Auxin biosynthesis and storage forms

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Received 15 January 2013; Revised 26 February 2013; Accepted 27 February 2013

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 (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), indoleacetamide (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
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., 2001; 2002; Sugawara et al., 2009). 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 nitrolyses (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 monoxygenases converts IPyA to IAA. Mutation of TAA1/SHADE AVOIDANCE3/WEAK ETHYLENE INSENSITIVE8/TRANSPORT INHIBITOR RESPONSE2 (TAA1/SAY3/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.

<table>
<thead>
<tr>
<th>Modified auxin form</th>
<th>Purpose</th>
<th>Species in which identified (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Chloroindole acetic acid (4-Cl-IAA)</td>
<td>Active auxin</td>
<td><em>Lens culinaris</em> (Engvild et al., 1981); <em>Lathyrus latifolius</em> (Engvild et al., 1980); <em>Lathyrus maritimus</em> (Engvild et al., 1981); <em>Lathyrus odoratus</em> (Engvild et al., 1981); <em>Lathyrus sativus</em> (Engvild et al., 1981); <em>Pinus sylvestris</em> (Ernstsen and Sandberg, 1986); <em>Pisum sativum</em> (Schneider et al., 1985; Barkawi et al., 2008); <em>Vicia amurensis</em> (Engvild et al., 1981); <em>Vicia faba</em> (Engvild et al., 1981); <em>Vicia sativa</em> (Engvild et al., 1981)</td>
</tr>
<tr>
<td>Phenylacetic acid (PAA)</td>
<td>Active auxin</td>
<td><em>Helianthus annuus</em> (Wightman and Rauthan, 1974; Wightman and Lighty, 1982); <em>Hordeum vulgare</em> (Wightman and Lighty, 1982); <em>Lycopersicon esculentum</em> (Wightman and Rauthan, 1974; Wightman and Lighty, 1982); <em>Phaseolus</em> (Okamoto et al., 1967); <em>Phaseolus vulgaris</em> (Okamoto et al., 1967); <em>Pisum sativum</em> (Wightman and Lighty, 1982); <em>Porphyra tenera</em> (Fries and Iwasaki, 1976); <em>Triticum aestivum</em> (Wightman and Lighty, 1982); <em>Tropaeolum majus</em> (Ludwig-Müller and Cohen, 2002); <em>Undaria pinnatifida</em> (Abe et al., 1974); <em>Zea mays</em> (Wightman and Lighty, 1982)</td>
</tr>
<tr>
<td>Indole-3-acetaldoxime (IAOx)</td>
<td>Auxin precursor</td>
<td><em>Arabidopsis thaliana</em> (Sugavara et al., 2009; Novák et al., 2012); <em>Brassica campestris</em> spp. <em>pekinesis</em> (Ludwig-Müller and Hilgenberg, 1988)</td>
</tr>
<tr>
<td>Indole-3-acetonitrile (IAN)</td>
<td>Auxin precursor</td>
<td><em>Arabidopsis thaliana</em> (Norman et al., 1993; Ilé et al., 1996; Zhao et al., 2002; Sugawara et al., 2009; Novák et al., 2012); <em>Brassica campestris</em> (Jones et al., 1952); <em>Brassica juncea</em> (Pedras et al., 2002); <em>Zea mays</em> (Bak et al., 1998; Park et al., 2003)</td>
</tr>
<tr>
<td>Indoleacetonitrile (IAM)</td>
<td>Auxin precursor</td>
<td><em>Arabidopsis thaliana</em> (Pollmann et al., 2002; Sugawara et al., 2009; Novák et al., 2012); <em>Cucurbita maxima</em> (Rajagopal et al., 1994); <em>Nicotiana tabacum</em> (Sugawara et al., 2009); <em>Oryza sativa</em> (Sugawara et al., 2009); <em>Zea mays</em> (Sugawara et al., 2009)</td>
</tr>
<tr>
<td>Indole-3-pyruvic acid (IPyA)</td>
<td>Auxin precursor</td>
