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Minireview

The ABC of auxin transport: The role of p-glycoproteins in plant development

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Abstract **A surprising outcome of the *Arabidopsis* genome project was the annotation of a large number of sequences encoding members of the ABC transporter superfamily, including 22 genes encoding the p-glycoprotein (PGP) subfamily. As mammalian PGP orthologs are associated with multiple drug resistance, plant PGPs were initially presumed to function in detoxification, but were soon seen to have a developmental role. Here, we summarise recent studies of plant PGPs indicating that PGPs mediate the cellular and long-distance transport of the plant hormone auxin. One class of PGPs, represented by AtPGP1, catalyze auxin export, while another class with at least one member, AtPGP4, appears to function in auxin import. Current models on the physiological role of PGPs, their functional interaction and their involvement in cell-to cell (polar) auxin transport are discussed.**

Keywords: p-Glycoprotein; ABC transporter; Auxin; Multidrug resistance; Plant development

1. Plant p-glycoproteins

One of the most remarkable results of the inventory of the *Arabidopsis thaliana* genome [2,3] was the identification of a large number of putative ATP-binding cassette (ABC) transporter genes (>120 members) compared to the number (~50) seen in other multicellular organisms of comparable genome size [2–5]. A similar number of ABC transporter genes were identified in the *Oryza sativa* (rice) genome [5,27]. A rationale for ABC gene expansion in plants is the need for sophisticated detoxification processes in sessile organisms that employ versatile metabolic processes to adapt to complex environmental conditions [3,4]. A proportional increase in genes encoding homologs of the mammalian ABCB/multidrug resistance/p-glycoprotein (MDR or PGP) subfamily is observed in the two genomes [1,3–5]. In *Arabidopsis*, 22 PGP genes (21 transcribed genes, 1 pseudogene) have been identified [1] (Fig. 1), while only 10 ABCB/PGP isoforms (9 genes, one pseudogene) have been identified in humans [6]. In rice and *Arabidopsis*, the PGP subfamily is the largest group of full-size ABC transporters containing two nucleotide-binding folds (NBFs) [4,5,27], and is the second largest ABC transporter subfamily overall.

PGPs were first identified in mammalian cancer lines because their overexpression confers MDR to chemotherapeutic cancer treatments [6]. In non-cancerous mammalian tissues, with the exception of ABCB8, which is apparently localised to mitochondrial membranes, PGPs are localised to the plasma membrane where they are thought to function as detoxifying efflux pumps. However, MDR3/ABCB4 has been shown to catalyze the translocation of phospholipids and defects in ABCB11 result in altered cholesterol metabolism [6]. Interestingly, yeast has no PGPs, making it an ideal model organism for the characterisation of plant PGPs [8,9]. However, STE6 – which is responsible for the export of the mating pheromone a-factor – shares some weak homology with PGPs and can be partially complemented by mammalian PGPs [7]. Further, some plant and most mammalian PGPs cannot be functionally expressed in yeast, presumably due to excessive N-linked glycosylation [8,19].

As in other eukaryotic organisms, plant PGP genes encode predicted proteins of ~1250 amino acid residues with a molecular weight of 125–140 kDa [3–5] and containing similar halves each consisting of a transmembrane domain (TMD) and a nucleotide-binding fold (NBF) [1,4]. The two similar halves are connected by a linker domain of ~60 amino acids, which characteristically shows a strong divergence in different PGPs [4,5].

Phylogenetic analysis of *Arabidopsis* PGPs (Fig. 1) indicates the presence of three clusters [5,27] around prototypes AtPGP1 (clade I), AtPGP4 (clade II) and AtPGP8 (clade III). In clade III, for which no members have been characterised so far, five PGP genes (AtPGP15–18, AtPGP22) show signs of transpositional duplication (Richards and Murphy, unpublished) and are highly similar, while AtPGP8 seems to be more distant. In clade II, AtPGP7 and AtPGP9 are closely related but separate from five other isoforms that are closer to the clade II prototype AtPGP4. Such clustering is not found in clade I, where close couples AtPGP1/19, AtPGP2/10 and AtPGP13/14 deeply branch.

A comparison with PGPs of the monocotyledon *O. sativa* suggests phylogentic and functional convergences and differ-

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Abbreviations: PGP, p-glycoprotein; MDR, multidrug resistance; PAT, polar auxin transport; IAA, indole-3-acetic acid; NPA, 1-*N*-naphthylphthalamic acid; TWD1, TWISTED DWARF1

