy metal tolerance and accumulation. Plant Cell Tiss Org Cult 107(1):69–77

Bhuiyan MSU, Min SR, Jeong WJ, Sultana S, Choi KS, Song WY, Lee Y, Lim YP, Liu JR (2011b) Overexpression of a yeast cadmium factor 1 (YCF1) enhances heavy metal tolerance and accumulation in Brassica juncea. Plant Cell Tiss Org Cult 105:85–91

Bovet L, Feller U, Martinoia E (2005) Possible involvement of plant ABC transporters in cadmium detoxification: a cDNA sub-microarray approach. Environ Int 31:263–267

Burkhead JL, Reynolds KA, Abdel-Ghany SE, Cohu CM, Pilon M (2009) Copper homeostasis. New Phytol 182:799–816

Chen S, Sánchez-Fernández R, Lyver ER, Dancis A, Rea PA (2007) Functional characterization of AtATM1, AtATM2, and AtATM3, a subfamily of Arabidopsis half-molecule ATP-binding cassette transporters implicated in iron homeostasis. J Biol Chem 282:21561–21571

Clemens S (2006) Evolution and function of phytochelatin synthases. J Plant Physiol 163:319–332

Clemens S, Palmgren MG, Kramer UA (2002) Long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7:309–315

Cobbett CS (2000) Phytochelatin biosynthesis and function in heavy-metal detoxification. Curr Opin Plant Biol 3:211–216

Delhaize E, Ryan PR, Hebb DM, Yamamoto Y, Sasaki T, Matsumoto H (2004) Engineering high-level aluminum tolerance in barley with the ALMT1 gene. Proc Natl Acad Sci USA 101:15249–15254

Fourcroy P, Sisó-Terraza P, Sudre D, Savirón M, Reyt G, Gaymard F, Abadía A, Abadia J, Alvarez-Fernández A, Briat JF (2013) Involvement of the ABCG37 transporter in secretion of scopoletin and derivatives by Arabidopsis roots in response to iron deficiency. New Phytol 201:155–167

Gaillard S, Jacquet H, Vavasseur A, Leonhardt N, Forestier C (2008) AtMRP6/AtABCC6, an ATP-binding cassette transporter gene expressed during early steps of seedling development and up-regulated by cadmium in *Arabidopsis thaliana*. BMC Plant Biol 8:22–32

Ghosh M, Shen J, Rosen BP (1999) Pathways of As(III) detoxification in Saccharomyces cerevisae. Proc Natl Acad Sci USA 96:5001–5006

Grill E, Loffler S, Winnacker EL, Zenk MH (1989) Phytochelatins, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific gamma-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase). Proc Natl Acad Sci U S A 86:6838–6842

Hanikenne M, Motte P, Wu MCS, Wang T, Loppes R, Matagne RF (2005) A mitochondrial half-size ABC transporter is involved in cadmium tolerance in Chlamydomonas reinhardtii. Plant Cell Environ 28:863–873

Huang CF, Yamaji N, Mitani N, Yano M, Nagamura Y, Ma JF (2009) A bacterial-type ABC transporter is involved in aluminum tolerance in rice. Plant Cell 21:655–667

Huang CF, Yamaji N, Ma JF (2010) Knockout of a bacterial-type ABC transporter gene, *AtSTAR1*, results in increased Al sensitivity in Arabidopsis. Plant Physiol 153:1669–1677

Huang CF, Yamaji N, Chen Z, Ma JF (2012) A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. Plant J 69:857–867

Hussain D, Haydon MJ, Wang Y, Wong E, Sherson SM, Young J, Camakaris J, Harper JF, Cobbett CS (2004) P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in Arabidopsis. Plant Cell 16:1327–1339

Kang J, Hwang JU, Lee M, Kim YY, Assmann SM, Martinoia E, Lee Y (2010) PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc Natl Acad Sci U S A 107:2355–2360

Kang J, Park J, Choi H, Burla B, Kretzschmar T, Lee Y, Martinoia E (2011) Plant ABC transporters. The Arabidopsis book. BioOne 9:e0153

Kim DY, Bovet L, Kushnir S, Noh EW, Martinoia E, Lee Y (2006) AtATM3 is involved in heavy metal resistance in Arabidopsis. Plant Physiol 140:922–932

Kim DY, Bovet L, Maeshima M, Martinoia E, Lee Y (2007) The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J 50:207–218