<td><em>Arabidopsis thaliana</em> (Tam and Norman, 1998; Mashiguchi et al., 2011; Won et al., 2011; Liu et al., 2012; Novák et al., 2012); <em>Lycopersicon esculentum</em> (Cooney and Nonhebel, 1989; Liu et al., 2012); <em>Pisum sativum</em> nodules (Badenoch-Jones et al., 1984)</td>
</tr>
<tr>
<td>Indole-3-acetaldehyde (IAA)</td>
<td>Auxin precursor</td>
<td><em>Arabidopsis thaliana</em> (Mashiguchi et al., 2011; Novák et al., 2012); <em>Pisum sativum</em> (Quittenden et al., 2009); <em>Pinus sylvestris</em> (Ernstsen and Sandberg, 1986)</td>
</tr>
<tr>
<td>Indole-3-butyric acid (IBA)</td>
<td>Precursor/storage</td>
<td><em>Arabidopsis thaliana</em> (Ludwig-Müller et al., 1993; Strader et al., 2010; Liu et al., 2012); <em>Daucus carota</em> (Epstein et al., 1991); <em>Medicago truncatula</em> (Campanella et al., 2008); <em>Nicotiana tabacum</em> (Sutter and Cohen, 1992); <em>Pisum sativum</em> nodules (Badenoch-Jones et al., 1984); <em>Solanum tuberosum</em> (Bloomer, 1954); <em>Tropaeolum majus</em> (Ludwig-Müller and Cohen, 2002); <em>Zea mays</em> (Epstein et al., 1989; Barkawi et al., 2008)</td>
</tr>
<tr>
<td>IBA-glucose</td>
<td>Precursor/storage</td>
<td><em>Arabidopsis thaliana</em> (Tognetti et al., 2013)</td>
</tr>
<tr>
<td>Methyl-IAA(MelAA)</td>
<td>Precursor/storage</td>
<td><em>Arabidopsis thaliana</em> (Narasimhan et al., 2003); <em>Vitis vinifera</em> (Gouthu et al., 2013)</td>
</tr>
<tr>
<td>IAA-glucose/IAA-glycan</td>
<td>Storage</td>
<td><em>Arabidopsis thaliana</em> (Tam et al., 2000); <em>Avena sativa</em> (reviewed by Cohen and Bandurski, 1982); legumes (Jakubowska and Kowalczyk, 2004); <em>Pinus pinea</em> (Rov and Gottlieb, 1980); Tobacco (Sitbon et al., 1993); <em>Zea mays</em> (reviewed by Cohen and Bandurski, 1982)</td>
</tr>
<tr>
<td>IAA-myco-inositol</td>
<td>Storage</td>
<td><em>Arabidopsis thaliana</em> (Luo et al., 2011); <em>Oryza sativa</em> (Hall, 1980); <em>Zea mays</em> (Nicholls, 1967; Chisnell, 1984)</td>
</tr>
<tr>
<td>IAA–Asp–glucose</td>
<td>Storage/inactivation</td>
<td><em>Oryza sativa</em> (Kai et al., 2007)</td>
</tr>
<tr>
<td>IAA–Glu–glucose</td>
<td>Storage/inactivation</td>
<td><em>Oryza sativa</em> (Kai et al., 2007)</td>
</tr>
<tr>
<td>IAA–Ala</td>
<td>Storage</td>
<td><em>Arabidopsis thaliana</em> (Kowalczyk and Sandberg, 2001); <em>Oryza sativa</em> (Kojima et al., 2009); <em>Pisidia abies</em> (Östín et al., 1992)</td>
</tr>
<tr>
<td>IAA–Asp</td>
<td>Inactivation</td>
<td><em>Arabidopsis thaliana</em> (Östín et al., 1991; Tam et al., 2000; Kowalczyk and Sandberg, 2001; Novák et al., 2012); <em>Ceratopteris richardi</em> (Sztein et al., 1999); <em>Citrus sinesis</em> (Chamarro et al., 2001); <em>Cucumis sativus</em> (Purves and Hol lenberg, 1982; Sonner and Purves, 1985); <em>Dilbergia dolichopetala</em> (Monteiro et al., 1988); <em>Daucus carota</em> (Sasaki et al., 1994); <em>Douglas fir</em> (Chiwocha and van Aerderkas, 2002); <em>Glycine max</em> (Cohen, 1982); <em>Heracleum lanicinatum</em> (Cohen and Ernstsen, 1991); <em>Oryza sativa</em> (Kojima et al., 2009); <em>Pinus pinea</em> (Rov and Gottlieb, 1980); <em>Pisum sativum</em> (Andreatte and Good, 1955); <em>Populus tremuloides</em> (Tuominen et al., 1994); Tobacco (Sitbon et al., 1993); <em>Triticum aestivum</em> (Martens and Franckenbeher, 1994); <em>Vigna radiata</em> (Nocini and Heuser, 1988)</td>
</tr>
<tr>
<td>IAA–Glu</td>
<td>Inactivation</td>
