

The biosynthesis of auxin: how many paths truly lead to IAA?

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Abstract The plant auxin indole-3-acetic acid (IAA) plays critical roles in plant growth and development. There are two main strategies proposed for plant synthesis of IAA: the Trp-dependent (TD) and the Trp-independent (TI) pathways. Four TD pathways, namely the indole-3-acetamide pathway, the indole-3-pyruvic acid pathway, the tryptamine pathway and the indole-3-acetaldoxime pathway, have been postulated, identified and extensively studied. On the other hand, neither genes nor mutants involved in the TI pathway have been identified to date. Interestingly, some bacteria have auxin synthesis pathways that are similar to those in plants, indicating conserved biosynthetic mechanisms. Over the past few years, genetic, biochemical and molecular studies have greatly advanced our understanding of auxin biosynthesis. This review both summarizes recent advances in genetic and molecular knowledge and addresses the unsolved questions regarding auxin biosynthesis pathways in plants.

Keywords Auxin biosynthesis · Trp-dependent (TD) pathway · Trp-independent (TI) pathway · IAA · IAM · IPyA · TAM · IAOx

Introduction

Auxin plays important roles in regulating plant development and growth, affecting such processes as cell division and elongation, tropisms, apical dominance, senescence, blooming and stress response (Mano and Nemoto 2012; Shi et al. 2014; Teale et al. 2006; Woodward and Bartel 2005; Yang et al. 2014). Generally, plants contain three naturally occurring, active auxins: IAA, 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA) (Enders and Strader 2015; Simon and Petrasek 2011). Of these, IAA is the most abundant and important auxin (Simon and Petrasek 2011). Maintaining auxin homeostasis is crucial for normal plant growth and development. Plants maintain auxin homeostasis through regulation of both its transport and biosynthesis and through modification of auxin molecules into active or inactive forms (Korasick et al. 2013).

Species within the plant kingdom have evolved both TD and TI pathways for auxin biosynthesis (Chandler 2009; Woodward and Bartel 2005). The TD pathway has been extensively studied, resulting in the identification of four pathways: the IAM pathway, the IPyA pathway, the TAM pathway and the IAOx pathway. These four TD pathways have been genetically and biochemically proven to be involved in IAA synthesis. A variety of abiotic IAA regulation factors, such as high- or low-temperature (Gray et al. 1998; Zhu et al. 2015) and wounding (Reverberi et al. 2005; Szein et al. 2002), have been demonstrated to function cooperatively and precisely in controlling free

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IAA levels. In addition, the TI biosynthesis pathway, by which IAA is directly synthesized from indole, may contribute to auxin homeostasis in higher eukaryotic plants (Normanly et al. 1993). However, no genes, mutants, or intermediate metabolites involved in the TI pathway have been identified to date (Ouyang et al. 2000; Zhang et al. 2008). Quite recently, a gene encoding indole synthase (INS) has been suggested to participate in the TI pathway (Wang et al. 2015).

Auxin biosynthesis is actively regulated. Over the past few years, some of the enzymes and regulators of IAA biosynthesis have been identified, especially in the IPyA pathway. In this review, we summarize the most recent discoveries in the auxin biosynthesis pathways, especially the TD pathways.

