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Correction: Learning from each other: ABC transporter regulation by protein phosphorylation in plant and mammalian systems

Bibek Aryal*, Christophe Laurent* and Markus Geisler*[1]
*Department of Biology – Plant Biology, University of Fribourg, Rue Albert Gockel 3, PER04, CH-1700 Fribourg, Switzerland

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Abstract

The ABC (ATP-binding cassette) transporter family in higher plants is highly expanded compared with those of mammalians. Moreover, some members of the plant ABCB subfamily display very high substrate specificity compared with their mammalian counterparts that are often associated with multidrug resistance (MDR) phenomena. In this review we highlight prominent functions of plant and mammalian ABC transporters and summarize our knowledge on their post-transcriptional regulation with a focus on protein phosphorylation. A deeper comparison of regulatory events of human cystic fibrosis transmembrane conductance regulator (CFTR) and ABCB1 from the model plant *Arabidopsis* reveals a surprisingly high degree of similarity. Both physically interact with orthologues of the FK506-binding proteins (FKBPs) that chaperon both transporters to the plasma membrane in an action that seems to involve Hsp90. Further both transporters are phosphorylated at regulatory domains that connect both nucleotide-binding folds. Taken together it appears that ABC transporters exhibit an evolutionary conserved but complex regulation by protein phosphorylation, which apparently is, at least in some cases, tightly connected with protein–protein interactions (PPI).

Key words: ATP-binding cassette (ABC) transporter regulation, ABCB, FK506-binding protein (FKBP), P-glycoprotein, protein phosphorylation.

Abbreviations: ABA, abscisic acid; ABC, ATP-binding cassette; ADL, adrenoleukodystrophy; CFTR, cystic fibrosis transmembrane conductance regulator; CK2, casein kinase II; ER, endoplasmic reticulum; FKBP, FK506-binding protein; Hsp, heat shock protein; IAA, indole-3-acetic acid; IBA, indolylbutyric acid; MDR, multidrug resistance; MDR, multidrug resistance; NBD, nucleotide-binding domain; PAT, polar auxin transport; PDR, pleiotropic drug resistance protein; PGP-1, P-glycoprotein 1; phot1, PHOTROPIN1; PID, PINOID; PIN, Pin-formed; PIS1, polar auxin transport inhibitor sensitive 1; PKA, protein kinase A; PKC, protein kinase C; PPI, protein–protein interaction; PPP2R3C, protein phosphatase 2A γ; SLC, solute carrier; SUR1/2, sulfonylurea receptor 1/2; TMD, transmembrane domain; TPR, tetratricopeptide repeat; TWD1, TWISTED DWARF1; VLCFA, very long chain fatty acid; Ycf1, yeast cadmium factor 1.

[1]To whom correspondence should be addressed (email markus.geisler@unifr.ch).

Importance of mammalian ABC transporters

ATP-binding cassette (ABC) transporters are integral membrane proteins ubiquitously present in all phyla [1]. They use the energy of nucleoside triphosphate (mainly ATP) hydrolysis to pump their substrates across the membrane [1]. The list of described substrates is long and diverse and includes metabolic products, sugars, metal ions, hormones, sterols, lipids and therapeutical drugs [1]. The ABC transport nano-machinery encompasses two transmembrane domains (TMDs) and two cytosolic nucleotide-binding domains (NBDs). ATP hydrolysis results in a conformational change of the NBDs, this in-turn brings the substrate across the membrane in a unidirectional fashion [2]. A total of 48 putative ABC transporters are encoded by the human genome and were shown to cluster into seven subfamilies (i.e. A–G) based on their domain assembly and phylogeny [3].

Most ABC transporters of the A subfamily, namely ABCA, are involved in lipid transport processes in different mammalian body locations. ABCA1 has a notable role in cholesterol efflux and Tangier disease which is due to harbouring a mutation in the ABCA1 transporter [4–6]. Further, member 4 of the A subfamily (ABCA4) acts as a transporter of *N*-retinylidene-phosphatidylethanolamine (NrPE). Malfunctioning of this transporter leads to the formation of lipid deposits in the macular region of the retina resulting in the Stargardt disease [7,8]. Mammalian ABCB1, also known as P-glycoprotein 1 (PGP1), is probably the best-characterized isoform of all ABCs: ABCB1 contributes to multidrug resistance (MDR) toward multiple cytotoxic chemotherapeutic agents when overexpressed in tumour cells [9,10]. ABCB2/TAP1 and ABCB3/TAP2 transporters are prominent members of the B subfamily, which are associated with the processing of antigens (TAPs) and function in the shuffling of peptides from the cytosol to the endoplasmic reticulum (ER) lumen [11,12].

