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Review

Spatiotemporal asymmetric auxin distribution: a means to coordinate plant development

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Abstract. The plant hormone auxin plays crucial roles in regulating plant growth development, including embryo and root patterning, organ formation, vascular tissue differentiation and growth responses to environmental stimuli. Asymmetric auxin distribution patterns have been observed within tissues, and these so-called auxin gradients change dynamically during different developmental processes. Most auxin is synthesized in the shoot and distributed directionally throughout the plant. This polar auxin transport is mediated by auxin influx and efflux facilitators, whose subcellular polar localizations guide the direction of auxin flow. The polar localization of PIN auxin efflux carriers changes in response to developmental and external cues in order to channel auxin flow in a regulated manner for organized growth. Auxin itself modulates the expression and subcellular localization of PIN proteins, contributing to a complex pattern of feedback regulation. Here we review the available information mainly from studies of a model plant, *Arabidopsis thaliana*, on the generation of auxin gradients, the regulation of polar auxin transport and further downstream cellular events.

Keywords. Indoleacetic acid, auxin transport, polar protein targeting, patterning, tropism, *Arabidopsis*.

Introduction

Auxin is a prominent plant hormone that plays critical roles in plant growth and development, including regeneration and adaptation responses. While the most common naturally occurring auxin is indole-3-acetic acid (IAA), other species include indole-3-butyric acid (IBA) and 4-chloroindole-3-acetic acid (4-Cl-IAA) [1]. Functionally analogous, synthetic auxins such as 1-naphthylacetic acid (1-NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) are metabolically more stable and, thus, they are more widely used in agricultural applications and biological studies. Interestingly, the exogenous application of each auxin species leads to a distinct but partially overlapping spectrum of effects [1]. This is possibly due to the specific transport properties for each type of auxin, and differences in tissue permeability and metabolic stability.

IAA is structurally related to and biochemically derived from tryptophan (Trp). However, Trp is not absolutely necessary for the synthesis of IAA, and several branching pathways, both dependent or independent of Trp, have been proposed for auxin biosynthesis [1,2]. As these biosynthetic pathways are redundant and show strong species- and tissue-specific differences, the contribution of each pathway is unclear. In addition, IAA can be inactivated or stored via conjugation to amino acids, sugars and peptides [1, 2]. Enhanced production of auxin *in planta* by the overexpression of bacterial [3, 4] or endogenous plant [5, 6] genes for auxin metabolism resulted in phenotypes similar to those induced by the exogenous application of auxin, indicating that the regulation of auxin biosynthesis and metabolism is important for plant growth and development.

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In addition, the intercellular transport of auxin has been shown to be crucial for most of the known effects of auxin. It is increasingly clear that polar auxin transport (PAT) is an important mechanism in plants, whereby auxin is channeled into specific plant tissues in order to trigger or maintain certain developmental responses. The effect of auxin overproduction or degradation, at least in some tissues such as embryonic cotyledons and root tips in *Arabidopsis thaliana*, can, to a large extent, be compensated by PAT [7]. Thus, the overall distribution of auxin *in planta* is determined by the net effects of auxin biosynthesis, metabolism and, by its directional transport. In this review, we discuss recent advances in the understanding of auxin distribution, its regulation by upstream elements and the induced downstream signaling cascades that together lead to diverse developmental responses.

Local auxin distribution patterns

The asymmetric distribution of auxin across plant tissues accompanies and mediates developmental responses from the earliest changes in embryo shape to large tropic responses in adult plants. However, the discovery of the importance of these auxin gradients first relied on the careful documentation of auxin distribution. A variety of methods have been successfully used, including immunolocalization with an anti-IAA antibody [8], direct measurements of endogenous auxin [9–12], by monitoring radioactively labeled auxin [13–16] and observation of the activity of auxin-responsive marker genes (e.g. synthetic promoter *DR5*; [10, 17–20]). Finding the consensus sequence TGTCTC (auxin response element, AuxRE) within the promoter regions of genes that are rapidly upregulated by auxin [21] (reviewed by [22]) was an important breakthrough for testing auxin distribution *in planta* with cellular resolution. The most widely used synthetic promoter, which is made up of multiple AuxRE repeats, is derived from the promoter of a primary auxin response gene from soybean, *GH3*, and is termed DR5 or DR5rev (another variant with inverse repeats). DR5 promoters are highly responsive to active auxins, and, therefore, the promoter activity can be used as an indirect indicator of endogenous auxin levels. The main advantage over natural auxin-inducible promoters is the lack of additional endogenous promoter elements that could potentially confer tissue-specific regulation. Expression of DR5 can be induced by exogenously applied auxin (e.g. 2,4-D) [8, 17, 20], and the DR5 pattern is altered by inhibition of PAT [8, 17–20]. Importantly, the activity of DR5 correlates well with the results obtained by direct measurements of auxin content within wild-type *Arabidopsis* roots [23]. Furthermore, the accumulation pattern of auxin, as inferred from anti-IAA immunolocalization patterns, reflects the pattern of DR5 activity [8]. Thus, despite limitations for the use of such synthetic promoters, including dependence on induction through a transcription pathway and a limited threshold of detection, it seems that DR5 activity can be used as a reasonable measure for auxin accumulation patterns, at least in embryos and root meristem. Nevertheless, more direct measurements of auxin *in vivo* (e.g. real-time monitoring using fluorescence resonance energy transfer (FRET)-based reporters [24]) would be an important subject for future study in this field. In the following sections of this review, auxin distribution patterns in *Arabidopsis thaliana*, inferred from the pattern of DR5 expression and detection by anti-IAA antibodies, are discussed.

Auxin distribution during embryogenesis

Embryogenesis is a fundamental process in plant development, in which the basic body plan, including apical-basal axis, radial and bilateral symmetries, as well as apical root and shoot meristems are established. Interestingly, auxin distribution dynamically changes at key steps of embryo development [20]. After fertilization, the single-celled zygote divides to form an apical cell, which develops into the embryo proper, and a basal cell, which develops into the suspensor. DR5 activity is higher in the apical cell and its daughter cells until the 32-cell-embryo stage. At later stages an inverse pattern of auxin maxima is observed [20] (Fig. 1a), whereby the highest activity is found in the uppermost suspensor cells, including the precursor to the root meristem – the hypophysis. Later still, DR5 activity is detected in cotyledon apices and in provascular cells. This pattern of auxin response maxima mirrors the dynamic IAA accumulation pattern verified by immunolocalization with an anti-IAA antibody [8, 20] and is abolished either by pharmacological or genetic interference with PAT [20], indicating that proper PAT underpins the generation of auxin asymmetry during embryogenesis.

Auxin distribution during post-embryonic organ formation

During post-embryonic development, the local application of auxin is sufficient to trigger leaf or flower formation in the shoot apex [25] or lateral root initiation [26], suggesting that auxin plays a crucial role in organ initiation. Under normal conditions, places of increased auxin levels also correlate with the initiation sites of organ primordia in both root and shoot [8, 27].

