Transport and Metabolism of the Endogenous Auxin Precursor Indole-3-Butyric Acid

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ABSTRACT  Plant growth and morphogenesis depend on the levels and distribution of the plant hormone auxin. Plants tightly regulate cellular levels of the active auxin indole-3-acetic acid (IAA) through synthesis, inactivation, and transport. Although the transporters that move IAA into and out of cells are well characterized and play important roles in development, little is known about the transport of IAA precursors. In this review, we discuss the accumulating evidence suggesting that the IAA precursor indole-3-butyric acid (IBA) is transported independently of the characterized IAA transport machinery along with the recent identification of specific IBA efflux carriers and enzymes suggested to metabolize IBA. These studies have revealed important roles for IBA in maintaining IAA levels and distribution within the plant to support normal development.

Key words: Auxin; auxin transport; indole-3-butyric acid; IBA.

INTRODUCTION

Auxin regulates many critical aspects of plant growth and development by directing cell division, elongation, and differentiation (reviewed in Perrot-Rechenmann, 2010). Levels of the active auxin indole-3-acetic acid (IAA) are tightly regulated (reviewed in Woodward and Bartel, 2005; Normanly, 2010), and the contributions of transport and de novo IAA synthesis to auxin homeostasis have been extensively studied. However, the importance of indole-3-butyric acid (IBA) transport and IBA-derived IAA in regulating auxin homeostasis has only recently been revealed.

The auxin precursor indole-3-butyric acid (IBA) was originally discovered as a synthetic compound that induced root initiation in a variety of plants (Zimmerman and Wilcoxon, 1935). IBA and IAA are nearly identical, but IBA carries a four-carbon side chain whereas IAA carries a two-carbon side chain (Figure 1). IBA was found as an endogenous constituent of potato tubers by paper chromatography (Blommaert, 1954), and subsequently has been identified by gas chromatography–mass spectrometry in a variety of plants, including pea, cypress, maize, carrot, tobacco, and Arabidopsis (reviewed in Ludwig-Müller, 2000). The occurrence of IBA in phylogenetically diverse Angiosperms suggests a conserved role for IBA in auxin homeostasis.

Early bioassays demonstrated that IBA application affects not only rooting, but also other auxin-regulated processes such as leaf epinasty, cell division, and stem bending (Zimmerman and Wilcoxon, 1935). These auxin responses, which were often distant from the site of application, hinted at the existence of an IBA transport system. This review explores our current understanding of the roles of IBA transport and IBA-derived IAA in plant development.

IBA TRANSPORT

Long-Distance Transport of IBA

The active auxin IAA moves in several distinct transport streams within the plant (see review by Peer in this issue). In stems, such as the hypocotyl and inflorescence stem, IAA moves basipetally from the apex towards the root. In the root, IAA moves not only acropetally from the root–shoot junction towards the root tip in the stele, but also basipetally from the root tip back towards the shoot in the epidermis.

Early reports suggested that IBA also moves long distances within the plant. However, these early studies relied on bioassays to monitor IBA movement, such as the ability of IBA to promote root formation distant from the application site (Went and White, 1938; Leopold and Lam, 1961; Yang and Davies, 1999). More recent studies using radiolabeled IBA demonstrated both acropetal and basipetal radiolabel movement...
Indole-3-acetic acid (IAA) is a naturally occurring auxin. Indole-3-butyric acid (IBA) is a naturally occurring IAA precursor.

in Arabidopsis stem cuttings (Ludwig-Müller et al., 1995). Interpreting these assays is complicated by the possibility that the transported compound may be IBA or a compound derived from IBA, such as IAA.

