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Structure and Function of Auxin Transporters

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Abstract

Auxins, a group of central hormones in plant growth and development, are transported by a diverse range of transporters with distinct biochemical and structural properties. This review summarizes the current knowledge on all known auxin transporters with respect to their biochemical and biophysical properties and the methods used to characterize them. In particular, we focus on the recent advances that were made concerning the PIN-FORMED family of auxin exporters. Insights derived from solving their structures have improved our understanding of the auxin export process, and we discuss the current state of the art on PIN-mediated auxin transport, including the use of biophysical methods to examine their properties. Understanding the mechanisms of auxin transport is crucial for understanding plant growth and development, as well as for the development of more effective strategies for crop production and plant biotechnology.

Contents

AUXIN AND POLAR AUXIN TRANSPORT

Auxins are a class of phytohormones that regulate almost every aspect of plant development and growth. Indole-3-acetic acid (IAA) is the most abundant and common auxin in most plant species, but other endogenous auxins, such as the IAA precursor indole butyric acid (IBA) as well as phenylacetic acid (PAA) or chlorinated IAA (4-Cl IAA), are typically found in lower concentrations in several plant species (83, 112, 122, 150). Some species prefer other auxins over IAA. For example, in legumes, 4-Cl IAA seems to be the principal auxin (83, 112, 113, 146). Over the years, many synthetic auxins have been developed, and they formed a major component of the green revolution (48). Several of these are still used in agriculture today, and synthetic auxins account for about 20% of all herbicide-treated farmland worldwide (24). IAA and several other auxins are directionally distributed in plant tissues, a phenomenon called polar auxin transport (PAT) (47, 55). By controlling local auxin concentrations, PAT regulates a variety of developmental responses, including gravitropic and phototropic organ growth, as well as embryogenesis, de novo postembryonic organogenesis, phyllotaxis, apical dominance, and organ development (15, 52, 78, 121, 140, 141). It is noteworthy that both endogenous and synthetic auxins have a carboxylic acid as a central component of their chemical structure (48, 122). The carboxylate of IAA ($pK_a = 4.75$) is partially protonated in the apoplast (pH ~5), allowing the acid to diffuse across the membrane into the cytosol (pH ~7), where it becomes deprotonated (110, 114). All auxins are believed to be imported and trapped to some extent in the cytosol by this ion trap mechanism, but protein-mediated uptake has been shown to also be a central contributor to auxin import (16, 105, 131, 133, 154). Within the cell, auxins are bound by intracellular receptors, such as F-box protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1) and its AUXIN SIGNALING F-BOX (AFB) paralogs, to initiate the cellular auxin response; transported across endogenous membranes, such as the membrane of the endoplasmic reticulum (ER) and the tonoplast; or exported out of the cell in a polar fashion to maintain PAT (34, 51, 95, 137).

Transporter: orders of magnitude slower than a channel; specificity is conferred by its substrate-binding site, sequentially exposed to the two sides of the membrane to facilitate transport (known as alternating access)

In this review, we revisit the biophysical principles underlying transport processes across membranes and discuss them in the context of auxin transport. We summarize the current knowledge about known auxin transporters and their biochemical properties and critically discuss the available data with respect to the biophysical properties and the limitations of the experimental approaches that were used to obtain the results. Finally, we review the recent progress that was made in our understanding of auxin export and its contribution to PAT by the recent solving of the structures of three different auxin exporters of the PIN-FORMED (PIN) family.

BIOPHYSICAL FUNDAMENTALS OF MEMBRANE TRANSPORT

Membrane Potential and Proton Motive Force

All living cells have a membrane potential ($\Delta\Psi$) that results from the uneven distribution of ions across membranes. The membrane potential is analogous to a battery, with the negative pole on the cytosolic side and the positive pole on the noncytosolic or extracellular side (94) (**Figure 1**). Typically, the animal plasma membrane resting potential ranges between −40 to −95 mV and is formed mainly by adenosine triphosphate (ATP) hydrolysis coupled to the generation of sodium and potassium gradients (96, 124). By contrast, plants and fungi use ATP hydrolysis to drive primary active proton pumps (discussed in the section titled Mechanisms of Transport) to acidify their external milieu (57, 125). This leads to the generation of a proton motif force (PMF) across the plasma membrane, *trans*-Golgi membrane, or tonoplast (vacuole membrane), (88, 89, 120). The PMF has two components: the chemical concentration gradient of protons (ΔpH or $\Delta[H^+]$) and the electrical component that is due to the charge of the protons that adds to the $\Delta\Psi$ originating from the uneven distribution of ions across the membrane. The plant and fungal plasma membrane potential is generally much more negative than that of animals and usually ranges between −110 and −160 mV, with potentials as low as −250 mV reported (21, 49, 90, 104). At ΔpH = 2 and $\Delta\Psi$ = –200 mV, the free energy (ΔG) yield from one proton passing through the membrane can be calculated to be −30.5 kJ/mol, with contributions from ΔpH of −11.2 kJ/mol and from the membrane potential of −19.3 kJ/mol ($\Delta G = G_{\text{chemical}} + G_{\text{electrical}}$, as reported in, e.g., 58). This is equal to about half of the free energy released by ATP hydrolysis under cellular conditions, highlighting the substantial energy available from the PMF for proton-coupled secondary active transport in plants (**Figure 1*b***).

$\Delta\Psi$ and ΔpH vary significantly across other cellular membranes. For example, $\Delta\Psi$ across the tonoplast is very low at −30 to 0 mV and across the ER is found to be 0 mV (54, 88, 120, 126). By contrast, the chemical gradient of protons can be significant in some internal organelles, with the extreme example of the citrus vacuole, which has a pH of around 1 to 2 with a ΔpH to the cytosol of about 5 pH units (134). This highlights that the ion distribution generating the $\Delta\Psi$ across endogenous membranes can be markedly different from the ion distribution generating the $\Delta\Psi$ across the plasma membrane. As a result, the contribution of $\Delta\Psi$ to the transport of charged substrates in these endogenous membranes is small, but the contribution of the chemical proton gradient is still considerable for proton-coupled transport processes.

The strong driving force of the PMF across the plasma membrane and its comparatively smaller driving force across some internal membranes due to lack of $\Delta\Psi$ have fundamental impacts on the transport rates and properties of structurally very closely related transporters that use the PMF, or one of its components, to concentrate their primary substrate using different transport mechanisms.

MECHANISMS OF TRANSPORT

Protein-mediated transport across membranes is commonly classified according to its energy requirements. Passive transport occurs with the electrochemical potential (ECP) gradient. The ECP is the sum of the concentration gradient of the primary substrate, as well as the $\Delta\Psi$ acting on the substrate if it is charged. By contrast, active transport is defined as transport against the ECP gradient of the primary substrate. It is important to appreciate that for uncharged substrates only the concentration gradient influences the ECP, whereas for charged substrates $\Delta\Psi$ also must be considered. As a result, charged substrates can be chemically concentrated on one side of a membrane even if the transport process itself is thermodynamically passive along the ECP gradient (71) (**Figure 1*c***).

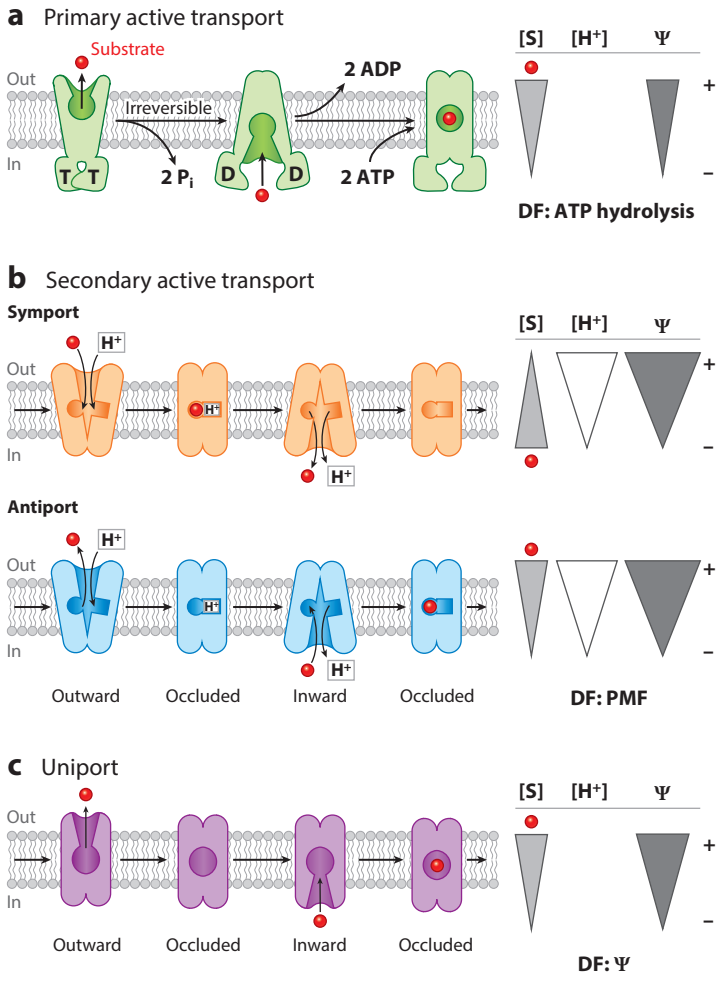

Figure 1

Schematic representation of three categories of substrate-concentrating mechanisms. (*a*) Primary active transport exemplified by an ABCB transporter (using analogy to the animal PGP exporter). In the ATP-bound state (T) the transporter is outward open and can release substrate (*red sphere*). ATP hydrolysis causes the rearrangement of the transporter into the inward-open configuration in which ADP (D) is still bound. Substrate binding triggers the exchange of ADP for ATP to form the occluded state and transport the substrate to the outside, thus completing the transport cycle. (*b*) Coupled transport. Symport and antiport catalyze the movement of a primary substrate against its ECP through a series of conformational states. Symporters and antiporters can support the movement of a primary substrate by utilizing both the $\Delta\Psi$ and the energy gained by moving protons down their ΔpH toward the cytosol (in). In symport, substrates are moved in the same direction. In antiport, substrate movement and proton movement take place in opposite directions. (*c*) Uniporters only move a primary substrate. If the substrate is charged, the membrane potential can result in increased substrate concentration. [S], H^+, and Ψ are represented schematically to the right. The DF that is used to concentrate the primary substrate is indicated for each example. Abbreviations: ABCB, ATP-BINDING CASSETTE group B; ADP, adenosine diphosphate; ATP, adenosine triphosphate; D, ATP; DF, driving force; ECP, electrochemical potential; [H^+], proton gradient; PGP, P-glycoprotein; P_i, inorganic phosphate; [S], substrate concentration; T, ATP-bound state; ΔpH, concentration gradient; $\Delta\Psi$, membrane potential; Ψ, electric potential.

Active transport is further divided into primary and secondary active or, more precisely, coupled transport (14, 45). In primary active transport, redox potential, light energy, or chemical energy, commonly in the form of ATP hydrolysis, is used to pump substrates against their electrochemical potential. The generation of the PMF across membranes is a major function of several primary active transporters. Proton P-type ATPases maintain the PMF at the plasma membrane, while V-type ATPases and pyrophosphatases (PPases) acidify internal organelles (43, 85, 100, 103, 119).

In plants, coupled transporters, that is, symporters and antiporters, couple the movement of protons along the PMF gradient into the cytosol to drive the transport of a primary substrate against its electrochemical gradient (**Figure 1*b***). As mentioned in the section titled Biophysical Fundamentals of Membrane Transport, the PMF (both ΔpH and $\Delta\Psi$) across the plant plasma membrane is considerable and will allow for the concentration of charged substrates, such as auxin, by several orders of magnitude, as illustrated in **Figure 2** (127).

