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Plant ABC Transporters

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ABC transporters constitute one of the largest protein families found in all living organisms. ABC transporters are driven by ATP hydrolysis and can act as exporters as well as importers. The plant genome encodes for more than 100 ABC transporters, largely exceeding that of other organisms. In Arabidopsis, only 22 out of 130 have been functionally analyzed. They are localized in most membranes of a plant cell such as the plasma membrane, the tonoplast, chloroplasts, mitochondria and peroxisomes and fulfill a multitude of functions. Originally identified as transporters involved in detoxification processes, they have later been shown to be required for organ growth, plant nutrition, plant development, response to abiotic stresses, pathogen resistance and the interaction of the plant with its environment. To fulfill these roles they exhibit different substrate specifies by e.g. depositing surface lipids, accumulating phytate in seeds, and transporting the phytohormones auxin and abscisic acid. The aim of this review is to give an insight into the functions of plant ABC transporters and to show their importance for plant development and survival.

I. INTRODUCTION

ABC transporters constitute one of the largest protein families and are present in organisms ranging from bacteria to humans (Henikoff et al., 1997). In most cases, functional ABC transporters act as ATP-driven pumps and consist of two transmembrane domains (TMD) hydrophobic domains, which constitute the membrane-spanning pore, and two cytosolic domains, which are referred to as the nucleotide-binding domains (NBD) or nucleotide-binding folds (NBF), as they contain the ATP-binding Walker A and B motifs (Martinoia et al., 2002).

In bacteria, ABC transporters catalyze the import of many primary metabolites, such as maltose, polyols, and histidine. They also export antibiotics, lipids, and proteins, such as proteases, lipases and the RTX (repeat in toxin) cytotoxin of *Vibrio cholera*. Initially, eukaryotic ABC transporters were thought to be involved exclusively in the extrusion of compounds from the cytosol, as they were originally identified as transporters that are involved in cellular detoxification. Later studies showed that ABC transporters are present in most cell membranes. Until recently, it was assumed that eukaryotic ABC proteins transport the substrate present at the side of the NBD to the other side of a membrane. However, recent findings show that at least plant ABC transporters can also act in the opposite direction. The first demonstration was provided by Yazaki and colleagues (Shitan et al., 2003). They showed that the benzylisoquinoline alkaloid berberine, which is synthesized in roots, is taken up in the rhizome by an ABCB-type transporter.

In plants, ABC proteins were originally identified as transporters involved in the final detoxification process, i.e., vacuolar deposition (Martinoia et al., 1993). Since this finding, numerous reports have shown that the functions of this class of transporters extend far beyond detoxification. ABC transporters have frequently been shown to be involved in such diverse processes as pathogen response, surface lipid deposition, phytate accumulation in seeds, and transport of the phytohormones auxin and abscisic acid. Therefore, ABC transporters play an important role in organ growth, plant nutrition, plant development, response to abiotic stress, and the interaction of the plant with its environment.

In this review, we provide an overview of the transport functions that ABC proteins fulfill in plants, focusing primarily on knowledge gained from studies in Arabidopsis, but also including a few, well-established examples of work carried out in other plant species. In Figure 1, we present the Arabidopsis ABC transporters that have been characterized to date. Soluble ABC proteins not involved in actual transport, such as the Suf complex (Fontecave et al., 2005) or the mitochondrial AtCCMs (Rayapuram et al., 2007),

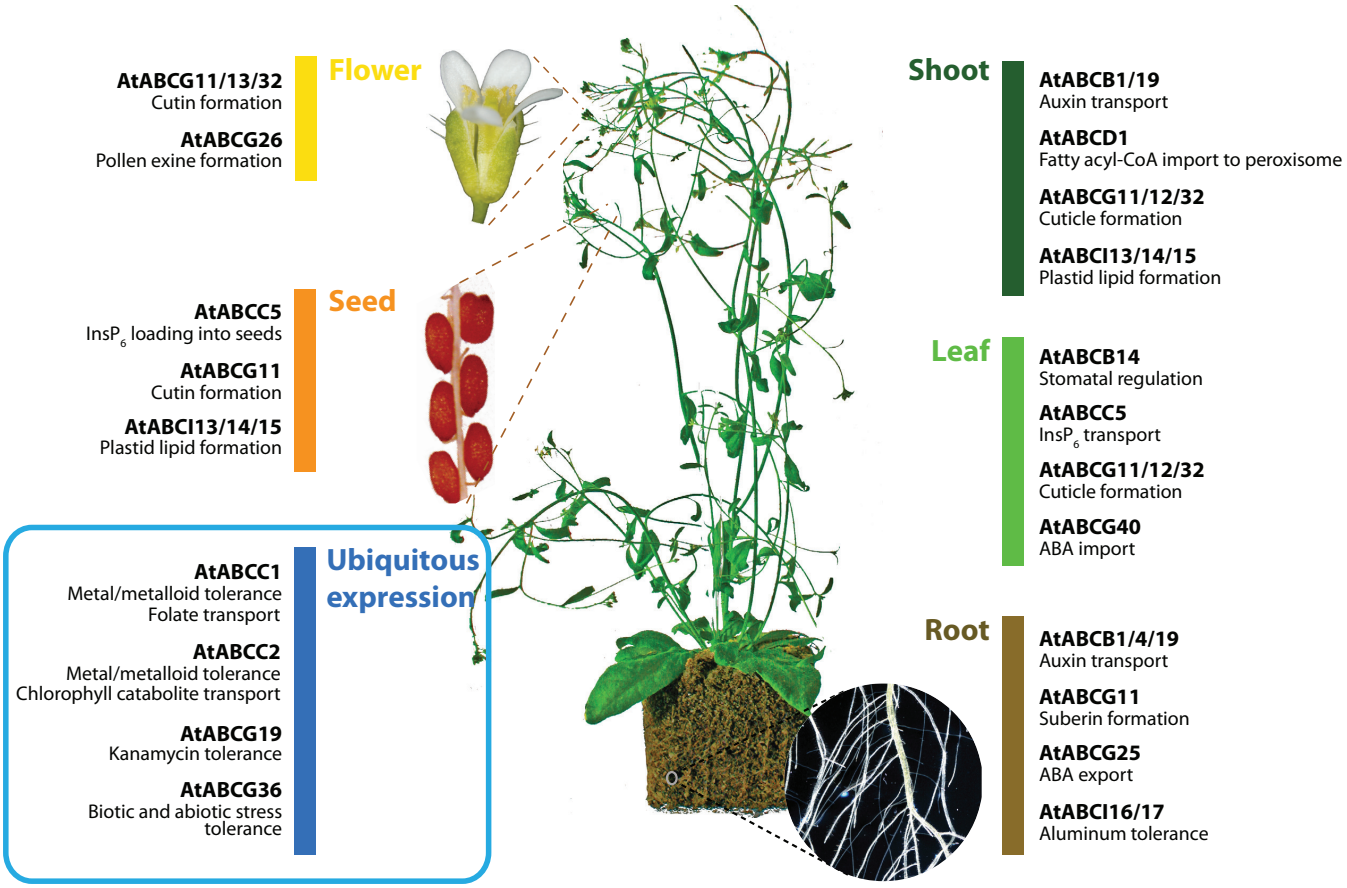

Figure 1. Overview of the Arabidopsis ABC transporters characterized to date.

ABC transporters whose functions and/or substrates have been reported are listed according to their tissue of action. Detailed information for each gene is described in the corresponding chapters. Figure modified from Kretzschmar et al. (2011).

will not be addressed here. Further information about this subset of proteins can be found in recent reviews by Rea (2007) and Yazaki et al. (2009).

II. STRUCTURAL AND PHYLOGENETIC RELATIONSHIPS OF ABC PROTEINS

Based on their domain structure and phylogenetic relationships the Arabidopsis ABC proteins are currently classified into eight subfamilies (Figure 2) in accordance with the nomenclature system for animal ABC proteins (Verrier et al., 2008). A common feature of ABC proteins is the presence of an ABC signature containing the amino acid sequence [LIVMFY]S[SG]GX3[RKA][LIVMYA]X[LIVFM][AG] as a consensus (Rea, 2007). This consensus sequence is the general case, but several exceptions have been reported (Rea, 2007).

Membrane-bound ABC proteins consist of four major subunits, two transmembrane domains (TMD) and two nucleotide-binding domains (NBD) (Higgins, 1992), which cooperate during ATP hydrolysis to drive active transport. These subunits are either encoded by individual genes (ABCI subfamily), by two genes each encoding one NBD and one TMD (half-size ABCs) that form heterodimers; by one gene encoding for one NBD and one TMD (half-size) that form homodimers or by a single gene (full-size ABCs). The subunits of ABCA to ABCD proteins have a so-called forward TMD-NBD domain organization, while those of the ABCG subfamily are characterized by reverse NBD-TMD organization. The soluble ABCE and ABCF subfamily of proteins consists only of two NBDs, whereas the ABCI subfamily comprises various genes that encode only one single domain, i.e. NBD, TMD or accessory domains. Some of these individual domains encoded by ABCIs have been shown to assemble into multi-subunit ABC transporters, in a manner similar to ABC proteins formed in prokaryotes (Verrier et al., 2008).

Within the ABCA subfamily, AtABCA1 (AOH according to the previous nomenclature) appears to be orthologous to mammalian ABC1, which is a full-size ABC transporter that harbors a large linker domain and represents the largest Arabidopsis ABC protein (Verrier et al., 2008). All of the remaining 11 Arabidopsis ABCA

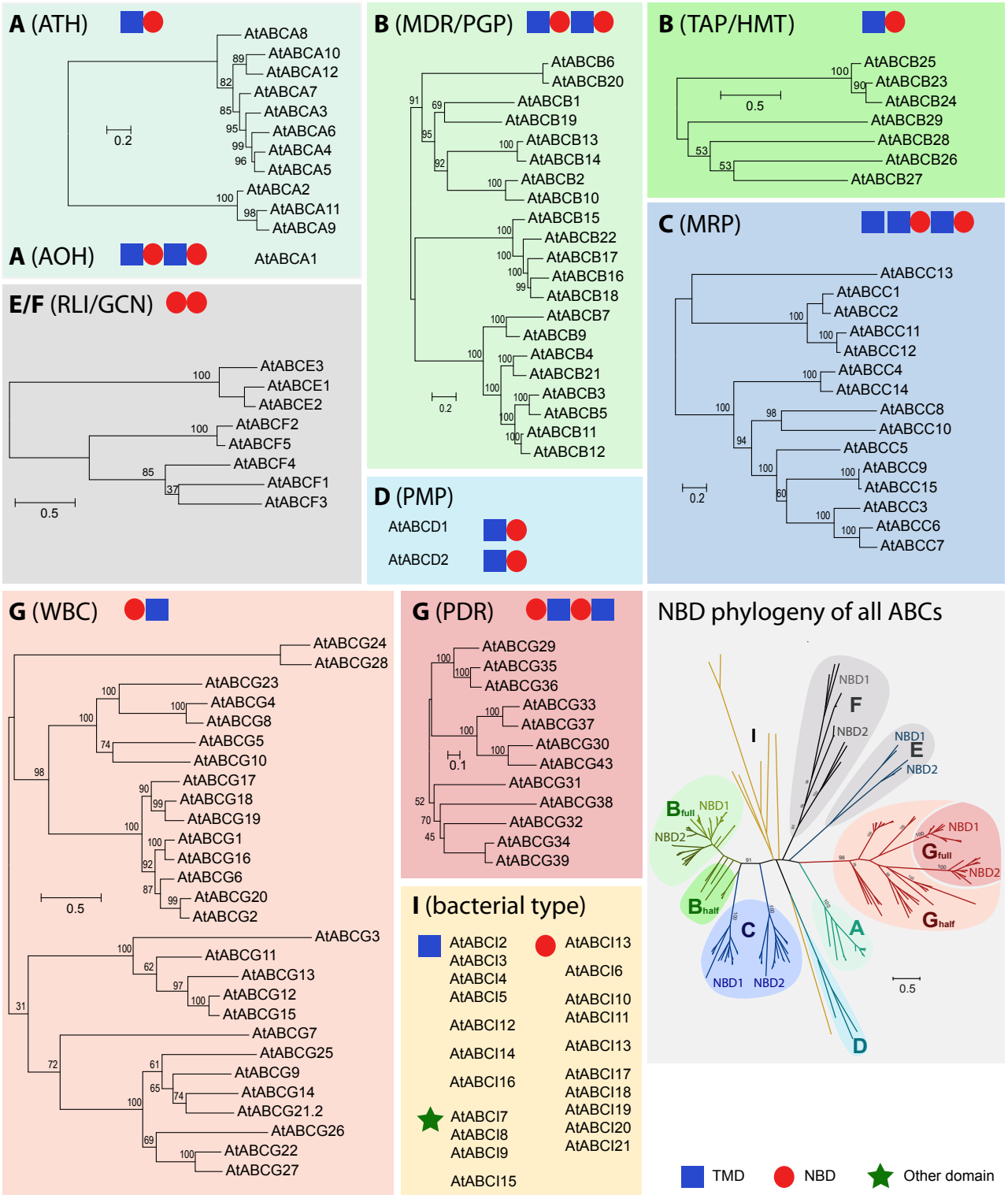

Figure 2. *Arabidopsis thaliana* ATP-binding cassette (ABC) protein subfamilies, with their maximum likelihood phylogenies, and a phylogeny of the NBDs of all Arabidopsis ABC proteins.

