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REVIEW PAPER

Roles for IBA-derived auxin in plant development

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Abstract

The plant hormone auxin is a central regulator of plant growth and development. Because auxin plays critical roles in cell division and cell expansion, plants use a number of cellular mechanisms to regulate auxin levels and response. Among these mechanisms is regulated input from the auxin precursor indole-3-butyric acid (IBA) toward the pool of active auxin [indole-3-acetic acid (IAA)]. In this review, we cover the mechanisms of IBA transport and conversion, and discuss specific roles for IBA-derived auxin in driving certain developmental events. We further discuss multiple open questions remaining for the IBA field.

Keywords: Auxin, IBA, plant development, root development, shoot development.

Introduction

The term auxin is derived from the Greek word 'auxein', which means 'to grow'. Because auxin is a potent regulator of cell division, cell expansion, and cell differentiation (reviewed in Enders and Strader, 2015), it is involved in nearly every aspect of plant development. Therefore, regulation of auxin levels and response is critical for normal plant form and function. Plants use a number of cellular mechanisms to regulate auxin levels and response, including transport, *de novo* biosynthesis, and management of inputs from various auxin precursors and storage forms (reviewed in Korasick *et al.*, 2013).

The predominant active auxin, indole-3-acetic acid (IAA), is transported long distances through plants via the combined action of distinct families of transporters (reviewed in Zazímalová *et al.*, 2010). The AUX1/LAX family of transporters act as IAA uptake carriers, whereas members of the ABCB and PIN family of transporters facilitate IAA efflux. Together, these transporters facilitate long-distance, directional transport of IAA through the plant to regulate numerous aspects of plant development.

The main auxin biosynthesis pathway uses the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1) and YUCCA family of enzymes (reviewed in Zhao, 2012). In this pathway, tryptophan is converted to indole-3-pyruvic acid (IPyA) through the activity of the TAA1 family of aminotransferase enzymes. The YUCCA family of flavin monooxygenase-like enzymes then converts IPyA to IAA. Conversion of IPyA to IAA is the rate-limiting step in this process; ovexpression of YUCCA family members results in elevated auxin levels. Further, tissue-specific expression of various YUCCA family members allows for *de novo* auxin biosynthesis to drive specific aspects of plant development (reviewed in Zhao, 2010).

In addition to biosynthesis of IAA via the IPyA pathway, the pool of active auxin can be modulated by inputs from additional storage forms and precursors, such as IAA conjugates and indole-3-butyric acid (IBA). These auxin inputs can drive distinct aspects of plant development (reviewed in Korasick *et al.*, 2013). In this review,

Abbreviations: ABCG36, ATP-BINDING CASSETTE G36; ECH2, ENOYL-COA HYDRATASE2; IAA, indole-3-actetic acid; IBA, indole-3-butyric acid;IBR1, IBA RESPONSE1; IBR3, IBA RESPONSE3; IBR10, IBA RESPONSE10; IPyA, indole-3-pyruvic acid.

we focus on specific roles for IBA-derived auxin in plant development.

IBA conversion and transport mechanisms

For decades, IBA was described as a 'synthetic auxin' that elicited auxin-like effects such as root initiation, stem bending, and leaf epinasty (Zimmerman and Wilcoxon, 1935). Indeed, IBA is the active ingredient in plant propagation media, such as Rootone®, used to induce adventitious rooting in stem cuttings. Later studies have demonstrated that IBA is an endogenous compound in a variety of examined plant species (reviewed in Korasick *et al.*, 2013).

The side chain in the 3 position on the indole ring of IBA has four carbons, as opposed to the two-carbon side chain of IAA (Fig. 1); this lengthened side chain results in a molecule that is probably unable to adopt a conformation for binding into the TIR1–Aux/IAA co-receptor pocket (Uzunova *et al.*, 2016). Indeed, surface plasmon resonance analysis suggests that IBA has no measured binding activity (Uzunova *et al.*, 2016), consistent with the genetic evidence that IBA activity is through its conversion to IAA (reviewed in Strader and Bartel, 2011).