Primary active transporters: use energy (typically by ATP hydrolysis) to transport one or more substrates against the ECP

Coupled transporter: symporter or antiporter that makes use of the coupled transport of at least one proton along the electrochemical potential to concentrate a substrate against its electrochemical potential

Symporter: possesses a proton-binding site and substrate-binding site; transports substrate and protons in the same direction (into cytosol)

Antiporter: proton- and substrate-binding sites can be shared; transports substrate and protons in opposite directions (protons into cytosol, substrate out of cytosol)

AUXIN TRANSPORTERS

The Advantages, Disadvantages, and Limitations of Experimental Approaches to Characterize the Biochemical and Biophysical Properties of Auxin Transporters

A challenge when characterizing membrane transporters in any experimental setup is that the amount of active protein is in most instances not known, but transport rates depend directly on this parameter. As a consequence, the maximal transport rate (analogous to the maximum velocity, V_{max}, in enzyme kinetics) cannot be determined precisely in any system. This hampers the detailed characterization of transport kinetics including reliable turnover numbers (k_{cat}). Often, therefore, the only reliable parameter is substrate affinity because it is independent of the protein amount. In analogy to enzyme kinetics, it can be defined as the substrate concentration needed for half-maximal activity and is normally called the Michaelis-Menten constant (K_m) or the half-maximal effective concentration (EC_{50}). This value is often reported but should be interpreted with some care. Often, transport involves more than one substrate (e.g., symport of the primary substrate and driving substrate), and the use of one-substrate kinetic models can become misleading (30).

Another key insight when trying to characterize the affinity of the substrate-binding site by measuring dissociation constants (K_d) is that the substrate-binding site of the transporter will sequentially be exposed to two different sides of a membrane, known as the alternating access model, which will be linked to structural changes in the binding site as well as different chemical environments (64). This often converts a high-affinity binding site to a low-affinity binding site and thus facilitates the release of a substrate in an environment with high substrate concentration, as exemplified by the SUC sucrose transporters in plants (10). The result is that both the association rate constant (k_{on}) and dissociation rate constant (k_{off}) are expected to differ between the inward- and outward-facing conformation of the transporter, and therefore also that the protein can have a conformation-dependent dissociation constant (K_d). Often when a K_d is reported in the literature, it is in fact the mixture of a minimum of two different K_d dissociation constants ($K_d^{inwardstate}$ and $K_d^{outwardstate}$) with an unknown ratio between them. Only if the transporter is fixed in one conformation (e.g., by mutagenesis or allosteric inhibitors) can the reported K_d be said to represent the true K_d of the substrate in that particular conformation. K_d values can be determined by several methods if purified membrane proteins are available. The most relevant are isothermal titration calorimetry, considered the gold standard for dissociation constants, and microscale thermophoresis (22, 108, 128).

Due to the challenges of obtaining useful quantities of any membrane protein with a high level of purity and quality, transporters are often characterized by expression in cell-based systems

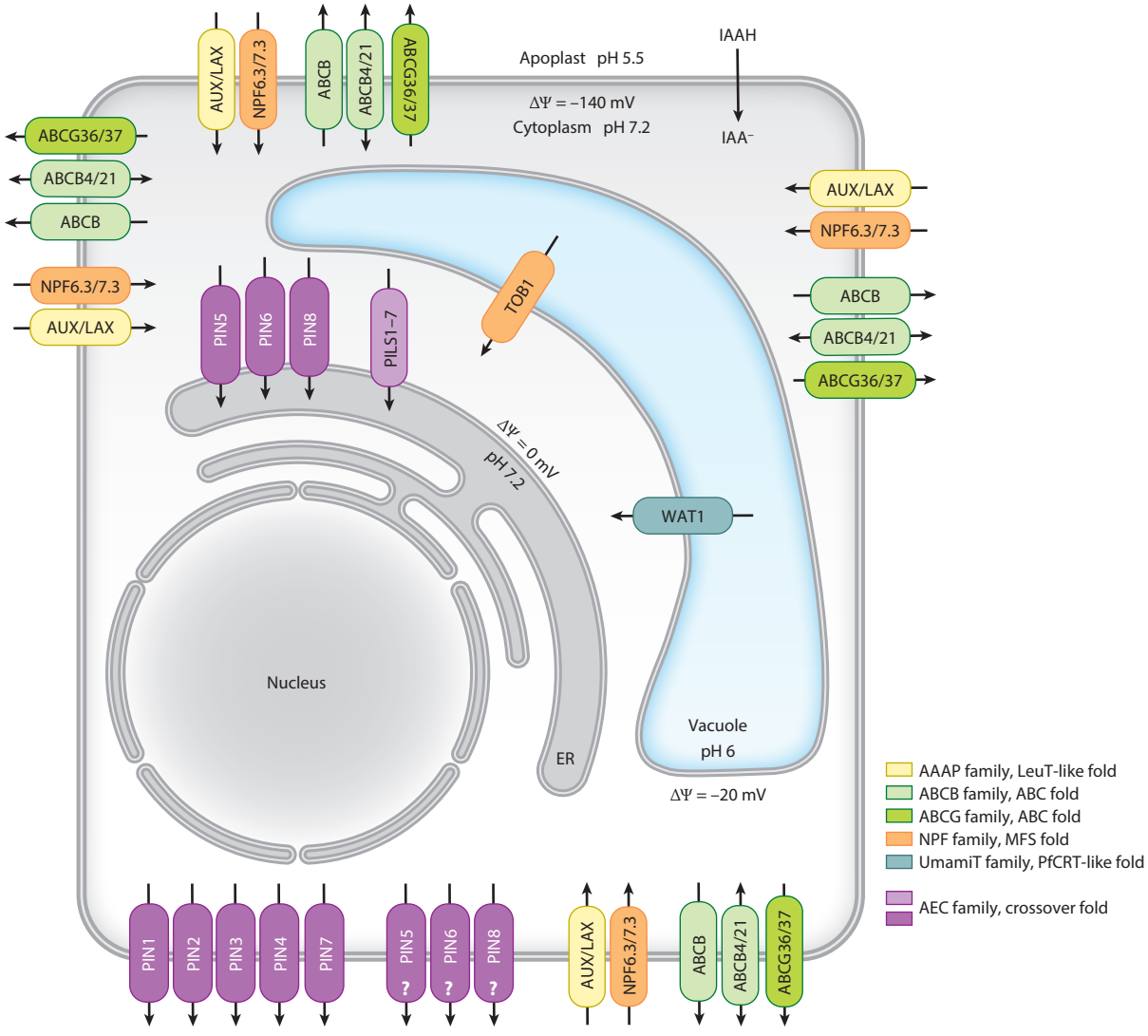

Figure 2

Schematic representation of auxin transporters identified to date in a plant cell. The localization of transporters in the plasma membrane or in internal membranes, as well as the direction of auxin transport (*arrows*), are shown. Colors represent the transporter families and the protein fold as indicated. ΔΨ and pH values are taken from references cited in the section titled Biophysical Fundamentals of Membrane Transport. Abbreviations: AAAP, AMINO ACID/AUXIN PERMEASE; AEC, AUXIN EFFLUX CARRIER; AUX/LAX, AUXIN RESISTANT/LIKE AUX1; ER, endoplasmic reticulum; IAA, indole-3-acetic acid; LeuT, LEUCINE TRANSPORTER; MFS, major facilitator superfamily; NPF, NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER family; PfCRT, *Plasmodium falciparum* CHLOROQUINE RESISTANCE TRANSPORTER; PIN, PIN-FORMED; PILS, PIN-LIKE; TOB1, TRANSPORTER OF IBA1; UmamiT, USUALLY MUTIPLE ACIDS MOVE IN AND OUT TRANSPORTER; WAT1, WALLS ARE THIN1; ΔΨ, membrane potential.

with low background transport activity, thus avoiding isolation and purification. Generally, yeast (*Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*), plant cells [typically tobacco Bright Yellow-2 (BY-2) cells or *Arabidopsis* cell culture], endosomal vesicles isolated from overexpressing cells, cultured animal cells [e.g., human embryonic kidney (HEK) cells], or *Xenopus laevis* oocytes are

Table 1 Overview over *Arabidopsis* auxin transporters and their properties

Transporters	Transport direction[a]	Known substrates	Transporter family[b]	Protein fold[b]	Functional characterization[c]	Biochemical properties	Reference(s)
AUX1, LAX2, LAX3	Import	IAA, 2,4-D	AAAP	LeuT-like	*Xenopus* oocytes, root hairs	High-affinity import[d]	36, 105, 131, 154
ABCB4/ABCB21	Import	IAA, NAA	ABC	ABC	*Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, root hairs	Unknown	28, 65, 115, 135, 153a
WAT1	Import	IAA	UmamiT	PfCRT-like	*Xenopus* oocytes, *S. cerevisiae*	Unknown	109
TOB1	Import	IBA, nitrate	NPF	MFS	*Xenopus* oocytes, *S. cerevisiae*	Low-affinity import[e]	92
NRT1.1/NRT1.5	Import	IAA, 2,4-D, nitrate	NPF	MFS	*Xenopus* oocytes, *S. cerevisiae*	Low-affinity import[e]	74, 148
ABCB1, ABCB4, ABCB14, ABCB15–21	Export	IAA, NAA	ABC	ABC	*S. pombe*, *S. cerevisiae*, protoplasts, ER vesicles	Unknown	24a, 46a, 65, 74a, 115, 135, 153a
ABCG36/ABCG37	Export	IBA	ABC	ABC	Protoplasts, *S. cerevisiae*, HeLa cells, *S. pombe*, root tips	Unknown	5a, 114a
PILS2, PILS5	Export	IAA, NAA	AEC	Crossover	Protoplasts	Unknown	7a
PIN1, PIN3, PIN8	Export	IAA, NAA	AEC	Crossover	Protoplasts, *S. pombe*, *S. cerevisiae*, ER vesicles, HeLa cells, *Xenopus* oocytes, ITC, SSM	Low-affinity export[e]	36a, 75a, 107, 129, 139, 155, 162
PIN2, PIN4, PIN5, PIN6, PIN7	Export	IAA, NAA	AEC	Crossover	Protoplasts, *S. cerevisiae*, ER vesicles, HeLa cells, *S. pombe*, *Xenopus* oocytes	Low-affinity export[e] (inferred)	1, 75a, 107, 147

[a]Import defined as transport into the cytosol and export as transport away from the cytosol.
[b]Classification according to references in the section titled Auxin Transporters.
[c]Listed in no particular order.
[d]High-affinity defined as $K_m < 10\ \mu M$.
[e]Low-affinity defined as $K_m > 10\ \mu M$.
Abbreviations: AAAP, amino acid/auxin permease; ABC, ATP-BINDING CASSETTE; ABCB, ABC transporter group B; ABCG, ABC transporter group G; AEC, AUXIN EFFLUX CARRIER; AUX1, AUXIN RESISTANT 1; ER, endoplasmic reticulum; IAA, indole-3-acetic acid; IBA, indole butyric acid; ITC, isothermal titration calorimetry; LAX, LIKE-AUX1; LeuT, Leucine transporter; MFS, major facilitator; NAA, naphthyl acetic acid; NPF, NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER; NRT, NITRATE TRANSPORTER; PfCRT, *Plasmodium falciparum* Chloroquine Resistance Transporter; PILS, PIN-LIKE; PIN, PIN-FORMED; SSM, solid supported membrane–based electrophysiology; TOB1, TRANSPORTER OF IBA1; UmamiT, USUALLY MULTIPLE ACIDS MOVE IN AND OUT TRANSPORTER; WAT1, WALLS ARE THIN1; 2,4-D, 2,4-dichlorophenoxyacetic acid.

used for this purpose, and all these systems have been applied to characterize auxin transporters (see references in **Table 1**).

Cell-based expression systems are well suited for the characterization of importers because the starting substrate concentration can be easily controlled. Linear transport rates over time at different experimental conditions allow for a detailed investigation of affinities for substrates and pH dependence, for example.

The characterization of exporters in cell-based systems, however, is challenging because the substrate needs to get into the cells before the efflux assay. This is generally done by incubating the cells in the substrate to allow it to permeate passively into the cells and then rapidly isolating the cells and putting them in a new, label-free solution to measure efflux. The main problem with this approach is that the initial internal substrate concentration cannot be controlled. As a result, the initial substrate concentration and thus the outward-directed driving force often vary considerably between different samples. To be able to compare transport rates directly between samples, identical starting concentrations of substrate must be used and absolute linear export rates provided. Nonlinear transport rates, expressed as values relative to the initial concentration, are

essentially of no use in understanding the transporter in question or in comparing the transport rates of different transporters.

