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Auxin Biosynthesis and Its Role in Plant Development

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Arabidopsis, tryptophan, YUCCA, TAA1, flavin monooxygenase

Abstract

Indole-3-acetic acid (IAA), the main auxin in higher plants, has profound effects on plant growth and development. Both plants and some plant pathogens can produce IAA to modulate plant growth. Although the genes and biochemical reactions for auxin biosynthesis in some plant pathogens are well understood, elucidation of the mechanisms by which plants produce auxin has proven to be difficult. So far, no single complete pathway of de novo auxin biosynthesis in plants has been firmly established. However, recent studies have led to the discoveries of several genes in tryptophan-dependent auxin biosynthesis pathways. Recent findings have also determined that local auxin biosynthesis plays essential roles in many developmental processes including gametogenesis, embryogenesis, seedling growth, vascular patterning, and flower development. In this review, I summarize the recent advances in dissecting auxin biosynthetic pathways and how the understanding of auxin biosynthesis provides a crucial angle for analyzing the mechanisms of plant development.

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Contents

INTRODUCTION

Auxin was identified as a plant growth hormone because of its ability to stimulate differential growth in response to light stimuli. The in vitro bioassay in which auxin-containing agar blocks stimulated the growth of oat coleoptile segments led to the identification of indole-3-acetic acid (IAA) as the main naturally occurring auxin in plants. Applications of IAA or synthetic auxins to plants cause profound changes in plant growth and development (6). Much of our knowledge of the physiological roles of auxin in plants is derived from studies on how plants respond to excess exogenous auxin. However, an equally important aspect of auxin biology is to characterize the developmental defects caused by auxin deficiency, which cannot be achieved without a clear grasp of auxin biosynthetic pathways.

In contrast to the great progress made in understanding auxin signaling and transport (40, 64), much less is known about how auxin is produced in plants. Elucidation of the molecular and biochemical mechanisms of auxin biosynthesis will have a great impact on defining the roles of auxin in plant development, understanding auxin transport, and studying the mechanisms of auxin in regulating plant development. Despite its apparent importance in auxin biology, auxin biosynthesis has remained an elusive target for plant biologists. Only recently have several key genes in de novo auxin biosynthesis been identified with molecular genetic approaches.

Auxin biosynthesis in plants is extremely complex. Multiple pathways likely contribute to de novo auxin production. IAA can also be released from IAA conjugates by hydrolytic cleavage of IAA-amino acids, IAA-sugar, and IAA-methyl ester (3, 36, 67, 72). Furthermore, although plants share evolutionarily conserved core mechanisms for auxin biosynthesis, different plant species may also have unique strategies and modifications to optimize their IAA biosynthesis. In this review, I discuss solely the mechanisms of tryptophan (trp)-dependent auxin biosynthesis. Discussions on the trp-independent auxin biosynthesis pathway and auxin conjugation/modification can be found elsewhere (3, 67). I focus on the auxin biosynthesis genes identified in *Arabidopsis*, but also briefly discuss relevant studies in other species. I also include two examples to demonstrate that knowledge of auxin biosynthesis provides a genetic basis for solving key plant developmental questions.

LESSONS FROM AUXIN BIOSYNTHESIS IN PLANT PATHOGENS

Plant pathogens such as *Agrobacterium* produce auxin to hijack plant cells for nutrient production. *Pseudomonas* and *Agrobacterium* use a tryptophan-2-monooxygenase called iaaM to convert tryptophan to indole-3-acetamide (IAM), which is subsequently hydrolyzed into IAA by the hydrolase iaaH (7, 13) (**Figure 1**). The iaaM/iaaH pathway is the only complete trp-dependent IAA biosynthesis

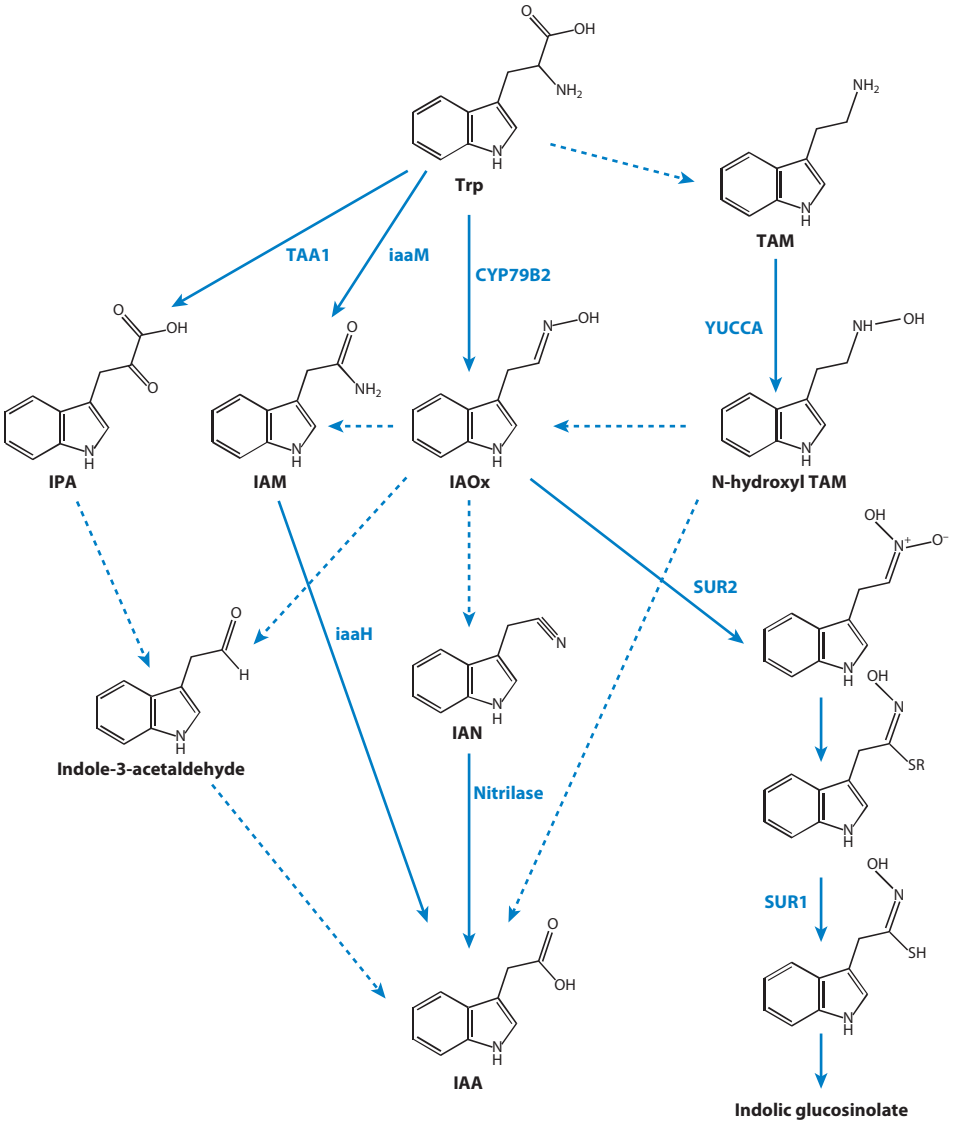

Figure 1

Trp-dependent auxin biosynthesis pathways. Solid arrows: genes responsible for the steps have been identified in plants or microorganisms. Dashed arrows: proposed steps, but genes for the steps have not been conclusively determined. Abbreviations: IAOx: indole-3-acetaldoxime; IPA: indole-3-pyruvate; IAM: indole-3-acetamide; IAN: indole-3-acetonitrile; TAM: tryptamine.

