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

Lucia C. Strader and **Bonnie Bartel**[1]

Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005, USA

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

[1] To whom correspondence should be addressed. E-mail bartel@rice.edu, fax 713-348-5154, tel. 713-348-5602.

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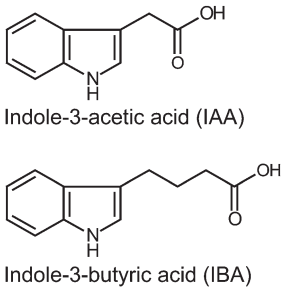

Figure 1. IAA and IBA.
Indole-3-acetic acid (IAA) is a naturally occurring auxin. Indole-3-butyric acid (IBA) is a naturally occurring IAA precursor.

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 [^{3}H]IAA and [^{3}H]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 [^{3}H]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 [^{3}H]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 [^{3}H]IAA, but not [^{3}H]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 (Poupart 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 [^{3}H]IBA (Růžička et al., 2010). Because IBA undergoes long-distance movement, and because none of the tested IAA efflux carriers

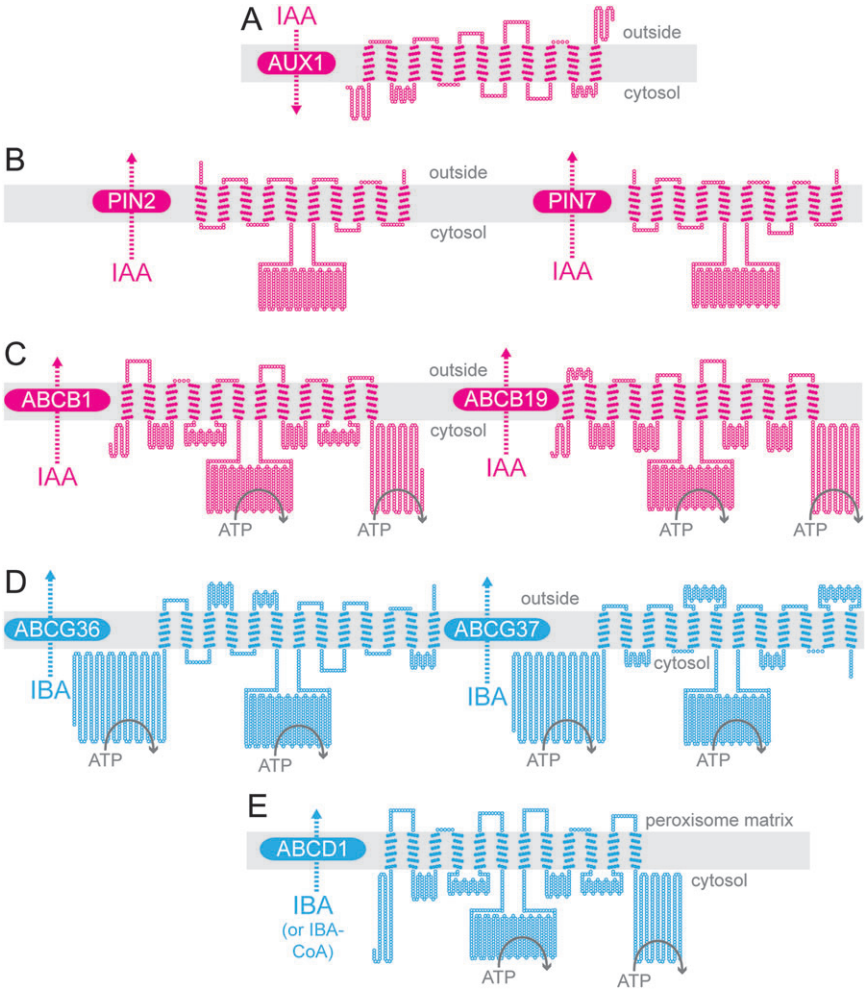

Figure 2. Predicted Topologies of IAA Carrier Proteins that Do Not Transport IBA (A–C) and Proteins Demonstrated (D) or Suggested (E) to Transport IBA.

