Essential Roles of Local Auxin Biosynthesis in Plant Development and in Adaptation to Environmental Changes

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Abstract
It has been a dominant dogma in plant biology that the self-organizing polar auxin transport system is necessary and sufficient to generate auxin maxima and minima that are essential for almost all aspects of plant growth and development. However, in the past few years, it has become clear that local auxin biosynthesis is required for a suite of developmental processes, including embryogenesis, endosperm development, root development, and floral initiation and patterning. Moreover, it was discovered that local auxin biosynthesis maintains optimal plant growth in response to environmental signals, including light, temperature, pathogens, and toxic metals. In this article, I discuss the recent progress in auxin biosynthesis research and the paradigm shift in recognizing the important roles of local auxin biosynthesis in plant biology.
INTRODUCTION

Auxin is an essential hormone for almost every aspect of plant growth and development. One of the predominant hypotheses in auxin biology is that auxin is a morphogen and that auxin gradients determine developmental outcomes (4, 30, 39, 42, 55, 91, 103). The effects of exogenous auxin treatments on plant growth and development are concentration dependent: Low concentrations of exogenous auxin usually promote growth, whereas high concentrations of exogenous auxin inhibit growth (19, 58). Petersson et al. (77) observed an auxin gradient and an auxin maximum in the Arabidopsis root apex by using high-resolution cell-specific auxin measurements. Analyses of auxin reporters DR5-GFP/GUS/Luciferase and DII-VENUS in Arabidopsis and other plant species also revealed that the expression of the auxin reporters was neither uniform nor ubiquitous (8, 20, 31, 88). Consistently, maxima and minima of the auxin reporter expressions were formed during embryogenesis, seedling development, and organogenesis, among other developmental processes (1, 5, 6, 32, 38). More importantly, there appears to be a correlation between an auxin gradient (defined by DR5 expression) and a particular developmental process. For example, an auxin maximum flanking an apical meristem seems to mark the incipient site of a leaf primordium (1, 47, 92). Furthermore, the auxin maximum at the tip of a lateral organ was proposed as a necessary determinant for primordium outgrowth (30, 55). Whereas peaks of auxin response are well correlated with organ initiation and outgrowth, auxin minima were proposed to have an essential role in axillary meristem formation and in specification of the valve margin separation layer in Arabidopsis fruit (24, 85, 105).

Because auxin gradients were proposed to have important roles in plant development, the generation and maintenance of auxin gradients have been studied extensively. The research activities in this regard have centered almost exclusively on the analyses of polar auxin transport. It was observed that exogenously applied auxin to plant segments moves predominantly from the apical
end to the basal end (shoot to root direction). This directional flow of auxin was termed polar auxin transport, which is achieved through plasma-membrane localized carrier proteins. Early studies found that the Arabidopsis pin-formed 1 (PIN1) mutants closely resembled those plants grown on media containing the polar auxin transport inhibitor N-1-naphthylphthalamic acid (NPA), suggesting that PIN1 plays an important role in auxin transport (34). Moreover, the PIN1 protein was located primarily to the basal side of the plasma membrane of parenchymatous xylem cells, consistent with the observation that auxin is transported directionally from the apical end to the basal end (34). Therefore, PIN1 and its homologs (PIN proteins) have served as a proxy for studying the directions and amplitude of polar auxin transport (34, 78, 106). PIN-based mathematical models were constructed to predict and explain developmental patterning such as vascular formation, phyllotaxis, lateral organ initiation, and root cell patterning (23, 38, 47, 70, 84, 92). For example, a model based on PIN-mediated transport generated an auxin gradient in a root tip with the auxin maximum focused at the stem cell niche (38). The model explained the development of sharply bounded meristematic and elongation regions. In this particular model, auxin transport (active transport and diffusion) alone is sufficient to generate an auxin gradient with an auxin maximum, which is able to guide root growth and the development of different cell types (38). The model does not require local auxin biosynthesis, degradation, or regulated auxin influx for the formation of an auxin maximum (38).

Although the studies of polar auxin transport have been valuable, one caveat is that the sources of auxin for a particular transport stream have not been defined. It has generally been assumed that auxin is ubiquitously produced. Some mathematical models suggest that polar auxin transport is so robust that the location of auxin production and degradation has little impact on the formation of auxin gradients (38). However, recent progress in elucidating the molecular mechanisms of auxin biosynthesis revealed that auxin biosynthesis is surprisingly not ubiquitous; rather, it is localized (13, 15, 16, 33, 79, 95, 99, 121). More importantly, disruption of auxin biosynthesis often causes only local developmental defects. For example, knockouts of the shoot auxin biosynthetic genes caused dramatic developmental defects in vascular patterning and flower development but few effects on root development (15). Conversely, disruption of auxin biosynthesis in roots greatly inhibited primary root elongation and caused agravitropic growth but had little effect on shoot growth (13). A growing body of literature demonstrates that local auxin biosynthesis is required for all major developmental processes (12, 15, 16, 79, 95, 99, 115). Plants also use local auxin biosynthesis to optimize their growth when the growth environment undergoes changes (9, 12, 28, 61, 69, 72, 76). In this article, I summarize the recent progress in auxin biosynthesis and highlight the essential roles of local auxin biosynthesis in facilitating various developmental processes and in optimizing growth patterns in response to environmental changes.