The common and severe disease, cystic fibrosis (also called mucoviscidosis), is caused by highly prevalent point mutations in the cystic fibrosis transmembrane conductance regulator, CFTR/ABCC7, an ATP-gated chloride ion channel [13–15]. ABCC2 is responsible for the transport of conjugated bilirubin across the plasma membrane. A mutation in ABCC2 leads to the accumulation of bilirubin

and its conjugates in the liver and hence results in the Dubin–Johnson syndrome (DJS) [3,9,16,17]. Further, ABCC8 (SUR1) and ABCC9 (SUR2) are sulfonylurea receptors, which are the molecular targets of the sulfonylurea class of anti-diabetic drugs. The mechanism of SUR1 and SUR2 is to promote insulin release from pancreatic beta cells and a mutation in SUR1 is associated with permanent neonatal diabetes [18,19].

Members of the ABCD subfamily are thought to localize in the peroxisomal membrane. A recent study showed that a dysfunction of the ABCD1 transporter is responsible for adrenoleukodystrophy (ADL). ADL is a X chromosome-linked disease, which is characterized by an impaired oxidation of very long chain fatty acids (VLCFA) in peroxisomes and hence the accumulation of VLCFA in tissues and body fluids [3,9,20].

Some members of the ABCG subfamily, namely ABCG5 and ABCG8, are known to transport sterols. Defects in these transporters are responsible for sitosterolemis, which is characterized by the accumulation of plant sterols including cholesterol [15,21,22]. In analogy to ABCB1/PGP1 and ABCC1/MRP1, the breast cancer resistance protein (BCRP/ABCG2), has been identified as MDR transporter and shown to transport diverse therapeutical drugs. Nevertheless, the physiological *in vivo* substrate remains unknown until now [9,23].

In analogy to human ABCB1, also yeast and bacterial ABC transporters have been shown to play an important role in the degree of pathogenicity of the organism and to create MDR phenomena [4,24–28]. For instance, the pleiotropic *Candida albicans* ABC transporter, Cdr1 (Candida drug resistance 1), has been reported to be involved in the export of various drugs and antifungals and is known as homologue to the *Saccharomyces cerevisiae* PDR5 [26,29,30].

In summary, the study of mammalian ABC transporters has decoded the causes of several human diseases and provided us with essential insights in ABC functionality, allowing us to develop a range of successful disease treatments.

Regulation of mammalian ABC transporters by protein phosphorylation

Being one of the most important post-translational modifications, protein phosphorylation plays also a significant role in the regulation of ABC transporters. This mode of action is fine-tuned by the antagonism between protein kinases and protein phosphatases adding or removing phosphate groups from the transporters respectively [31]. As such it is not surprising that point mutations of key phosphorylation sites are among the most prominent causes of some diseases, e.g. during cystic fibrosis [32]. However, not only mutations in the peptide sequence of the transporter itself are the cause of several conditions or diseases but also mutations in their regulators (e.g. protein kinases) can be the cause of grave physiological disorders [4,32].

The most common kinases known to be involved in human ABC transporter phosphorylation are protein kinase A (PKA), protein kinase C (PKC) and the CK2 [4]. PKA for instance is involved in the regulation of K_{ATP} channels by phosphorylating ABCC8 [33], whereas ABCB1 is a target of PKC [34,35]. Furthermore, ABCA1 has been shown to be regulated by CK2 [36]. These three kinases can act in concert, as was shown in the case of ABCC7/CFTR. Interestingly, although kinase mutations are suspected to be involved in several diseases [32], these events have not been associated with any ABC transporter related disease yet. This could be due to the fact that the majority of kinases regulating ABC proteins are also involved in apoptosis decisions [37–39]. Consequently, organisms lacking these regulators, or presenting mutated versions of these proteins, might not be viable.