In the primary root of *Arabidopsis thaliana*, a DR5 maximum is detected in columella initials and in the quiescent center (QC), with lesser activity in the mature columella root cap [17] (Fig. 1b). DR5 activity decreases gradually as the cells in the root differentiate and are displaced into maturing root tissues. However, the activity increases

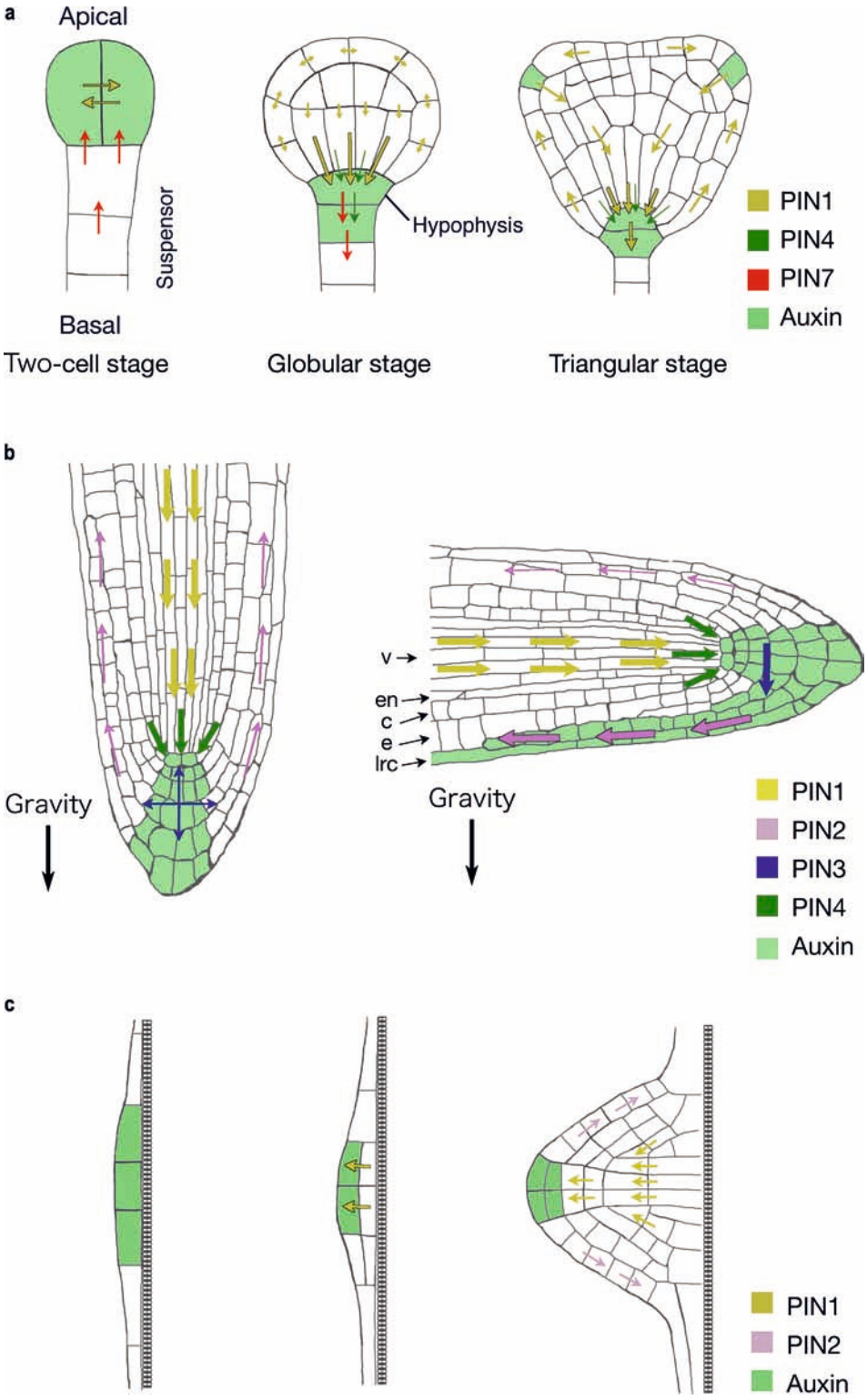

Figure 1. Schematic representations of PIN-mediated polar auxin transport. (*a*) Three subsequent stages of plant embryo development from the two-cell stage to the triangular stage, each of which exhibits distinct localizations of PIN proteins. (*b*) PIN-mediated polar auxin transport in the mature root and for gravitropic response. Auxin is transported in a radially symmetrical manner when the root grows along gravity vector (left). After a change in the gravity vector, auxin redistributes asymmetrically, allocating more to the lower side (right). (*c*) PIN-mediated polar auxin transport during lateral root primordial development. The polarity of PIN proteins and presumptive directions of auxin flow are indicated by arrows. The place of auxin accumulation is highlighted in green. lrc, lateral root cap; e, epidermis; c, cortex; en, endodermis; v, vascular bundle.

again in the presumptive founder cells of lateral root primordia in the pericycle [8]. Upon formation of initial cells by anticlinal cell divisions, DR5 activity is observed in all these cells. After periclinal divisions have occured to form inner and outer cells, the DR5 activity pattern gradually changes and is confined to central cells in both cell layers [8]. Later, a gradient with its highest activity at the tip is established (Fig. 1c).

The shoot apical meristem generates successive lateral organs in a predictable phyllotactic pattern. The site-specific microapplication of auxin can induce the generation of primordia in peripheral zone tissue closest to the site of application, suggesting that auxin can regulate the position and emergence of organ primordia in the shoot apical meristem [25]. Interestingly, under normal conditions, DR5 activity is detected in incipient primordia before it starts to bulge (e.g. position I_2), and then again later in young primordia at their apices [8, 27]. Similarly, DR5 activity is detected at the tips of developing floral organs [8]. Thus, despite the large morphological variety of lateral organs, a similar pattern of auxin distribution with its apex-localized maxima appears to underlie primordium development in various organs.

Once established, organs gradually become connected to the preexisting vascular tissue by newly formed vascular strands. In large organs such as leaves, a dynamic but typical pattern of vascular strands emerges ensuring an efficient exchange of nutrients, and assimilates throughout the organ. Interestingly, when vascular strands are disrupted by wounding, new vascular tissue can form, a process which can be also initiated by the local application of auxin [28]. In all tested instances, an increase in DR5 activity precedes and accompanies the formation of new vascular strands, thus suggesting that auxin may also play a role in the differentiation of vascular tissue [8, 29].

Auxin distribution during tropisms

Plants can change the direction of their growth according to the vectorial input of light (phototropism), gravity (gravitropism) or other signals (hydrotropism, thigmotropism). More than hundred years ago, Darwin's classical experiments on the phototropism of grass coleoptiles showed that while light is perceived at the tip, the differential elongation response occurs in the tissues spatially separated below. This indicated that there could be a mobile signal that regulates cell activity [30]. These observations eventually led to the emergence of the Cholodny-Went theory, which predicts that organ bending results from differential growth facilitated by the asymmetric distribution of auxin (summarized in [31]). Indeed, increased auxin levels were detected in various plant organs, including gravity-stimulated tobacco shoots (at the lower side) [32], light-stimulated maize coleoptiles (at the shaded side) [11], light- and gravity-stimulated *Arabidopsis* and *Brassica* hypocotyls [12, 18], and gravity-stimulated *Arabidopsis* roots (at the lower side) [19, 33, 34]. Auxin transport inhibitors interfere with both asymmetric auxin distribution and tropic growth, suggesting that PAT underlies plant tropism [18, 35]. In support of this, various mutants in auxin influx and efflux facilitators show defects in photo- and gravitropism [18, 36, 37].

