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Source: The Arabidopsis Book, 2014(12)

Published By: The American Society of Plant Biologists

URL: https://doi.org/10.1199/tab.0173

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First published on June 13, 2014: e0173. doi: 10.1199/tab.0173

Auxin Biosynthesis

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Indole-3-acetic acid (IAA), the most important natural auxin in plants, is mainly synthesized from the amino acid tryptophan (Trp). Recent genetic and biochemical studies in Arabidopsis have unambiguously established the first complete Trp-dependent auxin biosynthesis pathway. The first chemical step of auxin biosynthesis is the removal of the amino group from Trp by the TRYPTOPHAN AMINOTRANSFERASE OF *ARABIDOPSIS (TAA)* family of transaminases to generate indole-3-pyruvate (IPA). IPA then undergoes oxidative decarboxylation catalyzed by the YUCCA (YUC) family of flavin monooxygenases to produce IAA. This two-step auxin biosynthesis pathway is highly conserved throughout the plant kingdom and is essential for almost all of the major developmental processes. The successful elucidation of a complete auxin biosynthesis pathway provides the necessary tools for effectively modulating auxin concentrations in plants with temporal and spatial precision. The progress in auxin biosynthesis also lays a foundation for understanding polar auxin transport and for dissecting auxin signaling mechanisms during plant development.

INTRODUCTION

Auxin has long been recognized as a hormone essential for almost every aspect of plant growth and development (Zhao, 2010). However, an understanding of its biosynthetic mechanisms in plants had remained elusive until very recently. For a long time, the physiological roles of auxin were mainly inferred from studies on how plants responded to exogenous auxin treatments. These studies were also the foundation for elucidating the auxin signaling and polar transport mechanisms. However, to precisely define the physiological roles of auxin, we need to characterize auxin deficient mutants, a goal that becomes feasible only when we understand how auxin is synthesized in plants. Understanding of auxin biosynthesis will also reveal the sites of auxin production in plants, thereby allowing us to define auxin sources/sinks and to better understand polar auxin transport. Knowledge in auxin biosynthesis will greatly facilitate our understanding of the molecular mechanisms by which auxin controls various developmental processes. Progress in auxin biosynthesis research lays the foundation for improving agriculturally important traits such as branching and flower development by allowing us to regulate auxin levels in specific tissues/cells. Therefore, a clear understanding of auxin biosynthesis will ultimately have many significant impacts on agriculture and will also greatly extend our knowledge of fundamental plant biology.

Auxin biosynthesis can be divided into two general categories: *de novo* auxin biosynthesis and the release from auxin conjugates [see recent reviews (Normanly, 2010; Ludwig-Muller, 2011; Mano and Nemoto, 2012; Brumos et al., 2013; Ljung, 2013; Zhao, 2013; Tivendale et al., 2014)]. Indole-3-acetic acid (IAA), the main natural auxin in plants, exists in both free and conjugated forms. Free IAA is the active form of auxin and the conjugated auxins are considered storage forms or intermediates destined for degradation (Woodward and Bartel, 2005; Korasick et al., 2013). Free IAA can be released from IAA conjugates such as IAA esters, IAA-sugar, and IAA-amino acid conjugates by hydrolysis (Davies et al., 1999; Rampey et al., 2004; Ludwig-Muller, 2011; Korasick et al., 2013). Free IAA can also be produced from indole-3-butyric acid by a process similar to fatty acid β-oxidation in the peroxisomes (Zolman et al., 2000; Zolman et al., 2008). In this chapter, I focus on the recent progresses in *de novo* auxin biosynthesis. Mechanisms regarding the release of free auxin from conjugates and IBA have been reviewed elsewhere (Woodward and Bartel, 2005; Ludwig-Muller, 2011; Korasick et al., 2013).

Trp is a known precursor for auxin biosynthesis and it has been demonstrated that feeding plants with labeled Trp leads to the production of labeled IAA (Wright et al., 1991; Normanly et al., 1993). Two decades ago, isotope-labeling experiments in combination with using Trp biosynthetic mutants led to the proposal that IAA is also synthesized in a Trp-independent fashion (Wright et al., 1991; Normanly et al., 1993). So far, however, the molecular components of the Trp-independent pathway have not been identified. In this chapter, I will not discuss the Trp-independent auxin biosynthesis pathway. Instead, I will concentrate on the discovery of the first complete plant auxin biosynthetic pathway in which Trp is converted into IAA in two steps using indole-3-pyruvate (IPA) as the intermediate (Figure 1). This two-step auxin biosynthesis pathway plays an essential role in almost all of the major developmental processes including embryogenesis, seedling growth, root elongation, vascular patterning, gravitropism, and flower development. The pathway is highly conserved throughout the plant

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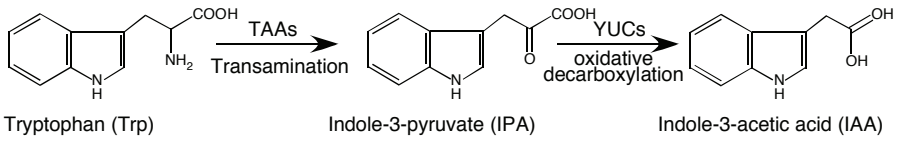


Figure 1. A complete tryptophan-dependent auxin biosynthesis pathway in plants.

Auxin is synthesized from the amino acid Trp in two chemical steps. The first step is the removal of the amino group by the TAA family of aminotransferases to produce IPA. The second step is the oxidative decarboxylation of IPA catalyzed by the YUC family of flavin-containing monooxygenases to generate IAA, CO_2 and water.

kingdom and has been functionally characterized in several plant species in both monocots and dicots.

Besides IPA, several other compounds including Indole-3-acetonitrile and Indole-3-acetamide have also been proposed as intermediates in auxin biosynthesis. Because the other pathways are less well defined and they have been reviewed extensively elsewhere (Woodward and Bartel, 2005; Zhao, 2010; Brumos et al., 2013; Korasick et al., 2013; Tivendale et al., 2014), I will not elaborate further on those alternative pathways. In this chapter, I discuss the genetic and biochemical data that led to the establishment of the first complete Trp-dependent auxin biosynthetic pathway. I also discuss the implications of the breakthrough in auxin biosynthesis in the context of studying polar auxin transport and auxin signaling. Knowledge of auxin biosynthesis allows us to manipulate auxin concentrations in plant cells, thereby enabling us to investigate the molecular mechanisms of auxin-regulated plant developmental processes from a different perspective.

GENETIC DISSECTION OF AUXIN BIOSYNTHESIS

Early auxin overproduction mutants and Trp biosynthesis mutants

Genetic approaches have been widely used to dissect the biosynthetic and signaling pathways of plant hormones. But genetic dissection of auxin biosynthesis has been a very long and difficult journey. Early attempts were centered on the characterization of Trp biosynthesis mutants, which display pleiotropic developmental phenotypes. Interestingly, free IAA concentrations in some of the Trp biosynthetic mutants do not differ significantly from those in wild type plants (Normanly et al., 1993). Some *trp* mutants actually contain elevated levels of total IAA (Normanly et al., 1993). The studies on Trp biosynthesis mutants led to the discovery of a Trp-independent auxin biosynthesis pathway, but revealed very little about how Trp is converted into IAA in plants.

The first major advance in the genetic dissection of auxin biosynthesis in plants was the isolation of the auxin overproduction mutants *superroot1* (*sur1: At2g20610*) and *superroot2* (*sur2: At4g31500*) in Arabidopsis (Boerjan et al., 1995; Delarue et al., 1998). When grown in light, both *sur1* and *sur2* have much longer hypocotyls than do wild type plants. Light-grown *sur1* and *sur2* plants also display epinastic cotyledons and true leaves. In total darkness, *sur1* and *sur2* seedlings lack an apical hook and have short hypocotyls (Boerjan et al., 1995; Delarue et al., 1998). The most striking phenotype of *sur1* and *sur2* is the development of adventitious roots from their hypocotyls. *SUR1* encodes a C-S lyase and *SUR2* encodes the Cytochrome P450 monooxygenase CYP83B1 (Barlier et al., 2000; Mikkelsen et al., 2004). Both SUR1 and SUR2 are now known key components of glucosinolate biosynthesis (Figure. 2). Disruption of either *SUR1* or *SUR2* blocks the biosynthesis of glucosinolates and leads to the accumulation of the metabolic intermediate indole-3-acetaloxime (IAOx), which can be converted into IAA through an undefined mechanism

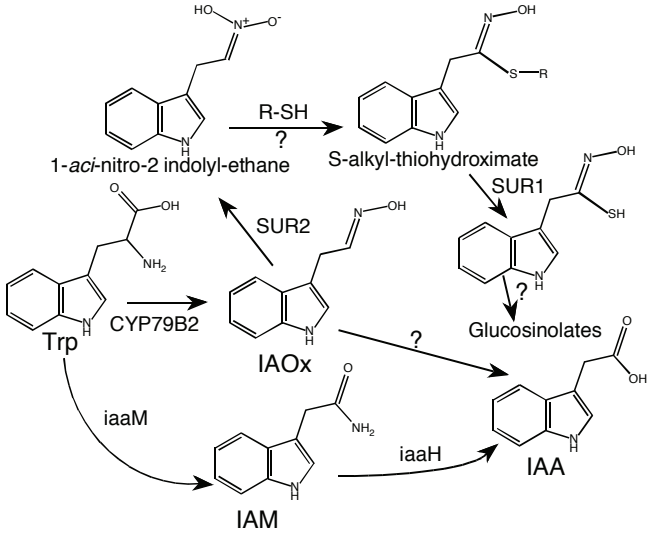


Figure 2. Two proposed metabolic pathways for the amino acid Tryptophan (Trp).