Nevertheless, protein kinase inhibitors, such as plant-derived flavonoids, have been investigated thoroughly in order to create new drugs against ABC transporter related diseases. Some flavonoids, like the flavonol quercetin [40,41], were shown to block protein kinase activities [40,41]. Therefore for long the research focus was laid on finding flavonoids that would inhibit the export activity of transporters involved in MDR in order to render anti-cancer drugs more efficiently [41]; this approach is nicely reviewed in [41]. In contrast, some flavonoids, like genistein, were previously thought to act on tyrosine kinases but were later shown to activate CFTR by directly interacting with the latter [42]. However, genistein was shown to be not suitable for the treatment of cystic fibrosis because it interacts with various other targets besides CFTR [43–45], whereas higher concentrations have an inhibitory action [42,43,46]. Linking inhibitory effects to protein structures might be helpful in designing flavonoid-based drugs with fewer side effects [43].

A plethora of studies in yeast and bacteria support the idea that protein phosphorylation might be an universal regulatory event of ABC transporters [47]. In the bakers yeast, *S. cerevisiae*, ABC transporters STE6, YCF1, PDR5 and CDR1 have been suggested to be regulated by phosphorylation [48–53]. A comprehensive list of identified and tested phosphorylation sites found in ABC transporters from the model species *Homo sapiens, S. cerevisiae and Arabidopsis thaliana* is provided in Table 1. From this list it gets clear that most investigated sites have an impact on the transport capacity of individual transporters, although it is not always obvious if phosphorylation leads to an activation or inhibition. A second group of phosphorylation events affects protein stability and/or protein dimerization. In the following, we will focus on human ABCB1 and CFTR/ABCC7 phosphorylation; for a more detailed description of the impact of protein phosphorylation on other ABC transporters, we refer to the excellent review by Stolarczyk et al. [4].

Several lines of clinical evidence suggest ABCBs as general targets for phosphorylation-dependent regulation in the so-called linker region. This linker region connects the N-terminal NBD1 of ABCBs with the TMD of the second half of the molecule (TMD2) and has been shown to regulate ABCBs by multiple phosphorylation events [54,55]. Linker

Table 1 | **List of experimentally confirmed ABC transporter phosphorylation sites in *S. cerevisiae*, *H. sapiens* and *A. thaliana* (n.i., not identified)**

ABC transporter	Organism	Phosphorylation site	Kinase	Effect	Selected reference
Ste6p	*S. cerevisiae*	n.i.	Yck1p	Protein stabilization	[48]
		n.i.	Yck2p	Protein stabilization	
Ycf1p	*S. cerevisiae*	S251	Cka1p	Negative regulation of transport activity	[49]
		S908	n.i.	Positive regulation of transport activity	[50]
		T911	n.i.	Positive regulation of transport activity	
MRP1	*H. sapiens*	T249	CK2α	Negative regulation of transport activity	[38]
ABCA1	*H. sapiens*	S1042	PKA	Positive regulation of transport activity	[117]
		S2054	PKA	Positive regulation of transport activity	
		T1242	CK2	Negative regulation of transport activity	[36]
		T1243	CK2	Negative regulation of transport activity	
		S1255	CK2	Negative regulation of transport activity	
		T1286	n.i.	Calpain-mediated degradation	[71]
		T1305	n.i.	Calpain-mediated degradation	
		n.i.	Cα(PKCα)	Structural stabilization	[70]
ABCB1	*H. sapiens*	S661	PKC	Regulation of transport activity	[34]
		S667	PKA; PKC	Regulation of transport activity	[34,35]
		S671	PKA; PKC	Regulation of transport activity	
		S683	PKA	Regulation of transport activity	[4,57]
		S683 (by consensus)	Pim-1	Prevents proteolysis and proteasomal degradation	[58]
ABCB2	*H. sapiens*	n.i.	Unnamed 40KD kinase	Negative regulation of transport activity	[118]
ABCB3		n.i.	Unnamed 40KD kinase	Negative regulation of transport activity	
ABCC7	*H. sapiens*	S660	PKA	Positive regulation of transport activity	[119–121]
		S670	PKA	Positive regulation of transport activity	[122]
		S700	PKA	Positive regulation of transport activity	[119–121]
		S712	PKA	Positive regulation of transport activity	[120,121]
		S737	PKA	Negative regulation of transport activity	[119–121]
		S753	PKA	Positive regulation of transport activity	[121]
		S768	PKA	Negative regulation of transport activity	[119–121]
		S795	PKA	Positive regulation of transport activity	[119–122]
		S813	PKA	Positive regulation of transport activity	
		S686	PKC	Unknown effect	[119,123]
		S790	PKC	Unknown effect	
ABCC8	*H. sapiens*	S1571	PKA	Regulation of the K_{ATP} channel	[33]
ABCC8	*H. sapiens*	n.i.	PKC	Regulation of the K_{ATP} channel	[124]
ABCC9	*H. sapiens*	S1387	PKA	Activation of the K_{ATP} channel	[125]
		T633	PKA	Activation of the K_{ATP} channel	[126]
		S1465	PKA	Activation of the K_{ATP} channel	
ABCD1	*H. sapiens*	n.i.	n.i. (tyrosine kinase)	Regulation of transport activity	[127]
ABCD3	*H. sapiens*	n.i.	n.i. (tyrosine kinase)	Regulation of transport activity	
ABCG2	*H. sapiens*	T362	Pim-1	Modulation of dimerization	[128]
ABCB1	*A. thaliana*	S634	PID	Negative regulation of transport activity	[103]
ABCB19	*A. thaliana*	n.i.	Phot1	Negative regulation of transport activity	[105]