Upstream of local auxin distribution: polar auxin transport

Genetic and pharmacological studies collectively suggest that asymmetric auxin distribution results mainly from intercellular auxin transport. Auxin is unique among plant hormones in that it is transported in a polar manner through the plant tissues. Experiments using radioactively labeled auxin showed that the translocation of auxin involves two physiologically distinct pathways: first, auxin is translocated rapidly with the mass flow of other metabolites in mature phloem, and second, auxin is transported from the shoot apex by a much slower, carrier-dependent, cell-to-cell polar transport (for overview, see [38]). PAT is specific for auxins and is predominantly unidirectional, running from the apical aerial tissues through vascular tissues towards the root tip. In the root apex, some of this auxin is then transported away from the tip through the outer lateral root cap and epidermis tissues [13, 16]. Once in the PAT system, two distinct carrier mechanisms control cellular influx and efflux separately. Both influx and efflux have been independently demonstrated in single plant cells and tissue segments [39–41]. In comparison to IAA, the other major naturally occurring auxin, IBA, is also transported in a polar manner, which can be uncoupled from polar transport of IAA [2]. It is interesting that the synthetic analogue, 1-NAA, while being an excellent substrate for cellular efflux, is only poorly actively transported by influx. The lack of influx is compensated by the passive diffusion of 1-NAA into cells (see next sections). On the other hand, another synthetic auxin, 2,4-D, is transported by influx but only poorly by efflux, resulting in its retention within cells [41]. The combination of greater metabolic stability and cellular retention probably accounts for the greater ability of 2,4-D to affect plant growth.

Cellular influx and efflux of auxin has also been discriminated pharmacologically using specific inhibitors for each pathway. Auxin efflux inhibitors [42] such as 1-N-naphthylphthalamic acid (NPA) and 2,3,5- triiodobenzoic acid (TIBA) have been used for more than half a century to study auxin-dependent plant development. The use of auxin efflux inhibitors has facilitated the physiological characterization of PAT and has established that auxin efflux is important for many aspects of plant development. Exogenously applied auxin efflux inhibitors

affect a number of processes, such as embryonic axis formation [43, 44], the initiation of lateral roots [23] and aerial organs [14, 25], root meristem patterning [17, 45, 46], vascular patterning [47], hypocotyl and root elongation in light [48], apical hook formation [35] and tropic responses [32, 42, 49]. On the other hand, inhibitors of auxin influx such as 1-naphthoxyacetic acid (1-NOA) and 3-chloro-4-hydroxyphenylacetic acid (CHPAA) were discovered only recently [50]. These auxin influx inhibitors have been used to demonstrate the roles of auxin influx in root gravitropism [51] and in correct leaf positioning [52]. Thus, cellular influx and efflux contribute to PAT-driven auxin distribution and are critical for many plant developmental processes.

Chemiosmotic hypothesis

Based on the chemical characteristics of auxin and the physiological nature of PAT, the chemiosmotic hypothesis was formulated to explain the cellular mechanism of auxin transport (for overview, see [38]). According to the model, plasma membrane-localized proton pumps generate a proton gradient across the plasma membrane. As some IAA in the acidic environment of the apoplast (e.g. approximately 16% at pH 5.5) exists in the protonated, hydrophobic form (IAAH), it is able to pass passively through the plasma membrane. However, as the cytosol is more basic, the majority of auxin there (e.g. more than 99% at pH 7.0) exists in the dissociated form (IAA^- anion), and is unable to pass through the plasma membrane by a simple diffusion. As a result, IAA becomes trapped within cells and can leave only by the action of efflux carriers. The directionality of auxin transport was explained by assuming that auxin efflux carriers are asymmetrically localized. While not accounted for in the original model, physiological experiments suggest that the cellular influx of auxin also participates in PAT [39, 41], presumably by increasing the efficiency of auxin transfer and/or directionality.

In support of the chemiosmotic hypothesis, Li and colleagues recently demonstrated that transgenic *Arabidopsis* plants overexpressing the H^+-pyrophosphatase *AVP1* (for *Arabidopsis* vacuolar pyrophosphatase) have an decreased apoplastic pH and exhibit an increased basipetal transport of IAA [53]. Conversely, in the *avp1* loss-of-function mutant, the apoplastic pH was more alkaline than in wild-type plants, and basipetal IAA transport was reduced [53]. These studies provide an important molecular support for the chemiosmotic hypothesis.

Molecular components

The chemiosmotic hypothesis proposed the existence of auxin carrier proteins. Mainly through molecular genetics using *Arabidopsis thaliana*, several classes of genes required for auxin influx, efflux and/or PAT have been identified.

AUX1

The *AUXIN1* (*AUX1*) gene encodes a transmembrane protein sharing significant similarity with plant amino acid permeases and was proposed to be involved in the influx of tryptophan-like IAA [37]. Plants mutant for *aux1* exhibit two major auxin-related phenotypes: resistance to 2,4-D-mediated inhibition of root growth, and agravitropic root growth. Several lines of evidence support the role of AUX1 in auxin influx in the root: First, resistance to the inhibition of root elongation by auxin is not observed with the membrane permeable auxin 1-NAA [49]. Similarly, the agravitropic root phenotype of *aux1* mutants can be rescued by exogenously applied 1-NAA [49, 54]. The restoration of gravitropism by 1-NAA seems to correlate with the recovery of basipetal auxin transport [55], which is defective in *aux1* [56]. Second, *aux1* roots incorporate approximately twofold less labeled 2,4-D than wild-type roots [49]. Third, auxin influx inhibitors, 1-NOA and CHPAA, phenocopy the root phenotype of *aux1* [51]. Recently, experiments that included the heterologous expression of *AUX1* in *Xenopus* oocytes directly demonstrated that AUX1 functions as a high-affinity, specific auxin uptake carrier [57]. AUX1 is expressed in various tissues, including protophloem cells, root columella cells, the lateral root cap and the shoot apical meristem [55, 58]. The *aux1* mutant root tip contains less auxin compared with wild-type, indicating that auxin supply to the root apex is defective, despite normal *AUX1* expression being limited to the root tip [55]. These data suggest that AUX1 could be involved in the loading of auxin into the root tip from the phloem [55], providing a potential mechanism for the connection between phloem-based, non-polar auxin transport and PAT pathways, which was previously demonstrated physiologically [59]. In the *Arabidopsis* genome, several *Like-AUX1* (*LAX*) genes were identified, but their functional characterization has not yet been established [60].

PIN proteins

The function of the *PIN1* gene in auxin efflux and transport was primarily surmised from the phenotypic analysis of *Arabidopsis pin-formed1* (*pin1*) mutants. The *pin1* mutants are progressively defective in organ initiation and phyllotaxy, which, in the end, results in pin-shaped inflorescence meristems devoid of flowers [14]. Similar defects can be phenocopied by culturing wild-type plants in the presence of auxin efflux inhibitors, and it was shown that PAT is dramatically reduced in *pin1* stem segments [14]. The *PIN1* gene encodes a protein with 10–12 putative transmembrane domains sharing a limited similarity to a group of bacterial transporters [61]. Mutations in a homologous gene *PIN2* (concurrently published as, *ETH-*

YLENE INSENSITIVE ROOT 1 and *AGRAVITROPIC 1*) cause a defect in root gravitropism [15, 36, 62, 63]. The other homologues, *PIN3*, *PIN4* and *PIN7*, are required for tropism, root meristem patterning and patterning in early embryogenesis, respectively [10, 18, 20]. The *Arabidopsis PIN* gene family consists of eight members in total. The functions of the remaining three members, *PIN5, 6, 8*, remain unknown.