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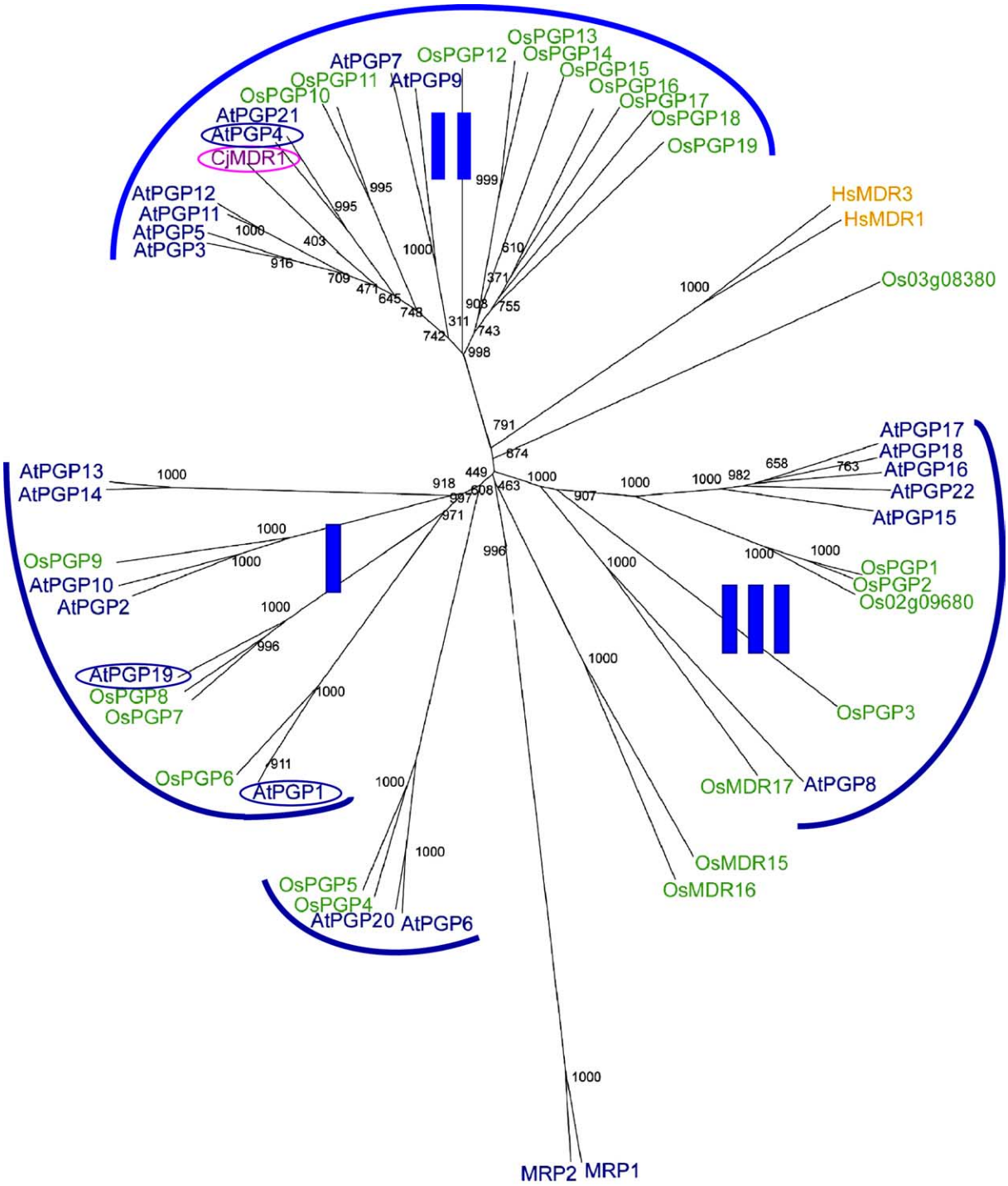

Fig. 1. Phylogenetic tree of *Arabidopsis* and rice p-glycoproteins. ClustalW version 1.83 was used to align full-length *A. thaliana* (At, blue) and *O. sativa* (Os, green) PGP amino acid sequences and subjected to phylogenetic analysis using ClustalW. The reliabilities of each branch point, as assessed by the analysis of 1000 bootstrap replicates, are given as bootstrap values. Accession numbers of rice and *Arabidopsis* PGPs can be taken from [41]. *Arabidopsis* AtMRP1 (At1g30400) and AtMRP2 (At2g34660), human (Hs, orange) MDR1 (ABCB1; P08183) and MDR3 (ABCB4; P21439) and *C. japonica* (Cj, pink) CjMDR1 (BAB63040) were added as out-groups. Plant PGPs that are characterised so far are circled.

ences. For all three *Arabidopsis* clades, rice PGPs were found that are apparently closer to *Arabidopsis* isoforms than to those present in rice. However, as with other ABC subfamilies [5], the number of orthologs between *Arabidopsis* and rice is low. Only 7 out of 24 rice isoforms clearly share significant homology to AtPGPs, among these are rice homologs of AtPGP1 and AtPGP19, AtPGP2 and AtPGP10, AtPGP6 and AtPGP20, and AtPGP8 (Fig. 1). Interestingly, as was the case for *Arabidopsis* PGPs, many of the rice PGPs of clades II and III (but not clade I) subcluster with themselves, suggesting that members of these subfamilies diverged by duplication after speciation events. Recent efforts to assign function to the

prototypical members of clade I and II, AtPGP1 and AtPGP4, indicate that at least some PGPs in these two clades function in plant developmental programs by directly transporting the plant hormone auxin.

