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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Abbreviations

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 Chiřiby 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 inverted 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 previous roots and the young man was set free.

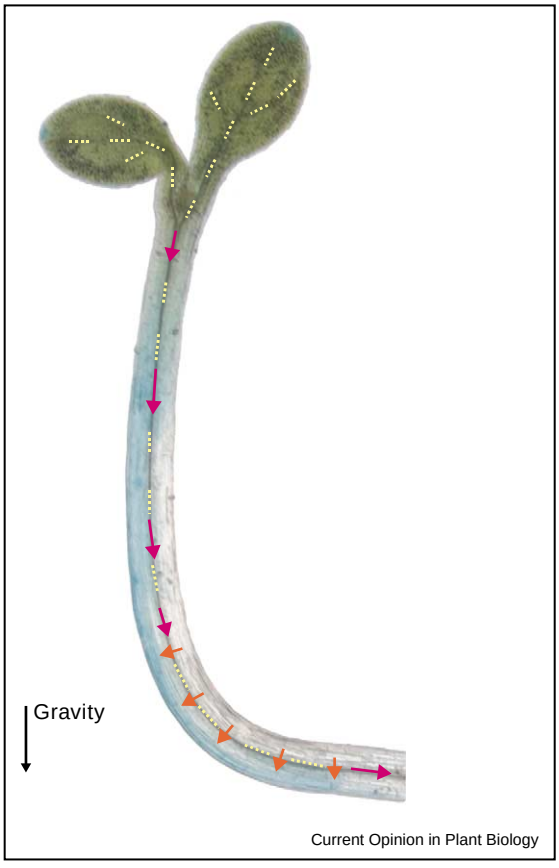
This rather romantic story demonstrates the fascinating plasticity of plant growth, which plants developed as an answer to their sessile fate and which became their major adaptation strategy. Special meristem tissues have evolved, which maintain the ability of plant cells to divide and differentiate throughout the life of the plant, and a number of differentiated cells keep their potential to elongate, forming the basis of plant tropisms. Thus, plants can flexibly change their shape and size to optimally adjust themselves to a changing environment. During the past century, an endogenous plant signal, auxin, and its distribution in the plant have been increasingly established as playing a central role in these complex adaptation responses. Recently emerging molecular data have shed light on the mode of auxin action and its regulated transport, and have begun to connect the plasticity of whole-plant development with processes at the cellular level.

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

Figure 1



Auxin response and transport in a gravistimulated *Arabidopsis* hypocotyl. Auxin transport throughout the plant involves both non-polar transport in phloem (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.

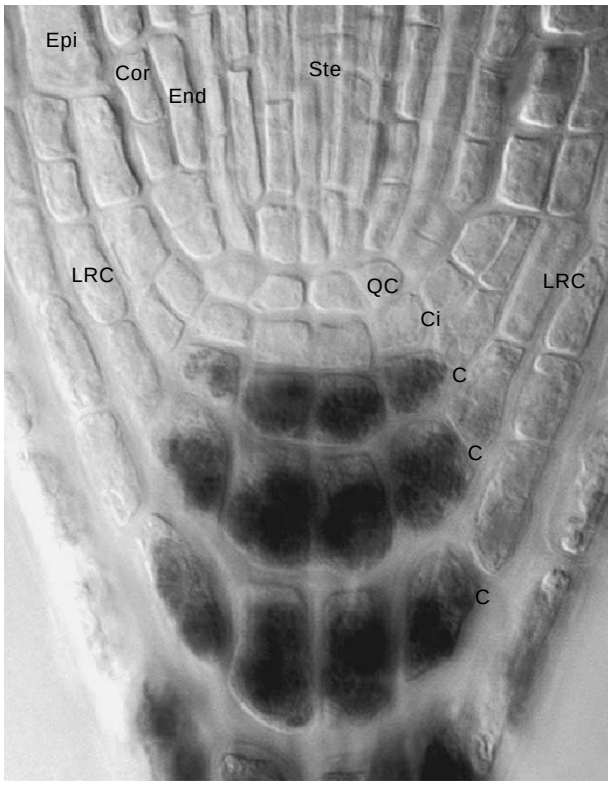
localized in different cells, and this localization impressively coincides with the known directions of auxin flux [8,9,10••,11•]. The AUX1 protein also localizes asymmetrically in root protophloem cells at the opposite cell side from PIN1 [12•]. These findings suggest that, at least in some tissues, 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-naphthylphthalamic 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 hormones 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;