Remarkably, all the reported phenotypes of *pin* mutants can be mimicked by auxin efflux inhibitors, suggesting that PIN proteins are involved in some aspect of auxin efflux. The *pin1* and *pin2* phenotypes are associated with reduction of PAT in the inflorescence stem and root, respectively [14, 16]. In addition, examination of auxin distribution based on DR5 activity and/or by direct measurement has revealed that the loss of *PIN2, PIN4* or *PIN7* activity affects local auxin distributions [10, 20, 36]. Furthermore, when *PIN2* was overexpressed in yeast, it retained less radioactively labeled auxin than control yeast and showed enhanced resistance to the cytotoxic IAA analogue 5-fluoro-indole [15, 36]. Recently presented experiments based on the heterologous expression of PINs in tobacco, mammalian and yeast cultured cells provide strong evidence for a direct role for PIN proteins in auxin efflux [64]. In addition, manipulation with PIN polar localization showed that, at least in meristematic tissues and for short-range transport, the polarity of PIN localization determines the direction of auxin flow [65]. Thus, a network of distinctly expressed and polarly localized PIN proteins provides a molecular basis for PAT in different parts of the plant [10, 18, 20, 61, 62] (Fig. 2).

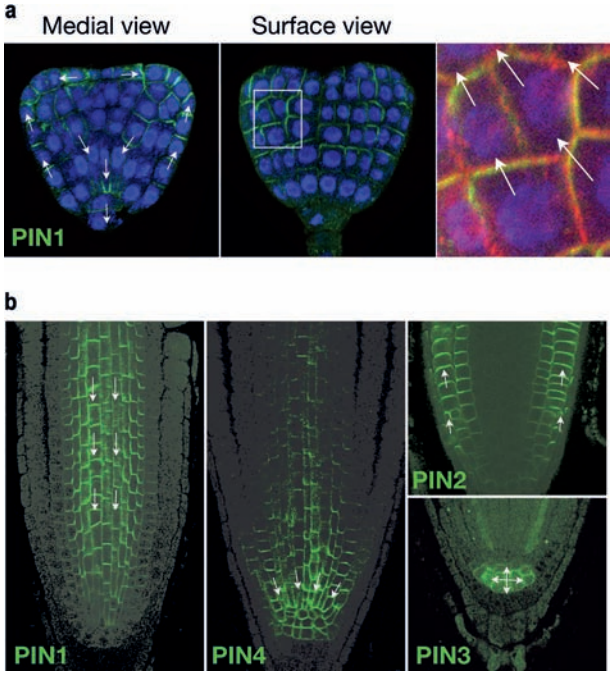

Figure 2. Polar localization of PIN proteins in *Arabidopsis* tissues. (*a*) PIN1 localization in the triangular-stage embryo. PIN1 (green) localizes basally in inner cells in a medial optical section (left) and points toward the tips of cotyledons in outermost cells (middle, right). (*b*) Localization of various PINs in the plant root. Arrows indicate the presumptive direction of auxin flow.

MDR/PGP subfamily of ABC transporters

Besides AUX1 and PIN protein families, members of the ATP-binding cassette (ABC) protein superfamily have been suggested to play a role in cellular efflux and influx of auxin. Members of the multi-drug-resistant/P-glycoprotein (MDR/PGP) subfamily, AtMDR1 (also know as AtPGP19), AtPGP1, AtPGP2, AtPGP4 and AtPGP10, have been isolated as proteins binding to the auxin transport inhibitor NPA [66, 67]. Loss-of-function mutations in several *PGP* genes cause diverse developmental defects, which can be interpreted as a consequence of altered auxin signaling or transport, but in most cases cannot be mimicked by treatment with auxin transport inhibitors. In addition, some of these mutants show aberrant auxin transport, including defective basipetal auxin transport in *pgp1, pgp4* and *pgp19* roots, reduced net efflux from *pgp1* and *pgp19* leaf protoplasts, and reduced auxin uptake into the *pgp4* root tip [67–69]. Furthermore, heterologous expression of PGP1 in HeLa cells and yeast results in the cellular export of auxin and the toxic IAA analogue, 5-fluoro-indol [68]. Conversely, expression of PGP4 in HeLa cells and yeast mediates cellular import of auxin and 5-fluoro-indol [67, 69]. PGP1 and PGP4 proteins are uniformly distributed at the surface of root apex cells with asymmetric localization in some other tissues, including the endodermal and cortex cells of the root elongation zone [67, 68]. However, the role of this polar PGP localization for directional auxin movement remains to be addressed. In summary, these data suggest that some plant ABC transporters are involved in both efflux and influx of auxin in different plant tissues. Whether PGP-mediated auxin transport is in any way related to PIN-dependent auxin transport, and what their possible functional interaction is, remains unknown.

Feedback regulations in auxin distribution

Numerous observations of the physiological effects of auxin, including its inductive effect on organ formation and vascular tissue differentiation, led to the formulation of the canalization hypothesis, which proposed that auxin has a feedback effect on both its own transport and the directional polarization of auxin flow [28]. A positive feedback effect on auxin transport could theoretically be driven by upregulation of auxin transport capacity and polarity, which could occur through auxin regulating the transcription, degradation and/or subcellular localization of its own transport machinery.

Regulation of transcription: PIN functional redundancy

Genetic analyses suggested that PIN proteins exhibit extensive functional redundancy, even though most members show only partially overlapping patterns of expression [8, 20, 70]. For example, *pin3* and *pin4* mutations enhance the patterning defects of early *pin7* embryos (e.g. in *pin1,3,4,7* quadruple mutants) [20, 71], although surprisingly the expression of PIN3 and PIN4 is only detected in later embryo stages in wild-type [10, 20]. These observations suggested that a special type of functional redundancy was involved, in which the compensatory ectopic expression of other *PIN* genes in *pin* mutant backgrounds could occur. Indeed, PIN4 was found to be expressed in the suspensor cells of young globular-stage *pin7* embryos, whereas in wild-type only PIN7, but not PIN4, is present at this stage [71]. Similarly, in roots, five members of the *PIN* gene family (*PIN1*, *PIN2*, *PIN3*, *PIN4* and *PIN7*) are expressed and play a collectively essential role in root meristem organization [70]. The systematic analysis of PIN protein expression in various *pin* mutant backgrounds has revealed upregulation of compensatory members [70, 71], providing additional evidence for extensive cross-regulation between members of the *PIN* gene family.

Since mutations in *PIN* genes disturb the local distribution of auxin, one possible mechanism for *PIN* cross-regulation could be that auxin itself changes the expression patterns of *PIN* genes. Indeed, auxin treatment influences the accumulation of transcripts from all tested members of the *PIN* gene family with different tissue specificity [71]. In addition, GUS reporter activity driven by the promoter from each *PIN* gene positively responds to auxin [71]. These changes to *PIN* expression patterns are regulated by auxin through Aux/IAA-dependent signaling (see below) [71]. These results show that the expression of *PIN* genes is either directly or indirectly regulated by auxin at the transcription level, providing a plausible explanation for the ectopic upregulation of *PIN* genes in *pin* mutants (Fig. 3). Interestingly, auxin seems also to affect PIN protein stability, at least in the case of PIN1, PIN2 and PIN7 [71, 72], providing an additional means of regulating cellular PIN levels. Furthermore, microarray analysis indicated that, in addition to *PINs*, expression of *AUX1*, *PGP1* and *PGP19* is induced by auxin [73]. These observations collectively indicate that the auxin-dependent transcriptional and post-transcriptional control of auxin transport components is involved in the fine tuning of auxin flow.