Kobae Y, Sekino T, Yoshioka H, Nakagawa T, Martinoia E, Maeshima M (2006) Loss of AtPDR8, a plasma membrane ABC transporter of *Arabidopsis thaliana*, causes hypersensitive cell death upon pathogen infection. Plant Cell Physiol 47:309–318

Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517–534

Kushnir S, Babiychuk E, Storozhenko S, Davey MW, Papenbrock J, De Rycke R, Engler G, Stephan UW, Lange H, Kispal G (2001) A mutation of the mitochondrial ABC transporter Sta1 leads to dwarfism and chlorosis in the Arabidopsis mutant starik. Plant Cell 13:89–100

Larsen PB, Cancel J, Rounds M, Ochoa V (2007) Arabidopsis ALS1 encodes a root tip and stele localized half type ABC transporter required for root growth in an aluminum toxic environment. Planta 225:1447–1458

Lee M, Lee K, Lee J, Noh EW, Lee Y (2005) AtPDR12 contributes to lead resistance in Arabidopsis. Plant Physiol 138:827–836

Leighton J, Schatz G (1995) An ABC transporter in the mitochondrial inner membrane is required for normal growth of yeast. EMBO J 14:188–195

Li ZS, Lu YP, Zhen RG, Szczypka M, Thiele DJ, Rea PA (1997) A new pathway for vacuolar cadmium sequestration in *Saccharomyces cerevisiae*: YCF1-catalyzed transport of bis (glutathionato)cadmium. Proc Natl Acad Sci U S A 94:42–47

Magalhaes JV, Liu J, Guimaraes CT, Lana UGP, Alves VMC, Wang YH, Schaffert RE, Hoekenga OA, Pineros MA, Shaff JE (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat Genet 39:1156–1161

Martinoia E, Maeshima M, Neuhaus HE (2007) Vacuolar transporters and their essential role in plant metabolism. J Exp Bot 58:83–102

Martinoia E, Meyer S, DeAngeli A, Nagy R (2012) Vacuolar transporters in their physiological context. Annu Rev Plant Biol 63:183–214

Meharg AA, Rahman MM (2003) Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environ Sci Technol 37:229–234

Meyer S, De Angeli A, Fernie AR, Martinoia E (2010) Intra-and extra-cellular excretion of carboxylates. Trends Plant Sci 15:40–47

Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P (2009) AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in Arabidopsis. Plant Physiol 149: 894–904

Morrissey J, Baxter IR, Lee J, Li L, Lahner B, Grotz N, Kaplan J, Salt D, Guerinot ML (2009) The Ferroportin metal efflux proteins function in iron and cobalt homeostasis in Arabidopsis. Plant Cell 21:3326–3338

Napolitano M, Rubio MA, Santamaria-Gomez J, Olmedo-Verd E, Robinson NJ, Luque I (2012) Characterization of the response to zinc deficiency in the Cyanobacterium Anabaena sp strain PCC 7120. J Bacteriol 194:2426–2436

Ortiz DF, Kreppel L, Speiser DM, Scheel G, McDonald G, Ow DW (1992) Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter. EMBO J 11:3491–3499

Ortiz DF, Ruscitti T, McCue KF, Ow DW (1995) Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. J Biol Chem 270:4721–4728

Palmer CM, Guerinot ML (2009) Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nat Chem Biol 5:333–340

Park J, Song WY, Ko D, Eom Y, Hansen TH, Schiller M, Lee TG, Martinoia E, Lee Y (2012) The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. Plant J 69:278–288

Preveral S, Gayet L, Moldes C, Hoffmann J, Mounicou S, Gruet A, Reynaud F, Lobinski R, Verbavatz JM, Vavasseur A, Forestier C (2009) A common highly conserved cadmium detoxification mechanism from bacteria to humans: heavy metal tolerance conferred by the ATP-binding cassette (ABC) transporter SpHMT1 requires glutathione but not metal-chelating phytochelatin peptides. J Biol Chem 284:4936–4943

Rea PA (2007) Plant ATP-binding cassette transporters. Annu Rev Plant Biol 58:347–375

Rodríguez-Celma J, Lin WD, Fu GM, Abadía J, López-Millán AF, Schmidt W (2013) Mutually exclusive alterations in secondary metabolism are critical for the uptake of insoluble iron compounds by Arabidopsis and Medicago truncatula. Plant Physiol 162:1473–1485