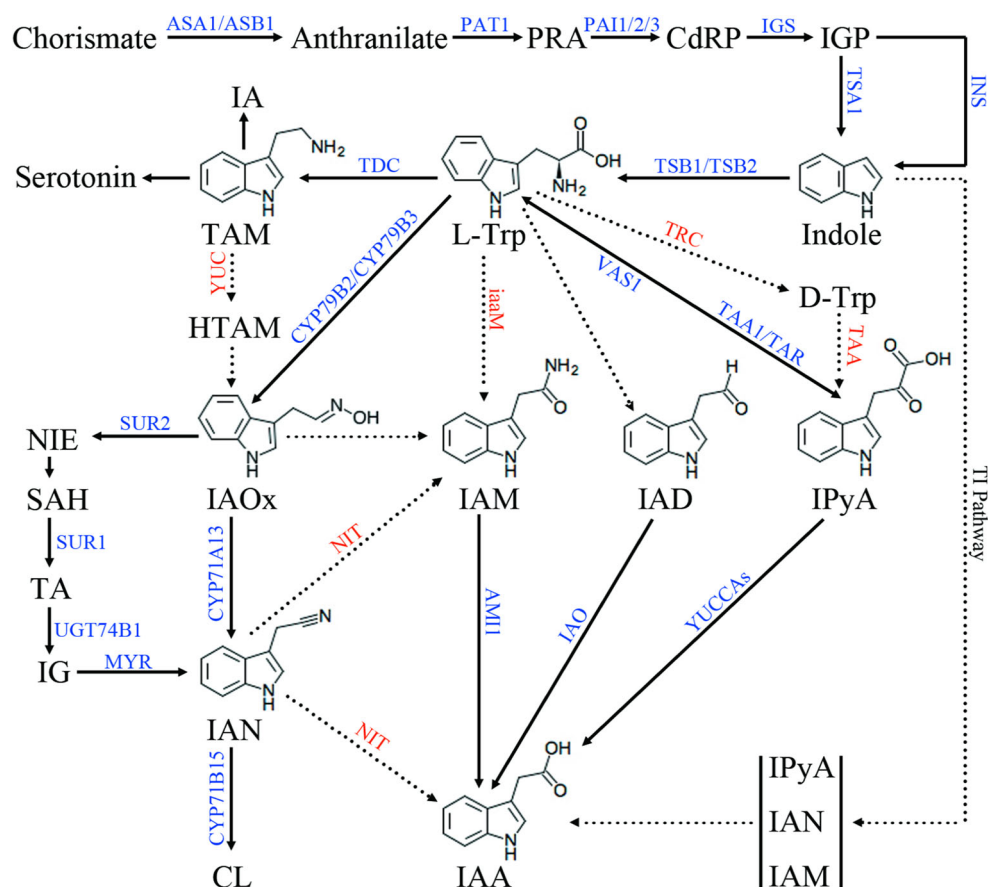
L-Trp biosynthesis pathway in plants

Plant cells synthesize Trp from chorismate via indole-3-glycerol phosphate (IGP) in the chloroplast (Radwanski and Last 1995). This process includes six critical steps (Fig. 1). In *Arabidopsis thaliana*, the *WEI2/ASA1* and *WEI7/ASB1* genes respectively encode the α and β subunits of anthranilate synthase (AS), which catalyzes the

conversion of chorismate to anthranilate and is a rate-limiting step in Trp biosynthesis (Fig. 1) (Niyogi et al. 1993; Stepanova et al. 2005). The α subunit binds to chorismate and catalyzes its aromatization, while the β subunit transfers an amino group from glutamine (Li and Last 1996). Anthranilate is converted to 1-(O-carboxylphenylamino)-1-deoxyribulose-5-phosphate (CdRP) by phosphoribosylanthranilate transferase (PAT1) and phosphoribosylanthranilate isomerase (PAI1/2/3) (Fig. 1) (Mano and Nemoto 2012). CdRP is then catalyzed by indole glycerol phosphate synthase (IGS) into IGP. IGP is a branch point that can lead to TI auxin synthesis directly from indole (Li et al. 1995; Ouyang et al. 2000; Wang et al. 2015).

To make Trp, IGP is converted by the Trp synthase complex (TS-complex), which consists of Trp synthase α (TSA1) and Trp synthase β (TSB) (Last et al. 1991; Radwanski et al. 1996). These two subunits form an $\alpha\beta_2$ heterodimer (Last et al. 1991; Radwanski et al. 1996). The *Arabidopsis* genome contains two closely related Trp synthase β genes, namely TSB1 and TSB2 (Last et al. 1991; Mano and Nemoto 2012), but only one chloroplast Trp synthase α gene (TSA1). However, there is a cytoplasmic isozyme of Trp synthase α named indole synthase (INS) (Last et al. 1991; Zhang et al. 2008).

Fig. 1 The proposed IAA biosynthesis pathways in *Arabidopsis*. There are four proposed Trp-dependent (TD) IAA biosynthesis pathways in *Arabidopsis*, namely the tryptamine (TAM) pathway, the indole-3-acetaldoxime (IAOx) pathway, the indole-3-acetamide (IAM) pathway, and the indole-3-pyruvic acid (IPyA) pathway, and one Trp-independent (TI) pathway. Enzymes and intermediate metabolites involved in TD and TI pathways are indicated. The solid lines and dashed lines indicate processes for which the genes have been and have not been identified, respectively. The blue colors and red colors indicate gene functions that have been determined and have not been determined, respectively. Genes in red are placed here through homology only. All metabolites shown have been purified from plants



Trp-dependent IAA biosynthesis pathways

Currently, the known TD pathways of IAA biosynthesis are the IAM pathway, IPyA pathway, IAOx pathway and TAM pathway. These pathways are delineated by genes involved in biosynthesis of Trp derivatives and known intermediate metabolites. However, not all proposed steps in each pathway have been identified in plants, calling into question whether the species analyzed to date synthesize IAA via these delineations of the pathways.

