Leading Edge
Review

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Auxin: A Trigger for Change in Plant Development

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The dynamic, differential distribution of the hormone auxin within plant tissues controls an impressive variety of developmental processes, which tailor plant growth and morphology to environmental conditions. Various environmental and endogenous signals can be integrated into changes in auxin distribution through their effects on local auxin biosynthesis and intercellular auxin transport. Individual cells interpret auxin largely by a nuclear signaling pathway that involves the F box protein TIR1 acting as an auxin receptor. Auxin-dependent TIR1 activity leads to ubiquitination-based degradation of transcriptional repressors and complex transcriptional reprogramming. Thus, auxin appears to be a versatile trigger of preprogrammed developmental changes in plant cells.

Introduction

Plants and animals have different solutions to the problem of survival in ever-changing and often adverse environments. Whereas animals focus mainly on behavioral responses, such as fighting or running away, plants have a remarkable repertoire of developmental tricks to shape their body and optimize their metabolism to specific environmental demands. This developmental flexibility involves permanent stem cell populations called meristems, de novo organogenesis, a remarkable capacity for regeneration, and directional growth responses to external cues (reviewed in Davies, 2004 and Tanaka et al., 2006).

Crucial questions in plant developmental plasticity concern how external cues are translated into specific developmental changes and how these are internally coordinated. Similar to animal hormones, small endogenous signaling molecules coordinate developmental and physiological processes in plants. As with their animal counterparts, plant hormones act at low concentrations and are often not synthesized where they act (reviewed in Davies, 2004). However, there are differences in the roles of hormones in plants and animals. For example, in animals, tissue morphogenesis often occurs through defined and invasive cell migration, whereas plant cells are immobilized due to rigid cell walls and thus have to rely on mobile signals to trigger comparable tissue reprogramming. In general, the synthesis of hormones in plants is not restricted to a particular, specialized tissue. Moreover, there is also extensive crosstalk between different hormonal and other signaling pathways that ultimately determine physiological outcomes (reviewed in Davies, 2004).

The plasticity of plant development and its responsiveness to a multitude of environmental situations suggests that regulatory mechanisms are very complex. Although numerous substances are recognized to function as plant hormones, plant shape is largely controlled by a rather simple indolic molecule—auxin (reviewed in Tanaka et al., 2006). An important level of regulation in auxin action is the differential distribution of auxin between cells of a tissue (auxin maxima or gradients). Auxin gradients are implicated in many auxin-mediated developmental processes (Figure 1). So far, the establishment of auxin gradients has been attributed to local auxin biosynthesis (Cheng et al., 2006, 2007; Stepanova et al., 2008; Tao et al., 2008) and directional intercellular auxin transport (reviewed in Tanaka et al., 2006), which are both controlled by diverse environmental and developmental signals. Thus the modulation of auxin distribution provides a means to efficiently integrate these signals.

Many, if not all, plant cells have the ability to interpret auxin signals through a short nuclear signaling pathway. This pathway involves a plant-specific receptor mechanism in which the ligand (auxin) does not trigger an allosteric change in the receptor (Tan et al., 2007). Instead, auxin binding stabilizes the interaction between the receptor, an F box protein called TIR1, and a group of transcriptional repressors called Aux/IAAs (Dharmasiri et al., 2005a; Kepinski & Leyser, 2005), targeting them for proteolysis (Gray et al., 2001). Using this short pathway, every cell has the capacity to execute a transcriptional response that is presumably preprogrammed. Thus, spatiotemporal control over local auxin distribution can select cells within a tissue to execute a specific change in developmental program. This mechanism seems to play an important role in conveying positional information during developmental processes such as embryogenesis, organogenesis, tissue patterning, and tropisms.

Auxin Distribution Patterns: Caught in the Act

One of the important discoveries of the last decade in the auxin field is the finding that auxin is distributed differentially within plant tissues (Figure 1), a feature that appears universally associated with auxin action (reviewed in Tanaka et al., 2006). In some cases auxin accumulates locally within a single cell or a small group of cells (auxin maxima); in other cases its distribution between cells