For the ABCI subfamily, only the encoded domain is indicated, as their divergence is too large to resolve their phylogenetic relationships. In the NBD phylogeny, both NBDs of full-size ABCs were included. NBDs of full-size ABCB show little divergence compared to NBDs of ABCCs and full-size ABCGs. Phylogenies were estimated using PhyML3.0 (Guindon et al., 2010) and the model LG+G4+I+F from a truncated protein sequence alignment generated with MUSCLE 3.8 (http://www.drive5.com/muscle). Gaps of more than 80% were removed (Capella-Gutiérrez et al., 2009). Branch support values correspond to non-parametric bootstrap values from 100 replicates. Domain organizations are indicated by colored symbols (key, bottom right).

subfamily proteins (ATH) are of the half-size type and lack the large domain found in ABCA1. Half-size ABCA proteins have only been identified in plants and prokaryotes (Peelman et al., 2003; Kovalchuk and Driessen, 2010). Further studies will be required to identify the subcellular localization of ABCA proteins.

The Arabidopsis genome encodes 21 full-size (Pgp/MDR) and seven half-size ABCBs, and has significantly more full-size ABCBs than humans, which contain only three (Vasiliou et al., 2009). So far, all characterized Arabidopsis full-size ABCB proteins have been shown to be localized at the plasma membrane (Blakeslee et al., 2007; Rea, 2007; Lee et al., 2008). Three half-size members of the ABCB subfamily (also called ATM) are localized to the mitochondria (Rea, 2007). Proteomic data indicate that AtABCB26 (TAP1) is localized to the chloroplast (Ferro et al., 2010), while AtABCB27 (TAP2) was detected in the vacuolar membrane (Jaquinod et al., 2007). The subcellular localization of the other half-size ABCBs still needs to be investigated.

The Arabidopsis ABCC protein subfamily consists only of full-size ABC proteins. Alignments and hydrophobicity profiles of TAIR10 gene models indicate that all ABCCs harbor the ABCC-specific additional N-terminal transmembrane domain (TMD0) (Tusnády et al., 2006; Klein et al., 2006). The function of TMD0 in plants is unknown; however, for certain human and yeast ABCCs this domain has been shown to be involved in protein targeting (Mason and Michaelis, 2002; Westlake et al., 2005). Most ABCC proteins localize to the vacuolar membrane (Rea, 2007; Nagy et al., 2009), and these are the only full-size ABC proteins found in the Arabidopsis tonoplast so far.

The ABCD subfamily is represented by one half-size and one full-size member, of which the latter, AtABCD1, has been shown to be located in peroxisomes (Hayashi et al., 2002).

Proteins of the Arabidopsis ABCE and ABCF subfamilies, which consist of three and five members, respectively, are thought to be soluble since they lack any detectable transmembrane domain. They probably function in processes other than transport, as is the case for their yeast and human orthologs, which participate in ribosome recycling and translational control (Vazquez de Aldana et al., 1995; Tyzack et al., 2000; Braz et al., 2004; Dong et al., 2004; Pisarev et al., 2010).

The largest ABC subfamily is ABCG, which contains 28 half-size (WBC) and 15 full-size (PDR) proteins in Arabidopsis (Verrier et al., 2008). All of them feature the characteristic, so-called, reverse organization of domains in the subunits (NBD-TMD). Genes encoding full-size ABCG proteins have only been identified in plants, fungi, oomycetes, brown algae and slime molds (Anjard and Loomis, 2002; Tyler et al., 2006; Cock et al., 2010). All characterized full-size and half-size ABCGs, except AtABCG19, localized to the plasma membrane (Lee et al., 2005; Stein et al., 2006; McFarlane et al., 2010; Kang et al., 2010b; Kuromori et al., 2010; Růžička et al., 2010; Choi et al., 2011).

The ABCH subfamily, which contains half-size transporters with a reverse domain organization, is phylogenetically unrelated to subfamily G and has not been identified in plants (Verrier et al., 2008).

Many of the 21 ABCI subfamily members are predicted to target to the chloroplast or mitochondria, and two are encoded by the mitochondrial genome (Verrier et al., 2008; Shimoni-Shor et al., 2010). ABCI19, 20 and 21, all of which encode for individual NBDs, have been shown to translate into cytosolic proteins (Marin et al., 2006) and form a distinct clade that roots to the center of the phylogenetic tree (Figure 2). The other ABCI members that encode for single NBDs also root to the center of the tree but appear to be unrelated to each other. ABCI members encoding other domains such as TMD or substrate binding domains were not included in the analysis.

In the phylogeny of NBDs from ABC genes, the sequences of two NBDs of full-size ABCB members appear to be more closely related to each other than NBDs of ABCC and full-size ABCG members (Figure 2).

III. ABC TRANSPORTERS INVOLVED IN DETOXIFICATION AND TRANSPORT OF CONJUGATED COMPOUNDS

Plants are exposed to a large number of potentially toxic compounds, such as the by-products of internal metabolic processes, certain minerals within the soil, toxins produced by pathogens, and anthropogenic compounds, such as herbicides and industrial waste. Since plants are limited in their ability to avoid toxins, they have developed versatile strategies to detoxify potentially toxic compounds. Modification of toxic compounds and their subsequent transport are well-known steps for detoxification in all living cells. Interestingly, a similar set of detoxification enzymes is used by plants for both endogenously produced organic compounds that may become toxic when accumulated within the cytosol and xenobiotics taken up from the environment. As the first step of detoxification, members of the cytochrome P450 family may catalyze the oxidation of potentially toxic endogenous and exogenous compounds, which are subsequently conjugated to a hydrophilic molecule, such as glucose or glucuronide (Kreuz et al., 1996). This conjugation step renders the potentially toxic compounds more hydrophilic and prevents the newly formed compounds from crossing membranes by diffusion. Similarly, xenobiotics are frequently conjugated to glutathione. This step is catalyzed by various glutathione S transferases (GSTs) and generally occurs without prior modification by electrophile substitution (Rouhier et al., 2008). As the last step, compound-conjugates are transported into the large central vacuole or released into the apoplast, a process known as internal or external excretion, respectively (Ishikawa, 1992; Martinoia et al., 1993). This process further reduces the toxicity of the compounds. The observations that the vacuolar transport activity for glucosylated luteolin, which is transported by ABC-type kinetics, is strongly reduced in a *Hordeum vulgare* (barley) mutant that does not synthesize this compound (Frangne et al., 2002) and that transcription of ABC-type transporters is up-regulated in a similar manner to other detoxifying enzymes in plants treated with xenobiotics suggest that modification and transport are parts of the general detoxification pathway (Gaillard et al., 1994).

III.A. Internal Excretion

Early studies suggested that xenobiotics were deposited within the central vacuole of plants (Schmitt and Sandermann Jr, 1982; Sandermann Jr, 1992). Since plants have only been exposed to industrial anthropogenic compounds for a few centuries, the question has been raised of how vacuolar transporters could recognize

such molecules. A widely distributed mechanism of detoxification in most living organisms is to conjugate a potentially toxic organic compound with the tripeptide glutathione through its thiol group (Meister, 1983). Therefore, Martinoia et al. (1993) considered the possibility that vacuoles may take up xenobiotics in their glutathionated form (GS-X), and observed that these compounds indeed efficiently accumulated in isolated barley vacuoles but only in the presence of ATP. Furthermore, they demonstrated that GS-X transport was efficiently inhibited by vanadate, an inhibitor of ABC-mediated transport processes, but not by inhibitors of the vacuolar V-ATPase or by dissipation of the electrochemical gradient generated by the latter. These observations showed that GS-X transport is not energized by the proton motive force, but is strictly ATP-dependent and is thus likely mediated by an ABC-type transporter.

III.A.1. Identification of vacuolar ABC transporters and their substrates

Based on the sequence of the human HsABCC1 protein that catalyzes the transport of GS-X (Cole et al., 1992; Jedlitschky et al., 1994), a first Arabidopsis GS-X transporter, AtABCC1/AtMRP1, was identified (Lu et al., 1997). Comparison of the peptide composition of Arabidopsis ABCC1 with that of human ABCC1 revealed a sequence identity of 41.5%. Using yeast vesicles expressing AtABCC1, the authors showed that this transporter exhibited similar transport properties to those described for isolated Arabidopsis vacuoles. This discovery was followed by the isolation and functional analysis of other members of the plant ABCC subfamily, such as AtABCC2 and AtABCC3 (Lu et al., 1998; Tommasini et al., 1998). These early publications extended the postulated range of substrates of tonoplast-localized ABC transporters from substrates of their human homologs to physiologically relevant substrates, such as chlorophyll catabolites. Chlorophyll pigments are degraded during senescence and at least the first intermediates of chlorophyll catabolism can still absorb light and transfer electrons, which results in oxidative stress if they are not efficiently detoxified. In plants, most secondary products as well as modified xenobiotics are either glucosylated or glutathionated. Compounds are rarely glucuronated, but there are some exceptions, such as the flavonoids produced in *Secale cereale* (rye) (Klein et al., 2000).

Early biochemical studies also provided insight into the transport kinetics of vacuolar ABC transporters. AtABCC2 transports both glutathionated compounds and glucuronated compounds (Klein et al., 1998; Lu et al., 1998). The two substrates do not compete for the transporter when both are present in the transport solution, but rather exhibit a trans-activation, which leads to an increase in GS-X transport by glucuronides and an enhanced affinity for glucuronides in the presence of GS-X (Liu et al., 2001). This result suggests that, when both substrates are present, the binding pocket becomes subject to steric changes that cause altered transport characteristics.

III.A.2. Inorganic compounds—Heavy metals and metalloids

A focal point regarding the study of detoxification mechanisms in plants has been heavy metal and metalloid detoxification. Contamination of agricultural soils has become a serious problem. For instance, more than 50 million people are exposed to toxic concentrations of arsenic in Bangladesh since aquifers providing drinking and agricultural water are contaminated with arsenic (Zhao et al., 2010). Similar to the P-type ATPases, proton co-transporters and proton antiporters involved in the uptake (Clemens et al., 1998; Vert et al., 2002) and vacuolar deposition (Hirschi et al., 2000; Morel et al., 2009) of several heavy metals and metalloids, ABC transporters have long been associated with heavy metal and metalloid detoxification. In *Saccharomyces cerevisiae*, it has been shown that an ABC transporter, YCF1 (Yeast Cadmium Factor1), contributes to heavy metal and metalloid tolerance (Szczypka et al., 1994; Li et al., 1997). This vacuolar transporter detoxifies bis-glutathione heavy metal/metalloid complexes, such as GS_2-Cd and GS_2-As. A similar mode of action has been postulated for human MRP1/HsABCC1, which partially complements the Cd-sensitive yeast mutant *ycf1* (Tommasini et al., 1996). Overexpression of ScYCF1 in Arabidopsis resulted in plants that were more tolerant to cadmium (Song et al., 2003), suggesting that the capacity of tonoplastic transport is a limiting factor in cadmium tolerance in this plant. In plants, some fungi, and animals, detoxification of heavy metals/metalloids is largely dependent on peptide-type chelators, the phytochelatins (PCs) (Grill et al., 1989; Clemens et al., 1999; Cobbett, 2000). These compounds are synthesized from glutathione by the heavy metal-activated phytochelatin synthase. A tonoplastic ABC transporter of *Schizosaccharomyces pombe*, HMT1, was identified as the first transporter of heavy metal-phytochelatin complexes (Ortiz et al., 1995). Functional homologs of SpHMT1 have thus far only been reported for *Caenorhabditis elegans* and Drosophila (Vatamaniuk et al., 2005; Sooksa-Nguan et al., 2009), while no homolog of this transporter was identified in the vacuolar membrane of plants.

