Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Plant Biology

Phosphorylation control of PIN auxin transporters

A. E. Lanassa Bassukas[a], Yao Xiao[a] and Claus Schwechheimer

Abstract

The directional transport of the phytohormone auxin is required for proper plant development and tropic growth. Auxin cell-to-cell transport gains directionality through the polar distribution of 'canonical' long PIN-FORMED (PIN) auxin efflux carriers. In recent years, AGC kinases, MAP kinases, Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE-RELATED KINASEs and receptor kinases have been implicated in the control of PIN activity, polarity and trafficking. In this review, we summarize the current knowledge in understanding the post-translational regulation of PINs by these different protein kinase families. The proposed regulation of PINs by AGC kinases after salt stress and by the stress-activated MAP kinases suggest that abiotic and biotic stress factors may modulate auxin transport and thereby plant growth.

Addresses

Plant Systems Biology, School of Life Sciences, Technical University of Munich, Emil-Ramann-Strasse 8, 85354, Freising, Germany

Corresponding author: Schwechheimer, Claus (claus.schwechheimer@wzw.tum.de)

[a] Equal contribution.

Current Opinion in Plant Biology 2022, **65**:102146

This review comes from a themed issue on **Growth and Development 2022**

Edited by **Moritz Nowack** and **Keiko Sugimoto**

For a complete overview see the Issue and the Editorial

Available online 30 December 2021

https://doi.org/10.1016/j.pbi.2021.102146

1369-5266/© 2021 Elsevier Ltd. All rights reserved.

Introduction

The phytohormone auxin regulates essentially every aspect of plant development from embryogenesis to fruit formation [1]. As plant body architecture determines a predominantly sessile lifestyle, developmental plasticity vastly controlled by the spatiotemporal distribution of auxin is key adaptive strategy of the plant as a response to environmental signals, as well as to abiotic and biotic stress factors.

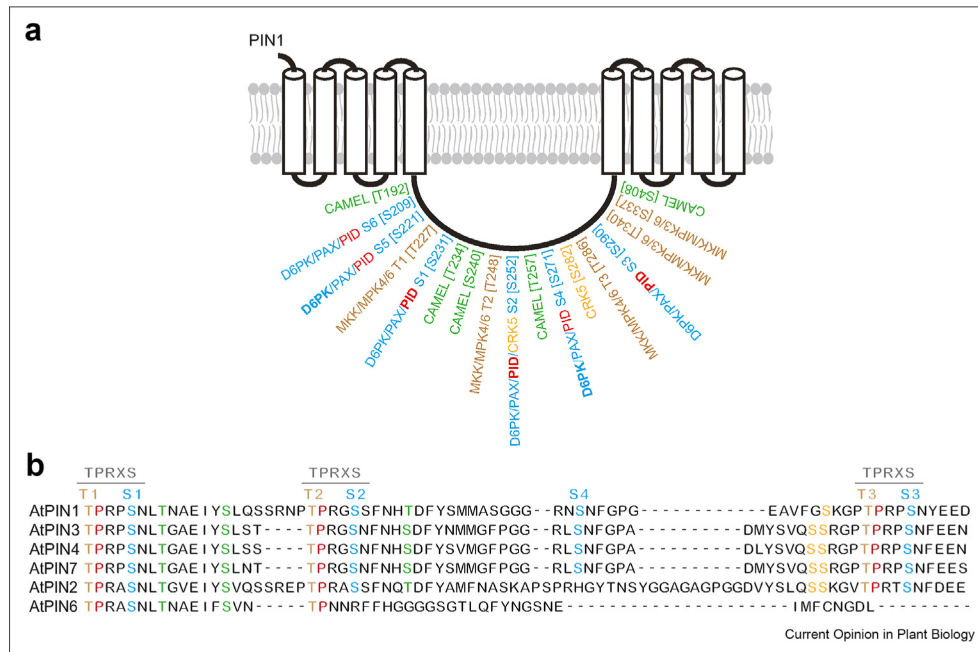
Auxin is dynamically transported through the plant and the directionality of its transport is achieved, to a large part, through the polar distribution of so-called 'long' or 'canonical' PIN-FORMED (PIN) proteins. These PINs are composed of 10 transmembrane helices and an approximately 320–360 amino acid-long cytoplasmic loop, which bears a number of phosphosites for the regulation of PIN activation, polar distribution and trafficking (Figure 1). At present, four classes of protein kinases have been implicated in PIN phosphoregulation and their functional roles are understood at varying detail: AGC1 and AGC3 kinases activate PINs through phosphorylation at, in part overlapping, serine residues, and AGC3 kinases can also alter PIN polar distribution (Figure 2) [2–4,**5,6]. MAP kinases phosphorylate threonine and serine residues and the known role of MAP kinases in abiotic and biotic stress responses suggest that MAP kinases may regulate plant growth via PINs in response to stress (Figure 2) [**7,8]. Finally, Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE-RELATED KINASEs (CRKs), as well as the auxin-regulated receptor CAMEL and its interactor CANALIZATION-RELATED RECEPTOR-LIKE KINASE (CANAR) phosphorylate PINs and may have a role in regulating PIN polar distribution at the plasma membrane (Figure 2) [*9–13]. In this review, the current understanding of these different PIN regulatory kinases is summarized with a focus on their effects on PIN function.