phosphorylation alters ABCB transport and associated ATPase activity [56]. An accumulation of serine residues was identified to be phosphorylated by PKC [54,55,57] that regulate the drug transport properties [56]. In addition to PKC, various publications suggest ABCB1 phosphorylation by PKA and CK2 but a significant conclusion has yet to be made [4,34,35]. Moreover, human ABCB1 was shown to be phosphorylated by Pim-1 kinase protecting ABCB1 from degradation and enabling its glycosylation and cell surface expression [58].

The regulatory R domain of ABCC transporters linking also NBD1 with TMD2 is a discrete domain unique for CFTR and its orthologues [59]. The R domain is another prominent target of their respective protein kinases [4,35,60]. The Cl^- channel ABCC7 has been shown to be largely phosphorylated all over the R domain by protein kinases,

PKA, PKC, CK2 and 5′-AMP-activated protein kinase (AMPK) [3,15,61,62]. Computational and NMR analyses indicate that phosphorylation results in a conformational change of the R domain and hence facilitates the opening of the core allowing ions to be transported [4,63,64]. Based on the well-studied regulation by PKA, it seems that there is the requirement for a specific phosphorylation pattern in the ABCC7 R domain [18]. This pattern needs to be formed in a specific series of phosphorylation events in order to activate the activity of ABCC7 successfully [18]. Furthermore, it has been shown that the different protein kinases are interdependent [61].

The regulatory subunit B of serine/threonine-specific protein phosphatase 2A γ (PPP2R3C) plays a major role in the negative regulation for both, the expression and function of HsABCB1 [65]. Furthermore, it seems that in order to exert its dephosphorylating function correctly, PPP2R3C needs to form a complex with a second serine/threonine-specific protein phosphatase, namely PP5 [65]. This example demonstrates that protein–protein interaction (PPI) is a second important event during the regulation of ABC transporters. However, PPI is also required in other non-phosphorylation events. One of these mechanisms, the regulation of the correct post-translational protein folding and the subsequent trafficking is best illustrated using ABCC7 as example: CFTR interacts with the heat shock protein Hsp90 and the FK506-binding protein 38 (FKBP38/FKBP8). ABCC7 mutations lead to an improper folding and trafficking as wells as loss of stability of the protein and finally to an endoplasmic reticulum-associated degradation (ERAD) [66]. CFTR, while localized on the ER, is retained by the chaperone function of Hsp90 [67]. Hutt et al. [66] provided evidence that subsequent to this interaction, FKBP38 is recruited to the complex through its Hsp90-interacting TPR domain and induces the liberation of CTFR from the Hsp90 complex [66,68]. Once having removed Hsp90 from the complex, FKPB38 is thought to interact with CFTR via its TPR domain before shifting the interacting domain towards its catalytic domain [66]. This interaction will probably allow FKPB38 to isomerize peptidyl prolyl bonds of CFTR, which will in return allow for the establishment of the final and functional protein structure of CFTR [66,69].

Phosphorylation of ABC transporters plays also an important role in protein stability [66]. This is supported by the finding that ABCA1 stabilization is dependent on protein kinase PKCα [4,70,71].

Taken together, ABC transporters exhibit a tight and complex regulation by protein phosphorylation, which apparently is, at least in some cases, tightly connected with PPI, although the exact interconnecting mechanisms are currently not well understood.

