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Minireview

ABC transporters involved in the transport of plant secondary metabolites

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Abstract **Plants produce a large number of secondary metabolites, such as alkaloids, terpenoids, polyphenols, quinones and many further compounds having combined structures of those groups. Physiological roles of those metabolites for plants are still under investigation, but they play, at least in part, important functions as protectants for plant bodies against herbivores and pathogens, as well as from physical stresses like ultraviolet light and heat. In order to accomplish these functions, biosyntheses and accumulation of secondary metabolites are highly regulated in a temporal and spatial manner in plant organs, where they can appropriately accumulate. In this mini-review, I introduce the mechanism of accumulation and membrane transport of these metabolites, in particular, focusing on ATP-binding cassette transporters involved.**

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Keywords: Secondary metabolite; Alkaloid; Terpenoid; Phenol; Plant ABC transporter; Primary transport

1. Introduction

Higher plants produce a vast number of secondary metabolites, in addition to primary metabolites, via complex pathways, which are regulated in highly sophisticated manners [1]. Many of them show strong biological activities, e.g., inhibition of DNA and protein synthesis, inhibition of the nerve system, cardiac activity, modulation of microtuble structure, etc. Bioactive secondary metabolites have been, therefore, utilized as natural medicines and often such plants containing those compounds have been used as medicinal plants and prescribed in many recipes as forms of crude drugs [2,3]. In most cases these bioactive natural compounds are found in particular organs, which are called ''medicinal part'' in pharmacognosy, and their contents in such organs are often seasonally regulated [4]. The physiological roles of these secondary metabolites for plants have not been completely elucidated, but reasonable explanations have been made for some secondary metabolites, i.e., they may function as biological protectants from herbivores, pathogen attacks and abiotic environmental stresses such as UV irradiation [5,6]. For instance, nicotine of tobacco or caffeine of coffee tree was reported to act as strong insecticides [7,8]. Some secondary metabolites are known to function as mediators necessary for the interaction with other organisms, as being allelopathic substances or insect attractants to facilitate pollination [9]. To achieve those functions, accumulation or secretion of those compounds has to be highly regulated, for instance, flavonoids acting as UV protectant are specifically accumulated in epidermal cells [10], and insect attractants are emitted from flower petals [11]. Biosynthetic genes responsible for the formation of those secondary metabolites may be highly expressed in such tissues where the metabolites are mainly accumulated, while translocation of natural compounds among plant organs often occurs as well, e.g., biosynthetic genes for nicotine, a pyrrolidine alkaloid of *Nicotiana* species, are mostly expressed in root tissues (source organ) whereas it is transported to the aerial part and accumulated in leaves (sink organ) [12]. The membrane transport of plant secondary metabolites is a newly developing research area [13], and it has been found that ATP-binding cassette (ABC) transporters are involved in some plant systems. In this mini review, I overview the involvement of ABC transporters for the membrane transport of endogenous secondary metabolites in plants and also those mediating the transport of plant products in heterologous systems as well. The comparison of ABC transporters in both systems is discussed.

2. Plant ABC transporters for endogenous secondary metabolites

2.1. Alkaloids

Alkaloids are nitrogen-containing low molecular weight compounds, which are found in about 20% of plant species. This diverse group implies most bioactive metabolites, and approximately 12000 compounds are elucidated to date [14]. Bioactive alkaloids, which influence for example the stability of chromosome structures or inhibit the DNA duplication, can be potentially toxic to plant cells but the producer plants seem to be insensitive to their own metabolites. For instance, when berberine was added to various plant cell cultures, it showed strong cytotoxicity to berberine-non-producing plant species like tobacco, while *Thalictrum minus* as well as *Coptis japonica*, both berberine producers, exhibited clear tolerance to this endogenous isoquinoline alkaloid [15,16]. Moreover, *C. japonica* cells revealed an ability to take up berberine from the medium against the concentration gradient when exogenously added to the culture medium, and the absorbed berberine was exclusively accumulated in the vacuoles [17] (Fig. 1).