Main section

The AGCVIII family of serine/threonine kinases

In vascular plants, the AGC1 – AGC4 kinases constitute subfamilies of the AGCVIII kinases (Figure 3) [14,15]. All AGCVIII kinases from land plants are evolutionarily derived from PHOTOTROPIN2-like proteins that are already present in algae [15,16]. In *Arabidopsis thaliana*, the two phototropin (phot) blue light receptor kinases form the AGC4 subfamily and regulate different processes related to light response, including phototropic stem bending [17,18]. Arabidopsis phot1 has an ascribed role in auxin transport regulation as negative regulator of the ATP-binding cassette B auxin transporter to inhibit auxin transport and prime lateral auxin movement during phototropic growth [19]. Members of the, in *A. thaliana*, 13-member AGC1 kinase family have acquired distinct regulatory functions in land plants. With regard to their evolutionarily descendance from PHOTOTROPIN2-like proteins, it is remarkable that they have maintained roles at the plasma membrane as regulators of auxin transport and tropic growth, cell

Figure 1



PIN1 can be phosphorylated at multiple phosphosites. a. Schematic representation of the *Arabidopsis thaliana* PIN1 auxin efflux carrier and its phosphorylation sites on the PIN1 intracellular loop and the respective targeting kinases. The phosphosites are not positioned proportionally to the length of the intracellular loop [27]. Bold lettering is used in cases where a phosphosite-preference for S1 – S5 had been reported for D6PK and PID, which may have functional relevance for PIN polarity control [2–6]. **b.** Sequence alignment of the five *Arabidopsis thaliana* 'canonical' PINs and 'intermediate' PIN6. Conserved AGC kinase-targeted S1 – S3 phosphosites as well as putatively MAP kinase-targeted T1 – T3 phosphorylation sites in the TPRXS motifs are shown, together with other phosphorylation sites identified in this part of the intracellular loop. The conserved prolines (P) of the TPRXS motifs, proposed to be the target of *cis/trans* prolyl isomerases, are marked in red [56].

elongation or related processes [20–22]. AGC3 kinases have acquired functions in PIN polarity control to accommodate needs of the development and differentiation of multicellular organisms [4–6]. AGC2 kinases, in turn, have only been discussed as nuclear proteins and are, thus, not directly implicated in the phosphoregulatory processes discussed here [23,24].

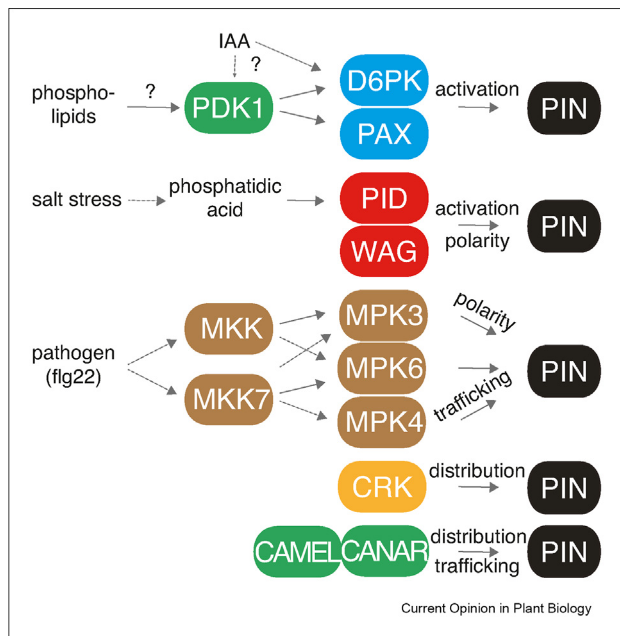
D6PK protein kinases from the AGC1 family

D6PK and the three related D6PK-LIKE1 – 3 are well-characterized AGC1 family members (Figure 3a). *d6pk d6pkl1* double mutants are defective in phototropic and negative gravitropic hypocotyl bending, which may be a result of the loss of auxin transport from the cotyledon to the hypocotyl [20,21]. *d6pk d6pkl1 d6pkl2* triple and *d6pk d6pkl1 d6pkl2 d6pkl3* quadruple mutants are defective in lateral root formation, have twisted rosette leaves and a spindly appearance [25]. *d6pk d6pkl1 d6pkl2 d6pkl3* quadruple mutants, furthermore, have severe embryo differentiation defects [26]. All mentioned phenotypes can be explained or correlated to the described biological functions of specific canonical PINs. In *d6pk d6pkl1 d6pkl2* mutants, the auxin maximum is broadened and shifted above the quiescent centre of the root when compared to the wild type and the formation of auxin maxima, required for lateral root initiation, is severely impaired [25]. Further, auxin transport is reduced in stems and hypocotyls of different *d6pk* backgrounds and the chemically induced functional impairment of D6PK results in differential auxin accumulation, as validated with auxin reporters, within 15–30 min [20–22].

D6PK and D6PKLs activate PINs through phosphorylation at four (PIN1 S1 – S4) or five (PIN3 S1 – S5) serine residues in RXS motifs within the PIN intracellular loop (Figure 1) [2]. In Arabidopsis PIN1, these motifs are clustered within an ∼80 amino acid region (Figure 1b) [27]. Phosphorylation of S4 appears to make a strong contribution to PIN activation by D6PK [25]. Besides S1 – S5, there are also other uncharacterized RXS motifs that may contribute to PIN activation by D6PKs. Although not studied in detail in every case, PIN phosphorylation regulation and the phosphosite preferences of D6PK/D6PKLs may also apply to AGC1 family members such as PAX (PROTEIN KINASE ASSOCIATED WITH BRX/AGC1-3), as discussed later in this review, and AGC1-12 (Figure 3a) [**3,22].

D6PK cycles rapidly to and from the basal plasma membrane, independently from its PIN substrates, such

Figure 2



Protein kinases targeting PIN auxin efflux carriers. Overview of the different protein kinases and kinase families that have been implicated in PIN phosphoregulation. Where known, upstream kinases and signals, as well as protein kinases having been implicated in PIN regulation and phosphorylation are shown, together with the described effects of the phosphorylations on PIN function or PIN distribution. Distribution refers to PIN distribution at the plasma membrane, trafficking refers to the observation of intracellular PIN accumulation.

that PIN activation can only occur after phosphorylation at the plasma membrane [21]. D6PK and other AGC1 kinases have an insertion loop between kinase subdomains VII and VIII with a lysine/arginine-rich domain (K/R) for phospholipid interactions and possibly counteracting adjacent serine residues, whose phosphorylation may neutralize the positive charges of the K/R-rich domain and contribute to kinase transport (Figure 3) [21]. Presently unknown is the role of the repeated CXX(X)P motifs occurring within the insertion loop in varying numbers and at varying positions in different AGC1 kinases [28].

The availability of heterologous PIN activation assays with D6PK has made it possible to examine the physiological function of the auxin transport inhibitor NPA, whose application to wild type seedlings leads to exact copies of *pin* mutant phenotypes [29]. In line with such observations, NPA was found to directly inhibit PIN function rather than inhibiting PIN-activating kinases [*30].

