Supporting information

Auxin-transporting ABC transporters are defined by a conserved D/E-P motif regulated by a prolylisomerase

Pengchao Hao^{1,4}, Jian Xia¹, Jie Liu¹, Martin Di Donato¹, Konrad Pakula^{2,5}, Aurélien Bailly³, Michal Jasinski^{2,6} and Markus Geisler^{1, *}

¹ University of Fribourg, Department of Biology, CH-1700 Fribourg, Switzerland

² Department of Plant Molecular Physiology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, PL-61-704 Poznan, Poland

³Institute for Plant and Microbial Biology, Zollikerstrasse 107, 8008 Zurich, Switzerland

⁴ present address: Shanghai Jiaotong University, School of Agriculture and Biology, Shanghai, China

⁵NanoBioMedical Centre, Adam Mickiewicz University, Umultowska 85, PL-61-614 Poznan, Poland

⁶Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Dojazd 11, 60-632 Poznan, Poland

* Corresponding author: Markus Geisler (markus.geisler@unifr.ch)

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Suppl. Fig. 1: Phylogenetic analysis of Arabidopsis ABCB transporter family members. Phylogenetic analysis was performed by constructing maximum likelihood trees using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) based on the amino acid sequences aligned using MUSCLE (Edgar et. al., 2004). Selected plant ABCBs from rice (Os), tomato (Si), sorghum (Sb) and maize (Zm) for that auxin transport was experimentally conformed are included. Clades of full-size ABCBs are coloured, half-size ABCBs are not. The presence of a conserved D/E-P1.008 motif (relative to AtABCB1) is indicated by green boxes; experimentally verified or falsified auxin transport is labelled with green or red dots, respectively.

