**TEACHING TOOLS IN PLANT BIOLOGY™: LECTURE NOTES**

# The Story of Auxin

Auxin is a remarkable little molecule that plays a role in nearly every aspect of plant growth and development. No mutants have been identified that can grow without auxin; it appears to be absolutely required for plant survival. Auxin is universally present in all plants and is found in green algae as well as the more distantly related red and brown algae, although its function in these organisms is not well characterized. In angiosperms, auxin synthesis or signaling mutants are frequently small, under-scoring auxin's role as a growth promoter. However, auxin's role is much more that merely a growth promoter; it is also necessary for the specification and maintenance of the root apical meristem, the initiation of lateral roots and leaves, and the formation of developmental patterns. In this article, we can only describe a few of auxin's functions; readers are encouraged to read some of the many excellent review articles listed below for more information. Classic studies on auxin are described in two of Kenneth Thimann's review articles from 1938 and 1974. For a superb and more recent historical perspective, see Abel and Theologis (2010), who capture the story of auxin research thus, "the century-long endeavor is a beautiful illustration of the power of scientific reasoning and human intuition, but it also brings to light the fact that decisive progress is made when new technologies emerge and disciplines unite."

## EARLY STUDIES

Auxin was the first plant hormone isolated, and it is probably the most thoroughly studied of all plant growth regulators. Many generations of plant scientists have contributed to our under-standing of how auxin works. Early botanists carefully described plant growth, development, and movement and even proposed the existence of mobile signals to coordinate these activities. In the late 19th and early 20th centuries, a series of elegant experiments into the nature of shoot phototropism (moving toward light) led directly to the identification of auxin as a mobile signal regulating cell elongation. Most famously, Charles Darwin and his son Francis studied phototropism in coleoptiles, a tissue in monocots that protects young leaves during germination. In 1880, they determined that light given from one side is perceived at the coleoptile tip but that "some influence is transmitted from the upper to the lower part, causing the latter to bend." In 1913, Peter Boysen-Jensen furthered these studies, observing that the "influence" can move through an agar block but not a solid substance. Subsequently, Arpad Paal (1919) showed that removing the tip of a dark-grown coleoptile and replacing the tip asymmetrically onto the coleoptile base could induce curvature in the absence of a light stimulus. Building upon these studies, Frits Went placed coleoptile tips onto agar blocks and showed

that these treated blocks were capable of promoting growth; they had captured the growth-promoting substance. Went's experiments led to the purification and identification of the auxin indole-3-acetic acid (IAA). Auxins in fact are a family of related compounds, some of which are entirely synthetic but mimic auxin effects, whereas others are low-abundance compounds or found in only some plant families. In most discussions, auxin is used synonymously with IAA, which is the most abundant naturally occurring auxin.

Once it was available in purified form, auxin's contributions to root initiation, fruit development, cell elongation, and the suppression of lateral buds by the shoot apex (apical dominance) were recognized, as were some of the fundamental properties that contribute to auxin action. In the 1930s, Kenneth Thimann observed that different tissues differ in their sensitivity to auxin, and H.G. Van der Weij discovered the polar nature of auxin transport. In the latter part of the 20th and early 21st centuries the emerging tools of molecular biology and *Arabidopsis thaliana* genetics finally revealed the underlying molecular basis for auxin action, although the story remains incomplete. Current studies incorporate genomics technologies, systems biology approaches, and computer modeling to explore the mechanisms by which auxin coordinates plant growth and development, including the as yet unresolved ways in which auxin signaling interacts with environmental inputs and other hormone signaling pathways.