To date, the detailed characterisation of only two *Arabidopsis* PGPs (AtPGP1 and AtPGP4) has been reported in the literature. From other plant species, potato *PMDR1*, wheat *TaMDR1*, barley *HvMDR2*, maize *ZmPGP1*/*BR2*, and *Coptis japonica CjMDR1* have been cloned and partially characterised [10,21,30,41,56]. CjMDR1 was identified as an alkaloid transporter in the medicinal plant *C. japonica* [30]. *PMDR1* cDNA was isolated in a screen of a potato expression library using calmodulin as probe, suggesting a direct Ca^{2+}/calmodulin interaction [10]. *TaMDR1* was isolated in an aluminium differential display screen, and its induction in planta by aluminium and calcium transport inhibitors was subsequently verified [56]. Only *ZmPGP1*/*BR2* from maize was identified as a gene involved in auxin transport, as *BR2* mutants are dwarves that exhibit reduced auxin transport [30].

2. Plant PGPs function in auxin transport

Auxin (primarily indole-3-acetic acid, IAA) is a hormone that plays a critical role in plant growth and development [11,12,45]. Although sufficiently cytotoxic at higher concentrations and therefore being evaluated as an anti-cancer therapeutic [13], IAA is essential to plant growth and is required for normal plant development. At an organismal level, auxin has been shown to be transported from cell to cell in a polar fashion [11,14] in a process thought to be motivated by the chemiosmotic gradient generated by plasma membrane H^+-ATPases. According to the current chemiosmotic model, polarised import and export of IAA is thought to be mediated by protein complexes that are characterised by members of the AUX1/LAX and PIN family, respectively [11,14,50]. In response, in part, to a lack of clear evidence of direct transport mediated by AUX1/LAX and PIN proteins, plant PGPs have recently been proposed as candidates for auxin transport [15,16].

AtPGP1 was the first plant PGP to be identified as part of an effort to isolate proteins that function in broad-spectrum herbicide resistance [17]. A link to auxin transport was suggested when hypocotyls of *AtPGP1* overexpression transformants were found to elongate under dim light in a manner similar to wild-type cells treated with low concentrations of auxin, and anti-sense lines exhibited reduced elongation similar to seedlings treated with auxin transport inhibitors like 1-*N*-naphthylphthalamic acid (NPA) [18]. Subsequently, interruption of the gene encoding AtPGP19/AtMDR1,[1] an auxin-inducible close homolog of AtPGP1, was found to result in partial dwarfism and reduced polar auxin transport (PAT) in hypocotyls and inflorescences [19]. Reductions in basipetal auxin transport were verified in *atpgp*1 and *atpgp19* hypocotyls [16] and *atpgp19* roots were shown to exhibit decreased NPA-sensitivity [52]. Auxin transport defects and dwarf phenotypes were also found to be more exaggerated in *Arabidopsis* double mutants, suggesting overlapping function [8,14,16]. The presence of severe growth phenotypes seen only in double mutants of these very closely related isoforms may explain why *Arabidopsis* PGPs did not show up in initial auxin-related mutant screens.

PGPs also function in monocot auxin transport. Mutations in PGP1 homologs of maize (*brachytic2*/*zmpgp1*) and sorghum (*dwarf3*/*sbpgp1*) result in reduced basipetal auxin transport and greatly reduced stalk height [21]. Mutations in genes encoding other PGPs in rice and maize have been identified (Richards and Murphy, unpublished), but have not been fully characterised. However, some monocot double mutants exhibit more severe growth phenotypes than single mutants, suggesting that overlapping function similar to that seen in *Arabidopsis* is also present in monocots.

Many light-regulated developmental processes, such as hypocotyl elongation, leaf expansion and phototrophic responses, are regulated by auxin [52]. PGP-mediation of hypocotyl elongation is light dependent [18,52] in a manner consistent with observed light dependence of NPA inhibition of hypocotyl growth [54]. AtPGP19 appears to acts downstream of the PhyA far-red photoreceptor, as elongation rates of *atpgp19 phyA* double mutant hypocotyls are similar to those of *phyA* mutants [52]. Destabilisation of PIN proteins observed in hypertropic *pgp19* mutants [20] and observation of similar PHOT1-dependent disruption of PIN protein localisation seen in dark-grown seedlings after blue light exposure suggest that PGPs may function in a photropin-regulated pathway as well [22]. As AtPGP19 was originally identified as a gene induced by the anion channel blocker NPPB [19], Ca^{2+}-modulated anion channels may function as signalling intermediates in the PGP-mediated blue light responses of *Arabidopsis* hypocotyls.