Recently, Song et al. (2010) succeeded in identifying the plant vacuolar phytochelatin transporters. The reason why these transporters remained undiscovered for such a long time is that two ABCC proteins, AtABCC1 and AtABCC2, exhibit a redundant function, rendering reverse genetic approaches unsuitable for their identification. Both AtABCC1 and AtABCC2 transport apoPC as well as As(III)-PC_2 when expressed in yeast. Interestingly, the transport activity of these proteins does not exhibit classical saturation kinetics, but rather has a sigmoid curve, with low transport activity when substrate concentrations are low. This characteristic of the transporters probably ensures that apoPCs accumulate in the cytosol. By preventing their vacuolar sequestration before reaching a certain threshold, a larger proportion of PCs can form complexes with heavy metals in the cytosol. Vacuoles isolated from *atabcc1 atabcc2* double knockout Arabidopsis plants exhibited only 10 to 15% residual As(III)-PC_2 transport activity, strongly suggesting that these two ABC transporters are the main PC transporters in Arabidopsis (Figure 3). Overexpression of the transporters alone did not result in plants with an increased As tolerance, and the additional co-expression of phytochelatin synthase was necessary to attain this desired As-tolerant phenotype. Subsequent research on these ABC transporters revealed their roles in tolerance to Cd and Hg(II) as well (Park et al., 2011). The *atabcc1 atabcc2* double knockout was highly sensitive to Cd(II) and Hg(II). Interestingly, while *atabcc1* single knockout mutant was more sensitive than the wild type to Cd(II) and Hg(II), *atabcc2* knockout mutant did not exhibit any dramatic difference

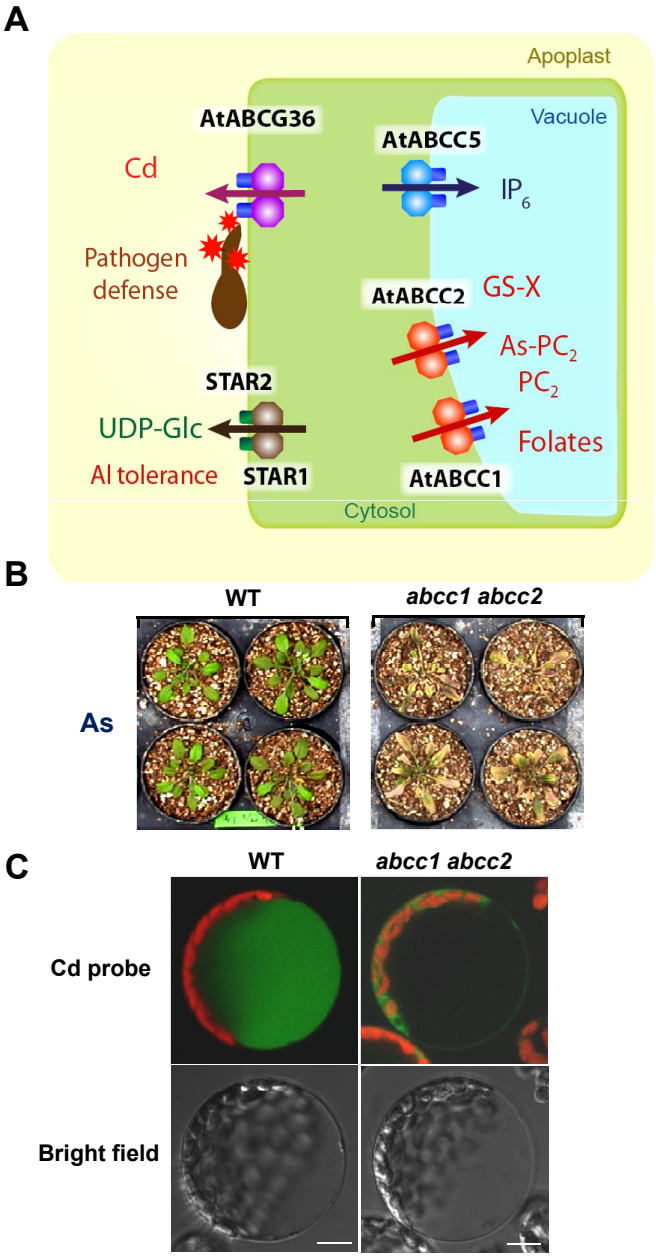

Figure 3. ABC transporters involved in cellular detoxification.

(A) At the plasma membrane, AtABCG36 mediates Cd export and is also involved in pathogen defense (Kobae et al., 2006; Stein et al., 2006; Kim et al., 2007; Bednarek et al., 2009; Clay et al., 2009). The bacterial-type ABC transporters, STAR1 and STAR2, confer aluminum tolerance by transporting UDP-glucose to the extracellular space (Huang et al., 2009; Huang et al., 2010). At the vacuolar membrane, AtABCC1 and AtABCC2 sequester arsenic-phytochelatin complexes in the vacuolar lumen and confer tolerance to toxic metals/metalloid (Song et al., 2010; Park et al., 2011). AtABCC1 is also implicated in folate transport (Raichaudhuri et al., 2009), while AtABCC2 is the major transporter of glutathione conjugate (Lu et al., 1998; Frelet-Barrand et al., 2008). AtABCC5 functions as a phytate transporter (Nagy et al., 2009).

(B-C) Loss-of-function of AtABCC1 and AtABCC2 resulted in arsenic hypersensitivity (Song et al., 2010) (B) and inhibition of vacuolar sequestration of Cd (Park et al., 2011) (C).

in the sensitivity from the wild type. These results suggest that both AtABCC1 and AtABCC2 contribute to Cd(II) and Hg(II) tolerance, and AtABCC1 can confer a significant level of tolerance to the divalent heavy metals in the absence of AtABCC2. The importance of AtABCC1 and AtABCC2 in vacuolar sequestration of Cd was clearly shown using a Cd-sensing fluorescent probe. The fluorescence was in the vacuole of wild-type cells, but mostly in the cytosol of *atabcc1 atabcc2* cells (Figure 3C). Overexpression of *AtABCC1* in Arabidopsis enhanced Cd(II) tolerance and accumulation.

AtABCC3 and AtABCC6 may also function in heavy metal tolerance. AtABCC3 has been reported to complement the Cd-sensitive phenotype of the *ycf1* mutant in *Saccharomyces cerevisiae,* suggesting that it can contribute to Cd tolerance (Tommasini et al., 1998). Growth of *atabcc6* loss-of-function mutants was slightly impaired in the presence of Cd (Gaillard et al., 2008). However, the mechanisms leading to the cadmium sensitivity or the substrates transported by AtABCC3 and AtABCC6 remain to be examined.

AtABCB25/AtATM3 is a mitochondrial ABC transporter involved in the biogenesis of iron-sulfur clusters in plants (Kushnir et al., 2001; Bernard et al., 2009), similar to its yeast homolog, Atm1p (Leighton and Schatz, 1995). In addition, AtABCB25 is also required for the biosynthesis of the molybdenum cofactor, a prosthetic group of molybdenum-containing enzymes (Teschner et al., 2010). Using a microarray for ABC transporters, Bovet et al. (2005) observed that AtABCB25 is strongly up-regulated 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). The observation that the *atabcb25* mutant produces more glutathione in the presence of cadmium than does the wild type also indicates that this mutant suffers higher oxidative stress. AtABCB25 is a close homolog of the vacuolar phytochelatin transporter of *Schizosaccharomyces pombe*, SpHMT1 (Ortiz et al., 1995). It is therefore tempting to speculate that AtABCB25 could transport glutathione-cadmium or cadmium-sulfur complexes as well as Fe-S clusters from the mitochondria to the cytosol, and thereby have multiple roles in biogenesis of iron-sulfur and molybdenum cofactor and heavy metal tolerance.

III.A.3. Anthocyanin transport: still an open debate

While Multidrug And Toxin Efflux (MATE)-type transporters are known to be responsible for the transport of several glucosylated flavonoids across membranes (Zhao et al., 2011), it is still debated which transport mechanism is responsible for anthocyanins accumulation within the vacuole. TT12, a MATE transporter, has been shown to catalyze the vacuolar transport of cyanidin-3-O-glucoside (Marinova et al., 2007) and epichatecin 3-O-glucoside (Zhao and Dixon, 2009). A similar substrate specificity has been shown to be mediated by Medicago MATE1, a close homolog of TT12 (Zhao and Dixon, 2009). A recent report provided evidence that transport of acylated anthocyanins in *Vitis vinifera* (grapevine) is also catalyzed by a MATE transporter (Gomez et al., 2009). In contrast, genetic evidence suggests that maize anthocyanins, which are not acylated, are transported by an ABCC protein, ZmMRP3 (Goodman et al., 2004). These observations raise the question of whether structurally different anthocyanins

are transported into the vacuole by different types of transporters or whether different plants use different transport systems. Anthocyanins are positively charged compounds and therefore not typical substrates for ABCC transporters. Furthermore, although glutathione transferases (GSTs) are apparently involved in anthocyanin transport (Alfenito et al., 1998; Mueller et al., 2000), there is no evidence that these compounds can form glutathione conjugates. However, it has been shown that animal ABCCs can transport positively charged alkaloids in the presence of the negatively charged glutathione (Versantvoort et al., 1995; Zaman et al., 1995). Alternatively, evidence has been presented that GSTs can act as ligandins of anthocyanins and it was therefore postulated they are involved in the delivery of anthocyanins to the transporter (Mueller et al., 2000). Hence, future work should address the important question of whether ZmMRP3 or its homologs can indeed catalyze the transport of anthocyanins and whether glutathione or GSTs are implicated in this process.

III.A.4. Transport of other glucosylated compounds

Besides plant secondary products, several other low molecular weight compounds, such as auxin, abscisic acid, salicylic acid, and modified xenobiotics, which were modified by cytochrome P450 in the first step of detoxification, are glucosylated within a plant cell. Salicylic acid glucoside has been shown to be taken up by an ABC-type transporter that is active in vacuolar vesicles isolated from *Glycine max* (soybean), but by a proton antiporter in *Beta vulgaris* (red beet; Dean and Mills, 2004). Similar differences were observed for two glucosylated sulfuron-based herbicides. While the transport of primisulfuron glucoside into barley vacuoles was driven by an ABC-type transporter, uptake of the closely related chlorsulfuron glucoside into vesicles isolated from red beet exhibited a proton antiport mechanism (Gaillard et al., 1994; Bartholomew et al., 2002). These results indicate that the uptake mechanism of several glucosylated compounds into the vacuole can differ between different plant species. In all of these cases, the transporters exhibiting these activities have not yet been identified and, to our knowledge, there has been no report demonstrating which ABC protein can transport glucosylated solutes.

III.A.5. Transport of antibiotics: AtABCG19 confers kanamycin resistance

A half-size member of the AtABCG family, AtABCG19, confers kanamycin resistance when overexpressed in plants (Mentewab and Stewart, 2005; Kang et al., 2010a). So far, this phenotype has only be found for AtABCG19, but not in other members in ABCG family. In the first report, AtABCG19 was proposed as a selectable marker in transgenic plants, since this gene specifically conferred resistance to kanamycin, but not to other aminoglycoside antibiotics, unlike the conventional antibiotics resistance gene, *nptII*. However, when expressed in hybrid aspen (*Populus tremuloides*), this gene conferred tolerance also to three other aminoglycoside antibiotics (Kang et al., 2010a). Mentewab and Stewart (2005) reported that AtABCG19 is targeted to the vacuolar membrane, which suggests that AtABCG19 removes kanamycin from the cytosol and stores it in the vacuole. However, this result awaits confirmation, since the N-terminal fusion of GFP used in this work might have masked the mitochondrial targeting sequence of the protein predicted from *in silico* analysis, and it therefore cannot be ruled out that this protein is localized in the mitochondrion.