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.

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; Růžička 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; Růžička et al., 2010). The IBA hypersensitivity phenotypes of mutants defective in these transporters suggest that IBA

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

Mutant	Gene product	Mutant phenotype	References
pdr8 / pen3 / abcg36	At1g59870 plasma membrane ABC transporter; IBA effluxer	IBA and 2,4-dichlorophenoxybutyric acid (2,4-DB) hypersensitivity; reduced IBA efflux from root tips; long root hairs	Strader and Bartel, 2009
pdr9 / eta4 / pis1 / abcg37	At3g53480 plasma membrane ABC transporter; IBA effluxer	IBA, 2,4-D, 2,4-DB, and TIBA hypersensitivity; reduced IBA efflux from root tips	Ito and Gray, 2006; Strader et al., 2008; Růžička et al., 2010
pxa1 / cts1 / acn2 / ped3 / abcd1	At4g39850 peroxisomal ABC transporter; moves IBA (and other substrates) into peroxisome	IBA resistance; reduced lateral root formation; decreased filament elongation; fatty acid β-oxidation defects	Hayashi et al., 1998; Zolman et al., 2001; Footitt et al., 2002; Hooks et al., 2004; Footitt et al., 2007
rib1	Unidentified	IBA and 2,4-D resistance; altered IBA transport; gravity response defects	Poupart and Waddell, 2000; Poupart et al., 2005

is a common substrate effluxed by both ABCG36 and ABCG37 (Figure 2D).

Root tips from *abcg36* and *abcg37* display wild-type [^{3}H]IAA accumulation but hyperaccumulate [^{3}H]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 [^{3}H]IBA efflux IBA slowly (Strader and Bartel, 2009), and root tips from the *abcg36 abcg37* double mutant accumulate even more [^{3}H]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 [^{3}H]IBA than control cells but accumulate [^{3}H]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 [^{3}H]2,4-D and [^{3}H]IBA (Růžička et al., 2010). Although direct demonstration of IBA efflux by heterologously expressed ABCG36 has not been reported, the IBA-hypersensitivity and reduced [^{3}H]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.

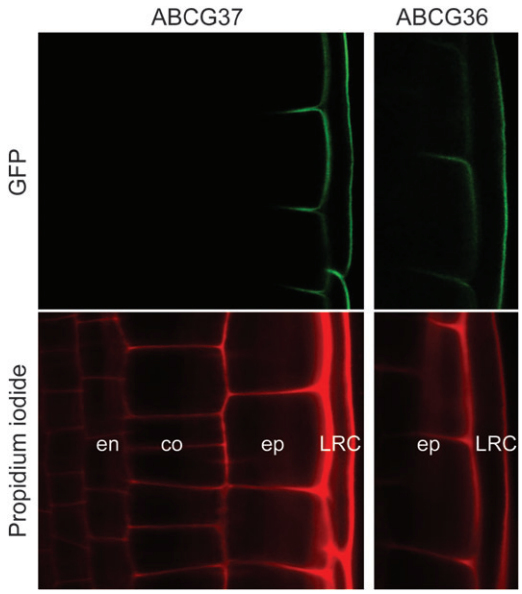

Figure 3. ABCG37 and ABCG36 Localize to the Outer Polar Domain of Root Epidermal and Lateral Root Cap Cells.

Confocal images of root tips of 5-day-old seedlings carrying *GFP–ABCG37* (*pis1-1* carrying *35S:GFP–ABCG37*) (Łangowski et al., 2010) and *ABCG36–GFP* (*pen3-1* carrying *PEN3:PEN3–GFP*) (Stein et al., 2006). The upper panels show GFP signal and the lower panels show propidium iodide staining of endodermal (en), cortex (co), epidermal (ep), and lateral root cap (LRC) cell walls. Both GFP–ABCG37 (Łangowski et al., 2010; Růžička et al., 2010) and ABCG36–GFP (Strader and Bartel, 2009) accumulate on the outward face of epidermal and lateral root cap cells, a polar domain termed the 'outer polar domain' (Łangowski et al., 2010), implying that these transporters move IBA from the root into the rhizosphere.