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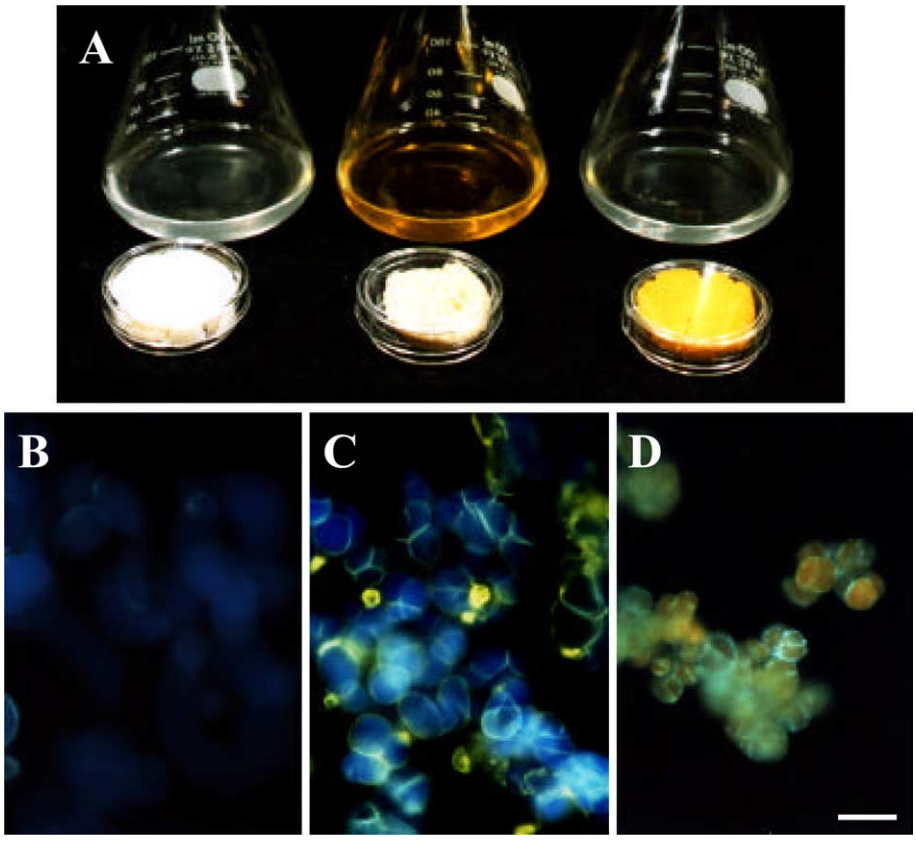

Fig. 1. Berberine producing plant cell cultures. (A) *T. minus* cell cultures without benzyladenine (BA) (left), those with BA (middle), and *C. japonica* cell cultures (right). Yielded cells (petri dishes) and their cultured media (conical flasks) are shown. Berberine is produced and secreted into the medium by *T. minus* cell cultures, whereas this yellow alkaloid is accumulated inside the cells of *C. japonica*. (B–D) Fluorescent micrographs of these cells. Large central vacuoles show blue fluorescence in *T. minus* (B,C) and the yellow fluorescence of berberine is observed at cell walls of the cultures with BA (C). The secreted berberine is often crystallized in the medium. Berberine is exclusively accumulated in the vacuoles of *C. japonica* (D).

In the cellular transport of berberine by *C. japonica* cells, two transport events are involved, i.e., uptake of berberine at the plasma membrane and efflux from the cytosol into vacuolar lumen at the tonoplast. Inhibitor experiments suggested the possible involvement of an ABC transporter in the cellular transport of berberine by the cultured cells [18]. Then, a multidrug resistance (MDR, or ABCB)-type ABC transporter was cloned from the *C. japonica* cell cultures via homology-based RT-PCR as a candidate of berberine transporter [19]. Functional analyses of the ABC transporter designated CjMDR1 was done with *Xenopus* oocytes, which showed that this ABC transporter recognized berberine as its substrate and transported it in an inward direction [20]. This was the first example of an eukaryotic ABC transporter mediating the uptake and not the efflux of a substance (Fig. 2).