IBA is likely to be converted to IAA in a process similar to fatty acid β-oxidation. Many plants convert IBA into IAA (reviewed in Epstein and Ludwig-Müller, 1993), including Arabidopsis (Strader *et al.*, 2010), hazelnut (Kreiser *et al.*, 2016), and elm (Kreiser *et al.*, 2016). In Arabidopsis, this process is peroxisome dependent (Strader *et al.*, 2010), and multiple mutants defective in peroxisome biogenesis and peroxisomal enzymes have been identified for IBA resistance while retaining sensitivity to the active auxin IAA (reviewed in Hu *et al.*, 2012). The PEROXISOMAL TRANSPORTER1/COMATOSE/ABCD1 (PXA1/CTS/ABCD1) transporter is likely to move IBA into the peroxisome for metabolism into active auxin (reviewed in Strader and Bartel, 2011; Michniewicz *et al.*, 2014). Whereas some peroxisomal enzymes, such as the PED1 3-ketoacyl-CoA thiolase, are likely to act in both fatty acid (Hayashi *et al.*, 1998) and IBA β-oxidation (Zolman *et al.*, 2000), other peroxisome enzymes appear to be specific to IBA β-oxidation. Specifically, the predicted short-chain dehydrogenase/reductase INDOLE-3-BUTYRIC ACID RESPONSE1 (IBR1) (Zolman *et al.*, 2008), the acyl-CoA dehydrogenase/oxidase-like IBR3 (Zolman *et al.*, 2007), the predicted enoyl-CoA hydratase IBR10 (Zolman *et al.*, 2008), and the predicted enoyl-CoA hydratase ENOYL-COA HYDRATASE2 (ECH2) (Strader *et al.*, 2011) are enzymes that may act solely in the conversion of the auxin precursor IBA to active IAA.

Similar to mechanisms regulating IAA levels (reviewed in Zazímalová *et al.*, 2010; Korasick *et al.*, 2013), mechanisms to regulate IBA levels include formation of IBA conjugates and IBA transport (Fig. 2). IBA exists in both amide- and ester-linked amide forms (reviewed in Woodward and Bartel, 2005; Bajguz and Piotrowska, 2009; Ludwig-Müller, 2011). Whereas some members of the Arabidopsis GH3 amino acid synthetase family, including GH3.4, GH3.5, GH3.6, and GH3.17, display adenylation activity with both IBA and IAA (Staswick *et al.*, 2005), enzymes specifically conjugating IBA to amino acids have not yet been reported. The hydrolases *Triticium aestivum* TaIAR3 (Campanella *et al.*, 2004), *Brassica rapa* BrIAR3 (Savić *et al.*, 2009), and *B. rapa* BrILL2 (Savić *et al.*, 2009) display higher affinity for IBA–amino acid conjugates than for IAA–amino acid conjugates, consistent with the possibility that IBA may be stored in amino acid conjugate form. Additionally, IBA is likely to be stored as conjugates to sugar. The enzymes UGT74E2 (Tognetti *et al.*, 2010) and UGT75D1 (Zhang *et al.*, 2016) promote the formation of IBA–glucose; overexpression of either *UGT74E2* (Tognetti *et al.*, 2010) or *UGT75D1* (Zhang *et al.*, 2016) results in elevated IBA–glucose levels. For example, determination that GH3.1, GH3.2, GH3.5, and GH3.17 conjugated amino acids to IAA led to examination of the respective knockout lines to determine the importance of IAA–amino acid

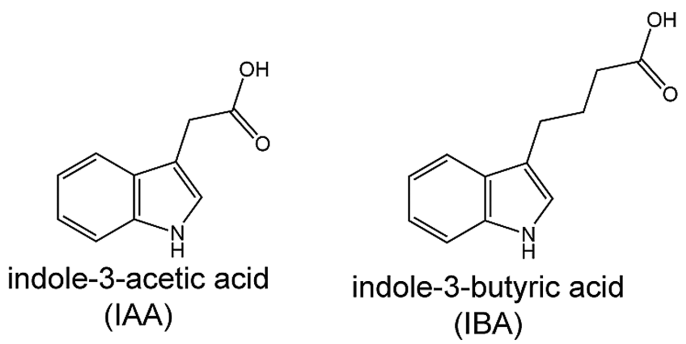

Fig. 1. IAA and IBA. The side chain in the 3 position on the indole ring of IBA has four carbons, as opposed to the two-carbon side chain of IAA.

