Auxin transport — shaping the plant

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Plant growth is marked by its adaptability to continuous changes in environment. A regulated, differential distribution of auxin underlies many adaptation processes including organogenesis, meristem patterning and tropisms. In executing its multiple roles, auxin displays some characteristics of both a hormone and a morphogen. Studies on auxin transport, as well as tracing the intracellular movement of its molecular components, have suggested a possible scenario to explain how growth plasticity is conferred at the cellular and molecular level. The plant perceives stimuli and changes the subcellular position of auxin-transport components accordingly. These changes modulate auxin fluxes, and the newly established auxin distribution triggers the corresponding developmental response.

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A century towards molecular players

Our linden tree had to wait more than 300 years before our curiosity was turned towards the mysterious mechanisms that so strangely affected its fate. At that time, a German botanist, Julius Sachs, proposed the existence of signaling substances that regulate coordinated plant growth, and Charles Darwin together with his brother Francis grew grass coleoptiles in unilateral light to demonstrate the existence of a transported signal that mediates plant phototropism [1,2]. Thus, the history of auxin began. During the century that followed, the chemical nature of auxin was uncovered, but we remained confused by the variety of apparently unrelated developmental processes that are regulated by such a simple molecule as indole-3-acetic acid (IAA).

Our attention was drawn to the transport of auxin, as its disruption interferes with almost all auxin-related developmental processes. We learned that auxin can be distributed via the phloem or by a directional, so-called ‘polar’, transport system (see Figure 1; [3]). The large amount of physiological and biochemical data on polar auxin transport has been integrated into the ‘chemiosmotic hypothesis’. This classical model explains the cell-to-cell movement of auxin by the action of specific auxin-influx and -efflux carriers. The asymmetric positioning of the latter at a particular side of the cell was proposed to determine the direction of auxin flux [4,5]. This model was reinforced by the identification and characterization of candidate proteins for auxin influx (AUXIN1 [AUX1]/LIKE-AUX1 [LAX] family) and efflux (PIN-FORMED [PIN] family) carriers [6–8]. Numerous pieces of circumstantial evidence demonstrate the role of these proteins in auxin transport despite the lack of rigorous proof for their function as carriers [3]. PIN proteins are asymmetrically

Abbriviations
AUX1 AUXIN1
IAA indole-3-acetic acid
MDR multidrug resistance
PIN PIN-FORMED
SGR2 SHOOT GRAVITROPIC2
ZIG ZIGZAG

Prelude — The linden tree of innocence

On the margin of the Chríby hills, an old mediaeval castle ‘Buchlov’ guards the wide valley of the South Moravian river ‘Morava’. There, on the terrace where the tribunal of the hunter’s court used to sit, and where the last farewells with the convicts were held, a famous linden tree of innocence stands as a witness of a local legend. It is told that early in the 16th century the lord of the castle was deceitfully slain during one of his frequent hunts. A young servant was accused of this murder and imprisoned. After long days of unavailing torture he was condemned to death on the castle terrace. At this, the young man rose and pulled out the young linden tree growing nearby. He set it incerted back into the soil with the words, “If next year this small tree will grow green, it will be a sign of God that you killed an innocent”. And indeed, in the spring, small green leaves flourished from the precious roots and the young man was set free.
Auxin response and transport in a gravistimulated *Arabidopsis* hypocotyl. Auxin transport throughout the plant involves both non-polar transport in xylem (dashed line) and an active, cell-to-cell polar transport (red arrows), which can transport auxin either basipetally (from the apex to the base) or laterally. During gravitropic or phototropic bending, increased auxin response (as indicated by DR5::GUS, displayed as blue staining) corresponding to higher auxin levels is found in the more elongated, outer side of bending hypocotyl. This asymmetric lateral auxin distribution appears to be established by lateral auxin transport and to trigger asymmetric growth.

