

DEVELOPMENT AT A GLANCE

PIN-FORMED and PIN-LIKES auxin transport facilitators

Michael Sauer^{1,*} and Jürgen Kleine-Vehn^{2,*}

ABSTRACT

The phytohormone auxin influences virtually all aspects of plant growth and development. Auxin transport across membranes is facilitated by, among other proteins, members of the PIN-FORMED (PIN) and the structurally similar PIN-LIKES (PILS) families, which together govern directional cell-to-cell transport and intracellular accumulation of auxin. Canonical PIN proteins, which exhibit a polar localization in the plasma membrane, determine many patterning and directional growth responses. Conversely, the less-studied non-canonical PINs and PILS proteins, which mostly localize to the endoplasmic reticulum, attenuate cellular auxin responses. Here, and

in the accompanying poster, we provide a brief summary of current knowledge of the structure, evolution, function and regulation of these auxin transport facilitators.

KEY WORDS: Auxin, Auxin transport, Phytohormone

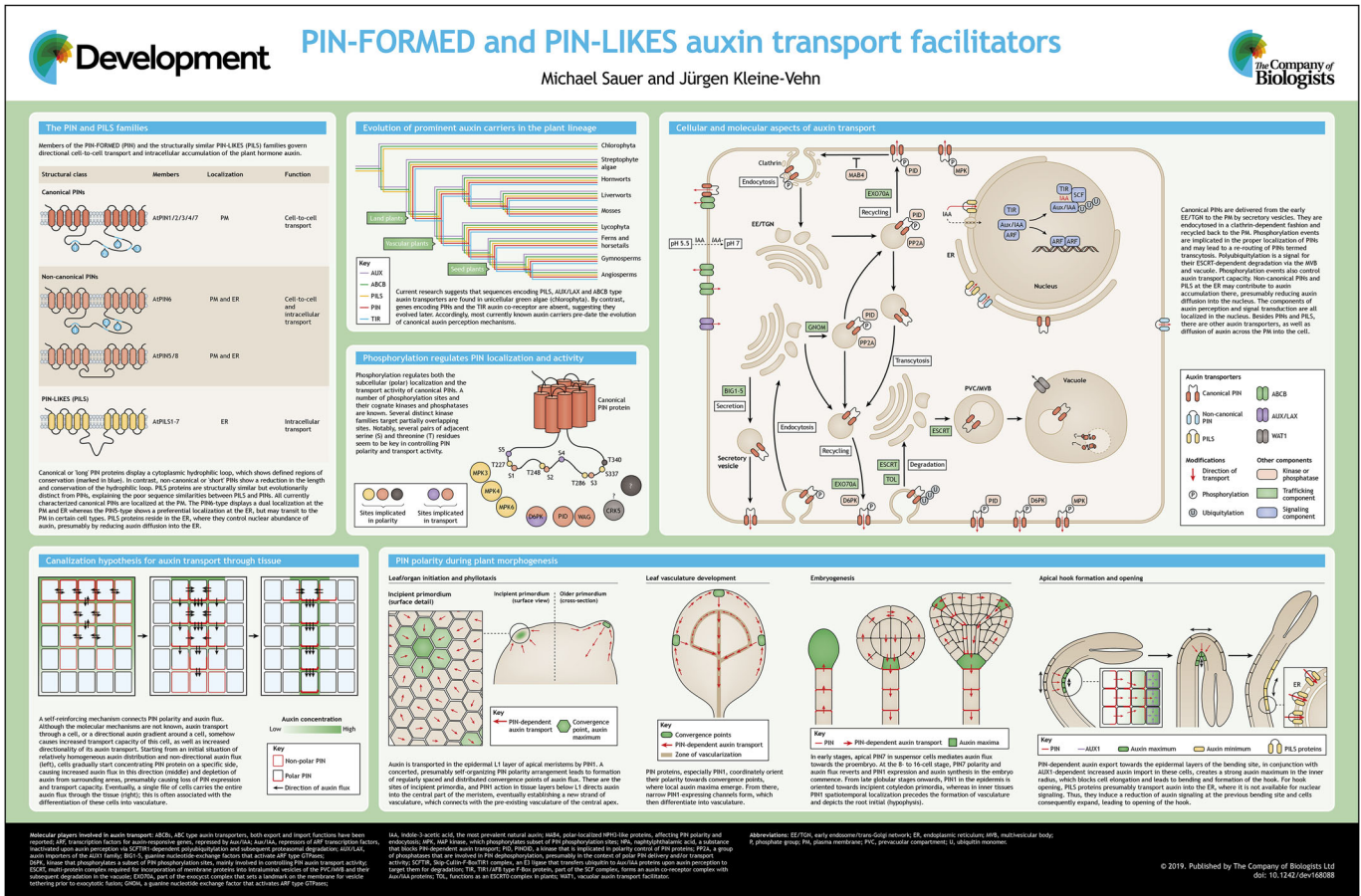
Introduction

Auxin, in particular its most abundant form indole-3-acetic acid (IAA), is the best-studied phytohormone. An immense body of literature has implicated auxin in nearly all growth-related aspects of a plant's life cycle. A characteristic feature of auxin is its complex inter- and intracellular transport, which gives rise to spatiotemporal variations in auxin concentrations and, consequently, auxin signaling that govern many aspects of plant development. Auxin transport is facilitated by proteins of the PIN family and the topologically similar PIN-LIKES (PILS) family. These proteins control cell-to-cell and intracellular auxin transport and thus constitute important determinants of plant architecture (Mravec et al., 2009; Barbez et al., 2012).