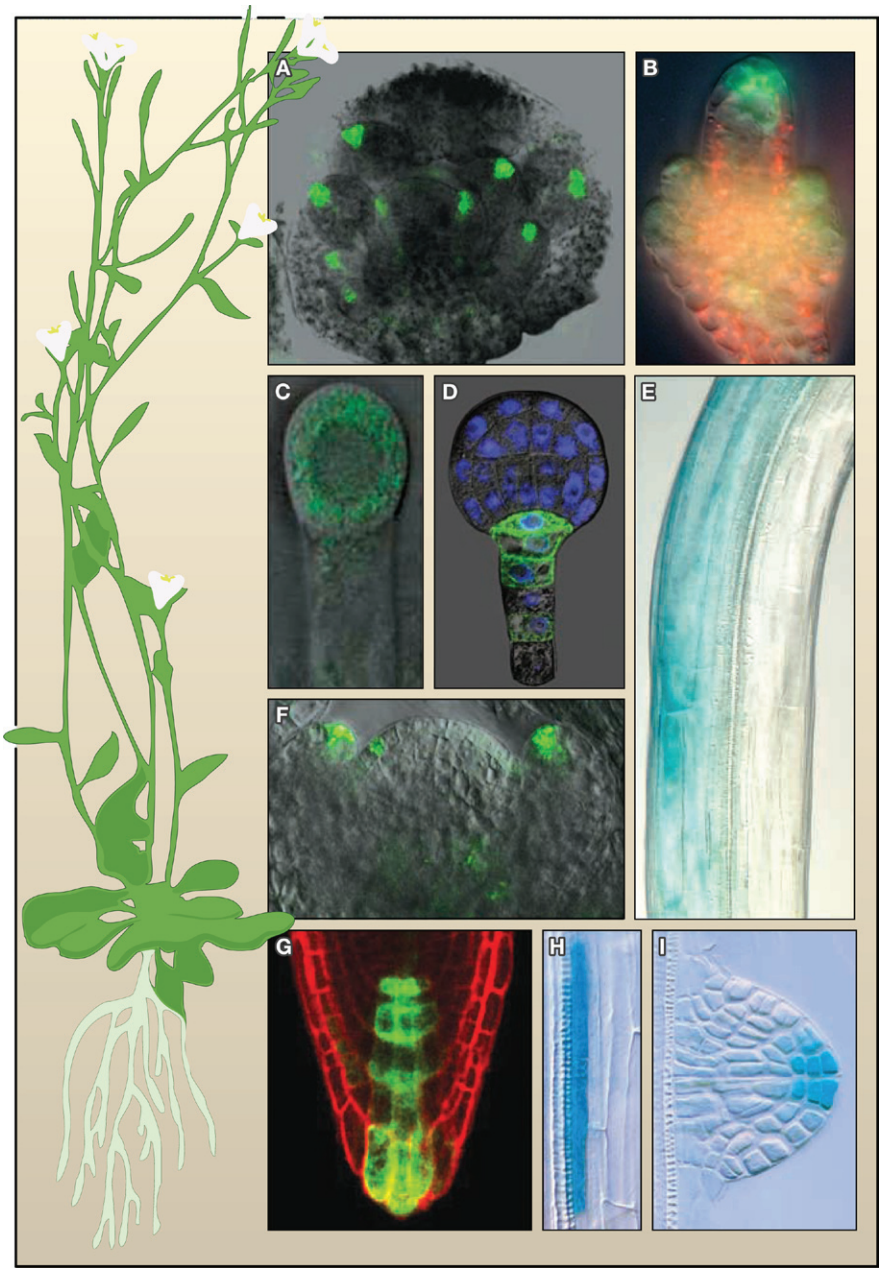

Figure 1. Patterns of Auxin Distribution during *Arabidopsis* Development

Distribution of auxin activity as visualized by the activity of auxin response reporters expressing green fluorescent protein (*DR5rev::GFP)* (A, B, C, D, F, and G) and *DR5::GUS* (E, H, and I). The following are locations of auxin activity: (A) tips of floral organ primordia within developing flowers, (B) the tip of the ovule primordium, (C) the apical cell of a divided zygote, (D) a root pole of the globular stage embryo, (E) the shade side of the photostimulated hypocotyls, (F) the position of incipient organ initiation and tips of flower primordia at the shoot meristem, (G) the root apical meristem, (H) lateral root initiation site, (I) and the tip of the emerging lateral root primordium. Image in (C) is reprinted with permission from Macmillan Publishers Ltd: Nature *426*, 147–153, copyright (2003). Image in (D) is reprinted with permission from Macmillan Publishers Ltd: Nature *415*, 806–809, copyright (2002).

is better described as a gradient (in this context the term "gradient" should be used according to its original meaning, namely as a graded distribution). Notably, not only auxin accumulation but also its depletion in some cells appears to play an important role in auxin-mediated development (Sorefan et al., 2009). It is important to note that most studies describing auxin distribution rely on indirect methods for visualizing auxin activity based on the expression of auxin-responsive genes. However, these patterns have been verified by more direct methods in the case of meristematic tissues, organogenesis, and auxin distribution during tropisms (reviewed in Tanaka et al., 2006).

The concept of differential auxin distribution emerged almost a century ago in studies that assessed the mechanisms underlying directional growth in response to environmental stimuli such as light or gravity (tropisms). Depending on concentration and tissue, auxin either stimulates or inhibits cell elongation (Thimann, 1938). Thus, a stimulus-induced differential auxin distribution across the organ, such as a root or stem, leads to differential growth resulting in organ bending. Based on this principle, roots and shoots redirect their growth during tropic responses in opposite ways. For example, gravistimulation leads to an increased auxin accumulation at the lower side of roots or shoots (reviewed in Tanaka et al., 2006). In roots, this auxin accumulation inhibits elongation and roots bend downward, whereas in shoots, it stimulates growth and shoots bend upward. Similarly, in unilateral light, auxin accumulates at the shaded side of shoots, causing them to bend toward the light (reviewed in Whippo and Hangarter, 2006). Besides tropisms, differential auxin distribution and response is also associated with differential growth during the formation of the apical hook of germinating plantlets—a structure that protects the delicate young apical meristem during soil penetration, with a maximum at the inner side of the hook (Friml et al., 2002b). Auxin also plays an important role during the so-called shade avoidance syndrome. When plants are overgrown by their neighbors and compete for sunlight, shade-induced changes in light quality trigger local auxin biosynthesis that accelerates stem elongation (Tao et al., 2008).

Besides fulfilling an important role in regulating growth in response to environmental stimuli, auxin has multiple functions in patterning and organogenic processes. In early embryogenesis, a highly dynamic pattern of auxin accumulation marks sites of major developmental decisions, such as specification of the apical cell and later establishment of the root pole and

cotyledons (Friml et al., 2003). During post-embryonic growth of plants, differential auxin activities form developmental hallmarks of de novo organogenesis for leaves, flowers, floral organs, and lateral roots (Benková et al., 2003). First, auxin accumulates at the location of organ initiation (Dubrovsky et al., 2008; Heisler et al., 2005), and next, an auxin gradient is established along the growth axis of the developing primordium with the maximum at its tip (Benková et al., 2003). Once a lateral or primary root meristem becomes functional, a stable auxin gradient forms with its maximum in the quiescent center and young columella cells (Sabatini et al., 1999; Friml et al., 2002a), which is required to maintain the pattern of the root meristem (Blilou et al., 2005). Moreover, differential auxin distribution appears to be important within developing leaves, where increased auxin activity corresponds with the formation of vascular tissue during leaf venation (formation of veins) (Mattsson et al., 2003; Scarpella et al., 2006).

These examples show that differential auxin distribution within plant tissues accompanies important developmental decisions (Figure 1). Moreover, they reflect the involvement of auxin in many, sometimes apparently unrelated, aspects of plant growth and development. The versatility of auxin is somehow discomforting as it is difficult to envision a common mechanism by which such a simple chemical substance can act as a specific signal in such a variety of processes.

Auxin Distribution Makes a Difference

Despite the impressive correlation between the occurrence of auxin maxima or gradients with the initiation of many auxin-dependent processes, crucial questions remain: What is the relevance of differential auxin distribution for development? Are they a causal factor in these processes or are they merely part of ongoing developmental programs that were triggered by another signal(s)? A number of studies suggest that differential auxin distribution is required for the corresponding developmental processes. For example, in vivo time-lapse imaging of organ initiation at shoot apical meristems (Heisler et al., 2005) and lateral root organogenesis (Dubrovsky et al., 2008) revealed an absolute spatial and temporal correlation of auxin maxima with developmental reprogramming and resulting organ initiation. Importantly, genetic or pharmacological interference with differential auxin distribution and response hinder all the developmental processes where auxin gradients have been observed, corroborating their requirement for these processes (reviewed in Tanaka et al., 2006).

Moreover, differential auxin distribution is not only a necessary part of developmental programs but also seems to be a sufficient signal to trigger them. Elegant experiments with microapplication of auxin to the shoot apical meristem demonstrate that a local increase in auxin is sufficient for initiation and complete formation of leaves or flowers (Reinhardt et al., 2003). Similarly, random stimulation of auxin biosynthesis in a single pericycle cell causes a localized increase in auxin that is sufficient to induce the initiation and formation of lateral roots from these competent cells (Dubrovsky et al., 2008). These studies indicate that auxin accumulation in a given cell is capable of modifying its developmental program. In other words, the timing and location of auxin accumulation determine spatio-temporal aspects of developmental reprogramming.

Auxin Biosynthesis: Homemade Auxin Counts

The differential auxin distribution within tissues reflects differences in auxin concentration between individual, neighboring cells. In principle, the cellular concentration of auxin can be controlled at multiple levels, such as biosynthesis, conjugation, deconjugation, degradation, and intercellular transport. Clearly, these multiple redundant pathways contribute to auxin production to a different extent, in different plant species, and at different developmental stages.