Facilitator: also known as uniporter; transports one substrate along the ECP, and can drive the substrate against the concentration gradient if an appropriate membrane potential is present

Xenopus oocytes are extremely well suited to express transporters and characterize their activity because they contain very few endogenous membrane proteins in their plasma membrane and allow transporters to be characterized with improved signal-to-noise ratio (23, 41). Their size of ~1 mm at stages V and VI, which are generally used for transport assays, makes them particularly well-suited to characterize exporters because defined amounts of substrate can be injected into the cells to reach identical initial conditions, thus eliminating the abovementioned problems associated with passive loading (39). *Xenopus* oocytes have been used to characterize auxin importers and exporters (1, 87, 139, 147, 149, 162). *Xenopus* oocytes are also used to perform voltage clamp experiments, a method that allows $\Delta\Psi$ to be controlled and has been used to characterize many transporters (145). This method, however, requires comparatively large amounts of charge movement due to the resolution limit of the instrumentation. The only auxin transporter characterized using this approach so far is the low-affinity nitrate/IBA TRANSPORTER OF IBA1 (TOB1) (92).

Purified membrane proteins can be reconstituted into liposomes to form proteoliposomes. The characterization of transport from such proteoliposomes represents the gold standard of transporter activity characterization since the results are derived solely from the activity of the transporter. This method has a further advantage in that lipid composition, known to impact transport properties, is experimentally adjustable and its effects can be characterized (for instance, see 60). In addition, both the inside and outside environments are controlled, thus allowing for total experimental control in principle. The main challenges are that large amounts of purified and stable protein are required, reconstitution of the transporter into liposomes is not trivial, and the orientation of the transporters in the liposomes can be mixed. To characterize transport properties, flux studies using labeled substrate and/or solid supported membrane (SSM)-based electrophysiology can be performed (11). SSM-based electrophysiology can be used to characterize charged substrates, but since detecting electrogenic events (i.e., charge movement in the protein due to substrate binding) is also possible, transport and binding of uncharged substrates by facilitators have also been characterized successfully (12). One challenge of SSM-based electrophysiology is that transport and binding events are often convoluted, requiring careful data analysis and proper controls to distinguish binding from transport (13).

Finally, for primary transporters utilizing ATP to drive substrate transport, proteoliposomes and even detergent-solubilized protein samples or isolated membrane fractions can be used to study ATPase activity as a function of substrate concentration. These assays provide valuable insights into the kinetics and mechanism of substrate translocation but have so far not been applied to ATP-BINDING CASSETTE group B (ABCB) and ABCG transporters from plants to demonstrate ATP hydrolysis in response to auxins as a substrate (80, 116, 117).

Mechanisms and Properties of Known Auxin Transporters from *Arabidopsis*

Auxin transporters' physiological role, mutant phenotypes, and subcellular localizations have been intensively studied, and numerous excellent reviews on these topics exist (2, 5, 51, 52, 132). We therefore limit our discussion strictly to general mechanisms and biophysical properties of published transporters with a focus on the cellular repertoire of *Arabidopsis* since these features are expected to be conserved in homologs from other species (**Figure 2**; **Table 1**).

A general challenge in the discussion of auxin transport properties is that the auxin concentrations in vivo are not well known. Contradicting reports of apoplastic versus cytosolic IAA concentrations have been published for tomato, squash, and cotton, and very low values in the

mid-nanomolar range were reported for the apoplast (53, 69, 70, 136). Single-cell studies using cell sorting to establish the internal IAA concentration in single cells of *Arabidopsis* roots found the intracellular concentrations to be in the nanomolar to lower-micromolar range (e.g., 106). It should also be noted that these values represent the whole cell and do not distinguish between organelles, possibly leading to the dilution of a large IAA concentration in one compartment when the concentration in other compartments is low. The ΔpH and $\Delta\Psi$ values of a generalized plant cell (**Figure 2**) make it clear that there is a strong driving force for IAA^- from the cell into the apoplast as has already been shown in Mary Goldsmith's milestone review on this topic (47). The driving force of the $\Delta\Psi$ has been calculated to be sufficient to passively accumulate IAA more than 1,000-fold toward the apoplast, strongly supporting the hypothesis that physiologically relevant auxin export levels can be reached by thermodynamically passive transport along the ECP gradient (127). The same calculation demonstrates that IAA import into the cytosol is mediated by active transport moving IAA against its ECP gradient. Transport between the cytosol and internal organelles will likewise depend on ΔpH and $\Delta\Psi$ values, which differ significantly from the values at the plasma membrane, and this means that auxin transporters can perform strikingly differently depending on intracellular localization (137).

Auxin import: AMINO ACID/AUXIN PERMEASE family. The AUXIN RESISTANT (AUX1) protein and its LIKE-AUX1 (LAX) homologs, LAX1, LAX2, and LAX3, were initially identified because of the *aux1* mutant's resistance to exogenously applied auxins (16). AUX1 is a member of the AMINO ACID/AUXIN PERMEASE (AAAP) family of transporters, and the AUX/LAX clade is a plant-specific protein family that can be traced back to the streptophyte and chlorophyte algae (32, 144, 151). The close phylogenetic relationship between the AUX/LAX family and the amino acid permeases makes it tempting to speculate that auxin import originated from the import of its metabolic precursor, tryptophan. AUX1 mediates high-affinity ($K_m \approx 1$ μM) IAA transport in *Xenopus* oocytes (154). It also transports the auxin herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) but not naphthyl acetic acid (NAA), shows a pH optimum at apoplastic pH (pH 5.5–6), and is sensitive to the IAA influx inhibitors naphthoxy-1-acetic acid (1-NOA) and naphthoxy-2-acetic acid (2-NOA) (154). The protein has a LEUCINE TRANSPORTER (LeuT)-like fold with 11 membrane-spanning α-helices (68, 73). AUX1 operates by a H^+-coupled symport mechanism, which is in line with the preexisting hypothesis that auxin transport is carrier mediated and depends on the PMF (36, 47, 82). Besides AUX1, *Arabidopsis* has three homologs, LAX1–LAX3. LAX3 has been shown to promote lateral roots and is a high-affinity ($K_m \approx 0.8$ μM) IAA importer in *Xenopus* oocytes, comparable to AUX1 (131). AUX/LAX proteins therefore very likely represent the major system using a symport mechanism to concentrate IAA in the cytosol against the outward-directed ECP.

Auxin import: the NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER FAMILY. The NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER FAMILY (NPF) is a part of the major facilitator superfamily (MFS), a very large and diverse superfamily of transporters (101). MFS transporters have a core of 12 transmembrane helices divided into two domains with a central substrate-binding site between them, and they catalyze uniport, symport, or antiport by a rocker-switch mechanism (38, 76, 101, 111, 157). The NPF transporters in plants are notorious for their highly diverse substrates (including nitrate, peptides, sugars, and hormones), and several members have been shown to transport a main substrate, generally a dipeptide or nitrate and additionally a hormone (27, 31).

NITRATE TRANSPORTER1.1 (NRT1.1)/NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER FAMILY6.3 (NPF6.3)/CHLORINA1 (CHL1) has long been recognized as one of the principal nitrate uptake systems, and its structure has been resolved (102, 130). NRT1.1

was shown to be able to import IAA in the absence of nitrate, which could be an elegant way to combine the input regarding nutrient and hormone availability at the level of plasma membrane transport (74). Kinetic parameters of IAA transport have so far not been established, but, similar to nitrate transport, the transport is against the ECP gradient and requires a proton-driven symport mechanism (102, 130).

Another nitrate transporter, NTR1.5/NPF7.3, has also been implicated in the transport of IAA and, in particular, its precursor IBA (148). The apparent K_m values, determined in yeast cells for both auxin substrates, were similar at 11 μM and 14 μM, but IBA transport appeared to be about 10 times as fast as IAA transport at saturating concentrations, indicating that IBA may be the preferred substrate for NRT1.5. Transport was dependent on an acidic external pH underlying the proposed proton-driven symport mechanism. Yet another nitrate-transporting NPF family member, TOB1/NPF5.12, was shown to be electrogenic and displayed proton-driven symport of IBA and nitrate into *Xenopus* oocytes with a very low affinity of ∼2 mM for IBA (92). The other transporters in this group that were found to transport auxin so far also transport nitrate with high μM affinity, indicating that substrate affinities are similar for both substrates. It will be interesting to see in further studies if more members of this family are also devoted to the transport of auxins in addition to their metabolic substrates and to investigate the physiological implications of this phenomenon.

Auxin import: USUALLY MUTIPLE ACIDS MOVE IN AND OUT TRANSPORTER family. The USUALLY MULTIPLE ACIDS MOVE IN AND OUT TRANSPORTER (UmamiT) family of transporters is part of the drug/metabolite transporter (DMT) superfamily (63, 109). UmamiTs are found in plant genomes in large numbers (e.g., 47 members in *Arabidopsis*), and were already present in early land plants and chlorophyte algae (161). The only UmamiT known to transport auxin is WALLS ARE THIN1 (WAT1). WAT1 localizes to the tonoplast and was shown to import IAA, its degradation product 2-oxoindole-3-acetic acid (oxIAA), and the synthetic auxin NAA, but not tryptophan, into yeast cells and *Xenopus* oocytes (109). This suggests that in vivo WAT1 transports IAA from the vacuole to the cytoplasm. Initially, some UmamiTs were identified as bidirectional amino acid transporters and recently also as transporters for glucosinolates, including indole glucosinolates (75, 98, 153). The investigations on WAT1 so far have not determined kinetic parameters and insights into its transport mechanism.

Auxin import and/or export: ATP-BINDING CASSETTE transporter family. In plants, the primary ATP-BINDING CASSETTE (ABC) transporters are divided into eight groups (37, 66). IAA transport has been described for several members of ABCB, and transport of IBA has been published for two members of the ABCG (specifically ABCG36 and ABCG37) (see references in **Table 1**). ABCB transporters are related to the well-studied multidrug efflux transporter P-glycoprotein (PGP) found in animals and therefore are expected to function by a similar mechanism (3). So far, plant ABC transporters have not been isolated and purified, and the measurement of ATP hydrolysis in response to substrate, a standard assay that is used to demonstrate substrate specificity and primary transporter activity, has not been conducted for any of these ABCB/ABCG transporters in response to auxin. Thus, it is unknown whether the identified transporters hydrolyze ATP, but from sequence analysis it is clear that all the structural elements needed for normal ABC transporter function are present. Therefore, the assumption that the ABCB/ABCG transporters use ATP for their transport function is reasonable.

The majority of the ABCB/ABCG transporters examined have been suggested to export auxin from the cell, as expected from the link to multidrug efflux transporters (**Table 1**). In virtually all of the studies that suggest ABCB-mediated auxin export, researchers used cell-based expression systems, with the associated shortcomings due to passive substrate preloading, and therefore the

biochemical properties of these transporters are still unknown. It remains unclear if IAA export is a specific physiological property or if these ABCB transporters, similar to their homologs in other kingdoms of life, are polyspecific proteins with a role in the general export of complex chemical compounds with a range of affinities for multiple compounds.

In addition to auxin export, conditional, expression system–dependent auxin import has been suggested for ABCB4 and ABCB21 (28, 65, 115, 135). Given that auxin import requires an active transport process due to the constrains of the membrane potential, auxin import by primary active transporters makes physiological sense, but it does pose a mechanistic conundrum: The mechanism employed by PGP and other ABC transporters from the multidrug efflux group involves an expected irreversible step, assuming that ATP molecules are hydrolyzed during transport, with a free energy, ΔG, of -50 to -70 kJ/mol per ATP hydrolyzed (81) (**Figure 1*a***). At face value, if these transporters can both import and export auxin, it would mean that either these particular ABCB transporters do not utilize ATP or the cytosolic domains are decoupled from the transport mechanism when running in reverse or are able to drive ATP synthesis by IAA transport. Overall, the determination of ATP hydrolytic activity in the presence of auxin will be pivotal to understand the role of ABC transporters in auxin transport.