CjMDR1 was localized to the plasma membrane of *C. japonica*. Berberine is biosynthesized in root tissues, and then translocated to the rhizome and trapped by the plasma membrane-localized CjMDR1, resulting in its accumulation in the rhizome. Since the rhizome is the sink organ also for starch, this plant accumulates the alkaloid having strong antimicrobial activity as a chemical defence against the soil-borne microorganisms. Contrary to the plasma membrane, the vacuolar transport of berberine in this plant cell is not mediated by an ABC transporter, but is dependent on the H^+-gradient across the tonoplast, suggesting that a H^+-antiporter is responsible for the berberine transport in vacuoles [21].

In another berberine producer, *T. minus* cell cultures, which secreted berberine to the culture medium, the possible involvement of an ABC transporter in berberine secretion was demonstrated [16,22] (Fig. 1). Interestingly the identified ABC transporter shared high similarity with CjMDR1 (our unpublished data). The regulatory mechanism which determines the direction of transportation is now under investigation.

For the transport of berberine, a vesicle-mediated mechanism was also proposed in a different plant [23]. The terminal steps of berberine biosynthesis takes place exclusively in specific intracellular vesicles in *Berberis*, which are probably derived from the endoplasmic reticulum (ER) and later fuse with the central vacuole. This scheme fits plants that produce and accumulate berberine in the same cells, but the carrier-mediated mechanism is appropriate for the plants whose sink and source organs are distant like in *C. japonica* [20].

One of the most known examples of long-distance transport is nicotine alkaloids in *Nicotiana* species. Nicotine is biosynthesized in root tissues, where it is specifically increased in the response to attacks by pathogens and herbivores, and the produced nicotine is translocated to the aerial part for accumulation [24]. Considering the translocation, this alkaloid should be loaded into xylem tissue and unloaded at mesophyl cells where nicotine is finally accumulated in the vacuoles [25]. This process implies transport of nicotine across at least three different membranes, plasma membranes in the root and in the leaf, and the vacuolar membrane of mesophyl cells, while no