During pollen formation, D6PKL3 localizes polarly at the sites of pollen aperture formation but this polarity-dependent process cannot be connected to PIN-mediated auxin transport [31]. This suggests that D6PK and D6PKLs may also have functions other than PIN activity control [31].

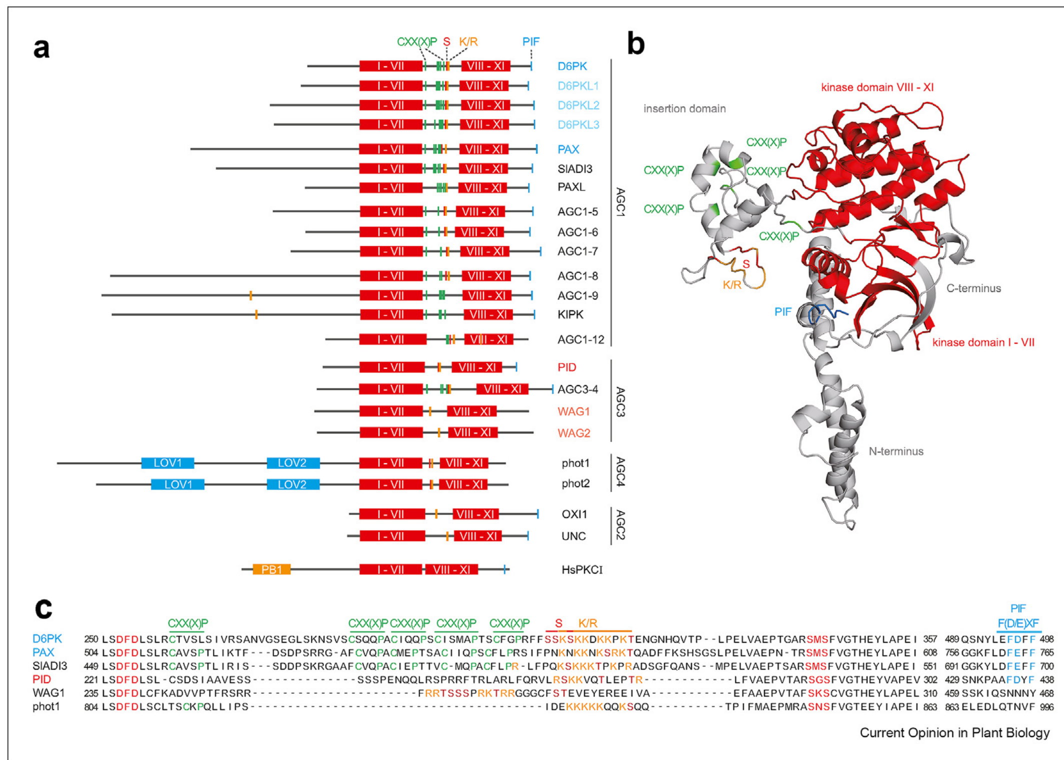
BRX regulation of the AGC1 kinase PAX

PAX is essential for protophloem development (Figure 3) [**3,32,33]. *pax* mutants share their protophloem differentiation phenotype with *brevis radix* (*brx*) mutants and BRX interacts with PAX [**3]. In auxin transport experiments conducted in *Xenopus laevis* oocytes, BRX attenuates PAX-mediated PIN auxin transport activation (Figure 4) [**3]. BRX exerts the same attenuating function on PAX- and on D6PK-dependent PIN activation but not on the AGC3 kinase PINOID (PID), and BRX may, thus, selectively act on AGC1 kinases [3].

AGC kinases contain a so-called activation loop and activation loop phosphorylation enhances kinase activity. In PAX, activation loop phosphorylation increases in an auxin-dependent manner and BRX relocates from the plasma membrane to the nucleus when auxin concentrations are high (Figure 4) [**3,32]. Indeed, PAX may regulate BRX plasma membrane localization since it phosphorylates BRX S228, a conserved serine residue required for auxin-dependent BRX nuclear targeting, at least *in vitro* [*32]. PAX and BRX function as positive and negative regulators of PIN activation where BRX reduces PIN-mediated auxin efflux at low auxin concentrations but relocates to the nucleus when auxin accumulates such that PAX can activate PINs and auxin efflux leading to a subsequent reset of the system (Figure 4) [**3]. This module is suitable to explain differences in auxin accumulation between the protophloem and adjacent cell files [**3]. A further component of this regulatory module is a class of phosphatidylinositol-4-phosphate 5-kinases (PIP5Ks), which catalyze the formation of phosphatidylinositol-4,5-bisphosphate (PI4,5P) [**33]. PIP5Ks interact with BRX and its targeting to the plasma membrane may serve to locally enhance PI4,5P distribution as part of a self-enhancing regulatory module (Figure 4) [**33,34].

Arabidopsis PAX shares 70% identity with ADI3 (AvrPto-dependent Pto-interacting protein 3) from tomato. ADI3 is phosphorylated by its interactor Pto kinase and, like PAX, also by 3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN KINASE1 (PDK1) [35,36]. Although ADI3 is, by all accounts, a tomato PAX orthologue, it has not been linked to auxin transport but rather seems to exert a critical function in the nucleus [37]. The K/R-rich domain implicated in phospholipid interactions in the case of AGC1 kinases, is essential for nuclear localization in the case of ADI3 [37]. Indeed, nuclear localization of AGCVIII kinases is not exclusive for ADI3, since nuclear accumulation and function of Arabidopsis AGC2 kinases OXI and UCN as well as maize PID-co-orthologue BARREN INFLORESENCE2 and rice OsPID have been reported [23,38–42]. Since other observations