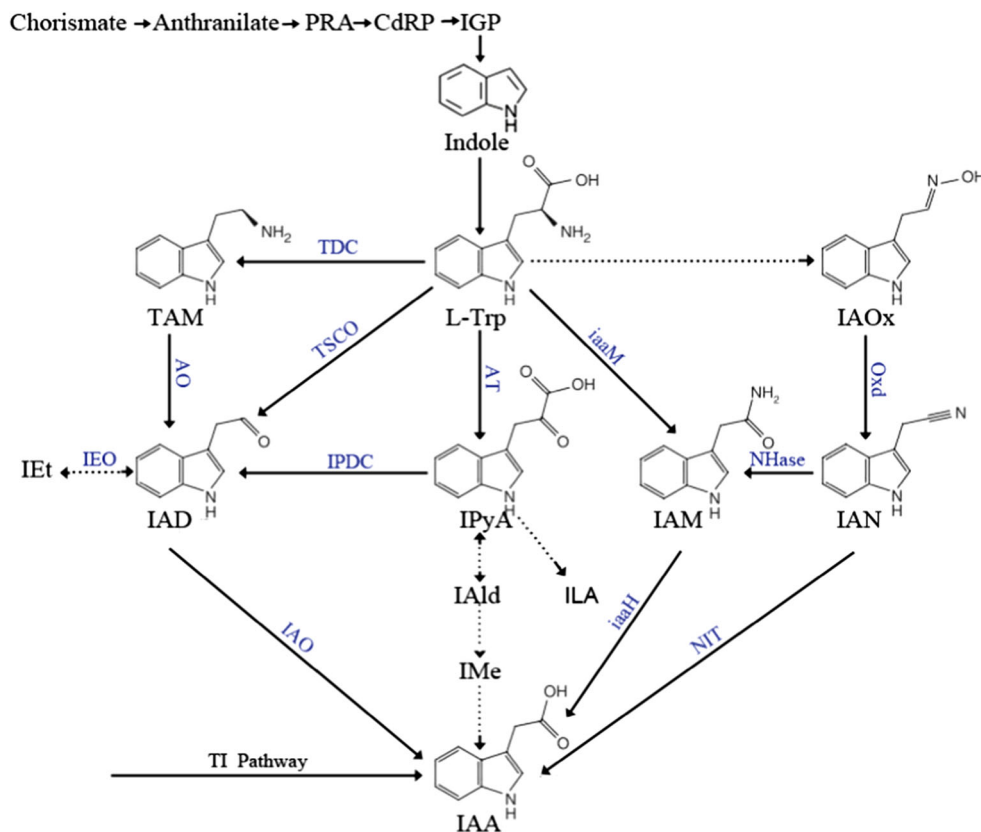
The indole-3-acetamide (IAM) pathway

The IAM pathway was first discovered in *Pseudomonas* (Magie et al. 1963) and later identified in other plant-associated bacteria (Pollmann et al. 2003). In bacteria, L-Trp is first converted to IAM by the tryptophan monooxygenase encoded by the *aux1/iaaM* gene (Fig. 2). IAM is further catalyzed by indole acetamide hydrolase (*aux2/iaaH*) to synthesize IAA (Fig. 2) (Casanova et al. 2005; Gaudin and Jouanin 1995). This pathway was thought to exist only in bacteria until this century. The identification of IAM and its metabolic conversion to IAA in *Arabidopsis* showed that an IAM-centered pathway functions in plants (Pollmann et al. 2002), however this pathway likely differs from those in bacteria.

The synthesis of IAM in different plants may occur through different strategies. In *Arabidopsis*, the IAM content decreases dramatically in *cyp79b2/cyp79b3* double mutants, which may cause failed conversion of L-Trp to IAOx (Fig. 1) (Mikkelsen et al. 2000; Zhao et al. 2002). When *cyp79b2/cyp79b3* double mutants were supplied with ¹³C6-labeled IAOx, labeled IAM and indole-3-acetonitrile (IAN) were detected, suggesting that IAM and IAN might be synthesized from IAOx (Fig. 1) (Sugawara et al. 2009). However, IAOx has not been identified in some plants which contain IAM, implying that there is an IAOx-independent pathway for IAM synthesis (Sugawara et al. 2009). Two different hypotheses regarding the IAOx-independent pathway have been stated: (1) IAM is synthesized from indole via a TI pathway (Fig. 1); or (2) IAM is catalyzed by an undiscovered tryptophan-2-monooxygenase, which would be a homolog of *aux1/iaaM* (Fig. 1) (Pollmann et al. 2006).