ABCB19	ABCB1	ABCB2	ABCB10	ABCB14	ABCB13	ABCB5	ABCB3	ABCB12	ABCB11	ABCB21	ABCB4	ABCB9	ABCB7	ABCB18	ABCB17	ABCB16	ABCB22	ABCB15	ABCB8	ABCB20	ABCB6	ABCB27	ABCB28	ABCB26	ABCB29	ABCB24	ABCB23	ABCB25		
KSLSLRSGSLRNL	NSVSSPIMTRNSSYGRSPYSRR	SR	PIT	VFSSRRTSSFRE-	VSSSRRTSSFRV-	SGSSRGNNSTRQD	RGSSRNIRTRVHD	ISREGSVISGGTSSFGNSSRH-	KSMEGTSSVGNSSRH-	KSSLSRSLSKR	KSSLGRSLSKGGSSRGNSSRH-	SSAMRRSVSRNSSSSRHS	IESSDSQNGIHS	SKDLKYSPKEF	SKDFKYSQHNS	RNDLDYNPRDL	NKDVKYSSRLS	SKDIRNSSRVS	NSVIRLSNRSS	PSSPKMIKSPSLQRGSGVFRPQ	PSSPKMAKSPSLORGHNVFRSO								(1/29) (1/29)	P635 P647
ABCB19	ABCB1	ABCB2	ABCB10	ABCB14	ABCB13	ABCB5	ABCB3	ABCB12	ABCB11	ABCB21	ABCB4	ABCB9	ABCB7	ABCB18	ABCB17	ABCB16	ABCB22	ABCB15	ABCB8	ABCB20	ABCB6	ABCB27	ABCB28	ABCB26	ABCB29	ABCB24	ABCB23	ABCB25		
IDPDDADA	IEP DDPDT	IVGETSEE	VVGDTGEE	IPP DOPNS	ISPDOPNS	IDSRDESG	IDSRDESG	IDSSDETG	IDSSDETG	IDPSDESG	IDPSVESG	IDSSSDEG	IDSSSEKG	IEPENPDG	IEPKNPDG	IEPENPDG	IEPEKPDG	IDPEDPDG	HENTNHGE	IEPDDNSA	IEPDDTSA	MSSSGDKC	AYGLERDIHTK	DQFISKG	VIERPEAL	IGDKDIDR	IGD KDTET	ITNTSDAK	(13/29)	P1008
ABCB19	ABCB1	ABCB2	ABCB10	ABCB14	ABCB13	ABCB5	ABCB3	ABCB12	ABCB11	ABCB21	ABCB4	ABCB9	ABCB7	ABCB18	ABCB17	ABCB16	ABCB22	ABCB15	ABCB8	ABCB20	ABCB6	ABCB27	ABCB28	ABCB26	ABCB29	ABCB24	ABCB23	ABCB25		
VDFAYPSRPDV.	IDFSYPSRPDI	VHFSYPSRPDV	VHFSYPSRPDV	VSFAYPTRPEI.	VSFVYPTRPEI	ISFTYQTRPDV	ISFTYQTRPDV	LSFTYPARPGI	LSFTYPARPDI	ISFKYPSRPDV	VSFKYPARPDV	VSFRYPMRPDV	VSFRYPMRPDI	VDFAYPTRPDV	VDFAYPTRPDV	VDFAYPTRPNM	VDFAYPTRPDV	VDFSYPTRPDV	IDFSYPNRPSI	VDFCYPTRPEI	IDFCYPTRPEV.	VWFAYPSRPSH	VHFAYPLRPDV	VSFSYPSRDEV.	ISFKYDE-NML	VHFSYLPER	VHFSYLPER	VHFSYLPER	(23/29)	P1034
ABCB19	ABCB1	ABCB2	ABCB10	ABCB14	ABCB13	ABCB5	ABCB3	ABCB12	ABCB11	ABCB21	ABCB4	ABCB9	ABCB7	ABCB18	ABCB17	ABCB16	ABCB22	ABCB15	ABCB8	ABCB20	ABCB6	ABCB27	ABCB28	ABCB26	ABCB29	ABCB24	ABCB23	ABCB25		
FISGLPEGYKT	FISALPEGYKT	FITSLPEGYST	FISSLPEGYST	FISRMEEGYMT	FIIKMBEGYKT	FISSIOKGYDT	FISSIDOGYDT	FISSIOQGYDT	FISSIQQGYDT	FISGLOOGYDT	FISGLOOGYDT	FISSLPQGYDT	FISSLPOGYET	FITSLSNGYDT	FITSLSNGYDT	FITSLSDGYDT	FIVTLSDGYDT	FITSLTEGYDT	FISAMEKGYKT	FISSLPHGYDT	FISSLPHGYDT	FIEAFPDKYNT	FIISLPOGYDT	FITALPNGYNT	FIRNLPEGYNT	TIMKFPDKYST	TIMKFPDKYST	TISNFPDKYST	(15/29)	P1149
ABCB19	ABCB1	ABCB2	ABCB10	ABCB14	ABCB13	ABCB5	ABCB3	ABCB12	ABCB11	ABCB21	ABCB4	ABCB9	ABCB7	ABCB18	ABCB17	ABCB16	ABCB22	ABCB15	ABCB8	ABCB20	ABCB6	ABCB27	ABCB28	ABCB26	ABCB29	ABCB24	ABCB23	ABCB25		
LV-SRPEGAYS	LLKNHPDGIYA	LV-LNKSGPYF	LV-ENKNGPYS	LV-SKSDGFYK	LV-SIPNGFYK	LI-NIEGGVYA	LI-NIEGGVYA	LI-KIDGGVYA	LI-KIEGGVYA	LI-NIKDGVYA	LI-NIKDGVYA	LM-KISGGAYA	LM-EISGGAYA	LLAKGPKGAYF	LLEKGPTGTYF	LLAKGPTGSYF	LLAKGPTGVYF	LLSKGPTGIYF	LKNIGGOFS	LAAKNGLYV	LAGKNGLYV	LLSLNGIYT	LVAQKGSYA	LLSKDGLYA	LSTHKDSLTSA	LLGKSGRYA	LLEKSGRYA	LLGKSGRYA	(8/29)	P1252

Suppl. Fig. 2: Sequence alignment of surface-exposed prolines identified in the linker and NBD2 of ABCB1 with all Arabidopsis ABCB transporter family members. Sequences were aligned using MUSCLE (Edgar et. al. 2004). Conservation of prolines (indicated with triangles) amongst all 29 Arabidopsis ABCBs is indicated.