Trp is oxidized into indole-3-acetaloxime (IAOx) by the Cytochrome P450 CYP79B2/B3. IAOx is a substrate for another P450, CYP83B1, which is also called SUPERROOT2 (SUR2). SUR2 converts IAOx into 1-*aci*-nitro-2 indolyl-ethane, which is further converted to Indol-3-ylmethyl S-alkyl-thiohydroximate by an unknown mechanism. SUPERROOT1 (SUR1) is a C-S lyase that converts S-alkyl-thiohydroximate to thiohydroximate. Both SUR1 and SUR2 are important for glucosinolate biosynthesis. Inactivation either *SUR1* or *SUR2* leads to the accumulation of IAOx, which is converted into IAA by an unknown mechanism. Trp can also be oxidized to indole-3-acetamide (IAM) by the bacterial auxin biosynthesis enzyme iaaM Trp-2-monooxygenase. IAM is hydrolyzed into IAA by the bacterial enzyme iaaH. Overexpression of *iaaM* or *CYP79B2* leads to auxin overproduction phenotypes. Question mark indicates that enzymes for the steps are not known.

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(Figure 2). IAOx is produced from Trp in Arabidopsis by the Cytochrome P450 monooxygenases CYP79B2 (AT4G39950) and B3 (AT2G22330) (Figure 2) (Hull et al., 2000; Zhao et al., 2002). Overexpression of *CYP79B2* leads to the accumulation of IAOx and consequently auxin overproduction phenotypes (Hull et al., 2000; Zhao et al., 2002). Characterization of *CYP79B2/B3* overexpression lines and the *sur1* and *sur2* mutants firmly establishes that IAOx can function as an auxin biosynthesis intermediate in Arabidopsis. However, it is now clear that IAOx is probably not a key auxin biosynthesis intermediate used throughout the plant kingdom (Zhao et al., 2002; Sugawara et al., 2009).

Although the analysis of the *sur1* and *sur2* auxin overproduction mutants and the *CYP79B2* overexpression lines did not uncover the key auxin biosynthesis mechanisms used by the majority of plants, these early studies established the phenotypic characteristics of auxin overproduction in Arabidopsis. Light grown auxin overproduction mutants have long hypocotyls and epinastic cotyledons. When grown in the dark, auxin overproduction mutants have short hypocotyls and lack apical hooks. These characteristic auxin overproduction phenotypes are also observed in transgenic plants in which the bacterial auxin biosynthesis gene *iaaM* is overexpressed under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter (Romano et al., 1995). The *iaaM* gene encodes the Trp-2-monooxygenase that catalyzes the conversion of Trp into indole-3-acetamide (IAM), which is subsequently hydrolyzed into IAA by the bacterial hydrolase iaaH (Figure 2) (Yamada et al., 1985). Interestingly, the auxin overproduction phenotypes observed in *sur1*, *sur2*, *CYP79B2* overexpression lines, and the *iaaM* overexpression lines are quite different from those displayed in plants treated with exogenous IAA, the synthetic auxin 1-naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). The prominent phenotype of Arabidopsis seedlings grown on IAA-containing media is that the plants develop short roots (Lincoln et al., 1990; Hobbie and Estelle, 1994). The characteristic phenotype of the auxin overproduction mutants is that they all have long hypocotyls and epinastic cotyledons.

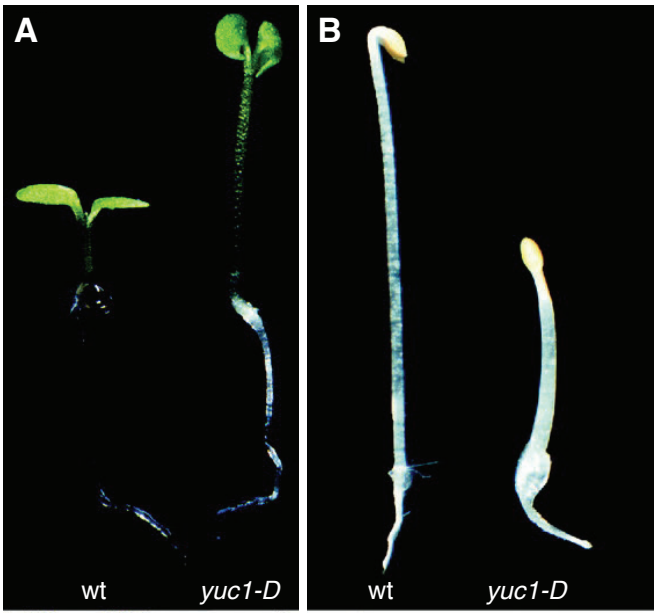


Figure 3. The characteristic phenotypes of auxin overproduction in Arabidopsis.

Overexpression of *YUC1* (*yuc1-D*) leads to long hypocotyl and epinastic cotyledons in light grown seedlings (A). In total darkness, *yuc1-D* has short hypocotyl and lacks an apical hook (B). This figure is reprinted with permission from *Science*. The original figure was published in Zhao, Y., Christensen, S.K., Fankhauser, C., Cashman, J.R., Cohen, J.D., Weigel, D., and Chory, J. (2001). A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* 291, 306-309.

Identification of *YUCCA* genes by activation tagging

The development of activation tagging technology in Arabidopsis in the late 1990s made it possible to identify important genes by isolating gain-of-function mutants (Weigel et al., 2000). When copies of the CaMV 35S enhancers are inserted near a gene, the enhancers cause elevated expression of the gene. Joanne Chory's group isolated long hypocotyl mutants by activation tagging in an attempt to identify genes involved in light signaling. One of the mutants named *yucca* (late renamed as *yuc1-D, At4g32540*) displays long hypocotyl and epinastic cotyledons when grown in light (Figure 3). In total darkness, *yuc1-D* has short hypocotyl and lacks an apical hook (Figure 3) (Zhao et al., 2001). The phenotypes of *yuc1-D* are almost identical to those observed in *sur1*, *sur2*, *CYP79B2* overexpression lines, and the *iaaM* overexpression lines, suggesting that *yuc1-D* phenotypes are probably caused by auxin overproduction. The *yuc1-D* mutants also have short primary roots, and develop longer and more root hairs than wild type plants (Zhao et al., 2001). Moreover, *yuc1-D* plants display increased apical dominance, which is often associated with elevated auxin levels. All of the growth and developmental phenotypes of *yuc1-D* plants indicate auxin overproduction (Zhao et al., 2001).

Physiological and genetic evidence demonstrates that *yuc1-D* indeed is an auxin overproduction mutant (Zhao et al., 2001). Explants of *yuc1-D* develop a large number of roots on plates containing Murashige and Skoog (MS) media. The *yuc1-D* explants develop into calluses on auxin-free media, suggesting that *yuc1-D* produces sufficient auxin to support the regeneration of an Arabidopsis plant. In *yuc1-D*, the known auxin inducible genes such as *GH3*, *Aux/IAA*, and the *SAUR* genes are up regulated. The auxin reporter *DR5-GUS* is also expressed at elevated levels (Zhao et al., 2001). Furthermore, the phenotypes of *yuc1-D* can be partially reversed by overexpressing the bacterial gene *iaaL*, which encodes an enzyme that conjugates free IAA with the amino acid lysine (Glass and Kosuge, 1986, 1988). Direct auxin measurements show that *yuc1-D* contains 50% more free IAA than wild type plants. All of the physiological, genetic, and analytic biochemical data provide evidence that *yuc1-D* phenotypes are caused by auxin overproduction (Zhao et al., 2001).