Further biochemical evidence for PGP function in auxin transport came when AtPGP1, AtPGP2, AtPGP4 and AtPGP19 were identified in high-affinity NPA-binding complexes, and specific NPA binding was found to be enhanced when *AtPGP19* was expressed in yeast [16,19,25,40,48]. NPA-binding complexes co-purified with two apparent GPI-anchored proteins (TWD1 and FAGP2) and exhibited detergent solubility characteristics consistent with localisation in sterol-enriched, detergent resistant microdomains (DRMs) [25,48,49]. As mammalian PGPs and GPI-anchored proteins have been shown to function in the maintenance of DRMs that are crucial for the formation of multi-protein complexes [49], a similar role has been suggested for PGPs in *Arabidopsis* [14]. Consistent with such a role, the PIN1 auxin efflux facilitator protein was subsequently shown to be destabilised from its normal polar localisation in xylem parenchyma cells of detergent-treated hypocotyls of *atpgp19* and *atpgp1 atpgpp19* mutants that exhibit hypertropic bending responses [20]. These results suggested that PGPs stabilised other components of membrane-localised transport complexes, but did not indicate whether PGPs directly transported auxin or modified membrane environments to enhance transport activity of other proteins.

2.1. AtPGP1 mediates direct auxin transport

Two mechanistic models have been proposed to explain PGP function: regulation of PIN proteins or direct transport of auxin [15]. In a recent study utilising whole plants, protoplasts, and heterologous expression systems, AtPGP1 was shown to directly catalyze primary active export of IAA, the synthetic auxin 1-NAA, and oxidative IAA breakdown products, but not the transport of common substrates of mamma-

[1] In order to aim a coherent nomenclature, we suggest using AtPGP19 instead of AtMDR1in the future.

lian PGPs [8]. AtPGP1-meditated transport was shown to be sensitive to auxin efflux and ABC transporter inhibitors.

Using a cmyc-tagged genomic construct, AtPGP1 was shown to exhibit non-polar expression in small, meristematic cells of the shoot and root apices where re-diffusion of auxin would inhibit directional auxin transport. Interestingly, in regions above the apex, AtPGP1 was found to be localised in a polar, predominantly basal [31] fashion (Fig. 2). This localisation was found to correspond well with both reduced auxin levels and Pro_{DR5}:GUS auxin reporter gene expression in *atpgp1* roots. As would be expected from the expression patterns and biochemical activity of AtPGP1, overexpression inverts root-shoot auxin ratios (Geisler, unpublished data).

When expressed in heterologous systems, PGP1-mediated auxin transport exhibited reduced auxin substrate specificity [8], suggesting that other factors contribute to the specificity of PAT seen in *planta*. Evidence that AtPGP1 that might function as ecto-phosphatase involved in multiherbicide tolerance [23,24] is consistent with a requirement for PGP interaction with other effectors in PAT.

Mutant phenotypes and reduced auxin transport observed in *atpgp19* suggest that AtPGP19 function is similar to that of AtPGP1. Mesophyll protoplasts from *atpgp19* export less IAA than *atpgp1* but export the artificial auxin 1-NAA at wild-type levels, suggesting comparable IAA but reduced 1-NAA affinity. Unfortunately, when expressed in yeast, AtPGP19 was not functional [19]. However, recent results indicate that AtPGP19 exhibits auxin transport activity and specificity similar to AtPGP1 when expressed in mammalian cell lines (Blakeslee, Lee, and Murphy, unpublished). Moreover, loss of *AtPGP19* function resulted in the greatest decrease in cellular IAA export from mesophyll protoplasts (*atpgp1 atpgp19* < *atpgp19* < *atpgp1* < wild-type) [8]. Taken together, these results suggest that AtPGP1 and AtPGP19 function as ATP-dependent hydrophobic anion carriers that are capable of exporting auxin in planta and when heterologously expressed.