¹Institute for Biochemistry and Biology, University of Potsdam, 14476 Potsdam, Germany. ²Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences Vienna (BOKU), Muthgasse 18, 1190 Vienna, Austria.

*Authors for correspondence (michael.sauer@uni-potsdam.de; juergen.kleine-vehn@boku.ac.at)

id M.S., 0000-0003-2790-2170; J.K., 0000-0002-4354-3756



Here, we provide an overview of PIN and PILS auxin transport facilitators. Other prominent auxin transporters, namely the AUX1/LAX type auxin importers, as well as importers and exporters of the ABCB/MDR type have been reviewed elsewhere (Singh et al., 2018; Geisler et al., 2017) and are not covered here.

The structure and evolution of PIN and PILS proteins

PIN orthologs are found in charophycean algae (Hori et al., 2014; Bowman et al., 2018) and thus pre-date the origin of land plants. Concomitant with the acquisition of more complex, three-dimensional body plans and the transition to land, the PIN family became bigger and diversified into different clades (Viaene et al., 2013; Bennett et al., 2014a). The unifying topology of all PIN proteins is a central hydrophilic loop of varying length, which is flanked by several, mostly conserved, N- and C-terminal transmembrane domains. Based on protein structure, PIN proteins can be further classified as either canonical or non-canonical (Bennett et al., 2014a).

Canonical PINs typically have longer hydrophilic loops that contain four highly conserved motifs (Bennett et al., 2014a). Although full structural analysis is still lacking, a topological study suggests that these proteins contain ten transmembrane domains and an orientation of the hydrophilic loop towards the cytoplasm, allowing access to kinases and phosphatases that regulate subcellular localization and activity (Nodzyński et al., 2016). Canonical PINs localize to the plasma membrane (PM) and function in cell-to-cell auxin efflux. This has been shown in various seed plant species, but also in the moss *Physcomitrella patens* (Bennett et al., 2014b; Viaene et al., 2014), suggesting a conserved role of canonical PIN proteins. In *Arabidopsis*, five out of the eight PIN family members, AtPIN1-4 and AtPIN7, are of the canonical type (Bennett et al., 2014a).

In contrast, non-canonical PINs are characterized by a more variable, typically shorter and less-conserved hydrophilic loop. The non-canonical PIN proteins found in *Arabidopsis* (AtPIN5, 6 and 8) localize to the endoplasmic reticulum (ER) to some extent and have been implicated in intracellular auxin transport (Mravec et al., 2009; Bennett et al., 2014a). Based on phylogenetic trait mapping, this intracellular PIN-dependent auxin transport was proposed as an ancestral function (Mravec et al., 2009; Viaene et al., 2014); however, more-extensive sampling has suggested that non-canonical PIN proteins in fact evolved independently (at least seven times) from canonical ancestors (Bennett et al., 2014a).

The higher variability in the hydrophilic loop and transmembrane domains of non-canonical PINs may translate to altered substrate specificity: indeed, the non-canonical AtPIN8 transports endogenous auxins, but also indole butyric acid and the synthetic auxin analog 2,4-dichlorophenoxyacetic acid, which are poor substrates for canonical PINs (Ding et al., 2012). Further studies are required to test whether non-canonical PINs and/or PILS can transport auxin metabolites, such as intermediates and conjugates, and thus could play additional roles in compartmentalized auxin metabolism (Barbez and Kleine-Vehn, 2013).

PILS proteins appear structurally similar to PINs but constitute a distinct protein family that evolved independently (Feraru et al., 2012). PILS proteins share little sequence identity with PINs despite their similar topology and their demonstrated function in intracellular auxin accumulation (Barbez et al., 2012). All PILS subfamilies in *Arabidopsis* (AtPILS1-7) have been shown to be ER localized and to contribute to intracellular auxin transport (Barbez et al., 2012; Béziat et al., 2017; Feraru et al., 2019). It is presumed that they transport auxin into the ER lumen, which would limit auxin diffusion into the nucleus and thereby restrict

the nuclear abundance and signaling of auxin (Barbez et al., 2012; Béziat et al., 2017; Feraru et al., 2019). In contrast to PINs, PILS orthologs are found in the chlorophyta clade (Barbez et al., 2012; Feraru et al., 2012), suggesting a more ancient role in plants.

PIN and PILS protein function

Canonical PIN proteins play an overarching role in plant development, which is best exemplified by the five canonical *Arabidopsis* PIN proteins, AtPIN1-4 and AtPIN7. These proteins are expressed throughout plant development, displaying distinct but also overlapping tissue distributions (reviewed by Zhou and Luo, 2018). They function largely redundantly, because the loss of a single family member often causes ectopic, transcriptional upregulation of another member to compensate (Vieten et al., 2005; Rosquete et al., 2013).