Růžička K, Strader LC, Bailly A, Yang H, Blakeslee J, Łangowski L, Nejedlá E, Fujita H, Ito H, Syōno K, Hejátko J, Gray WM, Martinoia E, Geisler M, Bartel B, Murphy A, Friml J (2010) Arabidopsis PIS1 encodes the ABCG37 transporter of auxinic compounds including the auxin precursor indole-3-butyric acid. Proc Natl Acad Sci USA 107:10749–10753

Ryan P, Tyerman S, Sasaki T, Furuichi T, Yamamoto Y, Zhang W, Delhaize E (2011) The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. J Exp Bot 62:9–20

Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301

Schmid NB, Giehl RN, Döll S, Mock H-P, Strehmel N, Scheel D, Kong X, Hider RC, von Wirén N (2014) Feruloyl-CoA 6′-Hydroxylase1-dependent coumarins mediate iron acquisition from alkaline substrates in Arabidopsis. Plant Physiol 164:160–172

Schwartz MS, Benci JL, Selote DS, Sharma AK, Chen AGY, Dang H, Fares H, Vatamaniuk OK (2010) Detoxification of multiple heavy metals by a half-molecule ABC transporter, HMT-1, and coelomocytes of Caenorhabditis elegans. PLoS One 5:e9564

Self WT, Grunden AM, Hasona A, Shanmugam KT (2003) Molybdate transport. Res Microbiol 152:311–321

Shikanai T, Müller-Moulé P, Munekage Y, Niyogi KK, Pilon M (2003) PAA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. Plant Cell 15:1333–1346

Shim D, Kim S, Choi Y-I, Song W-Y, Park J, Youk ES, Jeong S-C, Martinoia E, Noh E-W, Lee Y (2013) Transgenic poplar trees expressing yeast cadmium factor 1 exhibit the characteristics necessary for the phytoremediation of mine tailing soil. Chemosphere 90:1478–1486

Singh BR, Gupta SK, Azaizeh H, Shilev S, Sudre D, Song W-Y, Martinoia E, Mench M (2011) Safety of food crops on land contaminated with trace elements. J Sci Food Agric 91:1349–1366

Song WY, Sohn EJ, Martinoia E, Lee YJ, Yang YY, Jasinski M, Forestier C, Hwang I, Lee Y (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat Biotechnol 21:914–919

Song WY, Park J, Mendoza-Cozatl D, Suter-Grotemeyer M, Shim D, Hortensteiner S, Geisler M, Weder B, Rea P, Rentsch D, Schroder J, Lee Y, Martinoia E (2010) Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. Proc Natl Acad Sci USA 107:21187–21192

Song WY, Mendoza-Cózatl DG, Lee Y, Schroeder JI, Ahn S-N, Lee H-S, Wicker T, Martinoia E (2014) Phytochelatin-metal(loid) transport into vacuoles shows different substrate preferences in Barley and Arabidopsis. Plant Cell Environ 37(5):1192–1201

Sooksa-Nguan T, Yakubov B, Kozlovskyy VI, Barkume CM, Howe KJ, Thannhauser TW, Rutzke MA, Hart JJ, Kochian LV, Rea PA (2009) Drosophila ABC transporter, DmHMT-1, confers tolerance to cadmium: DmHMT-1 and its yeast homolog, SpHMT-1, are not essential for vacuolar phytochelatin sequestration. J Biol Chem 284:354–362

Stein M, Dittgen J, Sánchez-Rodríguez C, Hou BH, Molina A, Schulze-Lefert P, Lipka V, Somerville S (2006) Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell 18:731–746

Szczypka MS, Wemmie JA, Moye-Rowley WS, Thiele DJ (1994) A yeast metal resistance protein similar to human cystic fibrosis transmembrane conductance regulator (CFTR) and multidrug resistance-associated protein. J Biol Chem 269:22853–22857

Tennstedt P, Peisker D, Böttcher C, Trampczynska A, Clemens S (2009) Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. Plant Physiol 149:938–948

Teschner J, Lachmann N, Schulze J, Geisler M, Selbach K, Santamaria-Araujo J, Balk J, Mendel RR, Bittner F (2010) A novel role for Arabidopsis mitochondrial ABC transporter ATM3 in molybdenum cofactor biosynthesis. Plant Cell 22:468–480

Tommasini R, Evers R, Vogt E, Mornet C, Zaman GJ, Schinkel AH, Borst P, Martinoia E (1996) The human multidrug resistance-associated protein functionally complements the yeast cadmium resistance factor 1. Proc Natl Acad Sci USA 93:6743–6748