AN OVERVIEW OF THE CURRENT UNDERSTANDING OF AUXIN BIOSYNTHESIS

Indole-3-acetic acid (IAA), the primary natural auxin, is synthesized from both tryptophan (Trp)-dependent and Trp-independent pathways (7, 49, 51, 119, 120). The Trp-independent pathway was initially proposed decades ago on the basis of analyses of the maize Trp auxotrophic mutant orange pericarp (109). A subsequent analysis of Arabidopsis Trp biosynthetic mutants suggested that the Trp-independent pathway is used in dicots as well (74). It has been difficult to identify the genes and intermediates for the Trp-independent pathway. The cytosol-localized indole synthase (INS) is a key enzyme in Trp-independent IAA biosynthesis pathways, and auxin generated from Trp-independent pathways has an important role in apical–basal pattern formation during early embryogenesis in Arabidopsis (104). However, there is a lack of consensus on the exact role of INS in auxin biosynthesis and plant development (73).
Multiple Trp-dependent auxin biosynthetic routes have been proposed. Several recent reviews have detailed the evidence (or a lack thereof) supporting the various proposed Trp-dependent auxin biosynthesis pathways (49, 120). So far, only one complete Trp-dependent auxin biosynthesis pathway in plants has been firmly established (Figure 1) (21, 67, 96, 107). The TAA/YUC (TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS/YUCCA) pathway converts Trp to IAA in two consecutive chemical steps (Figure 1). First, Trp is metabolized into indole-3-pyruvate (IPA) by the TAA family of aminotransferases. Second, IPA undergoes oxidative decarboxylation catalyzed by the YUC family of flavin-containing monoxygenases to produce IAA (Figure 1). In this article, I focus on the TAA/YUC pathway because it is the primary auxin biosynthesis pathway and because it is required for all major developmental processes.

The role of the TAA/YUC pathway in auxin biosynthesis is well supported by genetic and biochemical evidence (21, 67, 96, 107). Moreover, the pathway is highly conserved throughout the plant kingdom. The pathway has been functionally characterized in Arabidopsis (21, 67, 96, 107), rice (Oryza sativa) (48, 108, 111, 115), maize (Zea mays) (33, 79), Brachypodium distachyon (75), petunias (101), liverwort Marchantia (25), and many other species (60, 62). Biochemically, the pathway is not complicated. The first reaction of the pathway uses pyridoxal phosphate (PLP) as a cofactor, and the catalytic mechanism of PLP-dependent transaminases is well understood (95, 99). PLP takes the amino group from Trp and transfers it to an α-keto acid. The most common acceptors of an amino group are pyruvate and α-ketoglutarate. Transaminase-catalyzed reactions...
are usually reversible and are determined by the availability of substrates. The TAA-catalyzed reaction is generally considered not rate limiting in auxin biosynthesis (95, 99). The second step of the TAA/YUC pathway requires oxygen and NADPH as co-substrates (Figure 1) (21, 67). The key activated intermediate of the YUC-catalyzed reaction is the C4a-hydroperoxyflavin (C4a-FAD), which can take two different routes (21). In the presence of the substrate IPA, the C4a intermediate facilitates the oxidation and decarboxylation of IPA to yield IAA. In the absence of IPA, the C4a intermediate decays back to the oxidized FAD and releases a molecule of H2O2 even if the IPA substrate is present. The intrinsic uncoupling reaction may provide a mechanism for deactivating YUC flavin-containing monooxygenases (21). Because the C4a intermediate is not stable and the decomposition product is H2O2, a reactive oxygen species, the YUC proteins should be produced only when needed.

The genetic complexity of the TAA/YUC auxin biosynthesis pathway prevented researchers from uncovering it for almost a century (15, 95, 99, 121). In this two-step pathway, the enzymes for each step are encoded by multiple homologous genes. In Arabidopsis, there are at least 4 TAA genes and 11 YUC genes (15, 95, 99, 121). Knockout of a single TAA or YUC gene in Arabidopsis usually does not cause dramatic developmental phenotypes. Simultaneous disruption of several TAA or YUC genes causes severe defects in almost all major developmental processes, which can be rescued by exogenous auxin or by auxin produced in plants with a bacterial auxin biosynthetic gene iaaM (13, 15, 95, 121). Inactivation of TAA genes or YUC genes leads to decreases in auxin concentrations. Furthermore, the taa mutants contained less IPA, whereas yuc mutants accumulated IPA, indicating that TAA aminotransferases are involved in IPA production and that YUC flavin monooxygenases use IPA as substrate (Figure 1) (67).