Suppl. Fig. 3: Logo representation (WebLogo 3) of NBD2 proline conservation (upper panels) in comparison with the homologous regions of NBD1 (lower panels) after multiple sequence alignment of Arabidopsis ABCBs using MUSCLE (Edgar *et. al.* 2004).



Suppl. Figure 4: Mutation of six surfaceexposed prolines in ABCB1 does not significantly alter PM location and expression on tobacco protoplasts.

Wild-type and proline mutants of ABCB1 are expressed on the plasma membrane revealed by confocal imaging of ABCB1-GFP of tobacco protoplasts prepared from leaves transfected with ABCB1-GFP and stained with PM marker, FM4-64; bar, 10 μ m.



Suppl. Fig. 5: Effect of mutation of surface-exposed prolines on ATPase activity.

ATPase activity of microsomal fractions prepared from tobacco leaves transfected with wild-type and indicated proline mutants of ABCB1 measured in the presence and absence of orthovanadate and ATP. Significant differences (unpaired *t* test with Welch's correction, p < 0.05) to vector control or ABCB1 are indicated by an 'a' or a 'b', respectively, (mean ± SE; n ≥ 4 transport experiments generated from independent tobacco transfections).



Suppl. Figure 6: Co-expression with TWD1 does not significantly alter expression of ABCB4.

Confocal imaging of ABCB4-GFP in the absence (upper row) and presence of TWD1-mCherry in tobacco leaves co-transfected with ABCB4-GFP and TWD1-mCherry; bars, 10 μ m.



Suppl. Figure 7: Benzoic acid (BA) export of protoplasts prepared from indicated Arabidopsis lines. *Abcb1-1* (A) or Col Wt (B) were transformed with Wt or indicated E-P1.008 mutations of ABCB1 expressed under native (A) or constitutive promoters (B).





Suppl. Figure 8: Conservation of the AtABCB1 P1008 in CFTR/ABCC7 and speculative model on the role of P1008 during ABCB1 regulation by TWD1. Surface models of AtABCB1 (A) and HsCFTR/ABCC7 (B) revealing surface exposure of P1.008 and P1.184, respectively. (C) P1.008 is conserved between AtABCB1 and CFTR/ABCC7 and AtABCC1/2. (D) P1.008 lies in between intracellular/coupling helices 2 and 3, IH2 and IH3, respectively, establishing the connection between intracellular loops, ICL2 and ICL3, and the NBD2. Further, P1.008 is in direct connection with an alpha-helix (in red) leading to TMH12 shown to be involved in IAA binding (Bailly *et al.* 2012). Colour code of functional domains can be deduced from Fig. 1.



Suppl. Figure 9: Putative model of posttranscriptional regulation of auxin-transporting ABCBs by TWD1/FKBP42.

TWD1 was shown to regulate biogenesis of auxintransporting ABCBs (ATAs) on the ER, supposedly with the involvement of a chaperone activity associated with the TPR domain (31). In the absence of TWD1, ATAs are retained on the ER and degraded (32-32), most likely by the ERAD pathway. Additionally, TWD1 is involved in ERto-PM trafficking (large arrow). Here, we show that TWD1 is probably also involved in regulatory events that alter ATA activity on the PM most likely by employing a *bona fide* PPIase activity acting on P1008. The impact of involved functional domains (FKBD, FK506-binding domain; TPR, tetratricopeptide repeat domain) on folding/ maturation or PPIase/ transport is specified by straight or interrupted lines; uncertain findings are indicated by a question mark.