## AUXIN HOMEOSTASIS

Auxin's effects are strongly dependent on its accumulation in the right place at the right time. The combined effects of regulated auxin transport (discussed below) and biosynthesis, conjugation, and degradation see that this happens. At one point it was thought that auxin was exclusively produced in young leaves, but we now know that auxin is synthesized throughout the plant by a set of tightly regulated biosynthetic pathways. Most of the catalytic steps are performed by enzymes encoded by multiple genes, ensuring resiliency, flexibility, and specificity. IAA is mainly produced from the amino acid Trp, although there is evidence for another, Trp-independent pathway that is not well characterized. There are several parallel but intersecting pathways for conversion of Trp to IAA, deduced through genetic and biochemical studies. These pathways are often referred to by their key intermediates; the IPA pathway converts Trp to IAA via indole pyruvic acid (IPA), the IAM pathway through indole-3-acetamide, and the IAOx pathway through indole-3-acetaldoxime. The IAOx pathway may be restricted to *Arabidopsis* and its close relatives.

The two-step IPA pathway is thought to account for the majority of auxin biosynthesis in plants. The first step is the

conversion of Trp to IPA via the activity of a Trp aminotransferase enzyme, encoded by the *TRYPTOPHAN AMINO TRANFERASE OF ARABIDOPSIS1* (*TAA1*) gene. The second, rate-limiting step is the conversion of IPA to IAA by the YUCCA flavin mono-oxygenase enzymes. Both gene families are conserved amongst plants. In *Arabidopsis*, YUCCA is encoded by 11 *YUC* genes that have different cell type-specific expression patterns. The regulation of the activities of these genes and enzymes is extremely important in regulating auxin accumulation in response to light, temperature, and nutrients. Recently an enzyme that catalyzes the reverse reaction to TAA1 was identified. This enzyme, VAS1, uses the ethylene precursor methionine as a substrate, and so it effectively coordinates auxin and ethylene biosynthesis. This new finding sheds further light on the many ways these two hormones interact, including their synergistic effects on shoot elongation in response to vegetative shading.

IAA can be conjugated to other molecules to allow its storage in a biologically inactive form and to trigger its degradation. *GH3* genes encode auxin conjugases and are strongly induced by auxin, implying that conjugation is part of a negative feedback mechanism to regulate auxin activity. Similarly, tissues with the highest levels of auxin also accumulate the highest levels of auxin degradation products, suggesting that these tissues have a rapid rate of hormone turnover.

## TOOLS IN AUXIN RESEARCH

Many of our recent insights into auxin action are a consequence of our ability to examine auxin accumulation, action, and transport at the cellular level. The traditional and most precise method for IAA quantification is tissue extraction followed by gas chromatography–mass spectroscopy. Until recently this method was unable to provide cell-specific information, but a refinement has been developed in which cells can be isolated from *Arabidopsis* roots and sorted based on their expression of cell type–specific green fluorescent protein constructs. Using this method, the auxin concentration of pools of homogenous cell types has been measured by gas chromatography–mass spectroscopy, greatly refining our knowledge of auxin concentrations throughout the root and confirming the presence of a significant auxin maximum at the quiescent center of the root. Auxin-specific antibodies also have been used with varying degrees of success to analyze auxin distribution using immunolocalization methods. Recently-characterized auxin synthesis inhibitors will help explore auxin's roles as well as help define the auxin biosynthetic pathway in different tissues.

Auxin responses have been examined using two artificial sensors. The first uses a synthetic auxin responsive promoter called *DR5*, fused to a reporter gene encoding β-glucuronidase (which cleaves a colorless substrate to produce a blue precipitate) or green fluorescent protein. As this sensor monitors transcription of auxin responsive genes, it is termed an "output sensor". A second sensor, called DII-VENUS, is a constitutively expressed protein consisting of a nuclear-localized yellow fluorescent protein fused to an amino acid sequence that promotes proteolysis in the presence of auxin, the degron domain II of Aux/IAA protein (see below). At high auxin levels,

the fluorescent protein is degraded, leading to non-fluorescent regions against a background of fluorescence. Often, the spatial patterns conferred by the *DR5* and DII-VENUS reporters are complementary.