More recent studies include demonstrations that most of the IBA remains intact during the timeframe of the transport experiment. For example, in a detailed comparison of [³H]IAA and [³H]IBA transport in Arabidopsis, Rashotte et al. (2003) found that roots transport both IBA and IAA both basipetally and acropetally. Also, similarly to IAA, IBA moves basipetally (towards the root) but not acropetally (towards the apex) in seedling hypocotyls (Rashotte et al., 2003). In inflorescence stems, however, IBA is not transported, whereas IAA moves basipetally (Rashotte et al., 2003). The directional transport of IBA in roots, hypocotyls, and other tissues suggested the existence of active IBA transporters, and the differences between IBA and IAA transport suggested that IBA might use transporters distinct from those used to move IAA.

Many IAA Transporters Do Not Transport IBA

Specialized influx and efflux carriers mediate IAA transport (reviewed in Vieten et al., 2007). Despite the chemical similarity of IAA and IBA (Figure 1), examination of many of these IAA carriers has revealed that they do not transport IBA.

Like IAA uptake, IBA uptake is saturable in Arabidopsis (Ludwig-Müller et al., 1995; Rashotte et al., 2003), suggesting the existence of protein carriers rather than passive diffusion. IAA influx into cells is mediated by the AUXIN RESISTANT1 (AUX1) family of proteins (reviewed in Vieten et al., 2007). The four members of the AUX1 family—AUX1, LIKE AUX1 (LAX1), LAX2, and LAX3—are similar to amino acid permeases and contain 11 transmembrane domains (Figure 2A; Swarup et al., 2004). Because both IAA and the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) are brought into cells by the AUX1 transporter, aux1 loss-of-function mutants are resistant to IAA and 2,4-D (Maher and Martindale, 1980; Yamamoto and Yamamoto, 1998; Marchant et al., 1999; Yang et al., 2006). In contrast, aux1 responds normally to NAA, which is not transported by AUX1 (Yamamoto and Yamamoto, 1998; Marchant et al., 1999; Yang et al., 2006). Although aux1 is moderately resistant to IBA-mediated inhibition of root elongation in long-term (multiple day) assays (Zolman et al., 2000), this resistance is likely due to aux1 resistance to IAA derived from IBA after import by a different carrier, as several lines of evidence suggest that IBA is not an AUX1 substrate. For example, root acropetal and basipetal IBA transport (Rashotte et al., 2003) and IBA accumulation in excised root tips (Strader and Bartel, 2009) are unaltered in aux1 mutants. Moreover, IBA does not competitively inhibit [³H]IAA uptake when AUX1 is heterologously expressed in Xenopus oocytes (Yang et al., 2006). Although it is unlikely that AUX1 is an IBA uptake carrier, other AUX1 family members are potential candidates for this role. For example, when LAX3 is heterologously expressed in Xenopus oocytes, IBA competes with [³H]IAA uptake, although not as well as IAA (Swarup et al., 2008). Whether LAX proteins or some other carriers mediate IBA uptake, the inability of AUX1 to transport IBA indicates that IAA and IBA influx are at least partially distinct.

As with influx, IBA efflux seems to use carriers distinct from those that efflux IAA. Two types of proteins facilitate IAA efflux: PIN-FORMED (PIN) proteins (Figure 2B) and members of the ABCB class of ATP-Binding-Cassette (ABC) transporters (Figure 2C). The eight members of the PIN family localize to either the plasma membrane or the endoplasmic reticulum membrane (reviewed in Grunewald and Friml, 2010). The plasma-membrane PIN proteins often display specific polar localization to a particular cellular face, suggesting that they determine the direction of IAA flow through cells and tissues (reviewed in Grunewald and Friml, 2010). Members of the ABCB/MULTIDRUG RESISTANCE/P-GLYCOPROTEIN family, such as ABCB1, ABCB4, and ABCB19, also localize to the plasma membrane but are more symmetrically localized than PIN proteins and may facilitate non-polar IAA efflux to contribute to long-distance IAA transport, potentially delivering IAA to the PIN proteins (Bandyopadhyay et al., 2007; Blakeslee et al., 2007; Bailly et al., 2008; Mravec et al., 2008).