The *yuc1-D* phenotypes are caused by the insertion of copies of CaMV 35S enhancers into Chromosome IV. The 35S enhancers lead to overexpression of the *YUCCA* (*YUC1: AT4G32540*) gene, which encodes a putative flavin-containing monooxygenase (Zhao et al., 2001). *YUC* belongs to a large gene family with 11 members in the Arabidopsis genome. *YUC* genes have

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been identified in all of the sequenced plant genomes, suggesting that *YUC*s are involved in an evolutionarily conserved auxin biosynthesis pathway. It has been shown that overexpression of *YUC* genes in Arabidopsis (Zhao et al., 2001; Woodward et al., 2005; Cheng et al., 2006; Kim et al., 2007; Hentrich et al., 2013;), tomato (Exposito-Rodriguez et al., 2011), tobacco (Zhao et al., 2001), petunia (Tobena-Santamaria et al., 2002), potato (Kim et al., 2012), strawberry (Liu et al., 2012), and rice (Yamamoto et al., 2007) leads to auxin overproduction.

The *YUC* genes are key auxin biosynthesis genes and essential for plant development

The *YUC* genes were not identified from previous genetic screens for loss-of-function mutants in Arabidopsis because of genetic redundancy among the *YUC* genes. Inactivation of a single *YUC* gene in Arabidopsis does not result in any obvious developmental defects. Characterization of various combinations of *yuc* mutants demonstrates that *YUC* genes are essential for embryogenesis, seedling growth, vascular pattern formation, and flower development (Figure 4) (Cheng et al., 2006, 2007a). The *yuc1 yuc4* (*yuc1*: *At4g32540*; *yuc4*: *At5g11320*) double mutants fail to make tertiary veins in leaves and produce discontinuous veins in flowers (Figure 4) (Cheng et al., 2006). The *yuc1 yuc4* phenotypes are further enhanced by disrupting *YUC2* (*AT4G13260*) and *YUC6 (AT5G25620)* (Cheng et al., 2006). The *yuc1 yuc4 yuc10 yuc11* (*yuc10*: *At1g48910*; *yuc11*: *At4g32540*) quadruple mutants do not have the basal part of the embryo (Cheng et al., 2007a), a phenotype that is also observed in auxin signaling mutants such as *monopteros (mp: At1g19850)* (Przemeck et al., 1996; Hardtke and Berleth, 1998), *bodenlos* (*bdl: At1g04550)* (Hamann et al., 1999), and *tir1 afb1 (tir1:At3g62980; afb1: At4g03190)* mutants (Dharmasiri et al., 2005). When *YUC3 (AT1G04610)*, *YUC5 (AT5G43890)*, *YUC7 (AT2G33230)*, *YUC8 (AT4G28720)*, and *YUC9 (AT1G04810)*, which form a clade in a phylogenetic tree, are simultaneously inactivated, the resulting quintuple mutants (*yuc*Q) develop very short and agravitropic roots (Chen et al., 2014).

Although overexpression of *YUC* genes causes auxin overproduction and disruption of *YUC* genes leads to dramatic developmental phenotypes, it was challenging to demonstrate that the developmental defects of the *yuc* mutants were caused by auxin deficiency. The *yuc1 yuc4* double mutants that displayed severe vascular and floral defects (Figure 4) could not be rescued by adding auxin to the growth media or by spraying auxin directly onto the plants (Cheng et al., 2006). It was hypothesized that auxin needs to be synthesized in specific cells at the right time in order for proper plant growth and development to proceed. The essential role of *YUC* genes in auxin biosynthesis was eventually demonstrated by using a genetic approach. Auxin biosynthesis by *YUC*s can be

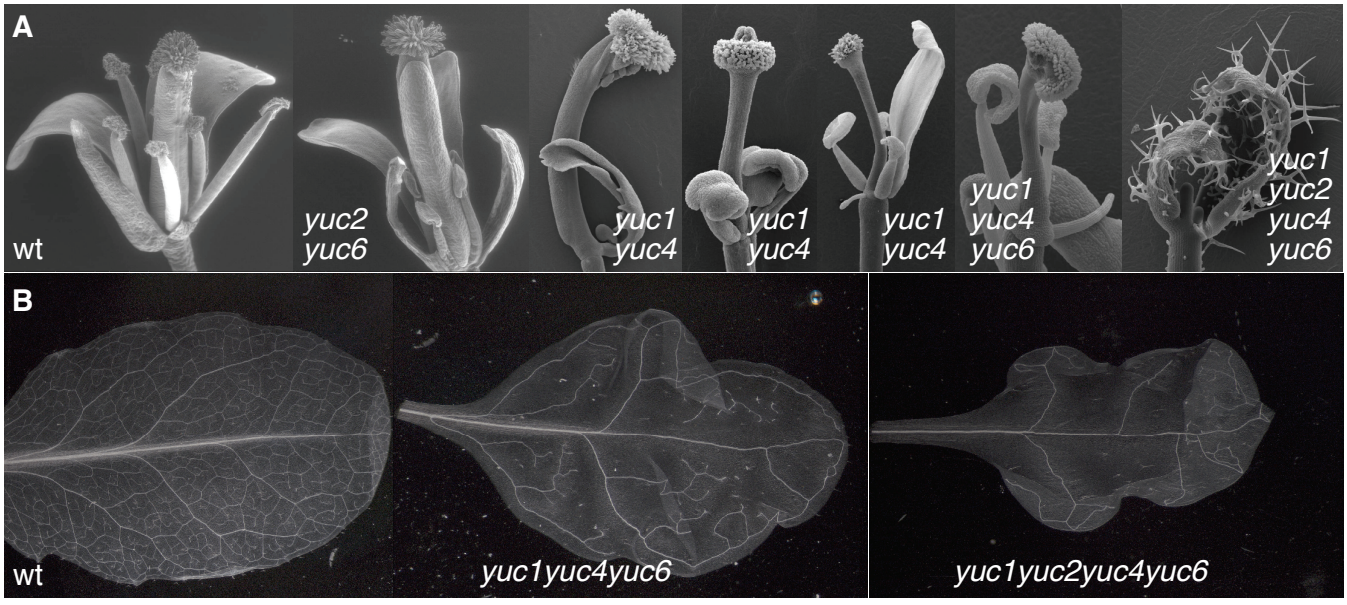


Figure 4. The *YUC* genes play essential roles in flower (A) and vascular development (B) in Arabidopsis.

Arabidopsis WT flowers usually have 4 sepals, 4 petals, 6 stamens, and two fused carpels (Note that 2 sepals and 2 petals are removed in WT to reveal the inner organs). Disruption of *YUC2* and *YUC6* leads to very short stamens. The *yuc1 yuc4* double mutants produce sterile flowers with dramatically reduced number of floral organs. In addition, *yuc1 yuc4* flowers display variations in terms of the type and number of floral organs. Note that the three *yuc1 yuc4* flowers shown are from the same plant and that they have different number and type of floral organs. The *yuc1 yuc2 yuc4 yuc6* quadruple mutants sometimes fail to produce flowers. Instead, pin-like structures are produced in the quadruple mutants. B) Inactivation of *YUC* genes prevents plants from forming tertiary and higher order veins in leaves. This figure is reprinted with permission from *Genes & Development.* The original figure was published in Cheng, Y., Dai, X., and Zhao, Y. (2006). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. Genes Dev 20, 1790-1799.

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mimicked by expressing the bacterial auxin biosynthetic gene *iaaM* under the control of a specific *YUC* promoter. Expression of the *iaaM* gene under the control of the *YUC1* promoter rescued the *yuc1 yuc4* double mutants (Cheng et al., 2006). *YUC6* promoter-driven *iaaM* expression was able to completely rescue the sterile phenotypes of *yuc2 yuc6* double mutants (Cheng et al., 2006). Recently, the *yuc*Q quintuple mutants were discovered to have dramatic defects in root development (Chen et al., 2014). Interestingly, the *yuc*Q phenotypes are rescued by adding a very low concentration (5nM) of IAA into the growth media (Chen et al., 2014). The difference in response to exogenous auxin between *yuc*Q and *yuc1 yuc4* double mutants may be caused by different auxin transport capacity among different tissues.

In summary, the essential roles of the *YUC* genes in auxin biosynthesis and in Arabidopsis development have been unambiguously demonstrated. Overexpression of *YUC*s leads to auxin overproduction and disruption of *YUC* genes causes dramatic developmental defects, which can be rescued by adding auxin in growth media (Chen et al., 2014) or by expressing the *iaaM* gene under the control of a *YUC* promoter (Cheng et al., 2006).