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pathway known to date. It is generally believed that plants do not use the iaaM/iaaH pathway to make IAA. However, IAM exists in plant extracts and has been suggested as a key intermediate in converting indole-3-acetaldoxime (IAOx) to IAA (58) (**Figure 1**). Furthermore, a family of amidases that can hydrolyze IAM into IAA has been identified in *Arabidopsis* (47), suggesting that IAM can be an intermediate for IAA biosynthesis in plants. However, the biochemical reactions for IAM production in plants have not been solved.

Elucidation of the iaaM/iaaH pathway has been key to recent progress in dissecting auxin biosynthesis in plants. The bacterial iaaM gene also provides a useful way to manipulate auxin levels in transgenic plants. Overexpression of the iaaM gene alone in petunia (32), tobacco (53), and *Arabidopsis* (49) leads to auxin overproduction phenotypes, suggesting that plants have enzymes for the hydrolysis of IAM. Transgenic *Arabidopsis* plants that overexpress the iaaM gene under the control of the CaMV 35S promoter are much taller than wild-type plants when grown in light (49). The iaaM overexpression lines define the characteristics of auxin overproduction in *Arabidopsis*, thus providing important traits for identifying plant auxin biosynthetic genes (see below). Tissue-specific expression of the iaaM gene enables the supply of auxin locally, which provides a key piece of evidence that demonstrates the roles of a family of flavin monooxygenases in auxin biosynthesis (see below).

IAA can also be produced from trp through the indole-3-pyruvate pathway (IPA) (**Figure 1**) found in some microorganisms. Unlike the iaaM/iaaH pathway, the IPA pathway has not been completely solved in microorganisms. The IPA decarboxylase, which catalyzes the conversion of IPA to indole-3-acetaldehyde, has been cloned from *Enterobacter cloacae* (33) and *Azospirillum brasilense* (14), but the genes responsible for converting tryptophan to IPA and enzymes for catalyzing indole-3-acetaldehyde to IAA have not been conclusively identified in microorganisms. Whether the IPA decarboxylase from microorganisms is functional in plants has not been investigated. Recently, enzymes involved in converting trp to IPA have been isolated in *Arabidopsis* (see below). The microorganism system may be useful for identifying the other genes involved in the IPA pathway in plants. For example, screening of plant cDNA libraries for genes that are able to complement the IPA decarboxylase function in microorganisms may help define the next step in the IPA pathway in *Arabidopsis*.

EARLY MOLECULAR GENETICS STUDIES ON AUXIN BIOSYNTHESIS

Early physiological studies on auxin biosynthesis have been comprehensively reviewed (3). Physiological and stable isotope labeling studies established that trp is a precursor for de novo IAA biosynthesis in plants. In addition, all the defined auxin biosynthesis pathways in microorganisms so far have been trp dependent. Therefore, it was logical that early molecular genetics studies on auxin biosynthesis were centered on analyzing *trp*-deficient mutants. Surprisingly, there were no differences in free IAA levels between wild-type and *trp* mutants (42, 69). In fact, the *trp* auxotroph mutants actually produced more IAA conjugates (42, 69). Consistent with the IAA measurement results, the *Arabidopsis trp* mutants used for the IAA analysis experiments did not show developmental defects as dramatic as those observed in some known auxin mutants including *pin1* (24) and *monopteros* (48). Further feeding experiments with [^{15}N]anthranilate and [$^{2}H_5$]tryptophan led to the hypothesis that IAA is mainly produced through a trp-independent pathway in *Arabidopsis* and in maize (42, 69). Although the early studies on *trp* mutants were informative, they did not identify the genes responsible for auxin biosynthesis in plants. Furthermore, interpretation of the experiments with *trp* mutants is not straightforward. It is difficult to determine whether the growth defects in the *trp* mutants are caused by problems in the synthesis of proteins, auxin, other trp metabolites, or by a combination of several processes. The *trp*

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mutants used in early auxin research were not true *trp*-deficient mutants because they still made some trp. The residual trp synthesis activity might complicate the interpretations of analytic biochemistry experiments. The flux of trp to different metabolic pathways may also be changed in a *trp* mutant.

AUXIN BIOSYNTHESIS PATHWAYS DEFINED BY *ARABIDOPSIS* GENETIC STUDIES

There have been no reported forward genetic screens conducted systematically for the purpose of isolating auxin-deficient mutants in any systems. Part of the reason is the lack of knowledge of the developmental consequences associated with auxin deficiency. Therefore, no robust auxin-deficient trait was known for genetic screens. Another difficulty is caused by genetic redundancy in auxin biosynthesis. Auxin can be synthesized from a trp-independent pathway as well as from several trp-dependent pathways. The recently identified auxin biosynthesis genes all belong to gene families, which explains why no auxin-deficient mutants came up from many screens for developmental defects in *Arabidopsis*. Some *trp* biosynthesis mutants including anthranilate synthase were isolated from screens for weak ethylene-resistant mutants and from the methyl jasmonate-insensitive mutant screens (55, 59). The hormone-resistant phenotypes of anthranilate synthase mutants (*asa1/wei2*, and *asb1/wei*7) were attributed to defects in auxin production. Recently, a rice tryptophan-deficient dwarf mutant, *tdd1*, which encodes the β subunit of anthranilate synthase, was found to have a reduced level of IAA and strong defects in floral and embryonic development (51). The identified anthranilate synthase mutants demonstrated that auxin plays a critical role in plant development, but did not solve the mechanisms of converting trp to IAA.

Identification of the first key trp-dependent auxin biosynthesis genes originated from the characterization of several auxin overproduction mutants in *Arabidopsis*. Analysis of the auxin overproduction mutants has led to the identification of two trp-dependent IAA biosynthesis routes (**Figure 1**). Recent characterization of mutants that are defective in shade avoidance and ethylene responses has identified an aminotransferase important for the production of indole-3-pyruvate (IPA) (**Figure 1**).

The IAOx and Glucosinolate Pathway

This pathway was defined by three auxin overproduction mutants, *superroot1* (*sur1*) (5), *superroot2* (*sur2*) (2, 16), and *CYP79B2* overexpression lines (29, 74). CYP79B2 and its close homolog CYP79B3 convert trp to IAOx (**Figure 1**), whereas SUR1 and SUR2 are involved in converting IAOx to indolic glucosinolates.