Neither the PIN family nor the ABCB family appears to facilitate IBA efflux. The polar auxin transport inhibitors naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) decrease auxin transport by blocking auxin efflux carrier complexes (Thomson et al., 1973; Cande and Ray, 1976). Including NPA or TIBA in transport assays effectively blocks polar transport of [³H]IAA, but not [³H]IBA, in roots and hypocotyls (Rashotte et al., 2003), suggesting that NPA- and TIBA-sensitive IAA efflux carriers are not required for IBA efflux. Moreover, auxin efflux deficiency causes pin2 roots to bend into agar containing substrates of the PIN2 effluxer, such as IAA and NAA (Utsuno et al., 1998). However, pin2 roots do not bend to enter IBA-containing agar (Poupard and Waddell, 2000; Zolman et al., 2000), suggesting that IBA is not a PIN2 substrate. In addition, root basipetal IBA transport is unaffected in a pin2 mutant (Rashotte et al., 2003) and PIN2, PIN7, ABCB1, and ABCB19 heterologously expressed in mammalian cells do not transport [³H]IBA (Růžička et al., 2010). Because IBA undergoes long-distance movement, and because none of the tested IAA efflux carriers...
appears to transport IBA, distinct carrier proteins likely facilitate IBA and IAA movement.

**ABCG/PDR IBA EFFLUX CARRIERS**

**ABCG36 and ABCG37 Promote IBA Efflux**

Genetic approaches in *Arabidopsis* have begun to identify molecular components facilitating IBA transport (Table 1). IBA efflux from root cells is promoted by at least two members of the PLEIOTROPIC DRUG RESISTANCE (PDR) subclade of the ABCG family of ABC transporters. Loss of *PDR8*/ABCG36 function increases sensitivity to IBA, but not IAA (Strader and Bartel, 2009). Similarly, loss of *PDR9*/ABCG37 function results in IBA hypersensitivity and wild-type IAA sensitivity (Strader et al., 2008; Ružička et al., 2010). Unlike *abcg36*, however, *abcg37* mutants also are hypersensitive to 2,4-D (Ito and Gray, 2006), and several auxin transport inhibitors (Fujita and Syōno, 1997; Ito and Gray, 2006; Ružička et al., 2010). The IBA hypersensitivity phenotypes of mutants defective in these transporters suggest that IBA

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**Figure 2.** Predicted Topologies of IAA Carrier Proteins that Do Not Transport IBA (A–C) and Proteins Demonstrated (D) or Suggested (E) to Transport IBA.

Schematic diagrams illustrating the predicted topologies of AUX1 (A), PIN2 and PIN7 (B), ABCB1 and ABCB19 (C), ABCG36 and ABCG37 (D), and ABCD1 (E) based on the outputs of ARAMEMNON (http://aramemnon.botanik.uni-koeln.de; Schwacke et al., 2003) and TOPO2 (www.sacs.ucsf.edu/TOPO2). Each amino acid residue is represented by a circle; filled circles represent residues predicted to span the membrane (gray rectangle). Positions of nucleotide-binding domains in the ABC proteins are schematized by ATP hydrolysis. For AUX1, the ARAMEMNON TmConsens prediction of transmembrane domains predicted 10 transmembrane domains with strong scores and one additional transmembrane domain with a weak score. Because AUX1 was experimentally shown to have 11 transmembrane domains (Swarup et al., 2004), all 11 transmembrane domains are depicted in the diagram. We used the ARAMEMNON TmConsens predictions for PIN2, PIN7, ABCB1, ABCB19, and ABCG37 to create the corresponding models, and the ARAMEMNON TmHMM_v2 prediction (Sonnhammer et al., 1998) to create the ABCG36 diagram. The ARAMEMNON MemSat_v3 (Jones et al., 1994) predicted 13 transmembrane domains for ABCD1. However, because the ATPase domains of ABCD1 are cytosolic (Nyathi et al., 2010), we did not include the first predicted transmembrane domain in the ABCD1 diagram.
is a common substrate effluxed by both ABCG36 and ABCG37 (Figure 2D).