YUC genes in petunia and maize have also been shown to play essential roles in development (Tobena-Santamaria et al., 2002; Gallavotti et al., 2008; LeCLere et al., 2010; Bernardi et al., 2012). Interestingly, the genetic redundancy of *YUC* genes appears to be less pronounced in petunia and in maize than in Arabidopsis. The single *yuc* mutant in petunia called *floozy* has defects in the formation of floral organ primordia and bracts at the base of the flower. Additionally, *floozy* mutants fail to produce secondary veins in leaves and bracts and display a decreased apical dominance in the inflorescence (Tobena-Santamaria et al., 2002). The *floozy* phenotypes are similar to those observed in Arabidopsis *yuc1 yuc4* double mutants. The maize *yuc* mutant *sparse inflorescence1* (*spi1*) has defects in the initiation of axillary meristems and lateral organs during both vegetative and reproductive development (Gallavotti et al., 2008). The maize mutant *defective endosperm18* (*de18*) is another *yuc* mutant (*Zmyuc1*) that has dramatically reduced free IAA levels and has approximately 40% less dry mass than the wild type (LeCLere et al., 2010; Bernardi et al., 2012).

Identification of TAA aminotransferases as key auxin biosynthetic enzymes

Three groups independently identified the *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1* (*TAA1*: *AT1G70560*) gene in Arabidopsis from three forward genetic screens (Stepanova et al., 2008; Tao et al., 2008; Yamada et al., 2009). In a genetic screen for mutants with altered responses to shade growth conditions, Joanne Chory's group isolated a mutant called *sav3* that had short hypocotyls in shade compared to wild type plants, which had elongated hypocotyls under the same conditions (Tao et al., 2008). Jose Alonso's group isolated a *weak ethylene insensitive 8* (*wei8*) mutant, which has elongated primary roots in the presence of the ethylene biosynthetic precursor ACC (Stepanova et al., 2008). It was later found out that *wei8* is allelic to the *sav3* mutants. Mark Estelle's group identified additional *sav3*/*wei8* alleles from a screen for mutants resistant to the auxin transport inhibitor NPA (*tir2* mutants) (Yamada et al., 2009). *SAV3/WEI8/TIR2* were renamed as *TAA1*, which encodes an aminotransferase that catalyzes the conversion of Trp to IPA.

TAA1 is the founding member of a large family of aminotransferases. Like the *YUC* genes, *TAA* genes also have overlapping functions. Although *taa1* mutants display altered responses to shade and are resistant to both ethylene and NPA, they do not show dramatic developmental defects under normal growth conditions. However, simultaneous inactivation of *TAA1* and its two close homologs *TAA RELATED1* (*TAR1: AT1G23320*) and *TAA RELATED 2* (*TAR2: AT4G24670*) leads to embryogenesis defects. The *wei8 tar1 tar2* triple mutants display *mp*-like phenotypes: the basal part of the embryo fails to develop (Stepanova et al., 2008). The *wei8 tar2* double mutants have dramatic defects in vascular and floral development and are completely sterile (Stepanova et al., 2008).

That the *TAA* genes are key auxin biosynthesis genes is supported by several observations. First, the *taa* mutants have lower auxin concentrations than do wild type plants (Stepanova et al., 2008; Tao et al., 2008). Second, the expression levels of the auxin reporter *DR5-GUS/GFP* are decreased in the *taa* mutants (Stepanova et al., 2008; Tao et al., 2008). Third, exogenous IAA can rescue the root phenotypes of *wei8* (Stepanova et al., 2008). The shade avoidance defects of *sav3* are also rescued by overexpressing the bacterial auxin biosynthetic gene *iaaM* or by using the synthetic auxin picloram (Tao et al., 2008).

TAA genes are highly conserved throughout the plant kingdom. The orthologs of the Arabidopsis *TAA1* in maize, in rice, and in Brachypodium have been shown to play important roles in auxin biosynthesis and in development (Phillips et al., 2011; Pacheco-Villalobos et al., 2012; Yoshikawa et al., 2014). The genetic redundancy of the *TAA* genes in monocots appears to be less than that of the *TAA* genes in Arabidopsis. Inactivation of a single *TAA1*-like gene in maize (*vt2* mutants) leads to dramatic defects in both vegetative and reproductive development (Phillips et al., 2011). Disruption of the rice *TAA1* gene (*fish bone* mutant) results in pleiotropic phenotypes including small panicles and abnormal vascular development (Yoshikawa et al., 2014). Mutations in the Brachypodium *TAR2*-like gene counter-intuitively cause an increase in auxin levels. Consequently the *Bdtar2* mutants have dramatically elongated seminal roots because of enhanced cell elongation (Pacheco-Villalobos et al., 2012). In *Bdtar2* mutants, several *YUC* genes are up regulated, which may account for the observed increase of auxin levels (Pacheco-Villalobos et al., 2012). Unlike overexpression of *YUC*s, overexpression of *TAA*s does not lead to any obvious developmental phenotypes, suggesting that TAAs-catalyzed reaction may not be a rate-limiting step in auxin biosynthesis (Stepanova et al., 2008; Tao et al., 2008).

Establishment of a complete two-step auxin biosynthesis pathway

TAA genes and *YUC*s were previously placed in two independent auxin biosynthesis pathways (Zhao et al., 2001; Stepanova et al., 2008; Tao et al., 2008). However, some *yuc* mutant combinations display phenotypes similar to those observed in the *taa* mutant combinations. The phenotypic similarities between *yuc* and *taa* mutants suggest that *TAAs* and *YUCs* may participate in the same auxin biosynthesis pathway (Strader and Bartel,

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2008; Mashiguchi et al., 2011; Stepanova et al., 2011; Won et al., 2011). Both *wei8 tar1 tar2* triple mutants and *yuc1 yuc4 yuc10 yuc11* quadruple mutants develop embryos without the basal part (Cheng et al., 2006, 2007a; Stepanova et al., 2008). Similar vascular and floral defects have also been observed in *wei8 tar2* and *yuc1 yuc4* double mutants (Figure 4) (Cheng et al., 2006, 2007a; Stepanova et al., 2008). In fact, *yuc* mutants display all of the characteristic phenotypes of the *taa* mutants (Won et al., 2011). For example, *yucQ* mutants are resistant to ACC and NPA in root elongation, a characteristic phenotype of *taa1* mutants (Won et al., 2011). The *yuc1 yuc4* double mutants have altered shade avoidance responses, a phenotype that is also observed in *taa1* mutants (Won et al., 2011). Interestingly, Arabidopsis plants appear to use different sets of *YUC* genes for auxin biosynthesis in the shoot and in the root despite using the same set of *TAA* genes in both shoots and roots (Won et al., 2011).

On the other hand, all of the characteristic phenotypes of *yuc* mutants are also displayed in *taa* mutants (Won et al., 2011). For example, inactivation of the protein kinase gene *PINOID* (*PID: AT2G34650*) in *yuc1 yuc 4* backgrounds completely abolishes the development of cotyledons (Cheng et al., 2007b). The *wei8 tar2 pid* triple mutants also fail to develop cotyledons (Won et al., 2011). The *NAKED PINS IN YUC MUTANTS* (*NPY)* (*NPY1: AT4G31820; NPY2: AT2G14820; NPY3: AT5G67440; NPY4: AT2G23050; NPY5: AT4G37590*) genes were identified from a genetic screen for *yuc1 yuc4* enhancers and the *yuc1 yuc4 npy1* triple mutants developed pin-like inflorescences (Cheng et al., 2007b, 2008). Disruption of *NPY1* in *wei8 tar2* background also leads to the formation of pin-like inflorescences (Won et al., 2011).

Several key pieces of evidence have unambiguously placed *TAA*s and *YUC*s in the same Trp-dependent auxin biosynthesis pathway. First, the auxin overproduction phenotypes displayed in *YUC* overexpression lines are dependent on the *TAA* genes (Mashiguchi et al., 2011; Stepanova et al., 2011; Won et al., 2011). Overexpression of *YUC* genes in *wei8 tar2* plants does not lead to the typical auxin overproduction phenotypes (Stepanova et al., 2011; Won et al., 2011). In contrast, overexpression of the *iaaM* gene in *wei8 tar2* still leads to long hypocotyls and epinastic cotyledons, two characteristic auxin overproduction phenotypes, indicating that *wei8 tar2* specifically block the auxin overproduction caused by overexpression of *YUC*s (Won et al., 2011). Second, the *taa* mutants contain a decreased concentration of IPA while *yuc* mutants have elevated concentrations of IPA, indicating that *TAA*s participate in IPA production while *YUC*s are involved in IPA consumption (Mashiguchi et al., 2011; Won et al., 2011). The hypothesis that YUCs function downstream of TAAs is also consistent with the findings that overexpression of *YUC1* can partially rescue the shade avoidance phenotypes of *taa1/sav3* (Won et al., 2011). Moreover overexpression of *YUC1* also partially rescues the fertility defects in the weak *wei8 tar2-2* mutants (Won et al., 2011). The conversion of the residual IPA in the weak *taa* mutants is probably accelerated in *YUC* overexpression lines, providing sufficient IAA for partially rescuing the weak *taa* mutant phenotypes. Genetic studies in maize also suggest that *TAA*s and *YUC*s participate in the same auxin biosynthesis pathway. The *taa1/vt2* mutants and the *yuc/spi1* mutants have similar phenotypes. *The spi1 vt2* double mutants are similar to *vt2* in terms of auxin levels and developmental phenotypes (Phillips et al., 2011).