Figure 2



Pattern of different cell types in a lugol-stained *Arabidopsis* root meristem. The quiescent center (QC) in the middle is surrounded by undifferentiated initial cells, such as columella initials (Ci) that give rise to differentiated lugol-stained layers of columella (C). Differentiated cell types such as epidermis (Epi), cortex (Cor), endodermis (End), stele (Ste) and lateral root cap (LRC) are indicated. The regular and invariant pattern of cell-fates correlates with the auxin gradient, which reaches its maximum in the columella initials.

second, it instructs responding cells directly (not through another signaling pathway or by signal relay); and third, a cell's response to a morphogen is dependent on morphogen concentration [22,23]. Does auxin fulfill these criteria? Using new analytical tools, a graded distribution of free auxin (i.e. an auxin gradient) has been demonstrated, for example, in Scots pine or along the *Arabidopsis* root [24,25]. Similarly, auxin reporters that are based on auxin-inducible promoters (e.g. DR5) have been used to indirectly confirm the existence of an auxin gradient within the root meristem, with its maximum in the columella initial cells (see Figure 2; [26]). The use of these reporters may be inadequate, however, especially when the throughput of the auxin signaling pathway becomes limiting; nonetheless, in tested situations, their activity correlates with direct auxin measurements [11•,25].

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

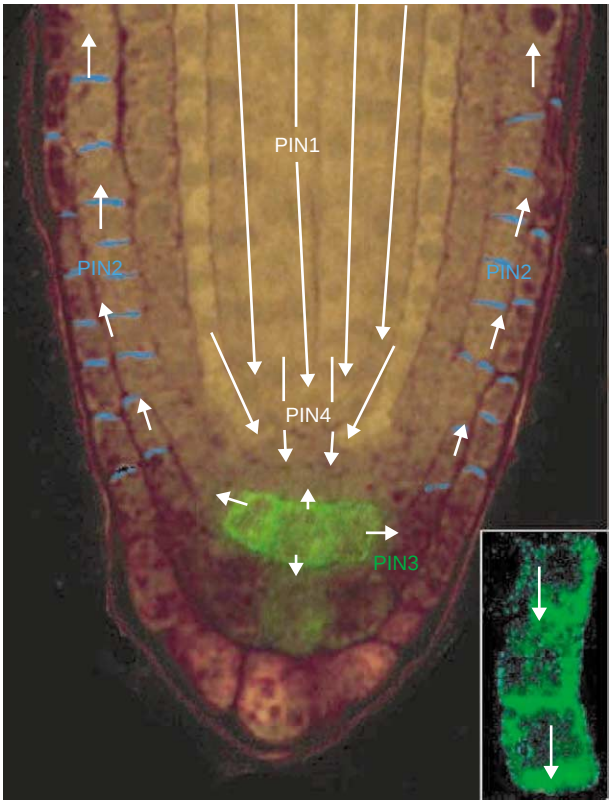
Another process that influences plant shape is directional bending with respect to an exogenous stimulus (mainly to light or gravity), which is called tropism. The role of auxin in tropisms was implied by the Cholodny–Went hypothesis, which suggests that unequal distribution of auxin between the opposite 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

Figure 3



Immunolocalization of the PIN3 protein in the *Arabidopsis* root apex and probable routes of polar auxin transport (white arrows). PIN3 (in green) is localized symmetrically in columella cells and apparently mediates lateral auxin distribution to all sides of root cap. After the root is turned by 90 degrees away from vertical (i.e. after a gravistimulus is applied), PIN3 rapidly relocates to the bottom side of columella cells (inset), and thus probably regulates auxin flux to the lower side of root. Auxin is further transported through lateral root cap and epidermis cells basipetally by a PIN2-dependent route (polar localization of PIN2 at the upper side of cells is depicted in blue) This basipetal transport also requires AUX1-dependent auxin influx. AUX1 is present in the same cells as PIN3 and PIN2. Auxin is supplied to the root cap by the PIN1- and PIN4-dependent acropetal route (which is depicted in white).

Morris and coworkers [31,32•] envisioned the rapid intracellular turnover of at least part of the auxin efflux complexes, 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 vasculature, 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.

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- • of special interest
- •• of outstanding interest

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The endodermis-specific expression of SGR2 and ZIGZAG (ZIG) complement the abnormalities in amyloplast sedimentation and shoot gravitropism of the *sgr2* and *zig* mutants, respectively. *ZIG* encodes AtVTI11, 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.

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