Canonical PIN proteins control directional cell-to-cell auxin transport at the PM. This shapes the distribution of auxin within tissues and organs, creating regions of low ('minima') and high ('maxima') auxin concentration that are crucially important to define positional cues for cellular fate changes within a developmental context (reviewed by Sauer et al., 2013). In this way, PIN proteins govern fundamental developmental and patterning aspects, such as apical-basal axis formation during embryogenesis, canalization/vascularization, *de novo* organogenesis, as well as stem cell and meristematic activity (reviewed by Sauer et al., 2013). Asymmetric tissue expansion in response to external triggers, as found in all sorts of tropisms, also requires dynamic, canonical PIN-dependent rearrangement of auxin distribution (reviewed by Sauer et al., 2013). In all of these developmental contexts, the defined polar localization of PIN proteins to certain sides of given cells determines the direction of auxin flux. Accordingly, understanding the mechanisms by which PIN proteins are delivered to membranes is key to describing and understanding plant development (Naramoto, 2017; Béziat and Kleine-Vehn, 2018).

Non-canonical PINs are mechanistically less well understood, but also contribute to diverse aspects of plant development, such as male gametophyte development, vascularization and organogenesis (Mravec et al., 2009; Ding et al., 2012; Simon et al., 2016; Ditengou et al., 2018). Similarly, the developmental role of PILS proteins is poorly understood. PILS proteins limit the rate of nuclear auxin signaling and play a role in integrating external signals, such as light and temperature, into auxin-dependent growth programs (Béziat et al., 2017; Feraru et al., 2019). PILS proteins impact *de novo* organogenesis as well as root and shoot organ growth rates (Barbez et al., 2012; Feraru et al., 2019). Similar to canonical PIN proteins, PILS proteins can also modulate differential growth responses along an organ; however, they do so by generating spatially defined auxin signaling minima (Béziat et al., 2017).

PIN and PILS membrane delivery and turnover

In general, the delivery and turnover of canonical PINs does not differ from that of other PM localized transmembrane proteins. They enter the secretory pathway during synthesis and pass through the ER, Golgi and trans-Golgi network before being delivered to the PM by secretory vesicles. Like many other PM-localized proteins, they undergo a cycle of constant endocytosis and subsequent recycling to the PM (Naramoto, 2017). The nature of the so-called recycling endosome that mediates this process is not clear, but seems to involve the ARF-GEF protein GNOM. In dividing cells, PINs are not sent to the PM but to the incipient cell plate, from which they are re-sorted upon entry into interphase (Men et al., 2008; Glanc et al., 2018).

A large and growing body of work has implicated numerous players in the delivery of PINs, including ARF-type small GTPases and regulators of the ARF-GEF and ARF-GAP classes, cytoskeletal components or vesicle tethering and docking machineries such as the SNARE and Exocyst complexes, as well as membrane lipid composition (reviewed by Naramoto, 2017; Singh and Jürgens, 2018; Vukašinović and Žárský, 2016). Not all canonical PINs follow the exact same pathway, presumably reflecting a partially distinct set of underlying regulators (Sauer et al., 2013).

Endocytosis of PM-localized PINs is clathrin dependent and involves AP2 adaptor complexes. Several compounds, including synthetic auxin (Paciorek et al., 2005), can slow down the rate of endocytosis although the exact mechanism and significance of this phenomenon is unresolved. Moreover, the ubiquitylation of certain residues leads to TOL- and ESCRT-dependent sorting of PINs into the intraluminal vesicles of multivesiculate bodies/late endosomes and eventual degradation in the vacuole (Korbei and Luschnig, 2013). It is currently not clear whether this ubiquitylation occurs directly at the PM or at an intermediate step after endocytosis.

Much less is known about the localization and delivery mechanisms of non-canonical PINs and PILS proteins. As multispan-membrane proteins, they are presumably co-translationally inserted into the ER membrane but the underlying retention mechanism that keeps them from escaping from the ER by bulk flow is unknown. Likewise, PILS protein turnover by post-translational mechanisms is a developmentally important phenomenon (Feraru et al., 2019) but is currently not understood at the molecular level.

Whereas PILS proteins have so far been reported to localize only at the ER (Barbez et al., 2012; Béziat et al., 2017; Feraru et al., 2019), the localization of non-canonical PINs appears to be more variable than originally anticipated. For instance, AtPIN6 displays dual localization at the ER and PM within the same cell (Simon et al., 2016; Ditengou et al., 2018), and AtPIN5 shows a cell type-dependent distribution, displaying localization at the PM in aerial tissues and intracellular localization in differentiated root vascular cells (Ganguly et al., 2014). PIN8 is specifically expressed in pollen and colocalizes with PIN5 at the ER; in this context, PIN5 and PIN8 seem to have opposing effects on gametophytic auxin homeostasis, possibly reflecting auxin import and export activity, respectively (Ding et al., 2012).