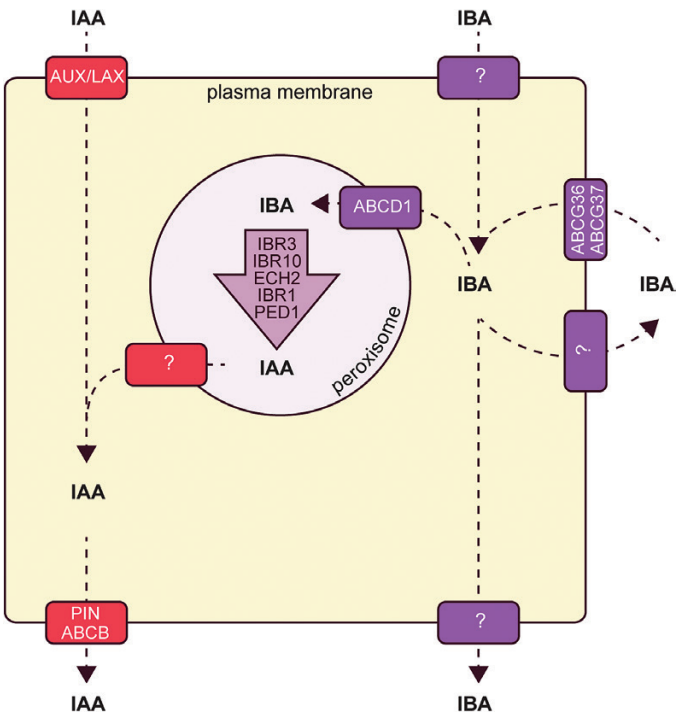

Fig. 2. Cellular model of IAA and IBA transport. IAA and IBA use distinct transporters for movement into and out of cells and into and out of the peroxisome.

conjugates in regulating IAA homeostasis *in planta* (Staswick *et al.*, 2005). Similarly, identification of ILR1, IAR3, ILL1, and ILL2 as IAA hydrolases was instrumental in understanding how IAA conjugates are cleaved and contribute to the free IAA pool *in planta* (LeClere *et al.*, 2002). Identification of the IBA-specific synthetases and hydrolases will similarly inform our understanding of how plants regulate their pools of free IBA.

Auxin distribution throughout the body of the plant is mediated by cellular auxin transport to achieve the long-distance movement of IAA. Similarly, IBA and/or IBA conjugates are thought to move long distances through the plant (reviewed in Strader and Bartel, 2011; Michniewicz *et al.*, 2014). Tracking of radiolabel in plants treated with [^{3}H]IBA allowed for the acropetal and basipetal movement of signal in *Cleopatra mandarin* midrib sections (Epstein and Sagee, 1992), and in various Arabidopsis tissues (Ludwig-Müller *et al.*, 1995*b*; Rashotte *et al.*, 2003). However, these radiotracer studies are complicated by IBA metabolism to IAA and to IBA conjugates because the identity of the moving molecule tracked is unknown; it is only certain that it is derived from the radiolabeled precursor. For example, later studies determined that most of the radioactive transported material was not the original [^{3}H]IBA, but rather [^{3}H]IAA derived from [^{3}H]IBA in an Arabidopsis columella cell transport assay (Růžička *et al.*, 2010). Likewise, in a study using heavy IBA feeding followed by analysis by MS, [$^{13}C_1$]IAA, ester–[$^{13}C_1$]IBA conjugates, or amide–[$^{13}C_1$]IBA conjugates derived from [$^{13}C_1$]IBA were predominantly transported through Arabidopsis hypocotyls, rather than the supplied [$^{13}C_1$]IBA itself (Liu *et al.*, 2012*a*). These studies are consistent with IBA conjugates being the major form of transported IBA.

Although IBA may be predominantly transported in the form of conjugates, uptake of the IBA molecule itself is a saturable process (Ludwig-Müller *et al.*, 1995*b*; Rashotte *et al.*, 2003), which suggests that IBA uptake into plant cells is carrier mediated. Further, examined transporters of IAA, including AUX1, PIN2, PIN7, ABCB1, and ABCB19, do not appear to facilitate the transport of IBA (reviewed in Michniewicz *et al.*, 2014), suggesting that other carriers act in the transport of IBA.

Several IBA transporters have been identified, although there are likely to be additional carriers that have yet to be identified (reviewed in Strader and Bartel, 2011; Michniewicz *et al.*, 2014). IBA efflux is promoted by ATP-BINDING CASSETTE G36/PLEIOTROPIC DRUG RESISTANCE 8/PENETRATION 3 (ABCG36/PDR8/PEN3) (Strader and Bartel, 2009), ABCG37/PDR9/PIS1 (Strader *et al.*, 2008; Růžička *et al.*, 2010), and possibly by additional members of the PDR subclade of the ABCG family (Michniewicz *et al.*, 2014). Mutants defective in ABCG29 (Michniewicz *et al.*, 2014), ABCG33 (Michniewicz *et al.*, 2014), ABCG36 (Strader and Bartel, 2009), or ABCG37 (Strader *et al.*, 2008; Růžička *et al.*, 2010) display increased sensitivity to the auxin precursor IBA and retain wild-type sensitivity to the active auxin IAA. Consistent with the IBA hypersensitivity displayed, root tips excised from mutants defective in either ABCG36 (Strader and Bartel, 2009) or ABCG37 (Strader *et al.*, 2008; Růžička *et al.*, 2010) hyperaccumulate [^{3}H]IBA, but not [^{3}H]IAA. The IBA hypersensitivity combined with the hyperaccumulation of [^{3}H]IBA in these mutants is consistent with roles for ABCG36 and ABCG37 in effluxing IBA from the root.