Our understanding of these processes is still fragmented. Nonetheless, based on presumptive identified intermediates, genetic studies, and in vitro assays, a sketch of the auxin biosynthetic pathways can be made. Thus far, one tryptophan (Trp)-independent and four Trp-dependent pathways for the IAA biosynthesis have been proposed, each of them designated for an intermediate that is a hallmark of the pathway. These are the indole-3-acetamide (IAM) pathway, the indole-3-acetaldoxime (IAOx) pathway, the tryptamine (TAM) pathway, and the indole-3-pyruvic acid (IPA) pathway (reviewed in Woodward and Bartel, 2005). Due to the unknown identities of some key enzymes and extensive functional redundancy, the importance of each of these pathways in the auxin biosynthesis has been difficult to assess. Thus far, only the TAM and IPA pathways have been highlighted as relevant to development in planta.

Rate-limiting for the TAM pathway are flavin monooxygenase-like enzymes of the YUCCA family (Zhao et al., 2001). They catalyze conversion of the Trp-derivative, TAM, to *N*-hydroxyltryptamine (Zhao et al., 2001), a precursor of IAOx that can be subsequently used in the biosynthesis of IAA. Mutation of multiple *YUCCA* genes with overlapping expression domains impairs local auxin accumulation to the extent that it results in severe developmental defects (i.e., defects in leaf venation, root pole specification, and floral organ patterning) (Cheng et al., 2006, 2007). These observations illustrate that YUCCA-mediated local auxin biosynthesis aids in the generation of auxin gradients in various developmental processes.

The IPA pathway has only recently been demonstrated in plants with the identification of a gene family encoding aminotransferases that catalyze the transamination of Trp to form IPA (Trp aminotransferase of *Arabidopsis*, TAA). In mutants defective in *TAA* genes, free IAA levels were reduced, demonstrating that the TAA-dependent IPA pathway contributes to IAA production (Stepanova et al., 2008; Tao et al., 2008). Importantly, these mutants have an impaired differential distribution of auxin that correlates with pronounced defects in gravitropism, embryogenesis, and vascular tissue differentiation (Stepanova et al., 2008). Given the similarities of some of the phenotypes between the mutants defective in the YUCCA- and TAA-dependent pathways, it will be interesting to test whether the TAM and IPA pathways represent independent or, at least partially, overlapping pathways for auxin production. Interestingly, diverse signals, such as light quality (Tao et al., 2008) and phytohormone ethylene (Stepanova et al., 2008), regulate TAA transcription and thus contribute to the control of auxin distribution and related development.

In summary, the characterization of mutants defective in rate-limiting enzymes of auxin biosynthesis together with the expression patterns of these enzymes, which are sometimes

highly localized, suggests an important role for local auxin biosynthesis in the generation of differential auxin distribution. However, this mechanism alone cannot account for the role of auxin as a coordinating signal between cells. In such a scenario, another intercellular signal would be needed to determine the spatio-temporal aspects of local auxin biosynthesis. On the other hand, intercellular transport could in theory act to coordinate tissue development, given that local auxin accumulation depends on the auxin transport activity of neighboring cells.

Auxin Transport: A Molecular Update on the Chemiosmotic Model

A unique feature that sets auxin apart from other plant signaling molecules is its ability to move between cells in a directional manner. Early experiments with pharmacological inhibitors of auxin transport revealed how crucial this process is for auxin action in regulating physiology and development. Although the mechanisms of action for inhibitors of auxin transport remain mostly unclear (Dhonukshe et al., 2008a), these compounds have been invaluable tools to demonstrate the developmental importance of auxin transport and to study its mechanism.

In plants two distinct major pathways to translocate auxin are used: One is for rapid, long-distance source-to-sink transport from young shoot tissues, which are biosynthetically highly active, toward sink tissues. This type of nonpolar auxin distribution occurs by loading of auxin into the major secondary metabolite highway, the mature phloem, by which it is passively distributed throughout the whole plant and unloaded again in sink tissues, such as the root (Marchant et al., 2002). The other, slower type of transport appears important for auxin distribution over shorter distances. In the latter, transport occurs in a cell-to-cell manner and depends on specific influx and efflux carrier proteins. One of the main features of this type of auxin transport is that its directionality is strictly controlled within a given tissue. In an attempt to grasp the mechanism of polar auxin movement, the chemiosmotic hypothesis (Figure 2) was derived from the biochemical and physiological data available at the time (Rubery and Sheldrake, 1974; Raven 1975). It proposed that differences in auxin lipophilicity in the apoplast and cytoplasm are the basis for the preferential accumulation of auxin in the cytoplasm. As auxins are weak acids (pKa = 4.75), a portion (~15%) becomes protonated in the relatively acid environment of the apoplast (pH 5.5), thereby becoming relatively lipophilic and able to diffuse through the cell membrane. Once in the neutral cytoplasm, auxins become deprotonated, can no longer permeate easily through the plasma membrane, and get "trapped" inside the cell. This diffusion-based mechanism is further aided by active auxin uptake by specific auxin influx carriers. To exit the cell, auxin must be transported actively through the plasma membrane necessitating the existence of specific efflux carriers. The chemiosmotic hypothesis also made the visionary prediction that if rate-limiting components of the transport, auxin efflux carriers, were localized differentially on one side of the cells, this would ultimately lead to a unidirectional flow of auxin within a field of cells.

Recent genetic studies indeed provided support for the predicted importance of apoplastic and cytosolic pH on auxin transport. In an *Arabidopsis* knockout mutant lacking