Role of ABC transporters in plants

The first role accredited to plant ABC transporters has been intracellular (vacuolar) detoxification in analogy to PM export in mammalian cells [72,73]. However, since then the function of different members of the vast plant ABC transporter family was quickly extended [73]. Besides the sequestering of xenobiotic conjugates into the vacuole, there is plethora of ABC transporter substrates ranging from phytohormones (IAA, IBA, ABA and cytokinins), heavy metals, lipids, terpenoids, lignols and organic acids [74–77]. As a consequence, the roles of the members of the eight ABC subfamilies found in plants vary from plant development, over important physiological cues to pathogen response [73]. Since the ABC family in plants is so highly expanded (the model plant *A. thaliana* contains more than 120 ABC transporter genes [73]) and with it their functional diversity, we are going to focus in this review mainly on described regulatory functions that were derived from *A. thaliana*. For a more exhaustive description we refer to the excellent review by Kang et al. [73].

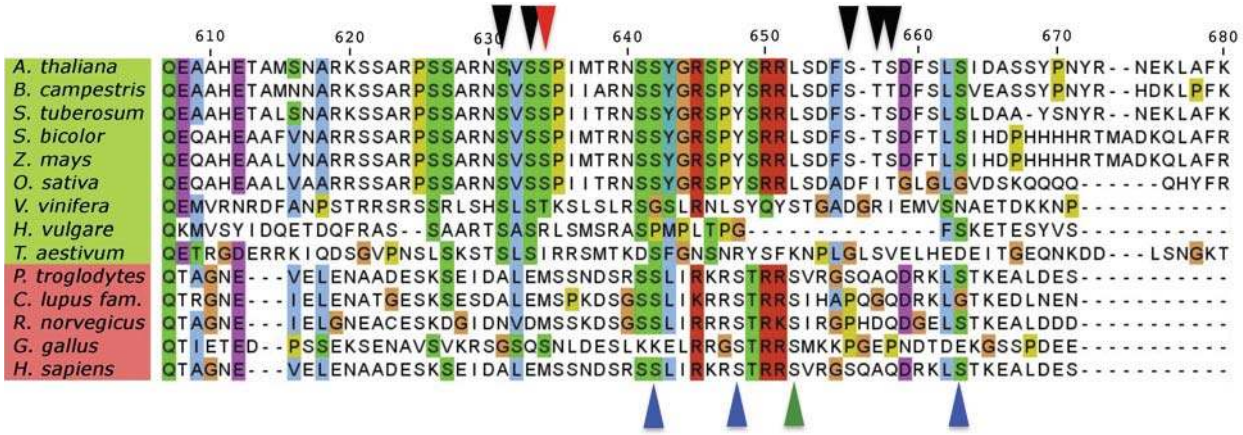
The classification of plant ABC transporters is in agreement with the nomenclature system known for their mammalian homologues and reflect their common evolutionary origin [73,78]. Hence, ABC proteins containing a TMD1–NBD1–TMD2–NBD2 domain signature are classified into the B family [78,79]. Well-studied examples of this subfamily in *A. thaliana* are ABCB1, ABCB19 and ABCB4, whereas initial reports concern also ABCB21. All four were show to transport the plant hormone, auxin [chemically indole-3-acetic acid (IAA)] [80–82]. Local gradients of IAA regulate plant growth and development and these gradients are created and maintained by the directional, cell-to-cell transport of IAA, in a process that is called polar auxin transport (PAT) [83]. This fascinating process is specific for higher plants and also not described for other plant hormones [84]. ABCB1 and ABCB19 were shown to function as IAA exporters functioning in the long-range transport of auxin [85], whereas ABCB4 and ABCB21 were characterized as facultative importers/exporters [81,82,86]. Interestingly, transport directionalities seem to be regulated by internal auxin concentrations [82]; a feature not yet described for any non-plant ABCB.

Another remarkable difference between plant and mammalian ABCBs lies in the fact that plant ABCB orthologues seem to own a far higher substrate specificity compared with their mammalian counterparts, especially upon overexpression in tumour cells [87]. Plant ABCB substrates are limited to a few native and synthetic auxins (and some organic acids) but do not transport any standard mammalian ABCB substrates even when they are expressed in mammalian cell cultures [75,85,88]. A molecular rationale for enhanced substrate specificities for plant ABCBs was recently given by the identification of kingdom-specific candidate substrate-binding regions within the translocation chamber formed by the TMDs of *Arabidopsis* ABCBs [89].