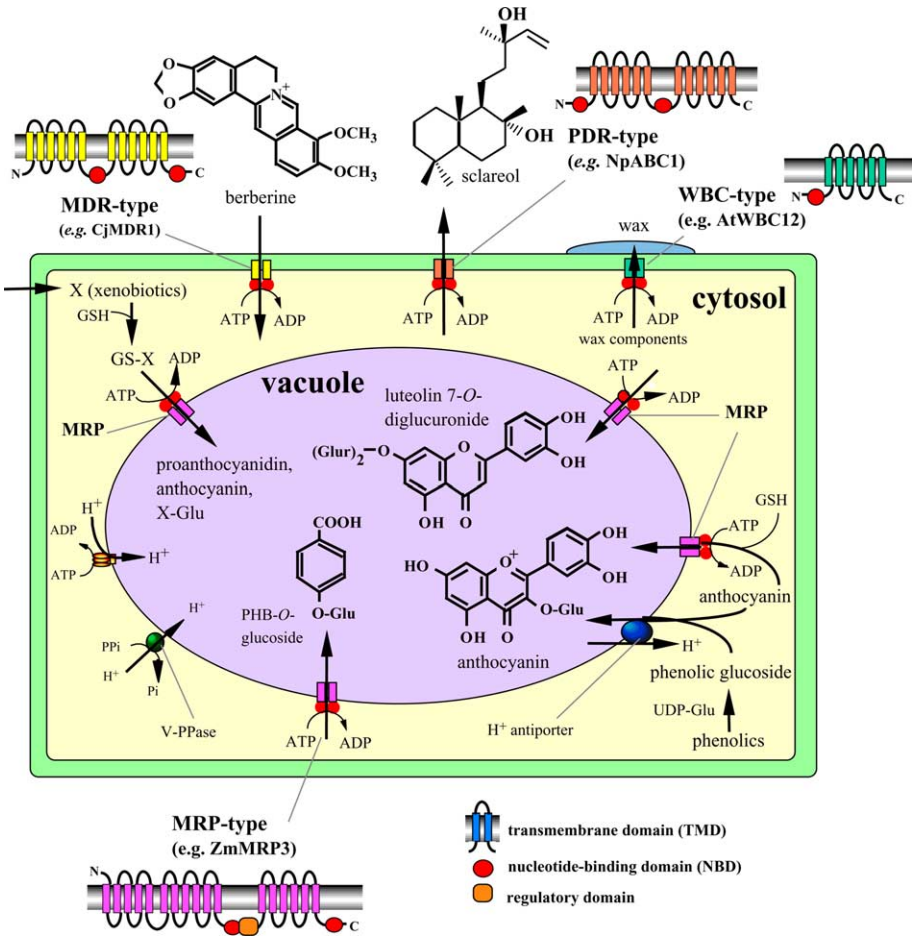

Fig. 2. Scheme of membrane transport of secondary metabolites and involved ABC transporters in plant cell. Representative natural products, which are proposed to be transported by plant ABC transporters, are drawn. Typical topologies of each ABC transporter subfamily are also drawn. Transport processes of each secondary metabolite mediated by ABC transporters are described in the text.

specific nicotine transporter is identified so far. ABC-type protein may take a role in a membrane transport event of this alkaloid.

Another isoquinoline alkaloid morphine, a major alkaloid in the latex of opium poppy, is accumulated in the large membranous vesicles of such latex. Immunofluorescence analyses using antibodies specific for five enzymes of alkaloid formation in opium poppy was recently reported [26]. In capsule and stem, two *O*-methyltransferases and an *O*-acetyltransferase were found predominantly in parenchyma cells within the vascular bundle, while codeinone reductase was localized to laticifers. Another group reported that three of those biosynthetic enzymes of morphine were localized in sieve elements of this plant [27]. In either case, the transport of the intermediate from specific cell-type of vascular tissue to laticifer was proposed, where an involvement of ABC transporter might be possible.

The early work by Zenk indicated that the vacuolar transport of indole alkaloids was mediated by a H^+-antiporter [28], although no endogenous transporter gene for indole alkaloids has been, to my knowledge, isolated thus far. Recent studies demonstrated that indole-3-acetic acid transport is mediated by some ABC transporters of MDR (ABCB)-type [29–32]. Moreover, the inhibitory activity specific to auxin was reported in indole alkaloids, such as brucine and yohimbine, as competitors [33]. These papers may suggest that an ABC transporter is involved in the transport of indole alkaloids in plants.

2.2. Terpenoids

Terpenoids are probably the most divergent secondary metabolites in the chemical structure. To date more than 25000 compounds were isolated and their structures were elucidated [14]. Terpenoids are biosynthesized by condensation of the monomeric C5 unit, dimethylallyl diphosphate and isopentenyl diphosphate, and they are classified according to the degree of condensation as hemi-, mono-, sesqui-, di-, sester-, tri, tetra- and polyterpenoids. Contrary to the phytochemical and biosynthetic studies, membrane transport of terpenoids is still largely unknown, except for the diterpene compound, sclareol. This antifungal compound is a dicyclic natural metabolites synthesized by *Nicotiana* species. As a study on the plasma membrane proteins that are inducible by sclareolide an antifungal analogue, a pleiotropic drug resistance (PDR)-type ABC transporter was identified [34]. The gene expression of this PDR member (NpABC1) was strongly induced in response to both sclareol and sclareolide in the leaves of *N. plumbaginifolia*, and the possible excretion of these diterpene derivatives to leaf surface were suggested by inhibitor experiments. An orthologue was isolated from tobacco [35], in which

the ABC transporter also showed a close relevance to the pathogen response. The *Arabidopsis* genome has 15 members of PDR subfamily, and one of them AtPDR12 was reported to be strongly induced by elicitor treatment, suggesting its direct involvement in the pathogen resistance processes by transporting antimicrobial metabolites [36]. Similar inducibility of a PDR orthologue was also reported in *Spirodela polyrrhiza* and the resistance against sclareol was revealed in both *Arabidopsis* [36,37] and *S. polyrrhiza* PDRs by the germination assay in which root elongation was evaluated [38]. These data are indicative that PDR members of *Arabidopsis* and *Spirodela* may recognize sclareol or other natural compounds of similar structure as the substrate and transport it in outward direction. However, it is yet unknown whether or not this diterpene is actually biosynthesized in those plant species. Further information about the PDR family in plants is available in a recent review [39] (Fig. 2).