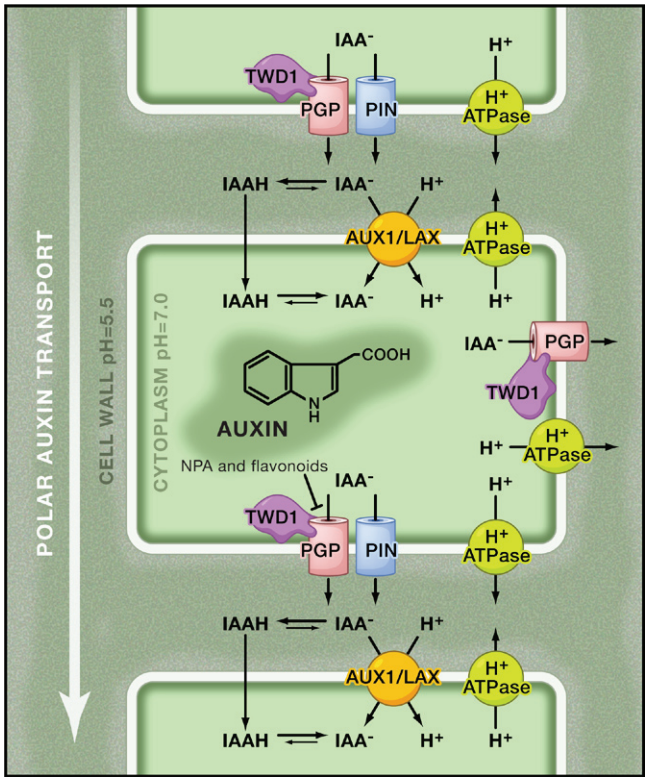

Figure 2. Chemiosmotic Hypothesis for Polar Auxin Transport

The low pH in the apoplast (cell wall) is maintained through the activity of plasma membrane H^+ ATPases. In the relatively acidic environment, a fraction of the weak acid, indole-3-acetic acid (IAA), the major form of auxin, becomes protonated. The protonated (IAAH) form is more lipophilic and can diffuse freely through the plasma membrane into the cell. Besides passive diffusion, auxin is also actively taken up from the apoplast by H^+/IAA^- symport mediated by AUX1/LAX influx carriers. Once inside the neutral cytosol, auxin is deprotonated and becomes trapped inside the cell. Auxin can leave the cell by auxin efflux carriers such as PIN-FORMED (PIN) proteins and P-glycoproteins (PGP) of the ATP-Binding Cassette family B (ABCB) transporter family. ABCB activity can be modulated by 1-naphthylphthalamic acid (NPA) and flavonoids that interfere with the interaction of ABCB and a protein that regulates it, TWISTED DWARF 1 (TWD1). The polar subcellular localization of PINs determines the direction of auxin flow out of the cell and thus the unidirectional auxin flow within tissues.

the H^+-pyrophosphatase AVP1, the acidity of the apoplast is decreased, which correlates with a reduction in polar auxin transport (Li et al., 2005). On the other hand, *AVP1* overexpression acidifies the apoplast and leads to increased polar auxin transport.

Components of auxin influx were identified by molecular analysis of the *auxin1* (*aux1*) mutant, which was originally found in a screen for roots that grew in an auxin-resistant manner (Pickett et al., 1990). Importantly, the *aux1* mutant is only strongly resistant to membrane-impermeable auxin, which requires active auxin uptake to get inside the cell, suggesting that the *aux1* mutation interferes with auxin uptake. Indeed, *AUX1* encodes an amino acid permease-like protein (Bennett et al., 1996) that acts as a H^+/IAA^- symporter (Yang et al., 2006). In total, the *Arabidopsis* genome encodes four of these high-affinity auxin influx carriers (AUX1/LAX) (Swarup et al., 2008), and they play important roles in processes such as gravitrop-

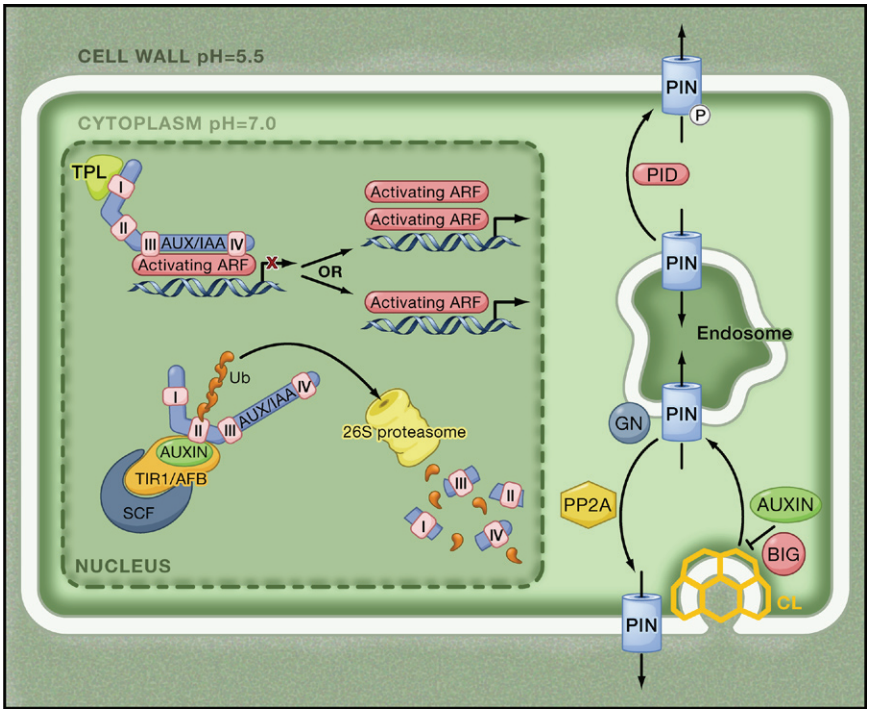

Figure 3. Cellular Auxin Signaling

At low auxin concentrations, Aux/IAA transcriptional repressors are more stable and dimerize through their domains III and IV with auxin response factor (ARF) transcription factors. Through their binding to ARFs, Aux/IAA transcriptional repressors recruit the transcriptional corepressor TOPLESS (TPL) to activating ARFs, by which they are rendered transcriptionally inactive. At higher concentrations, auxin serves as molecular glue between domain II of Aux/IAA transcriptional repressors and TIR1/AFB F box proteins, thereby stimulating Aux/IAA ubiquitination by $SCF^{TIR1/AFB}$ E3 ligase and causing subsequent targeting for proteolysis mediated by the 26S proteasome. Degradation of Aux/IAAs derepresses the ARF activity on transcription. It is not clear whether ARFs act as monomers, dimers, or both. Outside the nucleus, PIN auxin efflux carriers cycle continuously between endosomal compartments and the plasma membrane. The exocytotic step requires the activity of GNOM, an ADP-ribosylation factor GTPase guanine nucleotide exchange factor (ARF-GEF), whereas endocytosis occurs in a clathrin-dependent manner. The PIN phosphorylation status, controlled by counterbalancing activities of PINOID kinase (PID) and protein phosphatase 2A (PP2A), affects the affinity for the apical or basal targeting pathways. Auxin inhibits PIN endocytosis through an unknown mechanism that requires BIG protein, the function of which is unclear.

ism, phototropism, lateral root spacing, phyllotactic patterning (the regular arrangement of leaves or flowers on a stem), lateral root emergence, and root hair development (Bennett et al., 1996; Stone et al., 2008; Bainbridge et al., 2008; Swarup et al., 2008; Jones et al., 2009).

Other important components of the auxin transport machinery are P-glycoproteins of the ABCB transporter family (ABCB/PGP). This subgroup of ATP-binding cassette (ABC)-type transporters have been identified as interactors of the auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA) (Murphy et al., 2002). The best characterized are ABCB1/PGP1, ABCB4/PGP4, and ABCB19/PGP19 that mediate auxin efflux both in plant and non-plant systems (Geisler et al., 2005; Cho et al., 2007). Interestingly, synthetic and natural auxin transport inhibitors, such as NPA and flavonoids, directly affect ABCB activity by disrupting their interaction with the immunophilin-like FKBP42 TWISTED DWARF1 (TWD1) (Bailly et al., 2008). In auxin efflux, ABCBs act in concert with another group of auxin efflux carriers, the PIN proteins (Blakeslee et al., 2007; Mravec et al., 2008). PIN proteins are named after PIN-FORMED1, the prominent family member, whose loss of function leads to a characteristic needle-like inflorescence that is one of the hallmarks of defective auxin efflux (Gälweiler et al., 1998). Also other single and multiple *pin* mutants show typical defects in auxin-related processes, such as tropisms, embryo development, root meristem patterning, organogenesis, and vascular tissue differentiation (Luschnig et al., 1998; Friml et al., 2002a, 2002b, 2003; Benková et al., 2003; Reinhardt et al., 2003; Blilou et al., 2005; Scarpella et al., 2006). PIN proteins are plant-specific transmembrane proteins with a predicted topology reminiscent of carrier proteins. Importantly, as predicted by the chemiosmotic model for efflux carriers, PINs show polar, subcellular localizations that correlate with known directions of auxin flow. Indeed, PIN proteins are capable of mediating auxin efflux from plant, mammalian, and yeast cells (Petrášek et al., 2006). Detailed studies of *PIN* expression and localization as well as auxin distribution in *pin* mutants reveals that PIN proteins form the backbone of directional distribution networks that mediate auxin maxima and gradients during different developmental processes (reviewed in Vieten et al., 2007).