Tommasini R, Vogt E, Fromenteau M, Hortensteiner S, Matile P, Amrhein N, Martinoia E (1998) An ABC-transporter of *Arabidopsis thaliana* has both glutathione-conjugate and chlorophyll catabolite transport activity. Plant J 13:773–780

Ueno D, Yamaji N, Kono I, Huang CF, Ando T, Yano M, Ma JF (2010) Gene limiting cadmium accumulation in rice. Proc Natl Acad Sci USA 107:16500–16505

Vatamaniuk OK, Bucher EA, Sundaram MV, Rea PA (2005) CeHMT-1, a putative phytochelatin transporter, is required for cadmium tolerance in *Caenorhabditis elegans*. J Biol Chem 280:23684–23690

Verbruggen N, Hermans C, Schat H (2009) Mechanisms to cope with arsenic or cadmium excess in plants. Curr Opin Plant Biol 12:364–372

Verret F, Gravot A, Auroy P, Leonhardt N, David P, Nussaume L, Vavasseur A, Richaud P (2004) Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. FEBS Lett 576:306–312

Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briat J, Curie C (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. Plant Cell 14:1223–1233

Wang TL, Wu M (2006) An ATP-binding cassette transporter related to yeast vacuolar ScYCF1 is important for Cd sequestration in Chlamydomonas reinhardtii. Plant Cell Environ 29:1901–1912

Williams PN et al (2005) Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environ Sci Technol 39:5531–5540

Zhao FJ, McGrath SP, Meharg AA (2010) Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. Annu Rev Plant Biol 61:535–559

Zhu J-K (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53: 247–273

Phytate Transport by MRPs

Francesca Sparvoli and Eleonora Cominelli

Abstract Phytic acid is the main storage form for phosphate in plant seeds. From a nutritional point of view it decreases the seed value by chelating important minerals, such as iron, zinc, magnesium, and calcium. Therefore, the isolation of low phytic acid (*lpa*) mutants is considered a highly desirable objective in the genetic improvement of the nutritional quality of grain crops. On the other side phytic acid is a very important signaling molecule involved in development and hormonal regulation. The only phytic acid transporters characterized so far are proteins of the ABCC type. Some *lpa* mutants affected in these transporters have been isolated and characterized in Arabidopsis and in crops. Here we review advances in the characterization of these proteins and on the corresponding mutants. Moreover we propose an explanation on how mutations in these transporters may affect different aspects of cellular metabolism not only strictly related to phytic acid biosynthesis.

1 Phytic Acid

Phytic acid (*myo*-inositol-1,2,3,4,5,5-hexa*kis*phosphate; $InsP_6$) is a ubiquitous component of eukaryotic cells. In plants it is the most abundant form in which phosphorus is accumulated in seeds (up to 85 % of total phosphorus) and other plant tissues and organs such as pollen, roots, tubers, and turions. During germination, phytase enzymes remobilize the phosphorus stored as $InsP_6$ to support seedling growth (Raboy 2003). Due to its chemical structure (highly negatively charged at physiological pH), $InsP_6$ easily precipitates in the form of phytate salts binding important mineral cations such as iron, zinc, potassium, calcium, and magnesium.

F. Sparvoli (✉) • E. Cominelli
Institute of Agricultural Biology and Biotechnology, CNR, Milan, Italy
e-mail: sparvoli@ibba.cnr.it; cominelli@ibba.cnr.it

M. Geisler (ed.), *Plant ABC Transporters*, Signaling and Communication in Plants 22,
DOI 10.1007/978-3-319-06511-3_2, © Springer International Publishing Switzerland 2014

Since monogastric animals and humans lack phytases in their digestive tract, $InsP_6$ is poorly digested and decreases the nutritional value of the seeds by limiting phosphorus and mineral bioavailability. As a consequence, high amounts of undigested phytates are released with the animal waste into the environment, thus accentuating the phosphorus pollution from agriculture, whereas poor mineral bioavailability is ascribed as one of the most important causes of mineral deficiencies (mainly iron and zinc) in those populations whose diet is largely based on staple crops (Raboy 2001).