It was recently suggested that both PIN and ABCB transporters can exhibit channel-like properties, and an interactive effect was described (33). These observations are inconsistent with the PIN structures (see the section titled PIN Structure and Function), and they would be at odds with the basic principles of transporters, such as a defined binding site with alternating access.

Channel: fast (10^5–10^9/sec) transport along the electrochemical potential; the selectivity filter gives selectivity to ion/water channels, and others are normally nonselective

Auxin export: AUXIN EFFLUX CARRIER family. The AUXIN EFFLUX CARRIER (AEC) family contains the PIN auxin transporters and the structurally related PIN-LIKE (PILS) transporters as well as bacterial and fungal homologs (25, 86). PIN auxin transporters play a pivotal role in plant morphogenesis and adaptive tropic growth by establishing asymmetric auxin distribution and are the best studied of all the auxin transporters (15, 52, 78, 107, 140, 141, 143). They localize to the plasma membrane or ER to facilitate directional auxin transport and maintain auxin homeostasis. The first members of the PIN family were identified in *Arabidopsis* as genes underlying typical auxin export–related phenotypes (26, 46, 84, 97). *Arabidopsis* harbors eight annotated PIN transporters (PIN1 to PIN8), divided into two structural types: long-loop PINs, characterized by a central long autoinhibitory loop separating the two transmembrane domains, and short-loop PINs that do not have this long autoinhibitory loop (reviewed in 72) (discussed in the section titled The Regulatory Loop of PINs). The short-loop PINs and the PILSs are thought to transport IAA between the cytoplasm and the ER. Given the lack of a membrane potential across the ER membrane, they would be expected to transport IAA only in a concentration-dependent manner in this location (137).

Significant knowledge of PIN-mediated auxin transport and its regulation by kinases and other interacting proteins has accumulated over the years (reviewed in 8, 9, 51). Recently, it was shown that 1-naphtylphthalamic acid (NPA), an inhibitor of auxin efflux that has been instrumental in the characterization of PAT for decades, directly targets and inhibits PINs, an observation that now explains the phenotypic alignment of NPA treatment and the *pin1* shoot phenotype (1, 56, 99).

PIN Structure and Function

In 2022, the structures of *Arabidopsis thaliana* PIN1, PIN3, and PIN8 were published (129, 139, 155). For each, three different states—substrate-free, IAA-bound, and NPA-bound—were presented, for a total of nine structures. In 2023, a follow-up study presented an additional structure showing the binding of the human anti-inflammatory drug naproxen to PIN1 (152). All seven PIN1 and PIN3 structures, as well as one of the three PIN8 structures, adopt the same

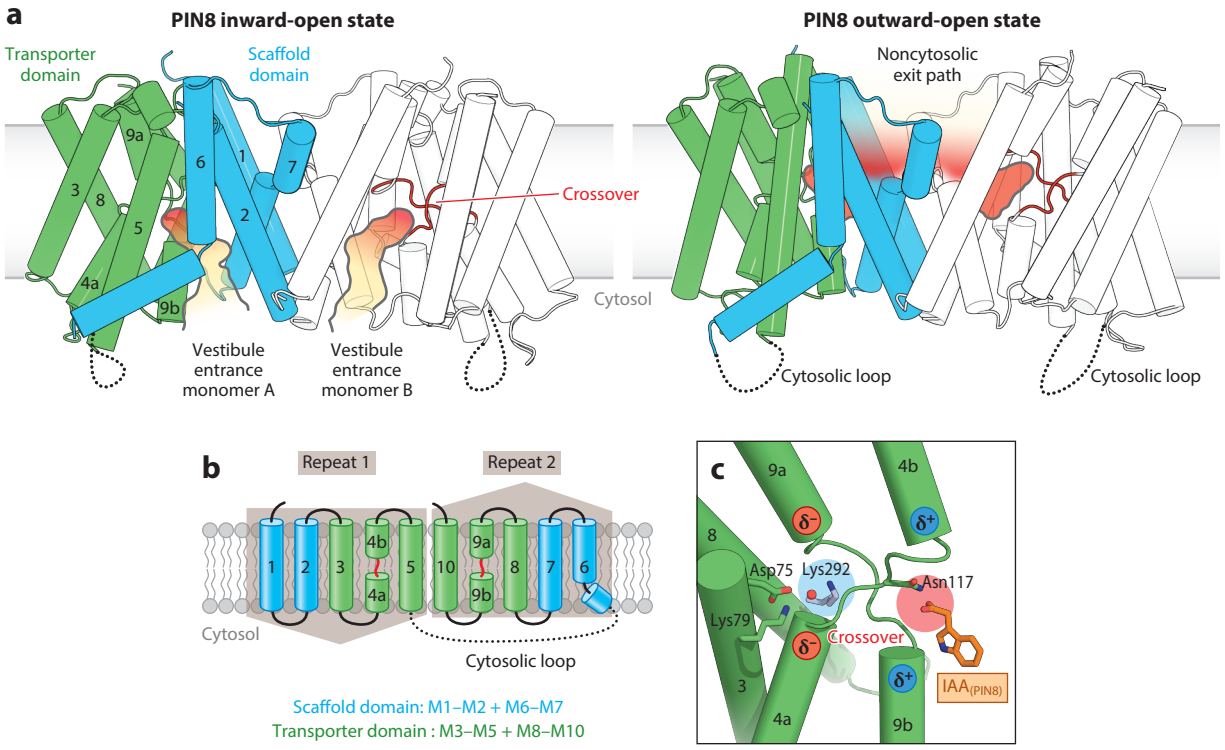

Figure 3

Inward- and outward-facing conformations of PINs. (*a*) Side view of the PIN dimer with M1–M10 labeled and colored by domain in monomer A. The central crossover is highlighted in red in monomer B, with the transporter domain in green, and the scaffold domain in blue. The cytosolic vestibule entrance is shown in yellow with the binding chamber in red. In the outward-open state, the binding chamber opens up directly to the noncytosolic side. The (long/short) cytosolic loop of PINs could not be resolved and is shown as a dotted line. PIN8 is used here as the representative structure since it was the only structure solved in both inward- and outward-facing conformations. (*b*) Membrane topology of the PIN monomer shows the inverted repeat of 5 transmembrane helices and their relation to the transporter and scaffold domains. (*c*) View of the crossover formed by M4 and M9, the position of IAA, the support site (*light blue*) with central residues highlighted, and the binding chamber (*light red*) in the PINs. Residue numbers correlate to PIN8 sequence. The crossover helix dipoles are highlighted in red and blue. Abbreviations: IAA, indole-3-acetic acid; PIN, PIN-FORMED.

inward-open conformation and are very similar, with a root-mean-square deviation (RMSD) of Cα atoms of only 1.0–1.2 Å between them. Interestingly, PIN8 was also solved in an outward-facing conformation in the substrate-free and IAA-bound states, providing the other central conformation found during transport, and this data allowed for a detailed description of the transport mechanism (139).

The PIN1, PIN3, and PIN8 structures are all homodimers in which each monomer comprises 10 membrane helices with two inverted repeats: M1–M5 and M6–M10 (**Figure 3*a*,*b***). The long loops of PIN1 and PIN3, as well as the short loop of PIN8, were disordered, and the structural data and analysis are focused on the transport mechanism of the transporter and not its regulation by the loop. Each of the PIN monomers can be divided into two: the scaffold domain and the transporter domain (**Figure 3*a*,*b***). The scaffold domain is formed by the first two helices of each repeat (M1–M2 and M6–M7) and forms the interface of the homodimer. The transporter domain comprises two three-helix bundles (M3-M4-M5 and M8-M9-M10) with helices M4 and M9 disrupted in the middle of the membrane around a conserved motif, leading to two interrupted

helices (M4a/M4b and M9a/M9b) that cross over each other (**Figure 3*a*,*b***). This crossover is a key structural feature of the PIN transporters, and the two three-helix bundles of the transporter domain are related by internal pseudosymmetry with an axis through the crossover. Essential for transport, the broken helices form positive and negative dipoles that are focused at two sites related to each other by the pseudosymmetry. The auxin-binding chamber is located on the side of the crossover where the positive dipoles are found, while a pseudosymmetrical site is present on the other side interacting with the negative dipoles. This pseudosymmetrical site is visible in all PIN structures but only described in the PIN8 work, where it was named the support site and was shown to be crucial for transport function (139) (**Figures 3*c*** and **4*a***).

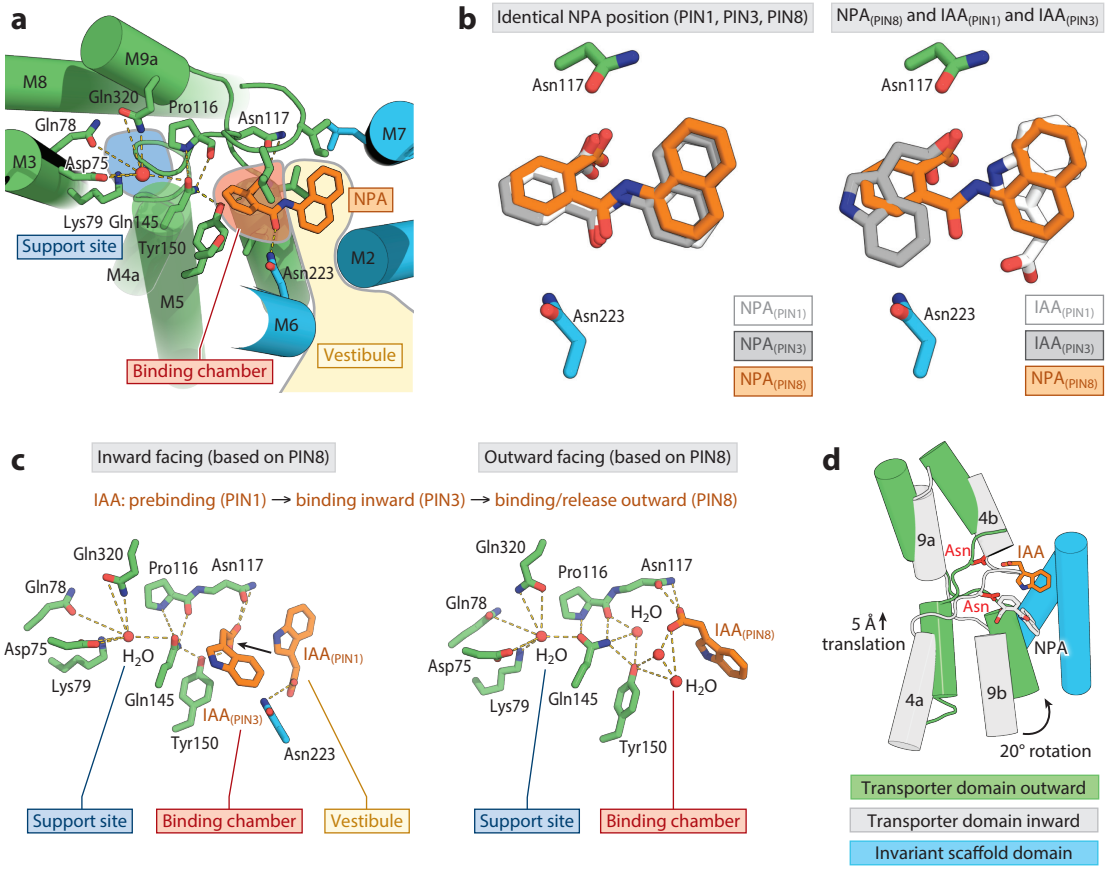

Figure 4

Transport of IAA in PINs. (*a*) View of the crossover and the position of NPA and the support site with central residues highlighted (PIN8 numbers). Dashed lines show the hydrogen bond network that links the support site (*blue*) to the binding chamber (*red*). The vestibule is shown in yellow. NPA binds in both the binding chamber and the vestibule and forms hydrogen bonds to both Asn117 and Asn223. (*b*, *left*) The overlay of NPA binding poses from PIN1, PIN3, and PIN8 shows that NPA is identical in all structures solved so far. (*Right*) The overlay of IAA binding position and poses from PIN1 and PIN3 in the inward state with NPA are shown for reference. (*c*) Overview of the hydrogen-bonding network linking the binding chamber to the support site through Gln145. The movement of IAA between current structures, from PIN1 to PIN3 and ending at PIN8, is shown. (*d*) Inward- and outward-facing structures superposed on the scaffold domain (*blue*) reveal the mechanism of transport: an elevator movement, with the substrate-binding site moving 5 Å. NPA and IAA positions are shown to highlight the change in position of the transporter domain and the binding site. Abbreviations: IAA, indole-3-acetic acid; NPA, 1-naphtylphthalamic acid; PIN, PIN-FORMED.