The control of PIN activity and polarity

In most cells, canonical PIN proteins exhibit a polar localization at the PM and thereby determine the directional, cell-to-cell transport of auxin. Intriguingly, the kinase-dependent phosphorylation of PINs appears to control both auxin transport activity and the polar delivery of PIN proteins, acting at partially overlapping phosphorylation sites. The so-called S1, S2 and S3 phosphorylation sites in the central hydrophilic loop of canonical PINs are targeted by two subfamilies of AGCVIII protein kinases, namely the D6 PROTEIN KINASES (D6PKs) as well as PINOID (PID) and the two closely related WAVY ROOT GROWTH (WAG) 1 and WAG2 kinases (Zourelidou et al., 2014; Weller et al., 2017; Friml et al., 2004; Dhonukshe et al., 2010). PINOID and WAG activity determines the polar delivery of PINs to the apical (shoot-ward) and basal (root-ward) side of cells (Friml et al., 2004; Kleine-Vehn et al., 2009; Grones et al., 2018). Accordingly, phospho-mimicking mutants for the S1-S3 sites exhibit a basal-to-apical shift in PIN proteins (Dhonukshe et al., 2010; Grones et al., 2018). In contrast, D6PK overexpression or loss of function has no effect on PIN polarity, but instead affects the auxin transport activity of PINs (Zourelidou et al., 2014). In agreement with the observed D6PK-

and PID-dependent control of S1-S3, phospho-specific antibodies for S1-S3 detect PIN proteins at the upper as well as lower sides of cells (Weller et al., 2017). These findings suggest that the phosphorylation status of the S1-S3 sites in PINs alone cannot explain the regulation of PIN polarity. It is therefore possible that additional characteristics of PID/WAGs impact on PIN polarity.

Phosphatases of the PP2A class counteract the PID-dependent phosphorylation of PIN proteins and, thereby, regulate apical and basal PIN targeting (Michniewicz et al., 2007). The impact of PID and PP2As on PIN phosphorylation determines the recruitment of PIN proteins to GNOM-dependent and GNOM-independent intracellular sorting pathways at the basal and apical cell sides, respectively (Kleine-Vehn et al., 2009). Thus, the phosphorylation status of PIN proteins inside an endosomal compartment (and not at the PM) could be decisive for its polar delivery. It is currently unknown whether PID or D6PKs feedback on PP2A activity (or other unknown molecular components), which could theoretically result in a differential impact on PIN phosphorylation status in endomembranes.

Besides the S1-S3 sites, five additional phosphorylation sites have been linked to the regulation of PIN polarity (reviewed by Barbosa et al., 2018; Zhou and Luo, 2018). It remains to be seen whether the phosphorylation status of one site also impacts on the regulation of others, and to what degree these sites jointly impact on PIN activity and polarity. In this regard, heterologous expression systems have proven to be instrumental for characterizing PIN auxin carrier activity. For example, the expression of PIN proteins in yeast and animal HeLa cells has been shown to be sufficient to induce auxin efflux capacity in these cells (Petrásek et al., 2006; Yang and Murphy, 2009). On the other hand, *Xenopus laevis* oocytes are particularly well suited as a heterologous system to assess the phosphorylation-dependent regulation of auxin transport, revealing that PIN proteins require the co-expression of a kinase, such as PID or D6PK, to induce auxin efflux (Zourelidou et al., 2014; Fastner et al., 2017). D6PK also targets the so-called S4 and S5 sites, which are seemingly not involved in PIN polarity control but are the most important sites for PIN activation by D6PK in oocytes (Zourelidou et al., 2014). Notably, phospho-mimicking versions of PINs are not sufficient to induce auxin transport activity in oocytes (Weller et al., 2017), which indicates that additional features of PID and D6PK contribute to their impact on PINs in oocytes. It is known that AGC kinases have diversified affinities for distinct interaction partners (Marhava et al., 2018), and it is therefore conceivable that additional interactors of PID/WAGs and D6PK contribute to the distinct, but partially overlapping, regulation of PIN polarity and activity.

Other molecular components and pathways may also target PIN proteins to modulate their phosphorylation-dependent characteristics. The Pin1At protein, for example, is a peptidyl-prolyl cis/trans isomerase that impacts on PIN1 protein conformation in the region of the phosphorylation sites. Pin1At may therefore affect PID- and PP2A-mediated regulation of PIN1 polar localization (Xi et al., 2016). Another example is selective degradation of PIN proteins at specific polar domains in a cytokinin-dependent fashion (Marhavy et al., 2014). This local depletion of PIN protein depends on its phosphorylation status (Marhavy et al., 2014), but how this regulation relates to auxin transport activities and/or phosphorylation-dependent polarity cues is currently unknown.

Conclusions and perspectives

Research efforts over the last three decades have provided us with a basic understanding of PM-localized, canonical PIN proteins and their functions in plant development. However, we know

comparatively less about the roles of non-canonical PINs (Mravec et al., 2009) and the evolutionarily distinct PILS proteins (Barbez et al., 2012). Both non-canonical PINs as well as PILS proteins likely sequester auxin in the ER and impact on cellular auxin homeostasis, highlighting a certain level of functional redundancy between these unrelated groups (Mravec et al., 2009; Barbez et al., 2012). On the other hand, non-canonical PIN proteins may also transit to the PM in certain cell types or conditions. Hence, the precise and possibly overlapping contribution of non-canonical PINs and PILS to intracellular auxin accumulation presents a challenging topic. In order to understand these proteins better, we will need more information about the role of the ER in intracellular auxin metabolism and auxin signaling.