Figure 3



AGC kinases target PIN auxin efflux carriers. a. Schematic representation of *Arabidopsis thaliana* AGCVIII kinases, as well as the PAX-orthologous tomato ADI3 (SlADI3) and human HsPKC1. Shown are the protein kinase subdomains that are, in the case of AGCVIII kinases, separated by an insertion loop. The insertion loop may contain CXX(X)P motifs (green), a K/R-rich polybasic motif for phosphoinositide interactions (orange), as well as serine residues (red) whose phosphorylation may neutralize the positive charge of the K/R-motif. The C-terminal PDK1-interacting fragment (PIF, blue) is the proposed PDK1 binding site [45]. LOV, light-oxygen-voltage light-sensing domain; PB1, Phox and Bem1p dimerization domain. Only two out of four Arabidopsis AGC2 kinases are shown. **b.** Model of the AGC1 kinase D6PK with sequence features as shown in A. The model was calculated using TrRefineRosetta [60]. **c.** Protein sequence alignment of the insertion loop of selected *Arabidopsis thaliana* AGC kinases with the different sequence features marked. The sequence variation in the PIF motif may have functional significance only for D6PK, PAX, and SlADI3, that are proposed PDK1 targets [36,43,**44].

related to AGC kinases suggest that they have functions other than PIN regulation, e.g. Ref. [31], it cannot be ruled out that, at least, some of these functions are related to phosphoregulation in the nucleus.

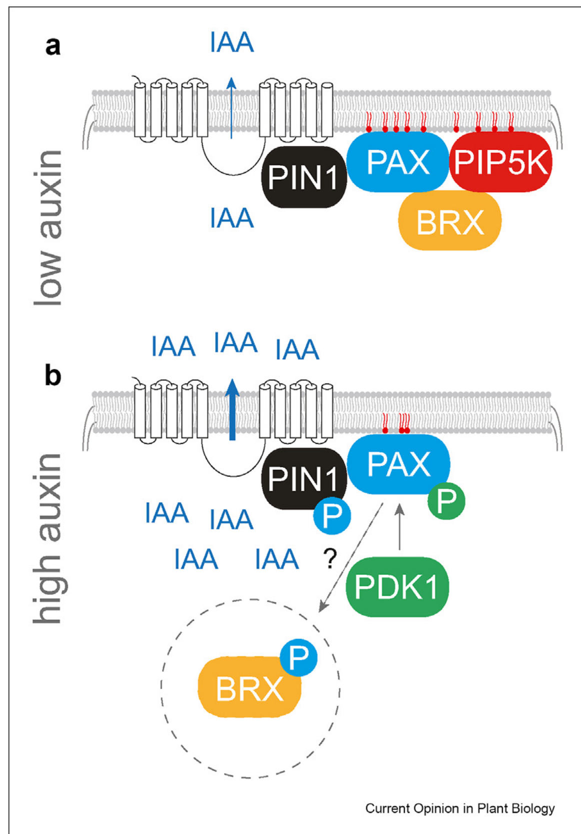
PDK1 kinases activate PAX

PAX displays strong PIN activation in heterologous auxin transport assays only when carrying a mutation mimicking its activation loop phosphorylation [**3]. PDK1, in Arabidopsis encoded by the paralogous *PDK1.1* and *PDK1.2*, phosphorylates the activation loops of PAX and D6PK and *pdk* double mutant phenotypes can be explained by reduced PAX and D6PK functions (Figure 1; Figure 4) [**43,**44]. Expression of a PAX variant with an activation loop phosphorylation-mimicking mutation from a *PDK1* promoter fragment can suppress the protophloem phenotype in a *pax* mutant. In turn, no phenotype reminiscent of *pid* mutants can be detected in *pdk1* mutants, suggesting that a previously proposed functional relationship between PDK1 and PID may not be relevant *in planta* [45]. Similarly, the proposed role of the PDK1 C-terminal pleckstrin homology (PH) domain for kinase activation was questioned as PDK1 variants lacking the PH domain could still complement the mutant phenotype [**44]. PDK1 is thus an upstream protein kinase regulating PAX, D6PK and possibly also other AGC1 kinases. How PDK1 activity is regulated and acts as a regulatory kinase in the context of BRX and PIP5Ks remains to be shown.

PIN phosphorylation by AGC3 kinases PID and WAG

Like AGC1 kinases, AGC3 kinases such as Arabidopsis PID, WAG1 and WAG2 phosphorylate PINs at RXS motifs [4,46]. *pid* mutants have a non-differentiated pin-shaped inflorescence and therefore resemble *pin1* mutants [47]. *wag1 wag2* mutants are defective in root growth control and display wavy roots [48]. Like AGC1 kinases, AGC3 kinases activate PINs. A K/R-rich motif in the AGC3 insertion domain is essential for phospholipid binding and phosphatidic acid-directed PID and PIN2 activation in response to salt stress has been described (Figure 3) [49,50].

Figure 4



Model for the regulation of PAX kinase by BRX, PDK1 and phosphoinositides. a. At low auxin concentrations, PAX-mediated PIN activation is attenuated by BRX interactions at the plasma membrane. BRX may also modulate relevant phosphoinositide composition at the plasma membrane by recruiting PIP5K and thereby positively enhance PAX binding. **b.** At elevated auxin concentrations, BRX translocates to the nucleus, in a BRX phosphorylation-dependent manner, allowing for PIN activation and auxin efflux. PAX activation loop phosphorylation by PDK1 is essential for PAX activation and PIN phosphorylation. PAX may be the protein kinase regulating BRX translocation.

Unlike AGC1 kinase overexpression, AGC3 overexpression correlates with a repolarization of PINs (Figure 2) [2,4,**5,51]. PIN1 S1 − S3 had initially been identified as AGC3-targeted sites and phosphorylation of these sites had been proposed to directly control PIN polarity [4,46]. However, PIN1 S1 − S3 phosphorylation is independent from PIN1 polar distribution and PIN polarity control must therefore be more complex than previously thought [52]. One potential explanation for the divergent effects of AGC3 and AGC1 kinases on PIN polarity regulation may reside in the phosphosite-preferences observed with PID and D6PK in *in vitro* phosphorylation experiments [21,22], which are also reflected in differences at the level of PIN activation in heterologous auxin transport assays (Figure 1) [2]. These distinct phosphosite preferences and activation mechanisms of AGC1 and AGC3 kinases, their differential regulation by upstream acting kinases [45] together with the distinct subcellular distribution of the apolarly distributed AGC3 kinases and the polarly localized AGC1 kinases, may account for their differential effects on PIN polar distribution.