In the inward-facing conformations of all three PINs, the inhibitor NPA is bound in an identical fashion, split between the binding chamber and a vestibule leading from the cytosol (**Figure 4*a*,*b***). The binding of NPA in both the vestibule and binding chamber explains why NPA is a competitive inhibitor and must bind from the cytosolic side, as demonstrated previously (1). NPA has multiple strong interactions in both the binding chamber and the vestibule that locks it in place and precludes the structural transition from inward- to outward-facing state.

By contrast, the pose and position of IAA in the three different PINs varies (discussed in detail in 138). PIN3 in the inward conformation and PIN8 in the outward conformation agree on IAA binding in the binding chamber with the carboxyl acid coordinated by a central and fully conserved asparagine (Asn117 in PIN8 and Asn112 in PIN1 and PIN3) and the positive dipole of the crossover. Notably, charge compensation from helix dipoles is seen in numerous membrane transporters and seems to be a motif in the PIN transporters also (59, 118). In PIN1, IAA is modeled outside the binding chamber in the vestibule in an unexpected, inverted pose with the carboxyl pointing away from the $Asn112_{(PIN1)}$ and the crossover (138) (**Figure 4*b*,*c***). However weak experimental map density produces uncertainty about this inconsistent binding pose for IAA in PIN1. Notably, in all the structures of PINs with inhibitors, the carboxylate of the inhibitor is oriented toward the crossover, as is observed for IAA in PIN3 and PIN8. Together, the structures support the idea that the coordination of the auxin carboxyl by the $Asn117_{(PIN8)}$ residue and a charge neutralization by the dipole of the broken α-helices of the crossover are key features of transport.

Combining the structural data suggests that IAA binding to the inward conformation could involve multiple steps with prebinding in the vestibule (as seen in PIN1) followed by binding in the binding chamber prior to transport (as seen in PIN3). In the outward-facing state, it seems that the indole ring is released by hydration (as seen in PIN8), while protonation of the carboxyl of IAA facilitates substrate release (**Figure 4*c***). However, further structural work is needed to confirm this.

Superposition of the inward and outward structures shows that the scaffold domain is largely unchanged (RMSD = 0.9 Å), while the transporter domain from each monomer undergoes an elevator-like movement associated with the transition during transport. This movement involves a rotation of about 20°, resulting in the translation of the crossover and the substrate-binding site by 5 Å away from the cytosolic side of the membrane (**Figure 4*d***).

The support site is located across from the binding chamber, on the pseudosymmetrical side of the crossover (**Figures 3*c*** and **4*a*,*c***). Mutations at this support site provide evidence that it plays a crucial role in transport. A positively charged and fully conserved residue (lysine or arginine), named the dipole-neutralizing residue, is in a position to be able to neutralize the negative dipole of the crossover and is central for transport function (137, 138). A glutamine residue (Gln145 in PIN8 and Gln140 in PIN1 and PIN3) is of interest as the only residue bridging the support site to the binding chamber, thus providing a site for coordination between the two positions (**Figure 4*c***).

In transport assays, PIN8 activity showed no evidence for Na^+ or K^+ dependence, displayed minimal pH dependence, and appeared to be insensitive to PMF decouplers (139). This was not examined for the other PINs, but the evidence so far supports the hypothesis that PIN transporters are capable of uniport to concentrate the negatively charged IAA, driven by $\Delta\Psi$ as described in the section titled Biophysical Fundamentals of Membrane Transport and elsewhere (4). Nevertheless, a role for proton binding to the support site or elsewhere cannot be excluded at this point. If any proton binds, it would be expected to follow the PMF gradient into the cytosol in an antiport transport scheme.

Possible cooperativity between the PIN monomers is an open question. Notably, the crossover elevator fold found in PINs is well known from several other transporter families where it mediates the symport or antiport of different substrates with either protons or sodium ions as the

driving substrate (138, 139). As with PINs, structures of these transporters are found to be mostly dimers and trimers and have so far been solved with the individual monomers of these oligomeric states in identical inward or outward conformations (40, 61, 62, 77, 79). Notably, superposing inward and outward conformations shows the scaffold domain to be invariant in all these cases. This supports the idea that protein-mediated communication between monomers is unlikely. However, it is not unreasonable to speculate that the annular lipids around the transport domain that move during transport can lead to local deformation of the membrane. This type of membrane deformation could easily be seen to affect the kinetics of the transport domain of the other monomer in the dimer, and whether this would enhance or decrease conformational cooperativity remains to be examined. The scaffold domain in PINs has distinct binding sites for lipids (139). It appears to be firmly anchored in the lipid bilayer, again reinforcing the elevator mechanism, where the transporter domain is moving with respect to the membrane, and thus further supporting the hypothesis that any deformations in the membrane caused by the transporter domain moving could be translated to the adjacent monomer.

The dimeric nature of the PINs raises the question of whether PIN heterodimers are possible. The scaffold domain of each monomer in all cases has a very large surface area bound to its corresponding monomeric neighbor. Thus, it seems that significant activation energy would be required to break this interface to create dynamic heterooligomeric states. However, the lipid environment might reduce this activating cost. Furthermore, while dynamic shift between different hetero- and homooligomers might be unlikely once the proteins are mature, individual monomers of PINs could possibly join together in heterodimers during maturation in the ER and Golgi apparatus. The PIN proteins are translated as monomers and search for a partner to dimerize with during this process. There are no distinct interaction features in the hydrophobic surfaces of the scaffold domain when comparing PIN1, PIN3, and PIN8, suggesting that PIN heterodimers could theoretically be possible. Notably, for PIN7, it has been shown that two splice isoforms exist and that these two isoforms very likely do come together to form PIN7 heterodimers (67). However, in this case the interface would be exactly identical for the two PIN7 isoforms.

This idea has implications also for regulation by the autoregulatory loop in the long-loop PINs. Since it is not known whether the loop will inhibit only its corresponding monomer or function by some other mechanism, for example, by cross-inhibiting the associated monomer in the dimer, one could imagine a situation where heterodimers could display a mixed regulation signal that is a combination of the regulatory responses expected from distinct homodimer populations. The possible complex transport patterns derived from heterodimer formation remain speculative and need to be tested.

There have also been suggestions of complex formation between ABCB transporters and PIN transporters that regulate PIN specificity. Recently, a modeling approach suggested that PINs would not transport IAA in roots on their own and that interaction with ABCBs would be required for codependent auxin transport (7, 20, 33, 91). The biochemical and structural data available so far do not support this notion. PINs are clearly able to function without ABCBs in biochemical assays, and there is so far no convincing evidence or argument why they should not be able to do so in plants. All PINs are dimers with no obvious interface that would allow for larger complex formation with ABCB transporters. If ABCB transporters should indeed modulate the substrate specificity of PINs, such a modulation would most likely necessitate the physical change of the substrate-binding site. So far, all structural and biochemical evidence points to substrate specificity being mediated by the binding chamber of PINs, and it is very hard to see how this binding chamber would be changed by any external protein complex partner, even if such a complex exists. It remains to be shown how any codependent auxin efflux of PINs and ABCBs can be explained alternatively.

The regulatory loop of PINs. The cytoplasmic loop between M5 and M6 in PINs is the distinguishing feature to nonphylogenetically classify the PINs into long-loop (or canonical) PINs and short-loop (or noncanonical) PINs. In *Arabidopsis* PINs, the long loop ranges between 297 aa (in PIN4) and 329 aa (in PIN2), while the short loop is 29 aa in PIN5 and 44 aa in PIN8. *A. thaliana* PIN6 possesses a loop of intermediate size (250 aa). The classification scheme based on loop length, while quite convenient, has some limitations. On a phylogenetic level, the PINs clearly do not cluster according to loop size. For instance, PIN8 (a short-loop PIN) is most closely related to PIN3 (a long-loop PIN) and not PIN5, the other short-loop PIN in *Arabidopsis*, which appears to be very distinct from other *Arabidopsis* PINs (17, 18, 137–139, 142, 144). This is also evident if one simply counts the number of identical residues in other long-loop PINs and PIN8. Here, PIN3 shares 204 identical residues with PIN8, while PIN5 shares only 139 identical residues with PIN8. Long- and short-loop PINs with comparable sizes can also be found in bryophytes (17, 18, 32, 42, 142, 144). The most ancient PIN characterized in some detail is from the charophyte alga *Klebsormidium nitens* (123).

No regulatory elements have been identified in short loops so far, while the long loops have clear regulatory roles. The long loops analyzed so far are autoinhibitory, and this inhibition can be released by the phosphorylation of multiple sites on the loop (129, 139, 155, 162). Since auxin transport is mediated by the transmembrane part of the protein, the loop can be seen as a separate functional unit, likely with multiple distinct functions, that can readily be interchanged between PIN proteins. This perspective is supported by studies showing that functional chimeric constructs of PINs where the loop is swapped between proteins will have transport properties that relate to the transmembrane sequence and regulatory features that are related to the loop sequence (158–160).

The long loops are predicted to be intrinsically disordered (19). In the structures of PIN1 and PIN3 this was also the case, except for a short ~40-aa β-sheet domain directly following M5. Presently the function of this domain is unclear, but from the structures it appears to interact with M6 of the scaffold domain. Immediately after the β-sheet domain, there is a conserved region that is found in all canonical PINs that contains at least five elements for PIN regulation by phosphorylation, which have been functionally characterized in *Arabidopsis*. The main five elements (called S1–S5) are key sites of phosphorylation, but several more specific phosphorylation sites have been proposed in the loops so far (9).

In vitro, the loop is directly phosphorylated by AGC1 and AGC3 kinases, MITOGEN-ACTIVATED PROTEIN (MAP) kinases, and Ca^{2+}/calmodulin-dependent protein kinase–related kinases (CRKs), as well as by CANALIZATION-RELATED AUXIN-REGULATED MALECTIN-TYPE RLK (CAMEL) together with CANALIZATION-RELATED RECEPTOR LIKE KINASE (CANAR) (see 9 for a recent review).

Among these, the phosphorylation of PINs by AGCVIII kinases, such as D6 PROTEIN KINASE (D6PK, a AGC1 kinase) and PINOID (PID, a AGC3 kinase), has received the most attention. PIN phosphorylation in vitro and PIN activation in oocyte transport assays are eliminated if the target serines on the S1–S5 regions are mutated to alanine. Notably, phosphomimicking mutations to aspartate or glutamate cannot rescue activation, indicating that phosphorylation of the residues is strictly required (149, 162). The same components of phosphoregulation are found in long-loop PINs from the moss *Physcomitrium patens* and the liverwort *Marchantia polymorpha*, and these PINs are most likely subject to the same activity regulation, indicating that this mechanism appeared early in the evolution of land plants (42).

Even though D6PK and PID phosphorylate the same phosphosites, mutations of specific serines have different effects on each kinase's ability to activate PINs in vitro and in oocyte-based auxin transport assays, suggesting a different mode of PIN binding (50, 162). Notably, PID also

regulates the polar distribution of PINs (29, 35, 44, 93, 156). Phosphatases that act antagonistically to the kinases have also been identified. At present, which features in the loop govern PIN localization and/or the interaction with other factors that control localization is unknown, and the loop clearly requires study to produce a better understanding of the biological context of PIN-mediated auxin export.