Based on the wealth of data accumulated since the formulation of the chemiosmotic hypothesis, a molecular update can now be assembled (Figure 2). Active apoplast acidification by proton pumps favors the protonation of IAA outside the cell. Protonated IAAH can diffuse through the plasma membrane inside the cells. In specific developmental situations, passive auxin uptake is additionally supported by the influx activity of AUX1/LAX uptake carriers. Once inside, IAA is deprotonated, thus becoming trapped in the pH-neutral cytosol. It can exit the cell by the activity of PIN and ABCB efflux carriers, which may or may not have a polar subcellular localization. The efflux activity of ABCBs, which is predominantly nondirectional, might mainly control the amount of auxin available in "auxin channels." In contrast, PIN proteins with their predominantly polar localization are responsible for the vectorial aspect of intercellular auxin movement.

Flow Directionality: PINs Show the Way

The prediction by the chemiosmotic hypothesis that subcellular positioning of auxin transporters determines the direction of auxin flow is a central point as it provides a connection between signaling at the level of the individual cell and directional signaling within tissues. In this view, each cell within the path of auxin flow has the option to integrate multiple signals

and translate them into changes in expression and subcellular targeting of auxin transport proteins, thereby deciding how much auxin will be transported and in which direction.

The observation that critical components of auxin efflux, the PIN proteins, show polar subcellular localizations that correlate with known directions of auxin flow (reviewed in Tanaka et al., 2006) provides support for this concept. Furthermore, manipulation of PIN polarity either by disruption of sequence-encrypted polar targeting signals (Wiśniewska et al., 2006) or by changing the phosphorylation status of PIN proteins, which depends on the counterbalancing activities of PINOID kinase (Friml et al., 2004) and protein phosphatase 2A (Michniewicz et al., 2007), led to predicted changes in auxin distribution. These results demonstrate the crucial importance of PIN polarity in cells for directionality of auxin flow.

Subcellular PIN localization is very dynamic because PIN proteins undergo constitutive cycles of clathrin-dependent endocytosis (Dhonukshe et al., 2007) and ARF GEF-dependent exocytosis (Geldner et al., 2001). This constitutive endocytic recycling (Figure 3) enables polar resorting of PIN proteins after each internalization step and provides a plausible mechanism for rapid reshuffling of PIN proteins between different sides of a cell, a process analogous to transcytosis in animal cells (Kleine-Vehn et al., 2008). Such a switch in PIN polarity means that auxin fluxes can be rapidly redirected, for example in response to environmental signals (as demonstrated for gravity responses; Friml et al., 2002b) or in response to endogenous signals yet to be identified. Thus, different signals can be integrated at the level of individual cells to control the directionality of auxin flow and thus the auxin distribution within tissues.

Auxin in Loops: Paving Its Own Path

It is not only external signals that modulate PIN-dependent auxin transport. Auxin itself provides feedback regulation of its own distribution (reviewed in Benjamins and Scheres, 2008). This is most apparent during processes that involve complex changes in tissue and cell polarities such as the formation of organ primordia and de novo formation or regeneration of vascular tissues. In general, for tissue polarization, the individual polarities of all cells within a tissue need to be coordinated, implying that the polarity of each cell integrates information derived from the polarities of adjoining cells, position and orientation within the tissue, and position of that tissue within the whole plant. The so-called "canalization hypothesis" proposes that auxin acts as a polarizing cue and that the auxin flow through the tissue will gradually polarize cells in a coordinated manner (Sachs, 1991). In this view, auxin is postulated to have the ability to promote the capacity and directionality of its own flow, allowing self-organizing reinforcement of auxin transport channels.

Indeed, the behavior predicted by the canalization hypothesis is observed during de novo formation or regeneration of vascular strands. A study on auxin distribution and PIN-dependent transport during vascular vein patterning in developing *Arabidopsis* leaves shows that PIN1 is initially broadly expressed at places of auxin accumulation and later, as the leaf grows to maturity, *PIN1* expression becomes progressively more polarized and more narrowly restricted, defining the pattern of future vascular differentiation (Scarpella et al., 2006). Similarly, when existing vasculature is interrupted by wounding, auxin accumulates above the wound leading to a broad *PIN* expression that becomes gradually narrower and more polarized until the new path of auxin flow around the wound is established and the new vascular strand is thus delineated. Auxin seems to be the primary signal inducing this vascularization process because local auxin application is sufficient to induce *PIN* expression, gradual PIN polarization, and formation of new vascular strands originating from the position of auxin application (Sauer et al., 2006).

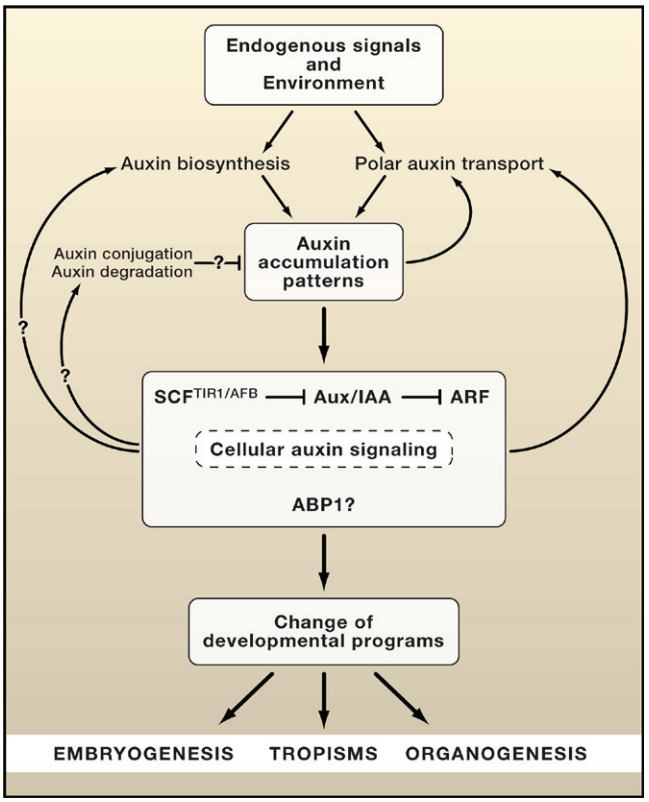

Figure 4. Feedback Regulation in Auxin Action

Endogenous and environmental signals modulate auxin biosynthesis and polar auxin transport. Auxin biosynthesis and intercellular transport, presumably in concert with auxin conjugation and degradation processes, determine auxin levels in individual cells and thus auxin distribution patterns within tissues. As a result of auxin accumulation, auxin signaling, dependent on cellular $SCF^{TIR1/AFB}$, Aux/IAA, and auxin response factors (ARFs), triggers predefined changes in developmental programs by activating expression of specific gene sets, including those coding for components of auxin transport and auxin synthesis. In addition auxin signaling provides feedback that regulates the rate and polarity of its own transport.