Root tips from abcg36 and abcg37 display wild-type [3H]IAA accumulation but hyperaccumulate [3H]IBA in a simplified auxin transport assay (Strader et al., 2008; Strader and Bartel, 2009), suggesting that ABCG36 and ABCG37 promote IBA efflux from root tips. Indeed, abcg36 root tips loaded with [3H]IBA efflux IBA slowly (Strader and Bartel, 2009), and root tips from the abcg36 abcg37 double mutant accumulate even more [3H]IBA than either parent (Růžička et al., 2010), consistent with partially redundant roles for ABCG36 and ABCG37 in promoting IBA efflux.

When expressed in heterologous systems, ABCG37 directly effluxes IBA. Schizosaccharomyces pombe cells expressing ABCG37 accumulate less [3H]IBA than control cells but accumulate [3H]IAA like control cells (Růžička et al., 2010), consistent with abcg37 mutant phenotypes of hyper-responsiveness to IBA and wild-type IAA responsiveness (Ito and Gray, 2006; Strader et al., 2008; Růžička et al., 2010). Additionally, mammalian cells expressing ABCG37 export both [3H]2,4-D and [3H]IBA (Růžička et al., 2010). Although direct demonstration of IBA efflux by heterologously expressed ABCG36 has not been reported, the IBA-hypersensitivity and reduced [3H]IBA efflux from root tips of the abcg36 mutant are consistent with ABCG36 acting as an IBA efflux carrier. Thus, at least two members of the PDR subclade of the ABCG transporters, ABCG36 and ABCG37, are likely to efflux the auxin precursor IBA from root cells.

### ABCG36 and ABCG37 Localize to the Outward Face of Root Epidermal Cells

Consistent with increased [3H]IBA retention in root tips from abcg36 and abcg37 mutants (Strader et al., 2008; Strader and Bartel, 2009), PDR8/ABCG36 and PDR9/ABCG37 localize to the outer face of root epidermal cells (Figure 3) (Strader and Bartel, 2009; Łangowski et al., 2010; Růžička et al., 2010). Because these proteins localize to the outward face of root cells regardless of the position or the apical–basal axis of a cell, they define a new polar region of the cell that has been termed the ‘outer polar domain’ (Łangowski et al., 2010). Intriguingly, maintaining these transporters in the outer polar domain does not require the same molecular components (i.e. the actin cytoskeleton, BFA-sensitive endocytosis, the ARF GEF GNOM, AXR4, the protein kinase PINOID, and the protein phosphatase PP2A) that are required to target IAA transporters to the apical and basal domains of the cell (Łangowski et al., 2010), suggesting the possibility of a novel mechanism that targets ABCG36 and ABCG37 to the proper cellular face. The outward-facing localization of ABCG36 and ABCG37 implies that plants efflux IBA into the rhizosphere.