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

Consistent with increased [^{3}H]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. 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 long- 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 phenoxyalkanoic 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 [^{3}H]IBA (Strader et al., 2008), but also [^{14}C]2,4-D (Ito and Gray, 2006) and [^{3}H]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 *Spirodela 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, also is 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; Růžička 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; Růžička 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.

Mutant	Gene product	Mutant phenotype	References
ech2	At1g76150 enoyl–CoA hydratase	IBA resistance	Strader et al., 2011
ibr1	At4g05530 putative short-chain dehydrogenase/ reductase	IBA resistance	Zolman et al., 2000, 2008
ibr3	At3g06810 putative acyl–CoA dehydrogenase	IBA resistance	Zolman et al., 2000, 2007
ibr10	At4g14430 putative enoyl–CoA hydratase	IBA resistance	Zolman et al., 2000, 2008
ped1	At2g33150 3-ketoacyl–CoA thiolase	IBA resistance; fatty acid β-oxidation defects	Hayashi et al., 1998; Zolman et al., 2000
UGT74E2OE	At1g05680 IBA glucosyltransferase	Overexpression leads to increased IBA glucosylation; increased shoot branching; abiotic stress resistance	Tognetti et al., 2010

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-oxidization to active IAA would allow for auxin responses.

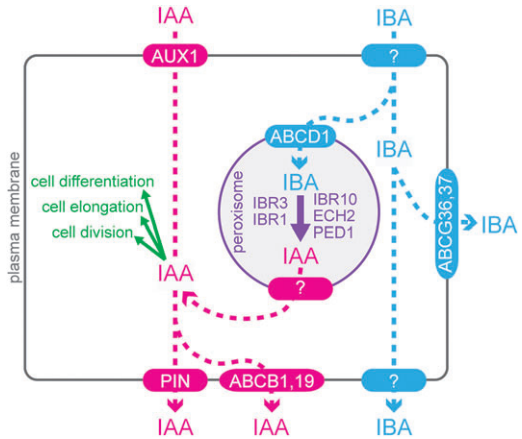

Figure 4. Cellular Model Showing Distinct IAA and IBA Transporters.

The auxin activity of IBA requires its conversion to IAA in peroxisomes, probably by β-oxidation enzymes encoded by *IBR1*, *IBR3*, *IBR10*, *ECH2*, and *PED1*.

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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REFERENCES

Bailly, A., Sovero, V., Vincenzetti, V., Santelia, D., Bartnik, D., Koenig, B.W., Mancuso, S., Martinoia, E., and Geisler, M.

(2008). Modulation of P-glycoproteins by auxin transport inhibitors is mediated by interaction with immunophilins. J. Biol. Chem. **283,** 21817–21826.

Bandyopadhyay, A., et al. (2007). Interactions of PIN and PGP auxin transport mechanisms. Biochem. Soc. Trans. **35,** 137–141.

Bednarek, P., et al. (2009). A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science. **323,** 101–106.

Blakeslee, J.J., et al. (2007). Interactions among PIN-FORMED and P-glycoprotein auxin transporters in *Arabidopsis*. Plant Cell. **19,** 131–147.

Blommaert, K. (1954). Growth- and inhibiting-substances in relation to the rest period of the potato tuber. Nature. **174,** 970–972.