Lessons from the PIN structure and the transport mechanism. Recent progress has opened the door to address the regulation of PINs, the possible interactions among PINs and between PINs and kinases, the physiological relevance of dimerization and cooperativity between the monomers, and substrate recognition. The transport mechanism and the biochemical properties have far-reaching consequences. PIN transport rates scale with protein levels and membrane potential. Firstly, this means that the cell can increase or decrease transport rates by increasing or decreasing the amount of transport competent protein in the membrane. For long-loop PINs this can be achieved quickly by activation through phosphorylation or deactivation by dephosphorylation. Another way is to control the amount of transporter in the membrane. Secondly, the dependency of PIN-mediated IAA export on $\Delta\Psi$ also implies that modulation of this parameter, most importantly by changing proton P-type ATPase activity, will inevitably lead to a change of IAA transport rate given permissive PIN phosphorylation. It may therefore be worthwhile to consider that effects of any treatment or stress on auxin export and cellular levels may be secondary and a result of changing the driving force rather than a direct effect on the transporter.

CONCLUSIONS

Polar auxin transport is an important process in plant biology, and many transporters apparently involved in this process have been identified as genes underlying mutant phenotypes. Here, we have focused on the relatively sparse biochemical data available on these transport proteins in order to change the focus from cellular-level and organism-level knowledge derived from the analysis of mutants in different plant species to the biophysical properties of the proteins. We note that there is clearly a gap between these two approaches. While genetic approaches are extremely powerful to address organism-level properties, they cannot answer biochemical questions about protein properties, and this is needed to better understand and refine plant growth and development. The number of transporter families linked to auxin transport in plants is large and growing. While significant progress has been made in some cases, we are still in the very early days of understanding the biophysics of auxin transport. In addition, to fully understand auxin fluxes it is also necessary to further investigate the role of plasmodesmata in symplastic auxin transport. While the role of plasmodesmata in auxin transport has been appreciated in many studies, the division between apoplastic and symplastic transport remains critically understudied (see 6 for a review). Auxin flux is a dynamic process, and modeling will be needed to address its complexity. For these models to work, parameterizing the transport steps as precisely as possible is a critical first step. This includes determining auxin concentrations on a subcellular scale and understanding the biophysics of the network of transporters involved in auxin distribution. Clearly, we need more and better structural, biochemical, and biophysical data for all transporter types, as well as for the symplastic transport pathway.

SUMMARY POINTS

1. The biochemical and biophysical data available for indole-3-acetic acid (IAA) transporters are scarce.

2. Auxin transport has evolved in members of many different and diverse families of transporters.
3. Based on the membrane potentials of the plasma membrane and endogenous membranes as well as the data available for the concentration gradients of IAA, the import of IAA requires active transport, whereas the export step does not.
4. PIN-FORMED (PIN) transporters form dimers and operate by a crossover elevator mechanism to export IAA from cells by a uniport mechanism.
5. The substrate affinities of all PINs determined so far are surprisingly low. This suggests that the amount of active transporter in the membrane is the decisive criterion by which the cell controls the IAA efflux rate.

FUTURE ISSUES

The characterization of IAA transporters has until recently relied on correlative evidence derived from the characterization of mutant phenotypes and transport data derived from the expression of the transporters in cell-based expression systems with low resolution. Advances in cryogenic electron microscopy and novel methods to characterize IAA transporters will and must fuel research on the characterization of their biochemical and biophysical properties. The following are the key questions to be answered:

1. How did substrate selectivity evolve in the diverse transporter families?
2. What are the different mechanisms by which these transporters work?
3. Can PINs work as monomers, and is the monomer/dimer status functionally relevant?
4. Do PIN heteromers exist, and, if so, do they have unique properties?
5. How does PIN autoinhibition through the loop domain work mechanistically?

NOTE ADDED IN PROOF

During the publication process of this review, an article that used substrate-induced ATP hydrolysis in ABCB19 was published (155a). The study demonstrated that BL stimulated ATP hydrolysis, but IAA did not. This underlines that the rudimentary biochemical characterization of ABCB transporters needs critical reevaluation that relies on something other than passive substrate preloading in cell-based export assays to show a convincing role in IAA transport.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. **Abas L, Kolb M, Stadlmann J, Janacek DP, Lukic K, et al. 2021. Naphthylphthalamic acid associates with and inhibits PIN auxin transporters. *PNAS* 118:e2020857118**
2. Adamowski M, Friml J. 2015. PIN-dependent auxin transport: action, regulation, and evolution. *Plant Cell* 27:20–32
3. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, et al. 2009. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* 323:1718–22
4. Andersen CG, Bavnhøj L, Pedersen BP. 2023. May the proton motive force be with you: a plant transporter review. *Curr. Opin. Struct. Biol.* 79:102535
5. Armengot L, Marques-Bueno MM, Jaillais Y. 2016. Regulation of polar auxin transport by protein and lipid kinases. *J. Exp. Bot.* 67:4015–37

5a. Aryal B, Huynh J, Schneuwly J, Siffert A, Liu J, et al. 2019. ABCG36/PEN3/PDR8 is an exporter of the auxin precursor, indole-3-butyric acid, and involved in auxin-controlled development. *Front. Plant Sci.* 10:899

6. Band LR. 2021. Auxin fluxes through plasmodesmata. *New Phytol.* 231:1686–92
7. Bandyopadhyay A, Blakeslee JJ, Lee OR, Mravec J, Sauer M, et al. 2007. Interactions of PIN and PGP auxin transport mechanisms. *Biochem. Soc. Trans.* 35:137–41

7a. Barbez E, Kubeš M, Rolčík J, Béziat C, Pěnčík A, et al. 2012. A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* 485:119–22

8. Barbosa ICR, Hammes UZ, Schwechheimer C. 2018. Activation and polarity control of PIN-FORMED auxin transporters by phosphorylation. *Trends Plant Sci.* 23:523–38
9. Bassukas AEL, Xiao Y, Schwechheimer C. 2022. Phosphorylation control of PIN auxin transporters. *Curr. Opin. Plant Biol.* 65:102146
10. Bavnhøj L, Driller JH, Zuzic L, Stange AD, Schiøtt B, Pedersen BP. 2023. Structure and sucrose binding mechanism of the plant SUC1 sucrose transporter. *Nat. Plants* 9:938–50
11. Bazzone A, Barthmes M, Fendler K. 2017. SSM-based electrophysiology for transporter research. *Methods Enzymol.* 594:31–83
12. Bazzone A, Zabadne AJ, Salisowski A, Madej MG, Fendler K. 2017. A loose relationship: incomplete H^+/sugar coupling in the MFS sugar transporter GlcP. *Biophys. J.* 113:2736–49
13. Bazzone A, Zerlotti R, Barthmes M, Fertig N. 2023. Functional characterization of SGLT1 using SSM-based electrophysiology: kinetics of sugar binding and translocation. *Front. Physiol.* 14:1058583
14. Beckstein O, Naughton F. 2022. General principles of secondary active transporter function. *Biophys. Rev.* 3:011307
15. Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, et al. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115:591–602
16. Bennett MJ, Marchant A, Green HG, May ST, Ward SP, et al. 1996. *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. *Science* 273:948–50
17. Bennett T. 2015. PIN proteins and the evolution of plant development. *Trends Plant Sci.* 20:498–507
18. Bennett T, Brockington SF, Rothfels C, Graham SW, Stevenson D, et al. 2014. Paralogous radiations of PIN proteins with multiple origins of noncanonical PIN structure. *Mol. Biol. Evol.* 31:2042–60
19. Bilanovičová V, Rýdza N, Koczka L, Hess M, Feraru E, et al. 2022. The hydrophilic loop of *Arabidopsis* PIN1 auxin efflux carrier harbors hallmarks of an intrinsically disordered protein. *Int. J. Mol. Sci.* 23:6352
20. Blakeslee JJ, Bandyopadhyay A, Lee OR, Mravec J, Titapiwatanakun B, et al. 2007. Interactions among PIN-FORMED and P-glycoprotein auxin transporters in *Arabidopsis*. *Plant Cell* 19:131–47
21. Blatt MR, Rodriguez-Navarro A, Slayman CL. 1987. Potassium-proton symport in *Neurospora*: kinetic control by pH and membrane potential. *J. Membr. Biol.* 98:169–89
22. Boudker O, Oh S. 2015. Isothermal titration calorimetry of ion-coupled membrane transporters. *Methods* 76:171–82
23. Broer S. 2010. *Xenopus laevis* oocytes. *Methods Mol. Biol.* 637:295–310
24. Busi R, Goggin DE, Heap IM, Horak MJ, Jugulam M, et al. 2018. Weed resistance to synthetic auxin herbicides. *Pest Manag. Sci.* 74:2265–76

24a. Chen J, Hu Y, Hao P, Tsering T, Xia J, et al. 2023. ABCB-mediated shootward auxin transport feeds into the root clock. *EMBO Rep.* 24:e56271

1. Demonstrates that PINs are the physiologically relevant targets of the NPA.

25. Chen JS, Reddy V, Chen JH, Shlykov MA, Zheng WH, et al. 2011. Phylogenetic characterization of transport protein superfamilies: superiority of SuperfamilyTree programs over those based on multiple alignments. *J. Mol. Microbiol. Biotechnol.* 21:83–96
26. Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson PH. 1998. The *Arabidopsis thaliana AGRAVITROPIC 1* gene encodes a component of the polar-auxin-transport efflux carrier. *PNAS* 95:15112–17
27. Chiba Y, Shimizu T, Miyakawa S, Kanno Y, Koshiba T, et al. 2015. Identification of *Arabidopsis thaliana* NRT1/PTR FAMILY (NPF) proteins capable of transporting plant hormones. *J. Plant Res.* 128:679–86
28. Cho M, Lee SH, Cho H-T. 2007. P-glycoprotein4 displays auxin efflux transporter–like action in *Arabidopsis* root hair cells and tobacco cells. *Plant Cell* 19:3930–43
29. Christensen SK, Dagenais N, Chory J, Weigel D. 2000. Regulation of auxin response by the protein kinase PINOID. *Cell* 100:469–78
30. Cleland WW. 1963. The kinetics of enzyme-catalyzed reactions with two or more substrates or products. I. Nomenclature and rate equations. *Biochim. Biophys. Acta* 67:104–37
31. Corratge-Faillie C, Lacombe B. 2017. Substrate (un)specificity of Arabidopsis NRT1/PTR FAMILY (NPF) proteins. *J. Exp. Bot.* 68:3107–13
32. De Smet I, Voss U, Lau S, Wilson M, Shao N, et al. 2011. Unraveling the evolution of auxin signaling. *Plant Physiol.* 155:209–21
33. Deslauriers SD, Spalding EP. 2021. Electrophysiological study of Arabidopsis ABCB4 and PIN2 auxin transporters: Evidence of auxin activation and interaction enhancing auxin selectivity. *Plant Direct* 5:e361
34. Dharmasiri N, Dharmasiri S, Estelle M. 2005. The F-box protein TIR1 is an auxin receptor. *Nature* 435:441–45
35. Dhonukshe P, Huang F, Galvan-Ampudia CS, Mähönen AP, Kleine-Vehn J, et al. 2010. Plasma membrane-bound AGC3 kinases phosphorylate PIN auxin carriers at TPRXS(N/S) motifs to direct apical PIN recycling. *Development* 137:3245–55
36. Dindas J, Scherzer S, Roelfsema MRG, von Meyer K, Müller HM, et al. 2018. AUX1-mediated root hair auxin influx governs $SCF^{TIR1/AFB}$-type Ca^{2+} signaling. *Nat. Comm.* 9:1174
36a. Ding Z, Wang B, Moreno I, Dupláková N, Simon S, et al. 2012. ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in *Arabidopsis*. *Nat. Comm.* 3:941
37. Do THT, Martinoia E, Lee Y, Hwang JU. 2021. 2021 update on ATP-binding cassette (ABC) transporters: how they meet the needs of plants. *Plant Physiol.* 187:1876–92
38. Drew D, Boudker O. 2016. Shared molecular mechanisms of membrane transporters. *Annu. Rev. Biochem.* 85:543–72
39. Dumont JN. 1972. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* 136:153–79
40. Fang S, Huang X, Zhang X, Zhang M, Hao Y, et al. 2021. Molecular mechanism underlying transport and allosteric inhibition of bicarbonate transporter SbtA. *PNAS* 118:e2101632118
41. Fastner A, Absmanner B, Hammes UZ. 2017. Use of *Xenopus laevis* oocytes to study auxin transport. *Methods Mol. Biol.* 1497:259–70
42. Fisher TJ, Flores-Sandoval E, Alvarez JP, Bowman JL. 2023. PIN-FORMED is required for shoot phototropism/gravitropism and facilitates meristem formation in *Marchantia polymorpha*. *New Phytol.* 238:1498–515
43. Forgac M. 2007. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nat. Rev. Mol. Cell Biol.* 8:917–29
44. Friml J, Yang X, Michniewicz M, Weijers D, Quint A, et al. 2004. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* 306:862–65
45. Gadsby DC. 2009. Ion channels versus ion pumps: the principal difference, in principle. *Nat. Rev. Mol. Cell Biol.* 10:344–52
46. Gälweiler L, Guan C, Muller A, Wisman E, Mendgen K, et al. 1998. Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* 282:2226–30
46a. Geisler M, Blakeslee JJ, Bouchard R, Lee OR, Vincenzetti V, et al. 2005. Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. *Plant J.* 44:179–94