To improve the nutritional value of seeds, plant breeders have spent many efforts to isolate and develop *low phytic acid* (*lpa*) crops and a number of such mutants, in which a 45–90 % reduction of phytic acid was achieved, have been obtained (Raboy 2009). A consistent number of these *lpa* mutants bears mutations in a gene coding for an ABCC-type transporter, orthologous to the Arabidopsis *AtMRP5* gene which recently was demonstrated to code for a high-affinity $InsP_6$ ATP-binding cassette transporter (Shi et al. 2007; Xu et al. 2009; Nagy et al. 2009; Gillman et al. 2009; Panzeri et al. 2011). Interestingly a screen for *lpa* mutations of a maize EMS mutagenized seed population revealed that the *ZmMRP4* locus, coding for such a transporter, is highly mutable with a rate nearly an order of magnitude greater than a typical rate (Raboy 2009) and that 10 % of the mutations in this locus were lethal (Raboy et al. 2001). Similar phenomenon may explain in part the high rate of mutation observed for this locus in different crops.

2 Structure, Expression, and Subcellular Localization of MRP Type ABC Phytic Acid Transporters

Phytic acid transporters are multidrug resistance-associated proteins (MRPs), belonging to the ABCC cluster of plant ABC transporters (Verrier et al. 2008). They are full-length ABC transporters containing the classical two membrane-spanning domains (TMD1 and TMD2, each containing six transmembrane α-helices), which constitute the membrane- spanning pore, tandemly arranged with two cytosolic domains, referred as nucleotide-binding domains (NBD1 and NBD2). These last contain the Walker A and B motifs together with the characteristic ABC signature. The domain arrangement is in the so-called forward orientation: TMD1-NBD1_TMD2-NBD2 (Fig. 1). $InsP_6$ transporters, as other members of the ABCC cluster, contain an additional extremely hydrophobic N-terminal extension (TMD0, containing five transmembrane α-helices), which is connected to the rest of the protein via a cytosolic loop (CL3), rich in charged amino acids. The role of this N-terminal TMD0 in plants is unknown; however, studies on some human and yeast ABCC type transporters suggest it might be important for appropriate protein trafficking and targeting (Mason and Michaelis 2002; Westlake et al. 2005; Bandler et al. 2008). As the cytosolic loop CL3 is concerned, it has been shown to play an important role in substrate recognition and transport (Gao et al. 1998).

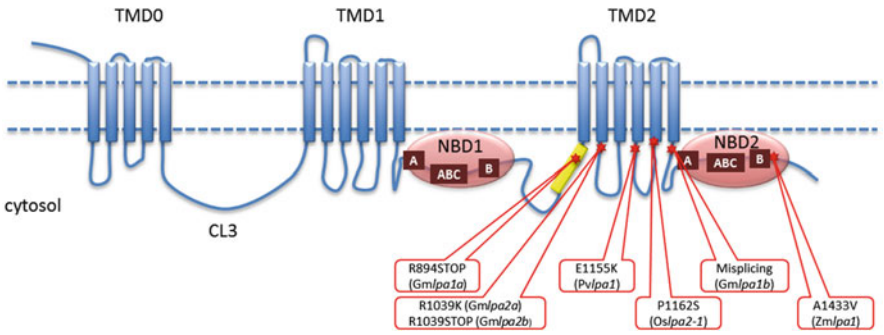

Fig. 1 Schematic representation of the structure of the MRP-type ABCC phytic acid transporter. The different domains are as follows: *red*, NBDs 1 and 2; *blue*, TMDs 0, 1 and 2; CL3, cytosolic loop domain; *dark red squares*, Walker A and B domains (A, B) and ABC signature (ABC); *yellow rectangle*, conserved stretch of lysine residues; *red stars*, position of the known mutations reported for *lpa*-MRP-type ABCC phytic acid mutants

The first evidence that an MRP-type ABC transporter was required for $InsP_6$ transport and accumulation in seed was provided by Shi and coworkers (Shi et al. 2007), using the maize insertional *lpa1* mutants in the *ZmMRP4* gene, while the biochemical demonstration was given by Nagy and coworkers using the Arabidopsis homolog AtMRP5 (also referred to as AtABCC5) (Nagy et al. 2009). They performed [^{33}P] $InsP_6$ transport uptake studies on microsomes obtained from a *ycf1* yeast mutant (a model system commonly used to investigate the function of ABC transporters) carrying the AtMRP5 transporter. Phytic acid transport was dependent on the presence of AtMRP5 and strictly dependent on the addition of ATP. The kinetics analysis of the transport showed V_{max} values of about 1.6–2.5 μmol min^{-1} mg^{-1} and a K_m ranging between 263 and 310 nm, indicating that AtMRP5 has a very high affinity for phytic acid. Further confirmation of the ATP-dependent activity of the transporter was provided showing that disruption by site directed mutagenesis of specific residues in the Walker B domain of NBD1 and NBD2 reproduced the *mrp5* phenotype (Nagy et al. 2009).