Although ABCG36 and ABCG37 appear to transport the auxin precursor IBA, but not active IAA, they probably transport additional substrates, as is common for members of the PLEIOTROPIC DRUG RESISTANCE family of transporters. In particular, ABCG37 is likely to transport the synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) (Ito and Gray, 2006; Strader *et al.*, 2008; Růžička *et al.*, 2010), the synthetic auxin precursor 2,4-dichlorophenoxy butyric acid (2,4-DB) (Strader *et al.*, 2008; Růžička *et al.*, 2010), 1-*N*-naphthylphthalamic acid (NPA) (Ito and Gray, 2006), the auxin breakdown product oxIAA-Hex (Peer *et al.*, 2013), and non-auxinic phenolic coumarin compounds (Fourcroy *et al.*, 2014). Further, ABCG36 probably transports the synthetic auxin precursor 2,4-DB (Strader and Bartel, 2009), oxIAA-Hex (Peer *et al.*, 2013), cadmium and cadmium conjugates (Kim *et al.*, 2007), coumarin (Fourcroy *et al.*, 2014), and a precursor to 4-*O*-β-D-glucosyl-indol-3-yl formamide (Lu *et al.*, 2015). Clearly, these transporters have roles outside of their regulation of cellular IBA levels.

Analysis of mutants with altered IBA to IAA conversion, altered management of storage forms, and altered transport have revealed roles for IBA-derived auxin in multiple specific developmental processes.

IBA-derived auxin drives aspects of root development

IBA-derived auxin has strong roles in various aspects of root development, including regulation of root apical meristem size, root hair elongation, lateral root development, and formation of adventitious roots. Mutations disrupting IBA metabolism and chemicals that affect IBA metabolism result in multiple root phenotypes, revealing specific roles for IBA-derived auxin in these processes.

The root apical meristem is a collection of undifferentiated cells at the root tip region that display indeterminate growth. The balanced cell division and differentiation in this tissue gives rise to new root tissue, while maintaining a small group of cells that undergo occasional cell division, called the quiescent center. Maintaining proper auxin levels and establishment of an auxin gradient in these tissues is essential to establish root patterning and meristem formation (reviewed in Iyer-Pascuzzi and Benfey, 2009). The *ech2 ibr10* double mutant, defective in β-oxidation enzymes required for IBA to IAA conversion, displays decreased *DR5-GUS* (β-glucuronidase) activity in root tips. Further the *ech2 ibr1 ibr3 ibr10* quadruple mutant, defective in multiple IBA conversion enzymes, has reduced free IAA levels in the root tip and displays a reduced meristem size (Strader *et al.*, 2011), consistent with IBA conversion to IAA acting as a major input into the auxin pool in this tissue.

Root hairs are long tubular outgrowths protruding from the epidermal cell layer of roots that aid in nutrient and

water acquisition by increasing the root surface area. Auxin affects the positioning of the root hair outgrowth site and promotes root hair elongation (reviewed in Honkanen and Dolan, 2016). Multiple lines of evidence suggest that IBA-derived auxin promotes root hair expansion. First, mutants defective in the ABCG36 or ABCG37 transporters, which act to efflux IBA out of the root, display longer root hairs (Strader and Bartel, 2009; Růžička *et al.*, 2010). Further, blocking IBA to IAA conversion suppresses the long root hair phenotype observed in *abcg36* mutants (Strader *et al.*, 2010), suggesting that elevated IBA-derived IAA levels in the *abcg36* mutant cause the elongated root hair phenotype. In addition, mutants defective in IBA conversion enzymes display root hairs that can be rescued with exogenous auxin (Strader *et al.*, 2010, 2011), consistent with the possibility that blocking IBA conversion can result in decreased auxin levels in root epidermal cells.

Lateral roots are post-embryonic organs originating from the primary root. The number and positioning of lateral roots is critical to establish the ideal root system for adaptation to local environments (reviewed in Rellán-Álvarez *et al.*, 2016). Auxin drives both lateral root initiation and lateral root emergence (reviewed in Laskowski and Ten Tusscher, 2017). Mutants defective in IBA conversion enzymes display greatly decreased production of lateral roots (Strader *et al.*, 2011). Further, treatment of seedlings with the compound naxillin results in increased lateral root production with limited effects on primary root elongation (De Rybel *et al.*, 2012). Naxillin activity requires an intact IBA to IAA conversion pathway (De Rybel *et al.*, 2012), consistent with the possibility that naxillin promotes IBA conversion to active IAA. Furthermore, IBA to IAA conversion occurs in the lateral root cap and contributes to the priming of lateral root pre-branch sites by setting up the amplitude and frequency of auxin oscillations through the root (De Rybel *et al.*, 2012; Xuan *et al.*, 2015). These oscillations are necessary to establish the lateral root pre-branch sites (Xuan *et al.*, 2015), pointing to an important role for IBA-derived auxin in driving lateral root development (Fig. 3). In addition to roles for IBA-derived auxin originating in the lateral root cap, IBA to IAA conversion in the lateral root primordia themselves is also likely to contribute to lateral root development. In particular, mutants defective in IBA conversion display decreased DR5-GUS staining in lateral root primordia, even after the addition of active auxins (Strader *et al.*, 2011), suggesting that IBA to IAA conversion acts to reinforce auxin responses in these tissues.

