REVIEW PAPER

Auxin biosynthesis and storage forms

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Abstract

The plant hormone auxin drives plant growth and morphogenesis. The levels and distribution of the active auxin indole-3-acetic acid (IAA) are tightly controlled through synthesis, inactivation, and transport. Many auxin precursors and modified auxin forms, used to regulate auxin homeostasis, have been identified; however, very little is known about the integration of multiple auxin biosynthesis and inactivation pathways. This review discusses the many ways auxin levels are regulated through biosynthesis, storage forms, and inactivation, and the potential roles modified auxins play in regulating the bioactive pool of auxin to affect plant growth and development.

Key words: auxin, auxin biosynthesis, auxin conjugates, development, IAA, IBA.

Introduction

Maintaining appropriate cellular levels of active auxin is important for regulating all aspects of plant growth and development. Cellular auxin levels can be altered by auxin transport, auxin biosynthesis, and interconversion of modified auxin forms. In this review, we focus on the various forms of auxin precursors and their roles in contributing to auxin homeostasis and plant development.

Many small molecules, when supplied exogenously, induce an auxin response. These compounds include naturally occurring active auxins (Fig. 1A), such as indole-3-acetic acid (IAA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA); naturally occurring inactive auxin precursors, such as indole-3-pyruvic acid (IPyA), indoleacetamine (IAM), indole-3-acetaldoxime (IAOx), indole-3-acetonitrile (IAN), and indole-3-acetaldehyde (IAAld); and naturally occurring auxin storage forms, such as indole-3-butyric acid (IBA), methyl-IAA (MeIAA), and auxins conjugated to amino acids or sugars. Additionally, synthetic compounds (Fig. 1B), such as 2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid (NAA), 3,6-dichloro-2-methoxybenzoic acid (dicamba), and 4-amino-3,5,6-trichloropicolinic acid (picloram), induce an auxin response. In this review, we focus on the various forms of naturally occurring active and inactive auxins and auxin precursors (Table 1), as well as roles for inputs from different modified auxins to the active auxin pool affecting plant growth and development.

Active auxins

IAA

IAA is the best-studied naturally occurring active auxin. IAA biosynthesis can occur through two major routes: tryptophan (Trp)-dependent and Trp-independent pathways (reviewed by Woodward and Bartel, 2005). Several Trp-dependent auxin biosynthesis pathways contribute to IAA levels, including the IAOx pathway, the IAM pathway, and the IPyA pathway (Fig. 2).
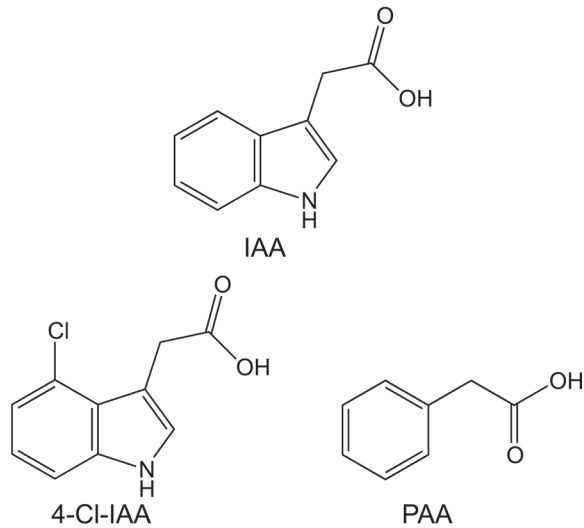
The IAOx pathway

The IAOx pathway has been suggested to occur only in crucifers (Sugawara *et al.*, 2009); however, IAN, a downstream
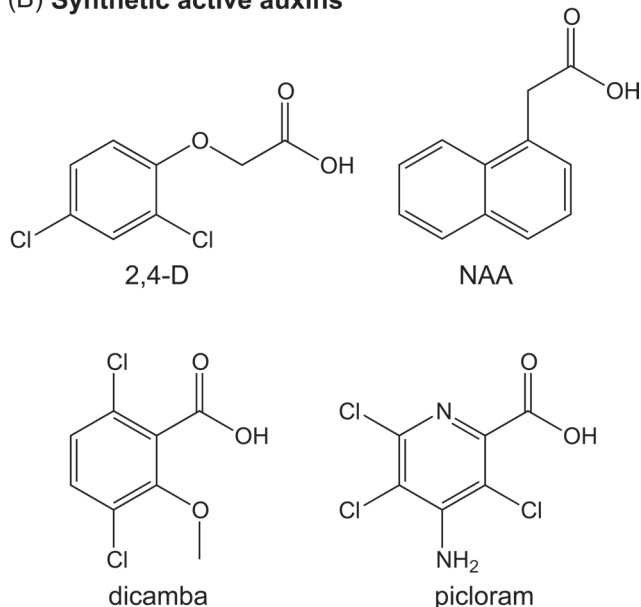
(A) Naturally-ocurring active auxins

IAA

4-Cl-IAA

PAA

(B) Synthetic active auxins

2,4-D

NAA

dicamba

picloram

Fig. 1. Naturally occurring and synthetic active auxins. (A) Indole-3-acetic acid (IAA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA) are naturally occurring active auxins. (B) 2,4-Dichlorophenoxyacetic acid (2,4-D), 1-naphthaleneacetic acid (NAA), 3,6-dichloro-2-methoxybenzoic acid (dicamba), and 4-amino-3,5,6-trichloropicolinic acid (picloram) are active synthetic auxins.