The mutant *sur1* is the first identified auxin overproduction mutant in *Arabidopsis* that displayed dramatic developmental defects (5). Light-grown *sur1* seedlings have long hypocotyls and epinastic cotyledons, whereas dark-grown *sur1* seedlings have short hypocotyls and lack an apical hook. In addition, *sur1* produces massive adventitious roots from hypocotyls. *SUR1* encodes a C-S lyase that catalyzes the conversion of *S*-alkylthiohydroximate to thiohydroximic acid, a key reaction in indolic glucosinolate biosynthesis (39) (**Figure 1**). Inactivation of SUR1 disrupts glucosinolate biosynthesis and likely leads to the accumulation of upstream intermediates including IAOx (**Figure 1**). Given that *sur1* is recessive and a loss-of-function allele, the auxin overproduction phenotypes of *sur1* are likely caused by funneling excess IAOx into IAA biosynthesis.

Like *sur1*, *sur2* also produces many adventitious roots from hypocotyls (16). Overall, *sur2* is phenotypically very similar to *sur1*. *SUR2* encodes the cytochrome P450 CYP83B1, an enzyme that synthesizes 1-*aci*-nitro-2-indolylethane from IAOx (2) (**Figure 1**). SUR2 defines the first step in making indolic glucosinolates from IAOx (**Figure 1**). Loss-of-function *sur2/cyp83B1* mutants block the production of glucosinolates from IAOx, leading to an increased IAOx flux for IAA biosynthesis.

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Unlike *sur1* and *sur2*, which are recessive and loss-of-function mutants, inactivation of *CYP79B2* does not cause auxin overproduction. Instead, it is the overexpression of *CYP79B2* in *Arabidopsis* that leads to auxin overproduction (74). CYP79B2 was isolated as an enzyme that metabolizes trp from a screen for *Arabidopsis* cDNAs that can confer resistance to 5-methyl trp in yeast (29). CYP79B2 catalyzes the conversion of trp to IAOx in vitro (29) (**Figure 1**). Overexpression of *CYP79B2* in *Arabidopsis* probably leads to an overproduction of IAOx, thus increasing the flux of IAOx to IAA biosynthesis.

Further evidence for the IAOx pathway comes from the observations that the *cyp79b2 cyp79b3* double loss-of-function mutants show measurably lower levels of free IAA than wild type and display phenotypes consistent with lower levels of auxin at high temperatures (74). Further evidence supporting the IAOx pathway is that *asa1/wei2* and *asb1/wei*7 mutants, which are defective in trp biosynthesis, partially suppress the phenotypes of *sur1* and *sur2* (55), presumably by decreasing the flux from trp to IAOx, and thereby restore IAOx to normal levels in *sur1* and *sur2*.

Production of IAOx from CYP79B2/B3 is probably not the main IAA biosynthesis pathway in plants for several reasons. First, IAOx was not detected in monocots such as rice and maize (58). Second, there are no apparent orthologs of CYP79B2 and CYP79B3 in rice and maize while the *Arabidopsis cyp79b2 cyp79b3* have no detectable levels of IAOx (58). Third, the phenotypes of *cyp79b2 cyp79b3* double mutants were very subtle compared to known auxin signaling or transport mutants.

IAOx can be converted to IAA in *Arabidopsis* on the basis of the observed phenotypes of *sur1*, *sur2*, and CYP79B2 overexpression lines, but the exact biochemical mechanisms that convert IAOx to IAA have not been worked out. In theory, IAOx can be used to make indole-3-acetonitrile (IAN) and indole-3-acetaldehyde, which can be further converted to IAA by nitrilases (43) and aldehyde oxidases (15, 52), respectively. Recent biochemical analysis of *cyp79b2 cyp79b2* double mutants suggests that IAM is probably also an important intermediate in converting IAOx to IAA, but the genes and enzymes for making IAM from IAOx are not known (58).

The YUC Pathway

The *Arabidopsis* mutant *yucca* (renamed *yuc1D*) was isolated from an activation-tagging screen for long hypocotyl mutants defective in light signaling (73). But it was soon realized that it was more likely that *yuc1-D* altered hormone homeostasis than light signaling because it had long hypocotyls in all light wavelengths. The phenotypes of *yuc1D* are very similar to those of known auxin overproduction mutants. Additional evidence further demonstrates that *yuc1D* is indeed an auxin overproduction mutant. Weak alleles of *yuc1D* contain 50% more free IAA than wild type and explants of *yuc1D* mutants produce massive roots in the absence of any exogenous plant hormones (73). Known auxin-inducible genes are upregulated in *yuc1D*. Overexpression of iaaL, which conjugates free IAA to the amino acid lysine, partially suppresses *yuc1D*. Furthermore, *yuc1D* is resistant to toxic trp analogs such as 5-methyl trp, suggesting that *yuc1D* overproduces auxin through a trp-dependent pathway (73).

The auxin overproduction phenotypes of *yuc1D* are caused by overexpression of a flavin monooxygenase-like (FMO) enzyme (73). Because *yuc1D* is a dominant and gain-of-function mutant, YUC1 likely catalyzes a rate-limiting step in auxin biosynthesis. In vitro assay indicates that YUC1 is capable of catalyzing the conversion of tryptamine into *N*-hydroxyl tryptamine, which can proceed to IAA through IAOx or other intermediates (**Figure 1**).

YUC1 belongs to a family with 11 members in *Arabidopsis*, and a subset of the members in the family may have overlapping functions (8). Overexpression of other members of the *YUC* family in *Arabidopsis* also leads to similar auxin overproduction phenotypes (8, 31, 38,

68), indicating that the *YUC*-like genes can participate in similar reactions. As expected, single loss-of-function mutants of any *YUC* gene do not display any obvious developmental defects. However, some combinations of *yuc* mutants in which two or more *YUC* genes are simultaneously inactivated display dramatic developmental defects (8, 9). For example, *yuc1 yuc4* double mutants produce abnormal flowers with no functional male or female floral organs. The *yuc1 yuc4* double mutants also make fewer vascular strains in leaves and flowers (8). Overall, the *YUC* genes appear to have both overlapping and unique functions during plant development. Analysis of different *yuc* mutant combinations clearly demonstrates that *YUC* genes are essential for embryogenesis, seedling growth, leaf and flower initiation, and vascular formation.

The *yuc* mutants are the first identified *Arabidopsis* mutants that are partially auxin deficient with dramatic developmental defects. In many ways, the developmental defects of *yuc* mutant combinations are very similar to those of known auxin signaling or transport mutants. For example, *yuc1 yuc4 yuc10 yuc11* quadruple mutants fail to make the basal part of the embryo during embryogenesis (9), a phenotype also observed in *monopteros/arf5* (27), *tir1 afb1 afb2 afb3* (17), and *pin* quadruple mutants (21). Inactivation of *YUC* genes leads to downregulation of the auxin reporter DR5-GUS. Loss-of-function *yuc* mutants display synergistic interactions with known auxin mutants such as *pin1* and *pid* (9). More importantly, the developmental defects of *yuc1 yuc4* are rescued by expression of the bacterial auxin biosynthetic gene iaaM under the control of the *YUC1* promoter, which presumably produces auxin in situ (8).