BIOCHEMICAL MECHANISMS OF AUXIN BIOSYNTHESIS

Biochemical mechanisms of the TAA-catalyzed reaction.

Recombinant TAA1 protein fused with either an N-terminal GST-tag or C-terminal His-tag is able to catalyze a PLP-dependent transaminase reaction *in vitro* (Stepanova et al., 2008; Tao et al., 2008). TAA1 catalyzes the transfer of the amino group from Trp to an α-ketoacid such as α-ketoglutarate to produce IPA and the amino acid Glutamate (Stepanova et al., 2008; Tao et al., 2008). TAA1 has an apparent K_M of 290 μM for Trp and a k_{cat} of 1.85 s^{-1} (Tao et al., 2008).

All 20 amino acids except Glu have been tested as amino donors in the TAA1-catalyzed reaction with α-ketoglutarate as the acceptor of the amino group (Tao et al., 2008). Besides Trp, TAA1/SAV3 can also use Phe, Tyr, Leu, Ala, Met, and Gln as an amino group donor (Tao et al., 2008). However, Trp appears to be the best amino donor because the K_M values for Tyr (4.7 mM) and Phe (9.35 mM) are much higher than that for Trp (0.29 mM). The k_{cat} values are similar when Trp, Phe, and Tyr are used as substrates (Tao et al., 2008). *D*ocking experiments using the TAA1/SAV3 crystal structures reveal that IPA has the best score in docking followed by Trp and then by Phe, Tyr, and His (Tao et al., 2008). Therefore, Trp appears to be the best amino donor among the 20 amino acids, a finding that is consistent with the physiological roles of TAA1 in Trp-dependent auxin biosynthesis.

The preference for the amino acceptor in the TAA1-catalyzed reaction has not yet been systematically analyzed. So far, only pyruvate and α-ketoglutarate have been tested as the *in vitro* amino-acceptor (Stepanova et al., 2008; Tao et al., 2008). A systematic analysis of the α-ketoacids corresponding to the 20 amino acids is still needed to determine the best amino acceptor for TAAs. Interestingly, IPA performed better than Trp in the docking experiments, suggesting that the release of the product IPA could be the rate-limiting step of the TAA1-catalyzed reaction (Tao et al., 2008). It is also likely that the TAA-catalyzed reaction may be subject to product inhibition (Tao et al., 2008). It will also be interesting to test whether TAAs can catalyze the transfer of the amino group from other amino acids to IPA to produce Trp and the other α-ketoacids. Although genetic studies have demonstrated that TAA1 participates in Trp-dependent auxin biosynthesis by producing IPA, it should be recognized that TAA1-catalyzed reaction is coupled with homeostasis of another amino acid/α-ketoacid. Disruption of *TAA* genes not only abolishes IPA production, but also affects the metabolism of other α-ketoacids and amino acids. Therefore, phenotypes of *taa* mutants may be caused by a combination of IAA deficiency and defects in homeostasis of other amino acids.

So far, the quantitative biochemical studies on TAA family proteins have been limited to the Arabidopsis *TAA1* (Stepanova et al., 2008; Tao et al., 2008, He et al., 2011). The TAAs (PsTAR1 and PsTAR2) from the pea plants (*Pisum sativum*) have been shown qualitatively to convert Trp to IPA and 4-chlora-Trp to 4-chlora-IPA (Tivendale et al. 2012). It will be interesting to determine whether TAA1 and TARs differ in substrate specificity and kinetic properties.

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The biochemical mechanisms of YUC flavin monooxygenases

YUC family proteins showed significant sequence homologies to the family of flavin-containing monooxygenases, which is one of the largest groups of monooxygenases that have been identified. Monooxygenases take one oxygen atom from a molecular oxygen and insert it into an organic compound (Cashman, 1995; Cashman et al., 1995). Flavin-containing monooxygenases play key roles in the synthesis of many physiologically important molecules in various organisms, and are responsible for the degradation of a large variety of aromatic and heteroatom-containing compounds. Flavin-containing monooxygenases usually use NADPH and FAD as cofactors and molecular oxygen as a co-substrate. YUC proteins contain two GXGXXG motifs that function as binding sites for FAD and NADPH (Hou et al., 2011). Mutations in the predicted FAD or NADPH binding sites completely abolish *YUC* functions in Arabidopsis, suggesting that YUCs probably also use FAD and NADPH as cofactors (Hou et al., 2011).

Flavin-containing monooxygenases are notorious for being very difficult enzymes for in vitro studies. Our current understanding of flavin-containing monooxygenases is mainly based on studies of the mammalian enzymes, which are purified from animal tissues (Cashman, 1995; Cashman et al., 1995). The fungal enzyme N^5-L-ornithine monooxygenase (OMO), a flavin-containing monooxygenase, was discovered over 25 years ago, but it was not until recently that the biochemical details of OMO were resolved, largely due to the recent success in expressing OMO in *E. coli* and purifying OMO to near homogeneity (Mayfield et al., 2010). In general, flavin-containing monooxygenases use a relatively stable C4a-(hydro)peroxyflavin intermediate to conduct oxidative reactions (Ziegler, 1988, 2002). Therefore, flavin-containing monooxygenases are usually promiscuous and the mammalian microsome flavin-containing monooxygenases are known to catalyze the hydroxylation of a large class of nitrogen and sulfur-containing compounds (Ziegler, 1988, 2002). In addition, mammalian flavin-containing monooxygenases have also been shown to catalyze Baeyer-Villiger type of reactions, in which the C4a intermediate makes a nucleophilic attack on a carbonyl group to form the Criegee intermediate, which then undergoes rearrangement to generate the final products (Lai et al., 2010). The difference between Baeyer-Villiger type of reactions and N-hydroxylation is that the C4a intermediate serves as a nucleophile in Baeyer-Villiger type of reactions while the C4a intermediate is an electrophile in N-hydroxylation reactions. Because of the catalytic mechanisms of flavin-containing monooxygenases, it is often very difficult to pin down the exact physiological substrates. Interestingly, some of the flavin-containing monooxygenases display remarkable substrate specificity. For example, OMO catalyzes hydroxylation of ornithine, but does not hydroxylate lysine, which has one more methylene than ornithine (Mayfield et al., 2010).

Solving the biochemical mechanisms of the YUC enzymes has been a challenging journey. Initially, it was proposed based on in vitro assays that YUCs catalyze the N-hydroxylation of tryptamine (Zhao et al., 2001; Expósito-Rodríguez et al., 2007, Kim et al., 2007; LeCLere et al., 2010). However, the early biochemical work had several caveats. In the early studies, YUC proteins with an N-terminal or C-terminal tag expressed in and purified from *E. coli* were not biochemically characterized. It was not clear what cofactors were required for the YUC-catalyzed reactions. The recombinant YUC proteins lacked any yellow color, an indication that the recombinant YUC proteins did not contain the flavin cofactor. The YUC proteins purified from *E. coli* were probably not folded correctly. Early enzyme assays of YUC proteins were all qualitative and only tryptamine was tested as a substrate (Zhao et al., 2001; Expósito-Rodríguez et al., 2007, Kim et al., 2007; LeCLere et al., 2010). In addition, the early enzymatic assays required the addition of free FAD to the reaction mixture, probably causing off-enzyme reactions. Recent studies no longer support the hypothesis that YUCs use tryptamine as a physiological substrate for auxin biosynthesis (Tivendale et al., 2010; Mashiguchi et al., 2011; Stepanova et al., 2011; Won et al., 2011; Kriechbaumer et al., 2012).