intermediate, has also been detected in maize coleoptiles (Bak *et al.*, 1998). The cytochrome P450 enzymes CYP79B2 and CYP79B3 convert Trp to IAOx (Hull *et al.*, 2000; Mikkelsen *et al.*, 2000; Zhao *et al.*, 2002). IAOx is largely used to produce defence compounds such as glucosinolates or camalexins (Bak *et al.*, 2001; Zhao *et al.*, 2002; Mikkelsen *et al.*, 2004) and is also used to produce IAA (Zhao *et al.*, 2002; Sugawara *et al.*, 2009). Overexpression of CYP79B2 results in increased levels of indole glucosinolates (Mikkelsen *et al.*, 2000; Zhao

et al., 2002), IAN (Zhao *et al.*, 2002), and free IAA (Zhao *et al.*, 2002), accompanied by long hypocotyls and epinastic cotyledons (Zhao *et al.*, 2002). Conversely, the *cyp79b2 cyp79b3* double mutant displays decreased IAOx (Zhao *et al.*, 2002; Sugawara *et al.*, 2009), IAN (Zhao *et al.*, 2002; Sugawara *et al.*, 2009), IAM (Sugawara *et al.*, 2009), and free IAA under normal (Sugawara *et al.*, 2009) or elevated (Zhao *et al.*, 2002) temperatures, suggesting that both IAN and IAM are downstream intermediates of the IAOx pathway. In addition, *cyp79b2 cyp79b3* displays slightly shorter petioles and smaller leaves (Zhao *et al.*, 2002), consistent with roles for IAOx-derived auxin driving these processes. Although IAN, an intermediate downstream from IAOx, was previously thought to be downstream of the glucosinolate pathway, *superroot1* mutants, defective in glucosinolate biosynthesis, display normal IAN levels (Sugawara *et al.*, 2009), consistent with IAN being produced independently of glucosinolates; however, the enzymatic steps between IAOx and IAN have yet to be identified. IAN can then be converted to active IAA through the activities of the NIT1 family of nitrolases (Schmidt *et al.*, 1996; Normanly *et al.*, 1997).

The IAM pathway
IAM, another source of auxin, is likely made from both IAOx and an additional unknown source (Sugawara *et al.*, 2009). IAM levels are decreased in the *cyp79b2 cyp79b3* mutant (Sugawara *et al.*, 2009), which is defective in conversion of Trp to IAOx (Mikkelsen *et al.*, 2000; Zhao *et al.*, 2002), consistent with IAOx contributing to IAM levels. In addition, synthesis of IAM from IAOx has been directly demonstrated in assays where *cyp79b2 cyp79b3* was fed $^{13}C_6$-labelled IAOx to generate $^{13}C_6$-labelled IAM (Sugawara *et al.*, 2009). Ratios of enriched IAM and IAN from these studies suggest that IAN and IAM are also produced independently from IAOx (Sugawara *et al.*, 2009). Additionally, IAM has been detected in many species in which IAOx has not been detected (Table 1), raising the possibility that IAM can also be produced in an IAOx-independent pathway. IAM can be converted to active IAA through the activity of AMIDASE1 (Pollmann *et al.*, 2003).

The IPyA pathway
The IPyA pathway appears to be the main contributor to free IAA (reviewed by Zhao, 2012) and is the only pathway in which every step from Trp to IAA has been identified (Fig. 2). Conversion of Trp to IAA via the IPyA pathway is a two-step process: the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) family of Trp aminotransferases converts Trp to IPyA, and the YUCCA (YUC) family of flavin monooxygenases converts IPyA to IAA. Mutation of *TAA1/SHADE AVOIDANCE3/WEAK ETHYLENE INSENSITIVE8/TRANSPORT INHIBITOR RESPONSE2* (*TAA1/SAV3/WEI8/TIR2*) results in decreased free IAA, and TAA1 has been shown to convert Trp to IPyA (Stepanova *et al.*, 2008; Tao *et al.*, 2008). *wei8 tar2* mutants, defective in *TAA1* and the related *TRYPTOPHAN AMINOTRANSFERASE RELATED2*, display altered meristem function and floral phenotypes suggestive of decreased