Accumulation of terpene compounds has been described in many plants, for example, monoterpenes in Labiatae plants are biosynthesized in secretory cells and accumulate in the epicuticular cavity of glandular trichomes [40], while terpenoids of woody plants are secreted into the resin duct [41]. For volatile mono- and sesquiterpenenoids, their emission from flowers of *Arabidopsis* [42], snapdragon [43], and from leaves of woody plants [44] has been reported. Further, dramatical increase in the emission of volatile terpenoids was demonstrated by insect attacks in maize leaves [45] and cotton flower buds [46], where biosynthetic genes were strongly induced under these condition. Excretion of higher terpenoid is also known, i.e., the hydrophobic triterpene bryonolic acid is highly accumulated in the apoplastic space of some plant cell cultures of Cucurbitaceae, and is probably attached to the cell wall [47]. To my knowledge, however, the transporter molecules involved in the secretion of those terpene compounds are not identified yet. Besides, for isoprene, which is a highly volatile hemiterpene (C5) emitted in a large amount from leaves of some plant species like poplar, no transporter seemed to be required for the emission [48].

2.3. Phenol

Phenolic secondary metabolites involve simple phenylpropanoids including coumarins and lignans, flavonoids, and also polyphenols of high molecular weight such as tannins. Many of these phenolic secondary metabolites are involved in plant pathogene interaction, protectants against abiotic stress, and in the formation of structural components like lignins. Phenylpropanoids and flavonoids are one of the most intensively studied plant secondary metabolites, not only for the chemical structures but also for the biological activities and the biosynthesis [49–51], in particular in the context of plant defense.

Many phenolic compounds are detected in glycosylated form in plants. Glucosidation plays a key role in detoxification of endogenous secondary metabolites and also xenobiotics in plants, and these glucosides often end up accumulated in the vacuoles. Multidrug resistance-associated protein (MRP or ABCC)-type ABC transporters are reported to be involved in the vacuolar sequestration of such glucosides, in addition to glucuronides and glutathione conjugates [52]. According to studies by Martinoia's group, there seemed to be an apportionment of transporter types either for the endogenous or exogenous substrates. For instance, a flavonoid glucoside, isovitexin, a native C-glucoside in barley, was transported into the isolated vacuoles of barley via electrochemical gradient-dependent secondary transport, whereas a herbicide glucoside of hydroxyprimisulfuron was taken up by directly energized primary transport mechanism [53]. They also reported that the uptake of the main barley flavonoid saponarin, an apigenin glucoside, into barley vacuoles occured via H^+-antiport, whereas the transport of saponarin into *Arabidopsis* vacuoles, a heterologous plant that did not produce this metabolite, displayed typical characteristics of an ABC transporter [54] (Fig. 2).

Studies on the transport mechanism of phenolic compounds have been probably most actively done for anthocyanins due to the fact that they play a central role in flower colour formation. Most anthocyanins are glycosylated and accumulated in the vacuoles, except for some anthocyanins that were found in apoplastic space, like riccionidin A in *Rhus javanica* [55]. The involvement of MRPs (ABCC) in the vacuolar transport of such phenolic glucosides was suggested in the *bronze-2* (*bz2*) muntant of maize [56]. This mutant, in which *bz2* encodes a glutathione *S*-transferase, was defective in the accumulation of anthocyanin in the vacuole. Because MRPs have a substrate preference for glutathione conjugates, and since their transport activity was often stimulated in the presence of glutathione, the involvement of MRPs in the vacuolar transport of anthocyanin was presumed. Similar results were also reported in dicots, such as petunia [57] *Arabidopsis* [58] and carnation [59]. Further strong evidence for the involvement of MRP proteins in anthocyanin accumulation was provided via reverse-genetic studies by Goodman et al. [60]. The maize ABC transporter, ZmMRP3, was localized to the tonoplast, and is required for the anthocyanin accumulation process in maize (Fig. 2).