An involvement of ABCB1 and ABCB19 in PAT was originally based on the phenotype of the *Arabidopsis abcb1 abcb19* double mutant, which combines strong dwarfism, a disoriented growth and a helical rotation of epidermal root

Figure 1 | Plant and mammalian ABCB1 linkers and phosphorylation sites are only weakly conserved

Relevant plant ABCB1 linker phosphorylation sites (green organism names) identified either by phosphoproteomics (black [116]) or experimentally (red [103]) are indicated by triangles above the alignment. Experimentally verified phosphorylation sites of mammalian ABCB1 orthologues (red organism names) that are target of PKC (blue) or PKA (green) are indicated below the alignment. Figure modified from [103]: Henrichs, S., Wang, B., Fukao, Y., Zhu, J., Charrier, L., Bailly, A., Oehring, S.C., Linnert, M., Weiwad, M., Endler, A. et al. (2012) Regulation of ABCB1/PGP1-catalysed auxin transport by linker phosphorylation. EMBO J. **31**, 2965–2980.



layers (twisting) [80]. In parallel, a similar phenotype was found for an *Arabidopsis* mutant lacking the functionality of the FKBP42, TWISTED DWARF1 (TWD1) [85,90]. In the last decade evidence was provided that ABCB1 and ABCB19 transport activity depends on the physical interaction with TWD1 [90,91]. As discussed above, similar interactions have been reported also for human ABCC7 and FKBP38; a mechanistic comparison will be addressed further down in this review. Interestingly, ABCB1 and ABCB19 were also shown to functionally interact with two permease-like members of the PIN (Pin-formed) family, PIN1 and PIN2 [88]. Both regulatory events stress the importance of PPI in the regulation of ABC transporters.

An additional vital function covered by the plant ABC transporter toolbox is their involvement in pathogen defense strategies. In *Arabidopsis*, member 36 of the ABC subfamily G (ABCG36/PDR8) is known for its pathogenic defense properties [92]. However, ABCG36 has also been reported for being involved in the transport of heavy metals, like cadmium [93]. A close ABCG36 homologue, ABCG37/PDR9/PIS1, was found to export a range of auxinic compounds, including the IAA precursor, indolylbutyric acid, IBA [94]. ABCG37 was shown also to be responsible for the export of phenolic compounds upon Fe deficiency [95]. Interestingly, both ABCG36 and ABCG37 reside on the outer lateral PM domain of epidermal root cells that defines the root and soil interface [94].

Recently, ABCG16 was reported to play a role in pathogen resistance against *Pseudomonas syringae* [96]. Furthermore, like for ABCG36, also for ABCG16 a second transport function was described that provides tolerance to the phytohormone, ABA (abscisic acid) [96]. Previously, ABCG25 was reported to export ABA from the cell, whereas ABCG40/PDR12 seems to mediate the import into cells [76,97]

Therefore the question arises whether the ABCG transporter class has a broader substrate specificity as described for its yeast orthologues [98–100]. An alternative explanation is that putatively identified ABCG substrates, such as the phytohormones IBA or ABA, might act as compounds that bridge pathogen-related and developmental roles [73,93].

Taken together, these two excerpts underline the impression that plant ABC transporters cover a very broad array of functions, although most of them still need to be uncovered compared with their mammalian counterparts.

Regulation of plant ABC transporters via protein phosphorylation

As for their mammalian counterparts, recent advancements in phosphoproteomics have enabled large-scale analysis of plant ABC phosphopeptides from complex protein mixtures by LC–MS/MS [101,102]. A recent comparison of plant and mammalian ABCB1 linker domains has revealed, however, a very low degree of conservation both on the sequence levels and in respect to the described phosphorylation sites [103] (see Figure 1). This might reflect – beside the overall similar concept of ABCB regulation by linker phosphorylation – an evolutionary split. This is obviously also supported by the finding that classical isoforms of PKA, PKC and PKG do not exist in plants [104].

Beside these proteomics data, until today only two studies have studied in detail plant ABC regulation by protein phosphorylation. Both studies suggest that AGC kinases, PHOTROPIN1 (phot1) and PINOID (PID), have

a direct impact on the auxin efflux activity of ABCB19 and ABCB1 respectively [103,105]. AGC kinases are plant orthologues of mammalian protein kinases A, C and G [104], however, evolutionary adaptations introduced specific structural changes within the AGC kinases that most likely allow modulation of kinase activity by external stimuli (e.g. light) [106].

The first report that plant ABCBs are regulated by protein phosphorylation came from the finding that auxin efflux transporter ABCB19 is an interactor of the ACG4 kinase, phot1, acting as a PM-located blue light receptor [105]. Co-expression of phot1 in Hela cells reduced ABCB19 mediated auxin efflux, which is further enhanced by blue light irradiation, whereas ABCB1 activity remained unchanged [105].