36. Uses several electrophysiological approaches to demonstrate proton couple IAA transport in AUX1.

47. Goldsmith MHM. 1977. The polar transport of auxin. *Annu. Rev. Plant Physiol.* 28:439–78
48. Grossmann K. 2010. Auxin herbicides: current status of mechanism and mode of action. *Pest Manag. Sci.* 66:113–20
49. Hafke JB, van Amerongen J-K, Kelling F, Furch ACU, Gaupels F, van Bel AJE. 2005. Thermodynamic battle for photosynthate acquisition between sieve tubes and adjoining parenchyma in transport phloem. *Plant Physiol.* 138:1527–37
50. Haga K, Frank L, Kimura T, Schwechheimer C, Sakai T. 2018. Roles of AGCVIII kinases in the hypocotyl phototropism of Arabidopsis seedlings. *Plant Cell Physiol.* 59:1060–71
51. Hammes UZ, Murphy AS, Schwechheimer C. 2022. Auxin transporters — a biochemical view. *Cold Spring Harb. Perspect. Biol.* 14:a039875
52. Han H, Adamowski M, Qi L, Alotaibi SS, Friml J. 2021. PIN-mediated polar auxin transport regulations in plant tropic responses. *New Phytol.* 232:510–22
53. Hartung W, Weiler EW, Radin JW. 1992. Auxin and cytokinins in the apoplastic solution of dehydrated cotton leaves. *J. Plant Physiol.* 140:324–27
54. Hedrich R. 2012. Ion channels in plants. *Physiol. Rev.* 92:1777–811
55. Hertel R, Evans ML, Leopold AC, Sell HM. 1969. The specificity of the auxin transport system. *Planta* 85:238–49
56. Hertel R, Lomax TL, Briggs WR. 1983. Auxin transport in membrane vesicles from *Cucurbita pepo* L. *Planta* 157:193–201
57. Higinbotham N, Graves JS, Davis RF. 1970. Evidence for an electrogenic ion transport pump in cells of higher plants. *J. Membr. Biol.* 3:210–22
58. Hille B. 1992. Potassium channels and chloride channels. In *In Ionic Channels of Excitable Membranes*, ed. B Hill, pp. 130–33 2nd ed.
59. Hol WGJ. 1985. Effects of the α-helix dipole upon the functioning and structure of proteins and peptides. *Adv. Biophys.* 19:133–65
60. Hresko RC, Kraft TE, Quigley A, Carpenter EP, Hruz PW. 2016. Mammalian glucose transporter activity is dependent upon anionic and conical phospholipids. *J. Biol. Chem.* 291:17271–82
61. Hu N-J, Iwata S, Cameron AD, Drew D. 2011. Crystal structure of a bacterial homologue of the bile acid sodium symporter ASBT. *Nature* 478:408–11
62. Hunte C, Screpanti E, Venturi M, Rimon A, Padan E, Michel H. 2005. Structure of a Na^+/H^+ antiporter and insights into mechanism of action and regulation by pH. *Nature* 435:1197–202
63. Jack DL, Yang NM, Saier MH Jr. 2001. The drug/metabolite transporter superfamily. *Eur. J. Biochem.* 268:3620–39
64. Jardetzky O. 1966. Simple allosteric model for membrane pumps. *Nature* 211:969–70
65. Kamimoto Y, Terasaka K, Hamamoto M, Takanashi K, Fukuda S, et al. 2012. *Arabidopsis* ABCB21 is a facultative auxin importer/exporter regulated by cytoplasmic auxin concentration. *Plant Cell Physiol.* 53:2090–100
66. Kang J, Park J, Choi H, Burla B, Kretzschmar T, et al. 2011. Plant ABC transporters. *Arabidopsis Book* 9:e0153
67. Kashkan I, Hrtyan M, Retzer K, Humpolíčková J, Jayasree A, et al. 2022. Mutually opposing activity of PIN7 splicing isoforms is required for auxin-mediated tropic responses in *Arabidopsis thaliana*. *New Phytol.* 233:329–43
68. Kazmier K, Claxton DP, McHaourab HS. 2017. Alternating access mechanisms of LeuT-fold transporters: trailblazing towards the promised energy landscapes. *Curr. Opin. Struct. Biol.* 45:100–8
69. Kojima K, Ohtake E, Yu Z. 2002. Distribution and transport of IAA in tomato plants. *Plant Growth Regul.* 37:249–54
70. Kojima K, Tamura Y, Nakano M, Han D-S, Niimi Y. 2003. Distribution of indole-acetic acid, gibberellin and cytokinins in apoplast and symplast of parthenocarpic tomato fruits. *Plant Growth Regul.* 41:99–104
71. Konings WN, Lolkema JS, Poolman B. 1995. The generation of metabolic energy by solute transport. *Arch. Microbiol.* 164:235–42
72. Krecek P, Skupa P, Libus J, Naramoto S, Tejos R, et al. 2009. The PIN-FORMED (PIN) protein family of auxin transporters. *Genome Biol.* 10:249

67. Provides evidence for the hypothesis that PIN heteromers with specific properties exist.

73. Krishnamurthy H, Gouaux E. 2012. X-ray structures of LeuT in substrate-free outward-open and apo inward-open states. *Nature* 481:469–74
74. Krouk G, Lacombe B, Bielach A, Perrine-Walker F, Malinska K, et al. 2010. Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. *Dev. Cell* 18:927–37
74a. Kubeš M, Yang H, Richter GL, Cheng Y, Młodzińska E, et al. 2012. The Arabidopsis concentration-dependent influx/efflux transporter ABCB4 regulates cellular auxin levels in the root epidermis. *Plant J.* 69:640–54
75. Ladwig F, Stahl M, Ludewig U, Hirner AA, Hammes UZ, et al. 2012. *Siliques are red1* from Arabidopsis acts as a bidirectional amino acid transporter that is crucial for the amino acid homeostasis of siliques. *Plant Physiol.* 158:1643–55
75a. Laňková M, Smith RS, Pešek B, Kubeš M, Zažímalová E, et al. 2010. Auxin influx inhibitors 1-NOA, 2-NOA, and CHPAA interfere with membrane dynamics in tobacco cells. *J. Exp. Bot.* 61:3589–98
76. Law CJ, Maloney PC, Wang D-N. 2008. Ins and outs of major facilitator superfamily antiporters. *Annu. Rev. Microbiol.* 62:289–305
77. Lee C, Kang HJ, von Ballmoos C, Newstead S, Uzdavinys P, et al. 2013. A two-domain elevator mechanism for sodium/proton antiport. *Nature* 501:573–77
78. Li L, Gallei M, Friml J. 2022. Bending to auxin: fast acid growth for tropisms. *Trends Plant Sci.* 27:440–49
79. Liu X-Y, Hou W-T, Wang L, Li B, Chen Y, et al. 2021. Structures of cyanobacterial bicarbonate transporter SbtA and its complex with PII-like SbtB. *Cell Discov.* 7:63
80. Livnat-Levanon N, Gilson AI, Ben-Tal N, Lewinson O. 2016. The uncoupled ATPase activity of the ABC transporter $BtuC_2D_2$ leads to a hysteretic conformational change, conformational memory, and improved activity. *Sci. Rep.* 6:21696
81. Locher KP. 2016. Mechanistic diversity in ATP-binding cassette (ABC) transporters. *Nat. Struct. Mol. Biol.* 23:487–93
82. Lomax TL, Mehlhorn RJ, Briggs WR. 1985. Active auxin uptake by zucchini membrane vesicles: quantitation using ESR volume and delta pH determinations. *PNAS* 82:6541–45
83. Ludwig-Müller J. 2022. Auxins in the right space and time regulate pea fruit development. *J. Exp. Bot.* 73:3831–35
84. Luschnig C, Gaxiola RA, Grisafi P, Fink GR. 1998. EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. *Genes Dev.* 12:2175–87
85. Maeshima M. 2000. Vacuolar H^+-pyrophosphatase. *Biochim. Biophys. Acta* 1465:37–51
86. Mansour NM, Sawhney M, Tamang DG, Vogl C, Saier MH Jr. 2007. The bile/arsenite/riboflavin transporter (BART) superfamily. *FEBS J.* 274:612–29
87. Marhava P, Bassukas AEL, Zourelidou M, Kolb M, Moret B, et al. 2018. A molecular rheostat adjusts auxin flux to promote root protophloem differentiation. *Nature* 558:297–300
88. Martinière A, Bassil E, Jublanc E, Alcon C, Reguera M, et al. 2013. In vivo intracellular pH measurements in tobacco and *Arabidopsis* reveal an unexpected pH gradient in the endomembrane system. *Plant Cell* 25:4028–43
89. McKay DW, McFarlane HE, Qu Y, Situmorang A, Gilliham M, Wege S. 2022. Plant trans-Golgi network/early endosome pH regulation requires cation chloride cotransporter (CCC1). *eLife* 11:e70701
90. Meharg AA, Blatt MR. 1995. NO_3^- transport across the plasma membrane of *Arabidopsis thaliana* root hairs: kinetic control by pH and membrane voltage. *J. Membr. Biol.* 145:49–66
91. Mellor NL, Voß U, Ware A, Janes G, Barrack D, et al. 2022. Systems approaches reveal that ABCB and PIN proteins mediate co-dependent auxin efflux. *Plant Cell* 34:2309–27
92. Michniewicz M, Ho C-H, Enders TA, Floro E, Damodaran S, et al. 2019. TRANSPORTER OF IBA1 links auxin and cytokinin to influence root architecture. *Dev. Cell* 50:599–609.e4
93. Michniewicz M, Zago MK, Abas L, Weijers D, Schweighofer A, et al. 2007. Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell* 130:1044–56
94. Mitchell P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 191:144–48
95. Morffy N, Strader LC. 2022. Structural aspects of auxin signaling. *Cold Spring Harb. Perspect. Biol.* 14:a039883