**Table 1.** Occurrence of modified auxin forms and precursors.

Modified auxin form	Purpose	Species in which identified (reference)
4-Chloroindole acetic acid (4-Cl-IAA)	Active auxin	*Lens culinaris* (Engvild et al., 1981); *Lathyrus latifolius* (Engvild et al., 1980); *Lathyrus maritimus* (Engvild et al., 1981); *Lathyrus odoratus* (Engvild et al., 1981); *Lathyrus sativus* (Engvild et al., 1981); *Pinus sylvestris* (Ernstsen and Sandberg, 1986); *Pisum sativum* (Schneider et al., 1985; Barkawi et al., 2008); *Vicia amurensis* (Engvild et al., 1981); *Vicia faba* (Engvild et al., 1981); *Vicia sativa* (Engvild et al., 1981)
Phenylacetic acid (PAA)	Active auxin	*Helianthus annuus* (Wightman and Rauthan, 1974; Wightman and Lighty, 1982); *Hordeum vulgare* (Wightman and Lighty, 1982); *Lycopersicon esculentum* (Wightman and Rauthan, 1974; Wightman and Lighty, 1982); *Phaseolus* (Okamoto et al., 1967); *Phaseolus vulgaris* (Okamoto et al., 1967); *Pisum sativum* (Wightman and Lighty, 1982); *Porphyra tenera* (Fries and Iwasaki, 1976); *Triticum aestivum* (Wightman and Lighty, 1982); *Tropaeolum majus* (Ludwig-Müller and Cohen, 2002); *Undaria pinnarifida* (Abe et al., 1974); *Zea mays* (Wightman and Lighty, 1982);
Indole-3-acetaldoxime (IAOx)	Auxin precursor	*Arabidopsis thaliana* (Sugawara et al., 2009; Novák et al., 2012); *Brassica campestris* spp. *pekinensis* (Ludwig-Müller and Hilgenberg, 1988)
Indole-3-acetonitrile (IAN)	Auxin precursor	*Arabidopsis thaliana* (Normanly et al., 1993; Ilić et al., 1996; Zhao et al., 2002; Sugawara et al., 2009; Novák et al., 2012); *Brassica campestris* (Jones et al., 1952); *Brassica juncea* (Pedras et al., 2002); *Zea mays* (Bak et al., 1998; Park et al., 2003)
Indoleacetamide (IAM)	Auxin precursor	*Arabidopsis thaliana* (Pollmann et al., 2002; Sugawara et al., 2009; Novák et al., 2012); *Cucurbita maxima* (Rajagopal et al., 1994); *Nicotiana tabacum* (Sugawara et al., 2009); *Oryza sativa* (Sugawara et al., 2009); *Zea mays* (Sugawara et al., 2009)
Indole-3-pyruvic acid (IPyA)	Auxin precursor	*Arabidopsis thaliana* (Tam and Normanly, 1998; Mashiguchi et al., 2011; Won et al., 2011; Liu et al., 2012; Novák et al., 2012); *Lycopersicum esculentum* (Cooney and Nonhebel, 1989; Liu et al., 2012); *Pisum sativum* nodules (Badenoch-Jones et al., 1984)
Indole-3-acetaldehyde (IAAld)	Auxin precursor	*Arabidopsis thaliana* (Mashiguchi et al., 2011; Novák et al., 2012); *Pisum sativum* (Quittenden et al., 2009); *Pinus sylvestris* (Ernstsen and Sandberg, 1986)
Indole-3-butyric acid (IBA)	Precursor/storage	*Arabidopsis thaliana* (Ludwig-Müller et al., 1993; Strader et al., 2010; Liu et al., 2012); *Daucus carota* (Epstein et al., 1991); *Medicago truncatula* (Campanella et al., 2008); *Nicotiana tabacum* (Sutter and Cohen, 1992); *Pisum sativum* nodules (Badenoch-Jones et al., 1984); *Pisum sativum* (Schneider et al., 1985); *Solanum tuberosum* (Blommaert, 1954) *Tropaeolum majus* (Ludwig-Müller and Cohen, 2002); *Zea mays* (Epstein et al., 1989; Barkawi et al., 2008)
IBA–glucose	Precursor/storage	*Arabidopsis thaliana* (Tognetti et al., 2010)
Methyl-IAA(MeIAA)	Precursor/storage	*Arabidopsis thaliana* (Narasimhan et al., 2003); *Vitis vinifera* (Gouthu et al., 2013)
IAA–glucose/IAA–glycan	Storage	*Arabidopsis thaliana* (Tam et al., 2000); *Avena sativa* (reviewed by Cohen and Bandurski, 1982); legumes (Jakubowska and Kowalczyk, 2004); *Pinus pinea* (Riov and Gottlieb, 1980); Tobacco (Sitbon et al., 1993); *Zea mays* (reviewed by Cohen and Bandurski, 1982)
IAA–*myo*-inositol	Storage	*Arabidopsis thaliana* (Luo et al., 2011); *Oryza sativa* (Hall, 1980); *Zea mays* (Nicholls, 1967; Chisnell, 1984)
IAA–Asp–glucose	Storage/inactivation	*Oryza sativa* (Kai et al., 2007)
IAA–Glu–glucose	Storage/inactivation	*Oryza sativa* (Kai et al., 2007)
IAA–Ala	Storage	*Arabidopsis thaliana* (Kowalczyk and Sandberg, 2001); *Oryza sativa* (Kojima et al., 2009); *Piscia abies* (Östin et al., 1992)
IAA–Asp	Inactivation	*Arabidopsis thaliana* (Östin et al., 1991; Tam et al., 2000; Kowalczyk and Sandberg, 2001; Novák et al., 2012); *Ceratopteris richardii* (Sztein et al., 1999); *Citrus sinesus* (Chamarro et al., 2001); *Cucumis sativus* (Purves and Hollenberg, 1982; Sonner and Purves, 1985); *Dalbergia dolichopetala* (Monteiro et al., 1988); *Daucus carota* (Sasaki et al., 1994); Douglas fir (Chiwocha and von Aderkas, 2002); *Glycine max* (Cohen, 1982); *Heracleum laciniatum* (Cohen and Ernstsen, 1991); *Oryza sativa* (Kojima et al., 2009); *Pinus pinea* (Riov and Gottlieb, 1980); *Pisum sativum* (Andreae and Good, 1955); *Populus tremula x Populus tremuloides* (Tuominen et al., 1994); Tobacco (Sitbon et al., 1993); *Triticum aestivum* (Martens and Franckenbeger, 1994); *Vigna radiata* (Norcini and Heuser, 1988)
IAA–Glu	Inactivation	*Arabidopsis thaliana* (Östin et al., 1998; Tam et al., 2000; Kowalczyk and Sandberg, 2001; Novák et al., 2012); *Citrus sinesus* (Chamarro et al., 2001); *Cucumis sativus* (Purves and Hollenberg, 1982; Sonner and Purves, 1985); Tobacco (Sitbon et al., 1993)
IAA–Gly	Unknown	*Helleborus niger* (Penčík et al., 2009)
IAA–Gln	Unknown	*Arabidopsis thaliana* (Barratt et al., 1999)
IAA–Leu	Storage	*Arabidopsis thaliana* (Kowalczyk and Sandberg, 2001); *Ceratopteris richardii* (Sztein et al., 1999); *Nitella* (Sztein et al., 2000); *Physcomitrella patens* (Ludwig-Müller et al., 2009)
IAA–Phe	Unknown	Postulated: *Arabidopsis thaliana* (Kai et al., 2007); *Funaria hydrometrica* (Sztein et al., 1999); *Helleborus niger* (Penčík et al., 2009); *Orthotrichum lyellii* (Sztein et al., 1999); *Oryza sativa* (Kojima et al., 2009)
IAA–Trp	Antagonist	*Arabidopsis thaliana* (Staswick, 2009)

Modified auxin form	Purpose	Species in which identified (reference)
IAA–Val	Unknown	Postulated: *Arabidopsis thaliana* (Kai et al., 2007); *Funaria hydrometrica* (Sztein et al., 1999); *Helleborus niger* (Penčík et al., 2009); *Marchantia polymorpha* (Sztein et al., 1999); *Pallavicinia lyellii* (Sztein et al., 1999); *Phaeoceros laevis* (Sztein et al., 2000); *Physcomitrella patens* (Ludwig-Müller et al., 2009); *Polytrichum ohioense* (Sztein et al., 1999); *Reboulia hemisphaerica* (Sztein et al., 1999)
Oxindole-3-acetic acid (oxIAA)	Inactivation	*Arabidopsis thaliana* (Östin et al., 1998; Kowalczyk and Sandberg, 2001; Novák et al., 2012); *Citrus sinesus* (Chamarro et al., 2001); *Lycopersicon esculentum* (Riov and Bangerth, 1992); *Marchantia polymorpha* (Sztein et al., 1999); *Phaeoceros laevis* (Sztein et al., 2000); *Pinus sylvestris* (Ernstsen et al., 1987); *Vicia faba* (Tsurumi and Wada, 1980); *Zea mays* (Reinecke and Bandurski, 1983)
IAA–peptide/IAA–protein		*Arabidopsis thaliana* (Walz et al., 2002); Postulated: *Avena sativa* (Percival and Bandurski, 1976); *Fragaria vesca* (Park et al., 2006); *Phaseolus vulgaris* (Bialek and Cohen, 1986)
Indolepropionic acid (IPrA)	Precursor/storage	Banana (Yan et al., 2012); *Chorella vulgaris* (Lu et al., 2010); *Curcurbita pepo* (Segal and Wightman, 1982); *Lycopersicon esculentum* (Aung, 1972); *Nicotiana glauca* (Bayer, 1969); *Nicotiana langsdorffii* (Bayer, 1969); *Pisum sativum* (Schneider et al., 1985); *Zea mays* (García Sánchez et al., 1996)