Employing co-immunoprecipitation/LC–MS/MS and bioluminescence resonance energy transfer (BRET) analyses, the AGC3 kinase, PID, was characterized as a physical interactor of the FKBP42, TWD1. PID was shown to phosphorylate S634 located in the linker domain of ABCB1 resulting in ABCB1 activation (see Figure 1). On the other hand, negative regulation of ABCB1 in the presence of TWD1 argues for a second, TWD1-specific ABCB1 phosphorylation by PID that does not essentially need to be part of the linker. As such TWD1 might function as a recruiting factor for ABCB1 phosphorylating protein kinases. Both modes of ABCB1 phosphorylation could take place in parallel or in competition resulting in the fine-tuning of ABCB1 as reported for mammalian ABCBs [18].

Interestingly, like mammalian ABCBs also plant ABCBs are inhibited by flavonoids [75,85]. This is of interest because flavonols were suggested to act as non-essential regulators of auxin transport [107]. Further, flavonols, including quercetin, were shown to efficiently disrupt *Arabidopsis* ABCB1–TWD1 interaction but not to bind to TWD1 [108], suggesting that plant ABCB regulation via flavonols might employ PPI. Obviously, in light of the fact that flavonols are also inhibiting protein kinases (such as PID) and the discussed ABCB1–TWD1 and TWD1–PID interaction, these findings indicate that ABCB regulation via PPI and protein phosphorylation might be interconnected.

Comparison of *H. sapiens* ABCC7 and *A. thaliana* ABCB1 regulation

As mentioned above, human ABCC7/CFTR is functionally interacting with Hsp90 and FKBP38 ensuring its correct folding [66] (see Figure 2). Moreover, chloride anion export activity and protein stability of ABCC7 is regulated via protein phosphorylation [66,109]. Finally, a third mode of regulation has been revealed by the correlation between defective CFTR proteins and decreased HCO_3^- concentrations in anion/bicarbonate transporting epithelia, resulting in mucin precipitation during cystic fibrosis [61]. Members of the solute carrier (SLC) family 26, such as SLC26A3 or SLC26A6, interact with ABCC7 and export bicarbonate by exchanging chloride provided by ABCC7 [61]. Interaction with the R domain of ABCC7 is provided by the STAS (sulfate transporter and anti-sigma factor antagonist) domain in the C-terminus of SLC26 [61]. In order to bring these two domains in proximity and hence mediate the interaction, both proteins need to bind PDZ (post-synaptic density 95/discs large/zona occludens 1) domain containing adapter molecules [61].

Despite the fact that both transporters belong to different ABC families and species, these models of human ABCC7 regulation draws some remarkable parallels to the one of *Arabidopsis* ABCB1. The setup we are facing here is also composed of an FK506-binding protein (here: the FKBP42, TWD1), a protein kinase (here: the AGC3 kinase PID) and a permease-like protein of the PIN-family (see Figure 2). Like FKBP38, TWD1 is thought to chaperone ABCB1 during its secretion from the ER towards the PM [18]. Subsequently, PID is thought to be recruited by TWD1 and to phosphorylate ABCB1 [18]. PID is regulating the activity of ABCB1 by phosphorylating specific residues (such as S634) localized in its linker domain, the equivalent to the R domain of ABCC7 [18,103]. If protein phosphorylation via PID alters also protein stability of ABCB1 is currently not known.

The ABCBs–PIN pairings have significant effects on auxin transport capacity of the efflux complex [88]. In this respect it is worth mentioning that ABCB19 was recently shown to own an anion channel activity when expressed in HEK cells that was inhibited upon co-expression with TWD1 [110]. Although it is until now unclear if this exported anion is in fact IAA, this functional analogy is striking and fuelled by the finding that *ABCB19* was isolated in genetic screen using an anion channel blocker [80].