Several built-in mechanisms ensure that TAA/YUC-mediated auxin biosynthesis does not get out of control (Figure 1). First, IPA is maintained at a steady concentration. If IPA levels are increased, the VAS1 aminotransferase converts IPA back to Trp with Met as the amino donor (Figure 1) (124). The VAS1-catalyzed reaction effectively couples the biosynthesis of ethylene and auxin, two important hormones (124) (Figure 1). Second, the TAA-catalyzed reaction is intrinsically reversible and can convert IPA back to Trp if IPA becomes more abundant (Figure 1) (35). The metabolite L-kynurenine, which competes with Trp for the binding sites in TAA aminotransferase, is a widely used auxin biosynthesis inhibitor (41). However, under certain conditions, kynurenine stimulates auxin biosynthesis by preventing TAA from converting IPA back to Trp (35). Third, the availability of YUC flavin monooxygenases is tightly regulated, and the conversion of IPA to IAA is the rate-limiting step in auxin biosynthesis (12, 26, 59, 98, 121). The expression levels of YUC genes correlate with auxin production in plants. Overexpression of YUC genes in Arabidopsis and rice causes growth and developmental phenotypes (111, 121).

**AUXIN BIOSYNTHESIS IS LOCALIZED AND IS TEMPORALLY AND SPATIALLY REGULATED**

When the TAA/YUC pathway was elucidated, it was obvious that auxin biosynthesis is not ubiquitous. RNA in situ hybridization results showed that an individual YUC gene is expressed only in small groups of discrete cells (Figure 2a) (15, 16). Analyses of the YUC promoter:GUS transgenic lines also revealed the highly localized patterns of auxin biosynthesis (13). Similarly, TAA genes are not expressed in all cells, although their expression patterns are broader than those of YUC genes (95, 99). The expression studies revealed that each YUC gene has a distinct expression pattern, and that some YUC genes also have overlapping expression patterns, suggesting that the YUC genes have overlapping or redundant functions (13, 15, 16). The TAA genes share overlapping
The $yuc_{1-11}$ quadruple mutants and $taa_{1-2}$ double mutants fail to make the basal part of the embryo. YUC10 and TAR1 are imprinted.

The $yuc_{2-6}$ double mutants do not produce viable pollen. The $yuc_{1}$ $yuc_{4}$ double mutants make fewer floral organs. The $yuc_{1-2}$ $yuc_{4}$ quadruple mutants produce pin inflorescences. The $taa_{1-2}$ double mutant flowers are sterile.

The $yuc_{1-2}$ $yuc_{4}$ $yuc_{6}$ quadruple mutants do not make tertiary veins. The $taa_{1-2}$ double mutants also make fewer veins.

Local auxin biosynthesis has essential roles in all major developmental processes. (a) The expression pattern of YUC4 in a mature embryo is shown as an example of localized expression of the auxin biosynthetic genes. RNA in situ hybridization results show that YUC4 is expressed only in the apical meristem and at the tip of cotyledons (black arrows). (b) Mutations in TAA genes or YUC genes result in defects in all major developmental processes. Note that different combinations of $yuc$ mutants affect different developmental programs. The mutant phenotypes usually correlate with the expression patterns of the auxin biosynthetic genes. (c) Auxin required for root development is synthesized mainly in roots, and auxin transported from shoots is not sufficient to support root development. The $yuc_Q$ mutants have short and agravitropic roots, which can be rescued by adding low concentrations of auxin to growth media. Auxin can be produced specifically in shoots by a two-component system. The shoot promoter $FRO6p$ is used to drive the expression of an artificial transcription factor XVE. In the presence of estradiol, XVE binds to OP-LexA, enabling inductive expression of YUC3. When YUC3 is expressed in shoots, it leads to auxin overproduction phenotypes, including long hypocotyls and epinastic cotyledons, but the root defects in $yuc_Q$ are not rescued. Abbreviations: MS, Murashige and Skoog; TAA, TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS; XVE, a chimeric transcription activator that is assembled by fusion of the DNA-binding domain of the bacterial repressor LexA (X), the acidic trans-activating domain of VP16 (V), and the regulatory region of the human estrogen receptor (E); YUC, YUCCA.

Expression patterns as well. Unexpectedly, both $TAA$ and $YUC$ genes are expressed in primary and lateral roots (13, 95, 99). It has long been hypothesized that auxin needed for root development originates from shoots and is transported downward through the polar auxin transport system (1, 38, 100). The emerging picture of auxin biosynthesis is that plants employ a group of $YUC$ and $TAA$ genes to control auxin biosynthesis locally. Precise local auxin production is achieved by expressing different paralogs of the auxin biosynthetic genes (13, 107).
LOCAL AUXIN BIOSYNTHESIS IS REQUIRED FOR ALL MAJOR DEVELOPMENTAL PROCESSES