Unlike the *CYP79B2/B3* genes that have so far been detected only in *Brassica*, the *YUC* genes appear to have a much broader existence. Genes homologous to *YUC1* have been identified in all of the plant genomes with available sequence data, including moss and rice. Some *YUC* genes in petunia (61), rice (66, 71), corn (23), and tomato (19) have been functionally characterized and they play similar roles in auxin biosynthesis and plant development. Therefore, the *YUC* pathway appears to be highly conserved throughout the plant kingdom.

YUC genes are evidently key auxin biosynthesis genes. Overexpression of *YUC* genes leads to auxin overproduction and loss-of-function *yuc* mutants display developmental defects that are rescued by in situ auxin production. However, the detailed biochemical mechanisms of YUC flavin monooxygenases are still not solved. *YUC* flavin monooxygenases clearly catalyze a rate-limiting step in a trp-dependent auxin biosynthesis pathway. YUC was tentatively placed in the step from tryptamine to *N*-hydroxyl tryptamine on the basis of in vitro biochemical studies (19, 31, 73). Because flavin monooxygenases have broad substrate specificities in vitro (75), further investigations are needed to determine whether tryptamine is the in vivo substrate for YUCs.

The IPA Pathway

Indole-3-pyruvate has long been suggested as an intermediate for IAA biosynthesis (**Figure 1**). However, only recently has the role of IPA in plant auxin biosynthesis and development been determined (56, 60). Three independent genetic studies have identified an *Arabidopsis* aminotransferase that can convert trp to IPA in vitro. Tao et al. initiated a genetic screen for *Arabidopsis* mutants defective in shade avoidance response, a process in which plants adapt to changes in light quality by elongating stems and petioles (60). The *shade avoidance* (*sav*) mutant *sav3* fails to elongate after being transferred to simulated shade conditions. SAV3 encodes a protein homologous to aminotransferases and later is shown to catalyze the production of IPA from trp; thus SAV3 is renamed as TAA1 (TRYPTOPHAN AMINOTRANSFERASE of ARABIDOPSIS). Several pieces of evidence indicate that TAA1 is involved in auxin biosynthesis. The *taa1* mutants

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contain 60% less free IAA and show a decreased IAA synthesis rate when transferred to shade conditions (60). Some known auxin-inducible genes are downregulated in *taa1* plants grown in shade conditions (60). Furthermore, *taa1* is partially rescued by a synthetic auxin picloram, and *taa1* is hypersensitive to the toxic trp analog 5-methyl tryptophan (60).

TAA1 was also isolated from a genetic screen for weak ethylene-insensitive mutants (*wei* mutants) in *Arabidopsis* (56). The *taa1/wei8* mutants have elongated roots in the presences of ACC, an ethylene biosynthesis precursor, whereas wild-type root elongation is inhibited under the same conditions. The *wei8* mutants show altered expression of the auxin reporter DR5-GUS, decreased levels of free IAA, and partial rescue of the ethylene defects with exogenous IAA. More importantly, simultaneously inactivating *TAA1* and two of its close homologs (*TAR1* and *TAR2*) leads to developmental defects similar to those in *monopteros* and *pinoid*, two well-known auxin mutants.

TAA1 was also identified as *tir2* (70), a mutant resistant to auxin transport inhibitor NPA that inhibits the elongation of *Arabidopsis* roots. The *tir2* mutant has short hypocotyls, a phenotype that can be rescued by IPA and IAA, indicating that *TIR2* may be involved in the IPA auxin biosynthetic pathway. Furthermore, *TIR2* is required for temperature-dependent hypocotyl elongation in *Arabidopsis*. Taken together, the TAA1 and its close homologs play important roles in auxin biosynthesis and plant development.

TAA1 is a PLP-dependent enzyme and appears to have a wide distribution throughout the plant kingdom, suggesting that the IPA pathway is also highly conserved. Functional characterization of TAA1-like genes in other species has not been reported. The step catalyzed by TAA1 may not be a rate-limiting step in auxin biosynthesis because overexpression of TAA1 under the control of the 35S promoter does not cause auxin overproduction phenotypes (56, 60). Alternatively, TAA1 may not be regulated at the transcriptional level.

RELATIONSHIPS AMONG THE PROPOSED AUXIN BIOSYNTHESIS PATHWAYS

The intermediate IAOx was proposed as a shared intermediate among the CYP79B2/B3, YUC, and glucosinolate biosynthesis pathways (74) (**Figure 1**). Recent biochemical analysis demonstrated that the bulk of IAOx is produced from the CYP79B2/B3 pathway in *Arabidopsis* (58). A caveat of this study is that the production of IAOx and conversion of IAOx to IAA may be coupled, although this may not be the case for IAOx to glucosinolate biosynthesis. In addition, *YUC* genes are mainly involved in local auxin biosynthesis (see below), and the analysis of bulk IAOx may not be indicative.

The relationship between the YUC pathway and the IPA pathway is even more intriguing. Inactivation of either *YUC* genes or multiple *TAA1* genes leads to similar phenotypes. For example, *yuc1 yuc4 yuc10 yuc11* quadruple mutants do not make hypocotyls and roots, a phenotype that is also displayed in *taa1 tar1 tar2* triple mutants (9, 56). The phenotypic similarities between *yuc* and *taa* mutants suggest that the two gene families may participate in the same pathway. One possibility is that *YUC* genes act downstream of IPA, but it is not obvious how a flavin-containing monooxygenase, which catalyzes the hydroxylation of hetero-atoms in organic compounds (75), may be involved in reactions downstream of IPA that do not contain any hetero-atoms such as nitrogen and sulfur. Alternatively, TAA1 could catalyze the reaction of converting IPA to trp, a reaction that may be important for intracellular trp transport and thus feed the YUC pathway. A bacterial trp aminotransferase, which reversibly catalyzes the transamination of trp to yield IPA, has a 138-fold-lower K_m value for IPA than for trp (34). Silico-docking experiments with *Arabidopsis* TAA1 crystal structures have also shown that IPA scored better than trp (60), demonstrating that IPA could be a better substrate than trp for TAA1.

The YUC pathway and the IPA pathway could simply be two independent pathways, as

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currently suggested. Some developmental processes such as vascular development may just need a threshold level of auxin (9). Disruption of either pathway could potentially lower the IAA levels below the threshold to cause similar phenotypes. Apparently, the two pathways are not redundant in other developmental processes such as shade avoidance (60). Further experiments are needed to address the relationship between the two pathways. Analysis of the various potential intermediates including IPA, IAN, IAM, and IAOx in various mutant backgrounds and feeding conditions will be essential. Conducting genetic interaction studies among various auxin mutants will be helpful as well.