What are the mechanisms by which auxin affects the capacity and polarity of its own transport? PIN-dependent auxin transport is regulated by auxin at multiple levels (Figure 4). Auxin can influence transcription, turnover, and plasma membrane localization of PIN proteins. Short auxin treatments activate transcription of different *PIN* genes in various tissues (Vieten et al., 2005). By comparison, long-term treatments with high concentrations of auxin lead to an increased turnover of some PIN proteins (Sieberer et al., 2000; Vieten et al., 2005). Furthermore,

auxin inhibits endocytosis by an unknown signaling pathway and thus inhibits internalization of PINs, stabilizing them at the plasma membrane (Paciorek et al., 2005). These mechanisms of PIN regulation differ in time and auxin concentration characteristics; for example, stabilization of PIN proteins at the plasma membrane is rapid and transient, whereas auxin-induced PIN internalization and degradation occurs more slowly (Abas et al., 2006). These opposing effects of auxin on PIN abundance and activity must be tightly coordinated, but the mechanisms of their crosstalk remain unknown.

It is difficult to imagine the mechanisms by which auxin influences PIN polarity and thus the direction of its own transport. Are PIN proteins polarized by auxin flow or by the gradient, i.e., the difference in auxin concentration between opposite sides of the cell? How can auxin flow or differential auxin levels be perceived and translated into changes in PIN polarity? These are fascinating unanswered questions. Clearly, several auxin-dependent mechanisms for PIN polarization exist. For example, during regeneration of root meristems, changes in the local auxin accumulation first alter cell fate and subsequently redefine PIN polarity (Xu et al., 2006). In contrast, auxin application to roots can directly influence PIN localization, possibly through auxin-dependent transcriptional regulation of modifiers of PIN polarity (Sauer et al., 2006). The inhibitory effect of auxin on PIN internalization (Paciorek et al., 2005) might be part of another mechanism for PIN polarization. In this scenario, higher auxin levels on one side of the cell lead to an increased PIN abundance, thereby creating a greater auxin efflux and higher PIN stabilization by a positive feedback loop at that side of the cell. This mechanism may account for the rapid polarization of PIN proteins toward auxin maxima, for example during initiation of aerial organs at the shoot apical meristem (Heisler et al., 2005). Though still largely unknown, the underlying mechanisms by which auxin regulates PIN polarization are intensively studied because they are a necessary part of many current models of patterning and organogenesis in plant development (reviewed in Merks et al., 2007).

Auxin Signaling in the Nucleus: Short and to the Point

Patterns of differential auxin accumulation within a field of cells determine spatial and temporal patterns of auxin-dependent developmental reprogramming (Sabatini et al., 1999; Friml et al., 2002a, 2002b, 2003; Benková et al., 2003). Nonetheless, in the end, the decision on the type of developmental output lies in the interpretation of these auxin accumulations at the level of the individual cell. Despite the diversity of cellular responses evoked by auxin, most of the effects of auxin come down to one relatively simple pathway that directly impinges on transcriptional regulation. In essence, it is the interplay between two classes of transcriptional regulators that represents the core of auxin signaling.

One class, designated Aux/IAAs, negatively regulate auxin signaling. Aux/IAAs were originally identified through the analysis of genes that were rapidly upregulated after auxin treatment (Theologis et al., 1985). There are 29 members in *Arabidopsis* (Abel and Theologis, 1996). Typically, their protein structure involves four highly conserved domains. The first domain contains an ERF-associated amphiphilic repressor (EAR) motif near their N terminus that is essential for its role in transcriptional repression (Tiwari et al., 2004). This motif is required for the recruitment of the transcriptional corepressor TOPLESS (TPL) (Szemenyei et al., 2008). Several auxin-insensitive dominant and semidominant mutants in Aux/IAAs have amino acid substitutions in domain II (Rouse et al., 1998; reviewed in Mockaitis and Estelle, 2008). Interestingly, this domain is essential for auxin-stimulated Aux/IAA proteolysis (Gray et al., 2001). Indeed, these amino acid substitutions in domain II dramatically increase the half-lives of these proteins by making their degradation insensitive to auxin (Dreher et al., 2006). The last two domains of Aux/IAAs are also essential for their function as transcriptional repressors because they are involved in homo- and heterodimerization and interaction with the other important class of transcriptional regulators, the auxin response factors (ARFs) (Kim et al., 1997; Hardtke et al., 2004).

The ARFs are a class of plant-specific transcription factors (B3-type) that mediate auxin-dependent transcriptional regulation. They bind to the so-called auxin-responsive element, a consensus sequence found in promoters of auxin-inducible genes (Ulmasov et al., 1997). Members of the ARF multigene family (23 members in *Arabidopsis*) can either activate or repress transcription, depending on the amino acid sequence in a nonconserved central domain (reviewed by Guilfoyle and Hagen, 2007). Activating ARFs are negatively regulated by interaction with Aux/IAAs, which recruit the transcriptional corepressor TPL (Szemenyei et al., 2008). As auxin stimulates Aux/IAA turnover, activating ARFs are released from TPL-mediated repression and can promote transcription of auxin-regulated genes (Figure 3).

This simple model does not take into account the intrinsic complexity of ARF activity. Indeed, besides interaction with Aux/IAAs, their two conserved domains allow homo- or heterodimerization with each other (reviewed in Guilfoyle and Hagen, 2007). The function of this pairing is not clear, but it is tempting to speculate that different sets of ARFs and Aux/IAAs are produced in a given tissue, thereby favoring the formation of specific combinations that can drive or inhibit transcription of a particular set of auxin-responsive genes depending on developmental context. The hypothesis that different Aux/IAA and ARF pairs can have specific sets of target genes is corroborated by the observation that expression of different stabilized Aux/IAA repressors in the same cell types did not always yield the same phenotypic changes (Weijers et al., 2005; Muto et al., 2007). The recent discovery that ARF activity can also be modulated by interaction with other factors, such as MYB77 (Shin et al., 2007), suggests there might be even greater combinatorial possibilities in auxin-dependent transcriptional regulation.

Auxin Perception: Ligand or Glue?