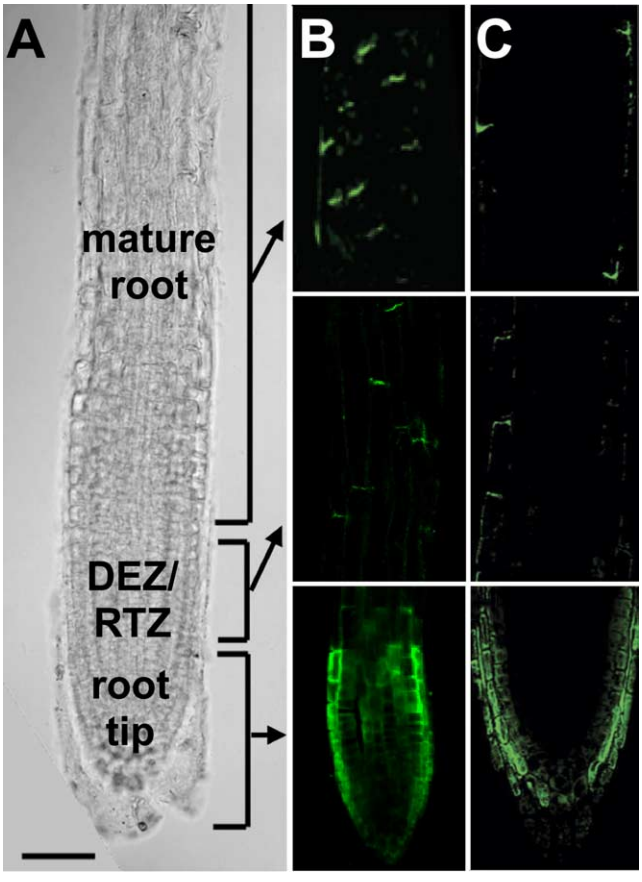

Fig. 2. Immunolocalisation of AtPGP1 and AtPGP4. Confocal images of immunohistochemical localisations of AtPGP1 (B) and AtPGP4 (C) in the root tip, the distal elongation zone/root transition zone (DEZ/RTZ) and the mature root. For better orientation, a DIC root image of a 5-d seedling is shown in A. Note the apolar location (see Ref. [31] for nomenclature) of AtPGP1 and AtPGP4 in the root tip, and the polar location in the RTZ (basal and apical for AtPGP1, basal for AtPGP4) and above (basal for AtPGP1 and apical for AtPGP4). Pictures were mainly taken from [8,25]. The bar corresponds to 100 μm.

2.2. AtPGP4 functions as auxin importer

AtPGP4 shares a 60% amino acid sequence identity with AtPGP1 and characterises phylogenetic clade II of *Arabidopsis* PGPs (Fig. 1). *AtPGP4* has been shown to function primarily in root development [9,25] and is expressed early in primary and lateral root development [9,53]. A role for AtPGP4 in auxin transport was suggested when *atpgp4* mutants were found to exhibit light-dependent alterations in lateral root number, reduced linear and gravitropic root growth, enhanced root hair formation, and decreased NPA growth inhibition [9,25,51]. *Atpgp4* mutants exhibit reduced basipetal root transport [25] and auxin influx [9,26] as well as altered free auxin levels [9,25]. Free IAA was found to be elevated in the root apex and the hypocotyl section from the mid-hypocotyl to the root-shoot junction [25]. Gene expression and immunolocalisation analysis indicated that AtPGP4 functions primarily in lateral root cap and epidermal cells [25]. Like AtPGP1, AtPGP4 exhibited apolar localisation in cells within the lateral root cap and polar localisation in epidermal cells above [25] (Fig. 2). Interestingly, AtPGP4 exhibits basal localisation in three stories of cells within the distal elongation zone and apical localisation in epidermal cells above (Fig. 2). These localisations are consistent with AtPGP4 function in basipetal redirection of auxin, as well as the growth phenotypes observed in *atpgp4* mutants. Analysis of auxin transport and contents in plants ectopically overexpressing AtPGP4 further suggests that AtPGP4 functions as an auxin uptake sink in the root cap [25]. The coincidence of AtPGP4 and AtPGP1 localisation at opposite ends of cells, even when that orientation is inverted in specific tissues suggests a pairing of both uptake and efflux activity mediated by AtPGP4 and AtPGP1.

Heterologous expression studies suggest that AtPGP4 functions in ATP-dependent influx. AtPGP4 confers hypersensitivity to IAA and the cytotoxic auxin analog 5-fluoroindole [29] in the hypersensitive *yap1-1* mutant [28] and wild-type, respectively [9]. Further, net IAA uptake was observed when AtPGP4 was expressed in the mammalian HeLa cell system, and this net uptake was reversed by NPA treatment [25]. Although PGPs are generally associated with substrate efflux, bacterial ABC transporters have been extensively shown to function as periplasmic uptake systems [28] while the product of the *CFTR* gene functions as a channel in both directions [29]. Moreover, AtPGP4 shares 71% amino acid sequence identity with CjMDR1 from the isoquinoline alkaloid producer *C. japonica* that functions as an ATP-dependent plasma membrane berberine importer [30].

Sequence comparisons between AtPGP4 and clade I PGPs indicate that AtPGP4 has a unique coiled-coil protein interactive domain at its N-terminus and exhibits substantial sequence divergence from AtPGP1 in the linker region adjoining the first nucleotide-binding fold [25]. Other functional differences between AtPGP1 and AtPGP4 are indicated by biochemical characterisations of the proteins [9,25]. While AtPGP1 and AtPGP4 are both effectively inhibited by auxin efflux inhibitors like NPA and flavonols [8,25,46,47], AtPGP4 seems to be less sensitive to inhibitors of ABC transporters such as cyclosporin A and verapamil [8,25]. Site-directed mutagenesis and domain swapping experiments are expected to elucidate the relative contributions of these sequence differences to AtPGP1 and AtPGP4 function.