Adventitious roots are similar to lateral roots in many regards, but are defined by their origination from aerial tissues, such as stems or leaves. Adventitious root formation is often a part of adaptive responses to stress, and shares both common and distinct regulatory mechanisms to lateral root formation (reviewed in Bellini *et al.*, 2014). Zimmerman and Wilcoxin first reported that IBA could stimulate adventitious rooting in cuttings of several species in 1935 (reviewed in Preece, 2003). Throughout the 1930s, IBA arose as the compound of choice for horticulturalists to induce adventitious roots on stem cuttings for plant propagation and is the active ingredient in many modern rooting compounds, such as Rootone® or Hormodin®. In Arabidopsis, IBA promotion of adventitious rooting requires its conversion to IAA; the *ech2 ibr10* mutant, defective in IBA to IAA conversion enzymes, fails to produce adventitious roots in response to IBA (Veloccia *et al.*, 2016). Indeed, cuttings from elm cultivars displaying higher levels of IBA to IAA conversion also display relatively high rates of adventitious rooting in response to rooting compounds (Kreiser *et al.*, 2016), suggesting that IBA conversion may be critical for plant propagation in certain species.

IBA-derived auxin drives aspects of shoot development

In addition to its varied roles in root development, IBA-derived auxin plays distinct roles in shoot development, with particular roles in cotyledon expansion and apical hook formation.

Altering IBA homeostasis or IBA conversion to IAA has striking effects on cotyledon expansion. For example, mutants defective in the ABCG36 transporter display larger cotyledons than the wild type (Strader and Bartel, 2009), consistent with its role in IBA efflux and suggesting that auxin levels are elevated in the cotyledons of this mutant. Combining the *abcg36* mutation with mutations in IBA conversion enzymes suppresses this large cotyledon phenotype (Strader *et al.*, 2010), suggesting that IBA-derived IAA, rather than IBA itself, drives the increased cotyledon expansion observed in the *abcg36* mutant. Further, a strong genetic block in IBA to IAA conversion results in dramatically reduced cotyledon expansion, concomitant with decreased cotyledon epidermal cell size (Strader *et al.*, 2011). Likewise, overexpression of the IBA glycosylating enzyme UGT75D1 results in decreased

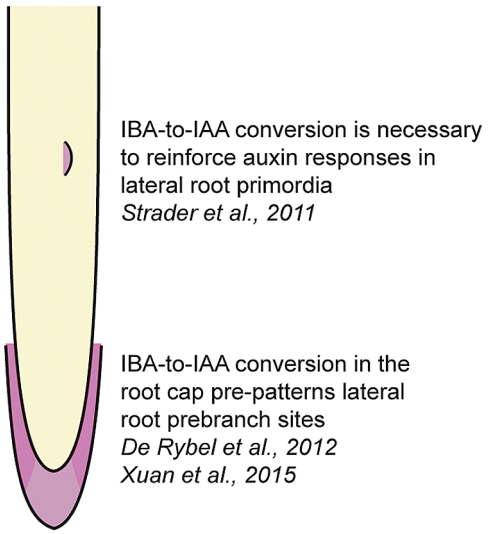

Fig. 3. IBA-derived auxin drives lateral root development. In addition to roles for IBA-derived auxin originating in the lateral root cap (De Rybel *et al.*, 2012; Xuan *et al.*, 2015), IBA to IAA conversion in the lateral root primordia themselves also likely contributes to lateral root development (Strader *et al.*, 2011). (This figure is available in colour at *JXB* online.)

cotyledon size (Zhang *et al.*, 2016), consistent with decreased contributions to the auxin pool in these overexpression lines. IBA-derived auxin also appears to play a role in compensated cell enlargement (CCE), a phenomenon that allows for increased cell expansion to occur when cell numbers are limited in order to achieve a 'normal' organ size in plants. Mutants defective in ECH2, an enzyme required for IBA to IAA conversion, are defective in CCE in cotyledons (Katano *et al.*, 2016), suggesting that IBA-derived auxin is important for driving cotyledon cell expansion not only during normal development but also under conditions where cell numbers are limiting.