Auxin biosynthesis is primarily a localized process, and its impact on plant growth and development is largely spatially restricted. Systematic analyses of taa and yuc mutants have demonstrated that local auxin production is required for all major developmental processes (Figure 2b). Local auxin biosynthesis is required for embryogenesis (16, 95), endosperm development (27), seed development (3, 18, 52), root development (13, 95, 99), seedling growth (15, 16, 99), vascular patterning (15, 16, 95), phyllotaxis (80), flower development (15, 16, 95), and de novo organogenesis (12, 90) (Figure 2). Overall, the auxin biosynthetic mutant phenotypes correlate well with the expression patterns of the auxin biosynthetic genes. For example, YUC1, YUC2, YUC10, and YUC11 are the primary YUC genes expressed during Arabidopsis embryogenesis (16). The yuc1 yuc4 yuc10 yuc11 quadruple mutants fail to develop the basal part of the embryo (Figure 2b) (16). Similar phenotypes are observed in taa1 tar2 double mutants (95). Local auxin biosynthesis has an essential role in vascular formation and patterning, a process that has been studied extensively. The predominant hypothesis for vascular patterning is the polar auxin transport–based canalization theory (89). In essence, auxin transport–mediated auxin flow determines the number, pattern, and size of the veins. Auxin biosynthetic yuc1 yuc4 double, yuc1 yuc2 yuc4 triple, and yuc1 yuc2 yuc4 yuc6 quadruple mutants produce progressively fewer veins, suggesting that vascular formation requires a threshold of auxin produced locally by the YUC flavin monooxygenases (15).

Generally, disruption of auxin biosynthetic genes causes developmental defects only in the cells and tissues in which the auxin biosynthetic genes are expressed. Moreover, the impact of a lack of local auxin biosynthesis on the expression of the auxin reporter DR5-GUS/GFP is limited locally. For example, YUC2 and YUC6 are the primary YUC genes expressed during pollen development (10, 11, 15). The yuc2 yuc6 double mutants fail to develop functional mature pollens, but other developmental processes in the mutants are essentially indistinguishable from those in wild-type plants (10, 11, 15). DR5 expression is detectable in a developing anther in which the two YUC genes are expressed. When the two YUC genes are mutated, DR5 expression in the developing pollen disappears as well (11). The correlation of DR5 expression with the expression patterns of YUC genes was also observed during seedling development. YUC1, YUC2, YUC4, and YUC6 are the main YUC genes expressed at leaf primordia. DR5 expression was reduced specifically in the emerging leaflets in the yuc1 yuc4 double mutants. Such a local reduction of DR5 expression correlates with the findings that the YUC genes mark the incipient sites of leaf development and that they are expressed in young leaves (16, 118).

Temporally and spatially regulated auxin biosynthesis controls Arabidopsis endosperm development (27). Before fertilization, the central cell does not divide because the expression of the auxin biosynthetic genes YUC10 and TAR1 is epigenetically blocked by the Polycomb group protein family (Figure 2b). Fertilization removes the epigenetic suppression and induces local auxin biosynthesis in the central cell. The newly synthesized auxin triggers central cell division and proliferation. Production of auxin in the central cell is necessary for proper endosperm development and is sufficient to trigger central cell division (27). In the early stages of endosperm development, locally produced auxin (not auxin transported from other cells or tissues) directs proper cell division events (27). YUC-mediated auxin biosynthesis is also required for endosperm development in maize (3).

The essential roles of auxin in root development are well established, but investigators have given conflicting accounts for the origin of auxin required for root development. Polar auxin transport is necessary for proper root development. For example, both aux1 and pin2, which encode an auxin influx carrier and an auxin efflux carrier, respectively, do not properly respond to gravity stimuli (2, 14, 64). Therefore, it was hypothesized that auxin required for root development...
is first synthesized in shoots and then transported downward through the central vascular tissues to the root tip (38). Then auxin is transported upward from the root tip through epidermal cells. The so-called fountain model of auxin transport has been used to account for root elongation and root gravitropic responses (100). Research conducted over the past few years has elucidated that auxin biosynthetic genes are expressed in roots and that auxin is synthesized in roots. The essential roles of local auxin biosynthesis in root development were revealed when the auxin biosynthetic mutants showed dramatic defects in roots (Figure 2b). Mutations in the TAA1 gene altered the root response to gravity, 1-aminocyclopropane-1-carboxylic acid (ACC), and N-1-naphthylphthalamic acid (NPA), and the phenotypes are caused by a reduction of auxin in roots (95, 99, 110). Because TAA1 is expressed in the shoot as well, it was difficult to determine whether the reduction of auxin concentration is caused by reduced auxin synthesis in roots, reduced transport from the shoot, or both.

The YUC3, YUC5, YUC7, YUC8, and YUC9 genes are expressed in roots, and inactivation of these five YUC genes (yucQ) leads to the development of short and agravitropic roots (Figure 2c). To determine the relative contributions of local auxin biosynthesis in roots and of polar auxin transport from shoots to roots, Chen et al. (13) used an inducible expression system to specifically overexpress a YUC gene in shoots in a yucQ mutant background. Whereas expression of a YUC gene in roots completely rescued the short root and agravitropic phenotypes of yucQ, overexpression of a YUC gene in shoots failed to rescue the root defects of yucQ (Figure 2c). Despite the obvious auxin overproduction phenotypes in shoots caused by overexpression of the YUC gene (Figure 2c), yucQ roots were still short and did not properly respond to gravity, demonstrating that auxin produced in shoots is not sufficient to support root development (13).