The crucial, but for long unresolved, question in auxin signaling concerned the mechanism of auxin perception and the identity of the auxin receptor. In initial genetic screens, several components of the protein ubiquitination machinery were identified, in particular components and regulators of a Skp1-cullin-F box protein (SCF) E3 ubiquitin ligase, such as the F box component

called TIR1 (Leyser et al., 1993; Ruegger et al., 1998), implying that regulation of protein stability is a crucial part of auxin signaling. Given the rapid auxin-dependent turnover of Aux/IAA repressors, it was reasoned that the stability of Aux/IAAs is regulated by ubiquitination-dependent protein degradation. In this scenario, Aux/IAAs are recruited by SCF^{TIR1} and interact directly through their domain II with TIR1 in an auxin-dependent manner; they are then ubiquitinated and thus marked for degradation (Gray et al., 2001). Although all components of the pathway had been identified, the mechanism by which auxin could activate SCF^{TIR1} and promote its interaction with Aux/IAAs remained unclear. Through step-by-step analysis of the components affecting the TIR1-Aux/IAA interaction, it has been shown that auxin is the only regulatory element in this equation. TIR1 binds auxin at physiologically relevant concentrations (K_d ~20–80 nM), and auxin binding to TIR1 is required to stimulate the interaction between TIR1 and Aux/IAAs (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005). Crystallographic analysis of this interaction shows that TIR1 contains a hydrophobic pocket in which auxin and various auxin analogs can bind. Interestingly, auxin does not change the conformation of TIR1 but instead acts as a "molecular glue" by filling a hydrophobic cavity at the interaction interface, thereby enhancing TIR1-Aux/IAA interactions (Tan et al., 2007). These observations demonstrate that TIR1 is the long elusive auxin receptor. Moreover, at least three other TIR1-related F box proteins (Auxin-Binding F box [AFB]) also interact in an auxin-dependent manner with Aux/IAAs and are to a large extent redundant with TIR1 in mediating auxin responses (Dharmasiri et al., 2005b). The importance of the SCF^{TIR1} pathway is illustrated by the fact that for all components homologs can be found in ancestral genomes of land plants, such as that of the moss *Physcomitrella patens* (Rensing et al., 2008).

In summary, when the auxin concentration is low, Aux/IAAs are more stable and can interact with ARFs to inactivate them by recruiting the TPL corepressor. At increased auxin concentration, auxin stabilizes the interaction between TIR1/AFBs and Aux/IAA proteins. In association with $SCF^{TIR1/AFBs}$, Aux/IAAs become ubiquitinated and are targeted for proteolysis, resulting in ARF derepression and modulation of transcription (Figure 3). Although this auxin signaling pathway is very short and simple, the number of genes that are involved at each level is large, providing plants with an impressive array of combinatorial possibilities for auxin-regulated transcription. Thus cells can have many different possible outputs of auxin signaling depending on the developmental context.

It's Not All TIRs

Although many auxin responses, in particular those that are clearly associated with a developmental output, can be explained by transcriptional regulation mediated by $SCF^{TIR1/AFBs}$, some rapid cellular auxin effects probably do not involve transcription, suggesting the existence of additional auxin signal transduction mechanisms. Examples of cellular responses that might be independent of $SCF^{TIR1/AFBs}$ include a rapid (within 5 s) auxin-induced increase in cytosolic Ca^{2+} (Shishova and Lindberg, 2004) and auxin-induced proton secretion that occurs in less than 10 min (Senn and Goldsmith, 1988). The pathway by which auxin inhibits endocytosis and thus internalization of proteins including PIN auxin efflux carriers is also unclear (Paciorek et al., 2005). Taking into account the time lag required for degradation, transcription, and translation, it is hard to imagine that these rapid responses are mediated at the transcriptional level. Nonetheless, it cannot be excluded that TIR1-related pathways could also mediate these processes by a nontranscriptional, ubiquitination-dependent mechanism.

A good candidate for an additional auxin receptor is the Auxin-Binding Protein1 (ABP1) that was detected by biochemical studies aimed at identifying high-affinity auxin-binding proteins (Hertel et al., 1972). In more than 30 years of research, a large amount of biochemical data has been compiled, showing that ABP1 binds auxin with high affinity and specificity in different plant species. Overexpression of ABP1 confers an auxin-dependent expansion in the size of differentiated cells that are normally nonresponsive (Jones et al., 1998). On the other hand, loss of ABP1 function causes embryonic arrest, associated with misorientation of cell division planes and defects in cell elongation (Chen et al., 2001). Moreover, conditional inactivation of ABP1 dramatically impairs plant growth through effects on cell growth and cell-cycle progression (Braun et al., 2008). Also some rapid cellular responses to auxin have been connected to the ABP1 function. For example, auxin-induced hyperpolarization of the plasma membrane is achieved by antibodies against a specific part of ABP1 or directly by ABP1 peptides (Leblanc et al., 1999). Unfortunately, the data on the function of ABP1 in auxin-mediated responses are still fragmented and no downstream components of the signaling pathway(s) have been identified. Thus, the physiological relevance of auxin binding to ABP1 remains a matter of debate.

Another event triggered by auxin is a rapid (within 5 min) but transient increase in MAPK (mitogen-activating protein kinase) activity (Mockaitis and Howell, 2000; Lee et al., 2009). A decrease in auxin-responsive transcription is caused by the induction of the MAPK activity through transient expression of constitutively active MAPKKK (ANP1) (Kovtun et al., 1998). Moreover, silencing of MPK12 renders roots hypersensitive to auxin (Lee et al., 2009). In general, MAPK activity is counterbalanced through dephosphorylation by dual-specificity phosphatases. Interestingly, a mutation in the dual-specificity phosphatase IBR5 leads to auxin-resistant phenotypes and reduced auxin-responsive transcription, even though Aux/IAA proteins do not accumulate as seen in mutants that affect SCF^{TIR1} function (Strader et al., 2008). Correspondingly, IBR5 can interact with MPK12 and dephosphorylate it in vitro. Moreover, MPK12 silencing partially reverses the auxin insensitivity of plants deficient in IBR5 (Lee et al., 2009). Thus, the MPK12-IBR5-regulated MAPK pathway represents a new pathway activated by auxin that is potentially TIR1 independent and that negatively affects expression of auxin-responsive genes. Similarly, in yeast mating pheromone signaling, a MAP kinase (Fus3) mediates a fast negative feedback on the downstream system response (Yu et al., 2008). This negative feedback mechanism allows a broader range of information about pheromone concentration to be transmitted. It will be of interest to see whether the MPK12-IBR5 pathway involves a similar "dose-response alignment" mechanism for improving the range and fidelity of auxin signal transduction.