III.B. External Excretion

An alternative strategy for plants to cope with toxic compounds is excretion from the cell. In particular, soil-born heavy metals that are taken up as stowaways during nutrient acquisition are excreted into apoplastic regions or directly back into the rhizosphere. External excretion can occur at a cellular level from the root epidermis, or excess compounds, such as Na^+, can be loaded into the phloem in the aboveground organs and transported back to the root (Berthomieu et al., 2003). The plasma membrane-localized full-size ABC transporter, AtABCG36/AtPDR8, which was previously connected to pathogen defense (see Chapter VII), was shown to be involved in cadmium resistance (Kim et al., 2007). Transgenic Arabidopsis plants overexpressing *AtABCG36* proved to be more tolerant to this highly toxic heavy metal. Measurement of Cd content revealed that accumulation of Cd was reduced in *AtABCG36* overexpressing plants, while it was increased in the loss-of-function mutants. Together, these results suggest that AtABCG36 excretes cadmium from roots (Figure 3A). Strong expression of *AtABCG36* in root epidermal cells (http://atted.jp/) supported this hypothesis. The direct involvement of AtABCG36 in the export of Cd ions or Cd complexes was demonstrated by flux assays using radio-labeled Cd and isolated mesophyll protoplasts. While an overexpressing line proved to be more efficient at extruding ^{109}Cd from the cell than the wild type, a silenced line was impaired in its export capacities. However, it remained unclear in which form Cd is transported by AtABCG36. Interestingly, AtABCG36 also conferred tolerance to toxic concentrations of Na^+ (Kim et al., 2010). This multi-specificity together with the involvement of AtABCG36 in pathogen defense raises the question of whether this ABC transporter exhibits broad substrate specificity, or whether AtABCG36 transports a hitherto unidentified common metabolite that links resistance to pathogens and abiotic stress.

In a screen for ABC transporters potentially involved in lead detoxification, Lee et al. (2005) observed that the transcript levels of *AtABCG40/AtPDR12* were up-regulated in the presence of lead. *AtABCG40* loss-of-function mutant plants were more susceptible to lead than the wild type, because they accumulated more of this toxic heavy metal. Accordingly, plants overexpressing *AtABCG40* were more tolerant to lead and contained less lead after exposure than the wild type. AtABCG40-mediated lead tolerance was not found to be related to glutathione-dependent detoxification mechanisms. It was hypothesized that AtABCG40 either acts as a reflux pump that extrudes lead or lead complexes directly or alternatively inhibits lead uptake into the root by excreting a chelating organic acid/agent into the rhizosphere that prevents lead uptake. The finding that AtABCG40 also acts as an ABA importer (see Chapter IV) raises the question of how these two functions are fulfilled by the same transporter. Future work is necessary to clarify whether AtABCG40 acts simultaneously and directly as a hormone importer as well as an exporter for sub-

strates involved in lead tolerance, or whether the lead tolerance is a secondary effect of the ABA transport activity of AtABCG40.

Aluminum (Al) is a toxic metal that greatly limits crop production in acidic soil. A well-known mechanism to cope with aluminum toxicity is the excretion of citrate and malate by MATE transporters (Magalhaes et al., 2007) and malate excretion by Aluminum Tolerance Transporters (ALMTs) into the rhizosphere (Delhaize et al., 2004; Meyer et al., 2010). These organic acids chelate Al, preventing the entry of toxic Al^{3+} into the root (Ryan et al., 2011). In a screen to identify genes involved in Al tolerance, several ABC transporters have been found to contribute to Al detoxification including *AtABCI16/AtALS3* and *AtABCI17/AtSTAR1* and two rice genes *OsSTAR1* and *OsSTAR2*. Mutation in *AtABCI16* resulted in hypersensitivity to Al and alteration in Al accumulation in roots (Larsen et al., 1997; Larsen et al., 2005). *AtABCI16* encodes one transmembrane domain homologous to a bacterial ABC protein, and may function to redistribute accumulated Al away from sensitive tissues, by transporting either Al directly or compounds involved in Al tolerance (Larsen et al., 1997; Larsen et al., 2005). *OsSTAR1* encodes the nucleotide-binding domain and *OsSTAR2* encodes the transmembrane domain of the transporter, which are homologous to bacterial-type ABC transporters (Huang et al., 2009). The combined ABC transporter complex is shown to localize mainly in membrane vesicles inside of root cells. Co-expression of *OsSTAR1* and *OsSTAR2* in oocytes revealed that they form a functional ABC transporter that is able to transport UDP-glucose (Figure 3A). This was a surprising result and the authors suggested that UDP-glucose may be used to alter the composition of the cell wall, and thereby prevent migration of Al into the plasma membrane. The same group recently reported that a close homolog of OsSTAR1 exists in Arabidopsis (Huang et al., 2010). The knockout mutant of *AtSTAR1* was also sensitive to aluminum. The observation that *OsSTAR1* could rescue the aluminum-sensitive phenotype of the Arabidopsis mutant indicates that the proteins encoded by these two genes exhibit similar functions. It is therefore likely that AtABCI16 and AtABCI17 make a functional transporter similar to the case of OsSTAR1 and OsSTAR2. The observation that *AtSTAR1* is expressed in the outer layers of root tips and developing leaves, however, raises the question as to whether this ABC transporter has an additional function, for instance, in pathogen resistance in Arabidopsis.

IV. TRANSPORT OF PHYTOHORMONES

IV.A. Auxin

Indole-3 acetic acid (IAA, auxin) is a phytohormone involved in a multitude of processes. It plays a role in embryogenesis, cell division, cell elongation, lateral root development, apical meristem dominance, gravitropism, phototropism, and other developmental and physiological processes (Benjamins and Scheres, 2008). Auxin is a somewhat unique growth regulator in that directional movement of the compound and is an essential component of the signaling mechanism. Tightly regulated and directed cell-to-cell transport of auxin leads to distinct auxin gradients created by asymmetric auxin distributions that are, to a large degree, responsible for the orchestration of auxin-dependent processes. Since auxin is mainly synthesized in leaf primordia and young leaves, it has to be transported to its sites of action. Polar transport of auxin from the shoot to the root apex and redirection at the root tip are essential for the programmed and plastic polarity of the plant form.

The control of differential growth by polar auxin streams was inferred from studies of tropic plant growth initiated by Charles and Francis Darwin (1880). A model for cellular auxin movement driven by chemiosmotic gradients was proposed by Rubery and Sheldrake (1974). This model predicted that auxin, which is present predominantly in the protonated, uncharged form in an acidic environment (*pKa* 4.75), can diffuse from the apoplast into a plant cell and is then released in its anionic form from this cell. The model also predicted that polarized carriers would direct the exit of auxin and, thus, the polar auxin streams. Subsequently, mutations that exhibited either reduced root gravitropism or “pinformed” inflorescences similar to those observed in Arabidopsis after treatment with the polar auxin transport inhibitor, naphthalene phthalamic acid (NPA), resulted in the identification of the PIN family of proteins (Chen et al., 1997; Gäelweiler et al., 1998; Luschnig et al., 1998). The PIN proteins exhibited a predominantly polarized cellular localization, as predicted, and were subsequently shown to exhibit auxin transport activity in heterologous systems (Petrášek et al., 2006). Earlier biochemical experiments had also predicted the presence of an auxin uptake symport activity, and this activity was subsequently associated with the AUX1/LAX family of proton symporters (Bennett et al., 1996; Yang et al., 2006). Together, PINs and AUX1/LAX proteins constitute a complex network that controls directional auxin fluxes (Křeček et al., 2009; Petrášek and Friml, 2009; Titapiwatanakun and Murphy, 2009).

IV.A.1. AtABCBs/PGPs as auxin transporters

Dudler and colleagues suggested that a subclass of ABC transporters might function in auxin transport, after observing that Arabidopsis ABCB1 localized to the plasma membrane, and that hypocotyl growth was reduced in *AtABCB1/AtPGP1* antisense lines, while *AtABCB1* overexpressing plants developed longer hypocotyls (Sidler et al., 1998). In a subsequent study, Noh et al. (2001) presented direct evidence that AtABCB1 and its closest homolog, AtABCB19/AtPGP19, participate in auxin transport. Arabidopsis *abcb1* and the more pronounced *abcb19* mutant exhibit reduced growth, decreased apical dominance, and impaired polar auxin transport (Figure 4A). *atabcb1 atabcb19* plants are very small, and the shoot basipetal auxin flux is reduced by more than 70%. Despite this strong reduction, the mutant does not exhibit the defects in organogenesis and tropic growth observed in plants treated with auxin efflux inhibitors as is the case in *pin* mutants. On the contrary, *atabcb19* hypocotyls are hyperphototropic and hypergravitropic (Noh et al., 2003; Lin et al., 2005), and *atabcb19* plants produce many more curvatures but are not agravitropic (Lewis et al., 2007). However, Arabidopsis ABCB1 and ABCB19 have been shown to act directly as auxin exporters in protoplast assays and in assays of yeast and mammalian cells expressing the Arabidopsis proteins (Geisler et al., 2005; Yang and Murphy, 2009). It is interesting to note that these transporters lost the

specificity for auxin when expressed in a heterologous system (Geisler et al., 2005). They may require some factors present in plant tissues to exhibit auxin specificity.

A third Arabidopsis ABCB transporter with an affinity for auxin, AtABCB4/AtPGP4, appears to function in auxin transport in the root, but this protein has been implicated in both auxin export and uptake activity (Santelia et al., 2005; Terasaka et al., 2005; Cho et al., 2007; Lewis et al., 2007; Wu et al., 2007). Loss of AtABCB4 function results in decreased shoot-ward auxin transport in the root (Terasaka et al., 2005; Lewis et al., 2007; Wu et al., 2007) and increased root hair elongation (Santelia et al., 2005; Cho et al., 2007). Overexpression of *AtABCB4* in root hair cells results in decreased elongation in a manner that is similar to what is seen upon overexpression of other auxin efflux transporters, and auxin efflux was enhanced in BY-2 cells overexpressing *AtABCB4* (Cho et al., 2007). However, auxin uptake activity was enhanced in *Saccharomyces cerevisiae* expressing Arabidopsis *ABCB4* (Santelia et al., 2005). When expressed in mammalian cells, AtABCB4 was shown to mediate IAA uptake at low concentrations, but to reverse to apparent efflux when treatment with NPA increased internal concentrations (Terasaka et al., 2005). A recent paper published by Yang and Murphy (2009) showed that AtABCB4 kinetics are different from those of AtABCB1 and AtABCB19. *Schizosaccharomyces pombe* cells expressing *AtABCB4* incubated in the presence of auxin initially accumulate auxin, but after a certain period of incubation, started to export auxin again. The primarily epidermal localization of AtABCB4 suggests that AtABCB4 may modulate the uptake of auxins produced by soil microorganisms (Figure 4B).

IV.A.2. Interaction of ABC transporters with PINs, PHOT1, and TWD1 modulate auxin transport activity

Evidence for the regulation of ABC protein-mediated auxin transport came from a yeast two-hybrid screen, in which the immunophilin-like TWD1 (FKBP42) was found to interact with AtABCB1. Later studies using the BRET (Bioluminescence Resonance Energy Transfer) technique provided evidence that auxin transport activity was modulated by the interaction between TWD1 and AtABCB1 (Bouchard et al., 2006; Bailly et al., 2008). Interestingly, the *twd1* loss-of-function mutant displayed a similar phenotype to the *atabcb1 atabcb19* double knock-out, except that, in addition to the dwarf phenotype, *twd1* plants exhibit a much more severe twist. NPA, an auxin transport inhibitor, as well as flavonoids, which are thought to act as endogenous auxin transport modulators, can disrupt the AtABCB1-TWD1 complex and thereby reduce the transport activity *in planta*.

Auxin transport is furthermore regulated by direct interaction between the two classes of auxin exporters, PINs and ABCBs. Localization and developmental studies suggest that these proteins mediate auxin transport independently. However, in some tissues, the two types of auxin transporters are co-localized and it was shown by a yeast two-hybrid assay as well as by co-immunoprecipitation that PIN1 can interact with AtABCB19. Similar results were obtained for PIN1 and AtABCB1. Analysis of the transport rates showed that, when present as a complex, PIN1

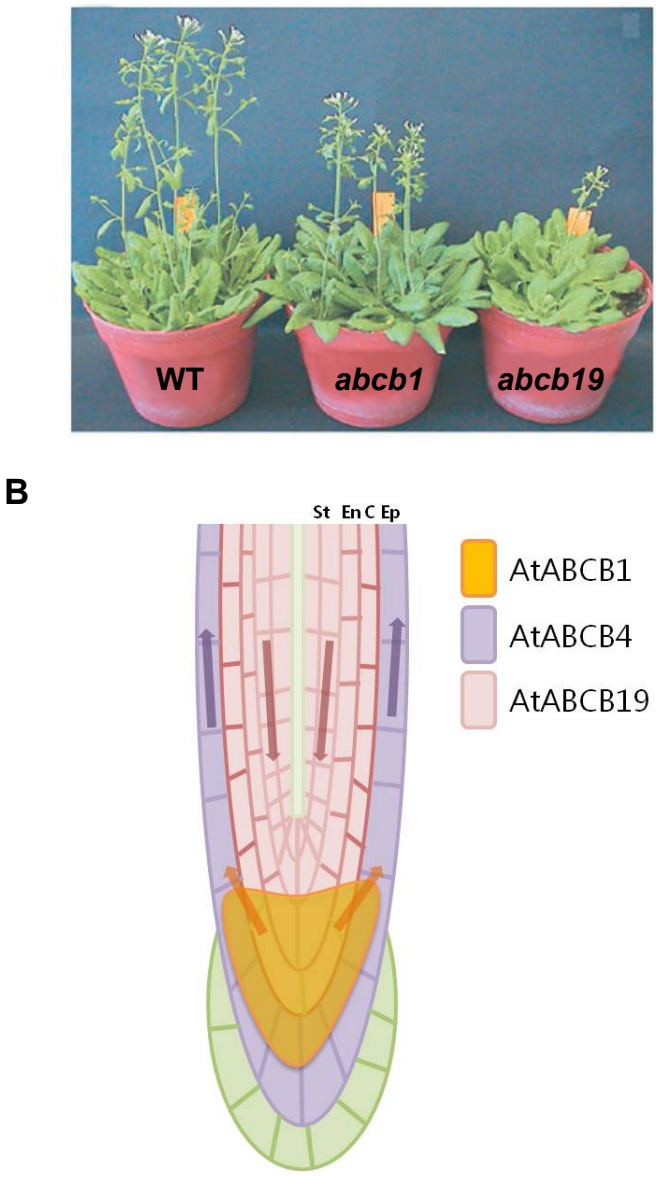

Figure 4. AtABCB1, AtABCB4, and AtABCB19 are auxin transporters.