Constitutive protein cycling: control of PIN localization

Not only is the expression of *PIN* genes exceptionally adaptable, several studies have indicated that the subcellular localization of PIN proteins is also very dynamic.

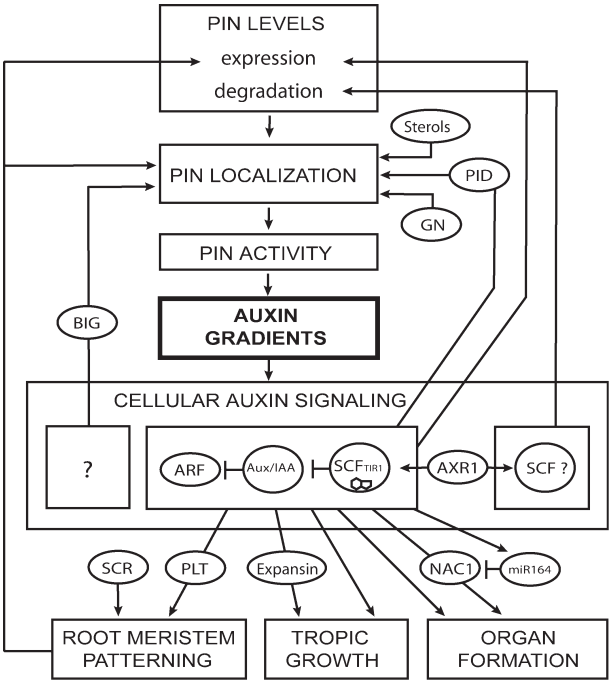

Figure 3. Possible feedback regulations in the generation of auxin gradients and auxin-related downstream events. Auxin modulates its own transport by upregulating *PIN* gene expression, PIN protein degradation and the redistribution of PIN proteins.

In response to the treatment of *Arabidopsis* roots with the fungal toxin brefeldin A (BFA), which inhibits exocytosis (movement to the plasma membrane) but allows endocytosis, PIN1 protein rapidly internalizes from the plasma membrane and accumulates into the so-called BFA compartments [74]. After BFA washout, PIN1 relocalizes back to the plasma membrane. PIN1 internalization occurs in this reversible manner even when new protein synthesis is inhibited, indicating that PIN1 protein constitutively cycles between the plasma membrane and endosomes [75]. In support of this, antibodies raised against a conserved peptide of *Arabidopsis* PIN1 protein not only labels the plasma membrane but also the Golgi network and endosomes, although the exact specificity of this intracellular labeling remains to be proved [76].

The primary cellular target of BFA, in terms of its effect on PIN1 localization, is GNOM [77], a GDP/GTP exchange factor for a small G protein of the ARF (adenosyl ribosylation factor) class [74], which is involved in the exocytosis of PIN1 [77]. Furthermore, PIN1 is mislocalized in *gnom* mutant embryos, such that cellular PIN1 polarity does not align with the embryonic axis [74]. These results indicate that proper vesicle trafficking plays an essential role in the normal localization of PIN1. In addition to PIN1, PIN2 and PIN3 cycle between the plasma membrane and endosomal compartment(s) in an actin-dependent manner [18, 75, 78]. It is now not so surprising that, consistent with the loss of PINs from the plasma

membrane, BFA rapidly interferes with auxin efflux [79, 80] and can also mimic the physiological effects of auxin efflux inhibitors such as inhibition of root gravitropism and lateral root initiation [75].

The function of the constitutive cycling of PIN is not yet clear. It is possible that it facilitates the redirection of auxin flux by rapidly repositioning PIN proteins to the required places at the plasma membrane in response to environmental stimuli. Indeed, PIN3 was shown to rapidly relocate in response to gravity stimulation [18]. The dynamics of the cycling of PIN proteins also potentially regulates protein localization at the membrane by controlling endo- and/or exocytosis themselves. Three key pieces of evidence support the idea that auxin modulates PAT by temporarily inhibiting the endocytosis of PIN proteins [33]. First, an increased level of auxin either by exogenous application, genetic manipulation or gravity stimulation reduced the endocytosis of proteins, including PIN1 and PIN2, in *Arabidopsis* roots [33]. Second, consistent with the stabilization of PINs at the plasma membrane, the efflux of NAA from tobacco BY-2 cells was increased by pretreatment with 2,4-D [33]. Third, *superroot1* mutant plants, which have an elevated level of auxin, are defective in PIN internalization [33] and exhibit increased PAT [81]. This novel effect supports the canalization hypothesis, where auxin, by inhibiting endocytosis, seems to positively regulate its own transport. The molecular mechanism by which auxin displays this effect is as yet unknown, and further dissection of this process by the analysis of proteins involved, including the Callosin-like protein BIG [33], is keenly awaited.

PIN polarity and direction of auxin flow

Increasing lines of evidence suggest that the asymmetric cellular distribution of PIN proteins is important for the directional guidance of auxin transport [82]. Notably, changes in the polarity of engineered PIN proteins are sufficient to determine the direction of auxin flow in the root epidermis [65]. In addition, the polarity of PIN proteins can be modified in response to external and developmental cues [8, 18, 20, 83]. Several factors required for the polar localization of PINs are described below.

The *Arabidopsis* mutant, *orc*, exhibits cell polarity defects, and the localization of PIN1 and PIN3 is disturbed in *orc* seedling roots [84]. Mutation in the *STEROL METHYLTRANSFERASE1* (*SMT1*) gene was found to cause the *orc* phenotype, providing a link between sterol biogenesis and the polarity of PIN localization [84]. Another link between sterols and PIN localization came to light from the finding that the sterol-specific fluorescent dye filipin co-localizes with the PIN2 protein [78]. These observations suggest that the sterol composition of membranes is important for the proper localization of PIN proteins.

To date, the only gene directly involved in the regulation of polar localization of PINs emerged from the analyses of *pinoid* (*pid*) mutants, which share phenotypic characteristics with *pin1* mutants [85]. The *PID* gene encodes a serine-threonine protein kinase [86], and its overexpression affects root development and tropism, seemingly due to altered auxin transport [87, 88]. Ectopic expression of *PID* causes a basal-to-apical shift in PIN1, PIN2 and PIN4 localization in seedling roots and developing embryos, leading to the loss of auxin gradients. This supports a direct role of PID in the regulation of PAT. Conversely, the loss of *PID* function causes an apical-to-basal shift in PIN1 localization at the inflorescence apex [88] and embryonic cotyledons [89]. This suggests that the activity levels of the PID kinase function as a binary switch for PIN polar localization and the regulation of PAT during organ formation. Interestingly, expression of *PID* itself is rapidly induced by auxin [73, 86, 87], indicating a potential feedback regulation of PIN polarity by auxin-mediated *PID* expression.

Thus, auxin itself modifies the direction of its own flow (Fig. 3). However, it remains to be addressed to which extent such feedback regulations contribute to auxin distribution in different tissues, including those in shoot and root meristem. For example, in regenerating root meristems, auxin affects the localization of PIN proteins only after cell-type specification or in the absence of developmental regulators responsible for cell differentiation [83].

Downstream: cellular auxin signaling

It seems that auxin accumulation in certain cells initiates a plethora of cell-specific developmental responses. It is increasingly apparent that the conversion of auxin accumulation patterns into specific physiological responses occurs through downstream auxin signaling.