localized in different cells, and this localization impressively coincides with the known directions of auxin flux [8,9,10*,11*]. The AUX1 protein also localizes asymmetrically in root protophloem cells at the opposite cell side from PIN1 [12*]. These findings suggest that, at least in some tissues, influx and efflux carriers in concert facilitate the vectorial movement of auxin. Recently, members of another protein family, namely multidrug resistance (MDR)-type ATP-binding cassette (ABC) proteins, have been implicated in auxin transport. Two of these proteins, AtMDR1 and *Arabidopsis thaliana* P-glycoprotein1 (AtPGP1), were originally identified as being functionally related to anion channels; nonetheless, the corresponding mutants and double mutants showed auxin-related phenotypes including a reduced rate of auxin transport [13]. Moreover, these proteins can bind 1-N-naphthylphthal-}

lamic acid (NPA), an inhibitor of auxin transport, thus providing an additional connection between MDRs and auxin delivery [14]. Although detailed information is still lacking, a century of studies on auxin transport have brought us the identity of a couple of players that are involved in auxin responses, and an image of a complex network of several transport systems that are involved in distributing auxin throughout the whole plant.

**Hormone or morphogen?**

Two theoretical concepts have greatly influenced the way that we think about auxin and its action: the concepts of a hormone and a morphogen. The mammalian hormone concept defines hormones as extracellular signaling molecules, which act on target cells distant from their localized site of synthesis [15]. Although recent studies have demonstrated the potential for IAA synthesis in a variety of *Arabidopsis* organs, its movement from its main source in young apical tissues throughout the whole plant has been proven many times [3,16*]. A known role for auxin in coordinating the development of organs, for example lateral roots, with the developmental stage of the shoot provides a functional meaning for this long-distance signaling [17,18]. Thus, auxin formally adheres to the classical definition of a hormone. However, the most-studied form of auxin transport, cell-to-cell polar transport, contrasts with the passive allocation of animal hormones through blood, which is more analogous to non-polar auxin distribution. Several arguments indicate that non-polar transport in phloem contributes to the movement of auxin from its main source in the apical tissues to the root. First, the known velocity of active transport (about 10 mm per hour) is too slow to execute efficient signaling, especially in larger plant species. Second, free auxin has been detected at relatively high concentrations (of about 1 μM) in phloem exudates [19]. And third, aux1 mutants, which are apparently impaired in loading auxin from leaves into the phloem and in unloading auxin from phloem into the root, display defects in their ability to distribute auxin between the shoot apex and the root [12*,20*]. Thus, the putative auxin permease AUX1 seems to act at both ends of the auxin route in phloem, connecting it at its lower end to the polar transport system in the root tip.