A recent study further showed that MAB4/MEL (MACCHI BOU4/MAB4 (ENP1)-LIKE) proteins, which are important for PIN polar localization, are recruited to the plasma membrane in a PIN- and PIN phosphorylation-dependent manner [53,**54]. Experimental evidence strongly suggests that MAB4/MEL proteins are recruited to the plasma membrane by PINs, that they interact with AGC3, as well as with AGC1 kinases and that MAB4/MEL proteins preferentially interact with phosphorylated PINs [**54]. The effects of these components and PIN phosphorylation on PIN lateral diffusion may explain changes in the plasma membrane distribution and could be part of a self-reinforcing module comprised of PINs, MAB4/MELs and AGC kinases for PIN polarity maintenance [**54].

Phosphorylation by MAP kinases

MITOGEN-ACTIVATED PROTEIN (MAP) KINASES (MPK) are protein kinases acting downstream of evolutionarily conserved MAP kinase pathway that includes upstream kinases such as MAP KINASE KINASE (MKK) and MAP KINASE KINASE KINASE (MKKK) [55]. The AGC kinase-targeted serines S1 − S3 reside within TPRXS motifs that are highly conserved among PINs and three times repeated within 'canonical' PINs (Figure 1) [4,46]. The threonines within the three TPRXS motifs, T1 − T3, are proposed MAP kinase target sites and their mutation to alanine essentially abolishes *in vitro* phosphorylation by the MAP kinases MPK4 and MPK6 (Figure 2) [*8]. Phenotypes of the Arabidopsis *MKK7* gain-of-function mutant *bushy and dwarf1* (*bud1*) were explained by altered PIN regulation (Figure 2) [**7]. Remarkably, *bud1* shoot and root mutant phenotypes can be selectively suppressed by introducing *mpk3* or *mpk6* loss-of-function mutations [**7]. Activation of the MAP kinase pathway by inducible *MKK7* expression or application of the pathogen-derived flg22 peptide, which activates the MAP kinase pathway, results in the MPK6-dependent intracellular accumulation of PIN1 in root cells, that may be the consequence of altered PIN1 trafficking (Figure 2) [*8]. Mutation of PIN1 T1 − T3 led also to altered intracellular PIN distribution when examined in transiently transformed protoplasts, suggesting that activation of the MAP kinase pathway may regulate PIN1 intracellular trafficking [*8]. A further relevant regulatory module at the TPRXS motifs may be *cis/trans* isomerization of the prolines (P) following T1 − T3 in the TPRXS motifs [56].

A further link to MAP kinase regulation comes from studies of PIN1 S337, whose phosphorylation was

associated with PIN polarity changes [57]. Finally, mutation of two proposed MAP kinase-targeted sites, T392 and T393, present in the intermediate-length non-canonical Arabidopsis PIN6, affected PIN cellular distribution [58]. T392 and T393 are, however, not conserved in long 'canonical' PINs [58]. Taken together, there are several indications that MAP kinase signaling regulates PIN cellular distribution. MAP kinase signaling is required for proper development but has also been extensively studied in signaling events downstream from abiotic and biotic stress [55]. Thus, modulation of auxin transport by MAP kinase signaling may be an important component of plant growth adaptation to stress. The underlying molecular mechanisms and responses need, however, to be understood at greater detail.

PIN2 regulation by CRKs

Most of the eight CRKs (Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE-RELATED KINASEs) from *A. thaliana* localize at the plasma membrane and phosphorylate PINs *in vitro* (Figure 2) [10–12]. Among all canonical PINs, specifically the distribution of PIN2 is altered in *crk5* mutants [*9]. In *crk5*, PIN2 is depleted from apical plasma membranes of epidermal cells and shows basal to apical localization in the cortex in the transition zone, and CRK5 phosphorylation may thus control PIN2 stability or degradation [*9]. In line with altered PIN2 distribution, *crk5* mutants have gravitropism defects [*9]. Somewhat surprisingly, comparable gravitropism defects were observed in single mutants of other Arabidopsis *CRK* genes [12]. Which role the different mapped phosphorylation sites play for PIN2 function more precisely and why *crk* mutants with defects in different genes display the same phenotype, remains to be investigated.

Receptor kinases CAMEL-CANAR phosphorylate PINs

The auxin-regulated receptor CAMEL (CANALIZATION-RELATED AUXIN-REGULATED MALECTIN-TYPE RECEPTOR KINASE) and its interactor CANALIZATION-RELATED RECEPTOR-LIKE KINASE (CANAR) are required for proper leaf venation, as well as for vasculature regeneration after wounding [**13]. CAMEL phosphorylates PINs *in vitro*, while CANAR lacks an RD motif found in many protein kinases and does not display kinase activity *in vitro*. CAMEL and CANAR may be part of a feedback regulatory system required for formation of the vascular system [**13]. Mutation of the CAMEL-CANAR phosphosites in PIN1 affects its polar distribution and trafficking, as well as its ability to fully rescue the *pin1* mutant phenotype [**13]. How precisely this PIN phosphorylation event regulates PIN cellular distribution remains to be seen.

Conclusions

In recent years, substantial progress has been made in understanding PIN regulation by phosphorylation. Molecular determinants for PIN activation by phosphorylation as well as for the function of regulatory kinases have been identified in the case of AGC1 and AGC3 kinases. The interplay of PAX kinases with other regulatory components can inspire research on other, as-yet less well understood kinases such as the CRKs and receptor kinases, which likely are similarly complex. Unraveling how these kinases regulate PIN distribution and trafficking will be important for understanding plant growth. The remarkable alignment of proposed MAP kinase with AGC kinase phosphosites could suggest an interplay between these kinase families in the regulation of PIN activity and distribution that remains to be understood. The role of MAP kinases as signal transducers downstream from abiotic and biotic stress responses could help understanding plant growth responses during stress.

Author contributions

CS wrote the paper, AELB and YX edited the paper and prepared figures.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work is supported by grants from the Deutsche Forschungsgemeinschaft to Claus Schwechheimer (SCHW751/12-2, SCHW 751/14-1, SCHW751/15-1, SCHW751/16-1) and a postdoctoral fellowship from the Alexander-von-Humboldt foundation (NLD 1216207 HFST-P) to Yao Xiao.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Weijers D, Wagner D: **Transcriptional responses to the auxin hormone**. *Annu Rev Plant Biol* 2016, **67**:539–574.