Auxin is a critical driver of pavement cell lobing (reviewed in Pan *et al.*, 2015). Auxin-driven intercalary growth results in lobes and indentations among neighboring cotyledon and leaf epidermal cells. The *ech2 ibr1 ibr3 ibr10* mutant, defective in IBA to IAA conversion, displays a strong defect in pavement cell lobing, with decreased lobe indentation and decreased pavement cell size, in addition to decreased cotyledon size (Strader *et al.*, 2011), consistent with IBA-derived auxin contributing to the lobing process. Additionally, overexpressing the IBA glycosylating enzyme UGT75D1 results in small cotyledon pavement cells (Zhang *et al.*, 2016) that appear to display decreased lobing. Further research will be required to understand how IBA to IAA conversion is regulated to affect pavement cell lobing.

Contributions to the auxin pool by IBA in shoot tissues are not limited to the cotyledons. In addition, IBA-derived auxin affects hypocotyls (Strader *et al.*, 2011), rosettes (Tognetti *et al.*, 2010), and shoot branching (Tognetti *et al.*, 2010). Dark-grown dicotyledonous seedlings display an apical hook and closed cotyledons as a mechanism to avoid damaging the shoot apical meristem during soil emergence (Goeschl *et al.*, 1966; Guzmán and Ecker, 1990). Mutant seedlings defective in IBA to IAA conversion enzymes display decreased apical hook curvature and also fail to maintain an apical hook for the same length of time as the wild type (Strader *et al.*, 2011). Further, DR5-GUS activity on the inner side of the apical hook is less in IBA conversion mutants than in the wild type (Strader *et al.*, 2011), suggesting that auxin responses in the region are lessened in the absence of auxin pool contributions by IBA. IBA-derived auxin appears important for apical hook formation and maintenance. In addition to playing roles in apical hook formation in dark-grown hypocotyls, IBA-derived auxin plays roles in high temperature responses in light-grown hypocotyls. Growth of seedlings at high temperature results in elevated auxin levels and increased hypocotyl elongation (Gray *et al.*, 1998). Under high temperature conditions, IBA conversion mutants display decreased hypocotyl elongation compared with the wild type (Strader *et al.*, 2011), suggesting that IBA to IAA conversion contributes to this process. Altering IBA homeostasis affects both shoot branching and rosette shape. In Arabidopsis, overexpression of *UGT74E2*, encoding an enzyme promoting IBA glycosylation, results in a compact rosette shape comprised of leaves with short petioles and dark-green leaves (Tognetti *et al.*, 2010). Further, these *UGT74E2* overexpression lines display decreased stature and increased numbers of apical branches (Tognetti *et al.*, 2010), suggesting roles for IBA homeostasis in regulating aspects of shoot architecture. The strong effects of IBA-derived auxin on multiple aspects of plant growth and development suggest that IBA is an important contributor to the auxin pool.

Considering developmental roles of IBA in both aerial and root tissue over the plant life cycle, a unifying theme is that IBA acts as an auxin reserve within the plant. Conversion of this auxin reserve pool is crucial for a variety of important developmental events, as enumerated above, but these processes may not represent the totality of IBA-dependent development. Although we understand the importance of IBA to IAA conversion in many facets of development, other questions about roles for IBA-derived auxin are sure to refine further our understanding of this important auxin. These open questions in IBA biology are expanded upon in the next section.

Open questions

Why do plants need IBA-derived auxin in addition to other pathways?

Auxin regulates a wide spectrum of developmental processes. The local distribution of auxin, modified by its transport or its metabolism, is critical for control of these developmental processes. In Arabidopsis, altering IBA contributions to the auxin pool results in a distinct set of developmental and growth effects, as detailed above, suggesting specific roles for this pathway in modulating auxin levels during seedling development and under stress conditions. Further, genes encoding enzymes required for IBA to IAA conversion are conserved throughout all examined plants (Strader *et al.*, 2011), suggesting that IBA contributions to the auxin pool may also be conserved. Thus, it seems likely that IBA conversion plays roles in plant growth and development that cannot be easily compensated for by other pathways; otherwise, we would expect this pathway to be lost, at least in some species.

Difficulties in detecting IBA

The auxin precursor IBA has been identified as an endogenous compound in numerous plant species, including various monocots and dicots (reviewed in Korasick *et al.*, 2013). However, multiple labs have had difficulty identifying IBA (personal communication), including a report that questioned its presence when it was undetected by GC-MS in samples from Arabidopsis, *Populus*, and wheat (Novák *et al.*, 2012). Further, IBA concentrations are often reported to be at lower levels than IAA concentrations (Sutter and Cohen, 1992; Ludwig-Müller *et al.*, 1993, 1997; Liu *et al.*, 2012*b*), and detection of IBA in maize kernels varies by variety examined (Epstein *et al.*, 1989; Ludwig-Müller *et al.*, 1993, 1997). However, IBA has been detected in Arabidopsis by MS (Ludwig-Müller *et al.*, 1993; Strader *et al.*, 2010; Liu *et al.*, 2012*b*). Further, mutants defective in enzymes required for IBA to IAA conversion display developmental phenotypes consistent with an auxin deficiency and decreased levels of