An important tool for examining patterns of auxin movement (from which some rough approximation of auxin levels can be inferred) comes from the study of auxin transport proteins, described below. Auxin movement through tissues is conferred to a large extent by the polar distribution of auxin influx and efflux proteins. For example, examining localization in neighboring cells of auxin efflux proteins of the PIN family gives an approximate picture of the direction of local auxin transport within a tissue. Using this method to monitor living tissues over time, very dynamic changes in PIN protein orientation have been revealed, as well as the important roles of auxin and auxin transport during development. Finally, computer models and simulations based on experimental data replicate and predict patterns of auxin accumulation and support the interpretation that auxin has a key role in developmental patterning.

## POLAR AUXIN TRANSPORT

Many hormones can be translocated through the plant by way of the xylem or phloem, but the directional movement of auxin between cells and tissues is particularly well described, and may be unusual in the extent to which it occurs. Polar auxin transport is fundamental to many of its functions in pattern formation, organogenesis, and directional growth responses. The Cholodny-Went theory proposed in the 1930s postulated that the asymmetries in growth rate in light- or gravity-responding organs are caused by an auxin gradient. After many years, this theory is now widely accepted, largely because of our ability to detect the proposed auxin gradient using the tools described above, and the identification of the chemical and cellular basis by which the auxin gradients are established and maintained.

Because IAA is a weak acid, it exists in a charged anionic form (IAA$^-$) in the neutral pH of the cytoplasm (pH ~7). In the more acidic cell wall environment (pH ~5.5), ~15% of the molecules are in the uncharged form (IAAH), which can transit through the plasma membrane. The pH differential between the cytoplasm and wall means that auxin can move into (as IAAH) but not out of plant cells. Changing the pH of the cell wall by overexpression or loss of function of a proton pump protein affects this chemiosmotic movement; when the apoplast is more acidic, a higher proportion of the IAA is uncharged, accelerating auxin transport.

Plants also employ specific transport proteins to move auxin precisely. Many auxin transporter proteins were identified through genetic screens for abnormal auxin responses, including agravitropism. The extremely agravitropic *aux1* mutant is deficient in polar auxin transport. *AUX1* encodes an auxin influx carrier that augments auxin's chemiosmotic influx into cells. *AUX1* and its related *LIKE-AUX1* genes seem to be particularly important for auxin influx in conditions when auxin efflux rates are high. The ATP Binding Cassette subgroup B (ABCB) transporters comprise a 21-protein family that contributes to auxin transport in diverse ways; some function in auxin influx and some in auxin efflux. (These were previously also known as multiple drug

resistance/P-glycoproteins.) Unlike PIN proteins, their cellular position seems to be relatively stable and they may interact with and stabilize PIN proteins at specific microdomains of the membrane. ABCB function is inhibited by plant flavonoid compounds, whose synthesis is increased upon wounding and environmental stresses; thus, these transporters may directly link auxin responses and stress responses.

The *PIN* genes (named for the *pin-formed* mutant) encode auxin efflux carriers with asymmetric, polar distributions on cell membranes. Through their polarity, PIN proteins contribute to the highly directional, polar transport of auxin that underlies developmental patterning and differential growth responses. In *Arabidopsis*, there are eight *PIN* genes with different tissue-specific expression patterns. Furthermore, the individual PIN proteins themselves can have different cellular distributions within cells. To some extent, these different family members are specialized for specific functions. For example, *PIN1* is expressed in the xylem parenchyma throughout the plant and has a major role in the polar transport of auxin from shoot tip to root tip. *PIN2* plays a key role in root gravitropism; loss-of-function mutants have a strongly agravitropic phenotype. Localization of the PIN3 protein changes upon a change in light or gravity orientation and is important for establishing the auxin gradients that mediate tropic growth responses, and PIN5 and PIN8 are localized to the endoplasmic reticulum and thought to be involved in intracellular active auxin transport.