Recent genetic studies have clearly indicated that YUCs and TAAs participate in the same Trp-dependent auxin biosynthesis pathway (Mashiguchi et al., 2011; Stepanova et al., 2011; Won et al., 2011). The findings that *yuc* mutants accumulate IPA and *taa* mutants are IPA deficient suggested that IPA was a substrate for YUC flavin-containing monooxygenases (Mashiguchi et al., 2011; Won et al., 2011). YUC enzymatic assays were then conducted to determine whether YUCs could use IPA as a substrate in vitro (Mashiguchi et al., 2011; Kriechbaumer et al., 2012). Recombinant GST-tagged YUC2, expressed in and purified from *E. coli*, was able to convert IPA to IAA in an NADPH-dependent manner (Mashiguchi et al., 2011). Interestingly, GST-YUC2 did not convert IPA into Indole-3-acetaldehyde, which had long been hypothesized as an auxin biosynthesis intermediate (Mashiguchi et al., 2011). Two isoforms of Arabidopsis YUC4, produced from in vitro transcription and translation using the PUREexpressTM kit, were also shown to use IPA as a substrate (Kriechbaumer et al., 2012).

In order to elucidate the catalytic mechanisms and the biochemical properties of YUC flavin monooxygenases, large quantities of YUC proteins are required. This was achieved for the Arabidopsis YUC6, which was expressed in and purified to near homogeneity from *E. coli* (Dai et al., 2013). The recombinant YUC6 displayed bright yellow color (Figure 5) and an UV-Visible spectrum resembled those of FAD and FMN (Dai et al., 2013). This was the first time that large quantities of a plant flavin-containing monooxygenase had been purified to near homogeneity with the flavin co-factor bound. Several factors contributed to the success: 1) The YUC6-expressing *E. coli* cells were grown at low temperature for two days before induction and two days after induction; 2) high concentrations of glycerol (30%) were added to the purification buffer to stabilize the YUC protein (Dai et al., 2013).

The availability of large quantities of the purified YUC6 enables the detailed characterization of the biochemical properties and the catalytic mechanisms of YUC6. YUC6 is an FAD-containing protein, as demonstrated by several pieces of experimental data: 1) The UV-Visible spectrum of YUC6 is very similar to those of FAD and FMN; 2) denatured YUC6 protein releases a small molecule that has the same retention time in HPLC as the standard FAD; 3) The flavin cofactor released from YUC6 has very little fluorescence, but upon treatment with a phosphodiesterase that converts FAD to FMN, the fluorescence increased more than 10 times (Dai et al., 2013). The experimental data also are consistent with sequence-based prediction that YUCs are FAD-containing enzymes (Dai et al., 2013).

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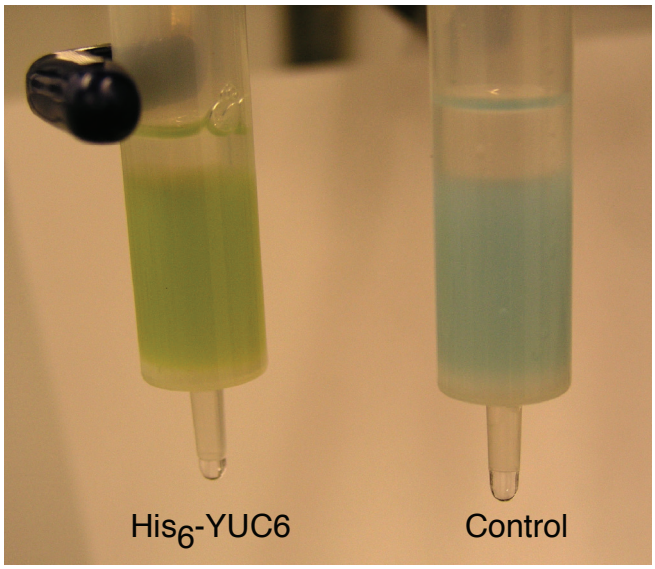


Figure 5. Purification of recombinant YUC6 with the flavin-cofactor bound.

YUC6 tagged with His_6 at the N-terminus was expressed in *E. coli* and purified with Nickel NTA agrose. Note that the flavin cofactor in YUC6 gave the bright yellow color.

Kinetic and spectroscopic characterization of the YUC6 protein has established the basic framework for YUC-mediated catalysis (Figure 6) (Dai et al., 2013). YUC6 as purified contains an oxidized FAD, which has peaks at 448 nm and 376 nm in the UV-visible spectrum (Dai et al., 2013). The FAD cofactor in YUC6 readily takes two electrons from NADPH to become FADH2, which no longer has the peak at 448 nm in the UV-visible spectrum (Dai et al., 2013). Reduction of FAD to FADH2 by NADPH is the first step of the YUC-catalyzed reaction (Figure 6). The second step is the reaction of FADH2 with molecular oxygen to form the C4a-(hydro)peroxyflavin intermediate (Dai et al., 2013). Mechanistically, flavin-containing monooxygenases use either a C4a-hydroperoxyflavin or a C4a-peroxyflavin as the species that carries out the oxygenation reaction. Because it is generally difficult to distinguish these two forms spectroscopically, the intermediate observed in YUC6-mediated catalysis is designated C4a-(hydro) peroxyflavin to reflect the uncertainty about the actual form of the intermediate. The YUC6 C4a-(hydro)peroxyflavin intermediate displays a distinct UV-Visible spectrum with a peak at 381 nm (Dai et al., 2013). The last step of the YUC6-catalyzed reaction is the oxidative decarboxylation of IPA by the C4a-(hydro)peroxyflavin to produce IAA (Figure 6). The proposed catalytic mechanism is well supported by the kinetic and spectroscopic results (Dai et al., 2013). The oxidized, reduced, and the C4a intermediate of YUC6 display characteristic UV-Visible spectra and have been observed experimentally. Overall, the YUC6-catalyzed reaction employs a catalytic strategy that is similar to those used by other well-characterized flavin-containing monooxygenases.

If the flavin-containing monooxygenases turn over NADPH without oxidizing the substrates, NADPH is wasted. There are several general strategies to prevent flavin-containing monooxygenases from becoming an NADPH oxidase: 1) Reduction of FAD by NADPH only takes place in the presence of the substrate; 2) Reaction of the reduced flavin with oxygen is regulated by substrate binding; 3) The C4a intermediate is stabilized in the absence of the substrate. However, the last strategy often leads to promiscuous enzymatic reactions. YUC6 is readily reduced by NADPH regardless of the presence of the substrate IPA. IPA also does not affect the rate of YUC6 reduction by NADPH (Dai et al., 2013). Furthermore, IPA does not affect the kinetic pattern and rate of formation of the C4a intermediate (Dai et al., 2013). In the presence of IPA, the decomposition of the C4a intermediate is greatly accelerated, suggesting that YUC6 uses the third strategy to minimize the NADPH oxidase activity. Interestingly, the C4a-intermediate in YUC6 appears much less stable than the counterpart intermediates in mammalian flavin-containing monooxygenases, which catalyze both N-hydroxylation and Baeyer-Villiger type reactions (Dai et al., 2013). The half-life values of the C4a intermediate in mammalian flavin-containing monooxygenases are usually more than 30 minutes whereas the half-life of the C4a intermediate of YUC6 is less than 20 seconds (Dai et al., 2013). Although it is not clear what significance of the short half-life of the YUC6 C4a intermediate, it probably increases the substrate specificity of YUC proteins.

YUC6 is a good NADPH oxidase with a turnover number of 2.4 per minute (Dai et al., 2013). The NADPH oxidase activity uses the electrons from NADPH to convert oxygen into hydrogen peroxide, which is a component of the reactive oxygen species (ROS). ROS has been implicated in many diverse physiological and pathological processes. The fact that YUC6 has NADPH oxidase activity indicates that YUC activities have to be tightly regulated.

Genetic studies have clearly demonstrated that YUCs use IPA as their physiological substrate. However, YUC6 can also use Phenylpyruvate (PPA) as a substrate in vitro (Dai et al., 2013). It still remains to be investigated whether YUCs can use all of the α-ketoacids as substrates. Because YUC6 forms the active C4a intermediate, it is reasonable to hypothesize that any α-ketoacids that can enter the active sites of YUCs probably will be able to react with the C4a intermediate. This raises the question of how substrate specificity of YUCs is achieved in plants. So far, only YUC6 has been analyzed both kinetically and spectroscopically. It is still a challenge to determine the biochemical properties of the other YUCs from Arabidopsis and other species.