96. Morth JP, Pedersen BP, Toustrup-Jensen MS, Sorensen TL, Petersen J, et al. 2007. Crystal structure of the sodium-potassium pump. *Nature* 450:1043–49
97. Muller A, Guan C, Galweiler L, Tanzler P, Huijser P, et al. 1998. *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. *EMBO J.* 17:6903–11
98. Müller B, Fastner A, Karmann J, Mansch V, Hoffmann T, et al. 2015. Amino acid export in developing *Arabidopsis* seeds depends on UmamiT facilitators. *Curr. Biol.* 25:3126–31
99. Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y. 1991. Requirement of the auxin polar transport system in early stages of Arabidopsis floral bud formation. *Plant Cell* 3:677–84
100. Palmgren MG. 2001. Plant plasma membrane H^+-ATPases: powerhouses for nutrient uptake. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52:817–45
101. Pao SS, Paulsen IT, Saier MH Jr. 1998. Major facilitator superfamily. *Microbiol. Mol. Biol. Rev.* 62:1–34
102. Parker JL, Newstead S. 2014. Molecular basis of nitrate uptake by the plant nitrate transporter NRT1.1. *Nature* 507:68–72
103. Pedersen BP, Buch-Pedersen MJ, Morth JP, Palmgren MG, Nissen P. 2007. Crystal structure of the plasma membrane proton pump. *Nature* 450:1111–14
104. Peña A, Uribe S, Pardo JP, Borbolla M. 1984. The use of a cyanine dye in measuring membrane potential in yeast. *Arch. Biochem. Biophys.* 231:217–25
105. Peret B, Swarup K, Ferguson A, Seth M, Yang Y, et al. 2012. *AUX/LAX* genes encode a family of auxin influx transporters that perform distinct functions during *Arabidopsis* development. *Plant Cell* 24:2874–85
106. Petersson SV, Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, et al. 2009. An auxin gradient and maximum in the *Arabidopsis* root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. *Plant Cell* 21:1659–68
107. Petrasek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, et al. 2006. PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* 312:914–18
108. Rajarathnam K, Rösgen J. 2014. Isothermal titration calorimetry of membrane proteins—progress and challenges. *Biochim. Biophys. Acta* 1838:69–77
109. Ranocha P, Dima O, Nagy R, Felten J, Corratge-Faillie C, et al. 2013. *Arabidopsis* WAT1 is a vacuolar auxin transport facilitator required for auxin homoeostasis. *Nat. Comm.* 4:2625
110. Raven JA. 1975. Transport of indolacetic acid in plant cells in relation to pH and electrical potential gradients, and its significance for polar auxin transport. *New Phytol.* 74:163–72
111. Reddy VS, Shlykov MA, Castillo R, Sun EI, Saier MH Jr. 2012. The major facilitator superfamily (MFS) revisited. *FEBS J.* 279:2022–35
112. Reinecke DM. 1999. 4-Chloroindole-3-acetic acid and plant growth. *Plant Growth Regul.* 27:3–13
113. Reinecke DM, Ozga JA, Magnus V. 1995. Effect of halogen substitution of indole-3-acetic acid on biological activity in pea fruit. *Phytochemistry* 40:1361–66
114. Rubery PH, Sheldrake AR. 1974. Carrier-mediated auxin transport. *Planta* 118:101–21
114a. Růžička K, Strader LC, Bailly A, Yang H, Blakeslee J, et al. 2010. *Arabidopsis PIS1* encodes the ABCG37 transporter of auxinic compounds including the auxin precursor indole-3-butyric acid. *PNAS* 107:10749–53
115. Santelia D, Vincenzetti V, Azzarello E, Bovet L, Fukao Y, et al. 2005. MDR-like ABC transporter AtPGP4 is involved in auxin-mediated lateral root and root hair development. *FEBS Lett.* 579:5399–406
116. Sarkadi B, Price EM, Boucher RC, Germann UA, Scarborough GA. 1992. Expression of the human multidrug resistance cDNA in insect cells generates a high activity drug-stimulated membrane ATPase. *J. Biol. Chem.* 267:4854–58
117. Schneider E, Hunke S. 1998. ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS Microbiol. Rev.* 22:1–20
118. Screpanti E, Hunte C. 2007. Discontinuous membrane helices in transport proteins and their correlation with function. *J. Struct. Biol.* 159:261–67
119. Serrano R, Kielland-Brandt MC, Fink GR. 1986. Yeast plasma membrane ATPase is essential for growth and has homology with ($Na^+ + K^+$), K^+- and $Ca2^+$-ATPases. *Nature* 319:689–93
120. Shen J, Zeng Y, Zhuang X, Sun L, Yao X, Pimpl P, Jiang L. 2013. Organelle pH in the *Arabidopsis* endomembrane system. *Mol. Plant* 6:1419–37

121. Shi B, Vernoux T. 2019. Patterning at the shoot apical meristem and phyllotaxis. *Curr. Top. Dev. Biol.* 131:81–107
122. Simon S, Petrášek J. 2011. Why plants need more than one type of auxin. *Plant Sci.* 180:454–60
123. Skokan R, Medvecká E, Viaene T, Vosolsobě S, Zwiewka M, et al. 2019. PIN-driven auxin transport emerged early in streptophyte evolution. *Nat. Plants* 5:1114–19
124. Skou JC. 1957. The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta* 23:394–401
125. Slayman CL. 1965. Electrical properties of *Neurospora crassa*: respiration and the intracellular potential. *J. Gen. Physiol.* 49:93–116
126. Somlyo AV, Gonzalez-Serratos H, Shuman H, McClellan G. 1981. Calcium release and ionic changes in the sarcoplasmic reticulum of tetanized muscle: an electron-probe study. *J. Cell Biol.* 90:577–94
127. Spalding EP. 2013. Diverting the downhill flow of auxin to steer growth during tropisms. *Am. J. Bot.* 100:203–14
128. Sparks RP, Fratti R. 2019. Use of microscale thermophoresis (MST) to measure binding affinities of components of the fusion machinery. *Methods Mol. Biol.* 1860:191–98

129. Provides three structures for PIN3; identifies the IAA-bound inward state.

129. Su N, Zhu A, Tao X, Ding ZJ, Chang S, et al. 2022. Structures and mechanisms of the *Arabidopsis* auxin transporter PIN3. *Nature* 609:616–21

130. Sun J, Bankston JR, Payandeh J, Hinds TR, Zagotta WN, Zheng N. 2014. Crystal structure of the plant dual-affinity nitrate transporter NRT1.1. *Nature* 507:73–77
131. Swarup K, Benkova E, Swarup R, Casimiro I, Peret B, et al. 2008. The auxin influx carrier LAX3 promotes lateral root emergence. *Nat. Cell Biol.* 10:946–54
132. Swarup R, Bhosale R. 2019. Developmental roles of AUX1/LAX auxin influx carriers in plants. *Front. Plant Sci.* 10:1306
133. Swarup R, Kramer EM, Perry P, Knox K, Leyser HM, et al. 2005. Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat. Cell Biol.* 7:1057–65
134. Tan YZ, Keon KA, Abdelaziz R, Imming P, Schulze W, et al. 2022. Structure of V-ATPase from citrus fruit. *Structure* 30:1403–10.e4
135. Terasaka K, Blakeslee JJ, Titapiwatanakun B, Peer WA, Bandyopadhyay A, et al. 2005. PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. *Plant Cell* 17:2922–39
136. Tsurusaki K-i, Masuda Y, Sakurai N. 1997. Distribution of indole-3-acetic acid in the apoplast and symplast of squash (*Cucurbita maxima*) hypocotyls. *Plant Cell Physiol.* 38:352–56
137. Ung KL, Schulz L, Kleine-Vehn J, Pedersen BP, Hammes UZ. 2023. Auxin transport at the ER: Roles and structural similarity of PIN-FORMED and PIN-LIKES. *J. Exp. Bot.* 2023:erad192. Erratum. *J. Exp. Bot.* 2023:erad322
138. Ung KL, Schulz L, Stokes DL, Hammes UZ, Pedersen BP. 2023. Substrate recognition and transport mechanism of the PIN-FORMED auxin exporters. *Trends Biochem. Sci.* 48:937–48

139. The first article providing PIN structures and a model of transport, and the only article providing outward states.

139. Ung KL, Winkler M, Schulz L, Kolb M, Janacek DP, et al. 2022. Structures and mechanism of the plant PIN-FORMED auxin transporter. *Nature* 609:605–10

140. van den Berg T, Yalamanchili K, de Gernier H, Santos Teixeira J, Beeckman T, et al. 2021. A reflux-and-growth mechanism explains oscillatory patterning of lateral root branching sites. *Dev. Cell* 56:2176–91.e10
141. Verma S, Attuluri VPS, Robert HS. 2021. An essential function for auxin in embryo development. *Cold Spring Harb. Perspect. Biol.* 13:a039966
142. Viaene T, Delwiche CF, Rensing SA, Friml J. 2013. Origin and evolution of PIN auxin transporters in the green lineage. *Trends Plant Sci.* 18:5–10
143. Vieten A, Vanneste S, Wisniewska J, Benkova E, Benjamins R, et al. 2005. Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. *Development* 132:4521–31
144. Vosolsobě S, Skokan R, Petrášek J. 2020. The evolutionary origins of auxin transport: what we know and what we need to know. *J. Exp. Bot.* 71:3287–95

145. Wagner CA, Friedrich B, Setiawan I, Lang F, Bröer S. 2000. The use of *Xenopus laevis* oocytes for the functional characterization of heterologously expressed membrane proteins. *Cellular Physiol. Biochem.* 10:1–12
146. Walter A, Caputi L, O'Connor S, van Pée K-H, Ludwig-Müller J. 2020. Chlorinated auxins—how does *Arabidopsis thaliana* deal with them? *Int. J. Mol. Sci.* 21:2567
147. Wang P, Shen L, Guo J, Jing W, Qu Y, et al. 2019. Phosphatidic acid directly regulates PINOID-dependent phosphorylation and activation of the PIN-FORMED2 auxin efflux transporter in response to salt stress. *Plant Cell* 31:250–71
148. Watanabe S, Takahashi N, Kanno Y, Suzuki H, Aoi Y, et al. 2020. The *Arabidopsis* NRT1/PTR FAMILY protein NPF7.3/NRT1.5 is an indole-3-butyric acid transporter involved in root gravitropism. *PNAS* 117:31500–9
149. Weller B, Zourelidou M, Frank L, Barbosa IC, Fastner A, et al. 2017. Dynamic PIN-FORMED auxin efflux carrier phosphorylation at the plasma membrane controls auxin efflux-dependent growth. *PNAS* 114:E887–96
150. Wightman F, Lighty DL. 1982. Identification of phenylacetic acid as a natural auxin in the shoots of higher plants. *Physiol. Plant.* 55:17–24
151. Wipf D, Ludewig U, Tegeder M, Rentsch D, Koch W, Frommer WB. 2002. Conservation of amino acid transporters in fungi, plants and animals. *Trends Biochem. Sci.* 27:139–47
152. Xia J, Kong M, Yang Z, Sun L, Peng Y, et al. Chemical inhibition of *Arabidopsis* PIN-FORMED auxin transporters by the anti-inflammatory drug naproxen. *Plant Commun*. 29:100632
153. Xu D, Sanden NCH, Hansen LL, Belew ZM, Madsen SR, et al. 2023. Export of defensive glucosinolates is key for their accumulation in seeds. *Nature* 617:132–38
153a. Yang H, Murphy AS. 2009. Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in *Schizosaccharomyces pombe*. *Plant J.* 59:179–91
154. Yang Y, Hammes UZ, Taylor CG, Schachtman DP, Nielsen E. 2006. High-affinity auxin transport by the AUX1 influx carrier protein. *Curr. Biol.* 16:1123–27
155. Yang Z, Xia J, Hong J, Zhang C, Wei H, et al. 2022. Structural insights into auxin recognition and efflux by *Arabidopsis* PIN1. *Nature* 611–15
155a. Ying W, Wang Y, Wei H, Luo Y, Ma Q, et al. 2024. Structure and function of the *Arabidopsis* ABC transporter ABCB19 in brassinosteroid export. *Science* 383:eadj4591
156. Zhang J, Nodzynski T, Pencik A, Rolcik J, Friml J. 2010. PIN phosphorylation is sufficient to mediate PIN polarity and direct auxin transport. *PNAS* 107:918–22
157. Zhang XC, Zhao Y, Heng J, Jiang D. 2015. Energy coupling mechanisms of MFS transporters. *Protein Sci*. 24:1560–79
158. Zhang Y, Hartinger C, Wang X, Friml J. 2020. Directional auxin fluxes in plants by intramolecular domain–domain coevolution of PIN auxin transporters. *New Phytol.* 227:1406–16
159. Zhang Y, Rodriguez L, Li L, Zhang X, Friml J. 2020. Functional innovations of PIN auxin transporters mark crucial evolutionary transitions during rise of flowering plants. *Sci. Adv.* 6:eabc8895
160. Zhang Y, Xiao G, Wang X, Zhang X, Friml J. 2019. Evolution of fast root gravitropism in seed plants. *Nat. Comm.* 10:3480
161. Zhao C, Pratelli R, Yu S, Shelley B, Collakova E, Pilot G. 2021. Detailed characterization of the UMAMIT proteins provides insight into their evolution, amino acid transport properties, and role in the plant. *J. Exp. Bot.* 72:6400–17
162. Zourelidou M, Absmanner B, Weller B, Barbosa IC, Willige BC, et al. 2014. Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. *eLife* 3:e02860

155. Provides three structures for PIN1 and identifies the IAA pre-bound inward state.