It has been shown that auxin conjugate hydrolases of the ILR1-like family reside in the ER (Sanchez Carranza et al., 2016). In order to be hydrolyzed, auxin conjugates are therefore likely to be transported into the ER. It remains to be seen whether PINs, PILS or yet-to-be-identified carriers can transport auxin conjugates and could thus contribute to compartmentalized auxin metabolism. Transport of auxin conjugates into the ER would presumably induce hydrolysis and would consequently increase free auxin levels, but on the contrary, both PIN5 and PILS5 induction correlate with higher levels of auxin conjugates. Moreover, there is growing evidence to support a role for PILS proteins in attenuating nuclear auxin signaling by restricting nuclear auxin availability (Béziat et al., 2017; Feraru et al., 2019; Sun et al., 2019 preprint). Hence, it is likely that PILS proteins deplete cytosolic auxin levels, reducing auxin diffusion from the cytosol into the nucleus. It has also been proposed, in contrast, that the ER acts rather as a positive factor for auxin signaling. This notion was inspired by the finding that the auxin transport probe NBD-NAA labels the ER but does not enter the nucleus by simple diffusion (Hayashi et al., 2014; Middleton et al., 2018). It is currently unclear whether NBD-NAA reflects the intracellular distribution of endogenous auxins. However, this finding motivated a computational study of auxin flux that proposed auxin transporters at the ER could play a dominant role in promoting nuclear accumulation of auxin (Middleton et al., 2018). The simulations predict a fundamental importance of the ER as the main conduit for nuclear auxin uptake, but such a mechanism awaits experimental validation.

Despite much progress in recent years, we also still lack a comprehensive understanding of the cellular events that define the polar delivery of canonical PIN proteins. As discussed above, a crucial question is how phosphorylation of the same sites contributes to both PIN polarity and/or the regulation of transport activity. Additional polarity regulators are likely involved and could interact with the underlying kinases and phosphatases. The mechanistic framework governing these interactions is unclear and will likely be complex. Besides phosphorylation-based mechanisms, other factors and mechanisms could be involved. Indeed, new players in plant cell polarity are emerging and may play a role in regulating PIN polar delivery. The recently discovered *DEEPER ROOTING 1 (DRO1)/NEGATIVE GRAVITROPIC RESPONSE OF ROOTS (NGR)/LAZY (LZY)* genes encode such factors. It was shown that in rice, *Medicago* and *Arabidopsis*, *dro/ngr/lzy* mutants impact on the directional redistribution of auxin and display a partially reversed direction of root and shoot gravitropism (Uga et al., 2013; Guseman et al., 2017; Ge and Chen, 2016; Taniguchi et al., 2017; Yoshihara and Spalding, 2017). In rice, *LZY* genes mediate gravity sensing, controlling PIN3 polarization and asymmetric auxin distribution in gravity-responding organs (Taniguchi et al., 2017). The precise mechanisms by which these proteins guide PIN polarization towards or away from gravity

remain to be determined, but the continued study of this novel family will very likely advance our understanding of the molecular events controlling PIN polarity. Another example of potential polarity regulators are the recently identified SOSEKI proteins, which localize in a polar fashion to various cell edges, seemingly integrating apical-basal and radial axis information (Yoshida et al., 2019). Interestingly, they are one of the few polarity-related proteins in plants that bear similarity to a known regulator of polarity in animals: they possess a DIX oligomerization domain related to that found in the animal polarity regulator Dishevelled (Yoshida et al., 2019). Whether SOSEKI proteins play a role in PIN polar delivery remains to be seen, but the discovery of this interesting protein family also opens up new avenues for the study of plant cell polarity.

Acknowledgements

We are grateful to Ulrich Hammes for valuable comments on the manuscript.

Competing interests

The authors declare no competing or financial interests.

Funding

This work was supported by the Vienna Science and Technology Fund (WWTF) (to J.K.-V.), Austrian Science Fund (FWF) (Projects: P26568-B16 and P26591-B16 to J.K.-V.) and the European Research Council (AuxinER - ERC starting grant to J.K.-V.).

Development at a Glance

A high-resolution version of the poster is available for downloading in the online version of this article at <http://dev.biologists.org/content/146/15/dev168088/F1.poster.jpg>