(A) Phenotypes of the loss-of-function mutants of *AtABCB1* and *AtABCB19*. Image reprinted Geisler et al. (2005) with permission from Wiley-Blackwell Publishing.

(B) Localization of AtABCB1, AtABCB4, and AtABCB19 in roots and auxin fluxes mediated by these three ABCB proteins. AtABCB1 is expressed in the root differentiation zone (orange, columella and root apical meristem; Sidler et al., 1998). AtABCB4 localizes mainly to the epidermis (purple; Terasaka et al., 2005; Wu et al., 2007). AtABCB19 is expressed from the stele to the cortex and weakly in epidermal cells of the root (pink; Wu et al., 2007; Blakeslee et al., 2007). St : stele, En: endodermis, C: cortex, and Ep: epidermis. The arrows represent auxin flux.

and AtABCB19 exhibit a synergistic effect, and the proteins are more sensitive to inhibitors (Blakeslee et al., 2007; Rojas-Pierce et al., 2007). In contrast, an interaction between PIN2 and AtABCB1 or AtABCB19 leads to the inhibition of auxin transport. Future research should analyze these interactions *in vivo* to resolve their temporal and spatial components. This is an ambitious goal, but probably the most promising approach to further unravel the modulation of auxin fluxes.

AtABCB19 is transcriptionally regulated by photomorphogenic mechanisms (Noh et al., 2001) and both *atabcb1* and *atabcb19* exhibit a hypersensitivity to far-red, red, and blue light inhibition of hypocotyl elongation (Noh et al., 2001; Lin and Wang, 2005; Wu, 2010), a result confirming the early observations by Sidler et al. (1998). AtABCB19 is a direct phosphorylation target of the PHOT1 photoreceptor, and transient inhibition of AtABCB19 transport by PHOT1 results in the pooling of auxin in the first step of phototropic bending (Christie et al., 2011). PHOT1 interacts with the C-terminus of AtABCB19, which has been shown to be a target of the inhibitory drug gravacin (Rojas-Pierce et al., 2007) and a site of interaction with the FKBP42 immunophilin TWD1.

IV.B. Auxigenic Compounds

Auxin precursors, such as indole-3-butyric acid (IBA) or amide- or ester-linked conjugates, can rapidly be metabolized to free auxin. Thus, they constitute a rapidly available auxin pool that does not depend on *de novo* synthesis. As is the case for auxin, the regulated transport of the precursors is crucial for the normal physiology of plant. The first transporter involved in IBA transport, AtABCD1/PXA1, was identified in a screen for IBA-resistant mutants (Zolman et al., 2001). The authors showed that loss-of-function of this peroxisomal ABC transporter confers resistance to IBA, but not to IAA. Further analysis of this peroxisomal ABC transporter, also called PED3p (Hayashi et al., 2002) or COMATOSE (Footitt et al., 2007), revealed that, besides transporting IBA, it is most likely responsible for the import of substrates for peroxisomal β-oxidation (see Chapter VI).

Three reports demonstrate that two members of the ABCG family are involved in the excretion of auxigenic compounds. Ito and Gray (2006) performed a screen to identify mutants that are resistant to 2, 4-D, a synthetic auxin analogue used as an herbicide. They found that a mutation in AtABCG37/AtPDR9 conferred resistance to 2, 4-D and other structurally related compounds. This was a surprising result, since ABC transporters were thought to excrete toxic compounds and hence mutations were expected to decrease resistance rather than to enhance it. The apparent contradiction was resolved by the finding that an Ala to Thr substitution at position 1034 confers increased stability to the AtABCG37 protein. Indeed, a T-DNA insertion in the corresponding gene leads to a 2, 4-D-sensitive phenotype, while overexpression enhances tolerance to a variety of auxigenic compounds.

In a similar screen, aimed at identifying genes that lead to IBA hypersensitivity, Růžička et al. (2010) identified AtABCG37 as a potential IBA transporter. They found that loss-of-function mutants of the gene were not affected in their sensitivity to auxin, but, using heterologous expression in yeast and animal cells as well as assays with root tips, they showed that AtABCG37 indeed exports IBA and some synthetic auxins, such as 2, 4-D, from the cell. Because of its specific plasma membrane localization at the soil-exposed face of root epidermal cells, this transporter is likely to release IBA and other auxigenic compounds into the soil. Since the expression of AtABCG36 and AtABCG37 partially overlaps, the authors investigated the double knock-out mutant and observed that the sensitivity to IBA was increased even further (Růžička et al., 2010). Some microorganisms, including symbionts, produce IBA; therefore, the authors hypothesized that AtABCG37 might be involved in the cross-talk between microorganisms and plants. However, AtABCG37 may be involved in the export of a much broader range of weak organic acids and hence have a similar function to yeast PDR12. In any case, detailed studies of how AtABCG36 and AtABCG37 function affect microbial communities in the rhizosphere are pending.

Strader and Bartel (2009) performed a screen to identify mutants that specifically restore IBA, but not auxin sensitivity in the auxin signaling mutant *ibr5*. The *ibr5* mutant is defective in a protein phosphatase, which renders it insensitive to endogenous and exogenously applied auxin. Mapping of a candidate mutation revealed that loss of AtABCG36 function restores IBA sensitivity in *ibr5*. Root tips of the *atabcg36* mutant exhibit increased IBA accumulation and reduced IBA efflux, suggesting that AtABCG36 promotes IBA efflux (Strader and Bartel, 2009).

IV.C. Abscisic Acid

Abscisic acid (ABA) is a plant hormone with profound effects on seed maturation, seed dormancy, stomatal closure, drought responses, and lateral root formation (Leung and Giraudat, 1998; Rock, 2000; Rohde et al., 2000). Under drought stress, the ABA level in the shoot can increase 50-fold compared to that under turgid conditions. ABA biosynthesis is highly induced by dehydration in the vascular parenchyma cells of roots and shoots, but not in guard cells (Zimmermann et al., 2004; Christmann et al., 2005; Endo et al., 2008; https://www.genevestigator.com/gv/). Consequently, ABA has to be exported from ABA-producing cells in the roots and leaves, redistributed, and directed to guard cells. A further important developmental process regulated by ABA is the maintenance of seed dormancy. Seed dormancy is the incapacity of a viable seed to germinate under unfavorable conditions (Finch-Savage and Leubner-Metzger, 2006). To maintain dormancy, the continuous synthesis of ABA in the seed coat (endosperm) and its subsequent transport to the embryo is required (Ali-Rachedi et al., 2004).

Recently, the PYR/RCAR family has been identified as ABA receptors (Ma et al., 2009; Park et al., 2009). Since they are localized in the cytosol, ABA has to cross the plasma membrane once it arrives at the target site. Despite the fact that ABA could passively accumulate in cells by diffusion, there is also evidence that ABA uptake is mediated by a transporter (Windsor et al., 1992 ; Daeter and Hartung, 1993; Wilkinson and Davies, 1997; Jiang and Hartung, 2008).

Two approaches have recently led to the simultaneous identification of an ABA exporter and an ABA importer (Figure 5). In a forward genetic approach, Shinozaki and collaborators performed a high-throughput screen for transposon lines affected in an ABA response (Kuromori et al., 2010). A mutant line exhibiting an ABA-sensitive germination phenotype was isolated and the authors showed that the responsible transposon insertion

disrupted the AtABCG25/AtWBC26 coding sequence. This ABC transporter corresponds to a half-size ABC transporter, formerly called AtWBC26. Promoter-GUS analysis revealed that AtABCG25 is predominantly expressed in the vascular bundle. Using an N-terminal YFP-fusion protein, it was shown that AtABCG25 is targeted to the plasma membrane. Biochemical analysis of membrane vesicles isolated from *AtABCG25*-expressing insect cells showed that AtABCG25 displays high affinity and high specificity for the transport of biologically active (+) ABA. *AtABCG25*-overexpressing plants had a higher leaf temperature, indicating reduced stomatal transpiration. These results are clear evidence that AtABCG25 acts as an ABA exporter. However, knockout lines did not display a stomatal phenotype, suggesting that there is some redundancy and that a second ABA exporter may exist and work in parallel with AtABCG25.

The rationale behind the second work (Kang et al., 2010b) was that, since plant ABCG transporters had previously been shown to transport terpenoids and since ABA is a tetraterpene-derived sesquiterpene, the ABCG proteins are strong candidates for ABA transporters (Campbell et al., 2003; Rea et al., 2007). In a screen using loss-of-function *atabcg* mutants Kang et al. (2010b) observed that *atabcg40* exhibited a decreased sensitivity to ABA-induced stomatal closure. Further analysis revealed that this mutant was more drought-sensitive and, when treated with ABA, had a higher leaf temperature than the wild type. These results indicated that stomata were not able to close efficiently in this mutant. Since it was also observed that *AtABCG40/AtPDR12* is preferentially expressed in guard cells and targeted to the plasma membrane, it was suggested that the corresponding protein might act as an ABA importer. Indeed, this hypothesis was confirmed with three independent transport assays, i) using protoplasts isolated from wild-type and mutant plants, ii) using *AtABCG40*-expressing yeast, and iii) using *AtABCG40*-expressing BY2 cells. AtABCG40 imports ABA with a high affinity and is specific for the biologically active form. It has long been assumed that ABA permeates into the cell passively, and thus an ABA importer may not be required in plants. However, it should be kept in mind that, during water stress, the pH in the apoplast rises from 5.5–6.0 to about 7, and hence the protonated, freely diffusible form of ABA is present only at very low levels. The observed phenotype and the demonstration that induction of ABA-inducible genes is strongly delayed in the *atabcg40* knock-out mutant, proves that ABA import is required for a rapid and efficient response during ABA-mediated stress reactions.

Another member of the ABCG family, AtABCG22/AtWBC23, which is implicated in stomatal regulation, was identified very recently (Kuromori et al., 2011). Mutants of this transporter had a lower leaf temperature and increased water loss. However, the substrate transported by this transporter could not be determined.

Although both *atabcg40* and *atabcg25* also exhibited a germination phenotype, the precise role of *AtABCG40* and *AtABCG25* in the complex process of seed dormancy and germination has yet to be elucidated. The seed coat produces and steadily releases ABA until conditions are favorable for seed germination. The embryo, on the other hand, has to take up ABA to remain dormant. How ABA efflux and import are coordinated and which transporters are involved in this developmental process remain to be established. The same holds true for ABA transporters implicated in lateral root formation.