LOCAL DEGRADATION AND INACTIVATION OF AUXIN ARE IMPORTANT FOR PLANT DEVELOPMENT

Conjugation of IAA to amino acids catalyzed by the Gretchen Hagen 3 (GH3) family of IAA–amido synthetases has an important role in regulating local concentrations of auxin (Figure 1) (40, 94). Zheng et al. (123, 124) conducted a genetic screen for second site mutations that can suppress the shade avoidance defects of taa1/sav3, which encodes a TAA aminotransferase for auxin biosynthesis (Figure 1). The taa1/sav3 mutants fail to elongate their hypocotyls in response to shade conditions (99). Two interesting suppressors, vas1 and vas2 (vas stands for the reversal of sav), were isolated from the genetic screen. VAS1 is an aminotransferase that specifically uses IPA and Met as the acceptor and donor, respectively, of the amino group (Figure 1) (124). VAS2 is the IAA–amido synthetase GH3.17, which catalyzes the conjugation of free IAA to Glu (Figure 1). IAA-Glu is considered inactive and destined to eventual degradation because exogenously applied IAA-Glu does not cause auxin over-accumulation phenotypes, and no amidohydrolase has been identified as responsible for the release of free IAA from the conjugate. Mutations in VAS2/GH3.17 lead to an increase of free IAA and a decrease of IAA–Glu (123). Consequently, vas2 has long hypocotyls when grown in both normal and shade conditions. VAS2/GH3.17 is expressed predominantly in hypocotyls and its expression in hypocotyls is regulated by light conditions. Shade treatments significantly downregulate VAS2 expression in the hypocotyl, which ensures that free IAA rapidly accumulates in hypocotyls to stimulate hypocotyl elongation. VAS2/GH3.17 functions locally, which is also supported by the observation that vas2 functions independently of polar auxin transport (123). The auxin transport inhibitor NPA can fully inhibit shade-induced hypocotyl elongation in Arabidopsis, presumably because free IAA in cotyledons is prevented from being transported to hypocotyls. However, under the same conditions, shade-stimulated hypocotyl elongation of vas2 mutants and vas2 sav3 double mutants was hardly affected by NPA. The vas2
mutant has longer hypocotyls under shade conditions even when polar auxin transport from cotyledons to hypocotyls is inhibited. Another interesting observation is that hypocotyl elongation in vas1 sav3 double mutants is fully inhibited by NPA. The observed difference in how vas1 and vas2 respond to treatments with NPA suggests that the accumulation of free IAA in vas2 mutant hypocotyls occurs independently of newly synthesized auxin in cotyledons (123).

VAS2 belongs to the GH3 family, which has eight IAA–amido synthetases in Arabidopsis. Interestingly, among the GH3 family members, only VAS2/GH3.17 and GH3.9, which is the closest paralog of VAS2, are not rapidly induced by exogenous auxin treatments. Induction of GH3 expression by IAA provides an effective means to prevent over-accumulation of free IAA. Although both VAS2 and GH3.9 can conjugate IAA to Glu in vitro, disruption of GH3.9 cannot suppress taa1/sav3, indicating that VAS2 and GH3.9 are not redundant in vivo. The phenotypic difference between vas2 and gb3.9 could be caused by the differences in their expression patterns. GH3.9 is expressed predominantly in siliques. Local activation of VAS2/GH3.17 effectively modulates auxin metabolism and rapid auxin spatial redistribution in response to environmental changes (123).

Recent findings demonstrated that auxin degradation has critical roles in maintaining local auxin homeostasis and normal plant development. IAA is oxidized into 2-oxoindole-3-acetic acid (OxIAA) in plants, the first step toward the complete degradation of IAA (Figure 1). Conversion of IAA to OxIAA is catalyzed by DAO (Dioxygenase for Auxin Oxidation), a 2-oxoglutarate-dependent-Fe (II) dioxygenase (122). DAO was first characterized in rice and then subsequently studied in Arabidopsis. In rice, mutations in the DAO gene lead to the development of parthenocarpic seeds filled with a sucrose-rich liquid. The dao flowers do not open and do not release any viable pollens (122). The dao mutants contain no detectable OxIAA in flowers and leaves, indicating that DAO is the primary enzyme that oxidizes IAA into OxIAA in rice (122). Consistent with the hypothesis that DAO converts free IAA to OxIAA, the dao mutants contain elevated concentrations of free IAA and show increased DR5-GUS expression (122). Moreover, the formation of parthenocarpic seeds is phenocopied by overexpressing a YUC gene in wild-type plants or by applying exogenous auxin to wild-type plants (122). DAO protein is also detected primarily in the extracts of wild-type anthers (122). The observable phenotypes in dao mutants correlate well with the expression patterns of DAO, suggesting that local degradation of auxin has important roles in plant development (122). In Arabidopsis, disruption of AtDAO1 resulted in only subtle phenotypes in shoot and root development (68, 81, 116, 117). Metabolic profiling showed that OxIAA concentrations in Atdao mutants decreased 50%, whereas the mutations increased IAA-Glu and IAA-Asp 438-fold and 240-fold, respectively, suggesting that auxin oxidation and conjugation are redundantly used to maintain local auxin homeostasis in Arabidopsis (68, 81, 116).