### Table 1. Arabidopsis Mutants Implicated in IBA Transport.

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<tr>
<th>Mutant</th>
<th>Gene product</th>
<th>Mutant phenotype</th>
<th>References</th>
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<tr>
<td>pdr8/+ pen3 / abcg36</td>
<td>At1g59870 plasma membrane ABC transporter; IBA effluxer</td>
<td>IBA and 2,4-dichlorophenoxybutyric acid (2,4-D) hypersensitivity; reduced IBA efflux from root tips; long root hairs</td>
<td>Strader and Bartel, 2009</td>
</tr>
<tr>
<td>pdr9/+ eta4 / pis1 / abcg37</td>
<td>At3g53480 plasma membrane ABC transporter; IBA effluxer</td>
<td>IBA, 2,4-D, 2,4-D, and TIBA hypersensitivity; reduced IBA efflux from root tips</td>
<td>Ito and Gray, 2006; Strader et al., 2008; Růžička et al., 2010</td>
</tr>
<tr>
<td>pxa1 / cts1 / acn2 / ped3 / abcd1</td>
<td>At4g39850 peroxisomal ABC transporter; moves IBA (and other substrates) into peroxisome</td>
<td>IBA resistance; reduced lateral root formation; decreased filament elongation; fatty acid β-oxidation defects</td>
<td>Hayashi et al., 1998; Zolman et al., 2001; Footitt et al., 2002; Hooks et al., 2004; Footitt et al., 2007</td>
</tr>
<tr>
<td>rib1</td>
<td>Unidentified</td>
<td>IBA and 2,4-D resistance; altered IBA transport; gravity response defects</td>
<td>Poupart and Waddell, 2000; Poupart et al., 2005</td>
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![Figure 3. ABCG37 and ABCG36 Localize to the Outer Polar Domain of Root Epidermal and Lateral Root Cap Cells.](image-url)
rhizosphere. This efflux is a facet of IAA homeostasis (discussed below); whether it also aids in plant communication with soil microbes and/or contributes to long-distance IBA transport remains to be determined. In any case, the outward-facing localization of ABCG36 and ABCG37 (Figure 3) is distinct from the apical/basal localization of the plasma membrane members of the PIN family of IAA effluxers, suggesting that additional components facilitating long-distance IBA movement may remain to be discovered.

Are Additional IBA Effluxers Found in the PDR Clade within the ABCG Family?

Within the 43-member ABCG subfamily of ABC transporters in Arabidopsis, the 15-member PDR group consists of full-sized transporters with two apparent nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs), each consisting of six membrane-spanning sequences (reviewed in van den Brule and Smart, 2002; Crouzet et al., 2006; Verrier et al., 2008). Interestingly, both the ABCB and ABCG subfamilies have full-sized and half-sized members, but only full-sized members of these subfamilies have demonstrated roles in IAA or IBA transport (Figure 2C and 2D). ABCG36 and ABCG37 are only 53% identical at the amino acid level, and yet each is implicated in IBA efflux. Because ABCG36 and ABCG37 are not closely related within the PDR group, and each promotes IBA efflux, additional PDR family members also may efflux IBA. ABCG36 is expressed in many plant organs and ABCG37 is highly expressed in roots (van den Brule and Smart, 2002); other PDR family members have diverse expression patterns (Crouzet et al., 2006), suggesting that if they function in IBA efflux, their roles may be distinct from PDR8 and PDR9 functions in moving IBA out of the root. It will be interesting to learn whether additional ABCG family members are polarly localized, efflux IBA or related molecules, or function in either short- or short-distance IBA transport.

Additional Substrates Transported by the PDR Clade of ABCG Family Members

Although ABCG36 and ABCG37 are implicated in IBA but not IAA efflux, these transporters do appear to be somewhat promiscuous. In addition to IBA hypersensitivity (Strader et al., 2008; Růžička et al., 2010), abcg37 loss-of-function alleles are hypersensitive to 2,4-D, other members of the phenylpropanoid acid family of herbicides (Ito and Gray, 2006), and auxin transport inhibitors (Fujita and Syōno, 1997; Ito and Gray, 2006; Růžička et al., 2010). Root tips from the abcg37 mutant hyperaccumulate not only [3H]IBA (Strader et al., 2008), but also [14C]2,4-D (Ito and Gray, 2006) and [3H]NPA (Ito and Gray, 2006) in simplified auxin transport assays, suggesting that these compounds—a naturally occurring auxin precursor, a synthetic auxin, and an auxin transport inhibitor, respectively—all may be ABCG37 substrates. Indeed, ABCG37 has been directly demonstrated to transport IBA and 2,4-D (Růžička et al., 2010). ABCG36, on the other hand, has been implicated in IBA efflux (Strader and Bartel, 2009), cadmium efflux (Kim et al., 2007), and pathogen response (Kobae et al., 2006; Stein et al., 2006). Additionally, abcg36 mutant shoots display altered callose (Clay et al., 2009), camalexin (Bednarek et al., 2009), and glucosinolate (Bednarek et al., 2009; Clay et al., 2009) accumulation in response to pathogens and their elicitors. Thus, both ABCG36 and ABCG37 are likely to efflux a range of substrates, a trait shared by many members of the PDR family (reviewed in Crouzet et al., 2006).