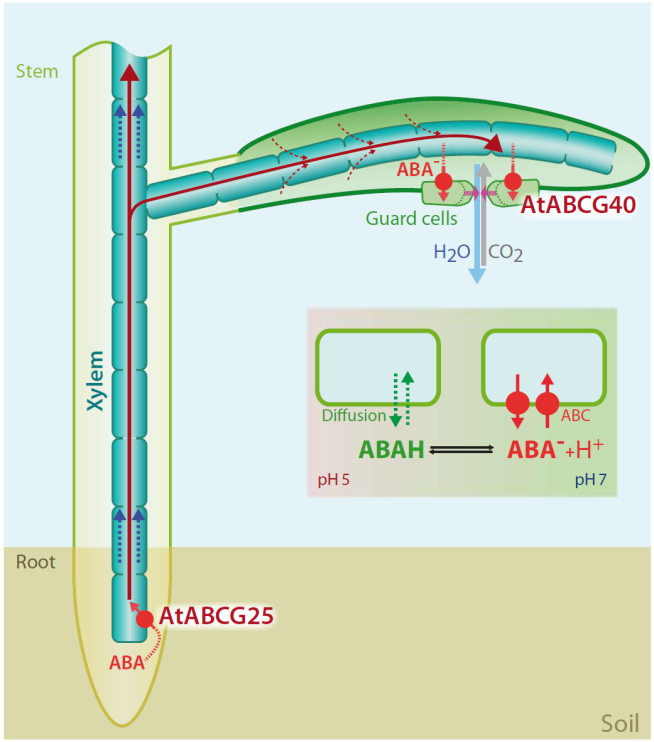

Figure 5. Abscisic acid (ABA) fluxes and the role of the ABA efflux and influx transporter.

AtABCG25 exports ABA from parenchyma cells in the vasculature into the xylem. ABA is directed with the transpiration stream to guard cells, where AtABCG40 mediates its uptake into guard cells.

V. TRANSPORT OF PRIMARY PRODUCTS

To allow for the optimal functioning of cellular metabolism, the concentration of most solutes in the cytosol and other metabolically active compartments are kept constant and transient accumulation of metabolites in excess is avoided. The large central vacuole, which exhibits only minimal metabolic activity, plays the major role as temporary storage compartment.

V.A. Folates and Folate Homologs Are Transported into the Vacuole by AtABCC1 and AtABCC4

Folates, also known as Vitamin B9 (Raichaudhuri et al., 2009), are enzymatic cofactors that are required for one-carbon transfer reactions, e.g., for amino acid and nucleotide biosynthesis. Transient accumulation of folates within the vacuole is important, primarily to maintain cytosolic concentrations of folate that are optimal for biochemical reactions. Most of the folate in the cell is conjugated to several glutamate molecules and hence bears a large number of negative charges. These can interact with cations, such as Ca^{2+}, in an unspecific manner, thus disturbing the cellular signaling pathways. Vacuolar membrane-localized ABC transporters, such as AtABCC1 (Raichaudhuri et al., 2009) and AtABCC4 (Klein et al., 2004), are involved in regulating cyto-

plasmic folate concentrations by transporting excess folates into the vacuole. Vacuoles isolated from *AtABCC1* T-DNA insertion mutants accumulated only approximately 50% of the antifolate methotrexate present in the wild type and the mutants were more sensitive when exposed to this compound. These results indicate that ABCC transporters, such as AtABCC1, are important for folate storage (Figure 3A). However, the mechanism by which folate is released from the vacuoles back into the cytosol when required remains unknown.

V.B. Phytate is Transported by AtABCC5

V.B.1. Cellular homeostasis

A similar tight regulation of cellular concentrations is also required for phytate (inositol hexa*kis*phosphate, $InsP_6$). As the main form of phosphate storage in seeds of many plant species, this compound is important for early plant growth. Phytate has six phosphate residues bound to an inositol ring, resulting in a molecule containing twelve negative charges. Consequently, phytate exhibits a considerable chelating capacity for positively charged compounds, particularly divalent cations. Therefore, the presence of high concentrations of this compound in the cytosol would adversely affect cellular metabolism. Based on sequence similarity to maize MRP4, which, when knocked-out causes a low phytate (*lpa*) phenotype in seeds (Shi et al., 2007), Nagy et al. (2009) showed that the knockout mutants of Arabidopsis AtABCC5/MRP5 also exhibit a low phytate phenotype. Transport experiments using vesicles isolated from yeast expressing *AtABCC5* confirmed that AtABCC5 acts as a phytate transporter. Several low phytate mutants, which have a mutation in homologs of AtABCC5 and ZmMRP4, have been described (Xu et al., 2009). The most drastic effect was observed in rice, where a mutation in an ABCC5 homolog is lethal (Turner et al., 2007). Manipulation of vacuolar phytate transport is of great interest to crop engineers, since reducing the phytate content in seeds would potentially allow the production of plants with a higher amount of bioavailable iron and zinc. Furthermore, a high $InsP_6$ content in seeds can have a negative impact on the environment. Monogastric animals that lack phytases in their digestive tract fail to process the phytates present in seed-based feed. As a consequence, high amounts of undigested phytates are released with the animal waste into nature, thus accentuating the phosphorus pollution from agriculture (Cromwell and Coffey, 1991).

V.B.2. Role of Inositol hexakisphosphate transport in guard cell signaling

Gaedeke et al. (2001) reported that loss-of-function mutants for *AtMRP5/AtABCC5* were no longer responsive to glibenclamide, a compound known to induce stomatal opening (Leonhardt et al., 1997). Further studies demonstrated that the guard cells of *atabcc5* mutants did not respond to ABA, Ca^{2+}, and auxin, but were still light sensitive (Klein et al., 2003). In addition, it was demonstrated that stomatal anion channel activity was reduced in *atabcc5* (Suh et al., 2007). However, it remains unclear how loss of function of a single ABC transporter could lead to such a complex stomatal phenotype. The report by Nagy et al. (2009), which demonstrates that AtABCC5 is a high affinity $InsP_6$ transporter, may provide a clue. Former studies showed that $InsP_6$ is an activator of vacuolar Ca^{2+} release and an inhibitor of K^+ influx (see references in Nagy et al. 2009). In guard cells, therefore, it was postulated that, if the $InsP_6$ signal is not readily removed from the guard cell cytosol, it would lead to the deregulation of Ca^{2+}-dependent signaling cascades and affect K^+ influx. The extremely high affinity found for this transporter (Km=0.3 µM) further supports the notion that guard cell-expressed AtABCC5 is indeed involved in the removal of this signaling compound. Further studies will be required to elucidate in detail how the integration of ABA, auxin, Ca^{2+}, and $InsP_6$ signals controls stomatal movement.

V.C. Malate Transport: Osmoregulation

In a study to identify the functions of ABC transporters highly expressed in guard cells, Lee et al. (2008) observed that loss-of-function mutants of the plasma membrane intrinsic protein AtABCB14 exhibited impaired stomatal regulation. In the presence of high levels of CO_2, stomatal closure was more pronounced in the mutant than in the wild type. This phenotype was not observed in isolated epidermal strips containing epidermal cells and guard cells only, suggesting that the aberrant stomatal movement of the mutant was not directly linked to CO_2. Based on the published literature, it is likely that the closing effect of CO_2 is on the one hand mediated directly by CO_2, and on the other by increasing malate levels in the apoplastic space (Hedrich and Marten, 1993; Hedrich et al., 1994; Hu et al., 2010). In line with these experiments, epidermal strips of *atabcb14* plants incubated in the presence of malate displayed a faster stomatal response than those of the corresponding wild type. Transport experiments using *E. coli* and HeLa cells expressing *AtABCB14* revealed that AtABCB14 is a malate importer. Since malate has dual functions as an osmoticum as well as a regulator of anion channels in guard cells (Hedrich and Marten, 1993), Lee et al. (2008) suggested that the phenotype observed for *atabcb14* was due to the accumulation of malate in the apoplast, which shifts the current-voltage curve of the guard cell anion channel, combined with the impaired import of malate into guard cells.

VI. TRANSPORT OF LIPIDS AND LIPOPHILIC COMPOUNDS

Lipids and lipophilic compounds are essential components of a plant. They are the building blocks of biological membranes, and constitute important energy reserves that are indispensable in the early phases of plant development. As surface lipids, they are also required to protect the plant from biotic and abiotic stresses.

VI.A. A Bacterial-Type ABC Transporter is Involved in the Transport of Polar Lipids from the ER to Plastids

Lipid synthesis occurs mainly in the chloroplast and endoplasmic reticulum. The plastidic stroma is the primary site for fatty acid synthesis. Fatty acids are used within the plastid for the synthesis of plastidic membranes, but are also exported to the ER, where they are used for the synthesis of building blocks for other membranes. Galactoglycerolipids, major components of plastidic lipids, are derived from phosphatidic acids by the exchange of

the phosphatidyl with a galactosyl group at the sn-3 position of the glycerol backbone. Interestingly, plastid lipids derived from phosphatidic acid are not only synthesized from plastidic phosphatidic acid, but also from phosphatidic acid assembled in the ER. Therefore, this compound has to re-enter the chloroplast.

In a screen originally aimed at identifying enzymes involved in alternative galactoglycerolipid synthesis pathways, Benning and co-workers discovered the so-called *tgd1* (trigalactosyldiacylglycerol) mutants, which exhibited a complex phenotype, including stunted growth, embryo abortion, a decrease in ER-derived plastid lipids, and accumulation of oligogalactoglycerolipids (TGDG), triacylglycerols, and phosphatidic acids in leaf tissues (Xu et al., 2005). Identification of the underlying genes revealed that *TGD1/AtABCI14* encodes a membrane-spanning protein. Two other *tgd* mutants, *tgd2* and *tgd3*, were isolated based on their altered lipid phenotypes, which resembled those of *tgd1*. *TGD2/AtABCI15* encodes a phosphatidic acid-binding protein anchored at the inner envelope of the plastid (Awai et al., 2006), and *TGD3/AtABCI13* encodes the catalytic domain of an ABC transporter located in the stroma of the plastid (Lu et al., 2007). These three subunits form a bacterial-type ABC transporter, which is proposed to be responsible for the import of phosphatidic acid into the plastid. The importance of this lipid transporter is reflected by the phenotype of the *tgd1* and *tgd2* mutants, which are consistently smaller and synthesize less chlorophyll than the wild type.

VI.B. AtABCD1 Is Required for the Import of Substrates for Beta-Oxidation

Beta-oxidation of fatty acids is an important catabolic process that is required for the generation of acetyl-CoA for entry into the citric acid cycle. In plants, this process occurs predominantly within the peroxisomes, and fatty acyl-CoAs must therefore be imported from the cytosol. At least four forward genetic screens identified an ABC transporter required for this process (Russell et al., 2000; Zolman et al., 2001; Footitt et al., 2002; Hayashi et al., 2002). In contrast to its animal and yeast counterparts, which consist of two half-size ABC proteins (Mosser et al., 1993; Shani et al., 1995; Hettema et al., 1996; Verleur et al., 1997; van Roermund et al., 2008), the plant peroxisomal ABC transporter is present as a full-size ABC protein. Arabidopsis loss-of-function mutants of *AtABCD1* are strongly impaired in several important metabolic and developmental processes, such as germination, fertility, seedling establishment, and root growth. These studies provided evidence that, besides fatty acyl-CoA, the plant peroxisomal ABC transporter can also import the auxin precursor indolbutyric acid (IBA) and precursors of jasmonic acid (Russell et al., 2000; Zolman et al., 2001; Footitt et al., 2002; Hayashi et al., 2002; Theodoulou et al., 2005). Furthermore, it was demonstrated that this transporter is also important for the peroxisomal uptake of acetate (Hooks et al., 2007). Performing a large-scale mutagenesis screen, Dietrich et al. (2009) showed that the transport activities of IBA and substrates of the β-oxidation can be separated locally. The reason for this might be that this full-length peroxisomal transporter contains two halves that are rather distinct, with each part being responsible for the recognition of certain substrates. All of these *in-planta* studies showed that β-oxidation is strongly affected in the absence of *AtABCD1*; however, no direct evidence for acyl-CoA fatty acid import was provided. To obtain direct evidence that these substrates are indeed imported by AtABCD1, Nyathi et al. (2010) expressed the Arabidopsis transporter in the yeast *pax1 pax2* mutant. This mutant yeast strain is deficient in the function of two homolgous genes of *AtABCD1* and thus fails to grow even when supplemented with exogenous fatty acids. The researchers demonstrated that the *AtABCD1*-expressing *pax1 pax2* mutant yeast was able to grow in the presence of a broad range of fatty acids. More importantly, by isolating yeast peroxisomal membranes, Nyathi et al. (2010) showed that ATP hydrolysis was stimulated in the presence of acyl-CoA fatty acids but not free fatty acids, providing direct evidence that AtABCD1 indeed has an acyl-CoA fatty acid transport activity.

VI.C. Members of the ABCG Family are Required for Deposition of Surface Lipids

Almost all plant organs are covered with hydrophobic compounds to protect the plant body against environmental stresses. As the precursors of surface lipids are mainly synthesized and modified in the endoplasmic reticulum, they must pass through the plasma membrane to reach their final destination, the cell wall, and this process requires the presence of lipid transporters at the plasma membrane. Surface compound-deficient mutants exhibiting severe defects in plant growth and plant resistance against various stresses were a promising starting point to identify transporters involved in this process. This approach led to the identification of some Arabidopsis ABCG transporters that participate in surface lipid formation.