For decades, substantial efforts were made to identify the auxin receptor and its downstream signaling components. In a biochemical approach, proteins that bind to auxin have been screened by affinity labeling and affinity purification. An auxin binding protein (ABP1) has been repeatedly identified in plant species, including maize and in *Arabidopsis*. Evidence for the role of ABP1 in cell elongation has been provided through functional analyses involving anti-ABP1 antibodies and by a genetic approach (for overview, see [90]). From studies in tomato, an additional member of the ABP1 pathway has been possibly identified as a protein of the cyclophilin class – diageotropica [91, 92]. However, to date, the role of ABP1 in a specific cellular process such as auxin-mediated regulation of gene expression has not been shown.

Pathway for auxin-mediated gene expression

Genetic approaches have been used to identify the molecular components of the auxin pathway. From screens

for auxin-resistant mutants, a number of auxin-related factors, such as AUXIN-RESISTANT1 (AXR1) and TRANSPORT INHIBITOR RESPONSE1 (TIR1), have been identified as being involved in ubiquitin-related modification of proteins, required for the regulated degradation of proteins [93]. *TIR1* encodes an F-box protein that forms a complex with AtCULLIN1 (AtCUL1) and a SKP1-like protein to form an SCF ubiquitin protein ligase E3 (SCF^{TIR1}) [94, 95] complex, which specifically ubiquitinates substrates for proteasome-dependent degradation. Substrates for the SCF^{TIR1}-mediated ubiquitination were originally identified from molecular biological techniques. The whole story began with a finding that auxin rapidly regulates the expression of multiple genes. Many of these genes were shown to be primary auxin-response genes, such as *GH3*, *SAUR* and *Aux/IAA* genes [22]. This led to the identification of an auxin-responsive *cis*-regulatory element (AuxRE) in the promoters of many auxin-responsive genes. Subsequently, AuxRE-binding proteins – the ARFs (auxin response factors) – and small, short-lived Aux/IAA proteins that bind to ARFs and repress their activity, have been isolated [22]. ARF proteins contain an N-terminal DNA-binding domain, middle region and C-terminal dimerization domain. The Aux/IAA proteins contain homologous dimerization domains (designated domains III and IV), which are used for their interaction with ARFs [96, 97]. Specific mutations in other domains (domain II) of Aux/IAAs leads to the stabilization of Aux/IAA proteins, often resulting in severe developmental defects and auxin-resistant phenotypes. Strikingly, Aux/IAAs directly interact with SCF^{TIR1} in an auxin-dependent manner, which provides a direct connection to the ubiquitination pathway [98]. Auxin enters this pathway by binding directly to the TIR1 F-box protein [99, 100]. Thus, the pathway for auxin-dependent regulation of gene expression appears to be remarkably straightforward: auxin binds to SCF^{TIR1}, facilitating the interaction of SCF^{TIR1} with Aux/IAAs. This leads to ubiquitination and degradation of Aux/IAA repressors and the release of ARF transcriptional regulators for the induction of auxin-dependent gene expression (overviewed by [101]).

The activity of the SCF^{TIR1} pathway is tightly regulated by several additional factors including AXR1, a protein related to the N-terminal half of ubiquitin-activating enzyme E1. AXR1, in concert with another activating enzyme, E1 C-terminal Related1 (ECR1), and RUB-conjugating enzyme1, RCE1, conjugate a ubiquitin-related molecule (RUB, also referred to as nedd8) to AtCUL1, a component of SCF^{TIR1} [102, 103]. AtCUL1 is also associated with a protein complex called the COP9 signalosome, which removes the conjugated RUB1 from AtCUL1 [104]. The cycle of RUB1 conjugation and its removal appears to be critical for the function of SCF^{TIR1} and might limit the capacity of auxin responsiveness in cells.

Auxin signaling can also be switched off, following its activation, by Aux/IAA repressors, which themselves are early auxin-inducible genes [22]. In addition, a microRNA-dependent mechanism seems to be involved in auxin signaling. The *NAC1* gene (encoding a NAM/ATAF/CUC-domain-containing transcription factor), which acts downstream of TIR1 and positively regulates lateral root formation [105], is downregulated by auxin-inducible miR164 [106]. Increasing lines of evidence point to the importance of auxin-mediated gene expression mediated by TIR1-related F-box proteins. However, in part because of redundancy in each component and early lethality associated by complete inhibition of the pathway [107], it remains unclear whether this pathway governs the whole of auxin-inducible gene expression.

Specificity of ARF- and Aux/IAA-dependent signaling

An important question remains as to how the rather simple cellular signaling of auxin, consisting of TIR1-, Aux/IAA- and ARF-dependent steps, can confer so many different developmental responses in different cell types [108]. There are 29 *Aux/IAA* genes and 23 *ARF* genes in the *Arabidopsis* genome [109, 110], theoretically providing sufficient molecular complexity, which could further be dramatically enhanced by specific combinations of ARF and Aux/IAA interactions. Accordingly, various cellular auxin responses could be facilitated through the specific expression of Aux/IAAs and ARFs in particular cells, specific interactions between particular Aux/IAAs and ARFs, and specialized roles for each ARF. Genetic analyses using loss-of-function *ARF* mutants have indeed revealed distinct although partially overlapping roles for *ARFs* in diverse auxin-related processes such as embryonic pattern formation [*ARF5/MONOPTEROS* (*MP*)] [111], lateral root formation and tropism [*ARF7/NON-PHOTOTROPIC HYPOCOTYL 4* (*NPH4*) *ARF19*] [110, 112], adaxial-abaxial axis specification in aerial organs (*ARF3/ETTIN* and *ARF4*) [113], seedling apical hook development (*ARF2*) [114], senescence (*ARF1,2,7* and *19*) [115, 116], floral organ development (*ARF1-3, 6* and *8*) [115, 117, 118] and auxin homeostasis (*ARF8* and *17*) [119, 120]. At least to some extent, different ARF proteins seem to have specific functions, since *ARF5/MP* rescued the *mp* mutant phenotype more efficiently than *ARF16* when expressed under the same promoter [121].

The pattern of *Aux/IAA* gene expression, at least at one level, contributes to their specific biological roles since the ectopic expression of stabilized Aux/IAA proteins in different cells can induce novel phenotypes [121, 122]. On the other hand, the functional specificity of *Aux/IAA* genes is also important, as they cause distinct phenotypes when expressed under the same promoter [121, 123], producing equivalent protein amounts [121]. For example,

iaa12/*bdl* mimicked the *mp* mutant phenotype more efficiently than *iaa3/shy2* when expressed under the control of the *BDL* promoter, suggesting that BDL/IAA12 and ARF5/MP are an optimized pair [121]. Thus, it appears that the specificity of auxin response in a specific tissue can be determined mainly by three levels: transcriptional regulation to limit the maximal action of ARFs and Aux/IAAs, the intrinsic function of ARFs and repression of ARF functions by an optimized Aux/IAA-ARF pair.

Downstream components of the ARF-dependent signaling cascade

The question arises next is, what are the factors present downstream of the ARF-dependent cellular auxin signaling that ultimately execute various developmental events? Some of the key factors converting auxin-regulated gene expression into developmental responses have been elucidated through genetic analyses and searching for auxin-inducible genes. For example, during organ formation and tropic responses, auxin induces the expression of genes regulating cell-fate determination [124] and cell elongation, respectively [12].