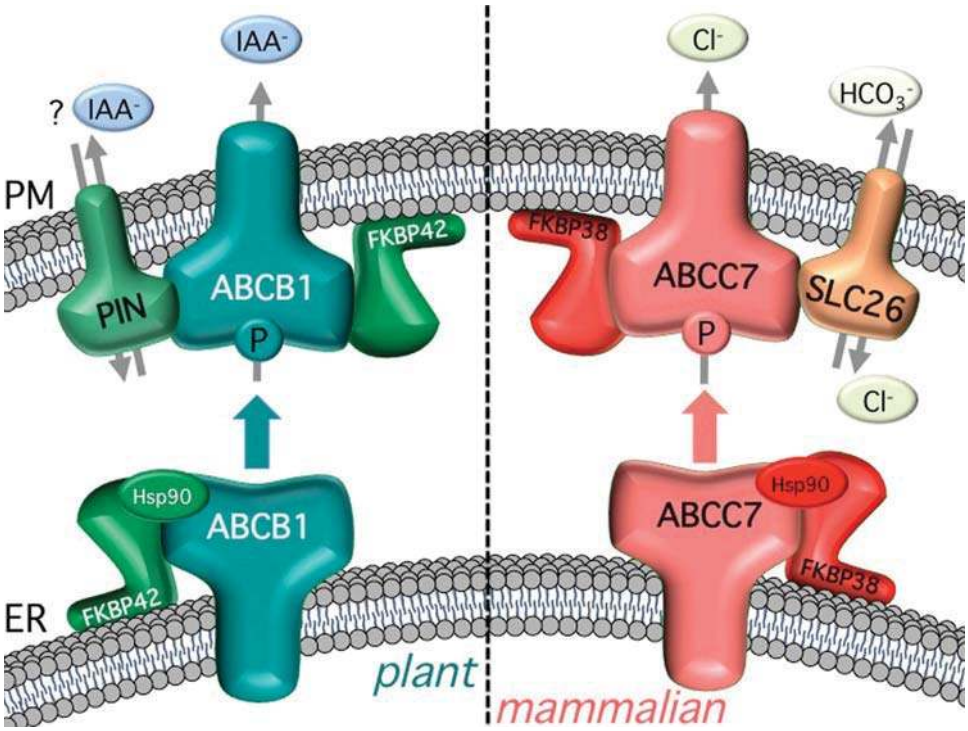
Another parallel is that like FKBP38 also TWD1 was shown to bind Hsp90 via its TPR domain [111], however, the relevance of this interaction is currently still unknown [112]. Further, a functional difference lies in the fact that TWD1 interacts with ABCB1 via its N-terminal FKBD [90] and not with its TPR domain as ABCC7. The finding that vacuolar TWD1–ABCC1/ABCC2 interaction is mediated by the FKBD of TWD1 [113] suggests that this interacting domain is ABCC specific.

This regulatory *Arabidopsis* ABCB1 circuit is even more complicated by the finding that PID phosphorylation of PINs decides for their basal or apical fate [114]. Further, it was shown that PIN phosphorylation by PID also activates PIN activity [115]. Although all these interaction and phosphorylation events still need functional elucidations, they again pinpoint to the question which component is in the end functioning as auxin exporter, PINs or ABCBs individually, or both together. In the latter case an auxin efflux complex consisting of PIN–ABCB (and eventually also TWD1) would allow obviously for a fine-tuning of auxin transport activity and directionality [18].

In summary, based on this comparison it appears that nature is employing despite obvious physiological and mechanistic differences similar blue prints for the regulation of evolutionary distant ABC transporters in plants and mammals. Finally, we believe that cross-kingdom evaluations

Figure 2 | Regulation of *Arabidopsis* ABCB1/PGP1 and human ABCC7/CFTR by FKBPs

Arabidopsis ABCB1 functionally interacts with PIN-like, secondary active auxin carriers on the PM. The FKBP42/TWD1 is essential for ABCB1 activity and PM presence and acts as a chaperon for the ER-to-PM delivery of ABCB1. In analogy, the human orthologue of FKBP42, FKBP38, was shown to chaperone ABCC7/CFTR to the PM. Here it functionally interacts with bicarbonate (HCO_3^-)–chloride (Cl^-) exchangers of the SLC26 family, leading to enhanced bicarbonate and thus fluid secretion. Note that phosphorylation of both linker and R-domains of ABCB1 and ABCC7 by the AGC kinase, PINOID and PKA, respectively, was shown to affect transport activity. Further, that the existence and identity of a counter-ion for PIN-mediated IAA transport is unclear; the same holds true for the involvement of Hsp90 shown to interact with the TPR domain of FKBP42. Figure modified from [18]: Geisler, M. (2014) It takes more than two to tango: regulation of plant ABC transporters. In Plant ABC transporters (Geisler, M., ed.), pp. 241–270, Springer, Switzerland.



are very powerful sources to identify not only functional similarities but also, and maybe even more importantly, operative differences.

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