Beyond the roles in IBA transport demonstrated for ABCG36 and ABCG37, other members of the PDR clade of ABCG family members are implicated in the transport of terpenoids, including the phytohormone abscisic acid (ABA). The first plant PDR family members to be characterized were SpTUR2 from Spirodea polyrrhiza (Smart and Fleming, 1996) and NpPDR1/NpABC1 from Nicotiana plumbaginifolia (Jasinski et al., 2001). SpTUR2 expression is up-regulated by ABA application (Smart and Fleming, 1996) and ectopic SpTUR2 expression in Arabidopsis confers resistance to sclareol, a diterpenoid antifungal agent (van den Brule et al., 2002). NpABC1 protein levels are increased by sclareolide and sclareol, and NpABC1 may promote secretion of these compounds to function in plant defense (Jasinski et al., 2001). AtPDR12/ABCG40, the closest Arabidopsis homolog of SpTUR2 and NpABC1, is also implicated in sclareol extrusion; abcg40 mutants are sclareol-hypersensitive (Campbell et al., 2003). More recently, PDR12/ABCG40 has been identified as a plasma membrane ABA influx carrier; heterologous expression of ABCG40 in yeast and BY2 cells promotes ABA uptake (Kang et al., 2010). abcg40 mutant plants display delayed ABA-responsive gene expression and impaired stress tolerance (Kang et al., 2010). In addition to its roles in transport of the terpenoids sclareol and ABA, ABCG40 is implicated in lead resistance (Lee et al., 2005). Future research will be necessary to disentangle the proposed roles of ABCG40 as both an effluxer of sclareol and lead and an influxer of ABA. The variety of phenotypes displayed by pdr/abcg mutants suggests that members of this family may act as carriers for a range of substrates, including IBA. It remains to be determined whether the apparent promiscuity of these ABCG transporters is regulated in different tissues, during development, or in response to specific environmental conditions.

**IBA-TO-IAA METABOLISM**

**Identification of Candidate IBA β-oxidation enzymes**

Numerous plants shorten the auxin precursor IBA into active IAA (Fawcett et al., 1960; reviewed in Epstein and Ludwig-Müller, 1993) and, at least in Arabidopsis, this metabolism is peroxisome-dependent (Strader et al., 2010). Genetic evidence suggests that auxin activity of IBA in Arabidopsis is completely dependent on its conversion to IAA through a multi-step process similar to fatty acid β-oxidation (Zolman et al., 2000). Indeed, some peroxisomal enzymes, such as the 3-ketoacyl–CoA thiolase encoded by PED1, may act not only in fatty acid
β-oxidation (Hayashi et al., 1998), but also in IBA β-oxidation (Zolman et al., 2000). In contrast, several peroxisomal enzymes appear to be dedicated to IBA β-oxidation; mutations in genes encoding these enzymes confer IBA resistance without altering IAA response or conferring dependence on exogenous fixed carbon sources to fuel growth after germination (Table 2). Candidates for dedicated IBA β-oxidation include 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 ENOYL–COA HYDRATASE2 (ECH2) (Strader et al., 2011). The ibr1 ibr3 ibr10 triple mutant displays additive IBA resistance (Zolman et al., 2008) and converts IBA to IAA inefficiently (Strader et al., 2010). Examination of ibr1, ibr3, ibr10, ech2, and higher-order combinations of these mutants has illuminated diverse roles for IBA-derived IAA in seedling development.