VI.C.1. AtABCG11, 12, 13, and 32 are involved in the transport of cuticular lipid precursors from epidermal cells to the surface for cuticle layer formation

The epidermis of aerial plant organs is covered with a hydrophobic cuticle that protects the plant body from detrimental environmental conditions, such as drought and pathogen invasion. Although there are species-dependent variations in the composition of the cuticle, it generally consists of two types of highly lipophilic materials, cutins and waxes. Cutin, which is deposited on the cellulosic cell wall, is a polymer consisting mainly of glycerol and ω- and mid-chain hydroxy and epoxy C16 and C18 fatty acids (Heredia, 2003; Nawrath, 2006). Recently, α,ω-dicarboxylic acids, components known to occur in suberin, were detected in cutin (Bonaventure et al., 2004). Wax, which is formed on the cutin layer, is a complex mixture of C20 to C60 straight-chain aliphatics and may contain secondary metabolites, such as triterpenoids, phenylpropanoids, and flavonoids (Jetter et al., 2007).

The main function of cutin is to confer resistance against mechanical damage and provide a docking structure for proper wax deposition. Wax, which is directly exposed to the environment, limits non-stomatal water loss, and prevents pathogen invasion by forming a physical barrier and by inducing defense signaling pathways upon pathogen invasion (Reina-Pinto and Yephremov, 2009). The cuticle layer is also important for plant development, as many mutants impaired in cuticule formation exhibit stunted growth and post-genital organ fusion (Nawrath, 2006).

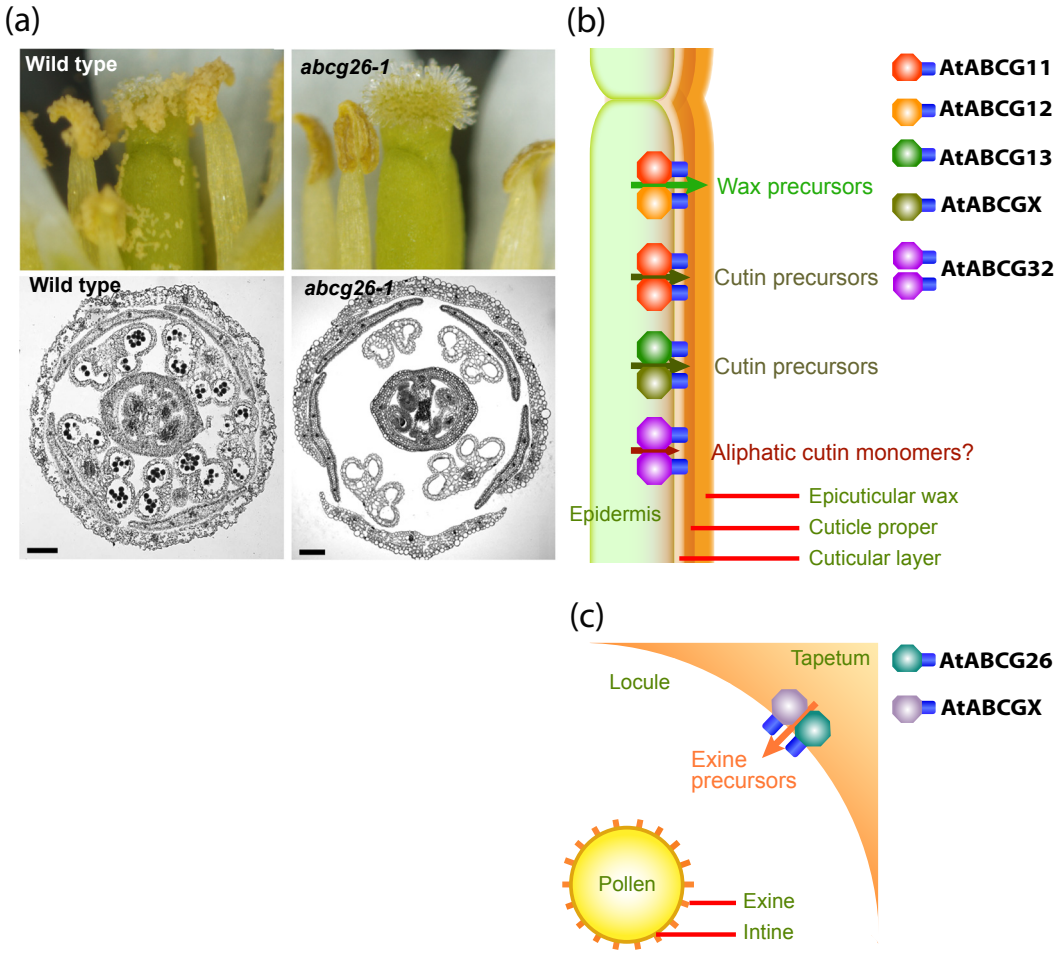

Figure 6. Surface lipids are secreted by ABC transporters of the ABCG family.

(A) Loss-of-function mutants in *AtABCG26* fail to self-pollinate due to defective pollen development caused by impaired sporopollenin formation. Scale bars = 100 µm. Figure taken from Choi et al. (2011).

(B) Members of the ABCG family that participate in cuticle formation by transporting components of the cuticular wax and/or cutin. See text for details.

(C) AtABCG26 is involved in pollen exine formation, possibly by transporting exine precursors from the tapetum to the locules.

The finding that *CER5*, a gene required for wax deposition at the outer surface of epidermal cells, encodes the half-size transporter AtABCG12 was the starting point to gain an in depth understanding of the mechanisms underlying wax deposition (Pighin et al., 2004, Figure 6B). *atabcg12* mutants contained less than 50% of the wax present in wild-type plants. AtABCG12 does not appear to be substrate-specific, since the amount of most wax components, such as alkanes, ketones, and primary and secondary alcohols, was reduced in the extracellular wax of *atabcg12* mutants. Interestingly, the strongly impaired export of these compounds did not lead to feedback inhibition of wax synthesis, as the total amount of waxes in epidermal cells and on the surface remained constant. Epidermal cells of the *atabcg12* mutant formed cytoplasmic protrusions that contained bundles of linear inclusions. These probably correspond to the precursors of cuticular waxes that cannot be exported in the absence of *AtABCG12*. AtABCG12 was localized to the plasma membrane and was specifically expressed in epidermal cells. In a subsequent study, Bird et al. (2007) showed that the *atabcg11* mutant exhibits a similar phenotype to *atabcg12*. In addition to the observations made for *atabcg12*, *atabcg11* loss-of-function mutants exhibited a stunted growth phenotype and showed post-genital organ fusions. Furthermore, the cuticle of *atabcg11* plants contained reduced amounts of both wax and cutin (Figure 6B), whereas that of *atabcg12* plants was reduced only in wax content (Panikashvili et al., 2007). The finding that the *atabcg12 atabcg11* double knockout did not display a stronger phenotype than either of the single knockouts suggests that these two half-size ABC transporters can form heterodimers. Phenotypic differences between the respective single mutants furthermore suggest that at least one of the proteins can also form a homodimer or interact with a third Arabidopsis ABCG protein. Similar studies on *atabcg11* were pre-

sented by Luo et al. (2007), Ukitsu et al. (2007), and Panikashvili et al. (2007). In an earlier study, it was shown that *AtABCG11* is responsive to stresses such as salt treatment, wounding, and ABA application (Alvarado et al., 2004). Together, these results indicate that AtABCG11 is an integral part of cuticle production and deposition, and that its activity may change in response to environmental conditions. In a subsequent study, Panikashvili et al. (2010) extended our knowledge of AtABCG11-mediated wax and cutin deposition by demonstrating that this protein also plays a role in petal and silique formation and prevents seeds from fusing during development. Furthermore, they showed that the absence of *AtABCG11* affected the expression of many genes implicated in cuticle metabolism and suberin formation in roots. Recently, McFarlane et al. (2010) provided convincing evidence that AtABCG11 and AtABCG12 indeed form heterodimers, while AtABCG11 can also form homodimers (Figure 6B). The dominant role of AtABCG11 is also reflected by the fact that AtABCG11 can traffic to the plasma membrane in the absence of AtABCG12, while the latter requires the presence of AtABCG11 to be correctly targeted.

AtABCG13, which is closely related to *AtABCG12*, is mainly expressed in petals and carpels. Accordingly, in the *atabcg13* mutant, a strong flower phenotype with inter-organ fusions was observed (Panikashvili et al., 2011). Interestingly, this ABC transporter exhibits a substrate specificity that differs from that of AtABCG11 and AtABCG12. Determination of surface compounds on flower petals revealed that flower-specific cutin components were not present in the cuticle of *atabcg13*-silenced lines, while the wax components remained unaltered (Figure 6B).

Recently, an additional member of the ABCG subfamily, AtABCG32/PEC1, a full-size ABCG protein, was reported to be involved in the formation of the cuticular layer (Bessire et al., 2011). The *atabcg32* mutant did not exhibit an obvious growth defect, and displayed organ fusions in only 2% of the population. The leaves and petals of *atabcg32* mutant plants were more permeable to toluidine blue staining, lost more water, and were hypersensitive to herbicide treatment, but resistant to infection by *Botrytis cinerea,* indicating that the mutant was defective in cuticle formation.

AtABCG32 was strongly expressed in the epidermis of expanding organs of the shoot, and the encoded protein displayed polar localization at the plasma membrane. In contrast to other mutants of *ABCG* genes that are involved in cuticle formation, the *atabcg32* mutant differed from the corresponding wild type only in the content of minor aliphatic cutin monomers (Figure 6B). The cuticles of the leaves and petals of the *atabcg32* mutant appeared to be normal; however, the cuticular layer, which is the inner boundary of the cuticle and cell wall, was more diffuse and less electron-dense. Nanoridges, which are a characteristic of the petal surface structure, exhibited an irregular shape, size, and distribution. The observation that the changes in cuticular lipids of the *atabcg32* mutant were different from those in other *abcg* cuticle mutants and that the expression of *AtABCG11* and *AtABCG13*, which are also expressed in the epidermis and involved in cutin formation, were not affected in the *atabcg32* mutant, suggests that AtABCG32 has a distinct substrate specificity and function in the cuticle formation. A homolog of *AtABCG32* was shown to be required for keeping transpiration low in barley as well as in rice (Chen et al., 2011).

VI.C.2. AtABCG26 is involved in the transport of sporopollenin precursors for pollen wall formation

Pollen grains are coated with lipophilic layers, which serve a similar protective function to the cuticle layer. The pollen wall is composed of the intine, which mainly consists of cellulose, and the exine, which mainly consists of sporopollenin, a polymer of fatty acid derivatives and phenylpropanoids. Pollen coats contain steryl ester as a main component, as well as flavonoids and alkanes as minor components (Hsieh and Huang, 2007). Most of the material in the pollen wall and coat are supplied by the tapetum, although the intine is supplied by the pollen itself. Many enzymes involved in the synthesis of sporopollenin precursors were discovered by identifying the genes responsible for pollen mutant phenotypes (Aarts et al., 1997; Morant et al., 2007; de Azevedo Souza et al., 2009; Dobritsa et al., 2009; Dobritsa et al., 2010). However, the mechanisms of the transfer of lipidic precursors have not been revealed until recently. Since the exine is formed before the tapetum degenerates by programmed cell death, it has been hypothesized that the lipophilic compounds are transported through the plasma membranes of the tapetal cells. Within the last two years, four papers were published that highlight the important role of AtABCG26 in this process (Quilichini et al., 2010; Xu et al., 2010; Choi et al., 2011; Dou et al., 2011).

Loss-of-function mutants of *AtABCG26* showed a dramatic decline in seed production. They failed to develop mature pollen (Figure 6A), which correlated with a defect in pollen exine formation. Similar to several genes encoding the synthetic enzymes of sporopollenin precursors, *AtABCG26* was exclusively expressed in the tapetum during early flower development, which corresponds with its role in pollen exine formation. In a study that identified AMS, an essential transcription factor for tapetal cell development and postmeiotic microspore formation (Xu et al., 2010), it was shown that *AtABCG26* is also regulated by this transcription factor. Locular inclusions, similar to those found in *atabcg11* and *atabcg12* mutants, were also observed in *atabcg26* (Quilichini et al., 2010). In the tapetum, electron-dense particles, which were interpreted as sporopollenin in previous reports on two other pollen wall mutants, *no exine formation* (*nef*) *1* and *defective in exine formation* (*dex*) *1* (Paxson-Sowders et al., 1997; Ariizumi et al., 2004), accumulated (Choi et al., 2011). Finally, AtABCG26 localized to the plasma membrane. Together, these observations strongly suggest that AtABCG26 exports sporopollenin precursors out of the tapetal cells and into the locules for pollen exine formation (Figure 6C). Several other ABC transporters expressed in anthers may play additional functions in pollen development or be responsible for the release of specific compounds required for the assembly of a functional sporopollenin complex.