The conversion of IAM into IAA is the second step in the IAM pathway and is catalyzed by acylamidohydrolases (AMI1). The catalytic domains of bacterial acylamidohydrolases share sequence homology with acylamidohydrolases from different organisms, such as AtAMI1 in *Arabidopsis* and NtAMI1 in tobacco (Nemoto et al. 2009; Pollmann et al. 2003). Both GST-AtAMI1 and GST-NtAMI1 fusion proteins expressed in *Escherichia coli* have

Fig. 2 The proposed auxin biosynthesis pathways in bacteria. There are five proposed Trp-dependent auxin biosynthesis pathways in bacteria: the indole-3-acetamide (IAM) pathway, the indole-3-pyruvic acid (IPyA) pathway, the tryptamine (TAM) pathway, the indole-3-acetonitrile (IAN) pathway and Trp side-chain oxidase (TSCO) pathway; and one Trp-independent pathway. This picture is partially adapted from Spaepen et al. 2007 and Patten et al. 2013. Enzymes and intermediate metabolites involved in these pathways are indicated. The *solid lines* and *dashed lines* indicate processes for which genes have been and have not been identified, respectively. The *blue color* indicates that gene functions have been determined for the shown gene



activities that convert IAM to IAA *in vitro*, suggesting conserved functions for the acylamidohydrolase family (Nemoto et al. 2009; Pollmann et al. 2003). Biochemical studies revealed that Ser137 of AtAMI1 and Ser136 of NtAMI1 are critical for enzymatic activity (Lehmann et al. 2010; Mano et al. 2010; Neu et al. 2007).

We know that the IAM pathway is an ancient and important pathway for IAA biosynthesis, through several additional lines of evidence. Firstly, we know that IAM and IAA are the only detectable auxins related to metabolism in the seaweeds *Ulva fasciata* and *Dictyotahumifusa* and that an AMI1-like enzyme in algae converts IAM to IAA (Stirk et al. 2009), indicating that the IAM pathway arose in an ancient photosynthetic lineage. Secondly, it has been shown that overexpression of the bacterial *aux1/iaaM* gene in the Ri plasmid keeps tobacco Bright Yellow-2 (BY-2) cells alive (Nemoto et al. 2009) and that overexpression of the *aux1/iaaM* gene in plants results in an auxin overproduction phenotype (Klee et al. 1987). Thirdly, IAM has been detected in many plants, including tobacco (Lemcke et al. 2000), *Arabidopsis* (Pollmann et al. 2002; Sugawara et al. 2009), maize and rice (Sugawara et al. 2009). Fourthly, genetic, biochemical, molecular and bioinformatic studies indicate that the IAM biosynthesis pathway exists widely in the plant kingdom (Lehmann et al. 2010; Mano et al. 2010). However, exactly how plants convert Trp into IAM is not clear.

The indole-3-pyruvic acid (IPyA) pathway

The IPyA pathway is a widely prevalent pathway in bacteria, such as in the rhizospheric *Azospirillum brasilense* and the enteric *Enterobacter cloacae*, which in soil promotes plant root growth (Costacurta et al. 1994; Patten and Glick 1996). In bacteria containing this pathway, L-Trp is converted to IPyA under the catalysis of tryptophan aminotransferase (AT) (Zhao 2014). IPyA is catalyzed by indole-3-pyruvate decarboxylase (IPDC) to indole-3-acetaldehyde (IAD), which is then oxidized by indole-3-acetaldehyde oxidase (IAO) to form IAA (Fig. 2) (Zhao 2014).

An IPyA intermediate is now included in plant IAA biosynthesis after characterization of mutants (Fig. 1). TAA1 (for TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1, also called SAV3, WEI8, TIR2 or CKRC1) catalyzes the formation of IPyA from L-Trp. During the past decade, mutant screening identified a variety of alleles of *taa1*, such as *sav3-1*, *wei8-1*, *tir2-1* and *ckrc1-1* (Stepanova et al. 2008; Tao et al. 2008; Yamada et al. 2009; Zhou et al. 2011). TAA1 is involved in regulating many aspects of plant growth and development, including hypocotyl elongation in different light wavelengths (Tao et al. 2008), tissue-specific ethylene response

(Stepanova et al. 2008), sensitivity to N-1-naphthylphthalamic acid (NPA) (Yamada et al. 2009), and crosstalk between cytokinin and auxin (Zhou et al. 2011). Recently, TAA1 was reported to regulate local auxin biosynthesis in the root-apex transition zone, mediating the aluminum-induced inhibition of root growth. The *Arabidopsis* genome contains four homologs of TAA1, namely, TAR1-4 (for TRYPTOPHAN AMINO TRANSFERASE RELATED 1–4). Genetic and biochemical analyses indicate that TAR1 and especially TAR2 coordinate with TAA1 in auxin biosynthesis (Stepanova et al. 2008).