PIN protein redistribution is critical for the movement of auxin that regulates pattern formation and organogenesis at the shoot apical meristem and during embryogenesis. Auxin maxima are required for and precede the initiation of lateral roots, leaves, and flowers at the shoot apical meristem and the embryonic formation of the radicle (embryonic root) meristem and cotyledons. It remains a fascinating and unresolved question how the PIN proteins themselves are properly positioned, but recent studies suggest that connections between the plasma membrane and cell wall may help maintain their polar distribution.

Some PIN proteins undergo continual movement between the plasma membrane and internal endosomal membranes through regulated endo- and exocytosis, which seems to be necessary for PIN repositioning at the cell surface. There are many different routes of intracellular PIN trafficking known. One way is via the molecule clathrin, which forms a polyhedral cage structure that encapsulates membrane vesicles. The PIN phosphorylation state also contributes to this membrane shuttling. *PINOID* encodes a protein kinase that phosphorylates PIN proteins in vivo. In the *pinoid* mutant or plants treated with protein kinase inhibitors, PIN proteins can accumulate in internal membranes. By contrast, mutants or inhibitors that interfere with protein phosphatases promote PIN localization to the plasma membrane. Auxin itself regulates PIN protein expression and membrane targeting, in some cases creating a robust and stable pattern of auxin flow (sometimes referred to as canalization).

Recently, a further gene family of seven members encoding PIN-LIKES (PILS) proteins was identified and characterized. The PILS proteins do not show extensive sequence similarity to PIN proteins, but they do show a similar protein topology to the PINs, consisting of a central hydrophilic loop flanked at each side by five transmembrane domains. The PILS proteins localize to the endoplasmic reticulum and are thought to regulate auxin homeostasis and intracellular auxin accumulation. Therefore, many different classes of auxin transporters regulate auxin flow within and between cells. The PIN proteins remain the best-characterized group and the expression, activity and localization of *PIN* genes and their encoded proteins are important targets for regulatory inputs from diverse signaling pathways, including light and gravity as well as ethylene and cytokinin. The complexities of polar auxin transport provide us with an engaging and fascinating puzzle and reveal that plants have developed very sophisticated systems to make sure that auxin goes where it needs to go.

## AUXIN PERCEPTION AND SIGNALING

Auxin seems to act through multiple types of receptor proteins. AUXIN BINDING PROTEIN1 (ABP1) was identified in the 1970s. Knock-down of ABP1 function has shown that it regulates cell division and expansion during postembryonic growth and is also necessary for the maintenance of the root meristem. ABP1 is membrane-localized and found in the endoplasmic-reticulum lumen or the outer surface of the plasma membrane. It is associated with auxin responses at the plasma membrane, including the activation of proton pumps and cell wall acidification and loosening. When auxin concentrations are low, ABP1 promotes the clathrin-dependent recycling of PIN proteins. At higher auxin concentrations, this effect is inhibited and so more auxin is transported through the cell-surface PIN proteins, which is a type of positive feedback. ABP1 also contributes to the regulation of the cytoskeleton and cell shape.

In 2005, the protein TRANSPORT INHIBITOR RESPONSE1 (TIR1) was identified as an auxin receptor, connecting auxin with the regulated proteolysis of auxin response repressors and continuing a story that had been emerging for more than 25 years.

TIR1 is an F-box protein, a component of an SCF (SKP1, CUL1, and F-box protein) ubiquitin ligase complex. Ubiquitin is a small protein that is conjugated to other proteins by ubiquitin ligase complexes, including SCF$^{TIR1}$. Because the F-box protein confers specificity to this complex by binding to the target proteins, SCF complexes are identified by their specific F-box protein component as indicated. Ubiquitinated proteins are proteolyzed by the 26S proteasome, which selectively degrades proteins, including regulatory proteins.