free IAA (Strader *et al.*, 2011), in agreement with endogenous IBA contributing to the auxin pool. These differences in detection of IBA in different labs and in different samples may reflect biological differences in IBA accumulation under different growth conditions (e.g. media used, light conditions, temperature) or may reflect differences in the methods used to extract and quantify IBA levels, although this seems less likely because recovery of the internal heavy IBA standard seems to be adequate in all reports of IBA quantification, whether endogenous IBA was detected or not. If differing growth conditions are truly the underlying basis for variability in detected IBA, this could lead to potentially interesting insight into how IBA homeostasis is influenced by environmental conditions.

Missing IBA transporters

IBA and IAA appear to use independent transport systems (reviewed in Strader and Bartel, 2011; Michniewicz *et al.*, 2014). Thus far, only two IBA carriers have been reported, ABCG36 (Strader and Bartel, 2009) and ABCG37 (Strader *et al.*, 2008; Růžička *et al.*, 2010). The *arm2* mutant in rice displays decreased IBA uptake and response and unaltered IAA uptake or response (Chhun *et al.*, 2005), suggesting that this mutant is defective in an IBA uptake carrier. Likewise, the *rib1* mutant in Arabidopsis displays IBA resistance and response and unaltered IAA uptake or response (Poupart and Waddell, 2000; Poupart *et al.*, 2005), suggesting that this mutant is also defective in an IBA uptake carrier. Because IBA uptake is a saturable process (Ludwig-Müller *et al.*, 1995*b*; Rashotte *et al.*, 2003), IBA uptake is likely to be a carrier-mediated process. Perhaps identification of the defective gene in *arm2* or *rib1* could provide insight into the molecular basis of IBA uptake. Identification of additional IBA carriers will be instrumental in understanding regulation of IBA homeostasis and contributions to the auxin pool.

Regulation of IBA-derived IAA in the auxin pool

Because IBA-derived auxin plays critical roles in plant development, mechanims to regulate IBA contributions to the auxin pool are likely to exist. Mechanisms to regulate these contributions could include regulated transport, formation and release from conjugates, and transcriptional control of IBA conversion enzymes. Evidence already suggests that regulation of IBA contributions to the auxin pool is important for stress responses. For example, overexpression of *UGT74E2* results in elevated IBA–glucose levels, increased tolerance to drought and salt stress, and increased shoot branching (Tognetti *et al.*, 2010). Similarly, overexpression of *UGT75D1* results in increased tolerance to osmotic stress (Zhang *et al.*, 2016). Additionally, eFP Browser-annotated (Schmid *et al.*, 2005; Winter *et al.*, 2007) expression of genes encoding the IBA conversion enzymes ECH2, IBR1, IBR3, and IBR10, although seemingly unaffected by treatment with various hormones (Fig. 4A), is up-regulated by several biotic (Fig. 4B) and abiotic (Fig. 4C, D) stresses, consistent with the possibility that IBA to IAA conversion plays roles in stress response. Future research will be needed to verify whether IBA conversion genes are indeed regulated by these stresses and to elucidate those conditions in which IBA contributions to the auxin pool affect growth and stress responses.