Campbell, E.J., Schenk, P.M., Kazan, K., Penninckx, I.A., Anderson, J.P., Maclean, D.J., Cammue, B.P., Ebert, P.R., and Manners, J.M. (2003). Pathogen-responsive expression of a putative ATP-binding cassette transporter gene conferring resistance to the diterpenoid sclareol is regulated by multiple defense signaling pathways in *Arabidopsis*. Plant Physiol. **133,** 1272–1284.

Cande, W.Z., and Ray, P.M. (1976). Nature of cell-to-cell transfer of auxin in polar transport. Planta. **129,** 43–52.

Clay, N.K., Adio, A.M., Denoux, C., Jander, G., and Ausubel, F.M. (2009). Glucosinolate metabolites required for an *Arabidopsis* innate immune response. Science. **323,** 95–101.

Crouzet, J., Trombik, T., Fraysse, A.S., and Boutry, M. (2006). Organization and function of the plant pleiotropic drug resistance ABC transporter family. FEBS Lett. **580,** 1123–1130.

Epstein, E., and Ludwig-Müller, J. (1993). Indole-3-butyric acid in plants: occurrence, synthesis, metabolism, and transport. Physiol. Plant. **88,** 382–389.

Fawcett, C.H., Wain, R.L., and Wightman, F. (1960). The metabolism of 3-indolylalkanecarboxylic acids, and their amides, nitriles and methyl esters in plant tissues. Proc. Royal Soc. London, Series B. **152,** 231–254.

Footitt, S., Dietrich, D., Fait, A., Fernie, A.R., Holdsworth, M.J., Baker, A., and Theodoulou, F.L. (2007). The COMATOSE ATP-binding cassette transporter is required for full fertility in *Arabidopsis*. Plant Physiol. **144,** 1467–1480.

Footitt, S., Slocombe, S.P., Larner, V., Kurup, S., Wu, Y., Larson, T., Graham, I., Baker, A., and Holdsworth, M. (2002). Control of germination and lipid mobilization by *COMATOSE*, the *Arabidopsis* homologue of human ALDP. EMBO J. **21,** 2912–2922.

Fujita, H., and Syōno, K. (1997). PIS1, a negative regulator of the action of auxin transport inhibitors in *Arabidopsis thaliana*. Plant J. **12,** 583–595.

Ganguly, A., Lee, S.H., Cho, M., Lee, O.R., Yoo, H., and Cho, H.T. (2010). Differential auxin-transporting activities of PIN-FORMED proteins in *Arabidopsis* root hair cells. Plant Physiol. **153,** 1046–1061.

Grierson, C., and Schiefelbein, J. (2002). Root Hairs: April 4, 2002. The Arabidopsis Book (Rockville, MD: American Society of Plant Biologists) doi: 10.1199/tab.0060:), www.aspb.org/publications/Arabidopsis/.

Grunewald, W., and Friml, J. (2010). The march of the PINs: developmental plasticity by dynamic polar targeting in plant cells. EMBO J. **29,** 2700–2714.

Hayashi, H., Nito, K., Takei-Hoshi, R., Yagi, M., Kondo, M., Suenaga, A., Yamaya, T., and Nishimura, M. (2002). Ped3p is a peroxisomal ATP-binding cassette transporter that might supply substrates for fatty acid β-oxidation. Plant Cell Physiol. **43,** 1–11.

Hayashi, M., Toriyama, K., Kondo, M., and Nishimura, M. (1998). 2,4-dichlorophenoxybutyric acid-resistant mutants of *Arabidopsis* have defects in glyoxysomal fatty acid β-oxidation. Plant Cell. **10,** 183–195.

Hooks, M.A., Turner, J.E., Murphy, E.C., and Graham, I.A. (2004). Acetate non-utilizing mutants of *Arabidopsis*: evidence that organic acids influence carbohydrate perception in germinating seedlings. Mol. Genet. Genomics. **271,** 249–256.

Ito, H., and Gray, W.M. (2006). A gain-of-function mutation in the *Arabidopsis* pleiotropic drug resistance transporter PDR9 confers resistance to auxinic herbicides. Plant Physiol. **142,** 63–74.