REGULATION OF AUXIN BIOSYNTHESIS

Auxin is synthesized in two chemical steps using IPA as the intermediate (Figure 1). *De Novo* auxin biosynthesis can be effectively regulated by using three strategies: 1) control of the IPA production; 2) Regulation of the conversion of IPA into IAA; 3) Diversion of IPA to other metabolic pathways

Metabolic regulation of auxin biosynthesis by VAS1

Recently, Zheng et al. isolated an aminotransferase gene named *VAS1* (*AT1G80360*) from a genetic screen for mutations that can suppress the defects of *sav3/taa1* in shade avoidance responses (Zheng et al., 2013). Under shade conditions, wild type

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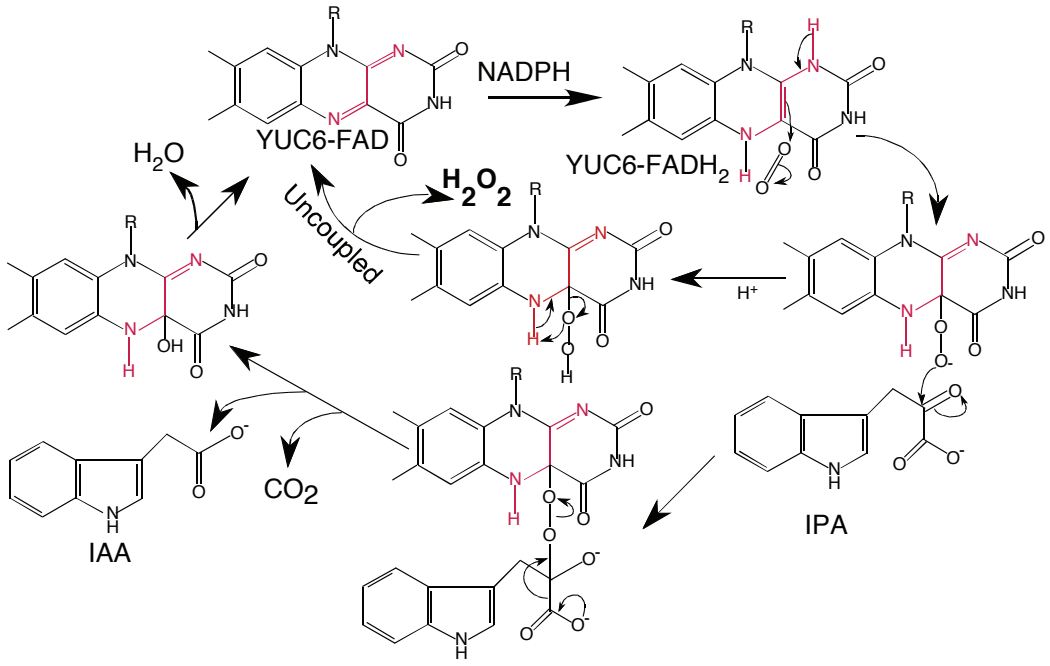


Figure 6. The mechanism of YUC6-catalyzed reaction.

YUC6 undergoes reduction and then binds to oxygen to form the C4a-(hydro)peroxyflavin intermediate. The C4a intermediate reacts with IPA to generate IAA, CO2 and water. The C4a intermediate can also decompose into H_2O_2. H_2O_2 produced from the uncoupled reaction may kill the enzymatic activities of YUC6. The reaction center of the flavin cofactor is marked red.

plants have elongated hypocotyls and *sav3* fails to elongate its hypocotyl. The *vas1 sav3* double mutants display long hypocotyl phenotypes under shade conditions. VAS1 is a predicted aminotransferase based on its sequence homology to known PLP-dependent aminotransferase. VAS1 has been systematically analyzed to identify its amino donors and acceptors (Zheng et al., 2013). It turns out that IPA is the most suitable amino acceptor for the VAS1-catalyzed transamination reaction. PPA or 4-hydroxyphenylpyruvate (two ketoacids from the amino acids Phe and Tyr, respectively) is only 3% as active as IPA in a VAS1-catalyzed reaction (Zheng et al., 2013). Other α-ketoacids including glyoxylate, pyruvate, 2-ketobutyrate, 2-keto-4-methyl-thiobutyric acid (KMBA), 2-oxoglutarate, and oxaloacetate, fail to function *in vitro* as amino acceptors in the VAS1 catalyzed transamination reaction (Zheng et al., 2013). For amino donors, VAS1 prefers Met to all other amino acids. The second most active amino donor is Phe. However, the relative activity with Phe as a substrate is only 21% that of Met. Other amino acids including Val, Ile, Tyr and Leu are less than 1% as active as Met *in vitro* (Zheng et al., 2013).

The *in vitro* biochemical analysis reveals that VAS1 has unusually high substrate specificity. It essentially only catalyzes the transfer of the amino group from Met to IPA (Zheng et al., 2013). Met and IPA are intermediates for ethylene biosynthesis and auxin biosynthesis, respectively. Therefore VAS1-catalyzed reaction metabolically links the biosynthesis of the two important plant hormones. Disruption of *VAS1* leads to the accumulation of IPA and IAA in Arabidopsis. Light grown *vas1* mutants have longer hypocotyls than wild type plants, a phenotype that is observed in auxin overproduction mutants. The other substrate of VAS1 is Met, which is converted into KMBA in the *VAS1* catalyzed reaction. Both Met and KMBA are precursors for ethylene biosynthesis in the Yang Cycle. The *vas1* mutants have elevated levels of ACC, the immediate precursor of ethylene biosynthesis (Zheng et al., 2013). It is reported that the elevated ethylene levels account for the exaggerated petiole elongation of the *vas1* mutants (Zheng et al., 2013). Trp is the other product of the VAS1-catalyzed reaction. It is not clear whether VAS1 also plays a key role in maintaining Trp homeostasis. The identification of VAS1 as a key component for maintaining the homeostasis of IPA, ethylene, and auxin demonstrated the complexity of regulating auxin biosynthesis.

Transcriptional regulation of auxin biosynthesis

One of the surprising findings in auxin biosynthesis is that the expression of auxin biosynthetic genes is often restricted to discrete groups of cells (Cheng et al., 2006, 2007a; Stepanova et al., 2008; Tao et al., 2008; Won et al., 2011). For a long time, it was believed that the location of auxin biosynthesis was not very important because auxin could reach any regions via the polar auxin transport system (Grieneisen et al., 2007). Computer models demonstrated that polar auxin transport is necessary and sufficient for generating auxin gradients for plant development (Grieneisen et al., 2007). However, recent progress in auxin bio-

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synthesis has unequivocally demonstrated that localized auxin biosynthesis also plays an essential role in virtually every major developmental processes including embryogenesis, seedling growth, vascular patterning, root development, phyllotaxis, and flower development (Cheng et al., 2006, 2007a; Stepanova et al., 2008; Pinon et al., 2013).

Auxin biosynthesis is regulated at the transcriptional level by developmental signals and environmental cues. The specific expression patterns of *TAA* genes and *YUC* genes are determined by transcription factors that bind to the regulatory regions of the genes. Several groups of transcription factors have been identified by their ability to bind directly to the regulatory regions of *TAA* genes and/or *YUC* genes. The SHORT INTENOTES/STYLISH (SHI/STY) (SHI: AT5G66350; STY1: AT3G51060) family of transcription factors plays an important role in leaf and flower development by regulating the expression of *YUC* genes (Sohlberg et al., 2006; Eklund et al., 2010). STY1, one of the SHI/STY proteins, binds directly to the short motif of ACTCTAC in the *YUC4* promoter activating the expression of *YUC4* (Eklund et al., 2010). STY1 also binds to the promoter region of *YUC8* and activates *YUC8* expression (Eklund et al., 2010). The NGATHA (NGA1: AT2G46870) family of transcription factors, which redundantly controls style development in a dosage-dependent manner, positively regulates *YUC2* and *YUC4* in the apical domain of *Arabidopsis* gynoecium (Alvarez et al., 2009; Trigueros et al., 2009). But it is not clear whether the regulation by NGA is direct or not. LEAFY COTYLEDON2 (LEC2: AT1G28300), a central regulator of embryogenesis, binds directly to the promoters of *YUC2, YUC4*, and *YUC10* to activate the expression of the *YUC* genes (Wojcikowska et al., 2013; Stone et al., 2008). The Arabidopsis *IDD14 (AT1G68130)*, *IDD15 (AT2G01940)*, and *IDD16 (AT1G25250)* are important regulators for lateral organ morphogenesis and gravitropism. It has been shown that the IDDs directly target *YUC5* and *TAA1* (Cui et al., 2013). Interestingly, the class III HD-Zip transcription factor REVOLUTA (REV: AT5G60690) also directly binds to the promoters of *TAA1* and *YUC5*, suggesting that part of REV's roles in shoot development and organ polarity is probably mediated by altering auxin biosynthesis (Brandt et al. 2012). PLETHORA transcription factors (PLT3: AT5G10510; PLT5: AT5G57390; PLT7: AT5G65510) control phyllotaxis in Arabidopsis by regulating the expression of *YUC* genes (Pinon et al., 2013).