The ABCG transporters involved in surface lipid formation are localized to the plasma membrane. Thus, it is still not clear how these lipidic compounds are transported from the endoplasmic reticulum to the plasma membrane, and then from the apoplastic space to the surface. Further, the existence of yet-to-be-identified lipid-binding and possibly also transporting families has been postulated. The next step in understanding the formation of surface lipids will include the identification of these components that cooperate with ABC transporters to bring about correct surface lipid deposition (Debono et al., 2009; Lee et al., 2009).

VII. ABC TRANSPORTERS INVOLVED IN PLANT-MICROBE INTERACTIONS

Anti-microbial plant secondary metabolites, such as phenolics, terpenoids and their derivates, alkaloids, glucosinolates, and cyanogenic glycosides form an important first line of defense against host and non-host pathogens (Osbourn, 1996). They inhibit the proliferation of fungal and bacterial microbes on aerial plant surfaces within the rhizosphere and in the apoplast around local infection sites. There is increasing evidence that the above-ground and below-ground secretion of such compounds is in part mediated by ABC transporters. Transcript profiling of ABCG full-size transporters of Arabidopsis revealed that the expression of about half of these transporters positively respond to jasmonic acid (JA) and/or salicylic acid (SA), two phytohormones that have been implicated in biotic stress responses. About half of the half-size ABCG transporters are induced by jasmonate, but only a fourth by salicylic acid (Genevestigator). Transcript levels of several ABCGs are up-regulated by jasmonate as well as salicylic acid, while others exhibit a specific induction by one of these compounds. This suggests that several ABCGs are involved in pathogen defense and/or in the cross-talk between the plant and microorganisms. This hypothesis is supported by the observation that the transcript levels of more than 50% of the ABCG transporters are up-regulated when treated with microorganisms. Indeed, loss-of-function of one full-size ABCG transporter, AtABCG30/AtPDR2, resulted in a drastic change in soil microflora in the rhizosphere that the authors attributed to an altered root-exudate composition in the mutant (Badri et al., 2008; Badri et al., 2009).

Plant-derived secondary compounds can also serve as powerful attractants of beneficial microbes, such as mycorrhizal fungi and rhizobacteria (Peters et al., 1986; Akiyama et al., 2005). Using plasma membrane vesicles from soybean, Sugiyama et al. (2007) provided evidence that the export of genestein, an isoflavonoid that acts as a plant-derived signaling molecule in the legume-rhizobia symbiosis, is catalyzed by an ABC-type transport mechanism. Since Arabidopsis does not form mycorrhizal associations and is not nodulated, insight into this fascinating topic requires that plant scientists work with other model plants that form this type of symbiotic interaction. In a study to identify genes required for correct arbuscular mycorrhiza (AM) development, Zhang et al. (2010) screened EMS mutagenized Medicago and identified a mutant producing tiny and shriveled mycorrhiza. They called this mutant *str1* for stunted arbuscules. Positional cloning revealed that the gene of interest codes for a half-size ABC transporter of the ABCG family. Interestingly, close homologues of this ABCG member are found in most plants but not in Arabidopsis, suggesting that it has a specific function in mycorrhization. The authors identified a close homologue, MtSTR2, and the *MtSTR2* RNAi plants exhibited a similar mycorrhizal phenotype to *str1*. Both genes are co-expressed and using BiFC the authors could show that they interact. However, the substrate of this transporter remains unknown.

Evidence for the involvement of a plant ABC transporter in the pathogen defense response was provided by the functional characterization of NpPDR1, a full-size ABCG protein of *Nicotiana plumbaginifolia* (Jasinski et al., 2001). NpPDR1 resides in the plasma membrane and is induced by the natural anti-fungal diterpenoid sclareol, which is also produced by *Nicotiana tabacum*. In isolated microsomes, NpPDR1 contributes to the transport of radio-labeled compounds that are closely related to sclareol, supporting the notion that sclareol is an *in vivo* substrate of NpPDR1. Transcript levels of this transporter were most abundant in the leaf epidermis and leaf trichomes (Stukkens et al., 2005), which constitute the first line of defense. Down-regulation of *NpPDR1* leads to a spontaneous and commonly lethal phenotype upon infection with the necrotropic fungus *Botrytis cinerea*. These plants were also highly susceptible to exogenously applied sclareol, indicating that NpPDR1 participates in basal plant defense. Studies on NpPDR1 have driven the idea that transporters of the ABCG subfamily are involved in the plant immune system.

In an extensive forward genetic screen of Arabidopsis mutants for increased susceptibility to the barley powdery mildew pathogen, Stein et al. (2006) identified AtABCG36/AtPDR8/PEN3 as a crucial factor in pre-invasive non-host resistance. The *AtABCG36* loss-of-function mutants were compromised in their capacity to prevent entry of two non-host biotrophs and one non-host necrotroph. On the other hand, they proved to be hyper-resistant to the compatible Arabidopsis powdery mildew pathogen, which the authors attributed to the hyperactivation of SA-dependent defense pathways observed in the mutant. Expression of an *AtABCG36-GFP* fusion construct under the control of the native promoter complemented the phenotype. The corresponding fusion protein was targeted to the plasma membrane, and displayed increased fluorescence intensity at infection sites. Two other loss-of-function mutants with a similar phenotype, *pen1* and *pen2,* were also recovered from the screen (Collins et al., 2003; Lipka et al., 2005). *PEN1* encodes a plasma membrane-located syntaxin, and *PEN2* encodes a myrosinase, a glucosyl hydrolyase implicated in the cleavage of glucose moieties from glucosinolates. By performing a detailed metabolite profiling, Bednarek et al. (2009) revealed that *pen2* mutants fail to accumulate two metabolites, the cysteine derivative, raphanusamic acid, and the tryptophan-derived indo-3-ylmethylamine. In contrast, two indole-derived glucosinolates, 4-methoxyindol-3-ylmethylglucosinolate (4MI3G), a putative precursor for raphanusamic acid and indo-3-ylmethylamine, accumulated to high concentrations in this mutant. The authors showed that cleavage of 4MI3G mediated by PEN2 is required to confer non-host-related pathogen defense. In a parallel paper, Clay et al. (2009) observed that Flg22-dependent callose deposition was strongly reduced in *pen2* and *pen3* mutants. Similarly, as in the work of Bednarek et al. (2009), Clay et al. (2009) found that the amount of 4MI3G was strongly increased in *pen2* as well as in the *pen3*/*atabcg36* mutant. These two publications showed for the first time that glucosinolates are produced not only to protect plants against herbivores, but also against pathogens. Also, these publications provide strong evidence that the cleavage product of 4-methoxyindol-3-ylmethylglucosinolate is a substrate of AtABCG36. However, if one considers that AtABCG36 is also required to protect plants against abiotic stress, such as heavy metal and salt stress (see chapter III), it is reasonable to speculate that AtABCG36 may be capable of transporting structurally unrelated compounds, as described for many ABC transporters.

Recently, a full-size ABCG transporter was identified as the responsible gene for a functional *LR34* (Leaf Rust 34) allele, which is characterized by a robust and durable pathogen resistance phenotype against leaf rust, stripe rust, and powdery mildew in

Triticum aestivum (wheat; Krattinger et al., 2009). *LR34* is predominantly expressed in adult foliar tissues, particularly of the flag leaf, and the highest transcript levels were found in the leaf tip, corresponding to the tissues that exhibit the phenotypic difference between the tolerant and susceptible wheat lines. Wheat varieties with functional *LR34* alleles can be distinguished phenotypically by the development of leaf tip necrosis in adult flag leaves. Despite its resistance-conferring properties, *LR34* is not responsive to pathogen inoculation, suggesting that it has constitutive rather than induced functions. In accordance, the expression of this gene is changed upon developmental stages not modulated by stress factors, unlike many other ABCG transporters. In contrast to NpPDR1 and AtABCG36, which are likely to be restricted to non-host resistance, LR34 is implicated in the defense against several compatible pathogens of fungal origin. LR34 is the only ABCG protein characterized to date that impedes the invasion and spread of compatible pathogens. It will be interesting to investigate whether a wheat- or *Gramineae*-specific compound is required for the tolerance, as described for PEN3.

Considering the participation of ABCG transporters in the secondary metabolite-based pathogen response, it is tempting to speculate that they also play a role in herbivore defense. This may include the deposition of a large variety of insect-deterring compounds on leaf surfaces. ABC transporters might have a role in this process, since jasmonic acid, a potent inducer of many full-size ABCGs, is also a major mediator of herbivory responses.

VIII. OUTLOOK

Throughout the last decade, different approaches such as phenotype analysis or targeted approaches have allowed considerable progress in the understanding of the role of ABC transporters in plants. However, in many cases the detailed transport studies are still missing, either due to technical problems or due to the fact that the desired radiolabeled compound is not available. Plant ABC transporters have so far been discovered in the membranes of most major plant organelles, where they contribute to a multitude of fundamental processes, such as detoxification, phytohormone transport, surface lipid deposition, and plant microbe-interactions. Thus, these transporters are indispensable for proper plant development and also for accurate signal transduction. Nevertheless, of the 130 ABC transporters annotated in the Arabidopsis genome (Verrier et al., 2008), only about 20 have been characterized on a functional level. As Arabidopsis harbors many members of this transporter family, some closely related members are predicted to have overlapping functions. In the case of ABC transporters implicated in surface lipid deposition, the mutants described still excrete a considerable amount of cutin and wax components, and it is therefore likely that additional ABCGs are involved in this important process. It is also likely that additional ABC proteins involved in auxin and abscisic acid transport are present in the Arabidopsis genome, since so far the transport of these hormones has been addressed only for some specific organs, tissues, or cell types. Despite the expected redundancies amongst the ABC transporters, we predict that many ABC proteins with novel functions will soon be discovered. Considering that there are differences in the substrates of surface lipid ABC transporters, and based on the large number of ABCGs and knowledge from the animal field, additional lipid transporters, such as sterol or brassinolide transporters, are expected to exist (Berge et al., 2000). Some of these functions will be revealed by specific screens, while for others, targeted approaches involving the creation of multiple knockouts will be required to identify a small group of genes with a specific function.

We are just starting to understand the role that ABC transporters play in plant-pathogen interactions. The data presented for PEN3/AtABCG36 and wheat LR34 indicate that ABC transporters could operate in a species-specific manner. However, the nature of the putative transported compound which is required for resistance is still elusive. On the other hand, there are some cases where biochemical analysis has demonstrated that ABC-type transport mechanisms are responsible for the transfer of certain substrates, but the corresponding transporter has not been identified. Examples of such cases include glucosylated solutes, peptides and monolignols (Gaillard et al., 1994; Bartholomew et al., 2002; Stacey et al., 2002; Dean and Mills, 2004; Miao and Liu, 2010; Ramos et al., 2011). The identification of the monolignol transporter is of particular interest, since it will not only allow for a better understanding of lignification, but may also have practical implications for biofuel production. Furthermore, it is tempting to speculate that, in some cases, ABCC transporters may be involved in the vacuolar transport of alkaloids, which can accumulate to very high concentrations in regions where it is questionable that the ion trap mechanism would be sufficient to create the predicted concentration gradient of alkaloids between the cytosol and the vacuole (Roberts et al., 1991; Martinoia et al., 2000). Xanthoxal, the precursor of ABA synthesized in the chloroplasts, is another potential substrate for the ABC transporters since it is exported to the cytosol where the last step in ABA synthesis occurs.

Finally, initial results have provided evidence that ABC transporters expressed in roots may have an impact on the interaction between plants and microorganisms. Further studies may provide insight into this cross-talk. The complex changes in root exudates observed in some ABC transporter knockout plants indicate that these transporters are integrated in the overall metabolism of the plant. Roles of many ABC transporters with subtle effects have not been recognized so far since their loss-of-function mutants do not exhibit any dramatic phenotype, but they may play an important role in the fine-tuning of metabolism. The importance of some ABC transporters for the plant survival under natural conditions might emerge only when plants with mutations in these ABC transporters are grown for several generations.

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