auxin levels (Stepanova *et al.*, 2008). Additionally, *tir2* mutants, defective in *TAA1*, display decreased temperature-dependent hypocotyl elongation, gravitropism, root hair formation, and lateral root development (Yamada *et al.*, 2009). The *wei8-1 tar2-1* double mutant accumulates less IPyA (Mashiguchi *et al.*, 2011) and less IAA (Stepanova *et al.*, 2008; Tao *et al.*, 2008) than wild type, whereas TAA1 over-expression lines accumulate more IPyA (Mashiguchi *et al.*, 2011) than wild type, consistent with roles for TAA enzymes in converting Trp to IPyA in an auxin biosynthesis pathway.

YUC enzymes convert IPyA to IAA. YUC was previously thought to converge on the IAOx pathway; however,
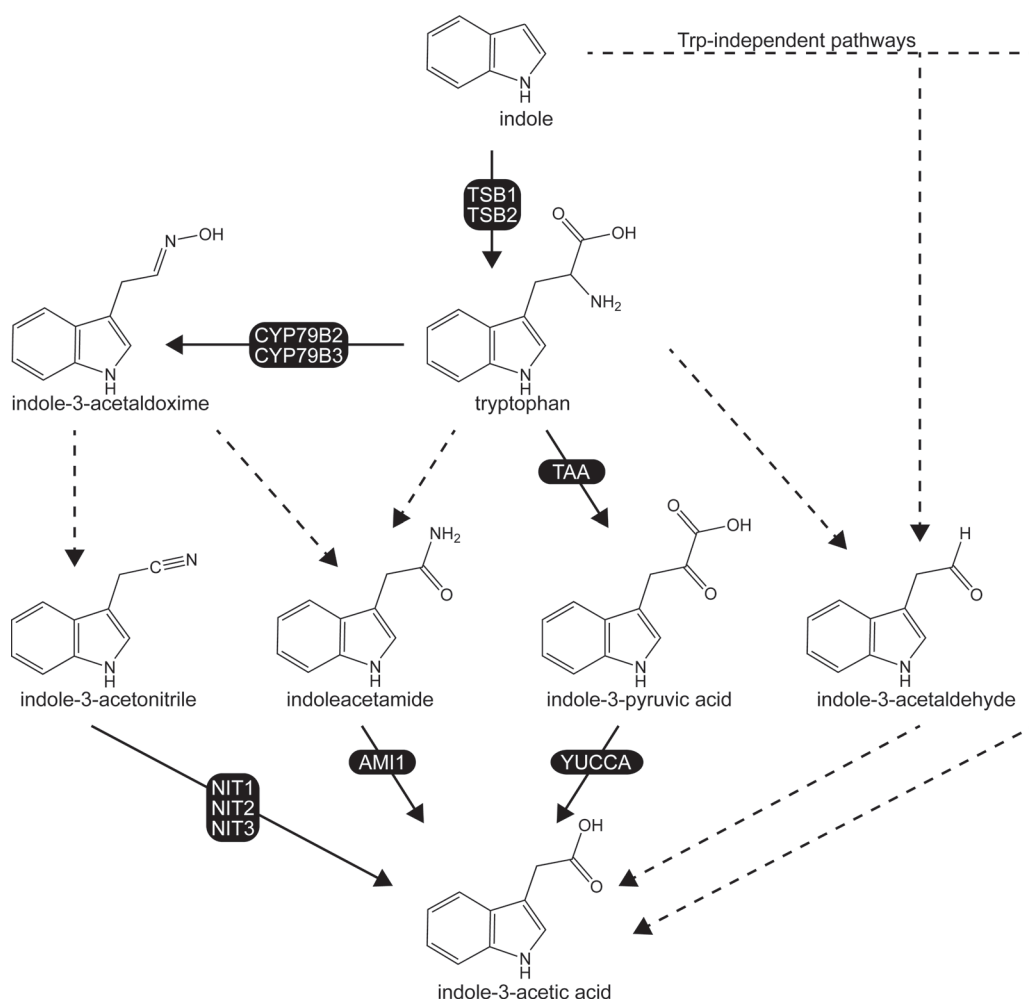
Fig. 2. Potential IAA biosynthetic pathways. Arrows in pathways for which enzymes have been identified are solid and arrows in pathways that have not been identified are dashed and may be single or multiple steps.

phenotypic similarities between *yucca* and *taa1* mutants raised the possibility that YUC and TAA1 act in the same pathway (Strader and Bartel, 2008), and non-additive phenotypes of *vanishing tassel2* (*vt2*) and *sparse inflorescence1* (*spi1*), maize homologues of TAA1 and YUC, respectively, support the possibility that TAA1 and YUC are in the same pathway (Phillips *et al.*, 2011). Further, the role of *N*-hydroxytryptamine, a proposed product of YUC enzymatic activity (Zhao *et al.*, 2001), has been called into question (Tivendale *et al.*, 2010). The YUC family is now known to convert IPyA into active IAA (Mashiguchi *et al.*, 2011; Stepanova *et al.*, 2011; Won *et al.*, 2011) using NADPH and oxygen in the conversion process (Dai *et al.*, 2013).