Contrary to those reports, the possible involvement of proton gradient-dependent transport for anthocyanin accumulation was also reported. The *Arabidopsis* gene *tt12* showed strong reduction in the proanthocyanidin deposition in vacuoles of endothelial cells [61]. The gene product of *TT12* was a secondary transporter-like protein belonging to the multidrug and toxic compound extrusion (MATE) family, suggesting that this protein might be responsible for the vacuolar transport of proanthocyanidin and anthocyanin via an antiport mechanism. A similar MATE-protein MTP77 was also reported in tomato [62], whereas further biochemical evidences are needed to prove the direct involvement of these antiporters in the anthocyanin transport.

The preference for a certain conjugated hydrophilic moiety for vacuolar transport of phenolic compounds, either glucose or glutathione, was analyzed using vacuolar membrane vesicles purified from red beet (*Beta vulgaris*) [52]. Whereas two phenol glucosides of *p*-hydroxycinnamic acid and *p*-hydroxybenzoic acid, were transported apparently by a H^+-gradient-dependent mechanism, the glutathione conjugate of a herbicide chlorsulfuron analogue appeared to be transported by an ABC transporter. Another experiment with phenylpropanoid derivatives showed that a glutathione conjugate of cinnamic acid was transported into the tonoplast vesicles via a GS-X pump, i.e., MRP-type ABC transporter [63]. These data suggested that the sugar moiety was a 'tag' to be recognized by the secondary transporters, while a glutathione moiety was a preferred 'tag' for MRP proteins functioning as primary transporters [64–66], although some glucosides seemed to be recognized by MRP-type ABC transporters. This indicates

that the combination between substrates and preferred transporter-types may fairly depend on plant species.

2.4. Wax

The plant body is covered by the cuticle, which is composed of cutin, polysaccharide and wax. The wax component is made of very long chain fatty acids and their derivatives. Recent finding showed that a half-size ABC transporter AtWBC12 (ABCG-type) in *Arabidopsis* was involved in wax secretion on the stem surface [67]. This member is in the reverse oriented subfamily of ABC transporter, and in the mutant plant (*cer5*) the wax components on the epidermal surface decreased to half compared to the wild type. Its localization at the plasma membrane was also revealed with GFP fusion protein. Since the substrates were very lipophilic, vesicle-mediated transport mechanism was also proposed for the wax secretion (Fig. 2). The putative interaction between the vesicle transport and ABC transporter will be a hot topic in near future.

3. Transport of secondary metabolites by non-plant ABC transporters

Several members of mammalian ABC transporters are known as efflux carriers of plant secondary metabolites especially those showing cytotoxicities, like alkaloids. Such transport studies have been intensively done in the field of cancer research, e.g., frequently used anticancer drugs, vincristine of *Catharanthus* species and paclitaxel (taxol) produced by *Taxus* species are preferred substrates of some human ABC transporters. Among many multispecific mammalian ABC transporters, the overlapping substrate specificities are observed, e.g., vincristine and vinblastine (indole alkaloids) are effluxed by human MDR1 (ABCB1) [68], MRP1 (ABCC1), and MRP2 (ABCC2) [69], but not by BCRP (breast cancer resistance protein, ABCG2) [70]; epi-podophyllotoxin and etoposide (lignans) are transported by human MDR1, MRP1 and MRP3 but not by MRP2; camptothecin derivatives (quinoline alkaloids) are preferred substrates of human MRP2 and BCRP but not of MDR1 or MRP1; taxol (diterpene derivative) is recognized by human MDR1 and BCEP (ABCB11) but not by MRP1 [68]; cardiac glycoside digitoxin (sterol glycoside) [71] and colchicin are also known substrates of MDR1 [72]. A similar substrate preference was also reported for the mouse *mdr1a* and *mdr1b* [73].