**PIF-YUC MODULE: REGULATING RESPONSES TO ENVIRONMENTAL SIGNALS**

**Shade Avoidance and Neighbor Detection**

When plants are grown under shade or foliar canopy, they undergo a series of growth and developmental changes, including elongation of hypocotyls, stems, and petioles and development of hyponastic leaves (9, 28, 43). The so-called shade avoidance syndrome (SAS) provides an adaptive advantage by enabling plants to grow quickly to reach light. A related process called neighbor detection allows plants to detect their competitors and to anticipate potentially unfavorable light conditions even before the actual shade conditions are developed. Both SAS and neighbor...
Important roles of local auxin biosynthesis mediated by the PIF-YUC module in adaptation to environmental changes. (a) The PIF-YUC module. PIF transcription factors interact with phytochromes. When in shade, phytochrome B is converted to the inactive Pr form, and PIF transcription factors are released to activate YUC transcription, resulting in localized auxin biosynthesis. (b) Localized shade-induced auxin biosynthesis mediated by PIF-dependent expression of the YUC genes is both necessary and sufficient to trigger leaf hyponasty. Local expression of a YUC gene at a leaf tip or application of exogenous auxin at the leaf tip (red spot) is sufficient to elevate the leaf angle (red trace). More importantly, the hyponastic response is restricted to the treated leaf only, demonstrating that local auxin biosynthesis triggers a local response. (c) Spot irradiation with FR is sufficient to trigger leaf hyponasty. Supplemental FR treatment to the whole plant simulates shade conditions and triggers petiole elongation and leaf hyponasty (middle plant). Spot irradiation with FR (red spot) at the leaf tip also triggers leaf hyponasty, but the response is restricted to the treated leaf only (right plant). Moreover, spot irradiation does not cause petiole elongation. FR treatments convert phytochrome B to the inactive Pr form and release PIF transcription factors that activate YUC-mediated local auxin biosynthesis. Abbreviations: FR, far-red light; IAA, indole-3-acetic acid, PIF, phytochrome-interacting factor; Pr, red light–absorbing form of phytochrome; Pfr, far-red light–absorbing form of phytochrome.

detection rely on the perception of the red (R)-to-far-red (FR) ratio (R:FR ratio) by phytochromes (9, 28). De novo auxin biosynthesis is required for SAS, as both taa mutants and yuc mutants are insensitive to shade conditions (50, 71, 99, 107). Recent studies have established a signaling pathway beginning where changes to the R:FR ratio are detected, which triggers extensive transcription reprogramming mediated primarily by the PIF (phytochrome-interacting factor) family of basic helix–loop–helix transcription factors (Figure 3a) (44, 57). Three PIF transcription factors, PIF4, PIF5, and PIF7, function downstream of the phytochromes during SAS and neighbor detection to regulate auxin biosynthesis by binding directly to promoters of YUC genes (44, 57, 69). Under normal light conditions, phytochrome B (phyB) stays in the active Pfr form and binds to PIF transcription factors, causing PIF transcription factors to become phosphorylated and degraded. Under shade conditions, phyB stays in the inactive Pr form (the red light–absorbing form of phytochrome), and consequently PIF transcription factors remain free and can activate auxin biosynthesis (Figure 3a).
Shade induces quick auxin production in cotyledons by the TAA/YUC pathway, and the newly produced auxin is then transported to the hypocotyl to induce its elongation. However, local auxin biosynthesis is necessary and sufficient to trigger leaf hyponasty in response to simulated shade conditions (Figure 3b) (69, 76). Moreover, local induction of auxin synthesis in one leaf leads to a hyponastic response in only that particular leaf and does not affect the development of other leaves on the same plant (Figure 3b) (69, 76). Michaud et al. (69) manipulated local concentrations of auxin in Arabidopsis leaves by local application of exogenous auxin and by local auxin biosynthesis mediated by YUC gene expression. They discovered that applying auxin or inducing YUC expression at the leaf tip phenocopies the hyponastic response induced by a low R:FR ratio (simulated shade). In contrast, applications of auxin or expressing the YUC genes at petioles did not elicit the hyponastic response, indicating that where auxin is produced has a profound impact on how plants respond to changes in light conditions (69).

In a complementary study, Pantazopoulou et al. (76) applied a 3.5-mm-diameter spotlight of FR irradiation to different regions of an Arabidopsis leaf (Figure 3c). Such a treatment lowers the local R:FR ratio, converts phyB to the inactive Pr form locally, and leads PIF transcription factors to accumulate at the spot (Figure 3a) (76). When an entire leaf is irradiated with FR (FRwhole), leaf hyponasty is observed as expected (76). Leaf hyponasty is also achieved when only the leaf tip is treated with FR (FRtip). In contrast, spot irradiation with FR at petioles does not cause leaf hyponasty (76). Interestingly, leaf hyponasty by FRtip appears to be even more pronounced. Moreover, FRwhole-induced leaf hyponasty can be completely reversed by applying R irradiation at the leaf tip, further supporting the finding that lowering the R:FR ratio at the leaf tip is necessary and sufficient to induce leaf hyponasty (76). Pantazopoulou et al. (76) also noticed that production of auxin in one leaf mediated by changing the local R:FR ratio does not trigger an auxin response in other rosette leaves, indicating that auxin synthesis is local and the response is also restricted locally.