<td><em>Arabidopsis thaliana</em> (Östín et al., 1998; Tam et al., 2000; Kowalczyk and Sandberg, 2001; Novák et al., 2012); <em>Citrus sinesis</em> (Chamarro et al., 2001); <em>Cucumis sativus</em> (Purves and Hol lenberg, 1982; Sonner and Purves, 1985); Tobacco (Sitbon et al., 1993)</td>
</tr>
<tr>
<td>IAA–Gly</td>
<td>Unknown</td>
<td><em>Helleborus niger</em> (Penčık et al., 2009)</td>
</tr>
<tr>
<td>IAA–Gln</td>
<td>Unknown</td>
<td><em>Arabidopsis thaliana</em> (Barratt et al., 1999)</td>
</tr>
<tr>
<td>IAA–Leu</td>
<td>Storage</td>
<td><em>Arabidopsis thaliana</em> (Kowalczyk and Sandberg, 2001); <em>Ceratopteris richardi</em> (Sztein et al., 1999); <em>Nitella</em> (Sztein et al., 2000); <em>Physcomitrella patens</em> (Ludwig-Müller et al., 2009)</td>
</tr>
<tr>
<td>IAA–Phe</td>
<td>Unknown</td>
<td>Postulated: <em>Arabidopsis thaliana</em> (Kai et al., 2007); <em>Funaria hydrometrica</em> (Sztein et al., 1999); <em>Helleborus niger</em> (Penčık et al., 2009); <em>Orthotrichum lyellii</em> (Sztein et al., 1999); <em>Oryza sativa</em> (Kojima et al., 2009)</td>
</tr>
<tr>
<td>IAA–Trp</td>
<td>Antagonist</td>
<td><em>Arabidopsis thaliana</em> (Staswick, 2009)</td>
</tr>
</tbody>
</table>
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 overexpression 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,

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**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 *taal* 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 inflorescencel* (*spil*), 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 to 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 (Szersen 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)-glucosyl-oxoglutarate: 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; Le Clerc et al., 2002; Rampey et al., 2004), suggesting that 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 (Öst in et al., 1998; Le Clerc 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 hydrolyses 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 HAGEN 3 (GH3) family of acyl amid 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; Le Clerc 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; Le Clerc et al., 2004; Rampey et al., 2006; Rampey et al., 2013). Interestingly, examined Arabidopsis conjugate hydrolyses display a high affinity for IAA–Leu and IAA–Ala and a low affinity for other IAA–amino acid conjugates (Le Clerc 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 (Öst in et al., 1998; Rampey et al., 2004). The triple ilrl 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 ilrl 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; Baguz 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 methylester 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) insertion 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.
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 (dfilD 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

We thank Lauren Gunther, Eric Hamilton, Marta Michniewicz-Paciorek, Julie Thole, and Corey Westfall for critical comments on the manuscript and helpful discussion. This work was supported by the National Institutes of Health (R00 GM089987-03 to L.C.S.) and the National
Science Foundation Graduate Research Fellowship Program (2011101911 to D.A.K.).

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