Environmental signals have a profound effect on auxin biosynthesis. *TAA1* was identified from a screen for mutants that were defective in shade avoidance responses, a light-mediated signaling process (Tao et al., 2008). Shade avoidance responses require the phytochrome photoreceptors and the *Phytochrome-Interacting Factors* (PIFs), which are bHLH transcription factors (Ballare, 1999). Recent studies have shown that part of the light signaling response is to modulate auxin homeostasis. Some of the auxin biosynthetic genes are direct targets of the PIFs (Li et al., 2012). Under shade conditions, auxin levels in Arabidopsis are elevated more than 50% compared to the levels in plants grown under normal white light conditions (Li et al., 2012). The increased auxin concentrations are caused by the up regulation of the expression of several *YUC* genes (Li et al., 2012). PIF7 (AT5G61270) is one of the key transcription factors downstream of the photoreceptor phytochrome B. Disruption of *PIF7* leads to short hypocotyls and expanded cotyledons under shade, suggesting that *PIF7* plays a positive role in shade avoidance response. It is reported that PIF7 binds to the promoters of *YUC2*, *YUC5*, *YUC8*, and *YUC9* and activates the expression of the *YUC* genes under shade conditions (Li et al., 2012).

In addition to participating in light signaling, PIFs have recently been shown to contribute to temperature-mediated and sugar-induced auxin biosynthesis (Franklin et al., 2011; Sun et al., 2012). It has been well documented that high temperatures stimulate hypocotyl elongation and auxin over-accumulation (Gray et al., 1998). The high temperature-induced developmental changes are mediated through PIF4 (AT2G43010). PIF4 binds directly to the promoters of *YUC8*, *TAA1*, and *CYP79B2,* thereby controlling auxin levels (Franklin et al., 2011; Sun et al., 2012). Sugar (glucose and sucrose) treatments cause elevated auxin biosynthesis, probably through PIF-dependent activation of *YUC* expression (Lilley et al., 2012; Sairanen et al., 2012). Circadian rhythm also regulates plant development through modulating auxin levels. For example, RVE1 (AT5G17300), a MYB-like transcription factor and clock-regulated transcription factor appears to positively regulate *YUC8* (Rawat et al., 2009).

The majority of transcription factors including STY1, PIFs, and REV1 that bind to promoters of auxin biosynthesis genes are positive regulators. In contrast, SPOROCYTELESS (SPL: AT4G27330) is a known negative regulator of auxin biosynthesis. Overexpression of *SPL* represses the expression of *YUC2* and *YUC6* (Li et al., 2008). LEAFY (LFY: AT5G61850) is another negative regulator of auxin biosynthesis. LFY binds directly to the *YUC4* promoter and inhibits *YUC4* expression (Li et al., 2013).

Control enzymatic activities of TAAs and YUCs

YUCs catalyze the rate-limiting step of auxin biosynthesis and the enzymatic activities of YUCs have to be tightly regulated. However, factors that regulate YUC activities are not understood. It is also not clear whether *YUC* protein stability is regulated. Interestingly, about 4% of the YUC6-catalyzed reaction is un-coupled (Figure 6) (Dai et al., 2013). The uncoupled YUC6-catalyzed reaction uses the electrons from NADPH to partially reduce oxygen to H_2O_2. Production of hydrogen peroxide by the uncoupled reaction potentially provides a built-in deactivation mechanism for YUCs. After several rounds of catalysis, H_2O_2 produced from the uncoupled reaction may deactivate the YUC enzymatic activities. Currently, very little is known regarding how TAA enzymatic activities are regulated.

MANIPULATION OF AUXIN CONCENTRATIONS WITH SPATIAL AND TEMPORAL CONTROL

The successful elucidation of the TAA/YUC auxin biosynthesis pathway provides tools for us to modulate auxin concentrations in plants with temporal and spatial control. Auxin concentrations in cells or tissues can now be increased or decreased by regulating auxin biosynthesis in plants. Auxin conjugation and degradation can also be manipulated to alter auxin concentrations in plants (Glass and Kosuge, 1986; Peer et al., 2013; Pencik et al., 2013;

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Zhao et al., 2013). In this chapter, I focus on discussing two general strategies to control auxin biosynthesis in plants: 1) use auxin biosynthesis inhibition by chemicals; 2) genetic activation or deactivation of auxin biosynthetic genes.

Inhibitors for auxin biosynthesis

Two inhibitors have been developed to block the activities of TAA aminotransferases. L-amino-oxyphenylpropionic acid (AOPP) was identified by a genomics-based approach as an effective inhibitor for Trp aminotransferases (Soeno et al., 2010). Plants grown on 50 μM L-AOPP displayed defects in root elongation, gravitropism, and root hair formation (Soeno et al., 2010). Adding exogenous auxin in the growth media largely reversed the phenotypes caused by L-AOPP. L-AOPP treatments lowered free IAA levels in plants (Soeno et al., 2010). Although L-AOPP is an effective inhibitor for the TAA family of aminotransferases, further characterization of the compound is still needed. L-AOPP targets PLP-dependent enzymes and it probably also inhibits other non-auxin biosynthesis enzymes.

The other characterized TAA/TAR inhibitor is L-Kynurenine, which is a degradation product of Trp in animal systems. L-Kynurenine is a competitive inhibitor of TAA with a Ki of 11.52 μM (He et al., 2011). L-Kynurenine treatments can phenocopy the seedling phenotypes of *wei8 tar2*. Results from molecular modeling and computational docking experiments suggest that L-Kynurenine is a specific and highly selective auxin biosynthesis inhibitor (He et al., 2011).

Recently, an inhibitor for YUC flavin-containing monooxygenases has been reported. The inhibitor named yucasin, 5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol, was initially identified as a potent inhibitor of auxin biosynthesis in maize coleoptile tips (Nishimura et al., 2013). Late yucasin was shown to suppress the auxin overproduction phenotypes displayed in *YUC1* overexpression Arabidopsis plants (Nishimura et al., 2013). It was shown that yucasin competitively inhibits the oxidative decarboxylation of IPA *in vitro* catalyzed by the YUC flavin monooxygenases (Nishimura et al., 2013). L-Kynurenine and yucasin synergistically inhibit auxin biosynthesis and plant growth (Nishimura et al., 2013). The inhibitors can be used to modulate auxin biosynthesis in plants with temporal control.

Modulate auxin biosynthesis by genetic approaches

IAA is synthesized from Trp in two chemical steps and the YUC-catalyzed reaction is the rate-liming step (Figure 1). Expression of one of the *YUC* genes under the control of an appropriate promoter can increase auxin levels in specific cells/tissues. If we are concerned that the YUC substrate IPA may not be available in certain cells, we can co-express both *TAA* and *YUC* genes under the control of the same promoter. The *YUC* and *TAA* genes can also be placed downstream of an inducible promoter so that auxin production can be temporally controlled. A two-component system can also be used to control the expression of auxin biosynthetic genes, thus providing precise temporal and spatial control of auxin biosynthesis.

RNAi, artificial microRNAs, and the recently developed CRISPR genome editing technology make it possible to disrupt auxin biosynthesis in specific cells/tissues. By choosing the appropriate promoters, *YUC* genes and *TAA* genes can be disrupted in the targeted cells/tissues such that auxin biosynthesis can be blocked both spatially and temporally. Previous studies analyzed the gene expression profiles in response to auxin treatments that led to elevated high auxin concentrations in cells. Disruption of auxin biosynthesis using genetic or chemical approaches will allow us to conduct gene expression profiling in response to auxin deficiency.

In summary, much progress has been made in the field of auxin biosynthesis. The two-step pathway from Trp to IAA catalyzed by the TAA aminotransferases and the YUC flavin-containing monooxygenases is the first identified complete auxin biosynthetic pathway, which is essential for almost all of the major developmental events. The identification of the TAA/YUC pathway offers novel tools to modulate auxin concentrations in plants and thus facilitates the elucidation of the molecular mechanisms by which auxin controls various developmental processes. The various auxin biosynthetic mutants are also very useful for further dissecting the roles of auxin in plant growth and development. The advancements in auxin biosynthesis reveal that localized auxin biosynthesis provides an essential and effective means to control auxin concentrations in plants.

ACKNOWLEDGEMENT

I would like to thank Dr. Hiroyuki Kasahara, Dr. Kiyoshi Mashiguchi, Dr. Yongxia Guo, Dr. Zuyu Zheng, and members of the Zhao lab for comments on the manuscript. Work in my lab is supported by the NIH (R01GM068631 to Y.Z.) and the NSF (Plant Genome DBI-0820729 to Y. Z.).

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