3. Do AtPGP1 and AtPGP4 function in PAT?

Chemiosmotic models of polar auxin transport have been extensively corroborated by molecular biological and physiological evidence [14,45]. In these models, lipophilic uptake of IAA and directional, carrier-mediated efflux driven by H^+ gradients determine the direction of auxin movement. In some tissues, accelerated uptake of anionic IAA is augmented by a biochemically characterised H^+ symport activity [55]. Analysis of mutant phenotypes, predicted protein topology, gene expression patterns, and subcellular protein localisations in *Arabidopsis* suggest that AUX1/LAX- and PIN family proteins characterise the respective uptake and efflux complexes, but direct biochemical verification of their function has been elusive [11,12,14,30].

If the same analysis is applied to the currently characterised *Arabidopsis* PGPs, these proteins must also qualify as valid auxin transporters. Reduction in basipetal auxin transport and free auxin levels in 5d old *atpgp1* roots, as well as both polar and apolar plasma membrane localisations of AtPGP1, are all consistent with current PAT models [8]. The same is true of defects in auxin transport, gravitropism and root development described for *atpgp19* [20] and *atpgp4* [9,25].

PGPs may fulfill an additional requirement for active import of auxin in some cells. At the apoplastic root pH of 5.5, only 16% of the IAA ($pK_a = 4.75$) present is protonated and able to diffuse over the plasma membrane [14]. Such an ATP-dependent uptake mechanism for anionic IAA might be expected to function in tissues where auxin is taken up rapidly, as is the case with epidermal cells that are more responsive to auxin-induced elongation. As is the case with efflux mediated by AtPGP1 and AtPGP19, other PGPs may mediate tissue-specific auxin uptake. So far, AtPGP21, a close homolog of AtPGP4, has not been characterised. Overlapping function of AtPGP4 and AtPGP21 would be indicated if *atpgp4 atpgp21* mutants show a more severe agravitrope phenotype similar to that seen in *aux1* [14,30].

The critical question that emerges is whether PGPs, AUX1/LAX, and PIN proteins function independently or cooperatively in auxin transport. Because auxin uptake proteins can be theoretically by-passed by lipophilic movement, but efflux carriers cannot, the cellular regulation of auxin efflux makes a greater contribution to PAT than influx [14]. In a complementary model of PGP, AUX1/LAX, and PIN action (Fig. 3A), auxin would enter the cell via diffusion or AUX1- or AtPGP4-homolog mediated import. Auxin then exits the cells via an efflux complex that is characterised by PIN proteins [32]. As has been described in detail recently [15], this model works in more mature tissues, in which cells are longer and re-diffusion is not a limiting factor. In meristematic tissues, where cells are smaller and auxin concentrations are higher, mathematical models predict that re-diffusion would inhibit vectorial auxin movement [33]. In these smaller cells, asymmetrically distributed transporters, like PGPs, could be essential to polar auxin movement [9,15].

It has been suggested that PIN–PGP pairings provide specificity and directionality to PAT [15]. In an additive model, PGPs and AUX1/LAX and PIN proteins, respectively, would function independently in the same cells (Fig. 3B), while in a synergistic model (Fig. 3C), individual components would co-function as import and efflux complexes. *AtPIN1* expression patterns partially overlap those of both *PGP1* and *PGP19*, while *AtPIN2* expression overlaps with *PGP1* and *PGP4* in some tissues; however, PIN–PGP interaction awaits molecular confirmation [15]. Comparative analysis of developmental phenotypes of *pin* and *pgp* mutants suggests that both PINs and PGPs function as transporters or transport activators in some way, with PIN proteins providing a basal transport vector and PGPs providing increased cellular loading and unloading.

However, the flexibility and directionality of auxin transport processes suggests that they depend on the continuous actin-dependent, endocytotic cycling of efflux and uptake complexes [11,12]. Mammalian PGPs are trafficked by similar mechanisms [14], and the co-purification of plant homologs of PGP-associated membrane trafficking proteins (i.e., APM1, β-adaptin, ADL1a/DRP1) in NPA-binding complexes from *Arabidopsis* suggest that plant PGPs may undergo similar cycling. However, a plant synaptic model suggests that PIN proteins reach their polar locations as a consequence but not as prerequisite of polar fusion of endocytotic vesicles [45]. In such a model, PGPs would most likely function as primary auxin transporters and PIN proteins as vectorial trafficking regulators.