When bound to auxin, TIR1 also specifically binds to Aux/IAA repressor proteins with the auxin holding the proteins together like a molecular glue, targeting them for proteolysis. Genes encoding Aux/IAA proteins were identified in the 1980s and were among the first auxin-induced genes to be identified through the newly developed tools of molecular biology. Aux/IAA proteins are short-lived, nuclear-localized proteins, whose rate of degradation is enhanced by auxin. Aux/IAA proteins have four conserved domains. A short amino acid sequence in domain II was identified as the "degron" and is necessary for auxin-induced instability. In the early 1990s, several research groups identified dominant, gain-of-function mutants in Aux/IAA genes; these mutations were mapped to amino acid changes in the

degron that interfere with auxin-induced protein degradation. Taken together, these results indicate that auxin signaling is dependent on the degradation of the Aux/IAA repressors and that stabilized mutant proteins confer an auxin-resistant phenotype because they are resistant to degradation.

Analysis of the promoters of several auxin-induced genes led to the identification of the auxin response element and a family of proteins that specifically bind to the auxin response element called auxin response factors (ARFs). *Arabidopsis* has 23 ARF-encoding genes. All ARFs have DNA binding domains; some have a transcriptional activation domain and function as transcriptional activators, whereas others function as transcriptional repressors.

ARFs and Aux/IAA proteins have homology at their C-terminal domains through which they can form homo- and heterodimers. At low levels of auxin, Aux/IAA proteins can accumulate and, through heterodimerization with ARF proteins, repress auxin responses. When auxin levels increase, SCF$^{TIR1}$ binds to auxin and to Aux/IAA proteins, initiating their ubiquitination and proteolysis by the 26S proteasome. Removal of Aux/IAA proteins relieves their repression of ARF protein function. Unrepressed ARF proteins can then exert their effects upon transcription.

The way that these two protein families interact has been illuminated by studies of the *Arabidopsis* embryonic patterning mutants *monopteros* (*arf5/mp*) and *bodenlos* (*iaa12/bdl*). These mutants have a very similar abnormal phenotype characterized primarily by their inability to form a primary root. The *arf5/mp* mutant has a loss-of-function mutation in *ARF5*, which encodes a transcriptional activator. The *iaa12/bdl* mutant has a gain-of-function mutation in *IAA12*. The increased stability of the IAA12 protein in the *iaa12/bdl* mutant has a similar effect as loss of the ARF5 transcription factor, highlighting IAA12's role as a repressor of ARF5 function. Overexpression of ARF5 can revert the *iaa12/bdl* mutant phenotype to the wild type, indicating that the relative abundance of these proteins is important in determining transcriptional outcomes.

In some ways, the auxin-mediated signal transduction pathway seems startlingly simple; auxin binds SCF$^{TIR1}$, which initiates proteolysis of Aux/IAA repressors, derepressing ARF transcription factors. If we contrast this to a canonical signal transduction pathway in which information is relayed from receptor through a series of intermediate proteins to effector proteins, we don't see the same opportunities for amplification or crosstalk that are inherent in longer signaling cascades. However, the *Arabidopsis* genome encodes 29 Aux/IAA proteins and 23 ARF proteins; it is likely that the complexity and specificity of the auxin response is conferred in part by the combinatorial interactions between these large protein families. Presumably, auxin's effects depend on the relative abundances of each of these proteins, their relative affinities for homo- and heterodimerization, and the binding affinities of the ARF proteins for the promoters of auxin-inducible genes. TIR1 is also a member of a protein family that consists of five additional proteins, AUXIN SIGNALING F-BOX PROTEIN1 (AFB1) through AFB5. As plants have evolved greater developmental and morphological complexity, the proportion of their genome that encodes auxin response genes has increased severalfold, from 0.14% of moss genes to 0.4 to 0.6% of angiosperm genes.

Among these, the Aux/IAA genes have been most dramatically amplified, from two genes in moss to 24 to 28 genes in the angiosperms. Although the auxin signaling pathway is short, it nevertheless provides ample opportunity for complexity.