In the root meristem, auxin is implicated in regulating the pattern of cell division and differentiation (see Figure 2), a short-distance activity that is related to the role of auxin as morphogen. The term morphogen was introduced as a purely theoretical term in mathematical models of self-organizing systems, and has evolved into a basic concept of developmental biology [21]. The least stringent definition refers to a morphogen as a substance that forms a concentration gradient and is involved in developmental patterning [21]. More rigorous definitions provide three critical conditions that bona fide morphogens must meet: first, a morphogen forms a stable concentration gradient;
How does the auxin gradient arise? In the root, the auxin efflux regulator PIN4 is asymmetrically distributed towards cells with increased DR5 response, and both pin4 mutations and the chemical inhibition of auxin transport disrupt the distribution of DR5 activity [11*,26]. These findings suggest that PIN4-dependent efflux-driven auxin transport actively maintains the auxin gradient. It is intriguing to speculate that auxin, in turn, influences the position and activity of the auxin efflux carriers; and thus, that the auxin gradient is stabilized by a feedback loop. But can such a gradient instruct patterning? The endogenous application of auxins or auxin-transport inhibitors, as well as the use of mutants that are impaired in auxin response or transport, have been used to establish a link between auxin distribution and patterning [11*,26]. Interestingly, changes in cell fate (inferred from the cell-specific markers) spatially correlate with changes in auxin gradients [11*]. Nonetheless, we know too little about auxin or auxin-gradient perception and downstream signaling to be able to pinpoint the direct connection between auxin gradients and instructed cells. Increasing intracellular auxin content by IAA treatment interferes much less with patterning than does the application of non-transportable 2,4-dichlorophenoxyacetic acid (2,4D). This may even mean that relative differences in auxin content between cells, rather than the absolute amount of intracellular auxin, are instructive. In this scenario, components of auxin transport such as PIN proteins might serve as auxin flux ‘counters’. Thus, our knowledge, especially on the interpretation of auxin gradients, is too scarce to allow us to decide whether auxin is a true morphogen, although it meets several of the descriptive criteria. Our notion of transport-driven auxin gradients also contrasts with the classical image of morphogens freely diffusing from a source [11*]. It is therefore questionable how much can we gain from grafting concepts that are derived from different experimental systems onto a plant-specific situation. Currently, the morphogen concept is also being revised in the animal field. In planar transcytosis models, morphogens such as decapentaplegic (DPP) or Wingless (WG) move actively through a field of cells and their gradient is maintained by vesicular trafficking [23]. It may well be that, as our experimental knowledge of both auxins and the morphogens increases, the theoretical concepts will converge and we will reach a common understanding about pattern formation in both plants and animals.

**Tropisms, transport, and traffic**

Another process that influences plant shape is directional bending with respect to an exogenous stimulus (mainly to light or gravity), which is called tropism. The role of auxin in tropisms was implied by the Cholodny–Went hypothesis, which suggests that unequal distribution of auxin between the opposite sides of a curving organ underlies differential growth, resulting in bending [27]. Differential auxin or auxin-response distribution within various
organs, with higher levels at the lower or less-illuminated side, has been correlated to the their bending in various experiments (see Figure 2; [3,7,10**,28,29]). Nonetheless, the question of how this asymmetry is achieved remains unanswered. Went originally proposed that cells change their polarity, which results in the lateral transport of auxin [27]; and experiments in which auxin efflux has been disrupted have indeed suggested that auxin transport mediates the lateral distribution of auxin [10**,29]. The search for molecular support for this concept brought about the identification of PIN3 [10**]. PIN3 is involved in hypocotyl and root tropisms, and is localized in the lateral endodermis of shoots, where it is perfectly positioned to regulate auxin redistribution in the lateral direction [10**]. The demonstration of a defect in lateral auxin redistribution in pin3 mutants, a more direct proof of this scenario, may be difficult as the rather subtle tropism defects of pin3 suggest that one or more other PIN proteins may functionally replace PIN3.

In roots, unlike in shoots, the locations of stimulus perception (in the root cap) and growth response (in the elongation zone) are remote from each other [28]. We do not know exactly where the asymmetry in auxin distribution is established in roots, but experiments using the DR5 reporter suggest that lateral auxin redistribution has already taken place in the root cap [26,29]. From there, auxin is translocated basipetally in an auxin efflux- and influx-dependent manner [3,6,12*,28,30]. AUX1 probably facilitates the uptake of auxin into the lateral root cap and epidermis region, and PIN2 probably mediates its directional translocation towards the elongation zone (see Figure 3). The next important question is that of how auxin transport is activated and regulated by a stimulus such as gravity. Gravity is perceived by the sedimentation of starch-containing organelles (i.e. statoliths) in the columella root cap and in shoot endodermis [28]. The presence of PIN3 in these cells raises the intriguing possibility that gravity perception and auxin redistribution are coupled via PIN3 [10**]. This scenario has been tested in gravistimulated Arabidopsis roots. Under normal conditions, most of the PIN3 protein is located symmetrically at the plasma membranes of columella cells. After gravistimulation, PIN3 changes its position within two minutes and relocates, presumably towards the new bottom of the cells [10**]. PIN3 is thus ideally placed to mediate an auxin flux towards the lower side of root (see Figure 3, inset). Interestingly, the auxin influx component AUX1 also shows strong subcellular dynamics in the columella cells [12*]. It is tempting to speculate that AUX1 mediates an influx of auxin into the columella after gravity stimulation, thereby creating a temporary pool of auxin that is needed for asymmetric relocation of auxin in a PIN3-dependent pathway.