4. Regulation of PGP-mediated auxin transport

Mammalian PGP activity is regulated by protein kinase A and C-dependent phosphorylation of the linker domain adjoining the first nucleotide-binding fold [6,34]. In human cells, protein kinase C (PKC) inhibitors reduce PGP phosphorylation and increase drug accumulation in a clinically relevant fashion during cancer treatment [35], while phorbole esters known to stimulate PKC activity have an opposite effect [35]. Mutational studies of putative PGP phosphorylation sites have been useful, but have not elucidated the mechanistic impacts of PKC phosphorylation on PGP-mediated drug transport [6]. Protein phosphorylation has been shown to play a role in the regulation of auxin transport [37], and recent phosphoproteomic data [36] suggest a direct link between auxin efflux and PGP phosphorylation status.

Flavonols such as quercetin and kaempferol are endogenous plant compounds that are widely studied for their activity as antioxidants and kinase inhibitors [39]. Flavonols appear to compete with NPA for identical sites as they reduce NPA binding to membrane protein complexes and antagonise NPA

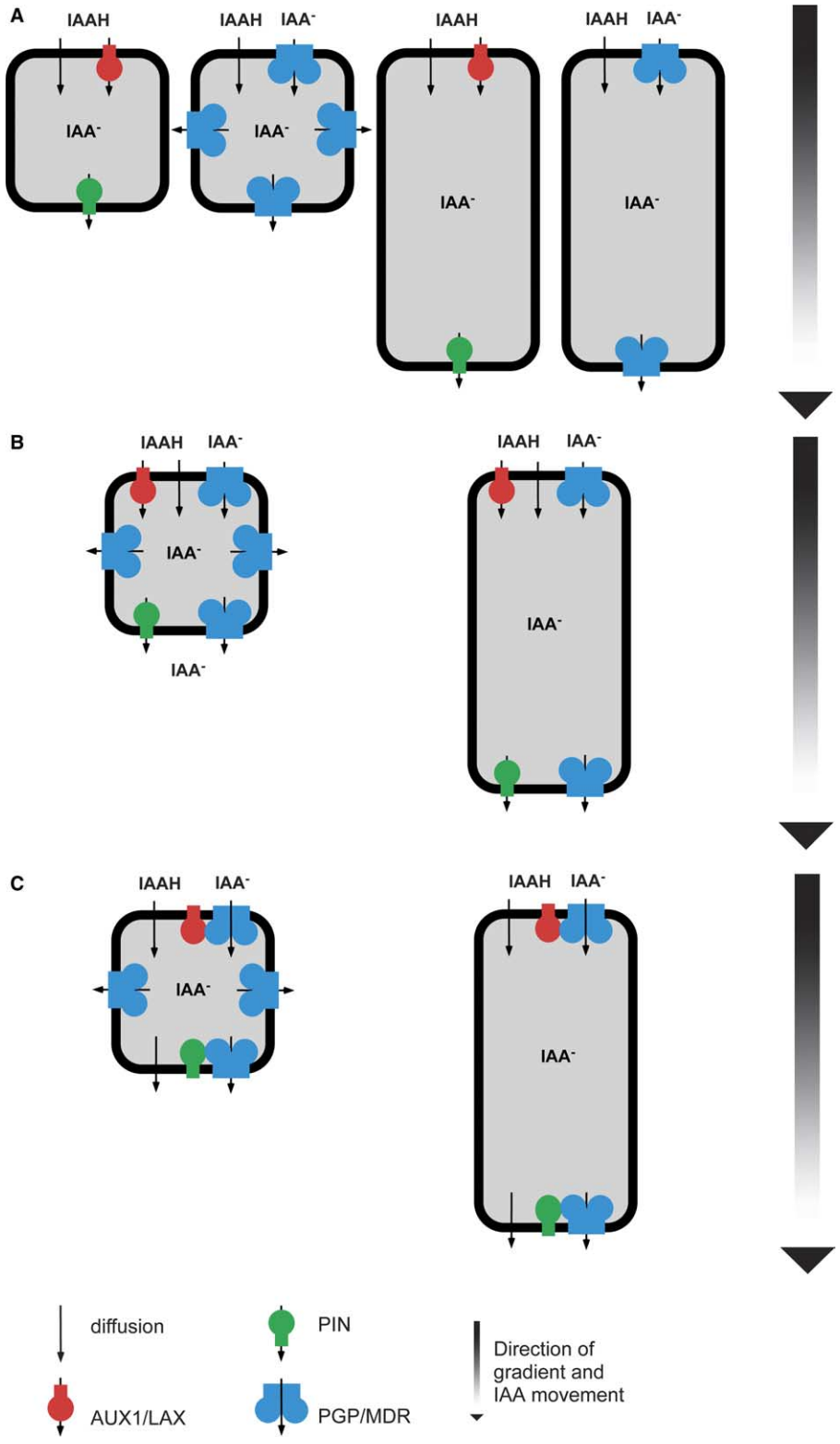

Fig. 3. Model of PGP-, PIN- and AUX1/LAX-mediated auxin transport. In a complementary model of PGP and AUX1/LAX and PIN action (A), auxin would enter the cell via diffusion or AUX1- or AtPGP4-like mediated import. Auxin then exits the cells via an efflux complex that is characterised by PIN proteins. This model works in mature tissues, in which cells are longer and re-diffusion is not a limiting factor. In meristematic tissues, where cells are smaller and auxin concentrations are higher, re-diffusion would inhibit PAT (see text for details). Therefore, in these smaller cells asymmetric distributed transporters, like PGPs, have been postulated to play an essential role in PAT. In an additive model (B), PGPs, and AUX1/LAX and PIN proteins, respectively, would function independently in the same cells. While in a synergistic model (C), individual components would co-function as import and efflux complexes. PIN–PGP pairings have been suggested to provide specificity and directionality to PAT [15] that would favor a synergistic mode of action compared to the additive model, which suggest the function of two independent efflux systems.

effects on auxin accumulation [38,48]. It has therefore been proposed that flavonols function as endogenous regulators of auxin export [38,46,47]. In apical tissues, kaempferol and quercetin negatively regulate auxin efflux, and *transparent testa* mutants, with decreased flavonoid production exhibit increased auxin transport and auxin-related phenotypes [39]. Interestingly, AtPGP1 and AtPGP4 have been shown to bind both NPA and flavonols, and their activity to be inhibited by both NPA and flavonols [8,25]. Moreover, quercetin aggregations have been shown to accumulate near the root tip when auxin concentrations increase. Similar aggregations were found in *atpgp4* roots [25].

Flavonols are widely utilised as inhibitors of mammalian PGPs in both experimental and clinical environments [43,44], suggesting that this mode of regulation has evolved early in evolution. Quercetin and kaempferol have been shown to act at the catalytic and regulatory phosphorylation sites, respectively, of human PGP1, suggesting specific modes of regulation for flavonols species in planta.