2. Zourelidou M, Absmanner B, Weller B, Barbosa IC, Willige BC, Fastner A, Streit V, Port SA, Colcombet J, de la Fuente van Bentem S, *et al.*: **Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID**. *Elife* 2014, **3**:e02860.

3. ** Marhava P, Bassukas AEL, Zourelidou M, Kolb M, Moret B, Fastner A, Schulze WX, Cattaneo P, Hammes UZ, Schwechheimer C, *et al.*: **A molecular rheostat adjusts auxin flux to promote root protophloem differentiation**. *Nature* 2018, **558**:297–300.
This cell biological study unravels the relationship of AGC1 kinases and BRX in protophloem development.

4. Dhonukshe P, Huang F, Galvan-Ampudia CS, Mahonen AP, Kleine-Vehn J, Xu J, Quint A, Prasad K, Friml J, Scheres B, *et al.*: **Plasma membrane-bound AGC3 kinases phosphorylate PIN**

auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. *Development* 2010, **137**:3245–3255.

5. ** Grones P, Abas M, Hajny J, Jones A, Waidmann S, Kleine-Vehn J, Friml J: **PID/WAG-mediated phosphorylation of the Arabidopsis PIN3 auxin transporter mediates polarity switches during gravitropism**. *Sci Rep* 2018, **8**:10279.

Here, the authors demonstrate the role of PID and WAG kinases in regulatig the dynamic polarity of PIN3 duing gravitropic bending.

6. Michniewicz M, Zago MK, Abas L, Weijers D, Schweighofer A, Meskiene I, Heisler MG, Ohno C, Zhang J, Huang F, *et al.*: **Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux**. *Cell* 2007, **130**:1044–1056.

7. ** Jia W, Li B, Li S, Liang Y, Wu X, Ma M, Wang J, Gao J, Cai Y, Zhang Y, *et al.*: **Mitogen-activated protein kinase cascade MKK7-MPK6 plays important roles in plant development and regulates shoot branching by phosphorylating PIN1 in Arabidopsis**. *PLoS Biol* 2016, **14**:e1002550.

The phenotypes of the MKK7 gain-of-function mutation may be explained by altered PIN1 cellular distribution, which may be caused by MPK activation and PIN phosphorylation.

8. * Dory M, Hatzimasoura E, Kallai BM, Nagy SK, Jager K, Darula Z, Nadai TV, Meszaros T, Juez EL, Barnabas B, *et al.*: **Co-evolving MAPK and PID phosphosites indicate an ancient environmental control of PIN auxin transporters in land plants**. *FEBS Lett* 2017, **592**:89–102.

In this largely biochemical study, the authors demonstrate the targeting of PIN1 T1 - T3 by MAP kinases and investigate to some extent the possible effects on PIN distribution.

9. * Rigo G, Ayaydin F, Tietz O, Zsigmond L, Kovacs H, Pay A, Salchert K, Darula Z, Medzihradszky KF, Szabados L, *et al.*: **Inactivation of plasma membrane-localized CDPK-RELATED KINASE5 decelerates PIN2 exocytosis and root gravitropic response in Arabidopsis**. *Plant Cell* 2013, **25**:1592–1608.

This study describes for the first time the regulation of PIN2 by CRK kinases. Further work from the group implies additional CRK family members in PIN control.

10. Baba AI, Valkai I, Labhane NM, Koczka L, Andrasi N, Klement E, Darula Z, Medzihradszky KF, Szabados L, Feher A, *et al.*: **CRK5 protein kinase contributes to the progression of embryogenesis of *Arabidopsis thaliana***. *Int J Mol Sci* 2019, **20**:6120.

11. Baba AI, Andrasi N, Valkai I, Gorcsa T, Koczka L, Darula Z, Medzihradszky KF, Szabados L, Feher A, Rigo G, *et al.*: **AtCRK5 protein kinase exhibits a regulatory role in hypocotyl hook development during skotomorphogenesis**. *Int J Mol Sci* 2019, **20**:3432.

12. Baba AI, Rigo G, Ayaydin F, Rehman AU, Andrasi N, Zsigmond L, Valkai I, Urbancsok J, Vass I, Pasternak T, *et al.*: **Functional analysis of the *Arabidopsis thaliana* CDPK-related kinase family: AtCRK1 regulates responses to continuous light**. *Int J Mol Sci* 2018, **19**:1282.

13. ** Hajny J, Prat T, Rydza N, Rodriguez L, Tan S, Verstraeten I, Domjan D, Mazur E, Smakowska-Luzan E, Smet W, *et al.*: **Receptor kinase module targets PIN-dependent auxin transport during canalization**. *Science* 2020, **370**:550–557.

During vascular development, CAMEL and CANAR may form a receptor kinase complex with a role in auxin-dependent PIN polarity control.

14. Bogre L, Okresz L, Henriques R, Anthony RG: **Growth signalling pathways in Arabidopsis and the AGC protein kinases**. *Trends Plant Sci* 2003, **8**:424–431.

15. Galvan-Ampudia CS, Offringa R: **Plant evolution: AGC kinases tell the auxin tale**. *Trends Plant Sci* 2007, **12**:541–547.

16. Li FW, Rothfels CJ, Melkonian M, Villarreal JC, Stevenson DW, Graham SW, Wong GK, Mathews S, Pryer KM: **The origin and evolution of phototropins**. *Front Plant Sci* 2015, **6**:637.

17. Briggs WR, Christie JM: **Phototropins 1 and 2: versatile plant blue-light receptors**. *Trends Plant Sci* 2002, **7**:204–210.

18. Ding Z, Galvan-Ampudia CS, Demarsy E, Langowski L, Kleine-Vehn J, Fan Y, Morita MT, Tasaka M, Fankhauser C, Offringa R, *et al.*: **Light-mediated polarization of the PIN3 auxin transporter for the phototropic response in Arabidopsis**. *Nat Cell Biol* 2011, **13**:447–452.