But what mechanism enables the rapid subcellular relocation of PIN3? Elegant physiological experiments by Morris and coworkers [31,32*] envisioned the rapid intracellular turnover of at least part of the auxin efflux complex, even before the molecular components of this complex were identified. These ideas have been corroborated by the demonstration that PIN1 and PIN3 continuously cycle in membrane vesicles along the actin cytoskeleton between the plasma membrane and the endosome [10**,33*]. If a decision about the targeting of PIN proteins takes place after each internalization event, such a recycling mechanism would be far more flexible than a mechanism involving a sequence of degradation→new protein synthesis→new targeting. It would provide a means of rapid retargeting and would...
explain why plants invest so much energy in the continuous recycling of proteins that are, in principle, only needed at the plasma membrane. There is only circumstantial evidence that the relocation of PIN3 regulates auxin redistribution, which leads to gravitropic bending. This evidence comes mainly from correlation of experiments that have shown that functional PIN3 is required for proper gravitropism, and that any disruption of the actin-dependent cycling of PIN3 (for instance by ‘auxin efflux inhibitors’, by the vesicle-trafficking inhibitor brefeldin A or by actin depolymerization) results in gravitropism defects [10*,28,33*]. Rigorous testing of the hypothesis that the relocation of PIN3 mediates auxin redistribution would require the replacement of PIN3 by a non-relocating but otherwise functional version, and the subsequent analysis of auxin redistribution. The presence of both statoliths and PIN3 in the shoot endodermis suggests that a similar mechanism, involving the relocation of PIN3 and/or other PIN proteins, operates during shoot tropisms, but this remains to be demonstrated. Recent studies on two other endodermis proteins, SHOOT GRAVITROPIC2 (SGR2) and SGR4, suggest a connection between membrane traffic, vacuole organization and shoot gravitropism. However, it seems that the sgr2 and sgr4 mutations interfere with gravity perception rather than with auxin redistribution as they are defective in the sedimentation of statoliths and display normal phototropism [34,35*].

One important question that remains is that of how the sedimentation of statoliths is connected to PIN3 relocation. Classical, as well as recent models, suggest that the actin cytoskeleton is reorganized due to statolith sedimentation [36,37]. Thus, the actin-dependent intracellular traffic of PIN3 would be redirected along the sedimentation routes, and PIN3 would preferentially accumulate at the cell bottom. It is probable that reality is more complex than our simplified, mechanistic idea and the exact elucidation of this issue remains a challenge for future investigations.

Conclusions
AUXIN distribution contributes to the plasticity of plant development and mediates a wide array of responses by which plants adjust their growth to changes in environment. In an effort to meet all of these demands, AUXIN appropriates the characteristics of a hormone, such as long-distance effects and distribution through the phloem vascularise, as well as features of a morphogen, having a gradient-dependent influence on patterning in the root meristem. Yet, classifying the effects of AUXIN has not significantly advanced our understanding of its molecular mechanism. Now that the intracellular dance of AUXIN transport components have been correlated to tropisms, a picture of how the plant shapes itself is beginning to emerge. In the scenario presented in this review, a plant perceives cues from its surroundings and accordingly changes the choreography of the intracellular movement of AUXIN transport proteins. This enables plants to modulate the direction of AUXIN fluxes and thus AUXIN distribution, which in turn triggers the appropriate response. Substantial work is needed to outline the details of this roughly shaped concept. More general validity should be demonstrated by extending this model to developmental processes other than root gravitropism. Loose ends, such as the connection between perception and protein relocation, as well as the downstream signaling of AUXIN distribution remain to be cleared up. Similarly, the input and integration of endogenous signals, which certainly modulate the whole process, are topics for future research. Plants are patient and some of them long living, maintaining our hope that the beauty of the linden tree at Buchlov castle will never be diminished in our eyes, even after losing its innocence.