Roles for IBA-Derived IAA in Plant Development

Consistent with a role for IBA efflux, mutants defective in ABCG36 and ABCG37 display developmental phenotypes suggestive of high auxin levels in certain cell types. Root hairs, the tubular outgrowths from specific root cell files, provide a single-cell expansion model that is highly sensitive to auxin response and levels (reviewed in Grierson and Schiefelbein, 2002). abcg36 and abcg37 loss-of-function mutants have lengthened root hairs (Strader and Bartel, 2009; Ružička et al., 2010), suggesting that root hair IAA levels increase when IBA efflux decreases. abcg36 seedlings also display enlarged cotyledons several days after germination (Strader and Bartel, 2009), a second phenotype suggesting increased cell expansion, because post-germinative Arabidopsis cotyledons grow by cell expansion without division (Mansfield and Briarty, 1996). The abcg36 root hair and cotyledon developmental phenotypes both are suppressed when combined with the ibr1, ibr3, and ibr10 mutations (Strader et al., 2010), suggesting that these abcg36 hyper-expansion phenotypes result from increased IBA-derived IAA in these cell types. In contrast to the expanded root hairs and cotyledons of IBA efflux mutants (Strader and Bartel, 2009; Ružička et al., 2010), mutants defective in IBA β-oxidation display reduced root hair and cotyledon cell expansion (Strader et al., 2010, 2011), confirming that IBA-derived IAA, rather than IBA itself, drives cell expansion in these cell types.

Developmental roles for IBA-derived IAA are not limited to cell expansion. ech2 ibr1 ibr3 ibr10 quadruple mutant seedlings display decreased free IAA levels and wide-ranging auxin-related developmental defects (Strader et al., 2011). In addition to defects in cotyledon and root hair cell expansion, ech2 ibr1 ibr3 ibr10 displays delayed development, reduced apical hook curvature, reduced high-temperature-induced hypocotyl elongation, decreased lateral root production, and smaller root meristems, defining multiple seedling developmental processes that depend on IBA-derived IAA. The high-auxin phenotypes displayed by mutants blocked in IBA efflux combined with the low-auxin phenotypes of mutants blocked in IBA-to-IAA conversion suggest that IBA-derived IAA is a significant auxin source during seedling development. In addition, increasing IBA glucosylation by overexpressing the UDP-glucosyltransferase UGT74E2 results in increased shoot branching and improved drought and salt tolerance (Tognetti et al., 2010), implying that roles for IBA are not confined to the seedling stage.

## PXA1/ABCD1 MAY TRANSPORT IBA INTO THE PEROXISOME TO BE METABOLIZED

Because IBA-to-IAA conversion is peroxisomal, a transporter is required to move IBA into the peroxisome. This transporter is likely the peroxisomal ABC transporter PXA1/ABCD1 (Figure 2E), because abcd1 loss-of-function mutants are IBA-resistant (Zolman et al., 2001) and do not efficiently convert IBA to IAA (Strader et al., 2010). In addition to functioning in IBA import into the peroxisome, ABCD1 also likely transports fatty acids (reviewed in Linka and Weber, 2010). As a result, abcd1 mutants β-oxidize seed storage lipids slowly and require exogenous fixed carbon to fuel seedling growth prior to