In humans, ABC transporters responsible for phytosterol efflux were identified. Two half-size ABC transporters ABCG5 [74] and ABCG8 [75], which were highly expressed in epithelial cells of intestine, were cloned via the analysis of the sterol storage disease sitosterolemia [76]. They were involved in the excretion of plant sterols derived from vegetables at the brush border of enterocytes as a heterodimer, resulting in decreased uptake of phytosterols. Their substrate specificity was described in a review [77]. While 29 genes are known to group in this subfamily in *Arabidopsis*, no member has been identified, which shows the function corresponding to ABCG5/ABCG8.

Fungal pathogenes are exposed to a variety of fungitoxic secondary metabolites produced by plants during pathogenesis [78]. ABC transporters can play an essential role in protection against those plant defense compounds during invasion. In the fungal pathogen *Magnaporthe grisea*, which caused rice blast disease, an ABC transporter similar to yeast multidrug resistance pump was identified [79]. The insertional mutant of this gene arrested the growth and the hypha died shortly after penetrating in epidermal cells of rice or barley, indicative that this ABC transporter was a pathogenicity factor. Its expression was indeed inducible by drugs and rice phytoalexin. Another ABC transporter gene acting as a virulence factor *MgAtr4* was identified in a wheat pathogen *Mycosphaerella graminicola* out of five similar genes of this fungi [80]. Disruption strain of *MgAtr4* displayed reduced intercellular growth in wheat leaves and less efficient colonization of substomatal cavities. The native substrate was not identified in both transporters yet, but the response of gene expression of fungal ABC transporter to plant metabolites might offer clues to find substrates. When gene expression was analyzed, two other members *MgAtr1* and *MgAtr2* responded to a phenylpropanoid eugenol and an alkaloid reserpine in their gene expression in a similar way as to the azole fungicides, suggesting that they were broad substrate drug efflux pumps [81].

Multispecific ABC transporters are particularly relevant to plant pathogens that have a broad host range since they are exposed to many plant defense compounds. *Botrytis cinerea* is an example of such a pathogen. A PDR-type ABC transporter *BcatrB* was isolated as a candidate drug efflux pump of broad substrate specificity [82]. *BcatrB* expression was upregulated by the grapevine phytoalexin resveratrol, a stilbene, as well as fungicides, and the gene replacement mutant became more sensitive to resveratrol. In gene expression analyses, some other fungal ABC transporters were also shown to respond to plant secondary metabolites [83,84].

A herbivor tobacco hornworm possesses a detoxification mechanism for nicotine. Transport activity similar to MDR1 (ABCB1) was reported in the Malpighian tubles of this insect, which excreted the alkaloid from the tissues [85]. The nicotine transport was inhibited by atropine, while vinblastine transport was suppressed by nicotine, indicative that the alkaloid transporter at the excretory Malpighian tubles recognized other alkaloids of different type. By immunostaining, the existence of a similar ABC transporter at the blood-brain barrier of insect for nicotine excretion was also suggested [86].

4. Modulation of ABC transporter by plant secondary metabolites

Secondary metabolites may play as endogenous modulators of plant ABC transporters. Some MDR (PGP or ABCB) members were reported as being auxin transporters [29–32], while flavonoid aglycones, such as kaempferol and quercetin, appeared to act as negative regulators of the auxin transport in *Arabidopsis* [31,87,88]. Possible function of flavonoids as endogenous modulators of plant MDRs are suggested.

Many compounds that modulate transport activity of mammalian drug efflux pumps are found among plant secondary metabolites. Camptothecin and its derivatives show strong inhibitory effect on topoisomerase I and are used as anticancer drugs of broad spectrum. BCRP (ABCG2) conferred resistance to these drugs by effluxing them, but the transport activity was inhibited by isoflavonoids like genistein and naringenin. The inhibitory effect by isoflavonoids seemed to be specific for BCRP since they did not influence the MDR1-mediated vincristine resistance or MRP1-mediated VP-16 resistance [89].