Unlike leaf hyponasty induced by producing auxin at the leaf tip or by lowering the R:FR ratio at the tip, petiole elongation is stimulated by FR treatment of the whole plant (FRwhole), whereas FRtip does not affect petiole elongation (76). Spot irradiation with FR at different regions changes the R:FR ratio and causes local auxin production mediated by PIF-dependent YUC gene expression (Figure 3a). In this process, PIF7 appears to have a predominant role. The pif7 mutant lacks any hyponastic response to either FRwhole or FRtip. The expression of both YUC8 and YUC9 in the lamina tip is also abolished in the pif7 mutant (76). The PIF7-YUC module in the leaf tip determines local auxin biosynthesis and responses to shade conditions.

Phototropism enables plants to grow toward a light source, and phototropins are the blue light receptors responsible for detecting directional light. The phototropic response is enhanced under shade conditions (36). The promotion of phototropism by shade is dependent on local auxin production mediated by the PIF-YUC module. Overexpression of PIF promotes phototropism, as does local induction of YUC gene expression (36). Moreover, induction of YUC3 in pif4 pif5 pif7 triple mutants rescued the inhibition of phototropism, demonstrating that regulated YUC expression by PIF transcription factors is a key regulatory step in phototropism (36).

**Thermomorphogenesis**

Thermomorphogenesis refers to a suite of morphological and architectural changes induced by elevated ambient temperatures (87). Developmental changes induced by high ambient temperatures are in many ways similar to those stimulated by shade (86). For example, both shade and high temperature stimulate the elongation of hypocotyls and petioles (37, 86). It is not surprising that shade avoidance and thermomorphogenesis may share similar molecular mechanisms.
PIF4, PIF3, and, to some degree, PIF5 have emerged as the regulatory hub of thermomorphogenesis, and pif mutants fail to elongate their hypocotyls at high ambient temperatures (29, 82, 83, 86). PIF4 is regulated transcriptionally and post-transcriptionally and by epigenetic modifications (17, 22, 46, 53, 102). Despite the complexity of PIF4 functions in thermomorphogenesis, the PIF-YUC module has a critical role in high-temperature-mediated hypocotyl elongation. High-temperature-induced hypocotyl elongation in *Arabidopsis* is caused by elevated concentrations of auxin (37). Several auxin biosynthetic genes, including *TAA1*, *YUC8*, and *CYP79B2*, are direct targets of PIF4 (29, 97). PIF4 interacts with phyB, which was recently proposed as a plant temperature sensor (54, 93). Warm temperatures reduce the abundance of the active phyB pool, thus freeing PIF4 to activate auxin biosynthesis. PIF4 also interacts with the blue light receptor CRY1 in a blue light–dependent manner (65). One consequence of the PIF4-CRY1 interaction is the inhibition of PIF4’s transcription activity, which partially explains why blue light inhibits hypocotyl elongation under both 22°C and 29°C. *YUC8* was upregulated in cry1 mutants, and CRY1 decreased PIF4-mediated YUC expression in a transient assay (65). The flowering time control protein FCA, which is also an RNA-binding protein, interacts with PIF4 and attenuates PIF4 activities, thus preventing *YUC8* from being induced at high temperature (53).

**Toxic Metals**

Toxic metals inhibit plant growth. For example, aluminum (Al) ions greatly inhibit root elongation in *Arabidopsis* (113). Al toxicity is dependent partially on auxin (59, 112). The auxin biosynthetic mutant *taa1* was less sensitive to Al than wild-type plants were, and the addition of auxin to growth media enhances the inhibitory effects of Al on root elongation (112). YUC flavin monoxygenases are also required for the full toxicity of Al on root growth. Interestingly, the expression of several YUC genes, including *YUC3*, *YUC5*, *YUC7*, *YUC8*, and *YUC9* (the root YUC genes), is induced when roots are treated with Al (59). PIF4 also appears to have a role in response to Al-induced stress. Disrupting PIF4 expression significantly alleviates the inhibition of root growth caused by Al-induced stress, whereas overexpression of PIF4 strengthened the inhibitory effects of Al. PIF4 binds directly to the promoters of *YUC5*, *YUC8*, and *YUC9* to promote local auxin biosynthesis in the root-apex transition zone when plants face Al-induced stress, thus demonstrating another example of the important roles of the PIF-YUC module (Figure 3b) (59).