After tropic stimuli, auxin is redistributed asymmetrically, resulting in the differential expression of auxin-inducible genes. Remarkably, included are genes coding for expansins, which loosen the cell wall and induce cell expansion [12]. In agreement with the proposed role of expansins in cell expansion, their transcript accumulation increases in the shaded or bottom sides of hypocotyls after phototropic or gravity stimulation, respectively. Induction of their expression depends on PAT and ARF7/NPH4, suggesting that auxin distribution generated by tropic stimulation triggers the expression of proteins that can directly regulate tropic growths [12].

During root patterning, the *PLETHORA1* (*PLT1*) and *PLT2* genes, which encode AP2-domain-containing transcription factors required for stem cell specification, are upregulated by prolonged treatment with auxin [124]. *PLT* expression spatially correlates with DR5 expression and depends on ARF activity [124], suggesting that auxin accumulation either directly or indirectly regulates the pattern of *PLT* transcript distribution. PLT, in turn, regulates the expression of PINs, possibly providing another feedback loop in auxin distribution that is essential for root development [70].

In the inflorescence meristem, expression of both DR5 and *PIN1* is detected in incipient and very early stages of primordia initiation [8, 27]. Marker genes derived from *SHOOTMERISTEMLESS* (*STM*) and *CUP-SHAPED COTYLEDON2* (*CUC2*), which are involved in the formation and maintenance of the shoot apical meristem and organ separation, are expressed in a complementary domain to the *PIN1*-expressing region [27]. Furthermore, Heisler and colleagues demonstrated by live imaging of PIN1::GFP that a developmentally regulated reversal of PIN1 polarity occurs in the boundary of primordia that correlates with the upregulation of *STM* and *CUC2* expression [27]. Consistently, *CUC2* expression is downregulated in cotyledon boundaries of *pin1* and *mp/arf5* embryos [125], suggesting that auxin transport and response are involved in the local upregulation of *CUC2*. Genetic evidence also suggests that auxin transport is required for the local downregulation of *CUC2* in the inflorescence meristem [126]. However, whether this regulation involves ARFs still remains elusive.

Model cases for auxin-dependent development

As the specific developmental processes triggered by transport-dependent auxin gradients differ considerably, we now discuss selected examples demonstrating this complex regulation.

Embryonic axis formation

Embryogenesis is a fundamental step in plant development, in which shoot and root meristems are generated along the apical-basal axis. Just prior to key events in embryonic patterning, the transport-dependent auxin accumulation pattern changes dynamically [20]. Following the asymmetric, anticlinal division of the zygote, *PIN7* becomes specifically expressed in basal cell and its derivatives (suspensor cells). Here, PIN7 localizes to the apical (upper) surface of the basal cell and directs auxin flow towards the apical cell, where auxin accumulates. This accumulation of auxin in the apical cell persists for several rounds of cell division in the subsequently formed proembryo cells [20] (Fig. 1a). From the first periclinal division of the apical cell, PIN1 comes into action, channeling auxin flow. It localizes at the two facing plasma membranes of the daughter cells (generated by the division of the apical cell), presumably to equalize the distribution of auxin between these cells. At the young globular stage, the developmentally regulated reversal of PIN polarities takes place. PIN1 starts to localize to the bottom side of the cells in the embryo proper, which face towards the suspensor cells. Concomitantly, PIN7 polarity changes in these cells to shift from the top to the bottom side. This rearrangement of PIN polarities results in the establishment of an 'apical-to-basal' flow of auxin. Thus, auxin accumulates in the basal part of the embryo and the upper suspensor cells, which later give rise to the root meristem [20] (Fig. 1a). Mutations in multiple *PIN* genes [20] as well as in the components of the auxin signaling pathway, including *TIR1*-related genes [107], *BDL/IAA*12 [127] and *MP/ARF*5 [111], cause severe misspecification of apical and basal embryonic structures. This shows that

PIN-dependent auxin accumulation and its cell-specific interpretation are critical for proper embryo patterning. As cotyledon initiation begins, PIN1 localization in the outermost cells of the apical part of the embryo is directed toward the apices of the incipient cotyledons [8] (Figs. 1a, 2a). The generation of cotyledons in the apical region also requires the activities of TIR1-related proteins [107] and ARFs [128] in addition to those of PINs [14, 20]. Collectively, these results demonstrate the central role of cellular auxin transport, auxin distribution and cellular auxin signaling in the specification of the apical-basal axis and organ formation during plant embryogenesis.

Phyllotaxis

Aerial organs such as leaves and flowers are generated by the shoot apical meristem in highly predictable phyllotactic patterns. For instance, *Arabidopsis* shoots produce leaves and then flowers in a spiral pattern. Microapplication experiments found that auxin can trigger new leaf and flower formation in the peripheral region of the shoot meristem [25]. It is now clear that PIN1-dependent PAT directs available auxin in the shoot meristem to specific locations for organ initiation. Studies into the localization patterns of PIN1 and AUX1 at shoot apices suggest that auxin is transported upwards into the meristem through the outer L1 layer of the shoot meristem [8, 58]. In an available space in the meristem, auxin presumably reaches a threshold concentration which changes PIN localization. PIN1 then, for the time being, localizes towards the initiation site, apparently pumping auxin in towards each new organ position. This corresponds to a temporary increase in DR5 expression in the initiation site, and the beginning of organ growth [8, 27, 58, 129, 130]. These results suggest that while movement of auxin into an incipient primordium triggers organ initiation, initiation is also inhibited in surrounding cells where auxin is temporarily depleted. Auxin is only able to increase again in cells at a specific distance from the absorbing influence of preexisting primordia. Thus, the positions of preexisting primordia, together with PIN-dependent auxin transport, and the specific dimensions of the meristem, appear to be sufficient to generate the distinct phyllotactic patterns that are seen in plants [27, 58]. It is interesting that computer simulations can generate phyllotactic patterns of auxin accumulation and organ formation using experimentally obtained parameters and the assumption that auxin distribution also affects PIN polarity [130, 131]. Although the auxin effect on the polarity of PINs has not yet been substantiated experimentally, it is possible that auxin impinges on the PIN polarity through the regulation of *PINOID* expression [87, 88] or through the effects of auxin on PIN trafficking [33].

Gravitropism

Gravitropism is a process which allows plants to sense the gravity vector and align their growth direction accordingly. Generally, roots show positive gravitropism – growing down into the soil – whereas shoots display negative gravitropism, growing away from the soil. In many plant species, including *Arabidopsis*, gravity perception primarily takes place in the endodermis in shoots and the columella root cap cells in roots, both of which contain starch-filled organelles called amyloplasts (also referred to as statoliths) [132]. As gravity perception and differential growth response take place in different tissues of the root (root cap and elongation zone, respectively), the mobilization of a signal from the perceiving to the responding tissue has been hypothesized. Similarly, the transport of such a signal from endodermis to epidermal cells in the shoot, where the control of shoot elongation occurs, has been proposed [132].