Recent work suggests that AtPGP1-mediated auxin efflux is regulated in part via protein–protein interactions [16]. The carboxy terminus of AtPGP1 was identified in a yeast two-hybrid screen as an interactor with the immunophilin-like protein TWISTED DWARF1 (TWD1) [16]. Mutations in *TWD1* result in severe developmental phenotypes, some of which are strikingly similar to those of *pgp1 pgp19* double mutants [16]. *Twd1* and *pgp1 pgp19* mutants exhibit similarly reduced auxin transport rates, elevated auxin levels and both reveal alterations in gravitropism (Bouchard and Geisler, unpublished data). AtPGP1/19-TWD1 interactions were verified using in vitro pull downs, co-immunoprecipitation and NPA- and TWD1-affinity chromatography [16]. Assays of auxin transport in heterologous systems co-expressing TWD1 and AtPGP1 indicate that TWD1 modulates the auxin transport activities of AtPGP1, while other related *Arabidopsis* FKBPs had no significant effect [41].

5. Conclusions

The year 2005 was a break-through year in auxin research. In addition to the identification of the TIR1/AFB family of auxin receptors [42], heterologous expression studies and mutant analysis have clearly demonstrated that AtPGPs function as primary active, catalytic components of auxin transport complexes. Surprisingly, individual members of phylogenetically distant PGP clades have been shown to catalyze auxin and import, respectively. Re-examination of the basic assumptions underlying chemiosmotic models of PAT PGP transport activity indicates that the PGP activity and *pgp* mutant phenotypes are consistent with and complement current models.

As is always the case, these discoveries open more new questions than they have answered. For example, it is not clear whether all 21 expressed *Arabidopsis* PGPs function as auxin transporters. Further, it is not clear whether or not members of clade II other than AtPGP4 function as auxin importers and little work has been done on the characterisation of the members of clade III. These questions can only be addressed by consequently analyzing and characterising the other members of the AtPGP family in *Arabidopsis*. An essential part of this process will be the construction of multiple knock-out mutants of closely related AtPGPs.

It is also still not clear how PGPs interact with PINs and AUX1/LAX proteins. *atpgp* mutant phenotypes are different from those of *pin* mutants and lack the severity of gravitropism defects seen in *aux1*. As has been seen with *pin* mutants, functional redundancy of closely related AtPGP isoforms complicates phenotypic analysis, as double *atpgp* mutants exhibit more severe phenotypes. However, severe defects in organismal polarity seen in double and triple *pin* mutants [12] have not yet been observed in *atpgp* mutants. This suggests that PGPs might contribute more to long-distance auxin transport and loading into transport streams than to the basal levels of vectorial auxin transport necessary for development. Biochemical elucidation of AUX1/LAX and PIN function and investigation of the suggested PIN–PGP pairings in tissue-specific auxin streams are required to determine the respective and combine contributions of these proteins to plant development.

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