Jasinski, M., Stukkens, Y., Degand, H., Purnelle, B., Marchand-Brynaert, J., and Boutry, M. (2001). A plant plasma membrane ATP binding cassette-type transporter is involved in antifungal terpenoid secretion. Plant Cell. **13,** 1095–1107.

Jones, D.T., Taylor, W.R., and Thornton, J.M. (1994). A model recognition approach to the prediction of all-helical membrane protein structure and topology. Biochemistry. **33,** 3038–3049.

Kang, J., Hwang, J.U., Lee, M., Kim, Y.Y., Assmann, S.M., Martinoia, E., and Lee, Y. (2010). PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc. Natl Acad. Sci. U S A. **107,** 2355–2360.

Kim, D.Y., Bovet, L., Maeshima, M., Martinoia, E., and Lee, Y. (2007). The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J. **50,** 207–218.

Kobae, Y., Sekino, T., Yoshioka, H., Nakagawa, T., Martinoia, E., and Maeshima, M. (2006). Loss of AtPDR8, a plasma membrane ABC transporter of *Arabidopsis thaliana*, causes hypersensitive cell death upon pathogen infection. Plant Cell Physiol. **47,** 309–318.

Łangowski, L., Růžička, K., Naramoto, S., Kleine-Vehn, J., and Friml, J. (2010). Trafficking to the outer polar domain defines the root–soil interface. Curr. Biol. **20,** 904–908.

Lee, M., Lee, K., Lee, J., Noh, E.W., and Lee, Y. (2005). AtPDR12 contributes to lead resistance in *Arabidopsis*. Plant Physiol. **138,** 827–836.

Leopold, A.C., and Lam, S.L. (1961). Polar transport of three auxins. In 4th International Conference on Plant Growth Regulators, Klein R.M., ed. (Iowa), pp. 411–418.

Linka, N., and Weber, A.P. (2010). Intracellular metabolite transporters in plants. Mol Plant. **3,** 21–53.

Ludwig-Müller, J. (2000). Indole-3-butyric acid in plant growth and development. Plant Growth Regul. **32,** 219–230.

Ludwig-Müller, J., Raisig, A., and Hilgenberg, W. (1995). Uptake and transport of indole-3-butyric acid in *Arabidopsis thaliana*: comparison with other natural and synthetic auxins. J. Plant Physiol. **147,** 351–354.

Maher, E.P., and Martindale, S.J.B. (1980). Mutants of *Arabidopsis thaliana* with altered responses to auxins and gravity. Biochem. Genet. **18,** 1041–1053.

Mansfield, S.G., and Briarty, L.G. (1996). The dynamics of seedling and cotyledon cell development in *Arabidopsis thaliana* during reserve mobilization. Int. J. Plant Sci. **157,** 280–295.

Marchant, A., Kargul, J., May, S.T., Muller, P., Delbarre, A., Perrot-Rechenmann, C., and Bennett, M.J. (1999). AUX1 regulates root gravitropism in *Arabidopsis* by facilitating auxin uptake within root apical tissues. EMBO J. **18,** 2066–2073.

Mravec, J., et al. (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature. **459,** 1136–1140.

Mravec, J., Kubes, M., Bielach, A., Gaykova, V., Petrasek, J., Skupa, P., Chand, S., Benkova, E., Zazimalova, E., and Friml, J. (2008). Interaction of PIN and PGP transport mechanisms in auxin distribution-dependent development. Development. **135,** 3345–3354.

Normanly, J. (2010). Approaching cellular and molecular resolution of auxin biosynthesis and metabolism. Cold Spring Harb. Perspect Biol. **2,** a001594.