AUXIN BIOSYNTHESIS IS LOCAL AND NONCELL AUTONOMOUS

De novo auxin production is highly localized and local auxin biosynthesis plays a key role in shaping local auxin gradients (8, 9, 56, 60). The predominant view in the auxin field has been that polar auxin transport is responsible for generating auxin gradients and auxin maxima, known to be essential for proper plant development. The location of auxin biosynthesis has been regarded as unimportant, and in some cases irrelevant (25). Mathematical modeling of auxin-regulated developmental processes including root development (25), vascular formation (18, 20), and phyllotaxis (4) is based exclusively on the analyses of auxin transport. However, recent findings demonstrate that auxin biosynthesis is regulated both temporally and spatially. The expression of both *YUC* genes and *TAA1* genes is restricted to a small group of discrete cells. For example, during embryogenesis both *YUC1* and *TAA1* are initially expressed in the apical region at the globular stage, then gradually are concentrated at the apical meristem at the heart stage, and finally are restricted only to the apical meristem in the mature embryo (9, 56, 60).

The location of auxin biosynthesis appears to be an important aspect of the overall regulation of auxin functions. Different combinations of *yuc* mutants have different phenotypes, which often correlate with the *YUC* gene expression patterns. The *yuc1 yuc4* double mutants have a decreased expression of DR5-GUS in the cells where *YUC1/4* are expressed, but DR5-GUS activities in non-*YUC1/4*-expressing cells are not affected, suggesting that auxin peaks are mainly generated locally (9). *TAA1* and its homolog *TAR2* are expressed in the root tips in *Arabidopsis*, and the DR5-GUS expression is dramatically reduced in the root tip of *taa1 tar2* double mutants (56). Local auxin biosynthesis was also recently shown to modulate gradient-directed planar polarity in root hair development in *Arabidopsis* (30).

The shoot was long believed to be the only source of auxin biosynthesis. It was also thought that the other parts of a plant were dependent on polar auxin transport to supply auxin. Now it is clear that both shoot and root can produce auxin (9, 46, 56). *TAA1* is expressed in both shoot and root (56). Inactivation of *TAA1* and its homologs affect both root and shoot development. *YUC* genes are also expressed in all organs including flowers, leaves, and roots in *Arabidopsis*. Each organ appears to be self-sufficient in terms of controlling auxin gradients for development. For example, auxin produced in other floral organs cannot compensate for the effects of inactivation of *YUC2* and *YUC6* in stamens (8). Within an organ, *YUC* genes appear to be noncell autonomous (61), which means that auxin synthesized by YUCs in one cell is certainly used by other cells.

The finding that auxin biosynthesis is local and the *YUC* genes are noncell autonomous raises interesting questions regarding the relationship between biosynthesis and polar transport and the relative contributions of the two processes to local auxin gradient generation and maintenance. These questions are difficult to address because of the existence of various regulatory loops. For example, auxin regulates PIN genes at the transcriptional level and affects intracellular vesicle traffic that is important for proper targeting of PIN proteins (44, 65). However, genetic studies of interactions between *yuc* mutants and auxin transport mutants have

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shed some light on this important question. The transport mutant *pin1* showed synergistic interactions with *yuc1 yuc4* double mutants (9). The triple mutants completely abolished the formation of true leaves, a phenotype not displayed in either *pin1* alone or in the *yuc1 yuc4* double mutants, demonstrating a complex relationship between auxin biosynthesis and transport.

REGULATION OF AUXIN BIOSYNTHESIS

Auxin biosynthesis is regulated by both environmental and developmental signals. For example, when plants are transferred from normal growth conditions to shade conditions, auxin levels and biosynthesis rates are upregulated (60). With the identification of several key auxin biosynthetic genes, it is also feasible to monitor auxin biosynthesis by analyzing gene expression changes in response to various signals or in different mutant backgrounds. Several reports document the regulation of *YUC* gene expression during plant development. A recent report identified a family of transcription factors (SHI, SHORT INTENOTES) that regulates the expression of *YUC4* (54). STY1 (STYLISH1), one of the SHI genes, was initially isolated from genetic screens for *Arabidopsis* mutants defective in style development (54). Inactivation of *STY1* leads to abnormal style morphology and vascular patterning. When the expression of *STY1* is induced, *YUC4* mRNA levels increase and auxin levels also increase accordingly (54). Still not clear is whether STY1 binds directly to the *YUC4* promoter.

Another family of transcription factors called NGATHA (NGA) was recently found to participate in regulating style development as well (1, 63). *NGA* genes act redundantly to control style development in a dosage-dependent manner, and the quadruple *nga* mutant completely abolishes the formation of style and stigma tissues. The *nga* mutant phenotypes were attributed partially to the failure of activating two *YUC* genes (*YUC2* and *YUC4*) in the apical domain of *Arabidopsis* gynoecium (63). LEAFY COTYLEDON2 (LEC2), a central regulator of embryogenesis, is also found to activate the auxin biosynthetic genes *YUC2* and *YUC4* (57). Moreover, LEC2 binds to the *YUC4* promoter as revealed by ChIP experiments, suggesting that *YUC4* may be a direct target of LEC2 (57). Unlike LEC2, NGA, and SHI, which are positive regulators of *YUC* expression, SPOROCYTELESS (SPL) appears to be a negative regulator (37). Overexpression of SPL led to a significant repression of the expression of *YUC2* and *YUC6*. The TAA genes display unique expression patterns, but the transcription factors responsible for *TAA* expression have not been identified.

FROM AUXIN BIOSYNTHESIS TO DEVELOPMENTAL MECHANISMS

Recent progress in auxin biosynthesis opens a new line of research into the mechanisms whereby auxin controls plant development. Understanding auxin biosynthesis provides tools to manipulate auxin levels in plants with temporal and spatial precision. The available auxin biosynthetic mutants also provide sensitized backgrounds for genetically isolating key components that are responsible for auxin-mediated development.

Auxin Biosynthesis Regulates Female Gametophyte Development in *Arabidopsis*

The female gametophyte in *Arabidopsis* is a seven-cell structure with four different cell types including an egg cell, a central cell, two synergid cells, and three antipodal cells (45). Fertilization of the egg cell is the first step in embryogenesis while fertilization of the central cell directs the development of the endosperm. Development of the female gametophyte in *Arabidopsis* consists of the specification of cell types and the formation of a particular pattern that dictates the relative positions of the cells within the embryo sac. The underlying mechanisms of female gametophyte development have remained elusive until recently

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when auxin gradients were discovered in the developing embryo sac (45). Normal auxin distribution and signaling are required for the proper pattern formation in the embryo sac and for the specification of gametic and nongametic cell identities (45). The auxin reporter DR5-GFP is asymmetrically distributed with the maxima located at the micropylar pole of the developing embryo sac (45). The observed auxin reporter gradient is not generated or maintained by the efflux-dependent auxin transport because the PIN genes are not expressed during the stages of gametophyte development (45). The identification of *YUC* genes as key auxin biosynthesis enzymes made it feasible to investigate whether the auxin gradient in the embryo sac is correlated with local auxin production. Indeed, the expression of both *YUC1* and *YUC2* genes localizes to the micropylar pole of the gametophyte (45), where the DR5-GFP maxima were observed. Furthermore, misexpression of *YUC1* in the embryo sac induces a cell identity switch between gametic and nongametic cells, providing strong evidence that auxin is a morphogenic signal (45).