## AUXIN ACTION IN WHOLE-PLANT PROCESSES

In spite of our remarkable progress in elucidating the mechanisms that control auxin homeostasis, transport, perception, and signaling, we still have a gap in our understanding of auxin-mediated processes downstream of its immediate effects on Aux/IAA turnover and ARF derepression. Many auxin-induced or auxin-repressed genes have been identified, which in some cases clarify how auxin effects are mediated (examples include tissue-specific transcription factors that contribute to organogenesis or enzymes that stimulate production or degradation of other hormones). In other cases, transcriptomic studies just lead to more questions; to understand auxin action in whole-plant processes, we will need to decipher these complex studies. We will also need to take into account auxin's roles beyond transcriptional regulation, namely, its extremely rapid effect in promoting cell elongation and its role as an important source of positional information that contributes to pattern formation and organogenesis.

## AUXIN'S ROLE IN CELL ELONGATION

Although auxin was initially purified through Frits Went's cell elongation assay, we still do not fully understand how auxin promotes cell or tissue elongation. Auxin-treated stem segments show a pronounced decrease in cell wall pH as a result of an auxin-induced stimulation of a plasma membrane proton pump. A stem segment incubated in an acidic buffer also elongates in an effect that is referred to as acid growth. Auxin-induced wall acidification may loosen the cell wall directly but also activates pH sensitive wall-loosening enzymes called extensins, which promote elongation when applied to a tissue. The model of auxin-induced acidification followed by extensin activation only accounts for short-term growth responses and does not explain all of the auxin-induced growth effects. It seems that this response is mediated by the auxin receptor ABP1. Plants overexpressing ABP1 produce significantly larger cells than control plants, suggesting that as we learn more about ABP1 and its downstream effectors, we will better understand the process of auxin-induced cell expansion. We still do not understand by what mechanism auxin acts differentially on cell growth in shoots and roots, promoting cell elongation in aboveground organs and inhibiting it belowground.

## AUXIN'S ROLES IN PATTERN FORMATION AND ORGANOGENESIS

The early part of the 21st century has brought new insight into the roles of auxin in establishing developmental patterns and in

organogenesis. Pattern formation is the process through which spatial differentiation emerges from a homogeneous material. Pattern formation occurs during plant and animal embryogenesis when the developing organism acquires its axes of polarity: top-to-bottom, in-to-out, and front-to-back. These axes often develop as a consequence of some sort of molecular gradient. In animal development, the term "morphogen" describes a mobile signal that forms a gradient within a developing structure and specifies cell identities and tissue and organ patterning in a concentration-dependent manner. For example, during vertebrate limb development, the "sonic hedgehog" morphogen is secreted from a zone of polarizing activity and specifies the formation of different digits at different positions depending on its concentration. Adding a second source of the morphogen causes a duplicated morphogen gradient and a duplicated, mirror image limb that develops from it.

In many of its effects auxin seems to act as a morphogen (although there are some differences between auxin and animal morphogens, including the fact that auxin does not form a gradient by diffusion but through polar auxin transport). Remarkably, an auxin gradient can be detected in a developing *Arabidopsis* embryo already after the first cell division. The gradient changes polarity to form a basal maximum at around the 32-cell stage. Formation of this gradient is necessary for proper embryonic root formation. Mutants in which auxin transport is interfered with (*pin1,3,4,7*) or auxin signaling is disrupted (*arf5/mp* and *iaa12/bdl*) are all unable to initiate a root during embryogenesis.

Pattern formation in plants also occurs outside of embryogenesis. Cell differentiation regulated by auxin gradient–specified positional information was first shown in the vascular cambium of pine (*Pinus sylvestris*). More recently, an auxin gradient was found to mediate pattern formation in the *Arabidopsis* female gametophyte. The most thoroughly characterized morphogen-like role of auxin is in the regulation of cell differentiation at the root apex, in which auxin accumulates in a strong and stable gradient with a maximum at the quiescent center. Cells are formed by divisions at the root apical meristem that encompasses the quiescent center. As cells are displaced away from the meristem, they stop dividing, elongate, and differentiate in a position-dependent fashion. Interfering with the auxin gradient through any of several means (mutation of *PIN*s or application of polar auxin transport inhibitors or exogenous auxin to name a few) disrupts the pattern of cellular activities in the root.