The classic Cholodny-Went hypothesis proposes that auxin is the major signal in gravitropic response and that asymmetric auxin distribution mediates differential growth, which results in the bending of gravistimulated organs [31]. It is interesting that after changes to gravity direction, organs consistently accumulate auxin at their lower side [12, 18, 32], even though the growth response may be different. Normally, cell elongation is stimulated in shoots, whereas in roots it is inhibited, allowing differential growth upwards and downwards, respectively. Auxin efflux inhibitors interfere with the generation of asymmetric auxin distribution and tropic growth, suggesting that PAT underlies gravitropism [12, 18, 32]. An important insight into the molecular link for gravity perception and generation of asymmetric auxin distribution was provided through study of the PIN3 protein [18, 133]. The *Arabidopsis pin3* mutant is partially defective in shoot and root gravitropism. Importantly, PIN3 protein has been detected in gravity-sensing cells – the columella cells of the root and endodermis cells of the shoot [18] (Fig. 2b). In columella cells PIN3 rapidly responds to the altered gravity vector by relocating to the new bottom side of the root, suggesting that statolith sedimentation is translated into changes in PIN3 polarity and PIN-mediated differential auxin accumulation [18] (Fig. 1b). In addition to PIN3, root gravitropism involves PIN2, AUX1, PGP4 and PGP19 [36, 37, 62, 67, 134]. Based on the capacity of tissue-specific expression of *AUX1* to rescue the agravitropic *aux1* root phenotype, AUX1 has been shown to be required in the lateral root cap and epidermis, where AUX1 in concert with PIN2 mediates basipetal auxin transport [34, 62] (Fig. 2b). Loss of PIN2 or AUX1 activities abolishes gravity-stimulated asymmetric auxin distribution and causes undirected root growth [19, 36, 65]. Signaling events downstream of auxin accumulation appear to be regulated by Aux/IAAs and ARFs [110, 112, 132]. Targeted expression studies using the dominant

mutation of *AXR3/IAA17* revealed that auxin response is specifically required in epidermal cells in the root elongation zone [34]. It has been reported that *pgp19* and *pgp4* mutants exhibit a hypergravitropic hypocotyl phenotype [134] and reduced gravitropic root bending [67], respectively. Although PGP4 and PGP19 are involved in auxin influx and efflux processes, it remains to be unambiguously demonstrated whether they are involved in asymmetric auxin distribution [67–68]. In summary, root gravitropic response involves gravity perception by statoliths in columella cells, rapid relocation of PIN3 in the same cells, generation of asymmetric auxin flow towards the elongation zone through the epidermis by AUX1 and PIN2, and transcriptional regulation of downstream factors by ARFs in the elongation zone. However, the cause-effect relationship between statolith sedimentation and PIN3 relocation has not been demonstrated so far, and this would be an interesting topic for future research.

In shoots, PIN3 localizes to the endodermis and exhibits asymmetric subcellular localization predominantly at the inner side of cells [18]. The loss-of-function *pin3* phenotype as well as localization of PIN3 protein suggests that PIN3 plays a similar role in the lateral distribution of auxin to the lower side of the shoot during the gravitropic response [18]. Accumulation of auxin at the lower side of hypocotyls causes expression of a set of genes in an ARF-dependent manner [12, 112]. The target genes include those coding for expansins, which can directly modify the cell wall and induce cell elongation, providing a likely mechanism connecting auxin distribution and differential growth [12].

It is possible that a mechanism involving changes in PIN polarity for asymmetric auxin distribution also underlies phototropic responses; however, a connection between light perception and PIN polarity changes has not been demonstrated so far.

PAT in non-*Arabidopsis* species

Molecular components potentially involved in PAT, auxin signaling and downstream events have been also recently characterized in plant species other than *Arabidopsis*. In this section, we summarize selected data with functional significance from the study of non-*Arabidopsis* plants and compare them with what is known from *Arabidopsis* research where appropriate.

Early studies of the effect of auxin on plant tropism were performed using plant species such as canary grass and oat coleoptiles [30, 31]. This tradition continued with studies demonstrating that auxin is asymmetrically distributed preferentially to the elongating side of tissues after tropic stimulation in both monocotyledonous and dicotyledonous plants, including maize and rice coleoptiles [135, 136] and *Brassica* hypocotyls [12]. Consistently, differential auxin-inducible gene expression is observed in various plant species, including light-stimulated maize coleoptiles [11], gravity- and light-stimulated tobacco stems [32] and *Brassica* hypocotyls [12]. In *Brassica* hypocotyls, accumulation of transcripts of genes, including expansins, increase at the bottom and shaded sides of hypocotyls stimulated with gravity and light, respectively [12]. Notably, differential auxin accumulation was not found in the rice mutant *coleoptile phototropism1* (*cpt1*) [136]. The *CPT1* gene encodes a protein orthologous to *Arabidopsis* NPH3, a signaling component that physically interacts with the photoreceptor and is involved in hypocotyl phototropism [137]. These results demonstrate that rice and *Arabidopsis* share a key component for light signaling involved in phototropism.

A genetic study involving RNA interference indicated that a rice homologue of *PIN1*, *OsPIN1*, is involved in the formation of adventitious roots [138]. The phenotype associated with suppression of *OsPIN1* messenger RNA (mRNA) accumulation is reminiscent of that of NPA-treated rice plants [138]. In rice, auxin accumulation appears to cause adventitious root formation through the ARF-dependent transcription of *CROWN ROOTLESS1*, which encodes a protein of the ASYMMETRIC LEAVES2/LATERAL ORGAN BOUNDARIES (AS2/LBD) family [139]. As some genes in the *AS2/LBD* family are induced by auxin in an ARF-dependent manner in *Arabidopsis* and the overexpression of *LBD16* promotes lateral root formation in the *arf7 arf19* double mutant [110], rice and *Arabidopsis* appear to share at least some downstream targets for ARFs. Regulation of *PIN*-gene expression appears to be shared between *Arabidopsis* and pea, as accumulation of transcripts of a pea homologue of *PIN* gene (*PsPIN1*) is upregulated by auxin [140].

In summary, increasing lines of evidence suggest that dicotyledonous and monocotyledonous plants share at least some key components for light and gravity perception, auxin transport and downstream factors. So far the available genetic and physiological data from non-*Arabidopsis* species suggest that PAT- and PIN-dependent asymmetric auxin distribution is a functional module, which also operates in pattern formation, organogenesis and tropisms in other higher plant species. Naturally, this does not necessarily mean that the details of this mechanism for each particular developmental process in other plant species are identical to *Arabidopsis*, as some processes such as embryogenesis or flower development are quite diverse in different plant species. It is, however, clear that growing knowledge from *Arabidopsis*, together with the recent availability of genetic and genomic resources, including rice and maize genomic DNA sequences [141–144], will accelerate our understanding of PAT-mediated regulation of plant growth and development in higher plants in general.

Conclusion and outlook

In summary, recent studies using *Arabidopsis thaliana* and other species have shown that the transport-dependent spatiotemporal distribution of auxin serves as a means to interpret environmental and developmental cues by modulating the concentration of auxin in order to trigger cellular growth responses. Such a system, which involves multiple feedback loops, together with the combinatorial capacity of downstream auxin signaling events, could account for the variety of auxin effects in plant development. However, as the molecular components that regulate the activity and polarity of auxin transport components are still poorly understood, identifying tissue-specific regulators and elucidating mechanisms that determine the polarity of transport proteins, including PINs, would be interesting topics to address. The accumulation pattern of auxin appears to be interpreted in cells through the binding of auxin with TIR1-related F-box proteins, followed by the degradation of Aux/IAA proteins and the subsequent release of ARF proteins, although the possibility of independent parallel pathways for auxin-inducible gene expression has not yet been excluded. In various development and growth processes, overlapping and distinct roles for different ARFs have been emerging. The increasing knowledge of the molecular components involved in PAT and downstream auxin signaling are greatly improving our understanding of the auxin-related regulation of each developmental process and how it is connected to the other plant signaling pathways.

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