References

- Barbez, E. and Kleine-Vehn, J. (2013). Divide Et Impera—cellular auxin compartmentalization. *Curr. Opin. Plant Biol.* **16**, 78–84. doi:10.1016/j.pbi.2012.10.005
- Barbez, E., Kubeš, M., Rolčík, J., Béziat, C., Pěňčík, A., Wang, B., Rosquete, M. R., Zhu, J., Dobrev, P. I. and Lee, Y. et al. (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* **485**, 119–122. doi:10.1038/nature11001
- Barbosa, I. C. R., Hammes, U. Z. and Schwechheimer, C. (2018). Activation and polarity control of PIN-FORMED auxin transporters by phosphorylation. *Trends Plant Sci.* **23**, 523–538. doi:10.1016/j.tplants.2018.03.009
- Bennett, T., Brockington, S. F., Rothfels, C., Graham, S. W., Stevenson, D., Kutchan, T., Rolf, M., Thomas, P., Wong, G. K., Leyser, O. et al. (2014a). Paralogous radiations of PIN proteins with multiple origins of noncanonical PIN structure. *Mol. Biol. Evol.* **31**, 2042–2060. doi:10.1093/molbev/msu147
- Bennett, T. A., Liu, M. M., Aoyama, T., Bierfreund, N. M., Braun, M., Coudert, Y., Dennis, R. J., O'Connor, D., Wang, X. Y., White, C. D. et al. (2014b). Plasma membrane-targeted PIN proteins drive shoot development in a moss. *Curr. Biol.* **24**:2776–2785. doi:10.1016/j.cub.2014.09.054
- Béziat, C. and Kleine-Vehn, J. (2018). The road to auxin-dependent growth repression and promotion in apical hooks. *Curr. Biol.* **28**, 519–525. doi:10.1016/j.cub.2018.01.069
- Béziat, C., Barbez, E., Feraru, M. I., Lucyshyn, D. and Kleine-Vehn, J. (2017). Light triggers PILS-dependent reduction in nuclear auxin signalling for growth transition. *Nat. Plants* **3**, 17105. doi:10.1038/nplants.2017.105
- Bowman, J. L., Briginshaw, L. N., Fisher, T. J. and Flores-Sandoval, E. (2018). Something ancient and something neofunctionalized—evolution of land plant hormone signaling pathways. *Curr Opin. Plant Biol.* **47**, 64–72. doi:10.1016/j.pbi.2018.09.009
- Dhonukshe, P., Huang, F., Galvan-Ampudia, C. S., Mähönen, A. P., Kleine-Vehn, J., Xu, J., Quint, A., Prasad, K., Friml, J., Scheres, B. et al. (2010). Plasma membrane-bound AGC3 kinases phosphorylate PIN auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. *Development* **137**, 3245–3255. doi:10.1242/dev.052456
- Ding, Z., Wang, B., Moreno, I., Dupláková, N., Simon, S., Carraro, N., Reemmer, J., Pěňčík, A., Chen, X., Tejos, R. et al. (2012). ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in *Arabidopsis*. *Nat. Commun.* **3**, 941. doi:10.1038/ncomms1941
- Ditengou, F. A., Gomes, D., Nziengui, H., Kochersperger, P., Lasok, H., Medeiros, V., Paponov, I. A., Nagy, S. K., Nádai, T. V., Mészáros, T. et al. (2018). Characterization of auxin transporter PIN6 plasma membrane targeting reveals a function for PIN6 in plant bolting. *New Phytol.* **217**, 1610–1624. doi:10.1111/nph.14923