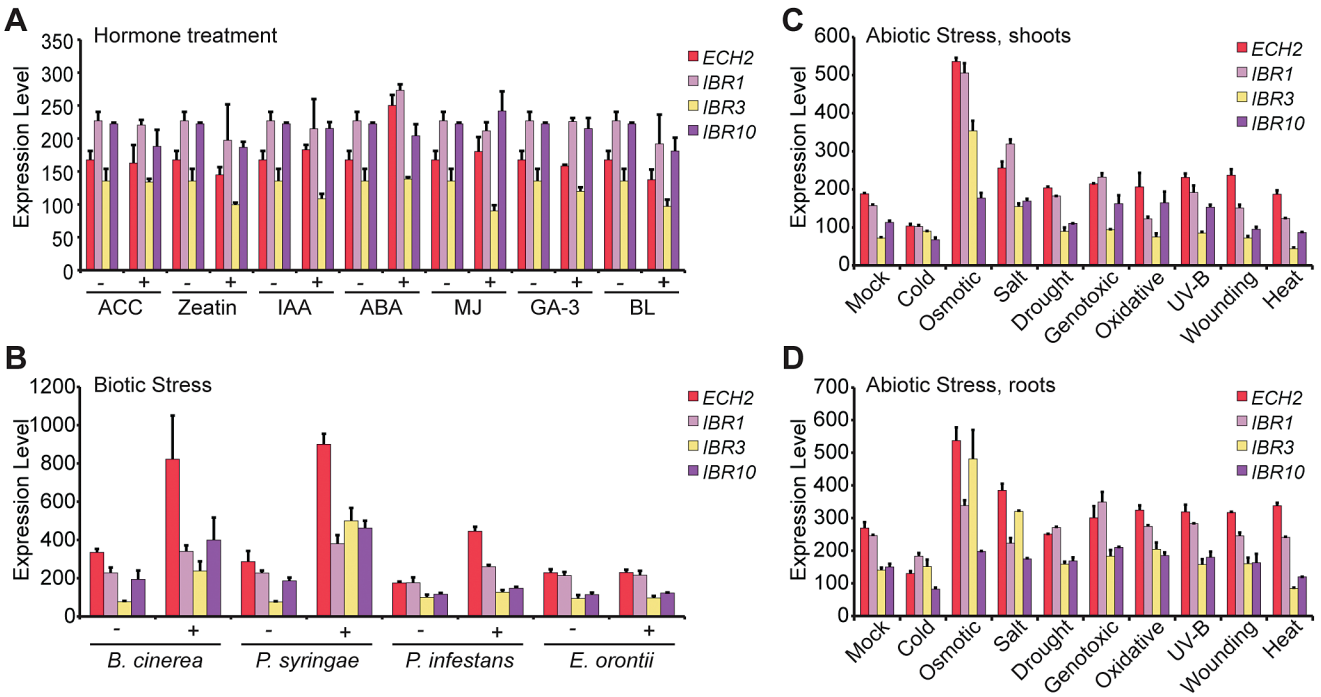

Fig. 4. Genes encoding IBA conversion enzymes are regulated by various stresses. Relative transcript levels of *ECH2*, *IBR1*, *IBR3*, and *IBR10* obtained from the eFP Browser database (Schmid *et al.*, 2005; Winter *et al.*, 2007) in response to (A) hormone treatment, (B) biotic stress, (C) abiotic stress in shoots, or (D) abiotic stress in roots.

IBA activity outside of its conversion to IAA

In the earliest studies of auxinic compounds in rooting and propagation assays, IBA was reported to be more effective than IAA (reviewed in Preece, 2003), causing speculation that IBA itself can act as a signaling molecule (reviewed in Ludwig-Müller, 2000). In addition, IBA is more effective than IAA at inducing crown roots in maize (Martínez-de la Cruz *et al.*, 2015). The *lrt1* mutant in rice displays decreased lateral rooting and decreased gravitropism. Application of IAA rescues the lateral root phenotypes of *lrt1*, but not agravitropic growth, whereas IBA application rescues both the lateral root and gravitropism phenotypes (Chhun *et al.*, 2003), consistent with the possibility that IBA plays some roles that IAA cannot. Additionally, some stress conditions caused increased accumulation of IBA, but not IAA (Ludwig-Müller *et al.*, 1995*c*). Further, inoculation of maize roots with arbuscular mycorrhizal fungi results in elevated IBA, but not IAA levels (Ludwig-Müller *et al.*, 1997). These conditions under which IBA levels are elevated, combined with the potency of IBA in rooting assays, provide some measure of support for roles in which IBA, rather than IBA-derived IAA, might act as a signaling molecule.

In contrast to these reports of roles for IBA as a signaling molecule, genetic data in Arabidopsis suggest that IBA has no discernible activity outside of its conversion to IAA (Zolman *et al.*, 2000, 2007, 2008; Strader *et al.*, 2010, 2011). Further, IBA is unlikely to adopt a conformation for binding into the TIR1–Aux/IAA co-receptor pocket and has no measured binding activity (Uzunova *et al.*, 2016). Potential explanations for the effectiveness of IBA in promoting rooting include the stability of IBA against degradation (Nordström *et al.*, 1991) and effects of nitric oxide produced during the IBA to IAA conversion process (Schlicht *et al.*, 2013), which contribute to lateral root formation. Although data in Arabidopsis are consistent with IBA activity caused by IBA-derived IAA, it remains a formal possibility that IBA could act as a signaling molecule under specialized conditions or in other organisms.

IBA synthesis

We do not currently know the molecular mechanism of IBA synthesis. IBA synthesis from IAA has been demonstrated in microsomal membrane preparations from maize (Ludwig-Müller *et al.*, 1995*a*) or Arabidopsis (Ludwig-Müller, 2007) seedlings when provided with acetyl-CoA and ATP. Identifying the enzymes required for IBA synthesis will be important in understanding IBA biosynthesis and its roles in the plant. Further, generating mutants defective in IBA synthesis will allow for experiments to understand roles for IBA-derived auxin, and perhaps IBA itself, in plant development.

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