Inhibitor studies on ABC transporter function suggested that human MDR1 might also recognize carotenoid derivatives of paprica, such as capsaithin and capsorubin in lymphoma cells [90], as well as quinone compounds like hyperforin (prenylated phlorogucinol derivative) and hypericin (naphthodianthrone derivative) of *Hypericum* (St. John's wort) [91]. These compounds might not be direct transport substrates of MDR1 but were capable of modulating the transport of substrates as suggested for curcumin I [92] and sesquiterpenes from Celastraceae [93]. These modulators seemed to bind the ABC transporter molecule [93,94]. Further modulating activities of MDR1 transport by many secondary metabolites from medicinal plants were reviewed by Zhou et al. [95], e.g., curcumin and ginsenosides acted as inhibitors while quercetin and some catechin derivatives stimulated the transport activity by interacting directly with the MDR1 polypeptide. The complexity of the modulatory action was demonstrated for flavonoids, e.g., (−)epicatechin inhibited rhodamine transport while it enhanced the transport of another marker LDS [96]. Moreover, these effects of flavonoids were ABC transporter species-specific, for instance chrysin inhibited BCRP-mediated topotecan transport in rats, but no influence was observed on its pharmacokinetics in mdr1a/1b (−/−) mice [97].

The binding affinity of flavonoid with ABC transporters varies among transporter members. One of the intensive works was done with BCRP, in which the most probable binding site with flavonoid inhibitors, e.g., quercetin, was the nucleotide-binding domain (NBD) [98]. In contrast, flavonoid binding to the MDR1 polypeptide appeared to involve the ATP-binding site, sterold-binding site and substrate-binding site [95,99]. Multiple binding sites for flavonoid were also reported for human MRP1 [100]. Furthermore, prenylation of the flavonoid molecule tends to increase the binding affinity [100,101]. It is, however, an open question whether or not flavonoids can be substrates of those mammalian ABC transporters, except for genistein, which was shown to be transported in its native form by BCRP (ABCG2) in transcellular transport assay using LLC-PK1 [89]. If plant secondary metabolites are conjugated either with glucuronic acid or glutathione in the cells, they may be more likely recognized by some ABC members to be effluxed in a similar manner as quercetin, which was transported as glucuronide by rat BCRP1 [102].

5. Substrate recognition

Due to the intensive studies on the roles of some mammalian ABC transporters in multidrug resistance in cancer cells, the simple assumption that ABC transporters could generally exhibit broad substrate specificity was widely accepted. However, recent studies have demonstrated that their functions are not only restricted to detoxification processes [103], but also involved in many specific biological activities, such as translocation of endogenous metabolites and cell signaling, in which they show narrow substrate specificity [20,30,31,38], and other divergent physiological functions [67,104]. It has recently been suggested that the ABC transporter family has evolved because of the necessity of transporting the specific substrates in each organism, and not as drug efflux pumps [105].

The molecular mechanism of substrate recognition is still largely unknown. The amino acid sequence identity between human MDR1 (ABCB1) and MRP1 (ABCC1) is only 17%, although they show overlapping in the substrates to large extent. On the other hand, MDR1 and MDR2 share 75% amino acid identity but they show very different functions, i.e., the former is a multiple drug efflux pump whereas the latter is a flippase for phosphatidyl choline while MDR1 cannot transport this phospholipid [106]. Comparing CjMDR1, a fairly specific alkaloid transporter for endogenous berberine, to human MDR1 recognizing many plant alkaloids, there is 35% amino acid identity with strong similarity in the hydropathy profile, whereas no significant feature is found to explain their difference in the substrate specificity and the transport direction. To argue these points, three dimensional structure analyses of ABC transporters will be necessary.

6. Conclusions

The large number of ABC transporter genes in *Arabidopsis* and the involvement of mammalian ABC transporters in the efflux of plant-derived products, led to the hypothesis that plant ABC transporters largely contribute to membrane transport of endogenous secondary metabolites in the plant body. However, there are still only limited examples of transport studies on secondary metabolites in plant cells to date. One reason is that *Arabidopsis* is a model plant suitable for genetics while it is not absolutely ideal for secondary metabolite studies, because phytochemical analysis of this plant has been still very limited and the amount of those natural products in *Arabidopsis* is usually low. More active phytochemical analyses in *Arabidopsis* is expected, and also other plant model systems appropriate for secondary metabolism research will provide important information for those transport mechanism in plants.

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