Overexpression of many *YUC* family members results in auxin overproduction phenotypes (Zhao *et al.*, 2001; Marsch-Martinez *et al.*, 2002; Woodward *et al.*, 2005; Cheng *et al.*, 2006; Kim *et al.*, 2007; Mashiguchi *et al.*, 2011). Higher-order *yuc* mutants have defects in floral patterning and vascular formation, and display decreased DR5–GUS activity (Cheng *et al.*, 2006) and the *yuc1 yuc4 yuc10 yuc11* quadruple mutant does not develop a hypocotyl or a root meristem (Cheng *et al.*, 2007). The *yuc1 yuc2 yuc4 yuc6* mutants hyperaccumulate IPyA whereas YUC6 overexpression lines hypoaccumulate IPyA (Mashiguchi *et al.*, 2011), consistent with roles for YUC family members in converting IPyA to IAA. Because overexpression of YUC family members results in auxin overproduction phenotypes (Zhao *et al.*, 2001; Marsch-Martinez *et al.*, 2002; Woodward *et al.*, 2005; Cheng *et al.*, 2006; Kim *et al.*, 2007; Mashiguchi *et al.*, 2011) and TAA1 overexpression lines resemble wild type (Tao *et al.*, 2008; Mashiguchi *et al.*, 2011), YUC is likely the rate-limiting step of the IPyA pathway.

Interestingly, IAAld has been identified in several plant species (Table 1) and has previously been hypothesized to be an auxin precursor (Seo *et al.*, 1998) and included as an intermediate in many previously proposed auxin biosynthetic pathways (reviewed by Woodward and Bartel, 2005). In addition, IAAld application results in increased free IAA levels (Larsen, 1949, 1951; Bower *et al.*, 1978; Koshiba *et al.*, 1996; Tsurusaki *et al.*, 1997), consistent with the possibility that IAAld is directly converted to IAA *in planta*. The ARABIDOPSIS ALDEHYDE OXIDASE1 (AAO1) enzyme has been suggested to convert IAAld into IAA (Seo *et al.*, 1998). However, roles for AAO1 in IAAld conversion to IAA have been questioned, because the *aba3* mutant, which fails to produce the molybdenum cofactor required for AAO activity (Schwartz *et al.*, 1997), displays no obvious auxin-related defects and does not hyperaccumulate IAAld (Mashiguchi *et al.*, 2011), suggesting that AAO members do not contribute to regulation of auxin homeostasis or regulation of IAAld-to-IAA conversion. At this time, IAAld is not hypothesized to be an intermediate in proposed auxin biosynthesis pathways, despite its natural occurrence and despite *in planta* conversion of supplied IAAld to IAA. Therefore, IAAld is an orphan intermediate in the currently proposed IAA biosynthetic pathways (Fig. 2); future studies will be necessary to identify enzymes required for IAAld-to-IAA conversion and to determine whether IAAld plays a role in auxin homeostasis through either the Trp-dependent or Trp-independent auxin biosynthetic pathways.

The Trp-independent pathway

In addition to the described Trp-dependent auxin biosynthetic pathways, Trp-independent auxin biosynthetic pathways might also contribute to auxin homeostasis (reviewed by Normanly *et al.*, 2004). Analysis of *trp* mutants in *Arabidopsis* and maize has revealed no differences in free IAA levels when compared with wild type (Wright *et al.*, 1991; Normanly *et al.*, 1993), consistent with the possibility that IAA can be synthesized in the absence of Trp. In addition, Trp-deficient mutants in both *Arabidopsis* and maize accumulate amide- and ester-linked IAA conjugates (reviewed by Normanly *et al.*, 2004; Woodward and Bartel, 2005). Furthermore, feeding assays with labelled Trp precursors support Trp-independent auxin biosynthesis (Normanly *et al.*, 1993). Little is known about potential intermediates in the proposed Trp-independent pathway, and none of the genes involved has been identified; the Trp-independent pathway is postulated to stem from either indole or indole-3-glycerol phosphate (Ouyang *et al.*, 2000).

Some studies have questioned the likelihood of a Trp-independent pathway. Non-enzymatic conversion of indole-3-glycerol phosphate, which hyperaccumulates in the *trp3-1* mutant of *Arabidopsis*, to IAA during extraction has been suggested to be the source of IAA in samples examined for auxin levels (Müller and Weiler, 2000). In addition, Trp is efficiently converted to IAA in maize kernels and is not competed by indole, suggesting that Trp-to-IAA conversion is the main driver of auxin homeostasis in these tissues (Glawischnig *et al.*, 2000). The molecular identification of enzymes required for Trp-independent IAA biosynthesis would clarify these differences.

4-Cl-IAA

Halogenated indole acetic acids are bioactive molecules and occur naturally in at least some higher plants. Specifically, the activity of endogenous 4-Cl-IAA has been studied in *Pisum sativum*, as well as multiple other legumes (reviewed by Reinecke, 1999). Although 4-Cl-IAA has yet to be found in *Arabidopsis*, it is an active auxin in *Arabidopsis* bioassays (reviewed by Reinecke, 1999). 4-Cl-IAA is likely synthesized through the IPyA biosynthetic pathway, e.g. chlorination of Trp, conversion to 4-chloroindole-3-pyruvic acid, followed by oxidation to 4-Cl-IAA (Tivendale *et al.*, 2012). Further analysis of the activities and potential storage forms of 4-Cl-IAA will shed light on its importance in auxin homeostasis.

PAA

PAA is a non-indolic, active endogenous auxin present at physiologically relevant levels in multiple higher plant species (Table 1). In addition to acting as an active auxin, PAA inhibits polar auxin transport in *P. sativum*, potentially regulating the effects of the free IAA (Morris and Johnson, 1987). The biological significance of PAA, however, is not completely understood. Future studies will contribute to understanding the physiological roles of PAA.

Inactive auxins

Only a small fraction of auxin exists as free, active signalling molecule. The auxin pool consists of a mixture of free auxin, conjugated auxins, the inactive auxin precursor IBA, and the inactive methyl ester form of IAA, MeIAA (Fig. 3). Cohen and Bandurski (1982) postulated that auxin storage forms exist to regulate auxin homeostasis in growth and development: to influence auxin sensitivity, transport, and compartmentalization. Although the presence of these storage forms

Fig. 3. Potential IAA storage form pathways. Arrows at steps for which enzymes have been identified are solid and arrows in pathways that have not been identified are dashed and may be single or multiple steps.

has been known for decades, the complex dynamics of this system are not yet fully understood, and whether any of these compounds act independently of conversion to IAA is under debate. Analysis of auxin conjugate composition profiles and examination of mutant plants deficient in different aspects of auxin homeostasis has allowed a more detailed understanding of the purposes and functions of inactive auxins.