There is possibly an alternative two-step Trp-to-IPyA pathway in which Trp racemase (TRC) first catalyzes L-Trp into D-Trp, which is then catalyzed by an unknown aminotransferase to form IPyA (Fig. 1) (Soeno et al. 2010). Recently, a suppressor of TAA1 (REVERSAL OF SAV3 PHENOTYPE 1, VAS1) was isolated, which encodes an aminotransferase (Zheng et al. 2013). Functional analysis indicates that VAS1 catalyzes the reverse synthesis of L-Trp from IPyA in plant, further influencing the equilibrium between IPyA, Trp and IAA *in vivo* (Fig. 1) (Zhao 2014; Zheng et al. 2013).

A comparative study based on bacterial conversion of IPyA to IAA by the enzymes IPDC and IAO identified 4 *Arabidopsis* homologs of IPDC (Kürsteiner et al. 2003). Functional analysis indicates that these plant IPDC genes participate in the synthesis of valine, isoleucine and in alcoholic fermentation (Ismond et al. 2003; Kürsteiner et al. 2003; Smith et al. 1989). However, no evidence for an IPDC enzyme with action on a Trp-derivative or with a product of IAD has been found. On the other hand, four indole-3-aldehyde oxidase (IAO) genes are predicted. In early studies, IAO1 was predicted to function in IAA biosynthesis (Seo et al. 1998). Other IAO genes have been shown to participate in abscisic acid (ABA) biosynthesis (Seo et al. 2004). Recently, IAO genes have been suggested to be irrelevant to IAA biosynthesis (Mashiguchi et al. 2011). So it remains unknown if the IAD present in plants is involved in the IPyA pathway to auxin biosynthesis.

The flavin monooxygenase gene family (YUCCA) has 11 predicted members, namely YUC1 to YUC11 (Cheng et al. 2006), which limit the conversion rate of IPyA to IAA (Cheng et al. 2006, 2007; Pacheco-Villalobos et al. 2013). The YUC and TAA1/TAR genes have been shown to function in the same pathway (Phillips et al. 2011), since the combination of reduced YUC and TAA1/TAR functions did not lead to additive phenotypes (Mashiguchi et al. 2011; Stepanova et al. 2011; Won et al. 2011). L-kynurenin (L-Kyn), a specific inhibitor of TAA1/TAR, inhibits IAA biosynthesis in mutants overexpressing YUC genes (Stepanova et al. 2011). IPyA homeostasis is regulated by both TAA1 and YUC (Mashiguchi et al. 2011; Won et al. 2011). Additionally, purified recombinant GST-YUC2 and YUC4

proteins catalyze the conversion of IPyA to IAA *in vitro* (Kriechbaumer et al. 2012; Mashiguchi et al. 2011). The most recent studies indicate that TAR1, TAR2, YUC3, 5, 7, 8 and 9 are required for high expression of HD-ZIP III and differentiation of the metaxylem (MX) in the vascular cylinder of the Arabidopsis primary root (Ursache et al. 2014).

A series of transcription factors that regulate the expression of TAA1 and/or YUCs have been identified and investigated, such as SHORT-INTERNODES 1/STYLISH 1 (SHI1/STY1) (Eklund et al. 2010; Staldal et al. 2012), TERMINAL FLOWER2 (TFL2) (Rizzardi et al. 2011), Phytochrome Interacting Factors (PIFs) (Franklin et al. 2011; Li et al. 2012; Sun et al. 2012), NGATHA (NGA) (Trigueros et al. 2009), Leafy cotyledon 2 (LEC2) (Stone et al. 2008; Wojcikowska et al. 2013), PLETHORA (PLT) (Pinon et al. 2013), and INDETERMINATE DOMAIN (IDD) (Cui et al. 2013). Importantly, studies have demonstrated the mechanism by which YUC catalyzes IPyA to IAA. YUC utilizes NADPH and O₂ to convert IPyA to IAA (Dai et al. 2013). Thus, the IPyA pathway is the first fully elucidated IAA biosynthesis pathway in plants (Fig. 1).