- Fastner, A., Absmanner, B. and Hammes, U. Z.** (2017). Use of xenopus laevis oocytes to study auxin transport. *Methods Mol. Biol.* **1497**, 259-270. doi:10.1007/978-1-4939-6469-7_21
- Feraru, E., Vosolsobě, S., Feraru, M. I., Petrášek, J. and Kleine-Vehn, J.** (2012). Evolution and structural diversification of PILS putative auxin carriers in plants. *Front. Plant Sci.* **3**, 227. doi:10.3389/fpls.2012.00227
- Feraru, E., Feraru, M. I., Barbez, E., Waidmann, S., Sun, L., Gaidora, A. and Kleine-Vehn, J.** (2019). PILS6 is a temperature-sensitive regulator of nuclear auxin input and organ growth in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* **116**, 3893-3898. doi:10.1073/pnas.1814015116
- Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P. B., Ljung, K., Sandberg, G. et al.** (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**, 862-865. doi:10.1126/science.1100618
- Ganguly, A., Park, M., Kesawat, M. S. and Cho, H.-T.** (2014). Functional analysis of the hydrophilic loop in intracellular trafficking of arabidopsis PIN-FORMED proteins. *Plant Cell*. **26**, 1570-1585. doi:10.1105/tpc.113.118422
- Ge, L. and Chen, R.** (2016). Negative gravitropism in plant roots. *Nat. Plants*. **2**, 16155. doi:10.1038/nplants.2016.155
- Geisler, M., Aryal, B., di Donato, M. and Hao, P.** (2017). A critical view on ABC transporters and their interacting partners in auxin transport. *Plant Cell Physiol.* **58**, 1601-1614. doi:10.1093/pcp/pcx104
- Glanc, M., Fendrych, M. and Friml, J.** (2018). Mechanistic framework for cell-intrinsic re-establishment of PIN2 polarity after cell division. *Nat. Plants* **4**, 1082-1088. doi:10.1038/s41477-018-0318-3
- Grones, P., Abas, M., Hajný, J., Jones, A., Waidmann, S., Kleine-Vehn, J. and Friml, J.** (2018). PID/WAG-mediated phosphorylation of the Arabidopsis PIN3 auxin transporter mediates polarity switches during gravitropism. *Sci. Rep.* **8**, 10279. doi:10.1038/s41598-018-28188-1
- Guseman, J. M., Webb, K., Srinivasan, C. and Dardick, C.** (2017). DRO1 influences root system architecture in Arabidopsis and Prunus species. *Plant J.* **89**, 1093-1105. doi:10.1111/tpj.13470
- Hori, K., Maruyama, F., Fujisawa, T., Togashi, T., Yamamoto, N., Seo, M., Sato, S., Yamada, T., Mori, H., Tajima, N. et al.** (2014). Klebsormidium flaccidum genome reveals primary factors for plant terrestrial adaptation. *Nat. Commun.* **5**, 3978. doi:10.1038/ncomms4978
- Hayashi, K., Nakamura, S., Fukunaga, S., Nishimura, T., Jenness, M. K., Murphy, A. S., Motose, H., Nozaki, H., Furutani, M. and Aoyama, T.** (2014). Auxin transport sites are visualized in planta using fluorescent auxin analogs. *Proc. Natl. Acad. Sci. USA* **111**, 11557-11562. doi:10.1073/pnas.1408960111
- Kleine-Vehn, J., Huang, F., Naramoto, S., Zhang, J., Michniewicz, M., Offringa, R. and Friml, J.** (2009). PIN auxin efflux carrier polarity is regulated by PINOID kinase-mediated recruitment into GNOM-independent trafficking in Arabidopsis. *Plant Cell* **21**, 3839-3849. doi:10.1105/tpc.109.071639
- Korbei, B. and Luschnig, C.** (2013). Plasma membrane protein ubiquitylation and degradation as determinants of positional growth in plants. *J. Integr. Plant Biol.* **55**, 809-823. doi:10.1111/jipb.12059
- Marhava, P., Bassukas, A. E. L., Zourelidou, M., Kolb, M., Moret, B., Fastner, A., Schulze, W. X., Cattaneo, P., Hammes, U. Z., Schwechheimer, C. et al.** (2018). A molecular rheostat adjusts auxin flux to promote root protophloem differentiation. *Nature* **558**, 297-300. doi:10.1038/s41586-018-0186-z
- Marhavý, P., Duclercq, J., Weller, B., Feraru, E., Bielach, A., Offringa, R., Friml, J., Schwechheimer, C., Murphy, A. and Benková, E.** (2014). Cytokinin controls polarity of PIN1-dependent auxin transport during lateral root organogenesis. *Curr. Biol.* **24**, 1031-1037. doi:10.1016/j.cub.2014.04.002
- Men, S., Boutté, Y., Ikeda, Y., Li, X., Palme, K., Stierhof, Y.-D., Hartmann, M.-A., Moritz, T. and Grebe, M.** (2008). Sterol-dependent endocytosis mediates post-cytokinetic acquisition of PIN2 auxin efflux carrier polarity. *Nat. Cell Biol.* **10**, 237-244. doi:10.1038/ncb1686
- Michniewicz, M., Zago, M. K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I., Heisler, M. G., Ohno, C., Zhang, J., Huang, F. et al.** (2007). Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell* **130**, 1044-1056. doi:10.1016/j.cell.2007.07.033
- Middleton, A. M., Dal Bosco, C., Chlap, P., Bensch, R., Harz, H., Ren, F., Bergmann, S., Wend, S., Weber, W., Hayashi, K. I. et al.** (2018). Data-driven modeling of intracellular auxin fluxes indicates a dominant role of the ER in controlling nuclear auxin uptake. *Cell Rep.* **22**:3044-3057. doi:10.1016/j.celrep.2018.02.074
- Mravec, J., Skúpa, P., Bailly, A., Hoyerová, K., Křeček, P., Bielach, A., Petrášek, J., Zhang, J., Gaykova, V., Stierhof, Y.-D. et al.** (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* **459**, 1136-1140. doi:10.1038/nature08066
- Naramoto, S.** (2017). Polar transport in plants mediated by membrane transporters: focus on mechanisms of polar auxin transport. *Curr. Opin. Plant Biol.* **40**, 8-14. doi:10.1016/j.pbi.2017.06.012
- Nodzyński, T., Vanneste, S., Zwiewka, M., Pernisová, M., Hejátko, J. and Friml, J.** (2016). Enquiry into the topology of plasma membrane-localized PIN auxin transport components. *Mol. Plant* **9**, 1504-1519. doi:10.1016/j.molp.2016.08.010