Understanding of Auxin Biosynthesis Provides New Opportunities to Genetically Dissect Developmental Mechanisms

Analyses of *Arabidopsis* mutants defective in auxin signaling or transport are instrumental in elucidating the molecular mechanisms of auxin action in plant growth and development (50, 64). Previous genetic screens for auxin signaling mutants in *Arabidopsis* have taken advantage of the observation that primary root elongation is greatly inhibited in the presence of exogenous auxin (35). Mutants defective in auxin uptake and signaling are less sensitive to exogenous auxin and thus have longer primary roots than wild type on auxin-containing media. Because the previous screens relied on the development of roots, some of the key auxin genes may have been missed if the genes were not expressed in the root, or were essential for root development. For example, *mp* and *bdl*, two key components of auxin signaling in specifying root meristem during embryogenesis, would not be isolated from a root-based auxin-resistant screen (26, 27). Some other known auxin mutants, including *pin1* (24) and *pid* (12), mainly affect shoot development and do not display auxin resistance in a root elongation assay.

Recent progress in auxin biosynthesis makes it possible to isolate mutants that can overcome or become oversensitive to partial auxin deficiency. Such screens offer an exciting opportunity to identify new components responsible for auxin action in plant development. Perhaps due to complications from polar auxin transport, exogenous IAA treatment and auxin overproduction differ phenotypically. All known auxin overproduction mutants have long hypocotyls, whereas the main phenotypic readout of IAA treatment is the inhibition of primary root elongation. Furthermore, the developmental defects of *yuc* mutants cannot be rescued by exogenous auxin treatments but are instead rescued by producing IAA from the iaaM gene under the control of a corresponding *YUC* promoter (8). Therefore, the auxin biosynthetic mutants provide a different angle for genetically dissecting auxin action in plant development.

The successful identification of the *yuc1 yuc4* enhancer, *npy1* (*naked pins in yuc mutants 1*), clearly demonstrates the power of using auxin biosynthesis mutants as starting materials for isolating new auxin components (10). The *yuc1 yuc4* double mutants produce abnormal flowers. When the *NPY1* gene is inactivated in the *yuc1 yuc4* background, the resulting *yuc1 yuc4 npy1* triple mutants develop pin-like inflorescences, a phenotype that is also observed in known auxin mutants *pin1*, *pid*, and *mp* (10). Formation of pin-like inflorescences is a hallmark of defective auxin-related processes. The *npy1* mutant is allelic to *enp1/mab4* that was isolated as an enhancer of *pid* (22, 62). The *npy1/enp1* and *pid* double mutants do not make cotyledons, indicating that NPY1/ENP1/MAB4 plays a general role in organogenesis. Furthermore, the *yuc1 yuc4 pid* triple mutants phenocopy *npy1 pid* double

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mutants. Genetic analyses have put YUC, PID, and NPY1 in a linear developmental pathway.

NPY1 is a novel protein, but it shares significant homology with NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3) (41). Both NPY1 and NPH3 belong to a plant-specific superfamily with 32 members in the *Arabidopsis* genome (11). The mechanisms of auxin-mediated organogenesis, particularly flower formation, appear to be analogous to those of phototropic responses. NPY1 is homologous to NPH3 and inactivation of NPY1 and its close homologs NPY3 and NPY5 leads to pin-like inflorescences (10, 11). Inactivation of PID, which is a ser/thr kinase and is homologous to the photoreceptor PHOT1, causes phenotypes similar to *npy1 npy3 npy5* triple mutants. Another similarity between these two pathways is that both require the auxin response factor *ARF5/MONOPTEROS*. Disruption of *ARF5* causes the formation of pin-like inflorescences, whereas inactivation of *ARF7* abolishes phototropic responses (28). Identification of NPY1 in a genetic screen for *yuc1 yuc4* enhancers demonstrates that genetic screens for modifiers of auxin biosynthesis mutants will probably lead to the discovery of additional components in the pathway.

CONCLUSIONS

Tremendous progress has been made in understanding auxin biosynthesis in plants over the past few years. Several key auxin biosynthetic genes and their roles in plant development have been discovered. It is clear that auxin biosynthesis takes place locally in response to both environmental and developmental signals. De novo auxin biosynthesis plays an essential role in many developmental processes. However, many questions regarding auxin biosynthesis and its role in plant growth and development still remain unsolved. Current understanding of auxin biosynthesis is still fragmented, and no single complete de novo biosynthesis pathway has been defined. It will likely take a combination of genetic studies and biochemical analyses of auxin biosynthetic intermediates with isotope labeling experiments to fill in the gaps in auxin production in plants. Also not clear is how the expression patterns of auxin biosynthesis genes are generated and how they are regulated.

To understand the molecular mechanisms by which auxin regulates plant development, findings from auxin biosynthesis, conjugation, transport and signaling must be integrated. Results from auxin biosynthesis probably should be incorporated into various mathematical models of plant development. Recent progress in auxin biosynthesis makes it practical to alter auxin levels with temporal and spatial precision, providing exciting tools with which to tackle complex questions regarding the mechanisms of how auxin controls plant development.

SUMMARY POINTS

1. Auxin biosynthesis in plants is complex and several pathways contribute to de novo IAA production.
2. De novo auxin biosynthesis plays an essential role in virtually every aspect of plant development.
3. Auxin biosynthesis is temporally and spatially regulated. Local auxin biosynthesis contributes to the generation and maintenance of local auxin gradients.
4. Understanding of auxin biosynthesis provides new tools for solving difficult questions about plant development.

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FUTURE ISSUES

1. There is still no single complete de novo auxin biosynthesis pathway in plants. Enzymes that function in the immediate upstream and downstream steps of the YUCs and TAAs need to be identified.
2. The relationship among the proposed tryptophan-dependent auxin biosynthesis pathways and the relative contribution of each pathway to overall IAA production need to be determined.
3. Identification of the transcription factors that bind to the promoters of the key auxin biosynthesis genes will help to understand how auxin biosynthesis is regulated by developmental and environmental signals.
4. Further investigations are needed to understand how plants integrate auxin biosynthesis, polar transport, and signaling in plant development.