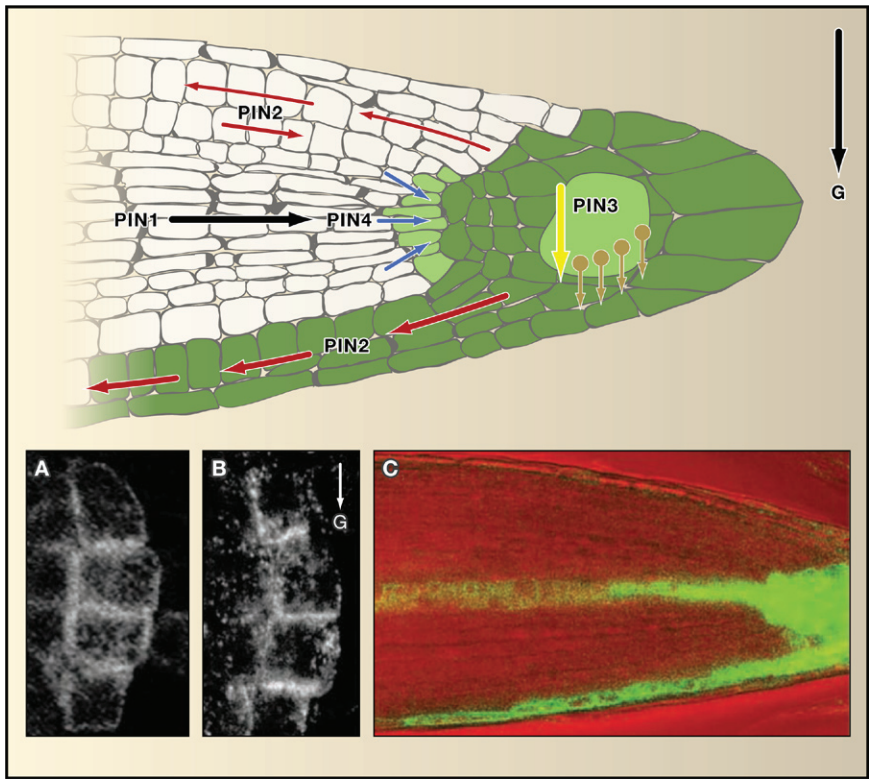

Figure 5. Root Gravitropism
PIN proteins control the distribution of auxin in the root. Within a vertically growing root, the auxin efflux carrier PIN3 is localized uniformly on all sides of root cap cells (A). Upon gravistimulation, starch-containing organelles (statoliths) sediment within these cells. (B) In a matter of minutes, PIN3 relocalizes to the bottom side of the cell. (C) As a consequence, auxin flow is redirected to the new lower side of the root tip, resulting in an auxin accumulation along the lower side of the root as visualized by an auxin response reporter (*DR5rev::GFP*). This differential auxin accumulation inhibits elongation and thus leads to downward bending of the root tip. Images in (A) and (B) are reprinted with permission from Macmillan Publishers Ltd: Nature *415*, 806–809, copyright (2002). Image in (C) is reprinted with permission from Macmillan Publishers Ltd: Nature *435*, 1251–1256, copyright (2005).

These examples demonstrate that some auxin-regulated processes cannot be easily attributed to the $SCF^{TIR1/AFB}$ pathway. Elucidation of the mechanisms of these alternative auxin signaling pathways and their eventual functional relation to the $SCF^{TIR1/AFB}$ pathway is an important task for future research.

Auxin as a Versatile Trigger of Developmental Change

The bewildering diversity of developmental responses mediated by auxin seems to be determined not only by the cellular signal transduction pathway but also by an additional level of regulation—the differential auxin distribution within tissues (auxin maxima and gradients). The temporal and spatial pattern of the auxin distribution is mediated by local auxin biosynthesis and directional intercellular auxin transport. In the cells where auxin accumulates, it triggers a change in the developmental program, a change that seems to be already preprogrammed in a given cell type. In other words, instead of instructing cells what to do, auxin accumulation selects a cell or cells at a particular position and at a particular time for a change in developmental program. This scenario explains the diversity of the effects of auxin. If auxin functioned as a versatile trigger of change, not surprisingly, the changes of many, otherwise unrelated developmental programs would depend on auxin. The developmental program initiated by auxin in a given cell can be preprogrammed, for example by different cell type-specific combinations of auxin-dependent transcriptional regulators. The potential number of distinct heterodimers between the 29 Aux/IAA transcriptional repressors and the 23 ARF transcription factors in *Arabidopsis* is very large and may provide sufficient diversity to convey the transcriptional changes needed for the myriad developmental programs that are controlled by auxin.

An important feature of the model based on auxin gradients is that developmental and environmental signals can be integrated and translated into a desired response through modulation of processes upstream of the differential auxin distribution. Indeed, local auxin biosynthesis and intercellular auxin transport can be modulated by environmental signals, such as light or gravity, as well as endogenous signals such as ethylene. Where the pattern of auxin-dependent development is preset by other signals, the local production of auxin is an effective way to accumulate auxin locally and to initiate and maintain the downstream developmental program. However, intercellular auxin transport has the additional capacity to mediate the communication between cells and coordinate the complex behavior of the whole group of cells in the course of a developmental process with the aid of self-regulatory loops.

An example of an environmental signal utilizing auxin distribution to execute a specific developmental program is root gravitropic bending (Figure 5). Gravity is perceived by the sedimentation of gravity-sensing organelles (statoliths) in the root tip. This process is closely followed by relocation of PIN3 auxin transporters to the lower sides of the same cells (Friml et al., 2002b), which in turn redirect the auxin flow to this side of the root, causing differential auxin distribution, asymmetric growth, and ultimately downward bending of the root. Another example of developmental regulation is the initiation of auxin biosynthesis in the proembryo and polarization of the PIN1 transporter to the lower sides of inner proembryo cells; the resulting downward flow of auxin from the proembryo signals the basal pole that a root meristem should be initiated (Friml et al., 2003). In this situation, auxin flow and auxin gradient formation coordinate the development of two clonally separated parts of the embryo, the apical parts and the root pole. If PIN polarization fails, the apical part of the embryo ectopically accumulates auxin giving a false signal for root initiation leading to the development of

root-like structures that are derived from embryonic leaf tissue (Dhonukshe et al., 2008b). Similar coordination of development occurs during the phyllotactic initiation of leaves or flowers at the shoot apical meristem. The combination of auxin sources and auxin sinks (represented by the already existing organ primordia) together with the auxin-dependent polarization of the PIN proteins leads to specific rearrangements, determining the position at which the next organ initiates (Reinhardt et al., 2003; Heisler et al., 2005). These examples clearly show the flexibility and versatility of developmental regulation that depends on auxin distribution.

The involvement of dynamic auxin gradients in cell fate determination during developmental programs raised the debate as to whether auxin acts as a morphogen (Friml, 2003). The morphogen concept, derived from the animal field, is not entirely strictly defined (Teleman et al., 2001; Entchev and González-Gaitán, 2002). Besides formation of a stable gradient throughout the tissue, the most important feature of a morphogen is that it elicits different cellular responses depending on its concentration. In some instances, the patterns of auxin distribution show very sharp boundaries and can be better described as local auxin maxima, whereas in others cases a clearly graded auxin distribution can be detected, such as across the wood-forming tissues of the Scott's pine (Uggla et al., 1996). What is highly relevant for plant biology, but currently unclear, is whether auxin can elicit cellular responses that differ depending on its cellular concentrations. If true, auxin would not only be a simple trigger of a change but would determine which of several predetermined developmental programs will be executed based on its cellular concentration, adding further to the impressive repertoire of mechanisms by which auxin regulates plant development.

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