Nyathi, Y., De Marcos Lousa, C., van Roermund, C.W., Wanders, R.J., Johnson, B., Baldwin, S.A., Theodoulou, F.L., and Baker, A. (2010). The *Arabidopsis* peroxisomal ABC transporter, comatose, complements the *Saccharomyces cerevisiae pxa1 pxa2*Δ mutant for metabolism of long-chain fatty acids and exhibits fatty acyl–CoA-stimulated ATPase activity. J. Biol. Chem. **285,** 29892–29902.

Perrot-Rechenmann, C. (2010). Cellular responses to auxin: division versus expansion. Cold Spring Harb. Perspect. Biol. **2,** a001446.

Poupart, J., and Waddell, C.S. (2000). The *rib1* mutant is resistant to indole-3-butyric acid, an endogenous auxin in *Arabidopsis*. Plant Physiol. **124,** 1739–1751.

Poupart, J., Rashotte, A.M., Muday, G.K., and Waddell, C.S. (2005). The *rib1* mutant of *Arabidopsis* has alterations in indole-3-butyric acid transport, hypocotyl elongation, and root architecture. Plant Physiol. **139,** 1460–1471.

Rashotte, A.M., Poupart, J., Waddell, C.S., and Muday, G.K. (2003). Transport of the two natural auxins, indole-3-butyric acid and indole-3-acetic acid, in *Arabidopsis*. Plant Physiol. **133,** 761–772.

Růžička, K., et al. (2010). *Arabidopsis PIS1* encodes the ABCG37 transporter of auxinic compounds including the auxin precursor indole-3-butyric acid. Proc. Natl Acad. Sci. U S A. **107,** 10749–10753.

Schwacke, R., Schneider, A., van der Graaff, E., Fischer, K., Catoni, E., Desimone, M., Frommer, W.B., Flügge, U.I., and Kunze, R. (2003). ARAMEMNON, a novel database for *Arabidopsis* integral membrane proteins. Plant Physiol. **131,** 16–26.

Smart, C.C., and Fleming, A.J. (1996). Hormonal and environmental regulation of a plant PDR5-like ABC transporter. J. Biol. Chem. **271,** 19351–19357.

Sonnhammer, E.L., von Heijne, G., and Krogh, A. (1998). A hidden Markov model for predicting transmembrane helices in protein sequences. Proc. Int. Conf. Intell. Syst. Mol. Biol. **6,** 175–182.

Stein, M., Dittgen, J., Sanchez-Rodriguez, C., Hou, B.H., Molina, A., Schulze-Lefert, P., Lipka, V., and Somerville, S. (2006). *Arabidopsis* PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell. **18,** 731–746.

Strader, L.C., and Bartel, B. (2009). The *Arabidopsis* PLEIOTROPIC DRUG RESISTANCE8/ABCG36 ATP binding cassette transporter modulates sensitivity to the auxin precursor indole-3-butyric acid. Plant Cell. **21,** 1992–2007.

Strader, L.C., Hendrickson Culler, A., Cohen, J.D., and Bartel, B. (2010). Conversion of endogenous indole-3-butyric acid to indole-3-acetic acid drives cell expansion in *Arabidopsis* seedlings. Plant Physiol. **153,** 1577–1586.

Strader, L.C., Monroe-Augustus, M., Rogers, K.C., Lin, G.L., and Bartel, B. (2008). *Arabidopsis iba response5* (*ibr5*) suppressors separate responses to various hormones. Genetics. **180,** 2019–2031.

Strader, L.C., Wheeler, D.L., Christensen, S.E., Berens, J.C., Cohen, J.D., Rampey, R.A., and Bartel, B. (2011). Multiple facets of *Arabidopsis* seedling development require indole-3-butyric acid-derived auxin. Plant Cell, in press.

Swarup, K., et al. (2008). The auxin influx carrier LAX3 promotes lateral root emergence. Nat. Cell Biol. **10,** 946–954.

Swarup, R., et al. (2004). Structure–function analysis of the presumptive *Arabidopsis* auxin permease AUX1. Plant Cell. **16,** 3069–3083.