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DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Alvarez JP, Goldshmidt A, Efroni I, Bowman JL, Eshed Y. 2009. The NGATHA distal organ development genes are essential for style specification in *Arabidopsis*. *Plant Cell* 21:1373–93
2. Barlier I, Kowalczyk M, Marchant A, Ljung K, Bhalerao R, et al. 2000. The SUR2 gene of *Arabidopsis thaliana* encodes the cytochrome P450 CYP83B1, a modulator of auxin homeostasis. *Proc. Natl. Acad. Sci. USA* 97:14819–24
3. Bartel B. 1997. Auxin biosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:51–66
4. Bayer EM, Smith RS, Mandel T, Nakayama N, Sauer M, et al. 2009. Integration of transport-based models for phyllotaxis and midvein formation. *Genes Dev.* 23:373–84
5. Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, et al. 1995. Superroot, a recessive mutation in *Arabidopsis*, confers auxin overproduction. *Plant Cell* 7:1405–19
6. Bonner J, Bandurski RS. 1952. Studies of the physiology, pharmacology, and biochemistry of the auxins. *Annu. Rev. Plant Physiol.* 3:59–86
7. Camilleri C, Jouanin L. 1991. The TR-DNA region carrying the auxin synthesis genes of the *Agrobacterium rhizogenes* agropine-type plasmid pRiA4: nucleotide sequence analysis and introduction into tobacco plants. *Mol. Plant Microbe Interact.* 4:155–62
8. Cheng Y, Dai X, Zhao Y. 2006. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. *Genes Dev.* 20:1790–99
9. Cheng Y, Dai X, Zhao Y. 2007. Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in *Arabidopsis*. *Plant Cell* 19:2430–39
10. Cheng Y, Qin G, Dai X, Zhao Y. 2007. NPY1, a BTB-NPH3-like protein, plays a critical role in auxin-regulated organogenesis in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 104:18825–29

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11. Cheng Y, Qin G, Dai X, Zhao Y. 2008. NPY genes and AGC kinases define two key steps in auxin-mediated organogenesis in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 105:21017–22
12. Christensen SK, Dagenais N, Chory J, Weigel D. 2000. Regulation of auxin response by the protein kinase PINOID. *Cell* 100:469–78
13. Comai L, Kosuge T. 1982. Cloning characterization of iaaM, a virulence determinant of *Pseudomonas savastanoi*. *J. Bacteriol.* 149:40–46
14. Costacurta A, Keijers V, Vanderleyden J. 1994. Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. *Mol. Gen. Genet.* 243:463–72
15. Dai X, Hayashi K, Nozaki H, Cheng Y, Zhao Y. 2005. Genetic and chemical analyses of the action mechanisms of sirtinol in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 102:3129–34
16. Delarue M, Prinsen E, Onckelen HV, Caboche M, Bellini C. 1998. Sur2 mutations of *Arabidopsis thaliana* define a new locus involved in the control of auxin homeostasis. *Plant J.* 14:603–11
17. Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, et al. 2005. Plant development is regulated by a family of auxin receptor F box proteins. *Dev. Cell* 9:109–19
18. Dimitrov P, Zucker SW. 2006. A constant production hypothesis guides leaf venation patterning. *Proc. Natl. Acad. Sci. USA* 103:9363–68
19. Expósito-Rodríguez M, Borges AA, Borges-Pérez AB, Hernández M, Pérez JA. 2007. Cloning and biochemical characterization of ToFZY, a tomato gene encoding a flavin monooxygenase involved in a tryptophan-dependent auxin biosynthesis pathway. *J. Plant Growth Regul.* 26:329–40
20. Feugier FG, Iwasa Y. 2006. How canalization can make loops: a new model of reticulated leaf vascular pattern formation. *J. Theor. Biol.* 243:235–44
21. Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, et al. 2003. Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. *Nature* 426:147–53
22. Furutani M, Kajiwara T, Kato T, Treml BS, Stockum C, et al. 2007. The gene MACCHI-BOU 4/ENHANCER OF PINOID encodes a NPH3-like protein and reveals similarities between organogenesis and phototropism at the molecular level. *Development* 134:3849–59
23. Gallavotti A, Barazesh S, Malcomber S, Hall D, Jackson D, et al. 2008. sparse inflorescence1 encodes a monocot-specific YUCCA-like gene required for vegetative and reproductive development in maize. *Proc. Natl. Acad. Sci. USA* 105:15196–201
24. Galweiler L, Guan C, Muller A, Wisman E, Mendgen K, et al. 1998. Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* 282:2226–30
25. Grieneisen VA, Xu J, Maree AF, Hogeweg P, Scheres B. 2007. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449:1008–13
26. Hamann T, Benkova E, Baurle I, Kientz M, Jurgens G. 2002. The *Arabidopsis* BODENLOS gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes Dev.* 16:1610–15
27. Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J.* 17:1405–11
28. Harper RM, Stowe-Evans EL, Luesse DR, Muto H, Tatematsu K, et al. 2000. The NPH4 locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial *Arabidopsis* tissue. *Plant Cell* 12:757–70
29. Hull AK, Vij R, Celenza JL. 2000. *Arabidopsis* cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. *Proc. Natl. Acad. Sci. USA* 97:2379–84
30. Ikeda Y, Men S, Fischer U, Stepanova AN, Alonso JM, et al. 2009. Local auxin biosynthesis modulates gradient-directed planar polarity in *Arabidopsis*. *Nat. Cell Biol.* 11:731–38
31. Kim JI, Sharkhuu A, Jin JB, Li P, Jeong JC, et al. 2007. yucca6, a dominant mutation in *Arabidopsis*, affects auxin accumulation and auxin-related phenotypes. *Plant Physiol.* 145:722–35
32. Klee H, Horsch RB, Hinchee MB, Hein MB, Hoffmann NL. 1987. The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. *Genes Dev.* 1:86–96
33. Koga J, Adachi T, Hidaka H. 1992. Purification and characterization of indolepyruvate decarboxylase. A novel enzyme for indole-3-acetic acid biosynthesis in *Enterobacter cloacae*. *J. Biol. Chem.* 267:15823–28