19. Christie JM, Yang H, Richter GL, Sullivan S, Thomson CE, Lin J, Titapiwatanakun B, Ennis M, Kaiserli E, Lee OR, *et al.*: **phot1 inhibition of ABCB19 primes lateral auxin fluxes in the shoot apex required for phototropism**. *PLoS Biol* 2011, **9**:e1001076.

20. Willige BC, Ahlers S, Zourelidou M, Barbosa IC, Demarsy E, Trevisan M, Davis PA, Roelfsema MR, Hangarter R, Fankhauser C, *et al.*: **D6PK AGCVIII kinases are required for auxin transport and phototropic hypocotyl bending in Arabidopsis**. *Plant Cell* 2013, **25**:1674–1688.

21. Barbosa IC, Zourelidou M, Willige BC, Weller B, Schwechheimer C: **D6 PROTEIN KINASE activates auxin transport-dependent growth and PIN-FORMED phosphorylation at the plasma membrane**. *Dev Cell* 2014, **29**:674–685.

22. Haga K, Frank L, Kimura T, Schwechheimer C, Sakai T: **Roles of AGCVIII kinases in the hypocotyl phototropism of Arabidopsis seedlings**. *Plant Cell Physiol* 2018, **59**:1060–1071.

23. Enugutti B, Kirchhelle C, Oelschner M, Torres Ruiz RA, Schliebner I, Leister D, Schneitz K: **Regulation of planar growth by the Arabidopsis AGC protein kinase UNICORN**. *Proc Natl Acad Sci USA* 2012, **109**:15060–15065.

24. Rentel MC, Lecourieux D, Ouaked F, Usher SL, Petersen L, Okamoto H, Knight H, Peck SC, Grierson CS, Hirt H, *et al.*: **OXI1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis**. *Nature* 2004, **427**:858–861.

25. Zourelidou M, Muller I, Willige BC, Nill C, Jikumaru Y, Li H, Schwechheimer C: **The polarly localized D6 PROTEIN KINASE is required for efficient auxin transport in Arabidopsis thaliana**. *Development* 2009, **136**:627–636.

26. Mergner J, Frejno M, List M, Papacek M, Chen X, Chaudhary A, Samaras P, Richter S, Shikata H, Messerer M, *et al.*: **Mass-spectrometry-based draft of the Arabidopsis proteome**. *Nature* 2020, **579**:409–414.

27. Hammes UZ, Murphy AS, Schwechheimer C: **Auxin Transporters-A Biochemical View**. In *Cold Spring Harb Perspect Biol,* edn 2021/06/16. Edited by: Cold Spring Harbor Laboratory Press; 2021:49–69.

28. Day IS, Miller C, Golovkin M, Reddy AS: **Interaction of a kinesin-like calmodulin-binding protein with a protein kinase**. *J Biol Chem* 2000, **275**:13737–13745.

29. Hadfi K, Speth V, Neuhaus G: **Auxin-induced developmental patterns in *Brassica juncea* embryos**. *Development* 1998, **125**: 879–887.

30. * Abas L, Kolb M, Stadlmann J, Janacek DP, Lukic K, Schwechheimer C, Sazanov LA, Mach L, Friml J, Hammes UZ: **Naphthylphthalamic acid associates with and inhibits PIN auxin transporters**. *Proc Natl Acad Sci USA* 2021, **118**. e2020857118.

Based on heterologous PIN auxin transport assays, the authors demonstrate that the well-known auxin transport inhibitor NPA targets PIN transporters directly.

31. Lee BH, Weber ZT, Zourelidou M, Hofmeister BT, Schmitz RJ, Schwechheimer C, Dobritsaa A: **Arabidopsis protein kinase D6PKL3 is involved in formation of distinct plasma-membrane aperture domains on the pollen surface**. *Plant Cell* 2018, **30**:2038–2056.

32. * Koh SWH, Marhava P, Rana S, Graf A, Moret B, Bassukas AEL, Zourelidou M, Kolb M, Hammes UZ, Schwechheimer C, *et al.*: **Mapping and engineering of auxin-induced plasma membrane dissociation in BRX family proteins**. *Plant Cell* 2021, **33**: 1945–1960.

Through domain swapping, the authors identify a protein domain and phosphosite required for the nuclear targeting of BRX.

33. ** Marhava P, Aliaga Fandino AC, Koh SWH, Jelinkova A, Kolb M, Janacek DP, Breda AS, Cattaneo P, Hammes UZ, Petrasek J, *et al.*: **Plasma membrane domain patterning and self-reinforcing polarity in Arabidopsis**. *Dev Cell* 2020, **52**: 223–235. e225.

The recruitment of PIP5 kinases to the plasma membrane through BRX may facility the binding of BRX-interacting AGC1 kinases as part of a self reinforcing mechanism.

34. Barbosa IC, Shikata H, Zourelidou M, Heilmann M, Heilmann I, Schwechheimer C: **Phospholipid composition and a polybasic motif determine D6 PROTEIN KINASE polar association with the plasma membrane and tropic responses**. *Development* 2016, **143**:4687–4700.

35. Devarenne TP, Ekengren SK, Pedley KF, Martin GB: **Adi3 is a Pdk1-interacting AGC kinase that negatively regulates plant cell death**. *EMBO J* 2006, **25**:255–265.

36. Gray JW, Nelson Dittrich AC, Chen S, Avila J, Giavalisco P, Devarenne TP: **Two Pdk1 phosphorylation sites on the plant cell death suppressor Adi3 contribute to substrate phosphorylation**. *Biochim Biophys Acta* 2013, **1834**:1099–1106.

37. Ek-Ramos MJ, Avila J, Cheng C, Martin GB, Devarenne TP: **The T-loop extension of the tomato protein kinase AvrPto-dependent Pto-interacting protein 3 (Adi3) directs nuclear localization for suppression of plant cell death**. *J Biol Chem* 2010, **285**:17584–17594.

38. McSteen P, Malcomber S, Skirpan A, Lunde C, Wu X, Kellogg E, Hake S: **Barren inflorescence2 encodes a co-ortholog of the PINOID serine/threonine kinase and is required for organogenesis during inflorescence and vegetative development in maize**. *Plant Physiol* 2007, **144**:1000–1011.