Theodoulou, F.L., Job, K., Slocombe, S.P., Footitt, S., Holdsworth, M., Baker, A., Larson, T.R., and Graham, I.A. (2005). Jasmonic acid levels are reduced in *COMATOSE* ATP-binding cassette transporter mutants: implications for transport of jasmonate precursors into peroxisomes. Plant Physiol. **137,** 835–840.

Thomson, K.-S., Hertel, R., and Müller, S. (1973). 1-N-naphthylphthalamic acid and 2,3,4-triiodobenzoic acid. Planta. **109,** 337–352.

Tognetti, V.B., et al. (2010). Perturbation of indole-3-butyric acid homeostasis by the UDP-glucosyltransferase *UGT74E2* modulates *Arabidopsis* architecture and water stress tolerance. Plant Cell. **22,** 2660–2679.

Utsuno, K., Shikanai, T., Yamada, Y., and Hashimoto, T. (1998). *AGR*, an *agravitropic* locus of *Arabidopsis thaliana*, encodes a novel membrane-protein family member. Plant Cell Physiol. **39,** 1111–1118.

van den Brule, S., and Smart, C.C. (2002). The plant PDR family of ABC transporters. Planta. **216,** 95–106.

van den Brule, S., Muller, A., Fleming, A.J., and Smart, C.C. (2002). The ABC transporter SpTUR2 confers resistance to the antifungal diterpene sclareol. Plant J. **30,** 649–662.

Verrier, P.J., et al. (2008). Plant ABC proteins: a unified nomenclature and updated inventory. Trends Plant Sci. **13,** 151–159.

Vieten, A., Sauer, M., Brewer, P.B., and Friml, J. (2007). Molecular and cellular aspects of auxin-transport-mediated development. Trends Plant Sci. **12,** 160–168.

Went, F., and White, R. (1938). Experiments of the transport of auxin. Bot. Gaz. **100,** 465–484.

Woodward, A.W., and Bartel, B. (2005). Auxin: regulation, action, and interaction. Ann. Bot. **95,** 707–735.

Yamamoto, M., and Yamamoto, K.T. (1998). Differential effects of 1-naphthaleneacetic acid, indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid on the gravitropic response of roots in an auxin-resistant mutant of *Arabidopsis, aux1*. Plant Cell Physiol. **39,** 660–664.

Yang, T., and Davies, P.J. (1999). Promotion of stem elongation by indole-3-butyric acid in intact plants of *Pisum sativum* L. Plant Growth Regul. **27,** 157–160.

Yang, Y., Hammes, U.Z., Taylor, C.G., Schachtman, D.P., and Nielsen, E. (2006). High-affinity auxin transport by the AUX1 influx carrier protein. Curr. Biol. **16,** 1123–1127.

Zimmerman, P.W., and Wilcoxon, F. (1935). Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7,** 209–229.

Zolman, B.K., Martinez, N., Millius, A., Adham, A.R., and Bartel, B. (2008). Identification and characterization of *Arabidopsis* indole-3-butyric acid response mutants defective in novel peroxisomal enzymes. Genetics. **180,** 237–251.

Zolman, B.K., Nyberg, M., and Bartel, B. (2007). IBR3, a novel peroxisomal acyl–CoA dehydrogenase-like protein required for indole-3-butyric acid response. Plant Mol. Biol. **64,** 59–72.

Zolman, B.K., Silva, I.D., and Bartel, B. (2001). The *Arabidopsis pxa1* mutant is defective in an ATP-binding cassette transporter-like protein required for peroxisomal fatty acid β-oxidation. Plant Physiol. **127,** 1266–1278.

Zolman, B.K., Yoder, A., and Bartel, B. (2000). Genetic analysis of indole-3-butyric acid responses in *Arabidopsis thaliana* reveals four mutant classes. Genetics. **156,** 1323–1337.