34. Koga J, Syono K, Ichikawa T, Adachi T. 1994. Involvement of L-tryptophan aminotransferase in indole-3-acetic acid biosynthesis in *Enterobacter cloacae*. *Biochim. Biophys. Acta* 1209:241–47
35. Leyser O. 2002. Molecular genetics of auxin signaling. *Annu. Rev. Plant Biol.* 53:377–98
36. Li L, Hou X, Tsuge T, Ding M, Aoyama T, et al. 2008. The possible action mechanisms of indole-3-acetic acid methyl ester in *Arabidopsis*. *Plant Cell Rep.* 27:575–84
37. Li LC, Qin GJ, Tsuge T, Hou XH, Ding MY, et al. 2008. SPOROCYTELESS modulates YUCCA expression to regulate the development of lateral organs in *Arabidopsis*. *New Phytol.* 179:751–64
38. Marsch-Martinez N, Greco R, Van Arkel G, Herrera-Estrella L, Pereira A. 2002. Activation tagging using the En-I maize transposon system in *Arabidopsis*. *Plant Physiol.* 129:1544–56
39. Mikkelsen MD, Naur P, Halkier BA. 2004. *Arabidopsis* mutants in the C-S lyase of glucosinolate biosynthesis establish a critical role for indole-3-acetaldoxime in auxin homeostasis. *Plant J.* 37:770–77
40. Mockaitis K, Estelle M. 2008. Auxin receptors and plant development: a new signaling paradigm. *Annu. Rev. Cell Dev. Biol.* 24:55–80
41. Motchoulski A, Liscum E. 1999. *Arabidopsis* NPH3: A NPH1 photoreceptor-interacting protein essential for phototropism. *Science* 286:961–64
42. Normanly J, Cohen JD, Fink GR. 1993. *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. *Proc. Natl. Acad. Sci. USA* 90:10355–59
43. Normanly J, Grisafi P, Fink GR, Bartel B. 1997. *Arabidopsis* mutants resistant to the auxin effects of indole-3-acetonitrile are defective in the nitrilase encoded by the NIT1 gene. *Plant Cell* 9:1781–90
44. Paciorek T, Zazimalova E, Ruthardt N, Petrasek J, Stierhof YD, et al. 2005. Auxin inhibits endocytosis and promotes its own efflux from cells. *Nature* 435:1251–56
45. Pagnussat GC, Alandete-Saez M, Bowman JL, Sundaresan V. 2009. Auxin-dependent patterning and gamete specification in the *Arabidopsis* female gametophyte. *Science* 324:1684–89
46. Petersson SV, Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, et al. 2009. An auxin gradient and maximum in the *Arabidopsis* root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. *Plant Cell* 21:1659–68
47. Pollmann S, Duchting P, Weiler EW. 2009. Tryptophan-dependent indole-3-acetic acid biosynthesis by 'IAA-synthase' proceeds via indole-3-acetamide. *Phytochemistry* 70:523–31
48. Przemeck GK, Mattsson J, Hardtke CS, Sung ZR, Berleth T. 1996. Studies on the role of the *Arabidopsis* gene MONOPTEROS in vascular development and plant cell axialization. *Planta* 200:229–37
49. Romano CP, Robson PR, Smith H, Estelle M, Klee H. 1995. Transgene-mediated auxin overproduction in *Arabidopsis*: hypocotyl elongation phenotype and interactions with the hy6–1 hypocotyl elongation and axr1 auxin-resistant mutants. *Plant Mol. Biol.* 27:1071–83
50. Santner A, Estelle M. 2009. Recent advances and emerging trends in plant hormone signaling. *Nature* 459:1071–78
51. Sazuka T, Kamiya N, Nishimura N, Ohmae K, Sato Y, et al. 2009. A rice tryptophan deficient dwarf mutant, tdd1, contains a reduced level of indole acetic acid and develops abnormal flowers and organless embryos. *Plant J.* 60:227–41
52. Seo M, Akaba S, Oritani T, Delarue M, Bellini C, et al. 1998. Higher activity of an aldehyde oxidase in the auxin-overproducing superroot1 mutant of *Arabidopsis thaliana*. *Plant Physiol.* 116:687–93
53. Sitbon F, Hennion S, Sundberg B, Little CH, Olsson O, Sandberg G. 1992. Transgenic tobacco plants coexpressing the *Agrobacterium tumefaciens* iaaM and iaaH genes display altered growth and indoleacetic acid metabolism. *Plant Physiol.* 99:1062–69
54. Sohlberg JJ, Myrenas M, Kuusk S, Lagercrantz U, Kowalczyk M, et al. 2006. STY1 regulates auxin homeostasis and affects apical-basal patterning of the *Arabidopsis* gynoecium. *Plant J.* 47:112–23
55. Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM. 2005. A link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in *Arabidopsis*. *Plant Cell* 17:2230–42
56. Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie DY, et al. 2008. TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* 133:177–91
57. Stone SL, Braybrook SA, Paula SL, Kwong LW, Meuser J, et al. 2008. *Arabidopsis* LEAFY COTYLEDON2 induces maturation traits and auxin activity: implications for somatic embryogenesis. *Proc. Natl. Acad. Sci. USA* 105:3151–56

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58. Sugawara S, Hishiyama S, Jikumaru Y, Hanada A, Nishimura T, et al. 2009. Biochemical analyses of indole-3-acetaldoxime-dependent auxin biosynthesis in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 106:5430–35
59. Sun J, Xu Y, Ye S, Jiang H, Chen Q, et al. 2009. *Arabidopsis* ASA1 is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. *Plant Cell* 21:1495–1511
60. Tao Y, Ferrer JL, Ljung K, Pojer F, Hong F, et al. 2008. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* 133:164–76
61. Tobena-Santamaria R, Bliek M, Ljung K, Sandberg G, Mol JN, et al. 2002. FLOOZY of petunia is a flavin mono-oxygenase-like protein required for the specification of leaf and flower architecture. *Genes Dev.* 16:753–63
62. Treml BS, Winderl S, Radykewicz R, Herz M, Schweizer G, et al. 2005. The gene ENHANCER OF PINOID controls cotyledon development in the *Arabidopsis* embryo. *Development* 132:4063–74
63. Trigueros M, Navarrete-Gomez M, Sato S, Christensen SK, Pelaz S, et al. 2009. The NGATHA genes direct style development in the *Arabidopsis* gynoecium. *Plant Cell* 21:1394–409
64. Vanneste S, Friml J. 2009. Auxin: a trigger for change in plant development. *Cell* 136:1005–16
65. Vieten A, Vanneste S, Wisniewska J, Benkova E, Benjamins R, et al. 2005. Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. *Development* 132:4521–31
66. Woo YM, Park HJ, Su'udi M, Yang JI, Park JJ, et al. 2007. Constitutively wilted 1, a member of the rice YUCCA gene family, is required for maintaining water homeostasis and an appropriate root to shoot ratio. *Plant Mol. Biol.* 65:125–36
67. Woodward AW, Bartel B. 2005. Auxin: regulation, action, and interaction. *Ann. Bot.* 95:707–35
68. Woodward C, Bemis SM, Hill EJ, Sawa S, Koshiba T, Torii KU. 2005. Interaction of auxin and ERECTA in elaborating *Arabidopsis* inflorescence architecture revealed by the activation tagging of a new member of the YUCCA family putative flavin monooxygenases. *Plant Physiol.* 139:192–203
69. Wright AD, Sampson MB, Neuffer MG, Michalczuk L, Slovin JP, Cohen JD. 1991. Indole-3-acetic acid biosynthesis in the mutant maize orange pericarp, a tryptophan auxotroph. *Science* 254:998–1000
70. Yamada M, Greenham K, Prigge MJ, Jensen PJ, Estelle M. 2009. The TRANSPORT INHIBITOR RESPONSE2 (TIR2) gene is required for auxin synthesis and diverse aspects of plant development. *Plant Physiol.* 151:168–79
71. Yamamoto Y, Kamiya N, Morinaka Y, Matsuoka M, Sazuka T. 2007. Auxin biosynthesis by the YUCCA genes in rice. *Plant Physiol.* 143:1362–71
72. Yang Y, Xu R, Ma CJ, Vlot AC, Klessig DF, Pichersky E. 2008. Inactive methyl indole-3-acetic acid ester can be hydrolyzed and activated by several esterases belonging to the AtMES esterase family of *Arabidopsis*. *Plant Physiol.* 147:1034–45
73. Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, et al. 2001. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* 291:306–9
74. Zhao Y, Hull AK, Gupta NR, Goss KA, Alonso J, et al. 2002. Trp-dependent auxin biosynthesis in *Arabidopsis*: involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes Dev.* 16:3100–12
75. Ziegler DM. 1988. Flavin-containing monooxygenases: catalytic mechanism and substrate specificities. *Drug Metab. Rev.* 19:1–32

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