Auxin conjugates

Three major forms of auxin conjugates exist in higher plants, including ester-linked simple and complex carbohydrate conjugates, amide-linked amino acid conjugates, and amide-linked peptide and protein conjugates (reviewed by Ludwig-Müller, 2011). Auxin conjugate forms are generally considered inactive; any observed auxin activity is attributed to conjugate hydrolysis for conversion to an active auxin (reviewed by Woodward and Bartel, 2005; Bajguz and Piotrowska, 2009; Ludwig-Müller, 2011). Interestingly, the composition of IAA conjugates varies between plant species. For example, the major conjugate form in maize kernels is ester-linked sugars (Bandurski *et al.*, 1995), whereas *Arabidopsis* and many other dicots primarily store IAA as amide-linked amino acid conjugates (reviewed by Bajguz and Piotrowska, 2009).

Ester-linked IAA–sugar conjugates have been identified in both monocots and dicots (Table 1). IAA–sugar conjugates can serve roles in auxin storage and in IAA inactivation (see below). UDP glucosyltransferases, such as UGT84B1 in *Arabidopsis* (Jackson *et al.*, 2001) and iaglu in maize (Szerszen *et al.*, 1994), conjugate IAA to glucose. Further conversion of IAA–Glc to IAA–*myo*-inositol in maize kernels occurs through the reversible activity of 1-*O*-(indole-3-acetyl)-glucose: *myo*-inositol indoleacetyl transferase (IAInos synthase), a serine carboxypeptidase-like acyltransferase that may also catalyse the hydrolysis of IAA–*myo*-inositol to generate free IAA (Kowalczyk *et al.*, 2003). Additionally, maize tissues can hydrolyze IAA–glucose isomers and IAA–*myo*-inositol to free IAA (Jakubowska and Kowalczyk, 2005), consistent with the possibility that hydrolysis of these compounds contributes to the bioactive auxin pool.

Many different amide-linked IAA-amino acid conjugates have been identified in higher plants (Table 1). Of these conjugates, the activity and function of IAA–Ala, IAA–Leu, IAA–Asp, IAA–Glu, and IAA–Trp are best understood. IAA–Ala and IAA–Leu both inhibit root elongation and are readily hydrolysable in *Arabidopsis* (Bartel and Fink, 1995; LeClere *et al.*, 2002; Rampey *et al.*, 2004), suggesting both IAA–Ala and IAA–Leu contribute to the active auxin pool in *Arabidopsis*. Conversely, IAA–Asp and IAA–Glu are not appreciably hydrolysed in *Arabidopsis* (Östin *et al.*, 1998; LeClere *et al.*, 2002; Rampey *et al.*, 2004) and are more likely intermediates in IAA catabolism (see Auxin inactivation pathways). Interestingly, IAA–Trp functions as an inhibitor of auxin-induced growth (Staswick, 2009).Although IAA-Asp is generally thought to be inactive, *Medicago truncatula* hydrolases readily hydrolyse IAA–Asp to free IAA (Campanella *et al.*, 2008), suggesting that different IAA–amino acid conjugates may play alternative

roles in different species. The distinct functions of these IAA–amino acid conjugates are consistent with the complexity and tight regulation of auxin homeostasis. Further work to unravel the importance of each conjugate is likely to lead to a more refined understanding of the contributions of each of these modified auxin forms to auxin homeostasis.

Investigation of IAA–amino acid conjugation enzymes has yielded a wealth of molecular data over the past decade. Group II of the GRETCHEN HAGEN3 (GH3) family of acyl amido synthetases conjugates IAA to amino acids (Westfall *et al.*, 2010). Recent crystal structures of IAA- and jasmonic acid-conjugating GH3 proteins (Peat *et al.*, 2012; Westfall *et al.*, 2012) and IAA–amino acid hydrolase proteins (Bitto *et al.*, 2009) have provided valuable insights into the molecular mechanism of phytohormone–amino acid conjugation and hydrolysis. Mutation of *GH3.1*, *GH3.2*, *GH3.5*, or *GH3.17* in *Arabidopsis* results in mildly increased sensitivity to IAA root elongation inhibition (Staswick *et al.*, 2005), and mutation of *GH3-1* or *GH3-2* in *Physcomitrella patens* results in mildly increased sensitivity to IAA in gametophore growth (Ludwig-Müller *et al.*, 2009). Further research may reveal whether GH3 enzymes have tissue-specific or developmental roles; understanding these roles may require the generation of higher-order mutants. The recent influx of biochemical data describing the action of IAA-conjugating proteins is an important and major step towards understanding the biochemical details of IAA homeostasis.

Several enzymes hydrolyse IAA–amino acid conjugates to free IAA: IAA–LEUCINE RESISTANT1 (ILR1; Bartel and Fink, 1995), ILR1 homologues, ILR1-LIKE1 (ILL1), ILL2 (Bartel and Fink, 1995; LeClere *et al.*, 2002), IAA–ALANINE RESISTANT3 (IAR3), ILL3, and ILL5 (Davies *et al.*, 1999). Mutant screens have also uncovered additional components necessary for IAA–amino acid conjugation, including transcription factors and metal transporters (Campanella *et al.*, 1996; Lasswell *et al.*, 2000; LeClere *et al.*, 2004; Rampey *et al.*, 2006 Rampey *et al.*, 2013). Interestingly, examined *Arabidopsis* conjugate hydrolases display a high affinity for IAA–Leu and IAA–Ala and a low affinity for other IAA–amino acid conjugates (LeClere *et al.*, 2002; Rampey *et al.*, 2004), suggesting that IAA–Leu and IAA–Ala are hydrolysable and contribute to the pool of free, bioactive auxin, whereas other conjugates may serve other functions, such as auxin catabolism (Östin *et al.*, 1998; Rampey *et al.*, 2004). The triple *ilr1 iar3 ill2* IAA–amino acid hydrolase mutant displays decreased light-grown hypocotyl elongation (Rampey *et al.*, 2004), decreased lateral root production (Rampey *et al.*, 2004), and decreased root hair elongation (Strader *et al.*, 2010), along with decreased IAA accumulation and increased IAA–Leu and IAA–Ala accumulation (Rampey *et al.*, 2004). The *ilr1 iar3 ill2* mutant does not display elevated IAA–Asp or IAA–Glu levels (Rampey *et al.*, 2004), suggesting that IAA–Asp and IAA–Glu may be involved in IAA degradation, rather than serving as storage forms.