The tryptamine (TAM) pathway

Tryptamine (TAM) has growth-regulating activity similar to IAA, however, its importance in plants is controversial (Winter 1966). TAM exists either at too high of a level to be a discreet signal or non-specifically throughout the plant in several species, including tomato (Cooney and Nonhebel 1991; Schneider et al. 1972), rice (Ishida et al. 2014), Arabidopsis (Sugawara et al. 2009), barley (Sugawara et al. 2009) and pea (Quittenden et al. 2009). TAM and IAA come from different Trp pools, and TAM is not the main precursor of IAA (Cooney and Nonhebel 1991). It is possible that some YUC family genes participate in IAA biosynthesis from TAM (Zhao et al. 2001). Currently, it is thought that tryptophan decarboxylase (TDC) catalyzes L-Trp into TAM, which is then converted into hydroxytryptamine (HTAM) by YUC catalysis. HTAM is in turn converted to IAOx, which is finally synthesized into IAA (Fig. 1) (Zhao et al. 2001). However, this synthesis pathway is debated based on the following aspects: (1) TDC genes are widely distributed in plants, but their reported functions are in biosynthesis of indole alkaloid (IA) and HTAM (De Luca et al. 1988; Guillet et al. 2000; Torrens-Spence et al. 2013); (2) Higher levels of TAM, but not IAA, accumulate when TDC is overexpressed in Arabidopsis (Facchini et al. 2000; Songstad et al. 1990); (3) Most of the IAOx is synthesized by CYP79B2/B3, whereas no YUC has been shown to produce IAOx (Sugawara et al. 2009); and (4) YUC can synthesize a stable 4- α -hydroperoxyl flavin from flavin adenine dinucleotide (FAD)

and acts on numerous substrates (Dai et al. 2013; Tivendale et al. 2010; Ziegler 1990), which decreases the possibility that YUC catalyzes the conversion of TAM to HTAM.

The placement of a TAM intermediate in IAA biosynthesis is currently not well-supported (Brumos et al. 2014), but the possibility of TAM as a precursor of IAA cannot be eliminated. In some instances, such as in the root of pea, TAM can be converted to IAA, although most TAM is converted to N- ω -acetyltryptamine (Quittenden et al. 2009). The TAM pathway is not expressed in the seeds of pea, in part explaining why plants can accumulate high levels of TAM (in certain tissues) but not IAA (Ross et al. 2012; Songstad et al. 1990). As such, it is highly possible that a TAM pathway exists in some plants or some organs, but not in all species or in all organs of the same species (Tivendale et al. 2014).

The indole-3-acetaldoxime (IAOx) pathway

Although IAOx was isolated from Brassica oleracea more than 40 years ago, its biosynthesis pathway remains unclear. IAOx has recently been identified in Arabidopsis, but not in tomato, rice, maize, pea, or tobacco (Mano and Nemoto 2012; Sugawara et al. 2009). In Arabidopsis, IAOx is synthesized from L-Trp by two cytochrome P450 enzymes, namely, CYP79B2 and CYP79B3 (Hull and Celenza 2000; Hull et al. 2000). Overexpression of CYP79B2 leads to an IAA-overproduction phenotype, including elevated levels of IAN and IAA, increased hypocotyl length, and enhanced cotyledon epinasty (Zhao et al. 2002). In contrast, *cyp79b2/b3* double mutant exhibits decreased IAOx, IAN, IAM and free IAA levels, shorter hypocotyl, and smaller leaves in high temperature (26 °C) (Zhao et al. 2002).

A series of studies indicate that both IAM and IAN are downstream intermediate metabolites of IAOx (Fig. 1) (Sugawara et al. 2009). Enzymatic activity *in vitro* and gene function *in vivo* demonstrate that CYP71A13 participates in the conversion of IAOx to IAN (Nafisi et al. 2007). The conversion of IAN to IAA is still undefined. Currently, it is proposed that a group of nitrilases (NITs) are involved in this process (Bartel and Fink 1994; Bartling et al. 1992). The Arabidopsis genome encodes four NIT genes (NIT1-4), of which NIT1-3 show substrate preference for phenyl propionitrile, allyl cyanide phenylthioacetoneitrile and methylthioacetoneitrile, respectively, indicating that NITs catalyze the conversion of IAN to indole glucosinolates (IG), but not of IAN to IAA (Vorwerk et al. 2001). NIT4 participates in nitrile detoxification (Piotrowski et al. 2001). Plants overexpressing NIT have no IAA-overproduction phenotype (Normanly 1997). All of these results indicate that IAN and the NITs participate in IG metabolism and nitrile detoxification, but not in IAA

biosynthesis (Su et al. 2011). However, disruption of IG accumulation commonly results in abnormal IAA homeostasis, such as in mutants of SUR1 (superroot 1) and UGT74B1 (a UDP-glucose: thiohydroximate S-glucosyltransferase), which have decreased IG and eventually reduced IAA levels (Delarue et al. 1998; Grubb et al. 2004; King et al. 1995; Tivendale et al. 2014).