### Table 2. Arabidopsis Mutants Implicated in IBA Metabolism.

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<tr>
<td>ech2</td>
<td>At1g76150 enoyl–CoA hydratase</td>
<td>IBA resistance</td>
<td>Strader et al., 2011</td>
</tr>
<tr>
<td>ibr1</td>
<td>At4g05530 putative short-chain dehydrogenase/reductase</td>
<td>IBA resistance</td>
<td>Zolman et al., 2000, 2008</td>
</tr>
<tr>
<td>ibr3</td>
<td>At3g06810 putative acyl–CoA dehydrogenase</td>
<td>IBA resistance</td>
<td>Zolman et al., 2000, 2007</td>
</tr>
<tr>
<td>ibr10</td>
<td>At4g14430 putative enoyl–CoA hydratase</td>
<td>IBA resistance</td>
<td>Zolman et al., 2000, 2008</td>
</tr>
<tr>
<td>ped1</td>
<td>At2g33150 3-ketoacyl–CoA thiolase</td>
<td>IBA resistance; fatty acid β-oxidation defects</td>
<td>Hayashi et al., 1998; Zolman et al., 2000</td>
</tr>
<tr>
<td>UGT74E2OE</td>
<td>At1g05680 IBA glucosyltransferase</td>
<td>Overexpression leads to increased IBA glucosylation; increased shoot branching; abiotic stress resistance</td>
<td>Tognetti et al., 2010</td>
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establishment of photosynthesis (Zolman et al., 2001; Footitt et al., 2002; Hayashi et al., 2002). Additionally, ABCD1 is thought to transport jasmonic acid (JA) precursors into the peroxisome; JA levels are reduced in abcd1 mutants (Theodoulou et al., 2005). Although ABCD1 is necessary for IBA response, direct transport assays, perhaps using a recently established yeast system for expressing ABCD1 (Nyathi et al., 2010), will be necessary to demonstrate which abcd1 phenotypes reflect loss of transport of ABCD1 substrates and which, if any, result from indirect effects. In any case, the subset of abcd1 defects that are rescued by auxin application, such as reduced lateral root formation (Zolman et al., 2001) and delayed stamen filament elongation (Footitt et al., 2007), suggest that the reduced IBA-to-IAA conversion observed when this transporter is impaired (Strader et al., 2010) has developmental consequences for the plant.

Because peroxisomal enzymes are required for IBA-to-IAA conversion, it follows that an unidentified IAA transporter is necessary to release IBA-derived IAA out of the peroxisome (Figure 4). Intriguingly, several newly characterized members of the PIN family target to internal membranes, including the endoplasmic reticulum (Mravec et al., 2009; Ganguly et al., 2010). Although no PIN proteins have been demonstrated to be peroxisomal, it will be interesting to learn whether the peroxisomal IAA effluxer is a member of this or some other protein family.

PERSPECTIVES: MISSING IBA TRANSPORTERS

The available evidence suggests that plants use distinct carriers to move IBA and IAA. AUX1 acts as an influx carrier for IAA, but not IBA. Similarly, PIN2, PIN7, ABCB1, and ABCB19 act as efflux carriers for IAA, but not IBA. Conversely, the PDR family proteins ABCG36 and ABCG37 appear to efflux IBA, but not IAA. These independent transport systems may provide a mechanism to specifically move an inactive precursor, thus avoiding auxin responses during transport (Figure 4). Once in a target cell, IBA β-oxidation to active IAA would allow for auxin responses.

Despite the recent progress, much remains unknown about IBA transport and metabolism. For example, how does IBA enter cells? IBA influx appears to be carrier-assisted. A possible carrier may be the unidentified RIB1, as rib1 mutants are IBA-resistant (Poupart and Waddell, 2000) and display altered IBA transport and normal IAA transport (Poupart et al., 2005). Once in the cell, IBA can be effluxed, conjugated for storage, or β-oxidized to IAA to provide active auxin activity. Peroxisomally localized candidate enzymes have been identified for β-oxidation of IBA–CoA to IAA–CoA (Table 2), but none of these enzymes has been biochemically characterized, and candidates have not emerged for the IBA–CoA ligase or IAA–CoA hydrolysis steps. Also, are there additional IBA efflux carriers? Because ABCG36 and ABCG37 localize to the outer polar domain and appear to function in auxin homeostasis to limit IAA levels, additional IBA efflux carriers may facilitate long-distance IBA transport. It remains to be determined whether these unidentified IBA effluxers will be other members of the ABCG family or members of the PIN or ABCB families that have not yet been tested with IBA. Finally, how is IBA efflux regulated? The intricate regulation of IAA efflux carrier levels and positioning is essential for normal development; it remains to be seen whether disturbing the polarity of IBA efflux carriers, such as the unusual outer polar domain of ABCG36 and ABCG37 in root epidermal cells, has developmental consequences. It seems certain that increasing our understanding of IBA transport and metabolism will continue to yield new insights into the complex mechanisms used by plants to control auxin homeostasis.

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