- Paciorek, T., Zazimalová, E., Ruthardt, N., Petrášek, J., Stierhof, Y. D., Kleine-Vehn, J., Morris, D. A., Emans, N., Jürgens, G., Geldner, N. and Friml, J.** (2005). Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature* **435**, 1251-1256. doi:10.1038/nature03633
- Petrášek, J., Mravec, J., Bouchard, R., Blakeslee, J. J., Abas, M., Seifertová, D., Wisniewska, J., Tadele, Z., Kubes, M., Covanová, M. et al.** (2006). PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* **312**, 914-918. doi:10.1126/science.1123542
- Rosquete, M. R., von Wangenheim, D., Marhavý, P., Barbez, E., Stelzer, E. H. K., Benková, E., Maizel, A. and Kleine-Vehn, J.** (2013). An auxin transport mechanism restricts positive orthogravitropism in lateral roots. *Curr. Biol.* **23**, 817-822. doi:10.1016/j.cub.2013.03.064
- Sanchez Carranza, A. P., Singh, A., Steinberger, K., Panigrahi, K., Palme, K., Dovzhenko, A. and Dal Bosco, C.** (2016). Hydrolases of the ILR1-like family of Arabidopsis thaliana modulate auxin response by regulating auxin homeostasis in the endoplasmic reticulum. *Sci. Rep.* **6**, 24212. doi:10.1038/srep24212
- Sauer, M., Robert, S. and Kleine-Vehn, J.** (2013). Auxin: simply complicated. *J. Exp. Bot.* **64**, 2565-2677. doi:10.1093/jxb/ert139
- Simon, S., Skúpa, P., Viaeane, T., Zwiewka, M., Tejos, R., Klíma, P., Černá, M., Ročlík, J., De Rycke, R., Moreno, I. et al.** (2016). PIN6 auxin transporter at endoplasmic reticulum and plasma membrane mediates auxin homeostasis and organogenesis in Arabidopsis. *New Phytol.* **211**, 65-74. doi:10.1111/nph.14019
- Singh, M. K. and Jürgens, G.** (2018). Specificity of plant membrane trafficking-ARFs, regulators and coat proteins. *Semin. Cell Dev. Biol.* **80**, 85-93. doi:10.1016/j.semcdb.2017.10.005
- Singh, G., Retzer, K., Vosolsobě, S. and Napier, R.** (2018). Advances in understanding the mechanism of action of the auxin permease AUX1. *Int. J. Mol. Sci.* **19**, 3391. doi:10.3390/ijms19113391
- Sun, L., Feraru, E., Feraru, M. I., Wabnik, K. and Kleine-Vehn, J.** (2019). PIN-LIKES coordinate brassinosteroid signalling with nuclear auxin input in Arabidopsis thaliana. *bioRxiv* doi:10.1101/646489
- Taniguchi, M., Furutani, M., Nishimura, T., Nakamura, M., Fushita, T., Iijima, K., Baba, K., Tanaka, H., Toyota, M., Tasaka, M. et al.** (2017). The Arabidopsis LAZY1 family plays a key role in gravity signaling within statocytes and in branch angle control of roots and shoots. *Plant Cell* **29**, 1984-1999. doi:10.1105/tpc.16.00575
- Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N. et al.** (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nat. Genet.* **45**, 1097-1102. doi:10.1038/ng.2725
- Viaeane, T., Delwiche, C. F., Rensing, S. A. and Friml, J.** (2013). Origin and evolution of PIN auxin transporters in the green lineage. *Trends Plant Sci.* **18**, 5-10. doi:10.1016/j.tplants.2012.08.009
- Viaeane, T., Landberg, K., Thelander, M., Medvecka, E., Pederson, E., Feraru, E., Cooper, E. D., Karimi, M., Delwiche, C. F., Ljung, K. et al.** (2014). Directional auxin transport mechanisms in early diverging land plants. *Curr. Biol.* **24**, 2786-2791. doi:10.1016/j.cub.2014.09.056
- Vieten, A., Vanneste, S., Wisniewska, J., Benková, E., Benjamins, R., Beekman, T., Luschnig, C. and Friml, J.** (2005). Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. *Development* **132**, 4521-4531. doi:10.1242/dev.02027
- Vukašinić, N. and Žárský, V.** (2016). Tethering complexes in the Arabidopsis endomembrane system. *Front. Cell Dev. Biol.* **4**, 46. doi:10.3389/fcell.2016.00046
- Weller, B., Zourelidou, M., Frank, L., Barbosa, I. C., Fastner, A., Richter, S., Jürgens, G., Hammes, U. Z. and Schwechheimer, C.** (2017). Dynamic PIN-FORMED auxin efflux carrier phosphorylation at the plasma membrane controls auxin efflux-dependent growth. *Proc. Natl. Acad. Sci. USA* **114**, 887-896. doi:10.1073/pnas.1614380114
- Xi, W., Gong, X., Yang, Q., Yu, H. and Liou, Y. C.** (2016). Pin1At regulates PIN1 polar localization and root gravitropism. *Nat. Commun.* **7**:10430. doi:10.1038/ncomms10430
- Yang, H. and Murphy, A. S.** (2009). Functional expression and characterization of Arabidopsis ABCB, AUX1 and PIN auxin transporters in *Schizosaccharomyces pombe*. *Plant J.* **59**, 179-191. doi:10.1111/j.1365-3113X.2009.03856.x
- Yoshida, S., van der Schuren, A., van Dop, M., van Galen, L., Saiga, S., Adibi, M., Möller, B., Ten Hove, C. A., Marhavý, P., Smith, R. et al.** (2019). A SOSEK1-based coordinate system interprets global polarity cues in Arabidopsis. *Nat Plants* **5**:160-166. doi:10.1038/s41477-019-0363-6
- Yoshihara, T. and Spalding, E. P.** (2017). LAZY genes mediate the effects of gravity on auxin gradients and plant architecture. *Plant Physiol.* **175**, 959-969. doi:10.1104/pp.17.00942
- Zhou, J. J. and Luo, J.** (2018). The PIN-FORMED auxin efflux carriers in plants. *Int. J. Mol. Sci.* **19**, 2759. doi:10.3390/ijms19092759
- Zourelidou, M., Absmanner, B., Weller, B., Barbosa, I. C., Willige, B. C., Fastner, A., Streit, V., Port, S. A., Colcombet, J., de la Fuente van Bentem, S. et al.** (2014). Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. *Elife* **3**, e02860. doi:10.7554/eLife.02860