Acknowledgements
We are grateful to Eva Benková and Dolf Weijers for helpful discussions and critical reading of the manuscript. We are deeply indebted to Karen Cornelis for her insuperable lesson on the use of articles in English. We acknowledge support by the VolkswagenStiftung.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


The authors present their characterization of a novel member of the family of auxin efflux regulators, APT13. pin3 mutants are defective in differential growth, including root and hypocotyl tropisms. PIN3 is positioned at the lateral side of endodermis cells, providing a candidate
component of the lateral auxin-transport system. PIN3 undergoes rapid subcellular movement in vessels along actin filaments. After stimulation by gravity, PIN3 in columella cells relocates towards the presumable new cell bottom, providing a means for lateral auxin redistribution after gravity stimulation.


This work focuses on an analysis of the auxin efflux regulator PIN4. PIN4 is asymmetrically localized towards the auxin response maximum in embryonic and seedling root meristems. ATP-binding cassette mutants are defective in the establishment and maintenance of an endogenous auxin gradient, and display patterning defects in both developing and mature roots. The authors propose a role for ATP-binding cassette in generating a sink for auxin below the quiescent center that is essential for auxin distribution and patterning.


This work localizes the putative auxin influx carrier AUX1 in the Arabidopsis root apex. The asymmetric localization of AUX1 at the upper side of protophloem cells suggests a connection between AUX1 and the phloem-based IAA transport pathway. Auxin analyses show that IAA content in the roots of the aux1 mutant is lower than that in wildtype roots, consistent with a phloem-unloading function for AUX1. AUX1 localization to columella and lateral root cap tissues rationalizes the agrativore phenotype of aux1.


The distribution and biosynthesis of IAA was investigated during early plant development in Arabidopsis. The youngest leaves exhibit the highest relative capacity for IAA synthesis. However, all other parts of the plant, including the cotyledons, expanding leaves and root tissues, are able to synthesize IAA de novo.


The authors present data that support the contribution of the putative auxin influx carrier AUX1 to loading of IAA from source tissues in leaves into the vascular transport system. The data also support a role for AUX1 in unloading IAA in the primary root apex and lateral root primordia. The authors suppose that auxin supplied from the apical source by this transport route mediates the development of lateral roots.


The authors use inhibition of Golgi-mediated protein traffic and protein synthesis, combined with measurements of the rate of IAA efflux, to demonstrate that an essential protein component of the auxin efflux carrier system is targeted to the plasma membrane. The protein is targeted through the secretory system and turns over rapidly without a requirement for concurrent protein synthesis.


The polar localization of PIN1 in root cells results from its rapid actin-dependent cycling between the plasma membrane and endosomal compartments. Auxin-transport inhibitors block PIN1 cycling and inhibit vesicle trafficking of other membrane proteins that are unrelated to auxin transport. In addition, brefeldin A mimics the physiological effects of auxin transport inhibitors. These data suggest that PIN1 cycling is required for auxin transport and that auxin efflux inhibitors act as inhibitors of vesicle trafficking.


The endodermis-specific expression of SGR2 and ZIG/ZAG (ZIG) complement the abnormalities in amyloplast sedimentation and shoot gravitropism of the sgr2 and zig mutants, respectively. ZIG encodes A VT11, which is a SNARE involved in vesicle transport to the vacuole. SGR2 encodes a phospholipase-like protein and localizes to the vacuole. These observations indicate that the vacuole participates in the early events of shoot gravitropism.