In addition to auxin–amino acid conjugates, high-molecular-weight auxin conjugates, such as IAA–polypeptide conjugates, have been identified. The first evidence of peptide-linked IAA was uncovered in the form of a 3.6 kDa peptide

from *Phaseolus vulgaris* covalently modified with IAA (Bialek and Cohen, 1986). IAA–PROTEIN CONJUGATE1, a protein related to a soybean seed maturation protein, is modified by IAA in a species-specific manner (Walz *et al.*, 2002) and IAA–protein conjugates have been identified in strawberry (Park *et al.*, 2006) and pea (Park *et al.*, 2010). This evidence collectively suggests that IAA–protein conjugates may exist either as a means of auxin storage or as a novel means of post-translationally influencing protein function or stability.

IBA and IBA conjugates

IBA is a naturally occurring auxin precursor in many plant species (Table 1) and, intriguingly, is transported independently of IAA (Rashotte *et al.*, 2003; Strader *et al.*, 2008; Strader and Bartel, 2009, 2011; Růžička *et al.*, 2010). The side chain in the 3 position on the indole ring of IBA contains four, rather than two, carbons. IBA-to-IAA conversion occurs in the peroxisome (Zolman *et al.*, 2000; Strader *et al.*, 2010). Several peroxisomal enzymes, including INDOLE-3-BUTYRIC ACID RESPONSE1 (IBR1; Zolman *et al.*, 2008), IBR3 (Zolman *et al.*, 2007), IBR10 (Zolman *et al.*, 2008), and ENOYL-COA HYDRATASE2 (ECH2; Strader *et al.*, 2011) appear to be dedicated to IBA β-oxidation (Fig. 3). Higher-order mutants deficient in IBA-to-IAA conversion display decreased IAA levels, small cotyledons, decreased root hair expansion, reduced apical hook curvature, decreased lateral root production, smaller root meristems, and delayed development (Zolman *et al.*, 2008; Strader *et al.*, 2010, 2011), which suggests that IBA-derived IAA is important for seedling growth and development in *Arabidopsis*. Additionally, the compound naxillin can be used to stimulate IBA-to-IAA conversion to drive lateral root initiation without affecting general auxin responses (De Rybel *et al.*, 2012), consistent with important roles for IBA-derived auxin in lateral root formation. Coupled with evidence that IAA is converted to IBA via the action of an unidentified IBA synthase (Ludwig-Müller and Epstein, 1994), these data indicate IBA is a significant storage form of auxin integral to plant growth and development.

Similar to IAA, IBA also exists in both amide and ester-linked conjugate forms (reviewed by Woodward and Bartel, 2005; Bajguz and Piotrowska, 2009; Ludwig-Müller, 2011). The purpose of IBA–amino acid conjugates, however, has not yet been elucidated. The wheat hydrolase TaIAR3 displays a high affinity for IBA–Ala and IBA–Gly without hydrolysing IAA–Ala or IAA–Gly (Campanella *et al.*, 2004) and the *Brassica rapa* hydrolases BrIAR3 and BrILL2 display a higher affinity for IPrA–Ala and IBA–Ala than for IAA–Ala (Savić *et al.*, 2009), consistent with the possibility that IBA–amino acid conjugates can serve as a storage form of auxin. In addition, UGT74E2 catalyses the formation of IBA–glucose in response to oxidative stress, and overexpression of *UGT74E2* results in elevated IBA–Glu levels, increased shoot branching, and heightened tolerance to both drought and salt stresses (Tognetti *et al.*, 2010), suggesting that roles for IBA are not confined to the seedling state. Further investigation of the physiological importance and enzymes responsible for the production of IBA conjugates will be necessary to understanding IBA functions.

MeIAA

In addition to conjugation to biomolecules, IAA can be converted to its methyl ester form, MeIAA. Conversion to MeIAA, likely by IAA CARBOXYMETHYLTRANSFERASE1 (IAMT1) in *Arabidopsis* (Zubieta *et al.*, 2003; Qin *et al.*, 2005), results in a non-polar modified auxin that is probably capable of transporter-independent movement (Li *et al.*, 2008; Yang *et al.*, 2008). Indeed, application of MeIAA to the *aux1* transport mutant results in partial rescue of the *aux1* mutant phenotype, thus implying a transporter-independent mechanism of MeIAA movement (Li *et al.*, 2008). However, the MeIAA molecule does not itself possess auxin activity (Li *et al.*, 2008; Yang *et al.*, 2008) and must be converted to IAA through the activities of a family of methyl esterases (Yang *et al.*, 2008).