Auxin has also been described as acting as a developmental trigger, eliciting a specific response at the site of a localized auxin maximum or minimum. A localized auxin maximum is sufficient to initiate the formation of leaves or flowers from the shoot apical meristem or lateral roots from the pericycle cells of the primary root, whereas a localized auxin minimum is necessary for the establishment of the cell separation zone in the *Arabidopsis* seed pod and also for establishing a competence window for lateral root founder cell specification. It is clear that throughout the life of a plant, from embryo to seed pod, and by acting as a morphogen or a trigger, auxin plays a critical role in conveying positional information.

## CROSSTALK AND INTERACTIONS WITH OTHER HORMONES AND SIGNALS

Now that many components of several hormonal and environmental signaling pathways have been identified, we can begin to address how these pathways overlap and influence each other. One of the first characterized examples of cross-regulation is the interaction between auxin and cytokinin. In the 1950s, Folke Skoog and colleagues showed that the undifferentiated cells of the interior tissue of tobacco (*Nicotiana tabacum*) stems (pith) could differentiate into shoots or roots depending on the hormones on which they were cultured and that the relative amounts of auxin and cytokinin were critical in the cellular responses. There are now many examples of how auxin pathways crosstalk with those of other hormones. Auxin-cytokinin interactions in several developmental contexts such as lateral root initiation and root development have been well-characterized. These show that there are multiple points of cross-regulation by cytokinin on auxin, including its biosynthesis, polar transport by affecting *PIN* expression and direct antagonistic responses on individual transcription factor promoters. (These interactions are described further in Teaching Tools in Plant Biology; Cytokinins.) Auxin and ethylene have a more synergistic interaction; they promote each other's biosynthesis, and ethylene can induce *PIN* expression. By contrast, jasmonates upregulate auxin biosynthesis but seem to attenuate auxin transport. Light, pathogens, nutrient availability, and stress all affect auxin accumulation, transport, or response. We are just beginning to learn how these diverse pathways are integrated and coordinated. Clearly, these studies are indispensible in deciphering auxin action at the whole-plant level.

## CONCLUSIONS AND FUTURE DIRECTIONS

It has long been recognized that auxin is critically and fundamentally important in the life of a plant and that its localized synthesis, transport, and response underlie plant growth and development. With each new tool in our inquiry toolbox, auxin researchers have pushed forward our understanding of these complex phenomena. Thanks to genomic sequence information from many plants, along with the powerful gene discovery approaches from *Arabidopsis* genetics, we now know the identity of many of the genes that contribute to auxin biosynthesis, transport, perception, and downstream signaling responses and some of the ways these genes respond to each other and other signaling pathways. Our ability to image auxin flow and response in vivo has shown us the phenomenally dynamic auxin distribution patterns that coordinate development. However, many questions remain: what coordinates these auxin fluxes? What is the role of the cytoskeleton and protein trafficking machinery in positioning them? What are the relative contributions of auxin synthesis and transport in pattern generation? If cellular responses are specified by ARF and Aux/IAA protein accumulation, what establishes their expression patterns? How do the functions of the AFB proteins compare with those of TIR1, and what mediates the ABP1 protein functions? The story of auxin, with its "beautiful

illustration of the power of scientific reasoning and human intuition," has chapters that have yet to be written.

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## RECOMMENDED READING

(*This is a representative list of sources to help the reader access a huge body of literature. We apologize in advance to those whose work is not included*.)

### Review articles

**Abel, S., and Theologis, A.** (2010). Odyssey of auxin. Cold Spring Harb. Perspect. Biol. **2**: a004572. doi:10.1101/cshperspect.a004572.

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