Together, these results indicate that IAOx-dependent IAA biosynthesis is not a common pathway in plants. It is highly possible that the IAOx pathway only plays roles in special developmental stages or environments (Brumos et al. 2014; Zhao et al. 2002).

Obscurities in IAA biosynthesis

Although our knowledge about IAA biosynthesis in plants has greatly advanced in the past decades, there are still a variety of unknowns remaining that require future study, including: (1) further confirmation of the role of IAO in IAA biosynthesis; (2) explanation for the YUC overexpression mutants, which have an IAA-overproduction phenotype but only slightly increased IAA level; (3) identification of more genes involved in the TI pathway, if any; and (4) further determination of the relationship between plants and bacteria in auxin biosynthesis.

The role of IAO in IAA biosynthesis

Early studies indicated that IAOs played roles in plants similar to those they play in bacteria, where IAOs mainly catabolize IAD into IAA (Fig. 2). However, recent evidence challenges the existence of this pathway in plants. Both YUC2 and YUC6 can directly catalyze the conversion of IPyA to IAA, implying that IAD and IAO may not participate in the IPyA pathway (Dai et al. 2013; Mashiguchi et al. 2011). Nevertheless, these results only show that the participation of IAD and IAO in the IPyA pathway is not absolutely necessary, but do not exonerate them completely of their roles in IAA biosynthesis. Isotope labeling indicated that IAD was produced from L-Trp and, therefore, may participate in other pathways of IAA biosynthesis (Mashiguchi et al. 2011). Meanwhile, some studies showed that IAO participated in the conversion IAD to IAA in Arabidopsis (Seo et al. 1998) and pea (Zdunek-Zastocka 2008), but did not show whether IAO participated in the IPyA pathway of these species (Fig. 1). It is possible that IAD and IAO function in other ways to contribute to IAA biosynthesis (Harrison et al. 2011; Normanly 2010; Woodward and Bartel 2005; Zhao 2010). To solve this problem, more accurate isotope labeling methods or other tracer techniques should be applied. Recently, a three-step conversion, Trp-IAOx-IAD-IAA,

has been identified in silkworms and sawfly. This finding may help us to understand the role of IAD in IAA biosynthesis (Suzuki et al. 2014).

YUC-overexpression results in a slight increase in IAA level but a strong IAA overproduction phenotype

A mutant overexpressing YUC1 exhibits an IAA overproduction phenotype, but the IAA content in the whole plant is only slightly increased (Stepanova et al. 2011). This may be attributable to the production of other auxins or substrates with auxin activity (Tivendale et al. 2010). Interestingly, recent studies indicated that 4-Cl-IAA and PAA were synthesized through the IPyA pathway (Dai et al. 2013; Korasick et al. 2013; Ross et al. 2012). On the other hand, phenotype differences may be explained by IAA having regulated and differential local accumulation that overexpression mutants obliterate (Tivendale et al. 2014), leading to strong phenotypes without gross changes in plant growth regulator (PGR) concentration. To understand this discrepancy in YUC1 overexpression phenotypes, it is necessary to determine if other auxin substrates are produced, to discover mutants with more discreet phenotypes, and to improve auxin measurement methods.

Trp-independent pathway

The TI pathway has been reported to exist in maize (Wright et al. 1991), Arabidopsis (Niyogi et al. 1993), carrot (Michalczuk et al. 1992; Ribnicky et al. 2002), Lemna gibba (Rapparini and Slovin 1999), Nicotiana tabacum (Sitbon et al. 2000) and tomato (Epstein et al. 2002). The TD and TI pathways function differently to maintain auxin homeostasis in plant. Generally, the TD pathway maintains the high levels of IAA that would initiate cell division during growth and development (Michalczuk et al. 1992), whereas the TI pathway is proposed to be responsible for maintaining basal auxin levels (Ribnicky et al. 2002). The relative contributions of each pathway to auxin content is likely differentially controlled by developmental stage and tissue identity in different species, such as in fruit development and the ripening process (Epstein et al. 2002), embryogenesis (Ribnicky et al. 2002) and embryos (Michalczuk et al. 1992), wounded tissue (Sztein et al. 2002) and hypocotyls (Ribnicky et al. 1996). Both the TI and TD pathways constantly operate in the plant. The TD pathway lacks significant feedback inhibition, whereas the TI pathway does have feedback inhibition (Michalczuk et al. 1992). Otherwise, IAA conjugation and degradation regulate basal IAA levels (Ribnicky et al. 2002).