39. Skirpan A, Wu X, McSteen P: **Genetic and physical interaction suggest that BARREN STALK1 is a target of BARREN INFLORESCENCE2 in maize inflorescence development**. *Plant J* 2008, **55**:787–797.

40. Skirpan A, Culler AH, Gallavotti A, Jackson D, Cohen JD, McSteen P: **BARREN INFLORESCENCE2 interaction with ZmPIN1a suggests a role in auxin transport during maize inflorescence development**. *Plant Cell Physiol* 2009, **50**: 652–657.

41. Xu M, Tang D, Cheng X, Zhang J, Tang Y, Tao Q, Shi W, You A, Gu M, Cheng Z, *et al.*: **OsPINOID regulates stigma and ovule initiation through maintenance of the floral meristem by auxin signaling**. *Plant Physiol* 2019, **180**:952–965.

42. Wu HM, Xie DJ, Tang ZS, Shi DQ, Yang WC: **PINOID regulates floral organ development by modulating auxin transport and interacts with MADS16 in rice**. *Plant Biotechnol J* 2020, **18**: 1778–1795.

43. ** Tan S, Zhang X, Kong W, Yang XL, Molnar G, Vondrakova Z, Filepova R, Petrasek J, Friml J, Xue HW: **The lipid code-dependent phosphoswitch PDK1-D6PK activates PIN-mediated auxin efflux in Arabidopsis**. *Native Plants* 2020, **6**: 556–569.

44. ** Xiao Y, Offringa R: **PDK1 regulates auxin transport and Arabidopsis vascular development through AGC1 kinase PAX**. *Native Plants* 2020, **6**:544–555.
The two studiese cited above demonstrate the dependency of AGC1 kinases D6PK and PAX on activation loop phosphorylation by PDK1.

45. Zegzouti H, Anthony RG, Jahchan N, Bogre L, Christensen SK: **Phosphorylation and activation of PINOID by the phospholipid signaling kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) in Arabidopsis**. *Proc Natl Acad Sci USA* 2006, **103**:6404–6409.

46. Huang F, Zago MK, Abas L, van Marion A, Galvan-Ampudia CS, Offringa R: **Phosphorylation of conserved PIN motifs directs Arabidopsis PIN1 polarity and auxin transport**. *Plant Cell* 2010, **22**:1129–1142.

47. Benjamins R, Quint A, Weijers D, Hooykaas P, Offringa R: **The PINOID protein kinase regulates organ development in Arabidopsis by enhancing polar auxin transport**. *Development* 2001, **128**:4057–4067.

48. Santner AA, Watson JC: **The WAG1 and WAG2 protein kinases negatively regulate root waving in Arabidopsis**. *Plant J* 2006, **45**:752–764.

49. ** Wang P, Shen L, Guo J, Jing W, Qu Y, Li W, Bi R, Xuan W, Zhang Q, Zhang W: **Phosphatidic acid directly regulates PINOID-dependent phosphorylation and activation of the PIN-FORMED2 auxin efflux transporter in response to salt stress**. *Plant Cell* 2019, **31**:250–271.
This study not only demonstrates the activation of PIN2 by AGC3 kinases but also the regulatory role of phosphatidic acid in the process during salt stress.

50. Simon ML, Platre MP, Marques-Bueno MM, Armengot L, Stanislas T, Bayle V, Caillaud MC, Jaillais Y: **A PtdIns(4)P-driven electrostatic field controls cell membrane identity and signalling in plants**. *Native Plants* 2016, **2**:16089.

51. Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PB, Ljung K, Sandberg G, *et al.*: **A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux**. *Science* 2004, **306**:862–865.

52. Weller B, Zourelidou M, Frank L, Barbosa IC, Fastner A, Richter S, Jurgens G, Hammes UZ, Schwechheimer C: **Dynamic PIN-FORMED auxin efflux carrier phosphorylation at the plasma membrane controls auxin efflux-dependent growth**. *Proc Natl Acad Sci USA* 2017, **114**:E887–E896.

53. Furutani M, Sakamoto N, Yoshida S, Kajiwara T, Robert HS, Friml J, Tasaka M: **Polar-localized NPH3-like proteins regulate polarity and endocytosis of PIN-FORMED auxin efflux carriers**. *Development* 2011, **138**:2069–2078.

54. ** Glanc M, Van Gelderen K, Hoermayer L, Tan S, Naramoto S, Zhang X, Domjan D, Vcelarova L, Hauschild R, Johnson A, *et al.*: **AGC kinases and MAB4/MEL proteins maintain PIN polarity by limiting lateral diffusion in plant cells**. *Curr Biol* 2021, **31**: 1918–1930.
The study provides cell biological and biochemical evidence for a complex consisting of PINs, AGC kinases and MAB4/MEL proteins controlling lateral PIN diffusion.

55. Meng X, Zhang S: **MAPK cascades in plant disease resistance signaling**. *Annu Rev Phytopathol* 2013, **51**:245–266.

56. Xi W, Gong X, Yang Q, Yu H, Liou YC: **Pin1At regulates PIN1 polar localization and root gravitropism**. *Nat Commun* 2016, **7**:10430.

57. Zhang J, Nodzynski T, Pencik A, Rolcik J, Friml J: **PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport**. *Proc Natl Acad Sci USA* 2010, **107**:918–922.

58. Ditengou FA, Gomes D, Nziengui H, Kochersperger P, Lasok H, Medeiros V, Paponov IA, Nagy SK, Nadai TV, Meszaros T, *et al.*: **Characterization of auxin transporter PIN6 plasma membrane targeting reveals a function for PIN6 in plant bolting**. *New Phytol* 2017, **217**:1610–1624.

60. Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, Wang J, Cong Q, Kinch LN, Schaeffer RD, *et al.*: **Accurate prediction of protein structures and interactions using a three-track neural network**. *Science* 2021, **373**:871–876.

Further reading

59. Yang J, Anishchenko I, Park H, Peng Z, Ovchinnikov S, Baker D: **Improved protein structure prediction using predicted inter-residue orientations**. *Proc Natl Acad Sci USA* 2020, **117**: 1496–1503.