At present, it is not known which amino acid residues are specifically involved in phytic acid transport. In fact, the amino acids compromised in known *lpa* mutants (red stars in Fig. 1) are conserved also in other ABCC proteins, indicating they should be involved in general proper functioning of ABCC transporters. This is clearly the case of the mutation A1433V found in the maize *lpa1-1*, which is located in the putative D-loop sequence (ASVD), adjacent to the Walker B motif (ILVLD), of the NBD2 domain (ILVLDEATA$_{1433}$SVD).

A detailed analysis of the multiple alignment of the amino acid sequence of MRP-type $InsP_6$ transporters associated with *lpa* mutants, compared to those of other Arabidopsis ABCCs, put in evidence some peculiarities, the most significant being a very conserved stretch of lysine residues (consensus K/RXIKEKKKX$_{4\text{-}5}$R/KKK, yellow rectangle in Fig. 1), located in the cytosolic loop linking NBD1 to TMD2. Moreover, a number of charged amino acid residues (mostly Lys and Arg)

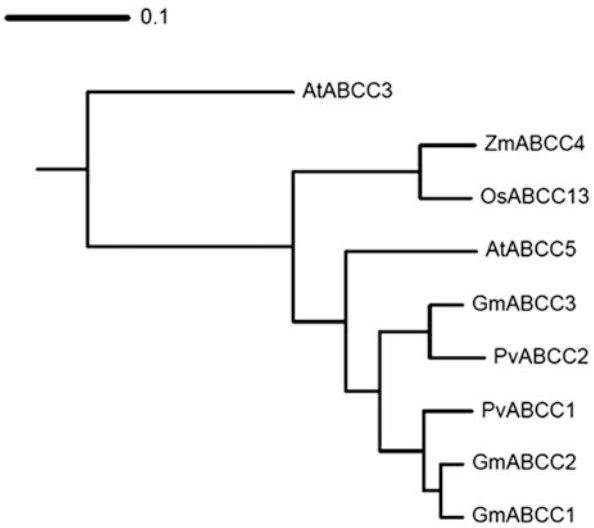

Fig. 2 Phylogenetic relationships among known MRP-type ABC phytic acid transporters associated to *lpa* mutations. The tree has been built comparing amino acid sequences of AtABCC5 (AtMRP5, Q7GB25); OsABCC13 (OsMRP5, NP_001048934); ZmABCC4 (ZmMPR4, ABS81429); GmABCC1 (GmMRP1, XP_003521316); GmABCC2 (GmMRP2 XP_003554305), GmABCC3 (GmMRP3, XP_003541373); PvABCC1 (PvMRP1, CBX25010); PvABCC2 (PvMRP2, CBX25011). The AtABCC3 (AtMRP3, Q9LK64) sequence not coding for a phytic acid transporter has been used as outgroup

are found in other conserved stretches located in TMD1 and TMD2 domains that might play a role in phytic acid transport.

Phylogenetic analyses of MRP type transporters indicate that $InsP_6$ transporters are represented by single copy genes (Klein et al. 2006; Wanke and Kolukisaoglu 2010; Kang et al. 2011); however it has been recently shown that soybean and common bean, two closely related legume species, bear a paralog copy (PvABCC2/PvMRP2 and GmABCC3/GmMRP3 in Fig. 2) (Panzeri et al. 2011).

Transgenic plants harboring the *GUS* reporter gene under the control of the *AtMRP5* promoter showed GUS staining mainly in vascular tissues of cotyledons, leaves (with the exception of xylem cells), roots (in the central cylinder, not in the root cortex and in the root tip) and anthers, in epidermal cells, particularly in guard cells and at the silique attachment site of the pedicel (Gaedeke et al. 2001). In the paper from Gaedeke and colleagues (2001) no data are available concerning GUS activity in the seeds. However from publicly available microarray data, it is clear that *AtMRP5* is expressed at different stages of seed development, particularly, from the pre-globular to the linear cotyledon stage it is mainly expressed at the chalazal seed coat, while at the maturation green stage it is very highly expressed in the entire seed, particularly in the embryo (http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi, Winter et al. 2007). Analysis of *AtMRP5* orthologs revealed a