It has been proposed that indole was more important than Trp in IAA biosynthesis (Bartel 1997). By using a

mutant that fails to synthesize L-Trp, a TI pathway was found, in which indole was synthesized from IGP through INS, and independent of the TS-complex (Ouyang et al. 2000; Wang et al. 2015).

However, no more mutants, enzymes or intermediate metabolites involved in the TI pathway have been identified to date, possibly because: (1) The TD pathway is dominant in plants, rendering it difficult to screen and obtain mutants involved in the TI pathway; (2) The possible intermediate metabolites of the TI pathway are IAN, IPyA and IAM (Bartel 1997; Mashiguchi et al. 2011), as in the TD pathway, rendering it difficult to distinguish metabolite origins; and/or (3) Model plants such as *Arabidopsis* and rice may not be the best materials for investigating the TI pathway. To solve these problems, it is worth combining Trp auxotrophic mutants with elaborate isotope labeling and hormone quantitation techniques. For instance, the tomato gene *SULFUREA*, which when mutated yields an auxin-deficient and yellow-leafed plant, may participate in the TI pathway (Ehlert et al. 2008). Identification of this gene function may help us further explore the TI pathway.

Auxin biosynthesis: similar but not the same in plants and microbes

Interestingly, comparison of the auxin biosynthesis pathways in plants and bacteria (Figs. 1, 2) shows that some metabolites are conserved or similar, but that the enzymes tend to differ. In early studies, some genes in plants, such as IPDC, IAO, NIT and AMI1, were obtained through homology analysis using bacterial auxin biosynthesis-related genes. However, most of these genes have proven to function in different ways, such as IPDC, IAO and NIT. It is even possible that other enzymes in addition to AMI1 catalyze the conversion of IAM to IAA in *Arabidopsis*. In addition, plants have evolved specific genes, such as TAA1 and the YUC family (Huang et al. 2014; Wang et al. 2014; Yue et al. 2014) that encode enzymes that catalyze reactions similar to those in bacteria. It is possible that IAA biosynthesis evolved independently in those bacteria that possess it and in plants (Yue et al. 2014). Further studies are required to identify the genes specifically participating in IAA biosynthesis in plants rather than obtaining homologs of bacterial genes.

Future directions

The auxin plant growth regulators, principally IAA, play fundamental roles in cell division, differentiation and expansion as well as in lateral root formation and flowering. Auxin biosynthesis, transport and signal transduction have

been extensively studied. It is clear that plants synthesize IAA through four TD pathways, in particular the IPyA and IAM pathways. On the other hand, the intermediates, enzymes, and metabolic flow of the TI pathway remain unclear.

In recent years, numerous studies have reported cross-links between auxin and other PGRs, such as cytokinins (Choi et al. 2014; Dello Ioio et al. 2008; Zhou et al. 2011; Zhu et al. 2015), abscisic acid (Zhao 2014), ethylene (Alarcon et al. 2014), brassinosteroids (Maharjan et al. 2014; Vandenbussche et al. 2013; Yoshimitsu et al. 2011), gibberellins (Chapman et al. 2012; Roumeliotis et al. 2012) and jasmonic acid (Cai et al. 2014; He and Zhao 2015; Hentrich et al. 2013). These results expand our knowledge of how plants not only maintain PGR homeostasis but also signal between the different hormone pathways. As studies continue, additional genes involved in these pathways will be newly identified, and known genes may be assigned new functions. Furthermore, new and improved research techniques, such as endogenous auxin content measurement methods (Yu et al. 2014), genetic screening, and imaging techniques, will serve to expand our studies on auxin biosynthesis and signal transduction. Additionally, careful study of available mutants of known or suspected auxin biosynthesis genes could be analyzed and stacked into mutants in order to study additive effects.

Recently, some laboratories have focused their efforts on screening for small molecules that inhibit the activities of specific enzymes involved in IAA biosynthesis. For instance, L-Kyn has been identified as inhibiting TAA1/TAR2 (He et al. 2011), and Yucasin [5-(4-chlorophenyl)-4 H-1,2,4-triazole-3-thiol] has been revealed to inhibit YUC enzymes (Nishimura et al. 2014). The newly synthesized inhibitor indole-3-oxoethylphosphonic acid (IOEP) can inhibit root hair growth in *Arabidopsis*, and the growth defect can be restored by treatment with IAA (Ishida et al. 2014). These and other new small molecules will be useful in future studies aimed at delineating the true pathway to auxin biosynthesis in plants.

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Conflict of interest The authors declare that they have no conflicts of interest.

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