Overexpression of *IAMT1* results in decreased IAA responsiveness and agravitropic growth, whereas *IAMT1* RNAi lines display leaf epinasty, decreased stature, and decreased fertility (Qin *et al.*, 2005), consistent with roles for IAMT1 in regulating auxin homeostasis. Conversely, *METHYL ESTERASE17* (*MES17*) insertional mutants display decreased sensitivity to MeIAA, remain sensitive to free IAA, and have increased hypocotyl elongation (Yang *et al.*, 2008). Overexpression of *MES17* results in hypersensitivity to MeIAA but not to IAA (Yang *et al.*, 2008). Although key players have been identified to suggest the roles of MeIAA in auxin homeostasis and mutant phenotypes suggest roles for MeIAA-derived auxin in various aspects of development, the extent to which MeIAA contributes to auxin homeostasis is not yet known.
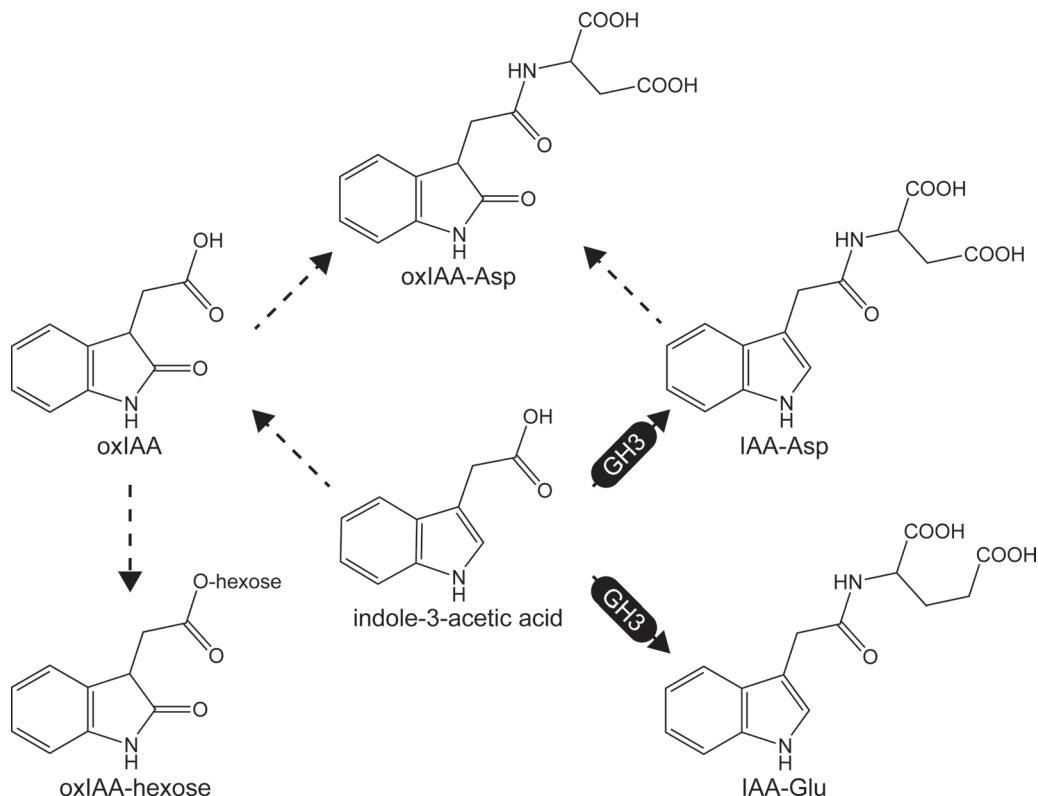
Auxin inactivation pathways

Many auxin storage forms can be converted back to the active auxin IAA. However, some storage forms appear to comprise an IAA inactivation pathway and cannot be converted back to active IAA (Fig. 4). These modified auxin forms are hypothesized to protect against auxin toxicity in the presence of excess auxin (Cohen and Bandurski, 1982; Woodward and Bartel, 2005). Auxin detoxification is carried out via an IAA catabolic pathway for irreversible modification of IAA.

Auxin conjugates

Ester-linked IAA–sugar conjugates can serve roles in both auxin storage and IAA inactivation (reviewed by Woodward and Bartel, 2005). Although IAA–sugar conjugates can be hydrolysed to free IAA (see above), plants overexpressing *UGT84B1* accumulate high levels of 1-*O*-IAGlc, are resistant to exogenous IAA, and are impaired in gravitropism (Jackson *et al.*, 2002), consistent with a potential role for glycosylation in IAA inactivation.

Fig. 4. Potential IAA inactivation pathways. Arrows in pathways for which enzymes have been identified are solid and arrows in pathways that have not been identified are dashed and may be single or multiple steps.

IAA–Asp and IAA–Glu accumulate at very low levels (<3% of all auxin levels) under normal growth conditions (Tam *et al.*, 2000) and rapidly increase upon auxin application (Östin *et al.*, 1998; Barratt *et al.*, 1999). Neither IAA–Asp nor IAA–Glu are well hydrolysed by amidohydrolases (reviewed by Ljung *et al.*, 2002), consistent with roles in IAA inactivation. Most IAA-specific GH3 proteins (across multiple species) are capable of conjugation of IAA to Asp and/or Glu (Staswick *et al.*, 2005; Westfall *et al.*, 2010; Böttcher *et al.*, 2011; Peat *et al.*, 2012), suggesting a bias toward auxin inactivation over creating hydrolysable auxin conjugates. Additionally, overexpression of GH3-6 (*dfl1-D* mutant plants), an amidosynthetase that generates IAA–Asp (Staswick *et al.*, 2005), results in dwarf plants with low auxin phenotypes (Nakazawa *et al.*, 2001).

Oxindole-3-acetic acid (oxIAA)

Inactivation of IAA occurs via the irreversible oxidation to oxIAA, the first precursor in the pathway responsible for catabolism of IAA (reviewed by Woodward and Bartel, 2005). The rapid accumulation of oxIAA after treatment with IAA (Östin *et al.*, 1998) suggests that oxIAA plays an important role in regulating bioactive auxin levels, and oxIAA and oxIAA derivatives have been identified in a number of species (Table 1). Further modification of oxIAA to di-oxIAA, oxIAA–hexose, oxIAA–sugar, oxIAA–Asp, oxIAA–Glu, di-oxIAA–Asp, or (di-)oxIAA–Asp/Glu–sugar are proposed next steps in the oxIAA non-decarboxylative catabolic pathway (Östin *et al.*, 1998; Ljung *et al.*, 2002; Kai *et al.*, 2007). In addition, IAA–Asp can be oxidized to oxIAA–Asp or di-oxIAA–Asp directly in many examined species (reviewed by Ljung *et al.*, 2002; Normanly, 2010). Future research on how IAA and IAA conjugates are oxidized and how the plant recycles these oxIAA molecules will deepen our understanding of auxin metabolism.

Future directions

Regulation of bioactive auxin levels is clearly complex and many questions about auxin biosynthesis and modified auxin forms remain unanswered. Chief among these questions is how the plant integrates and regulates local auxin biosynthesis, the generation of storage forms, release from storage forms, and transport to contribute to the generation and maintenance of auxin gradients. Understanding transcriptional regulation of auxin biosynthesis genes and the post-translational control of activity may provide some tools to answer these questions.

Acknowledgements

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