**PLANT-PARASITE AND PLANT-PATHOGEN INTERACTIONS REQUIRE LOCAL AUXIN BIOSYNTHESIS**

**YUC-Mediated Local Auxin Biosynthesis Regulates Haustorium Development**

A recent study of parasitic plant–host plant interactions demonstrates the importance of local auxin biosynthesis (Figure 4) (45). Parasitic plants can devastate agricultural productivity when they infect economically important crops such as corn. A key step during infection of a host plant by a parasitic plant is the formation of a haustorium, a multicellular organ that penetrates the host tissues (Figure 4) (45). Host-derived chemicals such as 2,6-dimethoxy-p-benzoquinone (DMBQ) can activate haustoria formation in parasitic roots in the absence of a host plant. Ishida et al. (45) used the facultative parasitic plant *Phtheirospermum japonicum* to study the molecular mechanisms by which haustoria formation is initiated. They identified and analyzed the *P. japonicum* genes induced by DMBQ. The gene *Pj-YUC3* clustered with the *Arabidopsis YUC3*, *YUC5*, *YUC7*, *YUC8*, and *YUC9*, which are the root YUC genes. *Pj-YUC3* was highly upregulated one day after treatment with host root exudate and was induced by more than 200-fold two days
Development of haustoria in the parasitic plant *Phtheirospermum japonicum* is controlled by YUC-mediated local auxin biosynthesis. (a) A haustorium is formed when the parasitic plant *P. japonicum* infects an *Arabidopsis* root. The vascular tissues in both host and parasitic plants are stained in red. Panel a was kindly provided by Dr. Ken Shirasu at RIKEN Japan. (b) Expression of *YUC3* is necessary and sufficient to induce the formation of a haustorium. During an infection, *YUC3* is expressed primarily within the epidermal and outer cortical layers of the region where haustorium growth is initiated (green dots), which is located at the interface between the host and parasitic roots.

After treatment. Upregulation of *Pj-YUC3* was also observed in haustorial tissues post infection. More importantly, the expression of *Pj-YUC3* is highly localized (Figure 4) (45). Its expression localizes to the haustorium apex and haustorial hairs. Spatially, *Pj-YUC3* was expressed primarily at the interface between the host and the parasitic root (45). The induction of *Pj-YUC3* expression also correlates with an increase in auxin concentration following root exudate treatment. Expression of *DR5* correlates with the patterns of *Pj-YUC3* expression during the early stages of haustorium development. Genetic studies demonstrated that local expression of *Pj-YUC3* and local auxin biosynthesis are necessary and sufficient to induce a haustorium-like structure in *P. japonicum* roots (45). Knockout of *YUC3* in *P. japonicum* roots significantly decreases the frequency of haustoria formation. On the other hand, ectopic expression of *Pj-YUC3* at the root epidermal cells stimulates haustorium-like structures to form in the absence of a host or without host root exudate treatment. The early stages of haustorium development appear to be controlled primarily by YUC-mediated local auxin biosynthesis, and the involvement of polar auxin transport is minimal (45).

**Herbivore Attack Rapidly Induces Local Auxin Production for Plant Defense**

Jasmonates (JAs) are recognized as the prominent hormone in plant defense against herbivores (56, 63, 114). However, a recent study has revealed that auxin may also have a key role in this process (66). Using the herbivore *Manduca sexta* (tobacco hornworm) and its host plant *Nicotiana attenuata*,
Machado et al. (66) discovered that herbivore attack or simulated herbivore attack (treatments with herbivore oral secretions and fatty acid conjugate elicitors) induces auxin to rapidly accumulate at the site of attack. The accumulation of IAA is so fast that it precedes the JA burst, a defensive response by plants against herbivores. Moreover, the accumulation of IAA does not require JA signaling (66). *N. attenuata* has at least nine *YUC*-like genes. Upon application of *M. sexta* oral secretions and fatty acid conjugates to the host plant, the expression of *NaYUCCA-like* 1, 3, 5, 6, and 9 genes is induced within 3 minutes. The induction of the *YUC* genes is likely responsible for the rapid accumulation of IAA upon herbivore attack (66), suggesting that de novo auxin biosynthesis has a critical role in plant defense against this herbivore.

**SUMMARY POINTS**

1. Auxin is produced in plants primarily by the two-step Trp-dependent auxin biosynthesis pathway catalyzed by the TAA family of transaminases and the YUC family of flavin monooxygenases. The pathway is conserved throughout the plant kingdom.
2. Both *TAA* genes and *YUC* genes are not expressed ubiquitously. Rather, their expression is localized. Where auxin is produced in the plant has a profound impact on plant growth and development.
3. Auxin required for root development is synthesized mainly in roots by the TAA/YUC pathway, and shoot-derived auxin is not sufficient to support root growth in *Arabidopsis*.
4. Local auxin biosynthesis is necessary and sufficient for several developmental processes, including endosperm development, leaf hyponasty under shade conditions, and some host–parasite interactions.

**DISCLOSURE STATEMENT**

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

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**LITERATURE CITED**


11. Investigates the contribution of auxin transported from shoots to root growth.


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59. Demonstrates that local auxin biosynthesis by YUC flavin monoxygenases is important for plants to respond to aluminum stress.

65. Investigates how cryptochrome 1 affects local auxin biosynthesis through the PIF-YUC module.

66. Reveals that local auxin biosynthesis is induced by herbivore attack prior to the production of jasmonate, a defense hormone.

69. Elegantly shows that local auxin production at leaf tip is necessary and sufficient for local leaf hyponasty.

76. Shows that changes in local auxin biosynthesis triggered by spotted light treatments are responsible for local leaf movements.


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123. Shows that local removal of auxin by conjugation is important for establishing an auxin gradient during shade avoidance response.
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Errata

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